ACTA CHEMICA SCANDINAVICA 4 (1950) 1–26

Kinetische Untersuchungen über die Umwandlung von 1,6-Dinitrito- in 1,6-Dinitrodipyridindiamminkobalt(III)-nitrat in festem Zustande

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Werner [1] hat vor etwa 40 Jahren gezeigt, dass von den 1,6-Dinitritodipyridindiamminsalzen, die neu dargestellt als mehr oder weniger rote Kristalle auftreten, das Bromid, Jodid, Nitrat und Dithionat beim Aufbewahren bei Zimmertemperatur in trocknem, festem Zustande allmählich ihre Farbe verändern und mit der Zeit immer gelber werden. Das hängt nach Werner mit der Isomerisation

$$\left[\begin{matrix}(1)\ \mathrm{ONO} & & \mathrm{py_2} \\ & \mathrm{Co} & \\ (6)\ \mathrm{ONO} & & (\mathrm{NH_3})_2\end{matrix}\right] \mathrm{X} \rightarrow \left[\begin{matrix}(1)\ \mathrm{O_2N} & & \mathrm{py_2} \\ & \mathrm{Co} & \\ (6)\ \mathrm{O_2N} & & (\mathrm{NH_3})_2\end{matrix}\right] \mathrm{X} \qquad (1)$$

zusammen. Nur bei dem Rhodanid fand er keine merkliche Farbenänderung.

Die Zeit zur vollständigen Isomerisation der obigen unbeständigen Dinitritosalze bei Zimmertemperatur wurde von Werner auf einige Wochen geschätzt. Die stabilen gelben Isomeren konnten durch Erhitzen während 1 bis 1 ½ Stunden auf 60° im Trockenschrank dargestellt und durch Umkristallisation gereinigt werden.

Mit der vorliegenden Arbeit wurde eine möglichst genaue Bestimmung der Geschwindigkeit der erwähnten Umwandlung bei dem festen Nitrat bezweckt. Sie wurde extinktiometrisch mit Hilfe von Licht der Wellenlänge $\lambda = 546$ mμ und bei den Temperaturen 20°, 30° und 40° C durchgeführt. Wenn man von immer älteren Proben des Salzes eine Lösung gegebener Konzentration bereitet, so nimmt nämlich die Extinktion der Lösung bei konstanter Schichtdicke mit der Zeit ab. Die Resultate waren mit denjenigen früherer

Untersuchungen über die Farbenänderungs- und Isomerisationsgeschwindigkeit von festem Nitritopentamminkobalt(III)-chlorid und -nitrat zu vergleichen [2, 3].

1. DIE VERWENDETE APPARATUR

Zur Mehrzahl der Extinktionsmessungen wurde die in einer vorigen Arbeit [4] beschriebene Apparatur mit zwei möglichst gleichen in Kompensationsschaltung arbeitenden Multiplikatorphotozellen benutzt. Zur Monochromatisierung diente ein Schottsches Didymglasfilter für die fragliche Wellenlänge. Da die in der erwähnten Arbeit besprochenen Philips' Zellen 3520 nicht mehr zu beschaffen waren, wurden sie mit zwei amerikanischen Multiplikatorphotozellen vom Typus RCA 1P 22 ersetzt. Diese fordern aber eine bedeutend höhere Betriebsanodenspannung (1250 Volt) und haben eine ganz andere Elektrodenanordnung (9-Stufentypus) als die früher verwendeten. Deshalb wurde ein neuer Spannungsstabilisator und Spannungsverteiler gebaut. Dessen Konstruktion verdankt unser Institut Herrn Assistenten L. Stigmark.

Um bei den neuen Photozellen die Proportionalität zwischen Photostrom und Lichtstärke zu prüfen, wurde mit ihnen die Abbleichungsgeschwindigkeit von Phenolphthalein in starker Natronlauge bei 20° C bestimmt. Die Messmethodik und die Versuchslösung (0.0003 *M* Phenolphthalein + 0,100 *M* NaOH + 0.100 *M* Na_2SO_4) wie die Wellenlänge $\lambda = 436$ mμ waren dieselben wie die von Bodforss und Adell [4] benutzten. Die Reaktion zwischen Phenolphthalein und Hydroxylionen führt zu einem Gleichgewichtszustande. Wenn dieser erreicht ist, sei die Extinktion der Versuchslösung bei der fraglichen Schichtdicke (hier 2,6 cm) gleich E_j. Für zwei zu den Zeitpunkten t_1 und t_2 gemessene Extinktionswerte E_1 und E_2 derselben Versuchslösung soll dann der Zusammenhang

$$k = \frac{1}{t_2 - t_1} \cdot \log \frac{E_1 - E_j}{E_2 - E_j} \tag{2}$$

gelten. k ist die Summe der monomolekularen Konstanten für die beiden entgegengesetzten Reaktionsrichtungen (Vgl. Bodforss und Leden [5]). Um die Gültigkeit der Gleichung (2) graphisch zu prüfen, kann man sie besser in der Form

$$\log\ (E - E_j) = \log\ (E_o - E_j) - k \cdot t \tag{3}$$

schreiben, wo E_o und E die zu den Zeitpunkten 0 und t gehörigen Extinktionswerte sind. $\log\ (E_o - E_j)$ soll somit eine Konstante und $\log\ (E - E_j)$ eine lineare Funktion von t sein.

Die Extinktion E der Versuchslösung wurde mit Intervallen von 1—2 Minuten bestimmt, und E_j konnte als der Grenzwert von E nach einigen Stunden gemessen werden. Es zeigte sich, dass die zusammengehörigen Werte von $\log\ (E - E_j)$ und t graphisch Punkte gaben, die sehr nahe an einer Gerade mit der Gleichung

$$\log\ (E - 0{,}1223) = 0{,}6612 - 1 - 0{,}0366 \cdot t \tag{4}$$

lagen. t war dabei in Minuten gerechnet.

In Tabelle 1 sind die nach (4) berechneten E-Werte mit den entsprechenden gefundenen zusammengestellt.

Tabelle 1. Die Abbleichung des Phenolphthaleins bei 20° C in der Lösung: 0,0003 M Phenolphthalein + 0,100 M NaOH + 0,100 M Na_2SO_4. λ = 436 mμ.

Zeit t in Min.	E		Zeit t in Min.	E	
	nach(4)ber.	gef.		nach(4)ber.	gef.
0	0,581	0,585	18,08	0,222	0,223
1,16	0,538	0,537	19,25	0,213	0,213
6,25	0,393	0,389	20,35	0,205	0,206
7,49	0,366	0,365	22,43	0,192	0,192
8,89	0,336	0,341	23,58	0,185	0,185
10,00	0,320	0,322	24,62	0,180	0,179
11,09	0,302	0,304	25,79	0,174	0,173
13,58	0,268	0,269	28,06	0,165	0,164
14,66	0,256	0,258	29,48	0,160	0,161
15,73	0,244	0,243			

Die Konstante k = 0,0366 in Gleichung (4) liegt der aus der Arbeit von Bodforss und Adell [4] hervorgehenden k = 0,0361 sehr nahe. Aus der guten Übereinstimmung der zusammengehörigen gefundenen und berechneten E-Werte der Tabelle 1 wurde auf eine genügende Proportionalität zwischen Lichtintensität und Photostrom der Zellen geschlossen.

Bei feuchtem und warmem Wetter (wie im Mai 1949) zeigte es sich aber schwierig, die Luftfeuchtigkeit in dem verhältnismässig kalten Arbeitszimmer mit der konstanten Temperatur 20,0° C hinreichend niedrig zu halten, und Isolationsschwierigkeiten konnten da eine erhebliche Unsicherkeit der Extinktionsmessungen mit diesem Apparate verursachen.

Dann wurden diese Messungen statt dessen mit Hilfe eines Beckman-Quarz-Spektrophotometers (Modell DU) ausgeführt. Das war im hiesigen Institut für kinetische Messungen in der Weise erheblich verbessert worden, dass die Lichtquelle etwa 50 cm von dem Küvettenhaus des Apparates entfernt worden war. Eine Temperatursteigerung der Versuchslösung während der Extinktionsmessungen wegen Wärmestrahlung aus der Lampe konnte so im wesentlichen eliminiert werden. Ein mit Thermostatenwasser durchspülbarer Kühlblock bildete ausserdem eine der Wände des Küvettenhauses. Um trotz der grossen Entfernung der Lampe eine hinreichend starke Beleuchtung der Eingangsspalte zu erhalten, war eine Sammellinse zwischen die Lampe und den Diagonalspiegel eingesetzt. — Mit dieser Apparatur wurden die Messungsreihen 26 a; 27 a und 28 a ganz, wie die Reihen 24 a und 25 a teilweise durchgeführt. (Vgl. die Tabellen 10, 7, 6, 15 und 11 unten).

2. DAS BENUTZTE INSTABILE SALZ UND SEINE ENTWÄSSERUNG

Das zu benutzende feste 1,6-Dinitritodipyridindiamminkobalt(III)nitrat. $[(ONO)_2Co\ py_2(NH_3)_2]NO_3$, $1H_2O$ wurde wie früher beschrieben [6] hauptsächlich nach Werner und Jörgensen dargestellt. Durch gravimetrische Bestimmung des Kobaltgehaltes wurde die Reinheit jedes Syntheseproduktes geprüft (Vgl. Adell und Thölin [3]).

Nun war es von Anfang an zu erwarten, und wurde durch Vorversuche auch bestätigt, dass es viel grössere Schwierigkeiten bereiten werde, der Isomerisation des festen kristallwasserhaltigen Salzes als der des kristallwasserfreien quantitativ zu folgen, denn das Salz muss ja während des Versuches längere Zeit im Thermostat trocken, aber mit unverändertem Kristallwassergehalt aufbewahrt werden. Eine Änderung des Kristallwassergehaltes könnte störend auf die Isomerisation des komplexen Kobaltions einwirken.

(Das feste 1,6-Dinitrodipyridindiamminkobalt(III)-nitrat hat auch ein Molekül Kristallwasser pro Molekül Komplexsalz. Das geht aus den Analysen dieser Arbeit hervor. Vgl. S. 8. In den Formeln Werners [1] ist das Wassermolekül weggefallen. Ein näheres Studium seiner Analysen zeigt aber, dass dies nur von einem Übersehen verursacht sein kann).

Es schien somit ratsam, in erster Hand die Umwandlung des kristallwasserfreien Dinitritonitrats zu untersuchen. Nach einigen resultatlosen Versuchen, dies Salz durch Ausfällen mit organischen Lösungsmitteln aus einem mit der wasserhaltigen Verbindung gesättigten, wasserarmen Aceton-Wassergemisch herzustellen, gelang es durch Entwässerung des fein gepulverten kristallwasserhaltigen Nitrats in einem Vakuumexsikkator über Phosphorpentoxyd bei einem Druck von 0,08—0,15 mm Hg. Der Exsikkator wurde mit einer Kapselölluftpumpe evakuiert. Tabelle 2 gibt den Zusammenhang zwischen der Entwässerungszeit und dem gefundenen relativen Gewichtsverlust des Salzes wieder.

Tabelle 2. Relativer Gewichtsverlust von $[Co(ONO)_2\ py_2(NH_3)_2]NO_3$, $1H_2O$ *bei Aufbewahrung über* P_2O_5 *und bei einem Druck von rund 0,1 mm Hg.*

Zeit Stdn.	Verlust %	Zeit Stdn.	Verlust %
1,17	1,25	16,0	4,25
2,00	2,02	17,0	4,24
3,00	2,85	17,0	4,26
3,92	3,52	17,4	4,26
5,50	4,16	18,2	4,24
10,75	4,11	19,3	4,26
12,8	4,20	20,3	4,28
14,8	4,24	20,6	4,25

Die vollständige Entwässerung soll theoretisch eine Gewichtsabnahme von 4,26 % mit sich bringen und ist, wie die Tabelle zeigt, nach etwa 17 Stunden durchgeführt. Das wasserfreie Salz hat eine blass gelbbraune Farbe, die derjenigen des Dinitronitrats ziemlich nahe kommt, und man könnte anfänglich vielleicht befürchten, dass die Entwässerung von einer mehr oder weniger vollständigen Isomerisation des komplexen Kobaltions begleitet sei. Das dies nicht der Fall ist, wurde durch folgenden Versuch gezeigt. Von einem neu hergestellten Präparat des kristallwasserhaltigen Salzes wurde ein Teil in der obenangegebenen Weise entwässert, während der Rückstand bei derselben Tem-

peratur wie jene Portion trocken aufbewahrt wurde. Mit dem wasserfreien Nitrat wurde dann eine Lösung von der Zusammensetzung: 0,00400 *M* Nitrat + 0,00988 *M* $HOCO \cdot CH_3$ + 0.00988 *M* $NaOCO \cdot CH_3$ und von der Temperatur 20° C bereitet. Ihre Extinktion bei 2,6 cm Schichtdicke und für Licht der Wellenlänge $\lambda = 546$ mμ konnte unmittelbar zu 0,427 bestimmt werden. Dann wurde aus dem kristallwasserhaltigen Nitrat schnell dieselbe Lösung wie oben bereitet. Sie zeigte (unter unveränderten Versuchsbedingungen) die Extinktion 0,415. Die die Entwässerung begleitende Extinktionsabnahme (2,8 %) war also ziemlich unbedeutend, und das wasserfreie Salz befand sich noch fern von dem Endzustand, wo es als Dinitronitrat in der obigen Lösung unter den fraglichen Umständen eine Extinktion von 0,1265 hätte zeigen sollen (Vgl. S. 9). (Die Lösung des kristallwasserhaltigen Salzes wurde etwa 25 Minute nach der des wasserfreien hergestellt, und die Umwandlung des ersteren Salzes in der Zwischenzeit kann man wegen ihrer kleinen Geschwindigkeit bei Zimmertemperatur unberücksichtigt lassen.)

3. BEREITUNG DER FESTEN PROBEN

Die Proben einer kinetischen Versuchsreihe liessen sich im allgemeinen auf folgende Weise bereiten. Das neu synthetisierte kristallwasserhaltige Nitrat wurde hinreichende Zeit, gewöhnlich über Nacht, in Vakuum entwässert. Dann wurde in jedes von 12—15 Gläschen eine berechnete Menge des wasserfreien Salzes eingewogen. Während des Wiegens, das rund $2\,^1/_2$ Stunden in Anspruch nahm, war die Hauptmasse des Salzes über Phosphorpentoxyd in einem Exsikkator aufbewahrt. Die Wägegläschen wurden so in einem Metallgestell aufgehängt, das seinerseits in einem mit Glasdeckel, Gummireifen und Metallklammer luftdicht zu verschliessenden Glaszylinder angebracht war. Der Zylinder war am Boden mit einer Phosphorpentoxydschicht beschickt und wurde schliesslich in einen Paraffinthermostat hineingestellt.

Bei dem obigen Verfahren hat man natürlich eine zu betrachtende Fehlerquelle darin, dass das wasserfreie Salz während des Einwiegens Wasser aus der Luft anziehen kann. Um den Einfluss von einem solchen Fehler zu erforschen, wurde eine offene Kristallisationsschale mit etwa 2,6 g des neu entwässerten, feinpulvrigen Nitrats in die Nähe der Wage gestellt. Ihr Inhalt wurde dann und wann umgerührt und ihr Gewicht von Zeit zu Zeit bestimmt. In Tabelle 3 sind die Resultate der ersten acht Wägungen gesammelt.

Tabelle 3. Relative Gewichtszunahme von entwässertem Komplexsalz an der Luft bei Zimmertemperatur.

Zeit (Stdn):	0,42;	1,0;	1,5;	2,0;	18,7;	19,7;	20,7;	21,7
Gewichtszunahme (Promille)	0,2;	0,5;	0,8;	1,1;	6,8;	7,2;	7,4;	7,7

Aus diesen Daten findet man graphisch, dass das Salz in offener Schale nach $2\,^1/_2$ Stunden etwa 1,5 $^0/_{00}$ an Gewicht hätte zunehmen sollen. Mit den Vorsichtsmassregeln, die beim Wiegen getroffen waren, muss die wirkliche Gewichtszunahme einer Probe durch Wasseraufnahme aber ganz zu vernachlässigen sein.

Natürlich hängt die Grösse einer derartigen Wasseraufnahme auch von dem Feuchtigkeitsgrad der Luft ab, und um von der fraglichen Fehlerquelle ganz frei zu werden, bin ich in den letzteren Kinetikreihen zu einem anderen Verfahren übergegangen, bei dem in jedes Gläschen eine berechnete Menge des kristallwasserhaltigen Salzes eingewogen wird. Alle Gläschen werden dann in den Vakuumexsikkator über Phosphorpentoxyd hineingestellt, und nach Entwässerung in gewöhnlicher Weise werden sie schnell in den obenerwähnten Glaszylinder übergeführt. Bei diesem Verfahren wie bei dem vorigen wurde immer die Zusammensetzung des Entwässerungsproduktes durch Bestimmung seines Kobaltgehaltes kontrolliert.

4. DIE MENGE ETWAIGER ZERFALLSPRODUKTE

In diesem Zusammenhang entsteht die wichtige Frage, ob sich das Komplexsalz, wenn man es besonders bei 30° und 40° C längere Zeit verwahrt, nicht in gewissem Grade zersetzt. Werner [1] hat zwar gezeigt, dass es nach Erhitzen etwa 1 Stunde auf 60° C und nach Umkristallisation ein gelbes Produkt gab, das fast denselben Stickstoff- und Kobaltgehalt hatte wie das ursprüngliche Salz und das er als das isomere Dinitronitrat deutete. Man kann aber aus seinen Daten keine Schlüsse auf die Menge von etwaigen Zerfallprodukten ziehen. Deshalb wurde nach dem letzteren von den beiden oben erwähnten Verfahren berechnete Mengen von dem kristallwasserhaltigen, neudargestellten Salz aufgewogen, entwässert und bei 40° C verwahrt. Mit angemessenen Intervallen von einigen Tagen wurden Proben ausgenommen und auf ihren Kobaltgehalt analysiert. Tabelle 4 gibt die Resultate an.

Tabelle 4. Der Kobaltgehalt längere Zeit bei 40° C verwahrter Proben des entwässerten Komplexsalzes.

Zeit in Tagen:	2,32	4,13	7,16	10,06	12,00	13,00
% Kobalt:	14,59	14,57	14,66	14,62	14,60	14,54

Während der theoretische Kobaltgehalt des wasserfreien Salzes 14,54 % ist, liegen die gefundenen Werte zwar ein wenig höher (im Durchschnitt bei 14,59 %), steigen aber nicht mit wachsender Erwärmungszeit an. Es scheint somit berechtigt zu schliessen, dass ein eventueller Zerfall unter diesen Umständen höchst unbedeutend sein muss. Die Kinetikreihen bei 40° C streckten sich nie länger als über etwa 4 Tage.

Ein 194 Tage altes bei Zimmertemperatur im Dunkeln verwahrtes Präparat des kristallwasserhaltigen Salzes zeigte weiter bei Analyse einen Wassergehalt von 4,28 % und einen Kobaltgehalt von 13,87 %, während die theoretischen Werte 4,26 % bzw. 13,93 % sind. Das spricht dafür, dass auch bei Zimmertemperatur die Farbenänderung mit einer fast reinen Isomerisation in Zusammenhang steht.

5. FERTIGSTELLUNG DER VERSUCHSLÖSUNG

Das Gewicht jeder festen Probe der Kinetikreihen war so gewählt, dass diese nach Auflösen in einer berechneten Menge einer auf 20° C temperierten Natriumacetat-Essigsäure-Pufferlösung 100 ml einer Versuchslösung der Zusammensetzung:

0,004 M Komplexsalz + a M $NaOCOCH_3$ + a M $HOCOCH_3$ geben sollte. a war dabei etwa 0,01. Um die zur Auflösung des Salzes nötige Zeit auf ein Minimum zu bringen, wurde die feste Probe mit dem Hauptteile der Pufferlösung in einen Erlenmeyerkolben gespült, dieser in den Wasserthermostat (mit der Temperatur 20,0° C) angebracht und das Gemisch mechanisch mit einem kleinen Glaspendel heftig umgerührt. Der Pendel war dabei an den Klöppel einer elektrischen Klingel befestigt. (Die Konstruktion dieses kleinen, gut funktionierenden Apparates verdanke ich unsrem Mechaniker, Herrn E. Hedenstjärna). Der Zeitpunkt des vollständigen Auflösens des Salzes wurde notiert und dann die Salzlösung mit dem Reste der Pufferlösung in einen Messkolben übergeführt und mit Wasser bis zu 100 ml Volumen verdünnt. Die Messküvette in dem auf 20° C temperierten Küvettenhaus konnte schliesslich mit der Versuchslösung gefüllt werden. In der älteren Apparatur mit Multiplikatorzellen war die lichtabsorbierende Schichtdicke 2,6 cm, in der Beckmanschen 3,0 cm.

6. DAS DINITRONITRAT UND DIE ENDEXTINKTION E_G

Es war von grundlegender Bedeutung für alle folgenden kinetischen Messungen, die Extinktion E_G einer Dinitronitratlösung mit der obigen Zusammensetzung und bei den genannten Schichtdicken für Licht der Wellenlänge $\lambda = 546$ mμ zu kennen. Zu diesem Zwecke wurde $[Co(ONO)_2py_2(NH_3)_2]NO_3$, $1\,H_2O$ umkristallisiert und in drei Portionen in einem elektrischen Ofen bei etwa 60° C während bzw. 1,5; 2,25 und 3,25 Stunden erwärmt. Das Produkt, in einen Glasfiltertiegel gebracht, wurde dann in wenigst möglicher Menge von 30-gradigem Wasser aufgelöst. Von der durchlaufenen Lösung wurde etwa die erste Hälfte für sich aufgenommen und die zweite für sich. Aus beiden konnte mit festem, feingepulvertem Natriumnitrat komplexes Kobaltnitrat ausgefällt werden. Die Niederschläge, mit Alkohol und Äther gewaschen und an der Luft getrocknet, wurden schliesslich mit Rücksicht auf ihren Kobaltgehalt (und in einigen Fällen) durch Entwässerung auf ihren Wassergehalt analysiert. Ein Teil von jedem wurde so zur Bereitung von Lösungen der oben genannten Zusammensetzung benutzt. Ihre Extinktion bei der Schichtdicke $d = 3{,}0$ cm und für $\lambda = 546$ mμ liess sich mit dem Beckman-Spektrophotometer bestimmen.

Ein Präparat von dem kristallwasserfreien Dinitritonitrat war weiter bei Zimmertemperatur in einer mit Urglas bedeckten Kristallisationsschale sich selbst während 89 Tage überlassen. Nach Auflösung in Wasser und Ausfällung mit festem Natriumnitrat wurde der Niederschlag auf seinen Kobaltgehalt analysiert und zu derselben Extinktionsmessung wie oben benutzt. Die Resultate finden sich in der Tabelle 5 wieder.

In dieser Tabelle sind diejenigen Präparate mit a oder b bezeichnet, die aus der zuerst bzw. zuletzt durchlaufenden Hälfte der Lösung des fraglichen Erhitzungsproduktes gefällt waren. Da das Dinitritonitrat leichter löslich als

Tabelle 5. Analysen und Extinktionsmessungen an erhitzten oder alten, umkristallisierten Präparaten.

Präp. Nr.	Erhitz. zeit Stdn.	Alter Tage	% Co	% H_2O	E_G für $d = 3{,}0$ cm
1a	1,5	—	—	—	0,163
1b	1,5	—	13,97	—	0,143
2a	2,25	—	13,95	4,36	0,148
2b	2,25	—	13,99	—	0,148
3a	3,25	—	13,95	4,21	0,152
3b	3,25	—	13,99	—	0,149
4	—	89	13,96	—	0,146

das Dinitronitrat ist (vgl. Werner [1]), so muss man erwarten, dass, falls bei der Erhitzung die Isomerisation nicht vollständig sei, die rötliche Dinitritoform sich in dem ersten Lösungsteil anreichen würde. Präparat 1a hat auch eine deutlich grössere Extinktion für das benutzte Licht als 1b. Bei 2a und 2b findet man keinen derartigen Unterschied. Mit der längeren Erhitzungszeit soll die Isomerisation auch vollständiger werden. Eine ähnliche Extinktionsdifferenz tritt zwar bei 3a und 3b wieder auf, ist aber hier so klein, dass man mit Rücksicht auf die Versuchsfehler an ihrer reellen Existenz zweifeln kann. Überhaupt stimmen die E_G-Werte der Präparate 1b bis 3b untereinander gut überein. Falls die Umwandlung nach 2,25 Stunden Erhitzung noch unvollständig sei, müsste E_G fallen statt ein wenig steigen, wenn man zu den Präparaten 3 übergeht. Aus diesen Umständen und aus dem, dass das vierte, alte Präparat den naheliegenden E_G-Wert 0,146 zeigte, scheint es gerechtfertigt zu folgern, dass eine 0,004-molare Lösung des stabilen Isomeren für $\lambda = 546$ mμ und die Schichtdicke 3,0 cm eine Extinktion von $0{,}148 \pm 0{,}001$ hat. Die Analyseresultate in der Tabelle 5 stimmen gut mit der Annahme, dass dies Isomere pro Molekül ein Molekül Kristallwasser enthält, was den Kobaltgehalt 13,93 % und den Wassergehalt 4,26 % fordert.

Eine nach einer Versuchsreihe übriggebliebene kleine Menge des kristallwasserhaltigen Nitrats wurde bei Zimmertemperatur und im Dunkeln mehrere Monate verwahrt. Nach 99 und 169 Tagen gab eine 0,004-M Lösung von diesem nicht umkristallisierten Salze unter den oben genannten Versuchsbedingungen die Extinktionswerte 0,140 bzw. 0,145. Eine 78 Tage alte Probe des bei 40° C gehaltenen, wasserfreien Salzes zeigte, zu derselben Konzentration aufgelöst und bei derselben Schichtdicke, die Extinktion 0,171.

Man hat also guten Grund anzunehmen, dass die Extinktion des Dinitritonitrats, unter festgelegten Versuchsbedingungen aufgelöst, sich mit wachsender Zeit derjenigen des Dinitronitrats nähert und dann innerhalb der Grenzen der Versuchsfehler konstant bleibt.

Für die kinetischen Berechnungen unten habe ich den Endwert E_G der Extinktion einer 0,004-molaren Lösung des Salzes bei der Schichtdicke $d =$ 3,0 cm gleich 0,146 gesetzt. (0,146 ist der Mittelwert von den letzten sechs Werten der Tabelle 5 nebst den beiden obigen 0,140 und 0,145). Für die mit den Multiplikatorphotozellen benutzten älteren Küvette mit $d = 2{,}6$ cm wird E_G dann gleich 0,1265. In einer früheren Arbeit [6] wurde für eine 0,004-molare Lösung in derselben Küvette der Grenzwert $E_G = 0{,}198$ verwendet. Das hängt damit zusammen, dass es damals richtig schien, ein Präparat des Salzes, das 3 Tage bei 30° C und 42 Tage bei 20° C verwahrt war, als vollständig isomerisiert zu betrachten. Die obigen Daten und die unten zu behandelnden Geschwindigkeitmessungen zeigen aber nun, dass bei Zimmertemperatur die Umwandlung des festen Salzes in ihrer zweiten Stufe äusserst langsam vor sich geht und dass eine Probe mit dem obigen Temperaturschicksal nicht unbedeutend von seinem Endzustand entfernt ist. (Die von Werner [1] auf einige Wochen geschätzte Zeit vollständiger Isomerisation ist also erheblich zu klein).

Die Extinktionsverhältnisse einer Lösung des Dinitritonitrats scheinen somit komplizierter zu sein als in der erwähnten vorigen Arbeit [6] angenommen wurde. Zwar nimmt die Extinktion derselben E_V mit der Zeit t nach einem monomolekularen Schema ab, erreicht einen Grenzwert E' und bleibt dann mehrere Tage konstant. Dieser Grenzwert ist aber grösser als die Extinktion einer gleichkonzentrierten Lösung des Dinitronitrats. Möglicherweise liegt im Endzustand ein Gleichgewicht der beiden Isomeren vor. Dieses Problem fordert aber ein eingehenderes Studium, das noch nicht durchgeführt ist.

7. DIE EXTRAPOLATION DER EXTINKTIONSWERTE AUF DEN ZEITPUNKT VOLLSTÄNDIGER AUFLÖSUNG DER FESTEN PROBE

Die Extinktion $(E_V)_T$ der immer 0,004-molaren Versuchslösung beim Zeitpunkte T der vollständigen Auflösung der festen Probe wurde durch graphische Extrapolation aus 10 bis 15 innerhalb etwa 40 Minuten und t Minuten nach T bestimmten Extinktionswerten $(E_V)_{T+t}$ der Versuchslösung ermittelt. Nach den Resultaten der oben besprochenen Arbeit [6] soll ja der Zusammenhang

$$\log\,[(E_V)_{T+t} - E'] = \log\,[(E_V)_T - E'] - kt \qquad (5)$$

gelten. E' ist der erwähnte Grenzwert der Extinktion. Die gefundenen $\log [(E_V)_{T+t} - E']$-Werte wurden gegen t eingezeichnet und durch die Punkte eine Gerade gezogen. Ihre Ordinate im Nullpunkt gab $\log [(E_V)_T - E']$ und damit $(E_V)_T$. (Vgl. Adell [2]). Für die Schichtdicken $d = 2{,}6$ und 3,0 cm wurde E' gleich 0,194 bzw. 0,224 gesetzt. Natürlich wäre es sehr vorteilhaft gewesen, wenn man wie bei den Nitritopentamminkobalt(III)-salzen [3] die Extinktionsänderung der Probelösung während der Messungen durch Zusatz von Salzsäure hätte verhindern können. Wie mehrere Versuchsreihen zeigten, ist dies aber hier nicht möglich. (Auf diese Reihen hoffe ich in einem späteren Aufsatz näher eingehen zu können). In salzsauren Lösungen mit der Konzentration $\leqq$ 0,02 Mol/Lit. fällt die Extinktion für $\lambda = 546$ mμ nämlich monomolekular und ebenso schnell oder schneller als in der fraglichen Natriumacetat-Essigsäure-Pufferlösung.

8. DIE PRIMÄREN VERSUCHSRESULTATE

Die Tabellen 6—17 enthalten die Resultate der kinetischen Messungen der Umwandlungsgeschwindigkeit des festen, wasserfreien Dinitritonitrats. Der Kürze wegen werden dort die zu dem Auflösungsaugenblick der festen Proben extrapolierten Extinktionswerte mit $(E_V)_{\text{gef.}}$ bezeichnet.

Tabelle 6. Versuchsreihe Nr. 28a. Temperatur 40,0° C. Die Extinktion mit Beckman-Spektrophotometer gemessen. Schichtdicke 3,0 cm. Formel:

$$E_V - 0{,}146 = 0{,}204 \cdot 10^{-0{,}118 \cdot t} + 0{,}201 \cdot 10^{-0{,}00425 \cdot t}$$

t(Stdn.)	$(E_V)_{\text{gef.}}$	$(E_V)_{\text{ber.}}$	t(Stdn.)	$(E_V)_{\text{gef.}}$	$(E_V)_{\text{ber.}}$
0,00	0,544	0,550	10,37	0,341	0,340
0,85	0,508	0,507	11,87	0,336	0,333
1,98	0,467	0,462	23,43	0,306	0,306
2,98	0,441	0,431	30,83	0,293	0,294
5,87	0,374	0,377	36,10	0,288	0,287
7,40	0,356	0,360	55,88	0,262	0,262
8,90	0,349	0,348			

Tabelle 7. Versuchsreihe Nr. 27a. Temperatur 40,0° C. Die Extinktion mit Beckman-Spektrophotometer gemessen. Schichtdicke 3,0 cm. Formel:

$$E_V - 0{,}146 = 0{,}208 \cdot 10^{-0{,}100 \cdot t} + 0{,}189 \cdot 10^{-0{,}00378 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,527	0,543	11,32	0,335	0,333
1,03	0,503	0,498	12,23	0,332	0,329
2,17	0,470	0,463	23,63	0,298	0,300
3,28	0,434	0,428	27,68	0,295	0,295
5,95	0,370	0,379	32,83	0,290	0,288
8,10	0,351	0,355	48,30	0,269	0,270
9,80	0,343	0,342	60,05	0,258	0,258

Tabelle 8. Versuchsreihe Nr. 18a. Temperatur 40,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}144 \cdot 10^{-0{,}100 \cdot t} + 0{,}131 \cdot 10^{-0{,}00368 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,401	0,402	12,98	0,256	0,251
0,71	0,383	0,380	15,22	0,251	0,246
0,97	0,370	0,372	18,73	0,239	0,238
2,18	0,345	0,343	20,77	0,234	0,237
3,17	0,327	0,324	24,58	0,232	0,233
4,42	0,303	0,305	31,23	0,228	0,227
9,28	0,264	0,265	46,00	0,218	0,215
10,24	0,268	0,260	65,97	0,199	0,201
11,30	0,260	0,256	84,95	0,191	0,190

Tabelle 9. Versuchsreihe Nr. 12a. Temperatur 40,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}147 \cdot 10^{-0{,}101 \cdot t} + 0{,}144 \cdot 10^{-0{,}00368 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,409	0,418	5,27	0,305	0,308
0,68	0,398	0,396	7,18	0,281	0,290
1,73	0,371	0,367	9,12	0,280	0,278
2,70	0,354	0,346	10,32	0,266	0,272
3,73	0,334	0,328	11,15	0,274	0,269

Tabelle 10. Versuchsreihe Nr. 26a. Temperatur 30,0° C. Die Extinktion mit Beckman-Spektrophotometer gemessen. Schichtdicke 3,0 cm. Formel:

$$E_V - 0{,}146 = 0{,}221 \cdot 10^{-0{,}0275 \cdot t} + 0{,}172 \cdot 10^{-0{,}00089 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,532	0,540	45,65	0,326	0,315
5,30	0,476	0,475	74,03	0,296	0,294
10,27	0,429	0,430	101,43	0,285	0,286
22,62	0,366	0,363	151,45	0,267	0,272
27,63	0,335	0,347	192,50	0,267	0,262
32,62	0,336	0,335	249,72	0.248	0,249

Tabelle 11. Versuchsreihe Nr. 25a. Temperatur 30,0° C. Die Extinktion der ersten fünf Probelösungen mit zwei Multiplikatorphotozellen, die der übrigen mit Beckman-Spektrophotometer gemessen. Alle Extinktionswerte auf die Schichtdicke 2,6 cm reduziert. Formel:

$$E_V - 0{,}1265 = 0{,}245 \cdot 10^{-0{,}0347 \cdot t} + 0{,}163 \cdot 10^{-0{,}00077 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,513	0,535	94,93	0,268	0,265
3,70	0,494	0,471	122,78	0,255	0,258
16,37	0,347	0,352	168,92	0,247	0,248
22,90	0,322	0,323	219,50	0,238	0,237
27,68	0,311	0,309	267,42	0,229	0,228

Tabelle 12. Versuchsreihe Nr. 23a. Temperatur 30,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}188 \cdot 10^{-0{,}0400 \cdot t} + 0{,}184 \cdot 10^{-0{,}00100 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,485	0,499	33,05	0,307	0,306
1,67	0,471	0,472	58,58	0,287	0,287
5,58	0,430	0,421	76,78	0,282	0,281
8,62	0,403	0,392	127,58	0,267	0,264
23,20	0,320	0,323	174,05	0,244	0,250
25,79	0,311	0,318	216,15	0,241	0,238
29,10	0,302	0,312			

Tabelle 13. Versuchsreihe Nr. 21a. Temperatur 30,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}194 \cdot 10^{-0{,}0300 \cdot t} + 0{,}157 \cdot 10^{-0{,}00089 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,472	0,477	34,37	0,294	0,291
1,65	0,456	0,456	46,53	0,277	0,277
5,15	0,423	0,418	56,37	0,274	0,270
8,97	0,386	0,385	71,17	0,261	0,264
22,73	0,315	0,317	78,22	0,258	0,261
25,92	0,306	0,308	96,18	0,252	0,256
30,38	0,297	0,298			

Tabelle 14. Versuchsreihe Nr. 16a. Temperatur 30,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}145 \cdot 10^{-0{,}0320 \cdot t} + 0{,}136 \cdot 10^{-0{,}00089 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,412	0,408	12,06	0,323	0,319
1,04	0,393	0,397	24,18	0,278	0,280
2,58	0,379	0,382	28,24	0,266	0,273
4,15	0,368	0,368	36,44	0,263	0,263
6,78	0,350	0,349	57,21	0,256	0,250
9,13	0,334	0,334			

Tabelle 15. Versuchsreihe Nr. 24a. Temperatur 20,0° C. Die Extinktion der ersten sieben Probelösungen mit zwei Multiplikatorphotozellen, die der übrigen mit Beckman-Spektrophotometer gemessen. Alle Extinktionswerte auf die Schichtdicke 2,6 cm reduziert. Formel:

$$E_V - 0{,}1265 = 0{,}199 \cdot 10^{-0{,}0096 \cdot t} + 0{,}187 \cdot 10^{-0{,}000256 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,495	0,513	189,51	0,294	0,297
17,93	0,451	0,446	232,53	0,290	0,291
45,12	0,394	0,382	315,80	0,285	0,282
70,00	0,350	0,348	406,83	0,268	0,274
84,33	0,332	0,335	498,08	0,268	0,266
112,72	0,333	0,318	616,43	0,257	0,256
168,13	0,294	0,301			

Tabelle 16. Versuchsreihe Nr. 22a. Temperatur 20,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}186 \cdot 10^{-0{,}0131 \cdot t} + 0{,}186 \cdot 10^{-0{,}000262 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,477	0,498	197,00	0,293	0,292
24,95	0,415	0,397	217,53	0,292	0,290
49,55	0,348	0,349	265,47	0,286	0,285
78,95	0,320	0,321	312,57	0,279	0,280
103,12	0,304	0,309	385,48	0,281	0,274
126,90	0,295	0,303	481,02	0,266	0,265
145,90	0,293	0,299	579,33	0,258	0,257
168,55	0,300	0,295			

Tabelle 17. Versuchsreihe Nr. 20a. Temperatur 20,0° C. Die Extinktion mit zwei Multiplikatorphotozellen gemessen. Schichtdicke 2,6 cm. Formel:

$$E_V - 0{,}1265 = 0{,}195 \cdot 10^{-0{,}0120 \cdot t} + 0{,}185 \cdot 10^{-0{,}000243 \cdot t}$$

t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$	t (Stdn.)	$(E_V)_{gef.}$	$(E_V)_{ber.}$
0,00	0,488	0,507	95,02	0,308	0,316
24,67	0,430	0,408	143,93	0,298	0,301
50,32	0,368	0,355	185,03	0,293	0,294
71,73	0,327	0,331	305,95	0,282	0,282

9. DIE METHODEN ZUR ERMITTLUNG DER GESCHWINDIGKEITSKONSTANTEN

Bei einem Versuche, die Isomerisation des festen 1,6-Dinitritodipyridindiamminkobalt(III)-nitrats theoretisch zu behandeln, liegt es nahe anzunehmen, dass diese in zwei Stufen mit dem Nitronitritonitrat als Zwischenglied, also nach dem Schema

$$\left[\begin{matrix} ONO & py_2 \\ & Co \\ ONO & (NH_3)_2 \end{matrix}\right] NO_3 \rightarrow \left[\begin{matrix} O_2N & py_2 \\ & Co \\ ONO & (NH_3)_2 \end{matrix}\right] NO_3 \rightarrow \left[\begin{matrix} O_2N & py_2 \\ & Co \\ O_2N & (NH_3)_2 \end{matrix}\right] NO_3$$

vor sich geht. (Vgl. Adell [6]). Schon wenn man den Zusammenhang zwischen den gefundenen E_V-Werten und der Umwandlungszeit t graphisch wiedergibt,

wird der Gedanke an eine zweistufige Umwandlung gelenkt, denn die durch die resultierenden Punkte gezogene ausgleichende Kurve hat einen ersteren ziemlich steil abfallenden Teil und einen zweiten sehr langsam sinkenden.

Beim Zeitpunkte der Auflösung der ersten festen Probe ($t = 0$) seien von den zu Bereitung eines Liters der Versuchslösung nötigen m Molen des Salzes nur a Mole unverändertes Dinitritonitrat, b Mole seien Nitronitritonitrat und c Mole Dinitronitrat. Es sei weiter angenommen, dass m Mole einer späteren Probe, die t Stunden nach der ersten eben aufgelöst werden, wegen der Isomerisation des festen Salzes in der Zwischenzeit aus $(a—x)$ Molen Dinitrito-, $(b + x—y)$ Molen Nitronitrito- und $(c + y)$ Molen Dinitronitrat bestehen. Man kann schliesslich den Ansatz machen, das sowohl das Dinitrito- wie das Nitronitritosalz sich in festem Zustande monomolekular isomerisiert. Das gibt die Gleichungen:

$$a + b + c = m \tag{6}$$

$$\frac{d\left(\frac{x}{m}\right)}{dt} = k_1 \cdot \frac{a — x}{m} \text{ oder } \frac{dx}{dt} = k_1 \cdot (a—x) \tag{7}$$

$$\frac{d\left(\frac{y}{m}\right)}{dt} = k_2 \cdot \frac{b + x — y}{m} \text{ oder } \frac{dy}{dt} = k_2(b + x — y) \tag{8}$$

wo k_1 und k_2 die Geschwindigkeitskonstanten der beiden Isomerisationsstufen sind.

Aus (7) und (8) berechnet man

$$x = a — a \cdot e^{-k_1 \cdot t} \tag{9}$$

$$y = a + b + a \cdot \frac{k_2}{k_1 — k_2} \cdot e^{-k_1 t} — \left[\frac{ak_1}{k_1 — k_2} + b\right] \cdot e^{-k_2 t} \tag{10}$$

Wenn man die lichtabsorbierende Schichtdicke der Versuchslösung mit d und die molekularen Extinktionskoefficienten der Dinitrito-, der Nitronitrito- und der Dinitroverbindung mit a_1, a_2 und a_3 bezeichnet, so kann die Extinktion der Versuchslösung E_V beim Zeitpunkte t auf folgende Weise geschrieben werden

$$E_V = d \cdot a_1 \cdot (a — x) + d \cdot a_2 \cdot (b + x — y) + d \cdot a_3 \cdot (c + y) \tag{11}$$

oder nach Einsetzung der x- und y-Werte oben und Vereinfachung:

$$\frac{E_V}{d} = (a+b+c)\alpha_3 + a\left(\alpha_1 - \alpha_2 \frac{k_1}{k_1 - k_2} + \alpha_3 \frac{k_2}{k_1 - k_2}\right) \cdot e^{-k_1 t} + \left(\frac{ak_1}{k_1 - k_2} + b\right) \cdot (\alpha_2 - \alpha_3) \cdot e^{-k_2 t} \tag{12}$$

(Vgl. Adell [6]). E_G sei die Extinktion einer m-molaren Dinitronitratlösung bei derselben Schichtdicke und für dieselbe Wellenlänge. Dann gilt:

$$\frac{E_G}{d} = (a+b+c) \cdot \alpha_3 \tag{13}$$

Aus (12) und (13) folgt

$$E_V - E_G = \tag{14}$$
$$= ad\left(\alpha_1 - \alpha_2 \frac{k_1}{k_1 - k_2} + \alpha_3 \cdot \frac{k_2}{k_1 - k_2}\right) \cdot e^{-k_1 t} + \left(\frac{ak_1}{k_1 - k_2} + b\right) \cdot d \cdot (\alpha_2 - \alpha_3) \cdot e^{-k_2 t}$$

oder falls man die in einer gegebenen Versuchsreihe konstanten Koeffizienten von $e^{-k_1 t}$ und $e^{-k_2 t}$ mit A und B schreibt

$$E_V - E_G = A \cdot e^{-k_1 t} + B \cdot e^{-k_2 t} \tag{15}$$

$$\text{mit } A = ad\left(\alpha_1 - \alpha_2 \frac{k_1}{k_1 - k_2} + \alpha_3 \frac{k_2}{k_1 - k_2}\right) \tag{16}$$

$$\text{und } B = \left(\frac{ak_1}{k_1 - k_2} + b\right) \cdot d \cdot (\alpha_2 - \alpha_3) \tag{17}$$

Geht man zu Zehnpotenzen über, so wird (15)

$$E_V - E_G = A \cdot 10^{-k_1 \cdot (\log e) \cdot t} + B \cdot 10^{-k_2 \cdot (\log e) \cdot t} \tag{18}$$

$$\text{oder } E_V - E_G = A \cdot 10^{-K_1 \cdot t} + B \cdot 10^{-K_2 \cdot t} \tag{19}$$

mit $K_1 = k_1 \cdot \log e$ und $K_2 = k_2 \cdot \log e$

Dass E_V und also E_V—E_G angfangs schnell, dann sehr langsam mit wachsendem t abnimmt, könnte man durch die Annahme erklären, dass K_1 erheblich grösser als K_2 sei. Dann wäre für grosse t-Werte $A \cdot 10^{-K_1 t}$ neben $B \cdot 10^{-K_2 \cdot t}$ zu vernachlässigen und log (E_V—E_G) in der Form

$$\log (E_V - E_G) = \log B - K_2 \cdot t \tag{20}$$

zu schreiben. Graphisch gegen t repräsentiert, würde log $(E_V—E_G)$ somit eine Kurve geben, die für grosse t-Werte in eine Gerade übergehe. Experimentell zeigte es sich auch, dass nach Versuchszeiten oberhalb einer gewissen Grenze die den gefundenen log $(E_V—E_G)$-Werten entsprechenden Punkte auf oder sehr nahe an einer Gerade lagen. (Dieser Grenzwert soll natürlich von der Zusammensetzung des festen Präparates beim Zeitpunkte $t = 0$ abhängen, war aber bei 40° C etwa 20, bei 30° C 70 und bei 20° C 190 Stunden). Die Ordinate im Nullpunkt und der Winkelkoeffizient dieser Gerade gaben die Werte von log B und $—K_2$. Graphisch oder nach der Methode der kleinsten Quadrate liessen sich so die Konstanten B und K_2 in den hinreichend langen Versuchsreihen der Tabellen 6—8; 10—12 und 15—16 ermitteln.

Wenn der oben gemachte theoretische Ansatz richtig ist, dann müssen weiter die mit bekannten B- und K_2-Werten aus den experimentellen Extinktionsdaten für kürzere Versuchszeiten berechneten Werte von

$$\log\ [E_V - E_G — B \cdot 10^{-K_2 \cdot t}] = \log\ A — K_1 \cdot t \qquad (21)$$

graphisch Punkte geben, die auf einer neuen Gerade liegen, deren Ordinate im Nullpunkt und Winkelkoeffizient log A bzw. $—K_1$ sind. Durch die gefundenen Punkte konnte man auch mit zufriedenstellender Annäherung eine Gerade ziehen. Das gilt besonders für die jüngeren Reihen 25a—28a (Tabellen 6—7 und 10—11), wo infolge der gewonnenen Erfahrung die Versuchstechnik besser als in den älteren ist. Man muss sich natürlich darüber klar sein, dass die Versuchsfehler die Werte von log $[E_V—E_G—B \cdot 10^{-K_2 \cdot t}]$ in bedeutend höherem Grade als die von log $[E_V—E_G]$ der vorigen Gerade beeinflussen müssen. Log A und K_1 wurden immer graphisch bestimmt.

(Die an und für sich nicht abweisbare Annahme, dass K_2 bzw k_2 vielmals grösser als K_1 bzw k_1 sei, würde in (16) und (17) eingesetzt, A-und B-Werte fordern, die nicht einmal an Vorzeichen mit dem empirisch gefundenen Zusammenhang Zwischen $E_V—E_G$ und t vereinbar seien.)

Für die kürzeren Versuchsreihen 12a, 21a und 16a (Tabellen 9, 13 und 14), die jede hauptsächlich nur die erste, schnell verlaufende Stufe der Umwandlung umfassten, mussten die Geschwindigkeitskonstanten K_1 und K_2 aber auf eine andere Weise als die oben angegebene ermittelt werden. Die Berechnungsmethode geht auf eine von Aigner und Flamm [7] zur Analyse von Abklingungskurven in der Radioaktivität ausgearbeitete zurück. Sie ist hier in erster Linie von Interesse, weil sie theoretisch es erlaubt, für Funktionen der Form

$$E_V—E_G = f(t) = A \cdot 10^{-K_1 \cdot t} + B \cdot 10^{-K_2 \cdot t} \qquad (22)$$

die Konstanten K_1 und K_2 aus dem empirisch gefundenen Zusammenhang zwischen $f(t)$ und t zu bestimmen, ohne dass man von vornherein die Faktoren A und B kennen muss. In einem Diagramm gibt man den genannten Zusammenhang graphisch wieder und zieht durch die resultierenden Punkte die bestmögliche ausgleichende Kurve. Die auf dieser Kurve zu den Abszissen t_1, $t_1 + \tau$ und $t_1 + 2\tau$ gehörigen Ordinaten seien a_1, a_2 und a_3. (τ ist ein beliebig wählbares Intervall). Weiter sei gesetzt

$$10^{-K_1 \cdot \tau} = \xi \text{ und } 10^{-K_2 \tau} = \eta \tag{23}$$

Dann gilt: $$f(t_1) = A \cdot 10^{-K_1 t_1} + B \cdot 10^{-K_2 t_1} = a_1 \tag{24}$$

$$f(t_1 + \tau) = A \cdot 10^{-K_1 t_1} \xi + B \cdot 10^{-K_2 t_1} \eta = a_2 \tag{25}$$

$$f(t_1 + 2\tau) = A \cdot 10^{-K_1 t_1} \xi^2 + B \cdot 10^{-K_2 t_1} \eta^2 = a_3 \tag{26}$$

Das Zusammenbestehen dieser drei Gleichungen erfordert

$$\begin{vmatrix} 1 & 1 & a_1 \\ \xi & \eta & a_2 \\ \xi^2 & \eta^2 & a_3 \end{vmatrix} = 0 \tag{27}$$

Diese Determinante gibt nach Ausrechnen und Ordnen der Glieder

$$(\eta - \xi) \cdot [a_3 - a_2 (\xi + \eta) + a_1 \cdot \xi \cdot \eta] = 0 \tag{28}$$

oder da $K_1 \neq K_2$ und also $\xi \neq \eta$,

$$a_3 - a_2 (\xi + \eta) + a_1 \cdot \xi \cdot \eta = 0 \tag{29}$$

Hier sind nicht nur A und B sondern auch die konstanten Grössen $10^{-K_1 t_1}$ und $10^{-K_2 t_1}$ eliminiert. Das bedeutet, dass die Gleichung (29) auch für drei zu den Abszissen t_2, $t_2 + \tau$ und $t_2 + 2\tau$ gehörige Ordinaten b_1, b_2 und b_3 gelten muss. (t_2 ist dabei eine andere beliebige Anfangszeit.) Man hat also

$$b_3 - b_2(\xi + \eta) + b_1 \cdot \xi \cdot \eta = 0 \tag{30}$$

Wenn man eine ganze Reihe von Ordinaten: a_1, a_2, a_3, a_4, a_5 --- u. s. w. für die Abszissen t_1; $t_1 + \tau$; $t_1 + 2\tau$; $t_1 + 3\tau$; $t_1 + 4\tau$ u. s. w. abgelesen hat, kann man die b-Ordinaten sehr wohl mit den a-Ordinaten teilweise zusammenfallen

lassen, z. B. $b_1 = a_2$; $b_2 = a_3$; $b_3 = a_4$ u. s. w. bzw. $b_1 = a_3$; $b_2 = a_4$; $b_3 = a_5$ u. s. w. oder $b_1 = a_4$; $b_2 = a_5$; $b_3 = a_6$ u. s. w. wählen. Das gibt die Gleichungen

$$a_4 - a_3 \cdot (\xi + \eta) + a_2 \cdot \xi \cdot \eta = 0 \tag{31}$$

$$a_5 - a_4 \cdot (\xi + \eta) + a_3 \cdot \xi \cdot \eta = 0 \tag{32}$$

$$a_6 - a_5 \cdot (\xi + \eta) + a_4 \cdot \xi \cdot \eta = 0 \tag{33}$$

u. s. w.

Aus der empirischen Kurve kommt man also zu einer ganzen Reihe von Gleichungen, in denen $(\xi + \eta)$ und $\xi \cdot \eta$ die Unbekannten sind. Theoretisch könnten sie, wie Aigner und Flamm hervorheben, z. B. nach der Methode der kleinsten Quadrate bestimmt werden.

Man könnte auch (29); (31) bis (33) auf folgende Weise schreiben

$$\frac{a_3}{a_1} = (\xi + \eta) \cdot \frac{a_2}{a_1} - \xi \cdot \eta \tag{29a}$$

$$\frac{a_4}{a_2} = (\xi + \eta) \cdot \frac{a_3}{a_2} - \xi \cdot \eta \tag{31a}$$

$$\frac{a_5}{a_3} = (\xi + \eta) \cdot \frac{a_4}{a_3} - \xi \cdot \eta \tag{32a}$$

$$\frac{a_6}{a_4} = (\xi + \eta) \cdot \frac{a_5}{a_4} - \xi \cdot \eta \tag{33a}$$

und dann die bekannten linken Glieder dieser Gleichungen $\frac{a_3}{a_1}, \frac{a_4}{a_2}$ --- als Ordinaten gegen die Koeffizienten $\frac{a_2}{a_1}; \frac{a_3}{a_2}$ --- als Abszissen in einem Diagramm benutzen. Durch die daraus sich ergebenden Punkte sollte man eine Gerade legen können, deren Winkelkoeffizient $\xi + \eta$ und Ordinate im Nullpunkt $-\xi\eta$ sich so graphisch bestimmen liesse. Aus den bekannten Werten von $(\xi + \eta)$ und $\xi\eta$ ist es leicht mit (23) K_1 und K_2 zu berechnen.

Wie man erwarten kann, stellt dies Verfahren aber so grosse Forderungen an die Messgenauigkeit bei Bestimmung der a-Werten, dass man es besonders bei den kürzeren Versuchsreihen nicht zur Berechnung von beiden Konstanten benutzen kann. Die Versuchsfehler üben mit anderen Worten einen

ganz zu grossen Einfluss auf die auf diese Weise berechneten K_1- und K_2-Werte aus. Das wurde z. B. an der Versuchsreihe 12a (Tabelle 9) geprüft.

Deshalb bin ich in einer anderen Weise vorgegangen. Wenn K_2 und damit $\eta = \eta_1$ bekannt wäre, so würde sich ξ aus (29) zu

$$\xi = \frac{a_3 - a_2 \cdot \eta_1}{a_2 - a_1 \cdot \eta_1} \tag{34}$$

berechnen lassen und (31) und (32) würde in entsprechender Weise

$$\xi = \frac{a_4 - a_3 \cdot \eta_1}{a_3 - a_2 \cdot \eta_1} \tag{35}$$

und

$$\xi = \frac{a_5 - a_4 \cdot \eta_1}{a_4 - a_3 \cdot \eta_1} \tag{36}$$

geben. Es wäre also möglich eine ganze Reihe von ξ-Werten aus der empirischen Kurve zu errechnen, deren Mittelwert K_1 nach (23) gäbe. Für die Versuchsreihen n:o 21a und 16a (Tabellen 13 und 14) habe ich in dieser Art mit $\tau = 5$ Stdn und K_2 gleich dem Mittelwert 0,00089 der graphisch bestimmten K_2-Werte der übrigen Reihen bei 30° C ξ zu 0,709 bzw. 0,615 und K_1 zu 0,032 bzw. 0,030 ermittelt. Diese K_1-Werte stimmen mit denen der Tabellen 10—12 0,028, 0,035 und 0,040 zufriedenstellend überein. — Ebenso wurde für die Reihe 12a (Tabelle 9) mit $\tau = 1$ Stunde und K_2 gleich 0,00368, dem Wert der Reihe 18a (Tabelle 8), bei 40° C $\xi = 0{,}792$ und $K_1 = 0{,}101$ gefunden (Die Reihen 12a und 18a waren unter sehr gleichartigen Bedingungen und mit fast demselben Anfangswert der Extinktion ausgeführt). Die übrigen K_1-Werte bei 40° C waren 0,118; 0,100 und 0,100. Wenn K_1 und K_2 bekannt sind, lassen sich die Konstanten A und B aus den experimentellen $(E_V—E_G)$-Werten nach Gleichung (15) graphisch oder mit der Methode der kleinsten Quadrate leicht ermitteln.

10. DIE GEFUNDENEN GESCHWINDIGKEITSKONSTANTEN, AKTIVIERUNGSENERGIEN UND AKTIONSKONSTANTEN

Für die längeren Versuchsreihen 28a; 27a und 18a (Tabellen 6—8) wurde ξ (und dann K_1) auch mit Hilfe der Gleichungen vom Typus (34) berechnet Der graphisch direkt bestimmte K_2-Wert gab dabei den nötigen η_1-Wert. Mit τ gleich einer Stunde wurde für die obigen drei Reihen K_1 gleich 0,107;

Tabelle 18. Übersicht der gefundenen Konstanten der Gleichung
$E_V - E_G = A \cdot 10^{-K_1 t} + B \cdot 10^{-K_2 \cdot t}$

bei verschiedenen Temperaturen. — Die Zeit in Stunden.

Versuchsreihe Nr.	Tabelle Nr.	Temp.	K_1	K_2	A	B
28a	6	40,0° C	0,118	0,0043	0,204	0,201
27a	7	40,0° C	0,100	0,0038	0,208	0,189
18a	8	40,0° C	0,100	0,0037	0,144	0,131
12a	9	40,0° C	0,101	(0,0037)	0,147	0,144

Mittelwerte: $K_1 = 0{,}105 \pm 0{,}004$; $K_2 = 0{,}0039 \pm 0{,}0002$

Versuchsreihe Nr.	Tabelle Nr.	Temp.	K_1	K_2	A	B
26a	10	30,0° C	0,028	0,00089	0,221	0,172
25a	11	30,0° C	0,035	0,00077	0,245	0,163
23a	12	30,0° C	0,040	0,00100	0,188	0,184
21a	13	30,0° C	0,030	(0,00089)	0,194	0,157
16a	14	30,0° C	0,032	(0,00089)	0,145	0,136

Mittelwerte: $K_1 = 0{,}033 \pm 0{,}002$; $K_2 = 0{,}00089 \pm 0{,}00007$.

Versuchsreihe Nr.	Tabelle Nr.	Temp.	K_1	K_2	A	B
24a	15	20,0° C	0,0096	0,000256	0,199	0,187
22a	16	20,0° C	0,0131	0,000262	0,186	0,186
20a	17	20,0° C	0,0120	0,000243	0,195	0,185

Mittelwerte: $K_1 = 0{,}012 \pm 0{,}001$; $K_2 = 0{,}000254 \pm 0{,}000006$.

0,097 und 0,107 statt graphisch 0,118; 0,100 und 0,100 gefunden. Die graphische Bestimmung beider Konstanten bringt sicherlich keine grössere Unsicherheit mit sich als die halb mathematisch-analytische oben und hat ausserdem den Vorteil eine viel schnellere Ermittlung der Konstante A zu erlauben. Deshalb wurde sie hier vorgezogen.

Die nach der über jeder der Tabellen 6 bis 17 angegebenen Formel berechneten Extinktionswerte $(E_V)_{ber.}$ finden sich in den dritten und sechsten Kolumnen wieder. Die mit Rücksicht auf die Versuchsfehler zufriedenstellende Übereinstimmung zwischen den berechneten und gefundenen Extinktionswerten scheinen die gemachte Annahme einer stufenweisen Isomerisation zu stützen.

Alle berechneten Konstanten sind in der Tabelle 18 zusammengestellt. Die Temperaturabhängigkeit von K_1 und K_2 lassen sich innerhalb der Grenzen der Messfehler durch die Formeln

$$\log K_1 = -\frac{4348}{T} + 12{,}891 \tag{37}$$

$$\log K_2 = -\frac{5400}{T} + 14{,}812 \tag{38}$$

wiedergeben.

Mit der Arrheniusschen Gleichung $K = \alpha \cdot e^{-\frac{q}{RT}}$ zusammengestellt gibt (37) für die erste Isomerisationsstufe die Aktivierungsenergie $q = 19{,}89$ kcal und die Aktionskonstante $\alpha = 7{,}8.10^{12}$ h^{-1} wie (38) für die zweite $q = 24{,}70$ kcal und $\alpha = 6{,}5.10^{14}$ h^{-1}. Sehr bemerkenswert ist, dass die Isomerisation in der ersten Stufe viel schneller als in der zweiten verläuft. Die erste Nitritogruppe wandelt sich also mit viel grösserer Geschwindigkeit als die zweite in eine Nitrogruppe um. Da die letztere Umwandlung eine höhere Aktivierungsenergie erfordert, nimmt der Quotient $\frac{K_1}{K_2}$ mit wachsender Temperatur ab und ist bei 20° C rund 47, bei 30° C 37 und bei 40° C 27. Rein statistisch wäre der Wert 2 zu erwarten.

Durch Tabelle 19 wird ein Vergleich ermöglicht zwischen den Werten der Geschwindigkeitskonstanten, der Aktivierungsenergie und der Aktionskonstante bei der Isomerisation des festen Isoxanthonitrats $[Co(ONO)(NH_3)_5]\,NO_3$ einerseits (vgl. Adell und Thölin [3]), und des wasserfreien, festen

Dinitritonitrats, $\begin{bmatrix} (1)\ ONO & & py_2 \\ & Co & \\ (6)\ ONO & & (NH_3)_2 \end{bmatrix} NO_3$, dieser Arbeit anderseits. Für die

erste Stufe des Dinitritonitrats sind alle Daten etwas niedriger als für das Isoxanthonitrat aber von derselben Grössenordnung. Für die zweite aber sind die Geschwindigkeitskonstanten infolge der grösseren Aktivierungsenergie 50—60

Tabelle 19. Die kinetischen Daten für die Isomerisation fester Nitrate mit einer und mit zwei Nitritogruppen.

	Isoxantho-nitrat [3].	Dinitritonitrat dieser Arbeit	
		Stufe 1.	Stufe 2.
Geschw.-konst. bei 20,0° C in min^{-1}	$0{,}26.10^{-3}$	$0{,}19.10^{-3}$	$0{,}42.10^{-5}$
» » » 30,0° C » »	$0{,}85.10^{-3}$	$0{,}55.10^{-3}$	$1{,}5.10^{-5}$
» » » 40,0° C » »	$3{,}2.10^{-3}$	$1{,}75.10^{-3}$	$6{,}5.10^{-5}$
Aktiv.-energie in kcal.	21,89	19,89	24,70
Aktionskonstante in min^{-1}	$5{,}7.10^{12}$	$1{,}3.10^{11}$	$1{,}1.10^{13}$

mal kleiner als für dieses Salz. Bei Isomerisationsreaktionen organischer Verbindungen kann man drei Gruppen mit Aktivierungsenergien von rund 65; 44 und 24 kcal unterscheiden. (Vgl. Adell [2], S. 326, Tabelle 10). Die Isomerisation des festen Dinitrito- und Nitronitritonitrats scheint wie die der Isoxanthosalze in dieser Hinsicht mit der letzten der obigen Gruppen am nächsten verwandt zu sein.

ZUSAMMENFASSUNG

1. Festes 1,6-Dinitritodipyridindiamminkobalt(III)-nitrat,

$$\left[\begin{matrix}(1)\ ONO & py_2 \\ & Co \\ (6)\ ONO & (NH_3)_2\end{matrix}\right] NO_3,\ 1\ H_2O$$

ist dunkelbraunrot. Beim Aufbewahren in trocknem Zustande wird seine Farbe mit der Zeit immer gelber. Das hängt nach Werner mit einer Umwandlung in das Isomere

$$\left[\begin{matrix}(1)\ O_2N & py_2 \\ & Co \\ (6)\ O_2N & (NH_3)_2\end{matrix}\right] NO_3,\ 1\ H_2O$$

zusammen.

2. Die Geschwindigkeit der Isomerisation bei 20,0°, 30,0° und 40,0° C wird extinktiometrisch untersucht.

3. Vor den extinktiometrischen Messungen wird das feingepulverte Salz über P_2O_5 in etwa 0,1 mm Vakuum bei Zimmertemperatur entwässert. Analysen zeigen, dass die Entwässerung nach etwa 17 Stunden vollständig ist. Zwischen den Messungen wird es über P_2O_5 in einem Paraffinthermostat verwahrt.

4. Besondere Versuche zeigen, dass das entwässerte Salz ohne erwähnenswerten Zerfall bei 40° C 13 Tage lang verwahrt werden kann. Für ein 194

Tage altes Präparat des kristallwasserhaltigen Salzes, das bei Zimmertemperatur und im Dunkeln gelegen hat, werden weiter bei Analyse Werte des Kobalt- und Wassergehaltes gefunden, die den theoretischen sehr nahe kommen.

5. Im voraus gewogene Proben des festen Salzes werden mit angemessenen Intervallen zur schnellen Bereitung von Versuchslösungen der Zusammensetzung 0,004 *M* Komplexsalz + *aM* $HOCOCH_3$ + *aM* $NaOCOCH_3$ benutzt ($a \simeq 0{,}01$).

6. Die Extinktion der Versuchslösung bei 20,0° C und konstanter Schichtdicke (2,6 bzw. 3,0 cm) wird für Licht der Wellenlänge $\lambda = 546$ mμ in der Mehrzahl der Fälle mit zwei Multiplikatorphotozellen vom Typus RCA 1P 22 bestimmt. Ein Schottsches Didymglasfilter wird zur Monochromatisierung verwendet. Um die Proportionalität zwischen dem Photostrom dieser Zellen und der Lichtstärke zu prüfen, werden mit ihnen vor den eigentlichen Extinktionsmessungen die Abbleichungsgeschwindigkeit von 0,0003 *M* Phenolphthalein in Gegenwart von 0,100 *M* NaOH gemessen. Die Resultate sind zufriedenstellend.

Die späteren Messreihen werden mit einem Beckman-Quarz-Spektrophotometer (Modell DU) durchgeführt. Um bei den Messungen konstante Temperatur der Versuchslösung zu sichern, werden besondere Vorsichtsmassregeln getroffen.

7. Die Extinktion jeder einzelnen Versuchslösung wird 10 bis 15 Mal innerhalb etwa 40 Minuten bestimmt. Ihr Wert E_V im Auflösungsaugenblicke des Salzes wird mit Hilfe einer logarithmischen Gleichung graphisch bestimmt.

8. Das Dinitronitrat wird durch Erhitzen des Dinitritosalzes während mehrerer Stunden auf 60° C dargestellt und dann umkristallisiert. Seine Extinktion E_G für $\lambda = 546$ mμ und die Schichtdicken d = 2,6 cm und 3,0 cm wird zu 0,146 bzw. 0,1265 bestimmt.

9. Mit wachsender Zeit t fällt E_V anfangs sehr schnell, nähert sich dann äussert langsam dem E_G-Wert.

10. Mit Ausgangspunkt von der Annahme, dass die Isomerisation stufenweise nach den Formeln

$$\left[\begin{matrix} ONO & py_2 \\ & Co \\ ONO & (NH_3)_2 \end{matrix}\right] NO_3 \rightarrow \left[\begin{matrix} O_2N & py_2 \\ & Co \\ ONO & (NH_3)_2 \end{matrix}\right] NO_3 \rightarrow \left[\begin{matrix} O_2N & py_2 \\ & Co \\ O_2N & (NH_3)_2 \end{matrix}\right] NO_3$$

vor sich geht, kommt man theoretisch zu dem Zusammenhang

$$E_V - E_G = A \cdot 10^{-K_1 t} + B \cdot 10^{-K_2 t}$$

$$\text{mit } A = ad\ (\alpha_1 - \alpha_2 \cdot \frac{K_1}{K_1 - K_2} + \alpha_3 \cdot \frac{K_2}{K_1 - K_2}) \text{ und}$$

$$B = (\frac{aK_1}{K_1 - K_2} + b) \cdot d \cdot (\alpha_2 - \alpha_3)$$

α_1, α_2 und α_3 sind die molekularen Extinktionskoeffizienten des Dinitrito-, des Nitronitrito- und des Dinitronitrates, a und b die Konzentrationen der beiden ersten Isomeren im Auflösungsaugenblicke, d die lichtabsorbierende Schichtdicke sowie K_1 und K_2 die Geschwindigkeitskonstanten für die erste, bzw. zweite Stufe der Isomerisation.

11. Die gefundenen Werte von log $(E_V - E_G)$ werden als Ordinaten gegen die entsprechenden t-Werte als Abszissen graphisch repräsentiert. Die durch die Punkte gezogene Kurve geht für hinreichend grosse t-Werte (bei 40°, 30° und 20° C nach etwa 20, 70 und 190 Stunden) in eine Gerade über. In dem Ausdrucke für $E_V - E_G$ ist dann $A \cdot 10^{-K_1 t}$ zu vernachlässigen. log B und $-K_2$ können als die Ordinate im Nullpunkt und der Winkelkoeffizient dieser Gerade ermittelt werden.

12. Für kleinere t-Werte geben die Werte von log $[E_V - E_G - B \cdot 10^{-K_2 t}]$ annähernd eine neue Gerade, mit deren Hilfe man log A und $-K_1$ in entsprechender Weise bestimmen kann.

13. Die Anwendbarkeit einer von Aigner und Flamm angegebenen Methode, aus der ausgleichenden $(E_V - E_G)$-t-Kurve K_1 und K_2 zu berechnen, wird erörtert.

14. Die Versuchsresultate scheinen also mit der Annahme einer stufenweisen Isomerisation des festen Salzes vereinbar zu sein. Für K_1 werden bei 20,0°, 30,0° und 40,0° C die Werte $0{,}19.10^{-3}$; $0{,}55.10^{-3}$ und $1{,}75.10^{-3}$ und für K_2 entsprechend $0{,}42.10^{-5}$; $1{,}5.10^{-5}$ und $6{,}5.10^{-5}$ gefunden. (Dekadische Logarithmen; Zeit in Minuten angegeben). Das gibt für die Aktivierungsenergie q bei der ersten und zweiten Stufe: $q = 19{,}89$ bzw. 24,70 kcal und für die Aktionskonstante a (in min^{-1}): log a = 11,11 bzw. 13,03. — Die Daten werden mit den für das feste Isoxanthonitrat $[Co(ONO)(NH_3)_5](NO_3)_2$ früher gefundenen verglichen.

Die Arbeit ist ganz im hiesigen Institut ausgeführt worden. *Statens Naturvetenskapliga Forskningsråd* hat mir Geldmittel zur Anstellung eines Assistenten bewilligt. Dafür spreche ich meinen besten Dank aus. Herr Professor Dr. S. Bodforss, der Vorstand des Instituts, hat die Untersuchung in mehreren Hinsichten durch grosses Entgegenkommen kräftig gefördert. Ich danke ihm herzlichst. Meinen Assistenten Rune Cigén und Brita Linderot verdanke ich schliesslich gute Mithilfe in der synthetischen und analytischen Arbeit.

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Eingegangen am 27. Oktober 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 27 – 38

Investigations of the Infra-Red Absorption Spectra of Some Amino-Acids between 2 and 15 μ

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This investigation has been made to valuate the possibility of determining directly the various amino-acids composing the proteins without preliminary chemical disintegration, as, for example, hydrolysis. This direct approach is important in the study of endocellular protein metabolism. The purpose of this study was to determine, whether the infrared absorption spectra of the amino-acids contained absorption bands specific for the different amino-acids, likewise whether these bands could be found again in the spectrum of a protein or peptide. From earlier investigations of the infra-red absorption spectra of amino-acids between 0.5 and 8.0 μ made by Heintz [1, 2] and Vlés [3], it appears that the difference between the spectra of the amino-acids in this range is not sufficient to allow their separate identifications. One might anticipate that the absorption bands specific for a substance are, however, to be found in a wavelength range above 8 μ. Heintz and Vlés examined the spectra of the amino-acids, dissolved in water or deposited as a thin powder layer on a rock salt base, the latter technique used also by Lenormant [4, 5, 6]. Because the infrared absorption of water is very great and coincides with that of the amino-acids, the former method cannot be considered satisfactory. The powder technique is also unsatisfactory in certain respects, which will later be treated. Many infra-red spectra of amino-acids have been recorded by Wright [7] in the range, 3 to 24 μ, for powder layers on potassium bromide plates using a potassium bromide prism. Such a prism, however, has relatively poor resolution particularly in the range below 10 μ.

In an attempt to meet above difficulties the infra-red absorption spectra between 2 and 15 μ, of five amino-acids, closely related compounds and one dipeptide, have been studied. Similar investigations have recently been made by Darmon and Sutherland [8, 9].

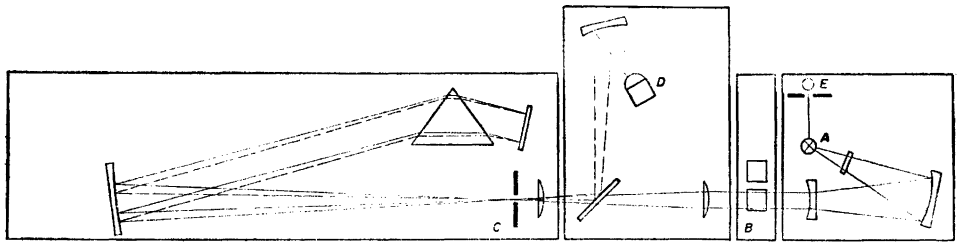


Fig. 1. Schematic optical path of the spectrophotometer. A. Radiation source. B. Liquid cell compartment. C. Slit. D. Photo-receiver, Bolometer. E. Glower regulating phototube.

APPARATUS

For these examinations a Beckman Infra-red Spectrophotometer was used to record spectra continuously and automatically. It was equipped with rock salt optics and rock salt prism. The latter is most suitable to this object, because it provides the best combination of dispersion and transmission range. A schematic drawing of the optical path of the spectrophotometer is shown in Fig. 1. The radiation source is an efficient Nernst glower, the radiation output of which is automatically held constant within 0.1 % by a special photoelectronic regulator.

The entire instrument is maintained constantly at the temperature of $25.0 \pm 0.1°$ C by means of integral coils and chambers through which oil from a constant temperature bath is circulated. With such control localized heating cannot occur, nor are temperature corrections necessary.

The reproducibility of the wavelength is about 0.005 μ, but at wavelengths shorter than 6 μ, the reproducibility decreases proportionately to the reduction in dispersion of its rock salt prism. The wavelength scale of the instrument was calibrated to the CO_2 band at 4.224 μ, after which check was made to establish that the scale coincided with the CO_2 band at 13.87 μ.

Because the radiation intensity successively decreases at longer wavelengths, it is necessary to compensate this decrease by increasing the slit width at given intervals. This was done according to the scheme below:

Wavelength interval in μ	Slit width in mm	Wavelength interval in μ	Slit width in mm
1.0—3.2	0.10	8.7—10.5	0.50
3.2—5.9	0.15	10.5—13.0	0.60
5.9—6.7	0.20	13.0—15.0	0.70
6.7—8.7	0.40		

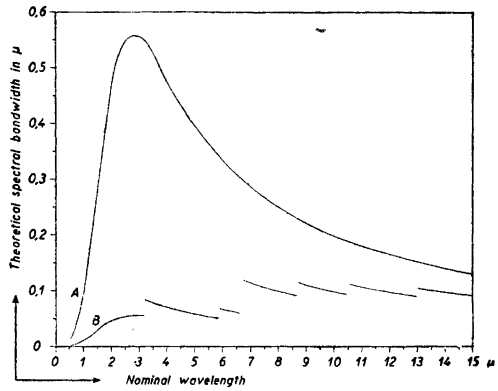


Fig. 2. Resolving power for Beckman spectrophotometer with rock salt prism. A. Theoretical curve for 1 mm slit width. B. From curve A calculated curve for the slit widths used in the examinations.

The resolving power, which is calculated from a theoretical curve for a Beckman spectrophotometer for 1 mm slit width, is illustrated in Fig. 2, which shows that the band width is about 0.1 μ in these examinations.

EXPERIMENTS AND RESULTS

For recording the infra-red spectra, it proved best to use suspensions of the amino-acids, because there are no suitable solvents for them. The powder technique, which was applied by Heintz [1,2], Vlés [3] and many others, has the disadvantage that the absorption curves vary markedly with the particle size [10]. If particle size is approximately the same as the wavelength of the radiation used for study, the radiation will be scattered rather than transmitted, and a false absorption maximum will appear.

The suspensions were prepared by grinding the substances in a powerful, mechanical glass mortar with paraffin oil, a very good suspension medium owing to its high viscosity and relatively weak absorption in infra-red. The particle size in the suspensions must be as small as possible, for in this instance also the particle size will, to a certain degree, influence the transmission. With the suspension method mentioned above the average particle size was about 1.2 μ, determined by microphotographic measurement. An attempt was made to suspend the amino-acids in paraffin oil with supersound at frequencies between 0.4 and 0.7 Mc/sec [11,12]. This failed, probably because the high viscosity of the paraffin oil impeded the cavitation effect. The amino-acid suspension, the concentration of which was about 5 %, was introduced with a record syringe into a rock salt cell. The light path length of the cell was 0.40 mm and the quantity of the suspension used to fill the cell was approxim-

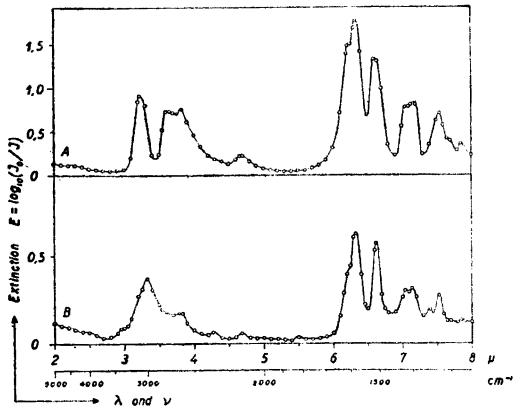


Fig. 3. Infra-red absorption spectra of L-*valine. A.* L-*Valine suspended in paraffin oil. B. Sublimated* L-*valine.*

ately 0.5 cc. The substances used were obtained from F. Hoffmann-La Roche & Co.

Paraffin oil absorbs strongly in the characteristic regions of the C-H frequencies (3.45 μ, 6.85 μ and 7.25 μ), but the remainder of the spectral range will be available. To ascertain the errors in the absorption curves, caused by the absorption of the paraffin oil itself, the absorption curve of suspended valine was compared with that of valine sublimated on a rock salt plate (layer thickness about 4 μ). Fig. 3 shows that the two curves differ only in the region between 3.35 and 3.55 μ. On the other hand no differences are detectable at 6.85 and 7.25 μ, which is very surprising. In spite of the small particle size, obtained by the sublimation of amino-acids, one cannot escape the disturbing effect of the scattering of the radiation. Above 8 μ the absorption of the paraffin oil is very weak.

Comparisons of the spectra of various compounds containing the same radical have shown that absorption bands occurring at certain frequencies can be correlated with specific atomic configurations within the molecule, as, for example; C—H, C = O, O—H, N—H, *etc.*[13, 14]. For simple or highly symmetrical molecules the frequencies of the absorption bands can be calculated mathematically, but when one considers structurally complicated compounds such as amino-acids, it is necessary to resort to the empirical method. By successive elimination of the radicals in a molecule it is often possible to discover the specific absorption bands and from these the corresponding frequencies or wavelengths. Actually only a few of the observed bands can be correlated in this manner.

Fig. 4 shows the infra-red absorption bands of five amino-acids, one amine and its hydrochloride, one carboxylic acid and its sodium salt. From these

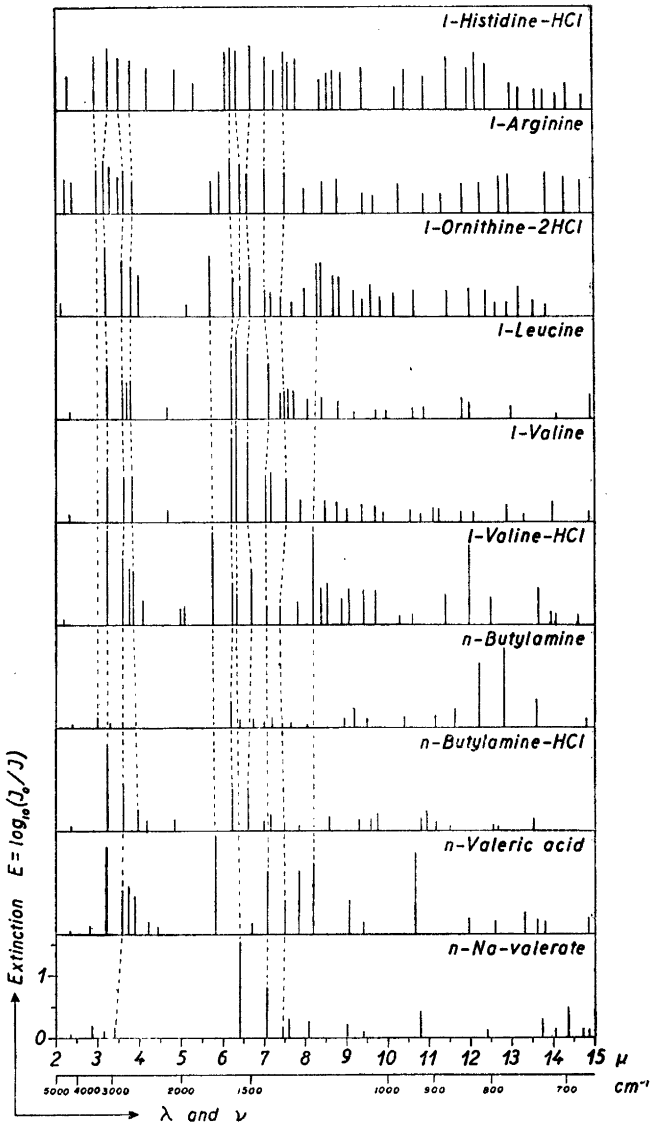
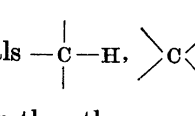
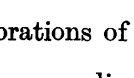
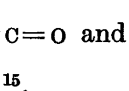


Fig. 4. Infra-red absorption bands of the five recorded amino-acids, one amine and its hydrochloride, one carboxylic acid and its sodium salt. The dotted lines between the bands indicate that these are common to the amino-acids and characteristic of a certain atomic configuration.

spectra one can observe the absorption frequencies of some common radicals and likewise the range within which the specific bands of the various amino-acids are to be found.

From the spectra, shown in Fig. 4, it is evident that the absorption bands characteristic of amino-acids are concentrated within two ranges: one between

2.9 and 4.0 μ, and the other between 5.7 and 7.6 μ. Within the first-mentioned range the absorption frequencies of the bondstretching vibrations of the radicals  and —O—H will occur. Within the other range there are absorption frequencies of the bondstretching vibrations of  and  and of the bondbending vibrations of the former radicals [15].

Absorption in the region 2.90—3.00 μ (3450—3330 cm^{-1}). Butylamine shows at 3.00 μ an absorption band, which recurs in the spectra of histidine hydrochloride at 2.94 μ and of arginine at 2.98 μ. This seems to be caused by the bondstretching vibrations in $\rangle$N—H.

Absorption in the region 3.15—3.30 μ (3180—3030 cm^{-1}). In this region butylamine hydrochloride has a strong absorption band identical to that in the following amino-acids: histidine hydrochloride (3.27 μ), arginine (3.17 μ), ornithine dihydrochloride (3.20 μ), leucine (3.23 μ), valine (3.23 μ) and valine hydrochloride (3.23 μ). This absorption arises from the bondstretching vibrations in the charged radical $\dot{-}^{+}NH_3$.

In the same region valeric acid also has a strong absorption band caused by the bondstretching vibrations in —O—H. This band, which is expected to occur likewise in the spectra of amino-acid hydrochlorides, cannot, however, be distinguished from the absorption of $\dot{-}^{+}NH_3$.

Absorption in the region 3.40—3.65 μ (2940—2740 cm^{-1}). In all the examined compounds a broad absorption band is present in this region. This can be assigned to the symmetrical and antisymmetrical bondstretching vibrations in $\rangle CH_2$ and $—CH_3$. The different bands cannot be discriminated, partly because they lie so close to one another and partly because of the disturbing absorption of the paraffin oil.

Absorption in the region 3.80—4.00 μ (2630—2500 cm^{-1}). This absorption seems to be due to the bondstretching vibrations in the charged radical $\dot{-}^{+}NH_3$, which appears from the absorption band of butylamine hydrochloride at 3.96 μ. For the amino-acids this band occurs at somewhat shorter wavelengths: histidine hydrochloride (3.81 μ), arginine (3.86 μ), ornithine dihydrochloride (3.81 μ), leucine (3.81 μ), valine (3.83 μ) and valine hydrochloride (3.86 μ).

Absorption in the region 5.70—5.85 μ (1750—1710 cm^{-1}). It has been shown that the carboxylic acids have an absorption band at approximately 5.8 μ, this arising from the bondstretching vibrations in the uncharged group —COOH. This band recurs in the spectra of the hydrochlorides of the amino-acids, because the amino group here is blocked, and no zwitterion can be

formed. For ornithine dihydrochloride this band lies at 5.72 μ and for valine hydrochloride at 5.77 μ. Histidine hydrochloride, however, lacks this band, because the proton here is bound to the nitrogen of the imidazole nucleus, and a charged carboxylic group is formed.

It should be noted that arginine also has an absorption band at 5.75 μ, but this cannot be caused by the carboxylic group.

Absorption in the region 6.15—6.30 μ (1615—1590 cm^{-1}). This band will be assigned to the bondbending vibrations in the charged radical $-\overset{+}{N}H_3$. This absorption recurs in the spectra of butylamine hydrochloride (6.24 μ), histidine hydrochloride (6.22 μ), arginine (6.20 μ), ornithine dihydrochloride (6.27 μ), leucine (6.22 μ), valine (6.22 μ) and valine hydrochloride (6.23 μ).

Butylamine also gives rise to an absorption band at 6.20 μ, which may be interpreted as the bondbending vibrations in >N—H. Since histidine hydrochloride and arginine contain >N—H as well as $-\overset{+}{N}H_3$ radicals, their spectra should show stronger absorption in this range, than that of the other amino-acids. Such is in fact the case.

Absorption in the region 6.30—6.45 μ (1590—1550 cm^{-1}). Sodium valerate shows here an absorption band at 6.42 μ as a result of the bondstretching vibrations in the charged group $-COO^-$. This band occurs in the amino-acids: histidine hydrochloride (6.35 μ), arginine (6.45 μ), leucine (6.34 μ), valine (6.33 μ), and, peculiarly enough, in ornithine dihydrochloride (6.44 μ) and valine hydrochloride (6.35 μ). In the latter two the carboxylic group is uncharged and for that reason should cause no absorption in this range. The absorption in these two last-mentioned compounds, however, is much weaker than in the others and may possible arise from another source.

Absorption in the region 6.60—6.70 μ (1510—1490 cm^{-1}). Histidine hydrochloride (6.70 μ), arginine (6.62 μ), ornithine dihydrochloride (6.68 μ), leucine (6.62 μ), valine (6.62 μ) and valine hydrochloride (6.70 μ) as also butylamine hydrochloride (6.62 μ) have in this region a rather strong absorption band, which seems to depend on the bondbending vibrations in $-\overset{+}{N}H_3$.

Absorption in the region 7.00—7.15 μ (1430—1400 cm^{-1}). There is in this region an absorption band common to all the amino-acids studied: histidine hydrochloride (7.06 μ), arginine (7.04 μ), ornithine dihydrochloride (7.03 μ), leucine (7.13 μ), valine (7.05 μ) and valine hydrochloride (7.06 μ) as likewise in the spectrum of valeric acid (7.08 μ) and sodium valerate (7.06 μ). This absorption is probably assignable to the bondstretching vibrations in the charged as well as uncharged carboxylic group.

Absorption in the region 7.40—7.55 μ (1350—1325 cm^{-1}). All the compounds examined show here an absorption band, which is to be referred to the bondbending vibrations in C—H.

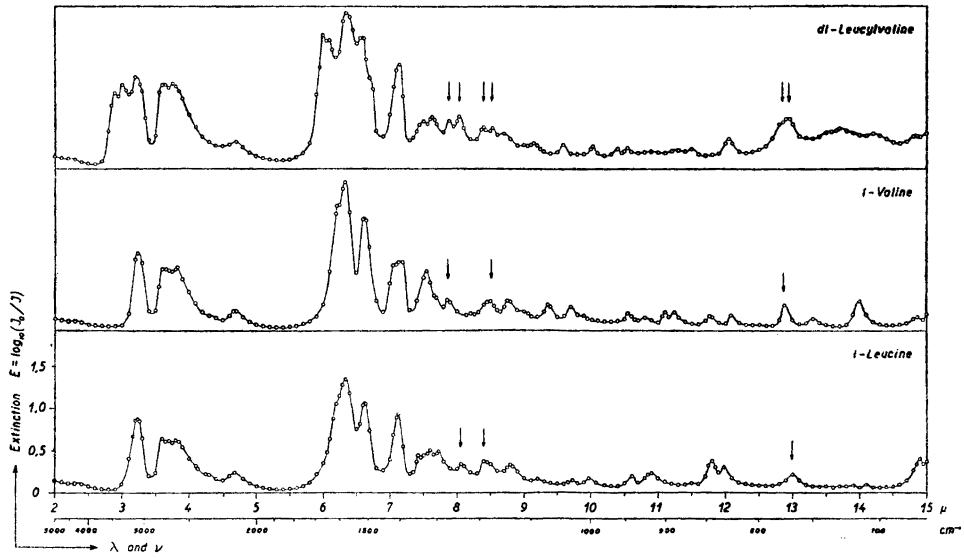


Fig. 5. *Infra-red absorption spectra of* DL-*leucylvaline,* L-*valine and* L-*leucine.* ***The arrows indicate the absorption bands specific for the two amino-acids.***

Absorption in the region 8.20—8.30 μ (1220—1200 cm⁻¹). Valeric acid shows at 8.20 μ a strong absorption band, which is found in valine hydrochloride at 8.21 μ and in ornithine dihydrochloride at 8.31 μ. (This band is displaced slightly to longer wavelengths because of fusion with a strong band at 8.40 μ). The absorption may possibly be caused by the bondbending vibrations in the radical —O—H.

It is known that the hydrocarbon compounds will absorb strongly at approximately 6.85 μ (1460 cm^{-1}) and 7.25 μ (1380 cm^{-1}), and that these absorptions are due mainly to various bondbending vibrations in the $-\overset{|}{\underset{|}{C}}-H$, $\diagup C\diagdown\!\!\diagup\!\!\diagdown$, and $-\overset{H}{\overset{|}{\underset{|}{C}}}-H$ groups. These absorption bands, which are likely to occur in the spectra of the amino-acids, have not been distinguishable in the examinations performed, because of the very strong absorption of the paraffin oil exactly in these ranges.

As mentioned before, only a few of the observed bands can be correlated with specific atomic configurations. The principal band components of the spectrum above 7.5 μ arise from normal modes of skeletal vibrations involving

the C—C and C—N bondstretching vibrations. These bands, which will usually differ among the various amino-acids, can successfully be used for the identification of each compound, as likewise the weak overtone bands and combination bands. By analysing these bands it was possible to trace two compounds as structurally similar as valine and leucine in the spectrum of leucylvaline. The result is seen in Fig. 5. The specific bands of leucine can be distinguished at 8.06 μ, 8.41 μ, 13.00 μ, and of valine at 7.88 μ, 8.48 μ, 12,88 μ. These respective bands occur in the spectrum of leucylvaline at 8.04 μ, 8.40 μ, 12.96 μ and 7.89 μ, 8.52 μ, 12.84 μ. The difference between the spectra of leucine and of valine appears most clearly at the absorption bands in the 8 μ region. The result must be regarded as satisfactory, when one considers, that the difference between the valine and leucine molecules consists only of an extension of the carbon chain with a $>CH_2$ group. For this reason no great differences are to be expected in their absorption spectra.

Besides the absorption bands characteristic of amino-acids there are bands specific for the peptide bond, —CO—NH—, in the leucylvaline spectrum. At 3.01 μ (3322 cm^{-1}) a band arising from the bondstretching vibrations in >N—H occurs, and at 6.00 μ (1667 cm^{-1}) a band, which is to be assigned to the bondstretching vibrations in the >CO group, is observed [9, 16].

SUMMARY

The apparatus and the technique for recording the infra-red absorption spectra of amino-acids in paraffin oil suspension have been described. Further the absorption bands characteristic of amino-acids between 2.9 and 4.00 μ (3450—2500 cm^{-1}) and those between 5.7 and 7.6 μ (1750—1320 cm^{-1}) have been discussed. A method for identifying leucine and valine in the spectrum of leucylvaline by analysing the bands specific for these two amino-acids between 7.5 and 15.0 μ (1330—670 cm^{-1}) has been demonstrated. Since other amino-acids likewise show specific absorption bands in this latter range, it is proposed that amino-acid components of peptides, other than leucylvaline, may be identified in the same way.

This study has been supported by grants from *Statens Naturvetenskapliga Forskningsråd* and *Medicinska Forskningsanslaget.*

Table 1. The infra-red absorption bands of L-*histidine hydrochloride,* L-*arginine,* L-*ornithine dihydrochloride,* L-*leucine,* L-*valine,* L-*valine hydrochloride and* DL-*leucylvaline.*

λ = Wavelength in μ
ν = Frequency in cm^{-1}
E = Extinction = $\log_{10}(I_0/I)$

L-Histidine-HCl			L-Arginine			L-Ornithine-2HCl		
λ	ν	E	λ	ν	E	λ	ν	E
2.30	4348	0.576	2.22	4505	0.576	2.13	4695	0.224
2.94	3401	0.878	2.40	4167	0.508	3.20	3125	1.128
3.27	3058	1.040	2.98	3356	0.692	3.58	2793	0.909
3.52	2841	0.844	3.17	3155	0.859	3.81	2625	0.816
3.81	2625	0.824	3.30	3030	0.765	3.99	2506	0.668
4.21	2375	0.684	3.50	2857	0.600	5.14	1946	0.196
4.88	2049	0.673	3.63	2755	0.700	5.72	1748	1.000
5.32	1880	0.465	3.86	2597	0.524	6.27	1595	0.697
6.09	1642	0.953	5.75	1739	0.520	6.44	1553	0.504
6.22	1608	1.011	5.94	1684	0.680	6.68	1497	0.792
6.35	1575	0.972	6.20	1613	0.881	7.03	1422	0.424
6.70	1493	1.068	6.45	1550	0.804	7.18	1393	0.404
7.06	1416	0.873	6.62	1511	0.648	7.44	1344	0.340
7.27	1376	0.656	7.04	1420	0.736	7.70	1299	0.244
7.52	1330	0.957	7.53	1328	0.672	8.00	1250	0.476
7.66	1305	0.780	8.00	1250	0.444	8.31	1203	0.872
7.80	1282	0.838	8.44	1185	0.532	8.40	1190	0.885
8.39	1192	0.492	8.80	1136	0.580	8.70	1149	0.656
8.56	1168	0.596	9.42	1062	0.352	8.84	1131	0.664
8.70	1149	0.644	9.67	1034	0.312	9.20	1087	0.432
8.91	1122	0.620	10.28	974	0.488	9.40	1064	0.280
9.40	1064	0.712	10.90	918	0.336	9.60	1042	0.540
10.22	988	0.372	11.31	884	0.328	9.84	1016	0.328
10.43	959	0.664	11.82	845	0.496	10.16	985	0.420
10.90	917	0.548	12.26	816	0.496	10.64	940	0.448
11.47	872	0.876	12.72	786	0.604	11.47	873	0.424
11.96	836	0.688	12.96	772	0.632	11.99	834	0.456
12.16	823	0.936	13.85	722	0.664	12.39	807	0.416
12.40	806	0.756	14.30	699	0.584	12.62	792	0.240
13.00	769	0.424	14.70	680	0.540	12.90	775	0.228
13.20	758	0.376				13.18	759	0.488
13.60	735	0.336				13.54	738	0.272
13.78	726	0.332				13.85	722	0.204
14.10	710	0.224						
14.35	696	0.420						
14.73	679	0.248						

L-Leucine

λ	ν	E
2.35	4255	0.120
3.23	3096	0.873
3.60	2778	0.640
3.70	2703	0.612
3.81	2625	0.628
4.68	2137	0.240
6.22	1608	1.112
6.34	1577	1.345
6.62	1511	1.064
7.13	1403	0.920
7.42	1348	0.440
7.51	1332	0.448
7.60	1316	0.504
7.73	1294	0.480
8.06	1241	0.332
8.41	1189	0.372
8.80	1136	0.324
9.20	1087	0.156
9.72	1029	0.144
9.97	1003	0.168
10.61	942	0.200
10.88	919	0.228
11.80	847	0.376
11.98	835	0.300
13.00	769	0.216
14.10	709	0.088
14.90	671	0.392

L-Valine

λ	ν	E
2.32	4310	0.112
3.23	3096	0.920
3.62	2762	0.728
3.83	2611	0.752
4.68	2137	0.232
6.22	1608	1.480
6.33	1580	1.760
6.62	1511	1.325
7.05	1418	0.777
7.17	1395	0.808
7.55	1325	0.712
7.88	1269	0.368
8.48	1179	0.344
8.77	1140	0.360
9.02	1109	0.224
9.37	1067	0.304
9.70	1031	0.284
9.90	1010	0.172
10.55	948	0.208
10.80	926	0.156
11.10	901	0.216
11.24	890	0.224
11.78	849	0.176
12.08	828	0.184
12.88	777	0.304
13.30	752	0.132
13.99	715	0.356
14.87	672	0.160

L-Valine-HCl

λ	ν	E
2.20	4545	0.100
3.23	3096	1.671
3.60	2778	1.088
3.76	2660	0.913
3.86	2591	0.880
4.09	2445	0.432
4.99	2004	0.272
5.08	1969	0.340
5.77	1733	1.632
6.23	1605	0.676
6.35	1575	0.536
6.70	1493	0.922
7.06	1416	0.312
7.40	1351	0.320
7.82	1279	0.388
8.21	1218	1.556
8.40	1190	0.592
8.53	1172	0.680
8.88	1126	0.436
9.06	1104	0.600
9.41	1063	0.588
9.70	1031	0.580
10.29	973	0.128
10.60	943	0.192
11.39	878	0.512
11.97	835	1.569
12.50	800	0.460
13.63	733	0.616
13.90	719	0.208
14.06	702	0.188
14.61	685	0.160

DL-Leucylvaline

λ	ν	E
2.26	4425	0.116
2.91	3436	0.896
3.01	3322	0.992
3.22	3106	1.088
3.63	2755	0.996
3.76	2660	1.000
3.85	2597	0.920
4.69	2132	0.312
6.00	1667	1.576
6.10	1639	1.520
6.35	1575	1.860
6.59	1517	1.556
6.76	1479	0.920
7.14	1401	1.232
7.50	1333	0.552
7.63	1311	0.608
7.89	1267	0.568
8.04	1244	0.616
8.40	1190	0.480
8.52	1174	0.476
8.72	1147	0.412
8.96	1116	0.280
9.15	1093	0.300
9.59	1043	0.288
10.03	997	0.268
10.38	963	0.236
10.55	948	0.232
11.30	885	0.220
11.50	870	0.228
12.05	830	0.356
12.84	779	0.520
12.96	772	0.588
13.54	738	0.440
13.70	730	0.468
14.10	709	0.412
14.81	675	0.388

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Received October 29, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 39–44

Die Bildungsweise der Chlorhydrine

VIII. Über die Addition von unterchloriger Säure an eine Doppelbindung

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Michael[1] hat einmal die Hypothese aufgestellt, dass die Reaktion zwischen unterchloriger Säure und einem Äthylenkörper in zwei Stufen vor sich ginge. Für den einfachsten Fall also folgendermassen:

$$H_2C : CH_2 + HOCl \rightarrow H_2C - CH_2 + \overset{+}{H} + \overset{-}{Cl} \qquad (1)$$

$$\underset{\diagdown O \diagup}{H_2C - CH_2} + \overset{+}{H} + \overset{-}{Cl} \rightarrow Cl \cdot CH_2 \cdot CH_2 \cdot OH \qquad (2)$$

Diese Hypothese sollte von einigen (doch nicht publizierten) Experimenten gestützt werden [2]. Befunde bei einer Untersuchung über die Darstellung von Äthylenchlorhydrin aus Äthylen und Chlor in wässeriger Lösung hat auch Frahm [3] veranlasst, sich dieser Auffassung anzuschliessen. Indessen haben Moureu und Dodé [4] gezeigt, dass im Falle des Äthylenchlorhydrins die Geschwindigkeit der Äthylenchlorhydrinbildung (also der Bruttoreaktion) grösser ist als die Geschwindigkeit der Teilreaktion 2, was ja mit der Hypothese Michaels nicht vereinbar ist.**

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* Manus von Smith.

** Zu der Arbeit von Moureu und Dodé möchte ich doch eine Bemerkung fügen. Obwohl die Richtigkeit ihrer Schlussfolgerungen nicht bezweifelt werden kann, scheint es ihnen entgangen zu sein, dass die Äthylenoxyde in verdünnter Salzsäure nicht nur Chlorwasserstoff, sondern auch Wasser unter Glykolbildung schnell addieren. Durch diese Reaktion wird die Chlorwasserstoffaddition bald zum Stillstande gebracht. Sie haben also ihre Messungen der Geschwindigkeit dieser Reaktion falsch gedeutet. Wären aber — nach der Auffassung Michaels — nennenswerte Mengen Äthylenoxyde in der Lösung anwesend, dann könnte man erwarten, dass bei der Chlorhydrinbildung aus Äthylenkörpern und unterchloriger Säure immer Glykole als Nebenprodukte gebildet würden. Dies ist doch, wie wir gefunden haben, nicht der Fall.

Bei unsymmetrischen Äthylenkörpern liegt die Möglichkeit vor, die Hypothese Michaels in besonderer Weise zu prüfen. Bei solchen Körpern (z. B. $R' \cdot CH = CH \cdot R''$) führt die Addition von unterchloriger Säure zu zwei verschiedenen Isomeren, nämlich $HO \cdot R'CH \cdot CHR''Cl$ und $Cl \cdot R'CH \cdot CHR'' \cdot OH$, und bei der Gültigkeit der genannten Hypothese würden die Proportionen zwischen den gebildeten Mengen dieser beiden Isomeren bei der Addition von unterchloriger Säure an $R' \cdot CH = CHR''$ dieselben sein wie bei der Addition von Chlorwasserstoff an die entsprechende Oxydoverbindung $R'CH{-}CH \cdot R''$.

In der Tat habe ich schon früher in der angedeuteten Weise einige Reaktionen untersucht, die die Unhaltbarkeit Michaels Hypothese beweisen. Es galt erstens die Bildung von Glycerinmonochlorhydrin (I), bzw. Glycerindichlorhydrin (II)[5]

$$\text{I}\quad \begin{cases} \text{A} & Cl \cdot CH_2 \cdot CH : CH_2 + HOCl \longrightarrow Cl \cdot CH_2 \cdot CH(OH) \cdot CH_2Cl \text{ „}\alpha\alpha\text{“} \\ \text{B} & Cl \cdot CH_2 \cdot CH{-}CH_2 + HCl \longrightarrow Cl \cdot CH_2 \cdot CHCl \cdot CH_2OH \text{ „}\alpha\beta\text{“} \end{cases}$$

I A gab die Proportion, $\%\,\alpha\alpha : \%\,\alpha\beta = 0{,}5 : 1$
I B » » » , » $\alpha\alpha$: » $\alpha\beta = 100 : < 1$

$$\text{II}\quad \begin{cases} \text{A} & HOCH_2 \cdot CH : CH_2 + HOCl \longrightarrow HOCH_2 \cdot CH(OH) \cdot CH_2Cl: \text{ »}\alpha\text{«} \\ \text{B} & HO \cdot CH_2 \cdot CH \cdot CH_2 + HCl \longrightarrow HO \cdot CH_2 \cdot CHCl \cdot CH_2OH: \text{ »}\beta\text{«} \end{cases}$$

II A gab die Proportion, $\%\,\alpha : \%\,\beta = 2{,}3 : 1$
II B » » » , » α: » $\beta = 7{,}9 : 1$

Die oben angegebenen Proportionen gelten für Reaktionstemperaturen in der Nähe von 0°.

Da einige dieser Bestimmungen zu einer Zeit ausgeführt worden sind, wo die analytische (kinetisch-analytische) Bestimmung der Zusammensetzung der entstehenden Isomerengemische noch nicht genügend entwickelt war, haben wir * Reaktionen I A und II A von neuem in derselben Hinsicht einer genauen Untersuchung unterzogen. Reaktionstemperatur — 5°. Bei einer Ausbeute von 77 % wurde für I A einen Prozentgehalt von 67,4 % α,β-Dichlorhydrin

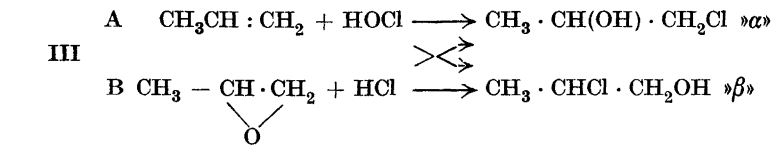
* Experimente von Sture Skyle. Seine vollständige Untersuchung wird später veröffentlicht.

und bei der Ausbeute 76 % für II A 26 % β-Monochlorhydrin erhalten. Die Proportionen werden also für I A 0,5 : 1 und für II A 2,8 : 1.

Die Übereinstimmung mit früheren Bestimmungen ist gut. Die Abweichung im zweiten Falle kann z. B. Verschiedenheiten in der Ausführung der Reaktion zugeschrieben werden oder vielleicht dem Umstande, dass bei meinen ersten Versuchen die Reinigung des Rohproduktes durch fraktionierte Destillation geschah. Dadurch können Veränderungen in der Zusammensetzung des Gemisches entstanden sein.

Für *Propylenchlorhydrine* (Reaktionssystem III) lagen auch alte Untersuchungen (*l. c.*) von mir vor für sowohl Reaktion A wie B. Indessen konnten meine Messungen früher nicht quantitativ verwertet werden. Erstens war die alkalische Zersetzungsgeschwindigkeit nur für die α-Isomere bekannt, und kinetische Analyse setzt ja die Kenntnis von beiden Konstanten voraus. Die Konstante der β-Verbindung ist jetzt [6] bestimmt worden und beträgt 2,65 (20°); $k_\alpha = 7{,}85$ (20°). Nimmt man dasselbe Verhältnis der Konstanten bei 25° wie bei 20° an, resultiert für jene Temperatur $k_\alpha = 14{,}3$, $k_\beta = 4{,}7$, woraus nach meinen früheren Messungen (bei 25°) für die Addition von *Chlorwasserstoff an Propylenoxyd* % α : % $\beta = 3 : 1$ * (B-Reaktion) berechnet wird.

Für die entsprechende »A-Reaktion» — Addition von unterchloriger Säure an Propylen — lassen sich trotz des Kenntnisses von k_β meine damaligen Versuche nicht gut auswerten, weil bei der genannten Reaktion die damals angewandte "unterchlorige Säure" [7], als Nebenprodukt Monochloraceton (etwa 25 %) gab, das auch sehr schnell (und viel schneller als die Propylenchlorhydrine) in alkalischer Lösung zersetzt wird. Die kinetische Analyse wurde dadurch zu kompliziert und unsicher. Doch schätzte ich damals [8] die Proportionen % α : % $\beta = 9 : 1$, was ich doch als wenig zuverlässig angab. Skyle hat in seinen fortgesetzten Untersuchungen über Addition von unterchloriger Säure an Doppelbindungen zeigen können, dass bei Verwendung von *reiner* unterchloriger Säure kein Monochloraceton entsteht. Er konnte dadurch die Proportionen $\alpha : \beta$ ziemlich genau bestimmen, die überraschenderweise mit den früher geschätzten übereinstimmten. Für Reaktion III somit:



III A gab die Proportion, % α : % $\beta = 10 : 1$
III B » » » , » α : » $\beta = 3 : 1$

* Bestätigt bei neueren, genauen Versuchen von Forsberg.

Über die Proportionen bei Addition von unterchloriger Säure an *zweifach* in β-Stellung substituiertes Äthylen (1,1-Dimethyläthylen) kann nichts ausgesagt werden, solange man in wässeriger Lösung mit chloridhaltiger unterchloriger Säure arbeitet, denn das isomere Chlorhydrin mit tertiärem Chlor wird schon durch Wasser zersetzt. Michael und Leigthon [9] meinen, es sei immerhin möglich, dass die genannte Verbindung »in sehr geringem Masse entsteht».*

Die Proportionen bei Addition von Chlorwasserstoff an 1,1-Dimethyläthylenoxyd sind dagegen bekannt [10]. Der Gehalt an β-Verbindung wird erhöht im Vergleich mit Propylenoxyd. Das Verhältnis % α : % β *sinkt* auf 0,8 : 1. Bei weiterer Substitution von H durch CH_3 (in α-Stellung) sinkt dieses Verhältnis abermals auf (0,5 : 1).

ANALOGE VERSUCHE MIT GLYCERINBROMHYDRINEN.

Diese Befunde bei Chlorhydrinen werden durch die Versuche mit vor allem *Glycerinbromhydrinen* gestützt, die von Laudon [11] ausgeführt worden sind. — Bei Wiedergabe seiner Resultate verwende ich Bezeichnungen, die analog mit den obigen (S. 40) sind: »BrIA» = Reaktion zwischen Allylbromid und unterbromiger Säure; »Brα» = α-Glycerinmonobromhydrin u.s.w.

Br	I	A	gab die	Proportion,	% Brα	: % Brβ	= 0,25 : 1
Br	I	B	»	»	»	: »	Brβ = 100 : <1
Br	II	A	»	»	»	: %	Br$\alpha\alpha$: % Br$\alpha\beta$ = 2 : 1
Br	II	B	»	»	»	: »	Br$\alpha\alpha$: » Br$\alpha\beta$ = 13 : 1

Die Übereinstimmung zwischen Chlor- und Bromverbindungen ist in beiden Reaktionstypen erstaunenswert gut.

ZUSAMMENSTELLUNG

			Bromhydrine
I A	$ClCH_2 \cdot CH : CH_2$ + HOCl;	% $\alpha\alpha$: % $\alpha\beta$ = 0,5 : 1	0,25 : 1
II A	$HO \cdot CH \cdot CH : CH_2$ + »	» α : » β = 2,8 : 1	2 : 1
III A	$CH_3 \cdot CH : CH_2$ + »	» α : » β = 10 : 1	

* Skyle greift dieses Problem unter Verwendung von *reiner* unterchloriger Säure an.

I B $Cl \cdot CH_2 \cdot CH(O)CH_2 + HCl$; % $\alpha\alpha$: % $\alpha\beta$ = 100 : <1 — 100 : <1

II B $HO \cdot CH_2 \cdot CH(O)CH_2 +$ » » α : » β = 8 : 1 — 13 : 1

III B $CH_3 \cdot CH(O)CH_2 +$ » » α : » β = 3 : 1

IV B $(CH_3)_2 \cdot C(O)CH_2 + HCl$; % α : % β = 0,8 : 1

V B $(CH_3)_2 \cdot C(O)CH \cdot CH_3 + HCl$; » α : » β = 0,5 : 1

SCHLUSSFOLGERUNGEN

Die Unterschiede in den Proportionen zwischen den "α"-und den "β"-Isomeren bei Addition von unterchloriger Säure an eine Doppelbindung und von Chlorwasserstoff an die entsprechende Oxydoverbindung sind im allgemeinen so erheblich, dass von einer allgemeinen Gültigkeit der Hypothese von Michael-Frahm nicht die Rede sein kann. Zu einem *geringen Betrage* wäre eine intermediäre Bildung von Oxydoverbindung immerhin möglich.

Die Verhältniszahlen »% α : % β» verändern sich in auffallender Weise in den A-Serien I—III und in den entsprechenden B-Serien in entgegengesetzter Richtung. Betrachtet man das Chloratom in HCl, bzw. HOCl als das dirigierende "Schlüssatom", kan dies nicht befremden, denn hierdurch wird *die entgegengesetzte Wirkung* von *H, OH und Cl auf einerseits* relativ *positives Chlor in HOCl und* anderseits *negatives in HCl* dargetan. Dieser Befund ist sehr interessant und, soweit ich weiss, bisher experimentell nicht in dieser Weise nachgewiesen. Die Wirkung dieses Ladungsunterschiedes sieht man vielleicht am deutlichsten beim Vergleich der Reaktionen IA und IB : in jener tritt das "positive" HOCl-Chlor zu $^2/_3$ in Nachbarstellung zum negativen Chloratom in Allylchlorid, in dieser das negative HCl-Chlor nur zu < 1 % in derselben Stellung zum Chloratom in Epichlorhydrin.

Markownikoffs Regel, nach welcher Halogen vorzugsweise an das wasserstoffärmere Kohlenstoffatom geht, gilt beim Übergang von III B zu IV B, von IV B zu V B dagegen nicht. Man sollte in dem letzten Falle eine Vermehrung des Gehaltes an Chlor in α-Stellung erwarten, während statt dessen eine Verminderung eintritt. Hier können stereochemische Verhältnisse eine Rolle spielen. Substitution (von H durch OH und Cl) an dem dem Ring benachbarten Kohlenstoffatom verändert indessen die »α : β»-Proportionen viel mehr als ein Ersatz von Wasserstoff durch Methyl an dem Ringkohlenstoffatom. Markownikoffs Regel wird hierdurch ungültig.

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Eingegangen am 3. November 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 45–48

Über die Reaktion zwischen Triäthyl-amino-silan und einigen primären Aminen

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Larsson und Mjörne[1] haben eine Methode verwendet, nach der man in Triäthyl-N-äthylamino-silan $(C_2H_5)_3Si \cdot NH \cdot C_2H_5$ die Gruppierung $-NH \cdot C_2H_5$ mit $-NH \cdot R$ ersetzen kann, wo R ein Kohlenwasserstoffrest ist. Man erwärmt einige Stunden ein Gemisch von Triäthyl-N-äthylamino-silan und dem Amin $R \cdot NH_2$, so dass das gebildete Äthylamin aus dem Reaktionsgemisch entfernt wird. Die darauf folgende Destillation ergibt die neue Verbindung $(C_2H_5)_3 Si \cdot NH \cdot R$. Der Umsatz findet nach der Formel

$$(C_2H_5)_3Si \cdot NH \cdot C_2H_5 + R \cdot NH_2 \rightarrow (C_2H_5)_3Si \cdot NH \cdot R + C_2H_5 \cdot NH_2 \qquad (1)$$

statt. In dieser Weise wurden *n*-Hexyl-, *n*-Heptyl-, Benzyl-, *α*-Phenäthyl- und *β*-Phenylisopropylamin mit Triäthyl-N-äthylamino-silan umgesetzt.

Im folgenden werden wir einige Umsätze zwischen Triäthyl-amino-silan und einigen primären Aminen nach

$$(C_2H_5)_3Si \cdot NH_2 + R \cdot NH_2 \rightarrow (C_2H_5)_3Si \cdot NH \cdot R + NH_3 \qquad (2)$$

beschreiben. Soweit man aus präparativen Versuchen beurteilen kann, reagiert ein Amin $R \cdot NH_2$ leichter mit Triäthyl-amino-silan nach (2) als mit Triäthyl-N-äthylamino-silan nach (1). Wir haben die folgenden Amine nach (2) mit Triäthyl-amino-silan reagieren lassen und in sämtlichen Fällen die erwartete neue Verbindung $(C_2H_5)_3Si \cdot NH \cdot R$ erhalten:

n-Butylamin	Anilin	Benzylamin
n-Hexylamin	*o*-Toluidin	Cyclohexylamin
n-Heptylamin	*p*-Toluidin	2-Aminothiazol

In zwei Fällen wurden Versuche ausgeführt, die zeigen, wie rasch ein Umsatz nach (2) stattfinden kann, wenn die Reaktion so ausgeführt wird, dass das gebildete Ammoniak aus dem Reaktionsgemisch entfernt wird, je nachdem es gebildet wird.

Durch ein Gemisch von 0,04 Mol Anilin und 0,02 Mol Triäthyl-amino-silan wurde ein mit Phosphorpentoxyd sorgfältig getrockneter Luftstrom durchgeleitet. Der Luftstrom passierte dann einen mit kaltem Wasser gekühlten Rückflusskühler, ehe sein Inhalt an Ammoniak in Salzsäure absorbiert wurde. Das absorbierte Ammoniak wurde titrimetrisch bestimmt. Wenn das Reaktionsgemisch bei Zimmertemperatur gehalten wurde, betrug die in der Salzsäure absorbierte Ammoniakmenge

nach	43	Minuten	0,010	Mol
»	90	»	0,016	»
»	135	»	0,018	»

In einem zweiten Versuch mit 0,04 Mol Cyclohexylamin und 0,02 Mol Triäthyl-amino-silan bei Zimmertemperatur wurde während 90 Minuten eine Ammoniakmenge von 0,0040 Mol erhalten. Die Temperatur des Reaktionsgemisches wurde dann auf etwa 150° erhöht. Während 240 Minuten bei dieser Temperatur wurden 0,012 Mol Ammoniak entwickelt. Insgesamt waren somit 0,016 Mol Ammoniak erhalten, einem Umsatz von 80 % entsprechend.

In beiden Versuchen wurden die Reaktionsgemische destilliert, nachdem das Hindurchleiten von Luft abgebrochen war. Dabei wurde in dem ersten Versuch Triäthyl-N-phenylamino-silan und in dem zweiten Triäthyl-N-cyclohexylamino-silan erhalten.

PRÄPARATIVE VERSUCHE

Triäthyl-amino-silan wurde nach Bailey, Sommer und Whitmore [2] aus Triäthyl-chlor-silan und flüssigem Ammoniak dargestellt.

Die Umsätze zwischen Triäthyl-amino-silan und dem primären Amin wurden so ausgeführt, dass ein Gemisch der beiden Verbindungen unter Rückflusskühlung in gelindem Sieden gehalten wurde. Wenn das Gemisch höher als 150° siedete, wurde es in einem Salz- oder Ölbade nur auf 150° erwärmt. Nach einigen Stunden wurde das Reaktionsgemisch unter vermindertem Druck destilliert. Der Umsatz zwischen Triäthyl-amino-silan und 2-Aminothiazol wurde jedoch bei 100° ausgeführt. Das Reaktionsprodukt konnte in diesem Falle nicht destilliert werden, sondern wurde in anderer Weise aufgearbeitet. Bei allen Operationen wurde darauf geachtet, Wasser fernzuhalten, da es die Bindung zwischen den Silizium- und Stickstoffatomen spaltet, wodurch die Ausbeuten erniedrigt werden und die gewünschten Verbindungen durch nicht immer leicht abtrennbare Verbindungen verunreinigt werden können.

Triäthyl-N-butylamino-silan

5,2 g (0,04 Mol) Triäthyl-amino-silan und 5,9 g (0,08 Mol) n-Butyl-amin ergaben nach 3 Stunden 5,4 g Triäthyl-N-butylamino-silan vom Sdp. 85—86° (12 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot C_4H_9 = C_{10}H_{25}NSi$ (187,4):

Ber.	N	7,5	Äquiv.-Gew.		187,4
Gef.	»	7,4	»	»	192,5

Triäthyl-N-hexylamino-silan

5,2 g (0,04 Mol) Triäthyl-amino-silan und 8,0 g (0,08 Mol) *n*-Hexylamin ergaben nach 2 Stunden 7,1 g Triäthyl-N-hexylamino-silan vom Sdp. 117° (15 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot (CH_2)_5 \cdot CH_3 = C_{12}H_{29}NSi$ (215,4):

Ber.	Si	13,0	Äquiv.-Gew.		215,4
Gef.	»	13,1	»	»	216,0

Triäthyl-N-heptylamino-silan

5,2 g (0,04 Mol) Triäthyl-amino-silan und 9,2 g (0,08 Mol) *n*-Heptyl-amin ergaben nach 3 Stunden 6,6 g Triäthyl-N-heptylamino-silan vom Sdp. 125° (13 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot (CH_2)_6 \cdot CH_3 = C_{13}H_{31}NSi$ (229,5):

Ber.	Äquiv.-Gew.		229,5
Gef.	»	»	230,1

Triäthyl-N-phenylamino-silan

6,7 g (0,051 Mol) Triäthyl-amino-silan und 9,9 g (0,106 Mol) Anilin ergaben nach 2 Stunden 6,5 g Triäthyl-N-phenylamino-silan vom Sdp. 139—140° (21 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot C_6H_5 = C_{12}H_{21}NSi$ (207,4):

Ber.	Si	13,5	N		6,8
Gef.	»	13,7	»		6,7

Triäthyl-N-*o*-methylphenylamino-silan

3,9 g (0,03 Mol) Triäthyl-amino-silan und 6,4 g (0,06 Mol) *o*-Toluidin ergaben nach 2 Stunden 3,5 g Triäthyl-N-*o*-methylphenylamino-silan vom Sdp. 137° (9 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot C_6H_4 \cdot CH_3{-}o = C_{13}H_{23}NSi$ (221,4):

Ber.	N	6,3
Gef.	»	6,3

Triäthyl-N-*p*-methylphenylamino-silan

7,8 g (0,06 Mol) Triäthyl-amino-silan und 3,2 g (0,03 Mol) *p*-Toluidin ergaben nach 7 Stunden 3,7 g Triäthyl-N-p-methylphenylamino-silan vom Sdp. 143° (12 mm).

6,7 g (0,05 Mol) Triäthyl-amino-silan, 10,7 g (0,1 Mol) *p*-Toluidin und 20 ml Benzol ergaben nach 3 Stunden 9,2 g Triäthyl-N-*p*-methylphenylamino-silan vom Sdp. 149° (17 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot C_6H_4 \cdot CH_3(-p) = C_{13}H_{23}NSi$ (221,4):

Ber.	Si	12,7	N	6,3
Gef.	»	12,6	»	6,0

Triäthyl-N-benzylamino-silan

5,2 g (0,04 Mol) Triäthyl-amino-silan und 8,5 g (0,08 Mol) Benzylamin ergaben nach 3 Stunden 5,2 g Triäthyl-N-benzylamino-silan vom Sdp. 133° (9—10 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot CH_2 \cdot C_6H_5 = C_{13}H_{23}NSi$ (221,4):

Ber.	Si	12,7	Äquiv.-Gew.	221,4
Gef.	»	12,8	»	» 222,9

Triäthyl-N-cyclohexylamino-silan

6,6 g (0,05 Mol) Triäthyl-amino-silan und 10,0 g (0,1 Mol) Cyclohexylamin ergaben nach 10 Stunden 6,1 g Triäthyl-N-cyclohexylamino-silan vom Sdp. 112—113° (10 mm).

Für $(C_2H_5)_3Si \cdot NH \cdot C_6H_{11} = C_{12}H_{27}NSi$ (213,4):

Ber.	Si	13,1	N	6,6
Gef.	»	13,2	»	6,4

Triäthyl-N-[thiazolyl-(2)-amino]-silan

5,2 g (0,04 Mol) Triäthyl-amino-silan und 2,0 g (0,02 Mol) 2-Amino-thiazol wurden zusammen während 2 Stunden auf 100° erwärmt. Danach wurde das unreagierte Triäthyl-amino-silan unter vermindertem Druck abdestilliert. Es wurden 2,1 g Triäthyl-amino-silan zurückgewonnen. Aus dem Rückstand wurde durch abwechselndes partielles Kristallisieren, Dekantieren und Schmelzen eine geringe Menge Triäthyl-N-thiazolyl-(2)-amino-silan erhalten. Kristalle.

Für $(C_2H_5)_3Si \cdot NH \cdot C_3H_2NS = C_9H_{18}N_2SSi$ (214,4):

Ber.	Si	13,1	N	13,1
Gef.	»	13,1	»	13,0

ZUSAMMENFASSUNG

Einige Verbindungen $(C_2H_5)_3Si \cdot NH \cdot R$ wurden durch Umsatz von $(C_2H_5)_3Si \cdot NH_2$ mit Aminen $R \cdot NH_2$ dargestellt.

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Eingegangen am 12. November 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 49 – 51

The Beta to Alpha Transformation of Fully Acetylated Glycosides by Alkali

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The mutarotation of sugars is known to be catalyzed both by acids and bases, but the analogous transglycosidation reaction has only been accomplished with the aid of acid catalysts. Wolfrom and Husted [1], however, have proved that fully acetylated sugars can be transformed from the β- to α-form, using alkali as catalyst. The acetylated sugar was dissolved in an inert solvent, preferably a mixture of dioxane and ether, and shaken with solid sodium hydroxide and a drying agent. Wolfrom and Husted believed that the active agent was a very mild alkalinity, but the present author considers it more probable that the reaction is an example of heterogenous catalysis, for when a mixture of solid sodium hydroxide and Drierite was shaken in dioxane-ether for ten hours and then filtered, no trace of alkalinity and no catalytic activity could be detected in the solution. The glycosides which would most closely resemble sugar acetates are the phenylglycosides, phenols being fairly strong acids. Therefore the effect of alkali in inert solvents on some phenyl glucosides has been investigated in this laboratory.

First the method of Wolfrom and Husted was improved. Instead of crushed solid sodium hydroxid, Ascarite was used. In this way more reproducible results could be obtained. Finally it was found that anhydrous pyridine was a more effective solvent than ether-dioxane. In this solvent the reactions were about 6—7 times faster than in ether-dioxane.

The β-glucoside of a very acid phenol, 2,4-dinitrophenol, was transformed into the α-form by this method. The α-glucoside tetraacetate melted at 182—182.5° and had a specific rotation of + 212° in chloroform. When morpholine was used as a solvent, no transglycosidation was observed but the glycosidic bond was broken and 2,4-dinitrophenyl-morpholine was formed.

Attempts to transform the β-glucosides of phenol, *o*-nitrophenol, and *p*-nitrophenol by this method were not successful. Other bases and more suitable solvents may, however, be found so that these glycosides also might be transformed. This would be of value not only from a theoretical but also from a synthetical point of view, as methods to transform phenyl β-glycosides into the α-glycosides are not known *.

EXPERIMENTAL

Transformation of β-glucose pentaacetate

A mixture of Drierite (10 g), Ascarite (3 g) and anhydrous pyridine (25 ml) was shaken mechanically in order to remove all traces of moisture. After one hour β-glucose pentaacetate (3 g) was added and the shaking continued for 45 minutes. The brown solution was filtered through a layer of kieselguhr into acetic acid (2 ml). The filter was washed with anhydrous dioxane (20 ml) and the filtrate poured into water (200 ml). White crystals slowly precipitated, which were collected and dried. Yield 1.6 g. M. p. 108—109°**. $[\alpha]_D^{20}$ + 101°. Recrystallization from methanol yielded pure α-glucose pentaacetate.

Using a shorter reaction time, equilibrium was not established, while a longer reaction time lowered the yield.

2,4-Dinitrophenyl α-glucoside tetraacetate

A mixture of Drierite (6 g), Ascarite (2 g) and anhydrous pyridine (20 ml) was shaken mechanically for one hour. Then 2,4-dinitrophenyl β-glucoside tetraacetate (2 g) was added and the shaking continued for three hours. The mixture was filtered through a layer of kieselguhr into a small amount of acetic acid, the filter washed with dioxane (20 ml), and the filtrate poured into water (200 ml). The yellow precipitate was collected and recrystallized from methanol, yielding 0.55 g of white crystals, melting at 181—181.5°. From the mother liquor a second crop of 0.1 g could be obtained. One further recrystallization from methanol yielded the pure substance. M. p. 182—182.5°. $[\alpha]_D^{20}$ + 212°.

$C_{20}H_{22}O_{14}N_2$ (514.4) Calc. N 5.45 Found N 5.47

2,4-Dinitrophenyl morpholine

When the above experiment was repeated using morpholine as solvent, a substance which crystallized from methanol in long yellow needles and melted at 118—119° was obtained. As the same product could be obtained by treating morpholine with 2,4-dinitrochlorbenzene, the substance evidently was 2,4-dinitrophenyl morpholine.

$C_4H_8ON-C_6H_3O_4N_2$ (253.2) Calc. N 16.6 Found N 16.4

* One rearrangement of this kind is reported. Montgomery, Richtmeyer and Hudson [2] have treated phenyl β-glucoside tetraacetate with anhydrous zinc chloride in phenol and obtained the α-glucoside. This method is of practical value only if the rearrangement is intramolecular, which, however, seems rather improbable.

** All melting points uncorrected. All specific rotations in chloroform, c = 2.

SUMMARY

A phenyl β-glycoside, 2,4-dinitrophenyl β-glucoside tetraacetate has been transformed into the corresponding α-glucoside with the aid of sodium hydroxide in absolute pyridine.

The author wishes to thank *Statens Naturvetenskapliga Forskningsråd* for financial support and Mr. L. Asp for skilful assistance.

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Received November 9, 1949.

ACTA SHEMICA SCANDINAVICA 4 (1950) 52 – 54

The Food Sparing Effect of Liver Extracts on Rats. II

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In two recent papers it was shown that the daily administration of about 0.2 g of a catheptic casein hydrolysate or 0.06 g of a liver extract to young rats significally increased the weight gain per g food eaten [1, 2]. In both investigations the active material was administered with pipettes and the weight of the rats at the beginning of the experiments was about 60—70 g. In an unpublished series of experiments on full-grown animals a food sparing effect was not noticed. Accordingly, it was of interest to observe the effect of a liver extract in later stages of growth than used in the previous experiments [1, 2]. It was also of importance to observe if the effect was present when the food was fortified with the extract.

EXPERIMENTAL

The animals, young albino rats, were kept in individual cages. Water and the commercial mouse bread described by Gard [3] were given *ad libitum*. Individual animal weight and food consumption were determined daily. Each ml of the liver extract used in this investigation contained 0.27 g of solids with a nitrogen content of 25 mg per ml. At the beginning of the first series of experiments the weight of the rats was about 80 g. The experimental time was 15 days and 0.3 ml of the liver extract was daily administered with pipettes. The results are given in Table 1.

In a second series the liver extract was added to the bread in amounts calculated to give each animal the content of 0.3 ml of extract when the daily food consumption was about 16 g. The same rats as in the previous experiment were used and the calculations were based on the figures obtained in that experiment. At the beginning of the new experiment the weight of the rats was about 135 g and the daily food consumption of the two groups of animals was about the same (22 g). The experimental time in this later experiment was 15 days. The results are given in Table 2.

Table 1. Growth of rats on a mixed diet with the addition of liver extract by means of pipettes.

Group	No. of animals	Average daily weight gain	Average daily food consumption	Weight gain per g food eaten
Liver	15	2.9 ± 0.20 *	12.7 ± 0.22 *	0.23 ± 0.015 *
Control	15	2.6 ± 0.15	19.6 ± 0.12	0.13 ± 0.008

There was no significant difference in daily weight gain between the liver and the control group in any of the two experiments. The food sparing effect of the liver extract was quite obvious in both series. The impression gained from this and the previous investigations [1, 2] is that the food sparing effect develops during the three first days of an experiment and remains for about five days when the treatment is discontinued.

A more complete interpretation of the results obtained in this and previous investigations must await the isolation and identification of the factor or factors responsible for the effect. As already mentioned liver extracts must contain several biologically active factors which are not identified (Ågren [4]). The food sparing effect could be related to some newly identified factor as vitamin B_{12} which is present in most liver extracts and not supplied in sufficient amounts with the mouse bread. However, the effect is also present in enzyme hydrolyzed casein which is not considered to be rich in vitamin B_{12}. Experiments performed with the pure vitamin or vitamin concentrates on chickens [5] and weanling pigs [6] showed that this factor stimulated the growth but the average daily food consumption of the animals given the vitamin increased or was unchanged. It may also be mentioned that Gard's mouse bread contains meat powder and milk both potential sources of vitamin B_{12}.

Table 2. Growth of rats on a mixed diet given as bread fortified with liver extract.

Group	No. of animals	Average daily weight gain	Average daily food consumption	Weight gain per g food eaten
Liver	15	2.2 ± 0.10 *	16.5 ± 0.14 *	0.13 ± 0.008 *
Control	15	1.6 ± 0.13	22.0 ± 0.16	0.07 ± 0.007

* The values in these columns are the means and the standard errors.

A possible explanation is that some factor or factors in the liver extract, either alone or in cooperation with the pituitary growth hormone, will maintain the nitrogen metabolism on a more efficient level by reducing the rate of oxidative disposal of amino acids. A similar mechanism has also been postulated by Totter *et al.*[7] in discussing the possible function of folic acid. A relationship to the function of vitamin A may also exist. Evidence was recently presented by Mayer and Krehl [8] that this vitamin participates in the control of the food utilization. Furthermore Cabell and Ellis [9] investigated the efficiency of certain A-deficient diets when supplemented with vitamin A and found the lack of a factor which could be furnished by liver extracts.

SUMMARY

Growing rats fed on mouse bread fortified with small amounts of a liver extract significantly increased the weight gain per g food eaten. After discontinuing the administration of liver the food sparing effect was of about five days duration.

The investigation was supported by a grant for medical research from the Swedish Medical Research Council. The technical assistance of Mr. Thore Persson and Mr. C. F. Skjöldebrand is greatfully acknowledged.

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Received November 18, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 55–59

Constituents of Pine Heartwood

XVII.* The Heartwood of *Pinus aristata* Engelm.

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Pinus aristata, 'Bristle-cone pine', is a *Haploxylon* pine growing at rather high altitudes on the southern Rocky Mountains in western North America. The botanists generally divide the section *Haploxylon* into two subsections, *Cembra* and *Paracembra*[1]. *P. aristata* is the first pine from the last-mentioned subsection, which has been investigated with regard to its heartwood constituents.

The ether extract of *P. aristata* heartwood (7.7 %) contained comparatively large quantities of phenolic substances, above all pinosylvin monomethyl ether but also the two flavones chrysin and tectochrysin, which have been isolated from all *Haploxylon* pines hitherto investigated. The acetone extract was divided into fractions in the usual way[2]. Small quantities of chrysin and pinocembrin were found in the 0.2 % sodium hydroxide fraction. This pinocembrin was partially racemised, $[\alpha]_D^{20}$—35° (in methanol). The pinocembrin isolated from other pines has always had a specific rotation of about —55°[2]. Since the isolation of pinocembrin has been carried out by similar methods in all cases, it seems probable that this substance has become racemised in the heartwood of *P. aristata* for some unknown reason.

The 4 % sodium hydroxide fraction of the acetone extract yielded tectochrysin (precipitated as its sodium salt) and a large amount of pinosylvin monomethyl ether.

The water-soluble part of the acetone extract consisted mainly of *l*-arabinose, which could be separated by precipitation with *p*-bromophenylhydrazine. The filtrate from this precipitation yielded only a very small quantity of a brown syrup, from which no crystalline products could be isolated. *P. aristata* thus differs from all *Haploxylon* pines hitherto investigated, which

* XVI. *Acta Chem. Scand.* 3 (1949) 1381.

contain pinitol along with *l*-arabinose. There are, however, some indications of the presence of very small quantities of pinitol even in *P. aristata*. As no specific reaction for pinitol was available, the methoxyl content of the crystalline sugar fraction and of the syrup left after the precipitation of arabinose by *p*-bromophenylhydrazine was determined. The former fraction contained 0.24 % OCH_3, and the latter 5.23 %. (Pure pinitol has 16.0 % OCH_3). These values show that some methoxyl-containing substance is heavily concentrated in the latter fraction. If that substance were assumed to be pinitol, the methoxyl values would correspond to a pinitol content of 1.5 % and 33 % respectively. Thus, the question of whether or not there is any pinitol in *P. aristata* cannot be definitely answered as yet. It would be of great interest to investigate other pines from the subsection *Paracembra* to see if pinitol is a characteristic heartwood constituent of the whole section *Haploxylon* or only of the subsection *Cembra*.

The total yields from 3.7 kg of air-dried heartwood were:

Substance	From the ether extract	From the acetone extract	Total yield
'Membrane substances'	—	4.0 g	4.0 g (0.11 %)
l-Arabinose	—	5.8 g	5.8 g (0.16 »)
Chrysin	4.7 g	0.5 g	5.2 g (0.14 »)
Pinocembrin	—	0.3 g	0.3 g (0.01 »)
Tectochrysin	3.0 g	2.3 g	5.3 g (0.14 »)
Pinosylvin monomethyl ether	11.3 g	25.5 g	36.8 g (1.0 »)
Neutral fraction	9.8 g	10.9 g	20.7 g (0.56 »)

Total weight of ether extract 287 g (7.7 %).

The specimen of *P. aristata* investigated here contained as much pinosylvin monomethyl ether as a rather good specimen of *P. sylvestris*. The yields of chrysin and tectochrysin were also high compared to other *Haploxylon* pines, but the content of pinocembrin was low. Pinosylvin, pinobanksin and strobopinin have not been isolated.

EXPERIMENTAL

The wood used for the investigation had grown at 3500 m altitude on the Rocky Mountains in California, U.S.A. The heartwood gave a dark red colour when stained with diazotised benzidine solution.

The air-dried, fine-ground heartwood (3.7 kg) was extracted with ether for 24 hours and then with acetone for 60 hours. Upon concentration, the ether extract deposited a yellowish brown precipitate, which was separated. It consisted of crude chrysin (m. p. 265°) and was combined with the chrysin found later on in the 0.2 % sodium hydroxide

fraction of the ether extract. The ether was then completely evaporated on the steam bath, yielding 287 g of a brown syrup. 20 g of this syrup were treated with 250 ml of light petroleum and the insoluble sticky residue separated from the solution. The residue was extracted three times with 200 ml of boiling water and the extracts cooled and extracted with ether. The ether solution was dried and concentrated, leaving 0.6 g of a brown semi-crystalline syrup. This quantity corresponds to 0.2 % of the wood, and it may be concluded that the content of phenols in the ether extract is large enough to necessitate a thorough examination of it.

Investigation of the ether extract

The ether extract was treated with light petroleum (1.5 l) and the insoluble residue dissolved in ether (1 l). The light-petroleum solution was concentrated to a yellow syrup (149 g) which deposited crystals, probably resin acids. This fraction was not further investigated.

The ether solution was divided into fractions by shaking with saturated sodium bicarbonate (3 × 100 ml, extract = EB), saturated sodium carbonate (3 × 100 ml, extract = EC), 0.2 % sodium hydroxide (3 × 200 ml, extract = EH_1) and 4 % sodium hydroxide (2 × 200 ml, extract = EH_2). The remaining ether solution, containing neutral substances, was concentrated to a brown oil with a strong fluorescence (9.8 g). The different fractions were acidified and taken up in ether, and the ether solutions dried over anhydrous sodium sulphate and then concentrated on the steam bath.

EB and *EC* yielded small amounts of brown oils, which did not crystallise.

EH_1 deposited yellowish-brown crystals of crude chrysin, melting about 260°. They were combined with the crude chrysin already found in the ether extract and purified by vacuum-sublimation and recrystallisation from ethanol. Yield, 4.7 g of chrysin, m. p. 274—276°*. The diacetate melted at 195—197° and gave no m. p. depression when mixed with an authentic specimen of chrysin diacetate.

EH_2: The 4 % sodium hydroxide extract deposited a yellow crystalline precipitate (EH_{21}), which was separated before the solution (EH_{22}) was acidified. EH_{21} was treated with dilute sulphuric acid, and the resulting pale yellow precipitate separated, dried, and then recrystallised from 50 % acetic acid and from chloroform-light petroleum (twice). Yield, 2.2 g of tectochrysin, m. p. 163—165°. 0.8 g of less pure product (m. p. 162—163°) were isolated from the mother liquors.

EH_{22} yielded a brown oil, which partly crystallised. It was distilled in a vacuum, yielding a yellowish brown distillate which crystallised on cooling. After two recrystallisations from 50 % acetic acid and one from chloroform-light petroleum, pure pinosylvin monomethyl ether (11.3 g) was obtained. M. p. 120—121°.

Investigation of the acetone extract

The acetone extract, after some days, deposited colourless crystals, melting gradually at 152—160°. They were soluble in water, and the solution reduced Fehling's solution. These crystals were combined with the W fraction (see below). The acetone was then evaporated on the steam bath, leaving a brown syrup and a small volume of water solution (about 20 ml). This unusually small quantity of water in the acetone extract explains

* All melting points uncorrected.

why the sugar precipitate appeared in the extract before concentration. The water solution (= W) was separated from the syrup, and the latter was treated with ether (1 l) to precipitate 'membrane substances'. These were separated and stirred with cold water (50 ml). The suspension was filtered, and the filtrate combined with W. Weight of 'membrane substances', after water treatment and drying, was 4.0 g.

The ether solution was then divided into fractions in the usual way [2]. The volumes of alkaline reagents employed for the extractions were: Saturated sodium bicarbonate 3 × 300 ml, saturated sodium carbonate 4 × 200 ml, 0.2 % sodium hydroxide 5 × 250 ml, 4 % sodium hydroxide 2 × 350 ml. The fractions are referred to as B, C, H_1, and H_2. The remaining ether solution was concentrated to a reddish-brown fluorescent oil (10.9 g).

W: The aqueous solution was concentrated by vacuum distillation to a syrup which crystallised when treated with ethanol. The crystals were collected and combined with the crystalline precipitate mentioned above, yielding 5.8 g of a colourless crystalline powder, which reduced Fehling's solution and gave a strong pentose reaction with phloroglucinol and hydrochloric acid. It contained 0.24 % OCH_3, corresponding to only 1.5 % of pinitol. Part of this fraction (4.0 g) was precipitated with *p*-bromophenylhydrazine in dilute acetic acid solution. The precipitate was collected, and the filtrate shaken with benzaldehyde and then with ether to remove excess *p*-bromophenylhydrazine. The water was then evaporated in a vacuum, leaving a small quantity of a brown syrup (about 0.2 g). No crystalline products could be isolated from this syrup. It gave a faint pentose colour reaction, indicating that all arabinose had not been precipitated. OCH_3 = 5.23 %, corresponding to 33 % of pinitol.

The *p*-bromophenylhydrazone was treated with benzaldehyde to liberate the arabinose again as described for *P. monticola* [3]. 2.7 g of *l*-arabinose, m. p. 158—160°, were obtained. $[\alpha]_D^{20}$ + 107° ± 1° (equilibrium rotation in water, c = 2.8).

B yielded a brown non-crystalline syrup (1 g).

C yielded a small amount of a brown oil, which deposited crystals of crude chrysin. This product was combined with chrysin from H_1.

H_1: The sodium hydroxide extract was acidified and extracted with ether, and the ether solution dried over anhydrous sodium sulphate and then concentrated to a brown oil which soon deposited reddish crystals. The oil was stirred with ether, and the crystals separated by filtration. After vacuum-sublimation and recrystallisation from ethanol they yielded 0.5 g of chrysin, m. p. 275—278°. The ether filtrate was concentrated to a syrup and extracted with boiling water several times. The aqueous extracts were cooled and extracted with ether. On concentration, the ether solution yielded a yellow syrup which deposited an insoluble precipitate when treated with methanol. This product melted at 185—189°. After two recrystallisations from 50 % acetic acid, it yielded 0.25 g of pure pinocembrin, m. p. 194—196°. $[\alpha]_D^{20}$ −35° ± 2° (methanol, c = 2.6). Mixed m. p. with pinocembrin from *P. Banksiana* 194—195°.

H_2: The sodium hydroxide solution deposited a crystalline yellow precipitate (= H_{21}), which was collected and treated with dilute sulphuric acid. The resulting pale yellow precipitate was dried and recrystallised from 60 % acetic acid and then from chloroform-light petroleum, yielding 2.3 g of yellow crystals, m. p. 165—167°. It gave no m. p. depression with tectochrysin. The acetate melted at 154—156° alone and on admixture of tectochrysin acetate.

After separation of H_{21}, the 4 % sodium hydroxide solution (H_{22}) was acidified and extracted with ether. On concentration, this extract yielded a large quantity of a brown

oil, which soon crystallised. Crystals of m. p. 114—118° could be separated by filtration, and the filtrate was distilled in a vacuum, yielding a distillate which crystallised on cooling. Both fractions were recrystallised from chloroform. Almost colourless crystals (25.5 g) were obtained. M. p. 119—121°, no m. p. depression with pinosylvin monomethyl ether.

SUMMARY

The heartwood of *Pinus aristata* Engelm. has been investigated. *l*-Arabinose, chrysin, tectochrysin, pinocembrin and pinosylvin monomethyl ether were isolated from it. Pinitol could not be isolated, but the presence of small quantities of a methoxyl-containing substance in the sugar fraction was demonstrated by analysis.

The author is indebted to Dr. N. T. Mirov, Placerville, California, U.S.A., for the supply of wood, to Mr. A. Misiorny for skilful experimental assistance, and to *Fonden för Skoglig Forskning* for financial support. Mrs. A. Timell has kindly carried out the methoxyl determinations.

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Received November 28, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 60 – 63

The Synthesis of the Monochloro Methoxyquinols and some Related Substances

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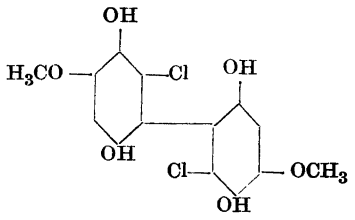
Quinones generally react with hydrogen chloride with the formation of monochloro quinols, which are rather stable compounds. By addition of hydrogen chloride to 4,4'-dimethoxydiquinone, however, Erdtman [1] obtained a substance (I), which possessed unexpected properties. On attempted recrystallization, it decomposed into hydrogen chloride and the original quinone. Contrary to expectation, this compound on acetylation yielded a diacetate, not a tetraacetate.



I ?

Since the structure of this peculiar addition product remains unelucidated, this problem deserves reinvestigation. To begin with, the three monochloro methoxyquinols have been synthesized* in order to compare the ultra-violet absorption of their actates with that of the acetate of the 'chlorophenol' (I).

The monochloro methoxyquinols were prepared by Dakin oxidation [2] of the corresponding monochloro vanillins. The chloromethoxyquinols were easily oxidized to the corresponding quinones with ferric chloride. Under

* According to private information from Dr. G. Castelfranchi similar reactions have been independentsy accomplished by A. Oliverio and G. Castelfranchi, Rome. This work will be published shortly in *Gazz. Chim. Ital.*

the same conditions methoxyquinol yields a diphenyl derivative [1]. The various substances prepared and their melting points are listed in Table 1.

*Table 1. Melting points * of the compounds described in this communication.*

Type of substance	2-Methoxy--3-chloro	2-Methoxy--5-chloro	2-Methoxy--6-chloro
Quinol	107—108	128—129	138—139
Quinol diacetate	80.5—81	125—126	80.5—81
Quinone	75—76	169—170	156.5—157

The results of the ultra-violet absorption measurements and further investigations of the 'chlorophenol' (I) will be published later.

EXPERIMENTAL

The three monochloro vanillins were prepared according to Raiford and Lichty [3].

2-Methoxy-3-chloroquinol

2-Chlorovanillin (2.5 g) was dissolved in 4 % sodium hydroxide (20 ml), and hydrogen peroxide (2.5 ml 30 % hydrogen peroxide in 20 ml water) was added. The temperature increased and the solution turned brown. After 15 minutes the solution was neutralized with concentrated hydrochloric acid and extracted continuously with ether over night. The ether solution was dried over calcium chloride and concentrated. The crystalline residue (1.75 g) was recrystallized from water. Two recrystallizations yielded the pure, colorless substances M. p. 107—108°.

$C_7H_7O_3Cl$ (174.6) Calc. OCH_3 17.8 Found OCH_3 17.5

2-Methoxy-3-chloroquinol diacetate

2-Chlorovanillin was oxidized as above, and then additional 4 % sodium hydroxide (20 ml) was added to the solution. This alkaline solution was shaken for 10 minutes with acetic anhydride (5 ml). The mixture was extracted with ether (2 × 50 ml) and the ether solution washed with dilute sodium carbonate and water, dried over calcium chloride and concentrated. The resulting oil (2.6 g) slowly crystallized and the crude product was recrystallized from methanol. Three recrystallizations yielded the pure colorless substance M. p. 80.5—81°.

$C_{11}H_{11}O_5Cl$ (258.7) Calc. OCH_3 12.0 Found OCH_3 11.9

* All melting points uncorrected.

2-Methoxy-3-chloroquinone

2-Chlorovanillin (2.5 g) was oxidized as above, and the solution acidified with concentrated hydrochloric acid (5 ml). Ferric chloride hexahydrate (4 g dissolved in a small amount of water) was added. The yellow quinone immediately precipitated and was collected by filtration. Yield 2.0 g. Two recrystallizations from ligroin yielded the pure substance, which crystallized in yellow needles. M. p. 75—76°.

$C_7H_5O_3Cl$ (172.6) Calc. OCH_3 18.0 Found OCH_3 17.7

Reductive acetylation with zinc dust and acetic anhydride yielded 2-methoxy-3-chloroquinol diacetate, identical with the substance described above.

2-Methoxy-5-chloroquinol

This substance was prepared in the same way as the 3-chloro derivative, 2.5 g 6-chlorovanillin yielded 2.0 g crude 2-methoxy-5-chloroquinol. Two recrystallizations from ethanol yielded the pure, colorless substance. M. p. 128—129°.

C_7H_7Cl (174.6) Calc. OCH_3 17.8 Found OCH_3 17.6

2-Methoxy-5-chloroquinol diacetate

This substance was prepared in the same way as the 3-chloro derivative. 2.5 g 6-chlorovanillin yielded 2.6 g crude 2-methoxy-5-chloroquinol diacetate. Two recrystallizations from methanol yielded the pure, colorless substance. M. p. 125—126°.

$C_{11}H_{11}O_5Cl$ (258.7) Calc. OCH_3 12.0 Found OCH_3 12.0

2-Methoxy-5-chloroquinone

This substance was prepared in the same way as the 3-chloro derivative. 2.5 g 6-chlorovanillin yielded 2.0 g of the crude 2-methoxy-5-chloroquinone. Two recrystallization from ethanol yielded the pure substance in the form of yellow needles. M. p. 169—170°.

$C_7H_5OCl_3$ (172.6) Calc. OCH_3 18.0 Found OCH_3 17.9

Reductive acetylation with zinc dust and acetic anhydride yielded the 2-methoxy-5-chloroquinol diacetate described above.

2-Methoxy-6-chloroquinol

This substance was prepared in the same way as the 3-chloro derivative. 2.5 g 5-chlorovanillin yielded 2.1 g crude 2-methoxy-6-chloroquinol. Two recrystallizations from benzene yielded the pure, colorless substance. M. p. 138—139°.

$C_7H_7O_3Cl$ (174.6) Calc. OCH_3 17.8 Found OCH_3 17.8

2-Methoxy-6-chloroquinol diacetate

This substance was prepared in the same way as the 3-chloro derivative. 2.5 g 5-chlorovanillin yielded 2.7 crude 2-methoxy-6-chloroquinol diacetate. Two recrystallizations from methanol yielded the pure, colorless substance. M. p. 80.5—81°.

$C_{11}H_{11}O_5Cl$ (258.7) Calc. OCH_3 12.0 Found OCH_3 12.0

2-Methoxy-6-chloroquinone

This substance was prepared in the same way as the 3-chloro derivative. 2.5 g 5-chlorovanillin yielded 1.8 g crude 2-methoxy-6-chloroquinone. Two recrystallizations from ethanol yielded the pure substance in orange-red needles. M. p. 156.5—157°.

$C_7H_5O_3Cl$ (172.6) Calc. OCH_3 18.0 Found OCH_3 18.1

Reductive acetylation with zinc dust and acetic anhydride yielded the 2-methoxy-5-chloroquinol diacetate described above.

SUMMARY

The three isomeric quinols, quinol diacetates and quinones with one chlorine and one methoxyl group have been prepared.

We are indebted to Professor H. Erdtman for suggesting this work.

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Received November 23, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 64 – 71

Influence of Nitrogen Nutrition on the Excretion of Protease by Gelatin-Liquefying Bacteria

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Virtanen and Tarnanen [1] and Virtanen and Suolahti [2] have shown that *Pseudomonas fluorescens* excretes into the nutrient solution a protein-hydrolyzing enzyme. This gave an explanation to the liquefaction of gelatin effected by the bacteria group called gelatin-liquefying bacteria. Peptidases are not excreted by these bacteria, but remain in the cells.

In connection with the studies made in this laboratory on the mutual relation of the nitrogen content and the enzymatic activity of the cells, we have also examined to what extent the formation of the protease excreted by *Ps. fluorescens* depends on the amount of nitrogen nutrition and, hence, on the N-content of the cells. At the same time we have also studied the dependence of the growth velocity of cells on the amount of N-nutrition and the percentage of the N-fraction insoluble in trichloracetic acid in cells with widely varying N-content. The results are reported in the present paper.

EXPERIMENTAL

The strain of *Pseudomonas fluorescens* used in the experiments was isolated from sewage. The selected strain dissolved gelatin with the greatest rapidity. Pepton broth was used for the stock culture in the laboratory. In the experiments proper, a mineral nutrient solution was used in which glycerin served as the only carbon source. The composition of the nutrient solution was: 50 g glycerin, 25 g K_2HPO_4, 15 g NaCl, and 0.2 g $MgSO_2 \cdot 7H_2O$ per 5 l tap water. Ammonium sulphate was used as the nitrogen source, its amount ranging from 0.05 to 25 g per 5 l. As it was important to induce as vigorous a growth of bacteria as possible, experiments were at first conducted in order to find out the conditions for the best growth. The growth was therefore compared in thin layers in Roux-flasks, and in thick layers in Kluyver-flasks where a strong current of air was passed through the solution by means of a compressor. The latter method, however, gave only about half of the bacterial yield obtained from the former, and therefore cultivation in the unmoving solution in the Roux-flasks was adopted.

Each Roux-flask contained 100 ml nutrient solution. An inoculation of 1 ml liquid culture of *Ps. fluorescens* was made into each flask, and ammonium sulphate was simultaneously added. Each experiment comprised seven different concentrations of ammonium sulphate, and for each concentration 10 Roux-flasks were employed (= 1 litre nutrient solution). The ammonium sulphate concentrations used were: 0.05 g, 0.1 g, 0.5 g, 5 g, 10 g, 15 g, and 25 g per 5 l nutrient solution.

It was attempted to maintain the pH of the nutrient solution at 6.8—7 throughout the growth period. It was controlled by means of indicator paper, and when the pH decreased NaOH solution was added.

The growth temperature was about 30° C, and the period of growth varied from 2 to 3 days.

When the growth was interrupted, the bacteria were separated from the solution by centrifugation, suspended in water, and separated again. All of the clear growth solutions with the same $(NH_4)_2SO_4$-concentration were combined as well as the bacterial masses, which were suspended in 30 ml of water. The growth of bacteria was determined both by the turpidity of the culture solution, using a Klett-Summerson photometer, and by determining the dry matter of the bacterial suspension obtained. In the lower bacterial concentrations, both methods gave a similar growth curve; but in higher concentrations, the photometric determinations gave too low values.

The nitrogen soluble in trichloracetic acid was determined by allowing the wet bacterial mass to stand for 24 hours in 20 % trichloracetic acid. After that time, it was centrifuged and the solution filtered through bacterial filter (Jena G 5 auf 3) for complete removal of bacteria. Nitrogen was determined in the clear solution.

The activity of the protease present in the growth solution was determined by examining its ability to decompose both casein and gelatin. Decomposition was followed by determining amino-N by the Cu-method of Pope and Stevens. Total N was determined by the Kjeldahl method introduced by Miller [4]. Ten per cent of casein was dissolved in water by adding NaOH to raise the pH to 9. This basal solution was added to the growth solution, to make it 2 per cent with regard to casein. In the experiments with gelatin, 2 per cent solutions were also used. The pH of the growth solutions was adjusted to 7. In all experiments, infections were prevented by means of toluene. A control, with distilled water instead of growth solution, was included in each experiment. The insignificant amount of amino-N formed therein was always subtracted from the results in the experiment proper.

The clear growth solution was used in the same experiment series in amounts that always corresponded to equal amounts of bacteria. If, for instance, the yield of bacteria was 762 mg dry substance in one litre nutrient solution with 10 g ammonium sulphate, and 132 mg bacteria in one litre with 0.1 g ammonium sulphate, the protease activity of the growth solution was determined by using, in the former case, 45.6 ml and, in the latter, 263.6 ml growth solution.

The increase of amino-N in casein and gelatin solutions is expressed in the results as mg per g total N of bacteria.

The peptidase activity of the bacterial mass was determined by following the increase of amino groups in a Witte-pepton solution. A known amount of bacterial mass was suspended in 2 % pepton solution, and the pH adjusted to 7.

The peptidase activity of bacteria grown in different ammonium sulphate concentrations was compared, in the same experiment series, by using the same amount of dry bacteria in each experiment. As the N-content of the bacteria was known, decomposition

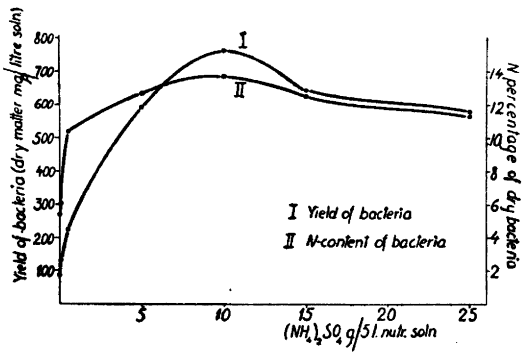


Fig. 1. Dependence of the growth and N-content of Ps. fluorescens on the ammonium sulphate concentration of the nutrient solution.

could be calculated per nitrogen in them. The increase of amino-N is expressed in the results as mg per g total N of bacteria.

RESULTS

In the preliminary experiments different problems were examined, especially the effect of ammonium sulphate concentration on the growth of bacteria, on the N-content of bacteria, and on the protease activity of the clear growth solution. After that, four complete experiments were made in which all of these questions were simultaneously examined. The dependence of growth and N-content of the bacteria on the ammonium sulphate concentration was similar in all experiments, as can be seen from Table 1 and Fig. 1.

The results show that 10 g $(NH_4)_2SO_4$ is optimum for the growth. The N-content of the cells also shows a maximum at this concentration. The decrease of N-content accompanying the lowering of $(NH_4)_2SO_4$ is very distinct. Table 2 shows the uptake of ammonium nitrogen by bacteria during the experiment.

Table 1. Dependence of the NH_4^+-concentration of the nutrient solution on the growth and N-content of Ps. fluorescens.

$(NH_4)_2SO_4$ g/5 l	Expt. 5 (growth 72 h)		Expt. 6 (growth 48 h)		Expt. 7 (growth 72 h)		Expt. 8 (growth 72 h)	
	Dry bact. mg/l	N % of dry bact.	Dry bact. mg/l	N % of dry bact.	Dry bact. mg/l	N % of dry bact.	Dry bact. mg/l	N % of dry bact.
25	504	12.9	259	10.5	577	11.3	516	11.5
15	—	—	—	—	646	12.5	556	11.8
10	—	—	—	—	762	13.7	824	12.4
5	550	11.1	391	11.2	595	12.7	544	11.9
1	294	10.2	—	—	—	—	—	—
0.5	141	10.0	209	10.7	224	10.4	237	10.1
0.1	—	—	112	6.1	132	6.1	137	6.1
0.05	57	5.4	76	5.6	87	5.4	94	5.4

Table 2. The amount of ammonium nitrogen present in the nutrient solution at the end of the experiment. Inoculation contained 4 mg N.

$(NH_4)_2SO_4$ g/5 l	N in bacteria mg/l	N remaining in solution mg/l
25	59	995
15	66	569
10	102	322
5	65	149
0.5	24	0
0.1	8	0
0.05	5	0

In an ammonium sulphate concentration of 0.5 g/5 l, the bacteria had consumed the entire nitrogen nutrition. The very distinct decrease in the nitrogen content of cells from this concentration on is evidently due to the fact that the cells are still able to form cell material to some extent although nitrogen nutrition from the outside is no longer available. Thus the decrease in the N-content of cells is natural. However, it is surprising that the nitrogen content of the cells is somewhat lower in the superoptimum ammonium sulphate concentration than in the optimum one.

In order to get a rough idea about to what extent the nitrogen compounds are similar in high-nitrogen and low-nitrogen cells, we determined, in experiments 6 and 8 the N-fractions soluble and insoluble in trichloracetic acid. The results appear in Table 3.

Table 3. N-fraction insoluble in trichloracetic acid in Ps. fluorescens with normal and low-nitrogen content.

$(NH_4)_2SO_4$ g/5 l	N insoluble in trichloracetic acid, % of total N	
	Expt. 6	Expt. 8
25	75 (10.5)	76 (11.5)
15	—	81.8 (11.8)
10	—	85.9 (12.4)
5	84.5 (11.2)	84.3 (11.9)
0.5	—	77.2 (10.1)
0.1	—	76.4 (6.1)
0.05	—	75.0 (5.4)

The parenthetical figures indicate the N-percentage of bacteria per dry matter.

It can be seen from the table that when the N-content of the cells is highest, the proportion of the so-called protein-N (insoluble in trichloracetic acid) to total N is also highest. In low-nitrogen cells this N-fraction is considerably lower. It is of special interest that the same applies also to cells grown in nutrient solutions with excess ammonium nitrogen, although their N-content is not much lower than that of the cells grown in optimum NH_4^+-concentration. The bacteria separated from cultures grown for 48 h and 72 h gave similar results. The age of the cells is, accordingly, not a decisive factor in the variations in the N-content of the cells or in the changes in the N-fraction insoluble in trichloracetic acid. A more detailed analysis of the N-compounds of the cells grown in nutrient solutions containing different amounts of N-nutrition was recently made by Virtanen and Miettinen [5]. They found that the lack of nitrogen nutrition had the greatest effect on the decrease of nucleic acid and protein fractions. Soluble nitrogen compounds other than nucleic acid did not at all decrease. The N-fraction from *Torulopsis* yeast soluble in trichloracetic acid contains, according to Roine [6], chiefly free amino acids and their amides (glutamic and aspartic acids, their amides, and alanine) and, in addition, nucleotides. Peptides are found, at most, in very small amounts. The composition of the soluble N-fraction was not examined quantitatively with *Ps. fluorescens*. If it is similar to that in *Torulopsis*, it is evident that in the low-nitrogen bacteria protein synthesis has not proceeded as far as in the normal-nitrogen cells.

The dependence of the activity of protease excreted into the nutrient solution was, in all four experiments, very similar. The results are therefore presented only from experiment 8.

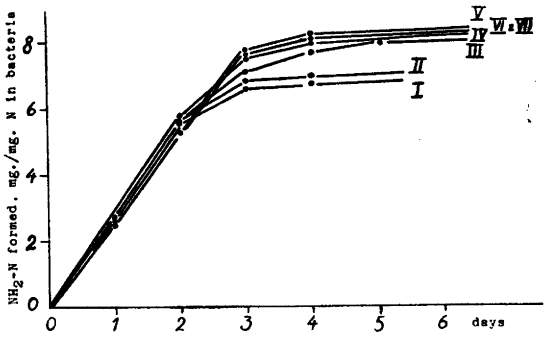
In each experiment, both with casein and gelatin, the quantity of the growth solution corresponded to 37.6 mg bacterial mass. Thus the experimental solutions had the following compositions (Table 4).

Table 4. Composition of the solution in experiments on the decomposition of casein by the extracellular protease of P s. f l u o r e s c e n s.

$(NH_4)_2SO_4$ g/5 l	Clear growth solution ml	Water ml	10 % casein solution ml
0.05	400	—	100
0.1	275	125	100
0.5	158.6	241.4	100
5	69.2	331.8	100
10	45.5	354.5	100
15	67.7	332.3	100
25	70.9	329.1	100
Control	—	400	100

Fig. 2. Influence of N-nutrition on the formation of amino-N from casein by excreted protease of Ps. fluorescens.

I.	$(NH_4)_2SO_4$	*0.05*	*g/5l*
II.	»	*0.1*	»
III.	»	*0.5*	»
IV.	»	*5.0*	»
V.	»	*10.0*	»
VI.	»	*15.0*	»
VII.	»	*25.0*	»



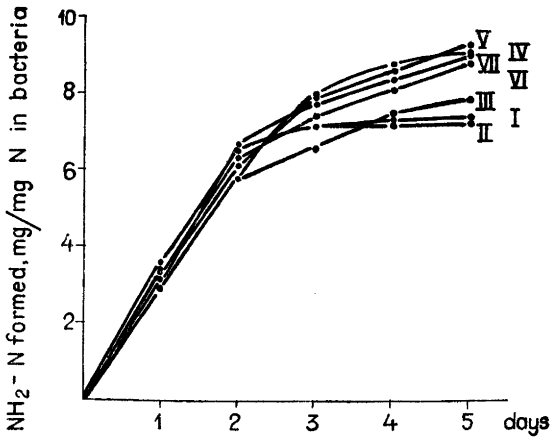
When gelatin was used as substrate, the experimental arrangement was the same. The results appear from the curves in Figs. 2 and 3.

The curves illustrate that the formation of amino-N, from both casein and gelatin, by the action of protease excreted into the culture solution, is practically independent of the ammonium sulphate concentration of the solution. Only in the lowest NH_4^+-concentrations is the hydrolysis of proteins by the growth solution retarded more rapidly than in higher concentrations. The formation of the extracellular protease in the cells is, on the whole, largely independent of the nitrogen content of the cells.

Fig. 4 shows the hydrolysis of pepton caused by the bacterial mass. The N-content of the bacteria has no noticeable effect even on this reaction. Virtanen and Winkler[7] have earlier noted that the proteolytic ability of *Escherichia coli* does not change as the N-content of the cells decreases from 13 to 9.5 %.

Fig. 3. Influence of N-nutrition on the formation of amino-N from gelatin by excreted protease of Ps. fluorescens.

I.	$(NH_4)_2SO_4$	*0.05*	*g/5l*
II.	»	*0.1*	»
III.	»	*0.5*	»
IV.	»	*5*	»
V.	»	*10*	»
VI.	»	*15*	»
VII.	»	*25*	»



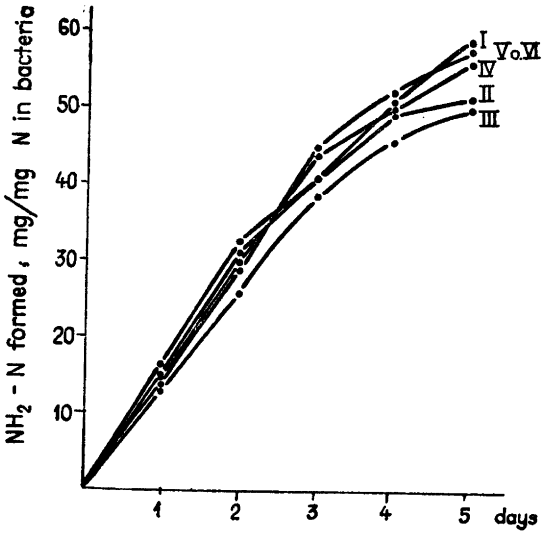


Fig. 4. Decomposition of pepton caused by Ps. fluorescens masses grown in different ammonium sulphate concentrations.

I.	$(NH_4)_2SO_4$	*0.05 g/5l*
II.	»	*0.1* »
III.	»	*0.5* »
IV.	»	*5* »
V.	»	*10* »
VI.	»	*15* »
VII.	»	*25* »

As the enzymes belong to the fraction insoluble in trichloracetic acid, it would be most natural to calculate the activity of the enzymes per nitrogen of this fraction and not per total nitrogen of the cells. The results, however, would not change in principle by this change of the calculation basis. We have therefore again used the total nitrogen basis since insoluble nitrogen was not determined in the earlier investigations with *E. coli*.

SUMMARY

The N-content of the cells of *Ps. fluorescens* was highest in the ammonium sulphate concentrations optimum to growth. The nitrogen content of the cells decreased as the N-nutrition was lowered and the growth weakened. When the ammonium nitrogen content of the nutrient solution was so low that it was rapidly consumed by the cells, the N-content of the cells decreased to less than half of the normal.

The percentage of the N-fraction insoluble in trichloracetic acid (so-called protein N) was highest in high-nitrogen cells in the ammonium concentration optimum to growth. As the N-content of the cells lowered, the percentage of this N-fraction decreased. The same occurred when the ammonium sulphate concentration of the nutrient solution was superoptimum. The activity of the protease excreted by *Ps. fluorescens* into the culture solution was largely independent of the ammonium sulphate concentration of the culture solution

and of the N-content of the cells. Both in the decomposition of gelatin and casein this fact could be noted. The enzymes which cause the hydrolysis of pepton and which are not excreted by the cells are also independent of the N-content of the cells.

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Received November 15, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 72 – 87

A New Principle for the Investigation of Complex Equilibria and the Determination of Complexity Constants

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The main methods for potentiometric determination of the complexity constants of a complex system are founded on measurements of the concentration of free ligand or of free central group. Such methods have been described by J. Bjerrum [1] and Leden [2]. In a previous treatise [3], the present author has developed methods of these two kinds and furthermore a third method, based on the use of cells without liquid junction. These three methods are suited for a determination of the complexity constants of the mononuclear complexes, irrespective of whether or not polynuclear complexes are formed, and in this respect they are general. Under favourable circumstances the constants of some of the dinuclear complexes can also be determined.

In practice, the choice of a measurement method will depend on the possibility of finding a suitable electrode. Many complex systems cannot be investigated by any of the methods mentioned, because there is no electrode of the first or second order, by means of which the concentration of free ligand or free central group can be determined. So *e. g.* the complex formation between Cu^{2+} and Cl^- cannot be investigated potentiometrically by means of a copper amalgam electrode or a silver chloride electrode, as both of them reduce Cu^{2+} to Cu^+ in the presence of Cl^-. In such a case it would be desirable that the measurements could be made via a second ligand, employing a non-reducing electrode.

The basic principle of the new method, described below, is the use of a three component complex system. Thus the solutions contain a central group M and two competing ligands A and B. It is thus possible to have the formation of complexes with A or B as ligands, and furthermore the formation of mixed complexes with both A and B as ligands. The affinity of M for B must

be greater than that of M for A, so that B will finally completely displace A as ligand, if the concentration of A is kept constant, while that of B is increased within a limited range. When the measurements give a direct determination of the concentration of free ligand [B] in the solutions, it will be shown below that it is possible to calculate the complexity constants of the mononuclear complexes MA, MA_2, . . ., MA_N. The complexity constants of mixed complexes of the type MA_jB ($j = 1,2, \ldots$) can also be determined. Naturally the constants of the B-complexes MB_j ($j = 1,2, \ldots$) can be calculated too, in the manner described in the previous treatise.[3, p. 14]

This method of determining the complexity constants of the A-complexes by means of another ligand will be called the "method of ligand displacement".

Deduction of the equations necessary for the calculation of the complexity constants

For the sake of brevity, we postulate that in the solutions, containing the central group M and the ligands A and B, only mononuclear complexes of the general type MA_jB_k are formed. As will be seen below, our equations easily can be generalized, so that they are also valid, when polynuclear complexes are formed.

For the known total concentrations of M and B we have the following expressions:

$$C_M = [M] + \sum_{j+k=1}^{N} [MA_jB_k]; \quad j, k \geqq 0 \tag{1}$$

$$C_B = [B] + \sum_{j+k=1}^{N} k[MA_jB_k] \tag{2}$$

We apply the law of mass action to the dissociation equilibria of the complexes and obtain *:

$$\beta_{j,k} = [MA_jB_k][M]^{-1}[A]^{-j}[B]^{-k} \tag{3}$$

As the measurements are performed at a constant ionic strength, we may presume that the complexity "constants" $\beta_{j,k}$ remain approximately constant, when the total concentrations C_M, C_A and C_B are varied within limited ranges. By substituting eq. (3) in (1) and (2), we obtain the expressions:

* The constants that we in the first place intend to determine are $\beta_{j,0}$, for which the notation β_j is used when the solutions contain only one sort of ligand.

$$C_M = [M]\,(1 + \sum_{j+k=1}^{N} \beta_{j,k}\,[A]^j[B]^k) \tag{4}$$

$$C_B = [B] + [M] \sum_{j+k=1}^{N} k\beta_{j,k}\,[A]^j[B]^k \tag{5}$$

For the sake of brevity we use the notation:

$$X([A],\ [B]) = 1 + \sum_{j+k=1}^{N} \beta_{j,k}\,[A]^j[B]^k \tag{6}$$

In the following we shall sometimes write only X in place of X ([A], [B]). By partial differentiation of X we get:

$$\frac{\partial X}{\partial [B]} = \sum_{j+k=1}^{N} k\beta_{j,k}\,[A]^j[B]^{k-1} \tag{7}$$

The ligand number $\bar{n}$, with respect to the ligand B, is defined in the usual way:

$$\bar{n} = \frac{C_B - [B]}{C_M} \tag{8}$$

Combining eq. (4—8) we obtain:

$$\frac{\partial \ln X}{\partial [B]} = \frac{\bar{n}}{[B]} \tag{9}$$

Here $\bar{n}$ is a function of [A] and [B]. Eq. (9) is deduced under the assumption that only mononuclear complexes exist. If polynuclear complexes are also formed, the expression for $\bar{n}$ will contain terms with [M], $[M]^2$ etc. as factors (*cf.* Fronæus [3, p. 19]). Then $\bar{n}$ will be a function of [A], [B] and C_M. But if we allow $C_M \to 0$ (that is $[M] \to 0$) these terms cancel, and we have the quite *general* equation:

$$\frac{\partial \ln X}{\partial [B]} = \left(\frac{\bar{n}}{[B]}\right)_{C_M = 0} \tag{10}$$

For the sake of clarity we put $(\bar{n})_{C_M = 0} = \bar{n}$ ([A], [B]). By $\Delta\bar{n}$ we mean the difference between the ligand numbers of two solutions, the first of which has [A] = 0, while [B] and C_M are the same in both solutions. Then we have: $\bar{n}$ (0, [B]) $- \bar{n}$ ([A], [B]) $= (\Delta\bar{n})_{C_M = 0}$. If [A] is kept at a constant value $[A]_m$, we obtain by integration of (10) with respect to [B]:

$$ln \frac{X([A]_n, [B]_n)}{X([A]_n, 0)} = \int_0^{[B]_n} \frac{\bar{n}([A]_n, [B])}{[B]} \cdot d[B] \qquad (11)$$

For $[A]_n = 0$ this equation is reduced to:

$$lnX\ (0, [B]_n) = \int_0^{[B]_n} \frac{\bar{n}\ (0, [B])}{[B]} \cdot d[B] \qquad (12)$$

Eq. (12) is identical with the eq. (24) of the previous treatise [3, p. 15]. From eq. (11) and (12) we derive:

$$ln\ X\ ([A]_n, 0) - ln \frac{X([A]_n, [B]_n)}{X(0, [B]_n)} = \int_0^{[B]_n} \left(\frac{\Delta\bar{n}}{[B]}\right)_{C_M = 0} \cdot d[B] \qquad (13)$$

The term of the polynomial X ([A], [B]) which is of the highest degree with respect to [B] is $\beta_{0,N} \cdot [B]^N$. Then we have presumed that the saturated complex MB_N, which cannot take up an additional ligand B, cannot take up a ligand A either. Hence it follows that:

$$\lim_{[B]_n \to \infty} \frac{X([A]_n, [B]_n)}{X(0, [B]_n)} = 1$$

As already mentioned, this means that if C_A is kept constant but C_B is increased, the higher M—B complexes become the predominant complexes in the solutions. Thus B will completely displace A as ligand, and the stronger a complex former (with respect to M) B is than A, the faster this displacement will proceed. The validity of this assumption can be examined experimentally, for if we choose a ligand B with the proper affinity for M, it will be possible to find a value b, within an appropriate concentration range, so that when $[B] \geqq b$ the function $(\Delta\bar{n}/[B])_{C_M = 0}$ is $= 0$, the random errors considered. If b is taken as the upper limit of integration in eq. (13), we consequently obtain:

$$ln\ X\ ([A]_n, 0) = \int_0^{b} \left(\frac{\Delta\bar{n}}{[B]}\right)_{C_M = 0} \cdot d[B] \qquad (14)$$

It is evident that the greater the value of $[A]_n$ is, the greater becomes the value of b which must be taken.

Determination of corresponding values of [B] and $\left(\frac{\Delta \bar{n}}{[B]}\right)_{C_M = 0}$

The calculation method is based on an experimental determination of [B] in the complex solutions. The measurements are arranged in the following way. In a series of measurements C_A and C_M are kept constant *, and C_B is varied. From the ligand measurement (regarding [B]) we obtain $\bar{n}/[B]$ which can be plotted against [B] with C_M (or its starting value in the series) and C_A as parameters. By repeating the measurements at different values of C_M, but still at the same value of C_A, the limiting function $(\bar{n}/[B])_{C_M = 0} = \bar{n}([A]_n, [B])/[B]$ can be obtained by extrapolation. At $C_M = 0$, $[A]_n$ is also constant and $= C_A$ at varying [B]. The function $\bar{n}(0, [B])/[B]$ at $C_A = 0$ is determined in the same way (*cf.* Fronæus [3, p. 16]). From the difference we get the function $(\Delta\bar{n}/[B])_{C_M = 0}$, and then the integration in eq. (14) is performed graphically and will give us a pair of values of $[A]_n$ and $X([A]_n, 0)$. After a sufficient number of corresponding values have been obtained in this way, the complexity constants of the complexes MA_j ($j = 1,2, \ldots$) can be calculated from the eq.:

$$X([A]_n, 0) = 1 + \sum_{j=1}^{N} \beta_j [A]_n^j \tag{15}$$

In (15) the complexity constants have been denoted by β_j instead of $\beta_{j,0}$ as no confusion is to be feared here.

If the complexity constants of the complexes MB_j are not known in advance, they can be computed from eq. (12).

Calculation of the complexity constants of mixed complexes of the type MA_jB

By combining eq. (6) and (10), we obtain the relation:

$$\lim_{[B] \to 0} \left(\frac{\bar{n}}{[B]}\right)_{C_M = 0} = \frac{\sum_{j=0}^{N-1} \beta_{j,1} [A]^j}{X([A], 0)} \tag{16}$$

* In order to reduce the number of titration solutions necessary, we may also allow C_M to vary in the series. Then C_M must be a known function of C_B (see eq. (18)).

At different values of [A], the limiting values of the left member of eq. (16) are estimated by graphical extrapolation of $(\bar{n}/[B])_{c_M=0}$ to [B] = 0. The corresponding values of the polynomial $X([A], 0)$ have already been calculated, and $\beta_{0,1}$, the constant of the complex MB, is determined in advance. Thus from eq. (16) we can compute as many of the constants $\beta_{1,1}$, $\beta_{2,1}$, $\beta_{N-1,1}$, as the concentration range of [A] will admit.

Estimation of the random errors

From the reproducibility obtained in the measuring of [B], the maximum random errors in the values of the functions $\bar{n}(0, [B])/[B]$ and $\bar{n}$ $([A]_n, [B])/[B]$ can be estimated as well as the corresponding maximum error in their difference. If the last-mentioned error is denoted by ε, and is considered to be practically constant at different values of [B], as it was in the measurements described below, the maximum error of $lnX([A]_n, 0)$, computed from (14), will be $\varepsilon \cdot b$.

Another error may arise from the fact that in eq. (14) we have a finite range of integration (0 to b instead of 0 to ∞). From the algebraical expression for the function $(\Delta\bar{n}/[B])_{c_M=0}$, it is easily seen that the function, at great values of [B], can be approximated by $\varphi([A]) \cdot [B]^{-2}$, as no mixed complex with N B-ligands exists. If no mixed complexes with $N-1, \ldots, N-k$ B-ligands are formed, we can use, at great values of [B], the approximation $\varphi([A]) \cdot [B]^{-k-2}$, and at increasing values of b the integral in eq. (14) then converges more rapidly. Thus, in the most disadvantageous case, we have as an approximate expression of the omitted integral R:

$$R = \int_b^\infty \frac{\varphi([A])}{[B]^2} \cdot d[B] = \frac{\varphi([A])}{b} \tag{17}$$

When [B] = b, the integrand is ~ 0 according to our measurements. Then on the basis of the estimated random errors, the true value can be ε at most.

$$\frac{\varphi([A])}{b^2} \leqq \varepsilon \; ; \; (R)_{max.} = \varepsilon \cdot b$$

Thus, the total maximum error of $lnX([A]_n, 0)$ is $2\,\varepsilon \cdot b$.

It is rather difficult to decide how the product $\varepsilon \cdot b$ depends on the strength of the M—B complexes. However, in order to secure approximately constant activity coefficients it is necessary that the value of b be considerably smaller than the chosen ionic strength.

A POTENTIOMETRIC INVESTIGATION OF THE $Cu^{2+}-SO_4^{2-}$ SYSTEM WITH Ac^-* AS THE DISPLACING LIGAND

In order to examine the applicability of the method described, it has been applied to the cupric sulphate system, which the present author has investigated in a previous work [3, p. 57] by potentiometric and spectrophotometric methods. In the latter work the potentiometric investigation was a central ion measurement, that did not presuppose anything regarding the composition of the complex system. These previous measurements proved that Cu^{2+} forms considerably weaker complexes with SO_4^{2-} than with Ac^-, and therefore the last-mentioned ion has been taken as the displacing ligand. In order to be able to compare the results, we have used the same ionic strength, 1 C (mols/litre), with sodium perchlorate as a neutral salt.

Chemicals used. The same preparations of cupric perchlorate, sodium perchlorate, perchloric acid, sodium sulphate, sodium acetate, acetic acid and quinhydrone were used as in the earlier investigations [3, p. 31]. The cupric perchlorate, however, was further purified by recrystallisation from water, so that the excess of perchloric acid in the preparation was negligible.

The measurements for the determination of $[Ac^-] = [B]$ were carried out with the quinhydrone electrode, as this gives great reproducibility and its applicability for this purpose has been examined in the previous work [3, p. 36]. The cells, the emf:s of which have been measured, were of the following type:

$$+ \text{RE} \;\Big\|\; \begin{matrix}\text{quinh. (s)}\\ \text{complex}\\ \text{solution}\end{matrix} \;\Big|\; \text{Au}-$$

The reference electrode RE had the same composition as before [3, p. 34], and the salt bridge consisted of 1 000 mC $NaClO_4$. The solution of the right half-cell was obtained by mixing two solutions S_1 and S_2, both of them of an ionic strength of 1 C.

$$S_1 = \begin{cases} a \text{ mC } Cu(ClO_4)_2 \\ C_A \text{ mC } Na_2SO_4 \\ (1000-3a-3C_A) \text{ mC } NaClO_4 \end{cases} \qquad S_2 = \begin{cases} C_A \text{ mC } Na_2SO_4 \\ 500 \text{ mC NaAc} \\ 250 \text{ mC HAc} \\ (500-3C_A) \text{ mC } NaClO_4 \end{cases}$$

Then for the measuring solution we have the following relation between C_M and C_B in mC:

---

* Ac^- = acetate ion.

$$C_M = a\left(1 - \frac{C_B}{500}\right) \tag{18}$$

The mixing was carried out in the electrode vessel, and good stirring was brought about by a stream of nitrogen gas, released from oxygen and led through 1 C $NaClO_4$ in order to obtain the correct water pressure. The cells were placed in a thermostat at 20.00 ± 0.02 °C. The emf:s measured were very steady and reproducible within 0.1—0.2 mV.

The emf in mV of the cell, given above, is denoted by E. At the same values of C_A and C_B, but at $C_M = 0$, the emf is called E_0. The value of E_0 is practically independent of C_B within the concentration range used, but varies somewhat with C_A (see Table 1). The difference $E_0 - E = E_B$ is the emf of a concentration cell, and we have

$$E_B = 58.16 \; log \frac{[H^+]}{[H^+]_0} \tag{19}$$

if we neglect the effect of somewhat different liquid junction potentials and activity coefficients in the two halves of the concentration cell. $[H^+]_0$ denotes the concentration of H^+ in the solution with $C_M = 0$. The concentration of undissociated acetic acid is:

$$0.500 \cdot C_B - [HSO_4^-] - [H^+] = 0.500 \cdot C_B - \vartheta$$

The correction term ϑ is calculated from the relation $\vartheta = [H^+]([SO_4^{2-}]/K_2 + 1)$, where K_2 is the secondary dissociation constant of the sulphuric acid. At $C_M = 0$, the correction term is called ϑ_0. For the calculation of ϑ and ϑ_0, we may put $[SO_4^{2-}] \simeq C_A$. For the dissociation of the acetic acid we have:

$$\frac{[H^+]\,[B]}{0.500 \cdot C_B - \vartheta} = \frac{[H^+]_0\,(C_B + \vartheta_0)}{0.500 \cdot C_B - \vartheta_0}$$

or approximately:

$$\frac{[H^+]}{[H^+]_0} = \frac{C_B - 2\,\vartheta + 3\,\vartheta_0}{[B]} \tag{20}$$

For the ligand number $\bar{n}$ (regarding B) the expression is:

$$\bar{n} = \frac{C_B + \vartheta - [B]}{C_M} \tag{21}$$

where $C_B + \vartheta$ is the corrected value of the total concentration of B. In order to compute the correction terms ϑ and ϑ_0, it is necessary to make an approximate determination of the dissociation constant K_2. For this reason the emf of the cell

$$+ \text{RE} \;\Big\|\; \begin{array}{l}\text{quinh. (s)} \\ C_H \text{ mC } HClO_4 \\ (1000 - C_H) \text{ mC } NaClO_4\end{array} \;\Big|\; \text{Au} -$$

was measured and gave a calibration curve, representing the relationship between the emf and $[H^+] = C_H$. A new series of measurements were then carried out, when the solution of the right half-cell had the composition C_A mC Na_2SO_4, C_H mC $HClO_4$, (1 000—$3C_A$—C_H) mC $NaClO_4$, and with the relation $8\ C_A + 3\ C_H = 1\ 000$ mC. From the emf, measured at different C_H, and the calibration curve, $[H^+]$ in these solutions was calculated without the liquid junction potentials influencing the measurements essentially. K_2 could then be computed, and at $C_H \leqq 100$ mC the value of K_2 was fairly constant and equal to $(8.4 \pm 0.5) \cdot 10^{-2}$ C. Thus we know all data that are necessary for the determination of [B] and $\bar{n}/[B]$ from eq. (19—21) and the values of E_0.

Table 1. Determination of $[H^+]_0$ *and* ϑ_0 *at different* C_A.

C_A mC	E_0 mV	$[H^+]_0$ mC	ϑ_0 mC
0	169.6	0.012	0.01
50	169.0	0.012	0.02
100	168.5	0.013	0.03
150	167.3	0.013	0.04

The values obtained from the potentiometric measurements of the cupric sulphate system are shown in Table 2. In the first four measurement series in the table (nos. 1—13) the factor a in eq. (18) has a value of 100 mC, and in the remaining four measurement series (nos. 14—27) a value of 50 mC. Every value of E_B is a mean value from at least two parallel measurements.

To obtain further proof of the applicability of the quinhydrone electrode, a measurement series ($C_A = 0$) was repeated with a glass electrode (Radio meter, type G 100), combined with a valve potentiometer (Radiometer, type PHM 3 h). The cells measured were of the following type:

$$+ \text{RE} \;\Big\|\; \begin{array}{l}\text{complex} \\ \text{solution}\end{array} \;\Big|\; \begin{array}{l}\text{glass} \\ \text{electrode}\end{array} -$$

Table 2. Potentiometric measurements on the cupric sulphate system with the acetate ion as a displacing ligand.

$M = Cu^{2+}$, $A = SO_4^{2-}$, $B = Ac^-$

No.	C_M mC	C_B mC	$C_A = 0$ mC	$C_A = 50$ mC	$C_A = 100$ mC	$C_A = 150$ mC
			E_B mV			
1	97.4	12.99	43.2	39.7	36.7	33.4
2	96.2	19.23	42.0	38.6	35.8	32.3
3	93.8	31.3	39.8	36.6	34.1	30.5
4	90.9	45.5	37.4	34.4	31.8	28.5
5	88.2	58.8	35.1	32.1	29.8	26.8
6	83.3	83.3	30.6	28.2	26.2	23.5
7	75.0	125.0	23.8	22.0	20.8	18.8
8	68.2	159.1	18.9	17.9	16.9	15.3
9	60.0	200.0	14.3	13.8	13.2	11.9
10	50.0	250	10.1	9.9	9.6	8.7
11	37.5	313	6.5	6.6	6.3	5.9
12	25.9	370	4.1	4.1	4.0	3.8
13	13.04	435	2.0	1.9	2.0	2.0
14	49.3	6.58	29.6	26.8	23.9	21.3
15	48.7	12.99	28.4	25.6	23.0	20.2
16	48.1	19.23	27.1	24.4	21.8	19.2
17	46.9	31.3	24.7	22.2	20.1	17.7
18	45.4	45.5	22.2	20.0	18.2	16.1
19	44.1	58.8	20.1	18.0	16.4	14.6
20	41.7	83.3	16.6	15.0	14.0	12.5
21	37.5	125.0	12.0	11.1	10.5	9.6
22	34.1	159.1	9.3	8.8	8.4	7.7
23	30.0	200.0	7.1	6.8	6.5	6.0
24	25.0	250	4.9	4.9	4.8	4.4
25	18.75	313	3.2	3.3	3.3	3.1
26	13.00	370	2.2	2.1	2.2	2.1
27	6.52	435	1.1	1.0	1.2	1.2

Concerning the determination of the slope of the glass electrode and experimental details, the reader is referred to the previous treatise[3, p. 40]. E_0 was determined before, as well as after, the measurement of E, in order to control that the asymmetry potential was a constant during a measurement series. Within the limits of the random errors, the measurements with the glass electrode gave the same values of E_B as the measurements with the quinhydrone electrode.

Table 3. Determination of $\bar{n}$/ [B] *as a function of* [B] *at different values of the parameters* C_A *and* a.

No.	$C_A = 0$			$C_A = 50$ mC			$C_A = 100$ mC			$C_A = 150$ mC		
	ϑ mC	[B] mC	$\frac{\bar{n}}{[B]}$ C^{-1}	ϑ mC	[B] mC	$\frac{\bar{n}}{[B]}$ C^{-1}	ϑ mC	[B] mC	$\frac{\bar{n}}{[B]}$ C^{-1}	ϑ mC	[B] mC	$\frac{\bar{n}}{[B]}$ C^{-1}
1	0.07	2.33	47.3	0.09	2.67	40.0	0.12	3.00	34.6	0.14	3.41	29.3
2	0.07	3.62	45.0	0.08	4.15	38.0	0.11	4.62	33.1	0.13	5.30	27.6
3		6.47	40.9	0.08	7.33	35.0	0.1	8.06	30.7	0.1	9.30	25.3
4		10.35	37.4		11.65	31.9		12.92	27.7		14.72	23.0
5		14.65	34.2		16.50	29.1		18.07	25.6		20.4	21.4
6		24.8	28.3		27.3	24.7		29.5	21.9		32.9	18.4
7		48.7	21.0		52.3	18.5		54.9	17.0		59.4	14.7
8		75.2	16.3		78.4	15.1		81.5	14.0		86.8	12.2
9		113.5	12.7		115.9	12.1		118.7	11.5		125.0	10.0
10		168	9.8		169	9.6		171	9.2		177	8.2
11		242	7.8		242	7.8		244	7.6		248	7.0
12		315	6.8		315	6.8		315	6.8		319	6.3
14	0.04	2.02	46.2	0.06	2.25	39.5	0.07	2.51	33.4	0.09	2.80	28.0
15	0.04	4.20	43.3	0.05	4.69	36.6	0.07	5.19	31.1	0.08	5.80	25.7
16	0.04	6.57	40.2	0.05	7.30	34.1	0.07	8.08	28.9	0.08	8.96	24.0
17		11.77	35.3		13.00	30.0		14.13	25.9		15.53	21.6
18		18.88	31.0		20.6	26.6		22.1	23.2		24.1	19.6
19		26.5	27.6		28.8	23.6		30.7	20.8		33.0	17.7
20		43.2	22.3		46.0	19.5		47.9	17.7		50.8	15.4
21		77.7	16.2		80.6	14.6		82.5	13.7		85.6	12.3
22		110.1	13.1		112.3	12.2		114.1	11.6		117.3	10.5
23		150.9	10.8		152.8	10.3		154.6	9.8		157.8	8.9
24		206	8.6		206	8.6		207	8.3		210	7.6
25		276	7.2		276	7.2		276	7.2		276	7.2

From Table 2 it is obvious that the values of E_B, at different C_A, gradually coincide when C_B is increased. In Table 3, [B] and $\bar{n}$/[B] of the solutions measured have been calculated. It is only necessary to compute the correction term ϑ at the smallest values of C_B, and it is evident that $[HSO_4^-]$ is quite negligible, compared with C_A.

Fig. 1 represents the measurements graphically at [B] $\leqq$ 55 mC. The function $\bar{n}$/[B] is dependent on the factor a in eq. (18). The reason for this is that in two solutions with the same values of C_A and [B] but with different values of C_M (that is of a), [A] is greatest in the solution that has the smallest

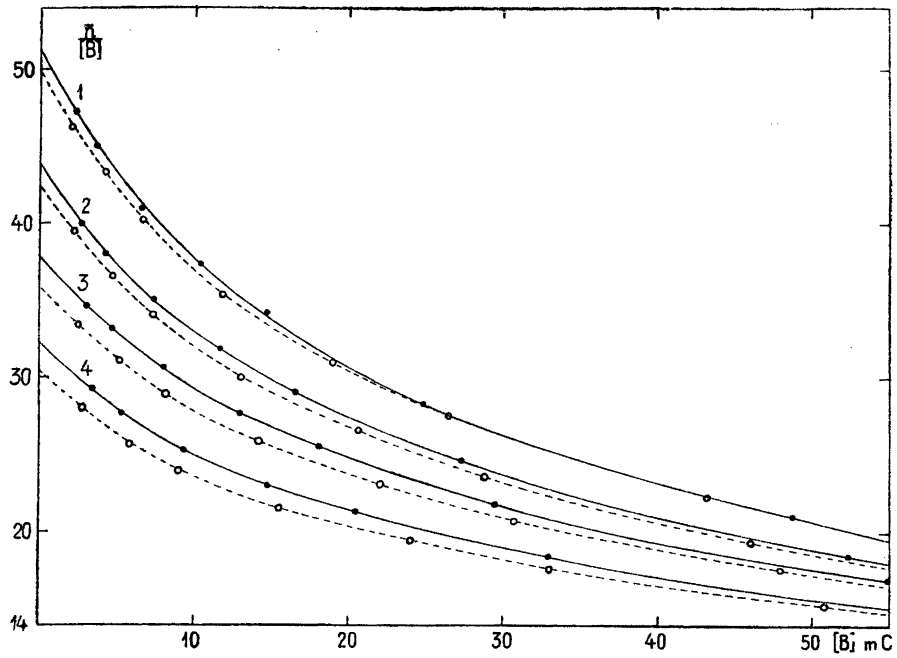

Fig. 1. $\bar{n}/[B]$ *as a function of* [B]. *1.* $C_A = 0$; *2.* $C_A = 50$ *mC; 3.* $C_A = 100$ *mC; 4.* $C_A = 150$ *mC. — Fulldrawn curves are valid at* $a = 100$ *mC (see eq. (18)); dashed curves at* $a = 50$ *mC.*

concentration C_M. Polynuclear complexes also may contribute to the dependence on a.

The definition of $\Delta\bar{n}$ has been given above (p. 74). In Table 4 the function $\Delta\bar{n}/[B]$ has been calculated at different values of [B]. The values in the columns 2—7 have been obtained from the graphical representation of $\bar{n}/[B]$. As potentiometric measurements have been performed at only two values of the parameter a, it has been presumed from the extrapolation of $\Delta\bar{n}/[B]$ to $a = 0$ (that is $C_M = 0$), that $\Delta\bar{n}/[B]$ is a linear function of a at a constant value of [B]. From Table 4 it will be seen that the dependence on a is rather small, and therefore this approximation is justified. In the columns 8—10 in Table 4, the extrapolated values of $(\Delta\bar{n}/[B])_{C_M=0}$ at $[A]_n = 50$, 100, and 150 mC are to be found.

As an upper limit of integration in eq. (14) for the three values of $[A]_n$ we may choose $b = 0.200$, 0.250, and 0.300 C, which appears from Table 4. The values of $ln\ X\ ([A]_n, 0)$ have been calculated by graphical integration and are given in Table 5.

Table 4. Determination of $(\Delta\bar{n}/[B])_{C_M=0}$ *as a function of* [B] *at different values of the parameter* $C_A = [A]_n$.

[B]	$C_M = 100 - \frac{C_B}{5}$ mC			$C_M = 50 - \frac{C_B}{10}$ mC			$C_M = 0$		
	$C_A = 50$ mC	$C_A = 100$ mC	$C_A = 150$ mC	$C_A = 50$ mC	$C_A = 100$ mC	$C_A = 150$ mC	$C_A = 50$ mC	$C_A = 100$ mC	$C_A = 150$ mC
mC	$\frac{\Delta\bar{n}}{[B]}$ C^{-1}			$\frac{\Delta\bar{n}}{[B]}$ C^{-1}			$\frac{\Delta\bar{n}}{[B]}$ C^{-1}		
0	7.2	13.3	19.0	7.5	14.1	19.5	7.8	14.9	20.0
5.0	5.9	10.3	15.2	6.1	11.0	15.9	6.3	11.7	16.6
10.0	4.7	8.5	12.7	4.9	9.1	13.3	5.1	9.7	13.9
15.0	3.9	6.9	10.7	4.2	7.6	11.5	4.5	8.3	12.3
20.0	3.3	5.8	9.2	3.6	6.6	10.0	3.9	7.4	10.8
30.0	2.5	4.6	7.3	3.0	5.3	8.0	3.5	6.0	8.7
50.0	1.8	3.0	4.9	2.3	3.6	5.3	2.8	4.2	5.7
75.0	1.0	1.8	3.2	1.3	2.1	3.4	1.6	2.4	3.6
100	0.6	1.2	2.4	0.9	1.5	2.6	1.2	1.8	2.8
150	0.3	0.6	1.6	0.5	1.0	1.8	0.7	1.4	2.0
200	0.1	0.4	1.0	0.1	0.4	0.9	0.1	0.4	0.8
250	0	0.2	0.6	0	0.1	0.4	0	0	0.2
300	0	0	0.4	0	0	0	0	0	0

The values of the function $\Delta\bar{n}/[B]$ at $C_A = 50$ mC are, on the average, 0.3 C^{-1} greater at $a = 50$ mC than at $a = 100$ mC within the main part of the range of integration (see Table 4). The greatest deviation from this average value is 0.2 C^{-1}. This means that the maximum error in the values of the function is about 0.1 C^{-1} in the two series of measurements. Then the values of the function at $a = 0$ may have a maximum error $\varepsilon = 0.2$ C^{-1}. The same value of ε is obtained at the other concentrations C_A. Thus according to the

Table 5. Corresponding values of $[A]_n$, $X([A_n, 0)$ *and* $(\bar{n}/[B])_{\substack{C_M=0 \\ [B]=0}}$.

$[A]_n$ mC	$lnX([A]_n, 0)$	$X([A]_n, 0)$	$(\bar{n}/[B])_{\substack{C_M=0 \\ [B]=0}}$ C^{-1}	$\sum_{j=0}^{N-1} \beta_{j,1} [A]_n^j$ C^{-1}
0			48.2	48.2
50	0.37 ± 0.08	1.45	40.8	59.2
100	0.65 ± 0.10	1.92	33.8	64.9
150	0.97 ± 0.12	2.64	28.6	75.5

calculation of errors, given above (p. 77), the maximum random error of $ln\ X$ is $= 0.4 \cdot b$.

From corresponding values of [A] and X ([A], 0) in Table 5, the constants of the mononuclear sulphate complexes have been computed from eq. (15), and the following values have been obtained:

$$\beta_1 = 9 \pm 2\ C^{-1};\ \beta_3 = 80\ C^{-3}$$

We get a small value for β_2, but since the uncertainty is great it is not justifiable to assign a numerical value. In the calculation of β_3 we may therefore put $\beta_2 \simeq 0$. It is hardly possible to mention an upper limit of the random error in the computed value of β_3. At an ionic strength of 1 C and with Ac^- as a displacing ligand, the sulphate concentration C_A cannot be increased materially above 150 mC, and therefore the value of $\beta_3 \cdot [A]^3$ is small compared with the value of the polynomial $X([A], 0)$. This fact is the cause of the difficulty in determining β_3 accurately.

The fourth column of Table 5, gives values of $(\bar{n}/[B])_{c_M = o}$ at $[B] = 0$. They have been obtained from Fig. 1 by linear extrapolation to $C_M = 0$ of the intercepts on the $\bar{n}/[B]$-axis, that correspond to a constant concentration C_A. The values of $\sum_{j=0}^{N-1} \beta_{j,1}\ [A]_n^j$, in the fifth column, have been calculated from eq. (16) The complexity constant of the mixed complex $CuSO_4Ac^-$ is:

$$\beta_{1,1} = 190 \pm 50\ C^{-2}$$

The measurements admit no determination of the remaining constants $\beta_{j,1}$.

Discussion

In the previous measurements by the present author [3, p. 62] with an amalgam electrode, the following values of the complexity constants of the mononuclear sulphate complexes were obtained at an ionic strentth of 1 C: $\beta_1 = 10.6\ C^{-1}$, $\beta_2 = 10$—$17\ C^{-2}$, and $\beta_3 = 200\ C^{-3}$. The value of β_1 has been confirmed in the present investigation, and the agreement shows the applicability of the new method. The divergence between the values of β_3 may be caused partly by the random errors, and partly by medium changes, since in the present measurements part of the sodium perchlorate must be exchanged for acetate buffer.

The complex formation in cupric sulphate solutions has been studied several times (Mecke and Ley [4], Ley and Heidbrink [5], Rouyer [6], Plake [7], Brintzinger

and Osswald [8], Davies [9], Owen and Gurry [10]). These investigations have been discussed in the previous treatise [3, p. 57]. Later Näsänen [11] made a spectrophotometric study of cupric perchlorate — litium sulphate solutions. It was assumed that only the complex $CuSO_4$ was formed, and by means of a Debye-Hückel equation the variation of β_1 with the ionic strength was investigated. At $I = 1$ C the value $\beta_1 = 4$ C^{-1} was obtained. However, a neglecting of the complex $Cu(SO_4)_2^{2-}$ must give too low values of β_1 in such measurements even at small concentrations of SO_4^{2-} (*cf.* Olerup [12, p. 60], Fronæus [3, p. 91]). In addition it must be emphasized that from the variation of β_1 with the ionic strength it is not justifiable to draw any conclusions about the variation when only the composition is changed but the ionic strength is kept constant by addition of sodium perchlorate.

SUMMARY

A new method for the investigation of the complex formation between a central group M and a ligand A is described. The method is based on the use of an auxiliary ligand B, the concentration of which can be measured in the complex solutions.

A competition for the central group arises between the ligands A and B, and B must form such strong complexes, that by increasing the concentration of B, the ligand A can be completely displaced.

The theoretical treatment shows that it is possible to calculate from the measurements the complexity constants of the mononuclear complexes MA_j ($j = 1,2, \ldots, N$), irrespective of whether or not mixed complexes and polynuclear complexes are formed. The method also admits a determination of the complexity constants of the mixed complexes of the type MA_jB ($j = 1,2, \ldots$).

In order to examine the method it has been applied to the cupric sulphate system with the acetate ion as the displacing ligand B. The ionic strength of the solutions has been kept constant at 1 C by addition of sodium perchlorate. As an acetate buffer has been used, it has been possible to determine [B] in the solutions by potentiometric measurements, employing the quinhydrone electrode. The measurements have been carried out at 20° C and the complexity constants of $CuSO_4$, $Cu(SO_4)_3^{4-}$ and $CuSO_4Ac^-$ have been computed and found to be

$$\beta_1 = 9 \pm 2 \text{ C}^{-1}; \qquad \beta_3 = 80 \text{ C}^{-3}; \qquad \beta_{1,1} = 190 \pm 50 \text{ C}^{-2}$$

The complexity constant β_2 of $Cu(SO_4)_2^{2-}$ is too small to be calculated from these measurements.

The results are in rather good agreement with previous measurements by the present author, and prove the applicability of the new method.

I am very much indebted to Professor S. Bodforss for his kind interest in this work and for the facilities he placed at my disposal.

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Received November 10, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 88–92

Preliminary Proposals for Notations for Solid Phases with Variable Composition ("Berthollides")

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This paper was presented to the Commission for Inorganic Nomenclature of the International Union of Chemistry at the Amsterdam meeting in September 1949. Inorganic Chemists, Metallographers, Mineralogists and others interested in this field are invited to communicate their opinion on Professor Hägg's proposals and possible improvements thereon as soon as possible to Prof. Arne Ölander, University of Stockholm, Sweden. It is intended that notation rules for berthollides be included in a set of Rules for Inorganic Nomenclature, which will be presented to the 1951 Conference of the Union in New York.

A. INTRODUCTION

Solid phases with variable composition, indicating solid solution, occur in non-metallic as well as metallic systems. Consequently, a practical method of notating such phases is of as great importance for chemistry in general as for metallography and mineralogy.

In connection with discussions of solid phases with variable composition the concept *characteristic* (or *ideal*) *composition* is frequently used. A definition of this concept seems to be lacking and as a matter of fact it seems impossible to obtain one single definition. In one case it may be necessary to use a definition based upon lattice geometry and in another to base the definition on the ratio of valency electrons to atoms. Sometimes one can state several characteristic compositions, and other times it is impossible to say whether a phase has a characteristic composition or not.

In spite of these difficulties it seems as if the characteristic composition can be used in its present undefined form when establishing a system of notation for phases of variable composition. It also seems necessary to use the

concept even if the characteristic composition is not included in the known homogeneity range of the phase. Thus for the iron oxide commonly written as FeO, this formula so clearly corresponds to a characteristic composition that it must be used as the basis for the phase notation in spite of the fact that it lies outside the homogeneity range hitherto observed.

Many years ago Kurnakow (*Z. anorg. Ch.* **88** (1914) 109, also Kurnakow and Glasunow, *J. Russ. Chem. Soc.* **44** (1912) 1007), proposed the word *berthollide* as a general denomination for such a phase with variable composition, for which no composition could be considered as characteristic. Later, Ölander (*Z. phys. Ch.* **A 165** (1933) 65), proposed that this word should be used for every solid phase with variable composition. This latter proposition seems to be very practical, particularly in view of the above mentioned difficulties of deciding whether a phase has a characteristic composition or not. The word berthollide in the sense proposed by Ölander also is a suitable antipode for the word *daltonide* proposed in the rules of 1940 for phases with a constant composition.

B. GENERAL PHASE NOTATION

1. For a phase where the variable composition is solely or partially caused by substitution, the following two alternatives 1a and 1b are proposed.

a. This is an extension of the notation which has long been in use, especially in mineralogy.

Atoms or atomic groups, which substitute each other are separated by a comma and placed in parentheses. If possible the formula ought to be written so that the limits of the homogeneity range are represented when either of the two atoms or groups are lacking.

Ex. (Cu,Ni) denotes the complete phase from pure Cu to pure Ni. (KBr, KCl) or better yet, K(Br,Cl) comprises the phase from pure KBr to pure KCl.

Substitution accompanied by the appearance of vacant positions (combination of substitutional and interstitial solution) receives an analogous notation. For example $(Li_2,Mg)Cl_2$ (which is probably better than $(Li,Mg_{\frac{1}{2}})Cl$) denotes the homogeneous phase from LiCl to $MgCl_2$ where the anion lattice is constant but where Mg^{+2} is substituted for 2 Li^+ (or more correctly where one vacant cation position appears for every substitution of Mg^{+2} for Li^+). $(Mg_3,Al_2)Al_6O_{12}$ will be the homogeneous phase from the spinel $MgAl_2O_4$ ($= Mg_3Al_6O_{12}$) to γ-Al_2O_3 ($= Al_2Al_6O_{12}$). In this case the phase is unstable at the end of the composition range richest in Al.

The solid solutions between CaF_2 and YF_3, where the cation substitution is accompanied by interstitial addition of F^-, would receive the symbol $(Ca,YF)F_2$. In this case the formal substitution of $(YF)^{+2}$ for Ca^{+2} does not

mean that $(YF)^{+2}$ takes the place of Ca^{+2}. It is probably quite clear that Y^{+3} takes the place of Ca^{+2} while at the same time one F^- is added interstitially.

The solid solutions between $LiFeO_2$ and Li_2TiO_3 where $Li^+ + 2\ Ti^{+2}$ are substituted for $3\ Fe^{+3}$ and where the oxygen lattice is constant, can be written $(Li_3Fe_3,\ Li_4Ti_2)O_6$ in order to show the constancy of the latter. A still better formulation, which shows the mechanism of the cation substitution more directly, seems to be $(Fe_3,LiTi_2)Li_3O_6$.

In many cases the notation may be improved by introducing the charges of the different ions. If several substitutions occur simultaneously it may be desirable to supply additional information or to use certain conventions in order to express the relations correctly. A notation for the phase represented by the plagioclases would be $(Na,Ca)(Si,Al)\ Si_2AlO_8$. Here it is necessary to note that a substitution of Ca^{+2} for Na^+ must be accompanied by a substitution of Al^{+3} for Si^{+4}. There is no proposition made for the notation of this fact.

In many cases the notation proposed above does not give any hints as to the structure of the phase or the function of the different atoms. This is a disadvantage which seems impossible to remedy in any simple way.

b. From a formal viewpoint a much better notation is one which involves a statement of the variables which define the composition. A phase involving simple substitution may be written A_xB_{1-x}, *e. g.* Cu_xNi_{1-x} and KBr_xCl_{1-x}. It is immediately evident that the total number of atoms in the lattice is constant. A combined substitutional and interstitial solution may be written in an analogous way. The homogeneous phase between LiCl and $MgCl_2$ becomes $Li_{2x}Mg_{1-x}Cl_2$. The phase between $MgAl_2O_4$ and Al_2O_3 can be written $Mg_{3x}Al_{2(1-x)}Al_6O_{12}$, which shows that it cannot contain more Mg than corresponding to $MgAl_2O_4 (x=1)$.

Another notation would be $Mg_{3x}Al_{2(4-x)}O_{12} (x \leqq 1)$. The other examples given under 1a will receive the forms $Ca_xY_{1-x}F_{3-x}$, $Li_{4-x}Fe_{3x}Ti_{4(1-x)}O_6$ and $Na_xCa_{1-x}Si_{2+x}Al_{2-x}O_8$ respectively.

The notation 1b evidently is more widely applicable and can give more information (*cf.* the unambiguous notation of the plagioclases) than 1a. It also makes the statement of definite compositions and solubility limits possible. (See below!) Furthermore it is hard to see how for example the γ phase in the AgCd system could be formulated with any notation other than 1b. In this phase, which has the characteristic formula Ag_5Cd_8, the Ag and Cd atoms can replace each other mutually to some extent. The notation would be $Ag_{5\pm x}Cd_{8\pm x}$. Phases of this kind are extremely frequent in alloy systems.

The disadvantage of notation 1b is that the formulae are sometimes more complicated to write than is the case with 1a and that they can not always be so rapidly interpreted as those of type 1a. If the composition is determined by more than one independent variable the expressions tend to be rather bulky. If the three atoms A, B, and C can substitute each other one obtains $A_xB_yC_{1-(x+y)}$. Perhaps the best way is to permit both notations 1a and 1b.

2. A notation, showing the extension of a homogeneity range, generally requires a statement of the corresponding temperature and pressure values. This reduces the practical use of such a notation.

The extension is most easily shown with the notation 1b. For example, the Ag phase in the system Ag—Cd could up to about 400° C be written $Ag_{1-x}Cd_x(x<0.42)$. If it is desirable to show that the variable denoted by x can only attain small values this may be done by substituting ε for x.

With the notation 1a it seems practical to show a limited solubility by means of the letter "p" (= partial) preceding the symbol of the component which is not included in the phase in question.

Ex. The Ag phase (α) in the system Ag—Cd will then be written as (Ag,pCd). The Cd phase (η) in the same system will be (Cd,pAg). The solid solutions of KI in KCl will be K(Cl,pI).

3. Interstitial solutions (whether of the addition or subtraction type) can be designated in a manner analogous to 1b.

Ex. $Fe_{1+x}Sb$, $Fe_{1-x}O$, $Fe_{1-x}S$, $Cu_{2-x}O$, $Na_{1-x}WO_3$ (sodium-tungsten-bronzes), $Mo_{2\pm x}C$. For $x = 0$ these formulae correspond to a characteristic composition. (In the second example the characteristic composition FeO lies outside the homogeneity range.) In several of these examples the range of the variable is small, which may be indicated by writing ε instead of x.

A solid solution of hydrogen in Pd phase can be written PdH_x. A phase of the composition M which has dissolved a variable amount of water (consequently dissolved as "zeolithic" water) can be written $M(H_2O)_x$.

In these cases the solubility limit is frequently determined by the available number of lattice interstices of a certain kind and may consequently be independent of rather large variations of temperature and pressure. In such cases it may be advisable to state the limiting value of x. For example a natrolite phase with 0—2 molecules H_2O for every formula unit can be written as $Na_2Al_2Si_3O_{10}(H_2O)_x(x\leqq 2)$. $A_{1-x}B(x\leqq\frac{1}{2})$ denotes a phase which is homogeneous from AB to $A_{\frac{1}{2}}B$ (AB_2).

4. In many cases (for example if the mechanism of solution is unknown) it may be convenient to have a general sign showing that a phase is a berthollide. In the 1940 rules it was suggested that a variable composition should be denoted by a bar placed above a formula corresponding to a characteristic

composition. A bar is rather inconvenient both in printing and typing and it is suggested here that it should be replaced by the letter "b" (= berthollide) preceding the formula and connected with it by means of a hyphen. Ex. b-Ag_5Cd_8, b-FeO.

5. Sometimes it will be necessary to denote the structure of a phase. This could be done by putting the structural type or some other indication after the formula.

Ex. FeC_x(type A1) or FeC_x(cubic face-centred) in order to indicate the solid solution of C in γ-Fe (austenite). This is a case where it would be appropriate to write FeC_ε.

C. NOTATION OF A CERTAIN COMPOSITION WITHIN THE HOMOGENEITY RANGE OF A BERTHOLLIDE

Using the notations of the type 1b and 3, a certain composition can be indicated by stating the actual value of the variable x. Probably the best way of doing this is to put the value in a parenthesis after the formula as has been done in 2 and 3 above.

Ex. $Li_{4-x}Fe_{3x}Ti_{4(1-x)}O_6$ $(x = 0.35)$.

If it is desirable to introduce the value of x into the formula the solution mechanism is more clearly understood if one writes $Li_{4-0,35}Fe_{3\,.\,0,35}Ti_{4(1-0,35)}O_6$ instead of $Li_{3,65}Fe_{1,05}Ti_{2,60}O_6$.

The above proposals have resulted from discussions between Professor S. Claesson, Uppsala, Professor A. Fredga, Uppsala, Professor A. Ölander, Stockholm and the author.

Received March 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 93–102

Oxidative Nitrogen Fixation in Ultrasonic Field

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The chemical mode of action of ultrasonic waves is not yet explained. The problem generally concerns reactions in aqueous solutions when the chemical changes are regarded as caused by formed hydrogen peroxide, nitrous acid, nitric acid [1–3] and, in addition, possibly ozone [4]. The presence of these substances in irradiated water was explained by Frenkel [5] and Bresler [6], by assuming that during the formation of the cavitation bubble the ionic charges are disrupted and free opposite charges appear on the inside wall of the bubble. These cause great potential differences, resulting in electrical discharge across the gases inside the bubble in diminished pressure.

It can be assumed that activation of gases, and hence chemical reactions between them, takes place in the cavitation bubbles. This activation has not been characterized more closely.* The observation of Loiseleur [7] that the formation of hydrogen peroxide in the ultrasonic field depends on the acidity of the solution is, in this connection, of special interest. According to him, the optimum acidity lies on both sides of the neutral point. pH 4 forms a limit, below which the formation is strongly diminished in proportion to the increase of hydrogen ion concentration.

In the present paper an attempt is made to examine the factors affecting the oxidative nitrogen fixation in the ultrasonic field.

METHODS

Ultrasonic waves were produced by means of a piezo-quartz with a frequency of about 300 kc/sec and with a radiating surface 42 mm in diameter giving a radiating intensity of $\sim$ 10 W/cm^2 in the radiating point. The quartz was completely immersed in an oil bath,

* *Addition to proof.* Results have also been recorded according to which the reactions in the ultrasonic field can be independent of cavitation. Die erste Ultraschalltagung in Erlangen, May 2–5, 1949 [*Naturwissenschaften* **37** (1950) 14].

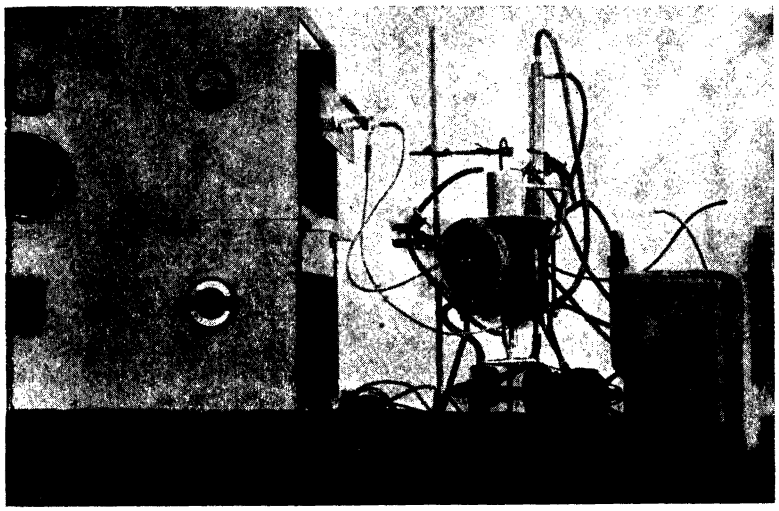


Fig. 1. The ultrasonic apparatus used in the experiments.

from which the vibrations were led into a test cylinder 64 mm in diameter. To avoid loss of energy at the junction, a 0.02 mm steel membrane was used as a bottom for the cylinder ($\lambda/2 \approx 0$). All the experiments with hydrogen were carried out in a glass cylinder to eliminate possible activation of hydrogen on the surface of the metal. The distance from quartz to the bottom membrane was always adjusted to a multiple of $\lambda/2$. All experiments were made at 18—20° C. Fig. 1 illustrates the apparatus used. It was constructed by Mr. J. Nukari in the laboratory of Technical Physics at the Finland's Institute of Technology.

Nitrite nitrogen was determined photometrically using the sensitive colour reaction with α-naphthylamine and sulphanilic acid according to Blom [8]. After 30 min, the reddish colour was estimated with a Klett-Summerson photometer, using the filter S50. The amount of nitrite nitrogen was read from a calibration curve constructed with known concentrations of nitrite.

Hydroxylamine nitrogen was determined colorimetrically according to the technique of Blom modified by Csáky [9].

Nitrate nitrogen was determined photometrically by the phenol disulphonic acid method according to Burström [10]. The yellow colour of the ammonium salt of nitrophenoldisulphonic acid was estimated with a Klett-Summerson photometer using the filter S42. Nitrate nitrogen was read from a calibration curve constructed with known concentrations of nitrate.

Hydrogen peroxide was determined by the titanium sulphate method [11]. The yellow colour was estimated with a Beckmann spectrophotometer (4100 Å). The amount of hydrogen peroxide was read from a calibration curve constructed with known concentrations of hydrogen peroxide.

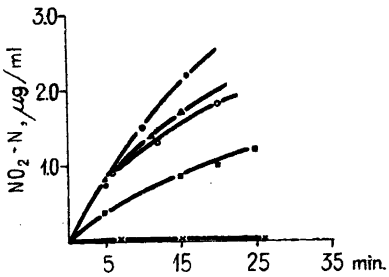


Fig. 2. The amount of NO_2-N found in aqueous solution after irradiation. Effect of pH on the formation of NO_2-N from N_2.

● *pH 11.20*
▲ » *8.00*
○ » *6.20*
■ » *3.30*
× » *1.62*

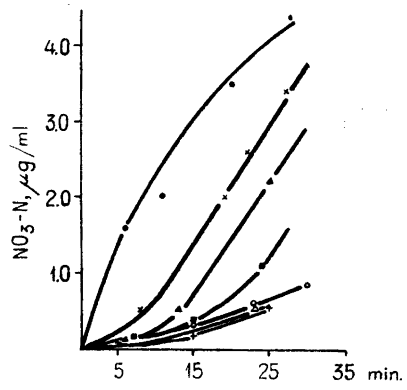


Fig. 3. The amount of NO_3-N found in aqueous solution after irradiation. Effect of pH on the formation of NO_3-N from N_2.

● *pH 1.25*
× » *1.85*
▲ » *2.40*
■ » *3.00*
○ » *3.40*
△ » *12.06*
\+ » *7.70*

Ferrous iron was determined by the $a-a'$-dipyridyl method [12]. The reddish colour was estimated with a Beckman spectrophotometer (5000 Å). The calibration curve used in the photometric reading was constructed with known concentrations of ferrous iron.

EXPERIMENTS AND RESULTS

100 ml of distilled water was used in the experiments, and a free access of air was allowed to the test cylinder. Thus, whenever this cylinder was used, the solution contained both nitrogen and oxygen regardless of whether some other gas was also led to the solution. The experiments were chiefly carried out in a steel cylinder. For the sake of control, one experiment was performed in a glass cylinder. The results were similar in both cases. Nitrogen fixation was examined in solutions, the pH values of which ranged from 1.25 to 11.8. The acidity was adjusted to pH between 1.25 and 3 by sulphuric acid, to pH between 3 and 5 by acetate buffer, to pH between 5 and 8 by phosphate buffer, and to pH over 8 by phosphate *plus* sodium hydroxide.

Hydroxylamine could not be found in any experiment.

The curves in Fig. 2 illustrate the formation of NO_2-N at different pH-values.

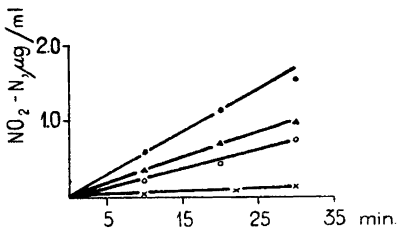


Fig. 4. Inhibitory effect of gaseous hydrogen on the formation of NO_2-N from N_2 during irradiation. pH of the solution in each experiment about 6.8.

● N_2 *(45.5 l/30 min)*
▲ $N_2 + H_2$ *(47.5 l/30 min 6.3% H_2)*
○ $N_2 + H_2$ *(54.5 l/30 min 11.0 % H_2)*
× $N_2 + H_2$ *(56 l/30 min 24.8 % H_2)*

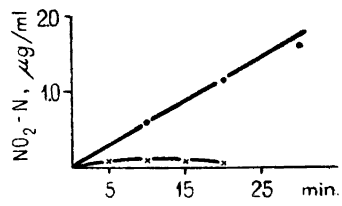


Fig. 5. Inhibitory effect of carbon monoxide on the formation of NO_2-N from N_2 during irradiation. pH of the solution in each experiment about 6.8.

● N_2 *(45.5 l/30 min)*
× *CO (12 l/30 min)*

The curves in Fig. 3 show the formation of NO_3-N at different pH-values.

Fig. 4 shows the inhibitory effect of hydrogen on the nitrogen fixation. In different experiments, hydrogen was led into the water (pH~6.8), kept in an open test cylinder in the ultrasonic field, either as pure hydrogen gas or as a mixture of nitrogen and hydrogen. The treatment of different experiments can be seen from the figure.

Curves in Fig. 5 illustrate the inhibitory effect of carbon monoxide on the nitrogen fixation.

Ether prevents nitrogen fixation completely even in small concentrations (0.08 ml/100 ml water). Ammonia weakens the fixation but does not entirely check it even in high concentrations.

The curve in Fig. 6 shows the reduction of ferric iron caused by hydrogen in the ultrasonic field (63.5 µg ferric iron/ml). When hydrogen was led through a ferric-salt solution, outside the ultrasonic field, no reduction was noted. Nor was ferric iron reduced in the ultrasonic field if hydrogen was not led to the solution.

In addition to hydrogen and carbon monoxide, the effect of argon on the nitrogen fixation was also examined. As can be seen from the curves in Fig. 7, the leading of argon to the reaction solution had no definite effect on the formation of nitrite. Smaller variations in the rate of nitrogen fixation between different experiments may be due to disturbances, caused by the bubbles of gas, to the ultrasonic field.

The curves in Fig. 8 show the dependence of the formation of hydrogen peroxide on the hydrogen ion concentration, in experiments where nitrogen

Fig. 6. Reduction of ferric iron to ferrous iron by the effect of ultrasonic waves in the presence of gaseous hydrogen. pH of the solution about 6.8.

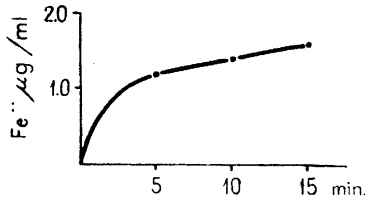


Fig. 7. The effect of gaseous argon and hydrogen on the formation of NO_2-N from N_2 during irradiation. pH of the solution about 6.8.
The full-line curves represent the results of a connected series of experiments, the interrupted curve a separate experiment with argon with maximum nitrogen fixation.
■ *H_2 (30 l/30 min)*
● *A (22 l/30 min)*
▼ *Control, no hydrogen, no argon*
○ *A (20 l/30 min)*

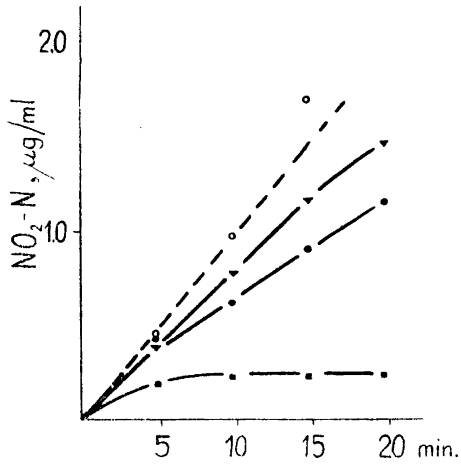
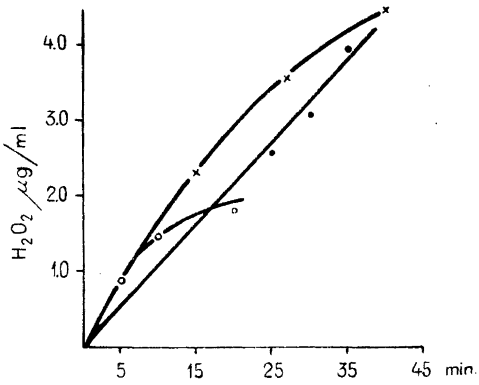


Fig. 8. Formation of hydrogen peroxide at different pH by the effect of ultrasonic waves in the presence of H_2 and CO.
× *H_2 pH 6.85*
● *H_2 » 1.25*
○ *CO » 6.50*



fixation was prevented by hydrogen or by carbon monoxide. Within 30 minutes 30 l of hydrogen or 12 l of carbon monoxide were led into the solution.

Nitrogen fixation is directly dependent on the gaseous oxygen in the solution. If the reaction cylinder is closed, so that oxygen can be removed by boiling and by leading nitrogen gas, washed with alkaline pyrogallol, through

the solution, the nitrogen oxides, formed by the effect of ultrasonic waves in the nitrogen atmosphere under normal pressure, amount only to about 8 % of those formed in the same test cylinder in ordinary air atmosphere under normal pressure. Even this minimum nitrogen fixation the authors are inclined to ascribe to residues of oxygen gas. The closed reaction cylinder was similar to that used by Schmid and Rommel [12b].

DISCUSSION

The results obtained in the experiments show that the fixation of molecular nitrogen in the ultrasonic field does not, at least essentially, depend on the hydrogen ion concentration of the solution, as far as the total amount of fixed nitrogen (nitrite *plus* nitrate N) is concerned. Instead, the mutual relation of nitrite and nitrate N is decided by the pH of the solution. In a strongly acid reaction (pH 1—2) the amount of nitrite-N is almost nil, while that of nitrate-N is high. As the acidity is lowered, nitrite nitrogen rises and reaches its maximum in a strongly alkaline reaction. On the other hand, the formation of nitrate nitrogen decreases as the pH rises. At pH 3, the drop is abrupt and at pH 3.4 the quantity of nitrate is already slight and remains so until a strongly alkaline reaction.

These results explain the observation of Loiseleur [7] on the rapid lessening of the formation of hydrogen peroxide below pH 4. The question is thus evidently not one of a decrease in the formation of hydrogen peroxide, but one of the consumption of hydrogen peroxide in the oxidation of nitrite to nitrate. This idea gained evidence by the findings on the inhibitory effect of hydrogen and carbon monoxide on the nitrogen fixation. When the formation of nitrite-N is prevented or strongly weakened, hydrogen peroxide is formed in almost equal quantities at pH 1.85 and 6.85. The hydrogen peroxide formed in the presence of hydrogen is stabile. The analytical values do then not change at the standing of the solution as regularly happens if the nitrogen fixation is not prevented. This instability of hydrogen peroxide was earlier considered to be due to the formation of radicals [6].

The formation of hydrogen peroxide, in the presence of hydrogen, explains also the findings of Mohren [13], according to which the reductive ability of ascorbic acid may rise up to 160 per cent in the presence of hydrogen in the ultrasonic field. The question is evidently one of the reducing effect of the hydrogen peroxide formed. The fixation of nitrogen to nitrite in the ultrasonic field must always be considered when experiments are made in the presence of air.

Our findings on the inhibitory effect of ether on the nitrogen fixation in the ultrasonic field is in agreement with the idea of Frenkel [5] of electric discharges across the gases inside the cavitation bubble in diminished pressure.

The inhibitory action of hydrogen on the nitrogen fixation in the ultrasonic field is of special interest to the authors, because in the aerobic nitrogen fixation by Azotobacter (Wyss and Wilson [14]) and by leguminous root nodules (Wilson and Umbreit [15]), hydrogen also prevents nitrogen fixation. Virtanen [16] has suggested that the first phase in the biological aerobic nitrogen fixation is oxidation: $N_2 \rightarrow N_2^+$ or $N_2 \rightarrow 2N \rightarrow 2N^+$. The transfer of electron to oxygen which possibly is effected by a hemin catalyst system would lead to the formation of nitrogen oxide. The preventive action of hydrogen would then depend on the competition between nitrogen and hydrogen for oxygen. Therefore, it was held important to find out the mechanism of the combination of nitrogen to oxygen in the ultrasonic field and the mode of action of hydrogen on this reaction.

It has frequently been stated already in the beginning of this century that the activation of the nitrogen in the arc process is the most important process [17-19] but it was suggested that parallel activation of oxygen was necessary. The opinion that only oxygen was activated was also presented [20]. Schwab and Loeb [21] considered that nitrogen became reactive after a collision with excited oxygen. Wansbrough-Jones [22] examined the reaction between nitrogen and oxygen in the triode when the purely thermal synthesis of nitrogen oxides was too slow to affect the results. By means of different gas mixtures and by following the diminishing of pressure during the reaction he arrived at the result that the primary reaction in the combination of nitrogen to oxygen is ionization of nitrogen molecule. Activation of oxygen is not necessary. No reaction occurs, unless the electron has an energy greater than 17 volts. Westhaver and Brewer [23] examined the formation of nitrogen dioxide in the glow discharge and also concluded that nitrogen alone is activated. The reaction is initiated entirely by N_2^+ ions. Argon, in the gas mixtures, has a pronounced retarding action on the synthesis of nitrogen dioxide. Westhaver and Brewer explain the influence of argon as follows. The ionization potential of argon lies between that of nitrogen and oxygen, hence, the possibility exists for an A^+ ion to transfer its charge to oxygen by collision of the second kind, i.e., $A^+ + O_2 = A + O_2^+$. This possibility does not exist with nitrogen. Argon, therefore, when added to a nitrogen-oxygen mixture, will markedly decrease the ratio of N_2^+ to O_2^+. The decrease in N_2^+ ion production corresponds to the decrease in the observed rate of synthesis.

Since the ionization energy is most important in the synthesis taking place in the electric discharges, some values for the ionization and dissociation

energies of the molecules used in the gas mixtures in the present work are recorded in Table 1.

Table 1. Ionization and dissociation potentials (eV) of hydrogen, oxygen, nitrogen, and argon.

$H_2 \rightarrow H_2^+$	15.4^{24} 15.4^{25}	$O_2 \rightarrow O_2^+$	12.1^{24} 11.7^{25}	$N_2 \rightarrow N_2^+$	15.6^{24} 15.8^{25}	$A \rightarrow A^+$	15.7^{23}
$H_2 \rightarrow 2H$	4.5^{24}	$O_2 \rightarrow 2O$	5.1^{24}	$N_2 \rightarrow 2N$	9.8^{24}		
$H \rightarrow H^+$	13.6^{24} 13.5^{25}	$O \rightarrow O^+$	13.6^{24} 13.6^{25}	$N_2 \rightarrow N^+$	14.5^{24} 14.5^{25}		
$H_2^+ \rightarrow H + H^+$	2.6^{24} 2.5^{25}	$O_2^+ \rightarrow O + O^+$	6.5^{24} 7.0^{25}	$N_2^+ \rightarrow N + N^+$	8.7^{24} 6.9^{25}		

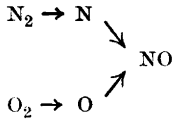
If the formation of nitrogen oxides in the ultrasonic field occurs as a result of the ionization of nitrogen in analogy with the ideas presented above, the inhibitory effect of hydrogen could be explained in the same way as Westhaver and Brewer explained the inhibitory effect of argon. The ionization potentials of argon and hydrogen lie near to each other.

The following reactions between nitrogen and oxygen in electric discharges should be possible according to the investigations of Wansbrough-Jones and Westhaver and Brewer:

$$N_2^+ + O_2 = 2NO \qquad N_2^+ + O_2 = NO_2^+ + N$$
$$N^+ + O_2 = NO_2 \qquad NO_2^+ + O_2^- = NO_2 + O_2$$
$$N_2^+ + O = N_2O \qquad N_2^+ + 2O_2 = (N_2O_4)^+$$
$$N^+ + O = NO \qquad (N_2O_4)^+ + e = 2NO_2$$

In our experiments in the ultrasonic field, however, argon does not, at least appreciably, prevent nitrogen fixation. Since, on the other hand, the inhibitory effect of hydrogen is very strong, and under the experimental conditions the hydrogen molecule has been proved to dissociate into atoms, as the reduction of trivalent iron to divalent one shows, the experimental material so far does not give any proof for the idea that the primary reaction in the combination of nitrogen to oxygen in the ultrasonic field is ionization of nitrogen molecule. Harteck [26] found that the atoms of hydrogen and oxygen are very active in many chemical reactions, i. e., they bring about a number of reductions or oxidations. Harteck and Roeder [27] showed that atomic nitrogen reacts with hydrogen peroxide at room temperature forming nitric acid. It is possible that the dissociation of nitrogen and oxygen molecules into atoms

in the ultrasonic field is sufficient to cause the combination of nitrogen to oxygen. Thus, the course of the reaction would then be



However, it cannot be concluded from our results to what extent the atoms or ions participate in the reaction *, although the combination of nitrogen atom to oxygen atom in the ultrasonic field is very possible.

Our observations on the indispensability of gaseous oxygen for the formation of nitrogen oxide strongly support the idea that nitrogen fixation takes place in the cavitation bubbles. The findings of Grabar [29] on the occurrence of different oxidation reactions in the ultrasonic field even in the complete absence of oxygen from the system do not fit into the formation of nitrogen oxide. The OH radicals arising secondarily from water are evidently unable to oxidize nitrogen.

In the absence of oxygen even hydrogen peroxide could not be detected in our experiments.

SUMMARY

The total amount of oxidatively fixed nitrogen (NO_2-N + NO_3-N) in the ultrasonic field does not essentially depend on the hydrogen ion concentration of the solution. In a strongly acid reaction nitrite-N is oxidized to nitrate-N, and therefore below pH 3 chiefly nitrate-N is formed. Hydroxylamine is not found at any acidity.

Hydrogen inhibits nitrogen fixation in the ultrasonic field, as does carbon monoxide. In the presence of hydrogen, hydrogen peroxide is formed in equal amounts below pH 4 as in neutral reaction, because hydrogen peroxide is then not consumed in the oxidation of nitrite-N.

Argon does not inhibit nitrogen fixation. — Ether inhibits already in low concentrations.

Gaseous oxygen is indispensable for the nitrogen fixation as also for the formation of hydrogen peroxide.

* Although the dissociation of hydrogen molecule into atoms should be theoretically possible by electrons of 4.5 volts' energy, Glockner, Baxter and Dalton (*J. Am. Chem. Soc.* **49** (1927) 58) found that the reaction begins at 11.4 volts. The dissociation of nitrogen in the ultrasonic field is thus also a natural reaction. Dissociation of oxygen appears from our finding that carbon monoxide prevents nitrogen fixation. According to Geib and Harteck [28] atomic oxygen oxidizes carbon monoxide.

Ferric iron is reduced to ferrous iron in the ultrasonic field in the presence of gaseous hydrogen. On the basis of this, it may be concluded that hydrogen is dissociated to atoms under such conditions. The dissociation of nitrogen is *ex analogia* probable.

The mechanism of nitrogen fixation occurring in the ultrasonic field has been discussed and compared with the corresponding mechanism of aerobic nitrogen fixation by micro-organisms.

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Received November 1, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 103–125

Physico-chemical Investigations on Micelles of Potassium Myristate and Laurate

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Following Mc Bain's [1] positive evidence that soaps in water solution form colloidal systems, intensive investigation has been directed toward the elucidation of the structure of these colloids. The ability of soap aggregates — the micelles — to bring water insoluble substances into solution, as well as their rôle as primary reaction loci in emulsion polymerization, has further increased the interest in their structure.

It is now certain that the association of soap into colloidal micelles occurs in steps [2–4]. Below a limiting concentration the solutions behave as ordinary electrolytes. Above this critical concentration (the concentration is reduced with increasing chain-length) lies a region of small, truly spherical, micelles. At still higher concentrations there are large roentgenographic micelles [5–7], which are aggregates of a lamellar structure, with water intercalated between sheets of polar groups.

The exact size and shape of the micelles existing above the critical concentration are still in question. Furthermore it has not been ascertained whether two different kinds of micelles really exist, or whether possibly only the degree of association is increased with increased concentration [8–10].

This paper reports an investigation of the molecular weights and shapes of the micelles of potassium myristate and laurate in salt solutions of various ionic strengths, as determined by sedimentation, diffusion and viscosity measurements.

DETERMINATION OF DEGREE OF SOLVATION

When using sedimentation and diffusion measurements to determine the degree of association of soaps it is quite necessary to have information about the density of the soap micelles. Soap dissolved in salt solution is always

more or less solvated and in order to calculate a correct molecular weight one has to know the partial specific volume of the complex, soap-water. The degree of solvation, k, was determined by the method of McBain and Jenkins [11], which is based on the assumption that the water of solvation is free of salt. In this method the soap solution is slowly pressed through an ultrafilter (viscose membrane) and the amount of salt in the filtrate measured. At any time during the filtration the concentration of salt in the filtrate is equal to the concentration of salt in the original solution exclusive of the amount of soap and solvation water; that is

$$c' = \frac{c}{1 - a\,(1 + k)} \tag{1}$$

where

c' = concentration of salt in filtrate in g/g of solution.
c = » » » » original solution in g/g of solution.
a = amount of soap in g/g of solution.
k = degree of solvation in g/g of soap.

Solving equation (1) for k gives

$$k = \frac{c' - c}{a \cdot c'} - 1 \tag{2}$$

At the high salt concentrations used here the amount of unassociated soap passing into the filtrate has been disregarded. The salt concentrations were determined gravimetrically. The following salt solutions were used:

Molar composition	Ionic strength
0.4 M KBr + 0.1 M K_2CO_3	0.7
0.8 M » + 0.1 M »	0.9
1.0 M » + 0.1 M »	1.1
1.6 M » + 0.1 M »	1.9

The soaps were prepared by neutralizing the corresponding acids (Schering-Kahlbaum "reinst") with fresh alcoholic potassium hydroxide and recrystallized from acetone. The results of these measuremets are given in Table 1. The values are averages of several determinations and refer to a soap concentration of 1.0 per cent by weight. The limits of the experimental errors have been calculated assuming that c and c' can be determined with an accuracy of 0.1 per cent and a with an accuracy of 0.2 per cent. The solvation shows a dependence on soap concentration, which is especially pronounced

Table 1. Degree of solvation of K-myristate and K-laurate at different ionic strengths.

Soap	μ	k g/g soap
K-myristate	0.7	1.7 ± 0.4
»	1.1	0.9 ± 0.2
»	1.3	0.8 ± 0.2
K-laurate	1.1	1.1 ± 0.2
»	1.9	0.7 ± 0.15

at low soap concentrations when the amount of salt is high, the k-values decreasing rapidly. Measurements in this region, however, are very unreliable.

PARTIAL SPECIFIC VOLUME

The calculation of the size and shape of the micelles requires knowledge of the partial specific volume of the components in the solution. The centrifugal force acting on an unsolvated particle is given by

$$M_1 (1 - \bar{V}_1 \varrho) \omega^2 x \tag{4}$$

where M_1 is the molecular weight of the particle, $\bar{V}_1$ is the partial specific volume, ϱ the density of the solution, ω the angular velocity of the centrifuge rotor and x the distance from the centre of rotation. Kraemer [12] has shown that in a case where the sedimenting particle is solvated, the centrifugal force is

$$M_c (1 - \bar{V}_c \varrho) \omega^2 x \tag{5}$$

where M_c and $\bar{V}_c$ respectively denote the molecular weight and the partial specific volume of the solvated particle. These two quantities are given by

$$M_c = M_1 (1 + \Sigma k_i) \tag{6}$$

$$\bar{V}_c = \frac{\bar{V}_1 + \Sigma k_i \bar{V}_i}{1 + \Sigma k_i} \tag{7}$$

k_i is the degree of solvation of component i expressed in g/g of substance. $\bar{V}_i$ is the partial specific volume of component i. If the partial specific volume of the unsolvated particle, $\bar{V}_1$, is used in moleculer weight calculations, the

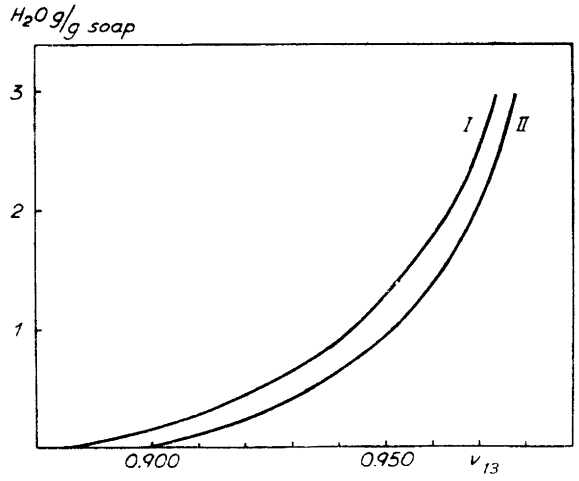

Fig. 1. Specific volume (v_{13}) vs. degree of solvation, according to formula (8).
I: K-myristate
II: K-laurate

error introduced in the molecular weight is eliminated by extrapolation to infinite dilution only in case of binary solutions (Kraemer [13]). Thus for all multicomponent systems the partial specific volume of the solvated particle must be used in the calculations. This is especially important for concentrated salt solutions, and when $\overline{V}\varrho$ is near to unity.

The conditions under which the sedimentation of soap micelles can be performed are limited. A concentrated salt solution must be used in order to increase the numerical value of the Archimedian factor, $1 - \overline{V}\varrho$, and because $\overline{V}$ for solvated micelles is near to unity. In this case especially an accurate calculation of the degree of association is impossible without information about the partial specific volume of the solvated micelles.

In some cases the specific volume of the solvated particles can be used instead of the partial specific volume. This is true when the specific volume of the solution is a linear function of the specific volumes of the components. In such a case the specific volume, v_{13} of the solvated micelle is given by the following formula according to Adair and Adair [14]

$$v_{13} = (v_1 + k/\varrho)/(1 + k) \tag{8}$$

v_1 is the specific volume of the unsolvated micelle and ϱ is the density of water.

The specific volumes of the solid soaps were measured pycnometrically in toluene. The values 0.833 cm³/g and 0.898 cm³/g were found for potassium myristate and laurate respectively. In Fig. 1 v_{13} from equation (8) is given as a function of the degree of solvation.

However, it is known that soaps in a solid and a dissolved state show remarkable differences in specific gravity. Thus a water solution of cetylpyridinium chloride has a lower density than pure water though this substance in the solid state is heavier than water [8]. Therefore, it is necessary to determine the partial specific volume of the soap in every medium used.

All determinations of partial specific volumes were made in a pycnometer containing about 44 cm³ of solution. The following notations are introduced:

c_1 = concentration of soap in g/g of solution.
c_2 = » » salt » » » »
c_3 = » » water » » » »
x_1 = amount of soap in g.
x_2 = » » salt » ».
x_3 = » » water » »
$\overline{V}_1$ = partial specific volume of soap in cm³/g.
$\overline{V}_2$ = » » » » salt » »
$\overline{V}_3$ = » » » » water » »
V = total volume of mixture in cm³.
v = specific volume of mixture in cm³/g.

Then

$$c_1 = \frac{x_1}{x_1 + x_2 + x_3}; c_2 = \frac{x_2}{x_1 + x_2 + x_3}; c_3 = \frac{x_3}{x_1 + x_2 + x_3}; \tag{9}$$

and consequently

$$c_1 + c_2 + c_3 = 1 \tag{10}$$

Furthermore

$$v = \frac{V}{x_1 + x_2 + x_3} \tag{11}$$

and by definition

$$\overline{V}_1 = \partial V/\partial x_1 ; \overline{V}_2 = \partial V/\partial x_2 ; \overline{V}_3 = \partial V/\partial x_3 \tag{12}$$

Introducing v instead of V in equations (12) we obtain

$$\overline{V}_1 = v + (x_1 + x_2 + x_3).\ \partial v/\partial x_1 \tag{13 a}$$
$$\overline{V}_2 = v + (x_1 + x_2 + x_3).\ \partial v/\partial x_2 \tag{13 b}$$
$$\overline{V}_3 = v + (x_1 + x_2 + x_3).\ \partial v/\partial x_3 \tag{13 c}$$

Eliminating x_1, x_2 and x_3 by means of equations (9) and taking equation (10) into account we finally obtain

$$\bar{V}_1 = v + (1 - c_1) \cdot \partial v/\partial c_1 - c_2 \cdot \partial v/\partial c_2 \quad (14\,a)$$
$$\bar{V}_2 = v - c_1 \cdot \partial v/\partial c_1 + (1 - c_2) \cdot \partial v/\partial c_2 \quad (14\,b)$$
$$\bar{V}_3 = v - c_1 \cdot \partial v/\partial c_1 - c_2 \cdot \partial v/\partial c_2 \quad (14\,c)$$

If we want to determine only $\bar{V}_1$, it is advantageous from an experimental point of view to keep the ratio c_2/c_3 constant. Then

$$\bar{V}_1 = v + (1 - c_1) \frac{dv}{dc_1} \quad (15)$$

which is the same formula as for a binary system.

For a ternary system where a complex is formed between components 1 and 3 we are interested in the partial specific volume, $\bar{V}_{13}$, of the complex. Using the symbols

k = amount in g/g of component 3 bound to component 1.
$y_{13} = x_1 (k + 1)$ = amount of complex in g.
$y_2 = x_2$ = amount of component 2 in g.
$y_3 = x_3 - k \cdot x_1$ = amount of component 3 in g.
c'_1, c'_2 and c'_3 = concentrations of components in g/g of solution.

In the same way as before

$$\bar{V}_{13} = \partial V/\partial y_{13} = v + (1 - c'_1) \cdot \partial v/\partial c'_1 - c'_2 \cdot \partial v/\partial c'_2 \quad (16)$$

In this case the ratio y_2/y_3 is obviously not constant even if the x_2/x_3 is constant. Thus the system cannot be treated as a binary one. Using the relations

$$c'_1 = c_1 (k + 1);\ c'_2 = c_2;\ c'_3 = c_3 - k.c_1 \quad (17)$$

the concentrations c instead of c' can be introduced in equation (16)

$$\bar{V}_{13} = v + [1 - c_1 (k + 1)] \frac{\partial v}{\partial c_1} \frac{1}{k + 1} - c_2 \frac{\partial v}{\partial c_2} \quad (18)$$

From equations (14a), (14c) and (18) it is easily seen (Kraemer[13]) that

$$\bar{V}_{13} = \frac{\bar{V}_1 + k \cdot \bar{V}_3}{k + 1} \quad (19)$$

which is a special case of equation (7).

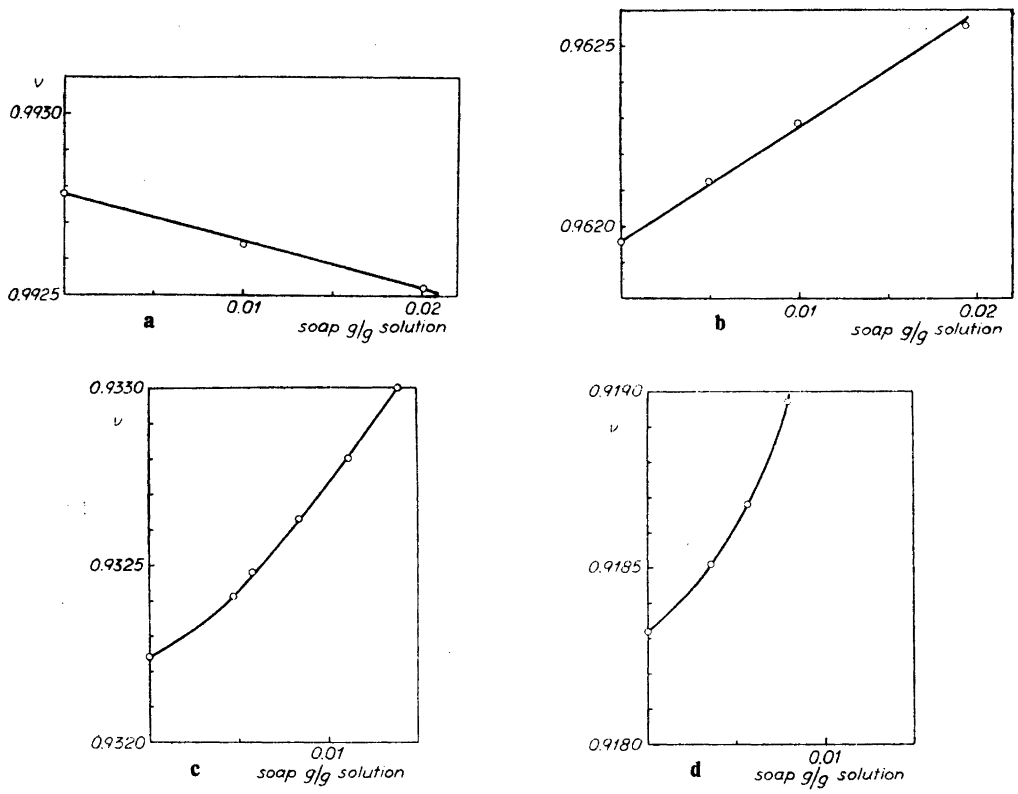

Fig. 2. Specific volume of the soap solution vs. concentration of soap.

a. K-myristate in 0.1 M K_2CO_3
b. » » 0.4 » KBr + 0.1 M K_2CO_3
c. K-myristate in 0.8 M KBr + 0.1 M K_2CO_3
d. » » 1.0 » » + 0.1 » »

Fig. 2 illustrates the change of the specific volume of some solutions as a function of the soap concentration in various media. In these measurements the ratio, salt/water, was kept constant. But as the measurements have been made at different salt concentrations it has been possible to calculate $\partial v/\partial c_2$ and $\partial v/\partial c_1 = dv/dc_1 + c_2/(1 - c'_1) \cdot \partial v/\partial c_2$ at different values of c_2. Equations (14a), (14c) and (18 or 19) then give the required partial specific volumes. These are given in Table 2.

It is evident from Fig. 2 that v is not always a linear function of the soap concentration, in which case the partial specific volume will also be dependent on concentration. The values given in Table 2 refer to the concentration range 0.005—0.01 g/g of solution.

Table 2. Partial specific volume of soap solutions of various ionic strengths.

Soap	μ	$\frac{\partial v}{\partial c_1}$	$\bar{V}_1$	$\bar{V}_3$	$\bar{V}_{13}$
K-myristate	0.3	-0.022 ± 0.001	0.9798 ± 0.0001	1.0021 ± 0.0004	
»	0.7	-0.008 ± 0.001	0.9940 ± 0.0001	1.0020 ± 0.0005	0.999 ± 0.0007
»	0.9	-0.020 ± 0.001	0.9826 ± 0.0001	1.0027 ± 0.0005	0.994 ± 0.0007
»	1.0	-0.011 ± 0.002	0.9916 ± 0.0001	1.0025 ± 0.0006	0.997 ± 0.0009
»	1.1	-0.005 ± 0.005	0.9970 ± 0.0001	1.0020 ± 0.0010	1.000 ± 0.0008
»	1.3	-0.002 ± 0.010	1.0000 ± 0.0002	1.0020 ± 0.0020	1.001 ± 0.0010
K-laurate	1.1	-0.031 ± 0.001	0.9705 ± 0.0001	1.0017 ± 0.0005	0.987 ± 0.0012
»	1.9	-0.030 ± 0.001	0.9713 ± 0.0001	1.0020 ± 0.0010	0.984 ± 0.0014

Instead of the partial specific volume, $\bar{V}$, in the expression $1 - \bar{V}\varrho$ Mc Bain [28] used the density of the sedimenting particle, which can be determined from a graph of sedimentation velocity versus the density of the medium by extrapolation to zero sedimentation velocity. As pointed out by Lansing and Kraemer [29] this method is reliable only if the change in density of the medium has no influence on the sedimenting substance. From the measurements obtained here it is obvious that the partial specific volume of the solvated particle changes with the density of the medium and hence the method of McBain cannot be used in soap investigations.

SEDIMENTATION MEASUREMENTS

As far as the author knows no valuable sedimentation measurements on soaps have been published. The partial specific volume of the solvated micelles is very near that of water; hence the sedimentation velocity is low and the uncertainty of the measurements is high. Furthermore the light absorption method previously used to follow the sedimentation is in this case unsuitable since the soap solutions do not obey Beer's law.

The introduction of Lamm's scale method [15], however, has made possible a more accurate study of the sedimentation properties of the soap micelles. The use of a medium consisting of low molecular electrolytes at a high concentration facilitates the measurements. Such a medium was used by Lamm [17] in his diffusion measurements on soaps. It has two advantages: firstly the density of the medium will be higher and secondly the critical concentration [18] for micelle formation and probably also the transformation to roentgenographic micelles will be shifted towards lower soap concentrations.

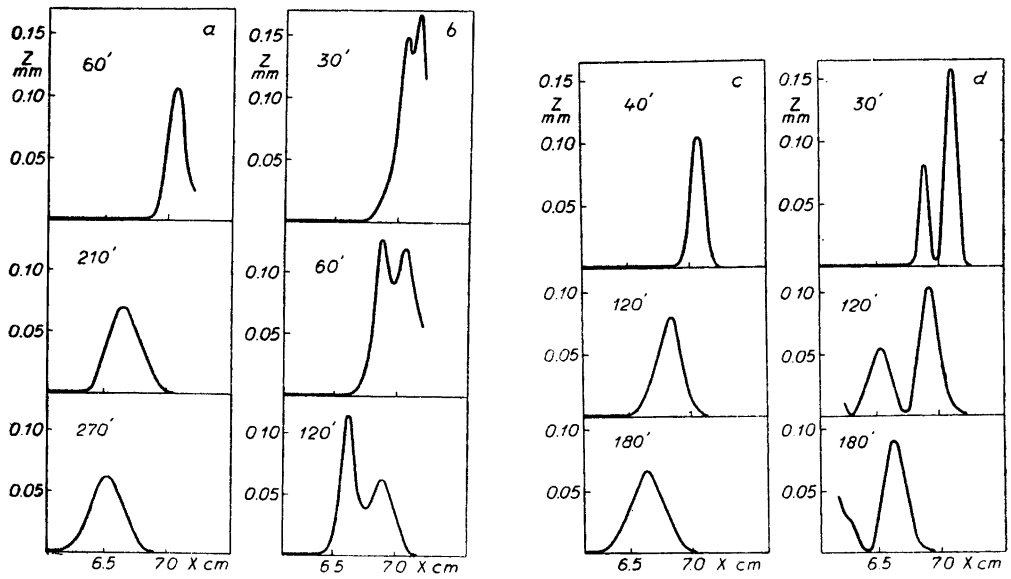

Fig. 3. Sedimentation diagrams of K-myristate in various media.

a. 0.27 % K-myristate in 0.6 M KBr + 0.1 M K_2CO_3
b. 0.9 » » » 0.6 » » + 0.1 » »
c. 0.11 » » » 0.8 » » + 0.1 » »
d. 1.07 » » » 0.8 » » + 0.1 » »

In the sedimentation runs (Svedberg and Pedersen [16]) a rotor speed of 65 000 r.p.m. and a cell distance of 6.5 cm from the axis were used in all measurements.

Since an alkaline medium increases the solubility of potassium soaps [17], measurements were made in a mixture of 0.1 *M* K_2CO_3 and 0.4—1.6 *M* KBr, under which conditions a sufficient range of soap concentrations was possible even at high salt concentrations. The temperature was 28° C. Reference scales were always taken on the pure medium.

The data about the sedimentation experiments are given in Figs. 3—4. The *s*-values are expressed in S-units and refer to the actual medium at 20° C. Reference to pure water can hardly be made because of the difficulties encountered in determining the partial specific volume of the complex soap-water in a salt-free medium. Correction has been made for the viscosity of the medium. A negative sign indicates that the sedimentation occurs towards the centre of rotation. It has been possible to extrapolate the sedimentation constants to zero concentration with a fairly high accuracy. This procedure is an attempt to correct for the interaction between the

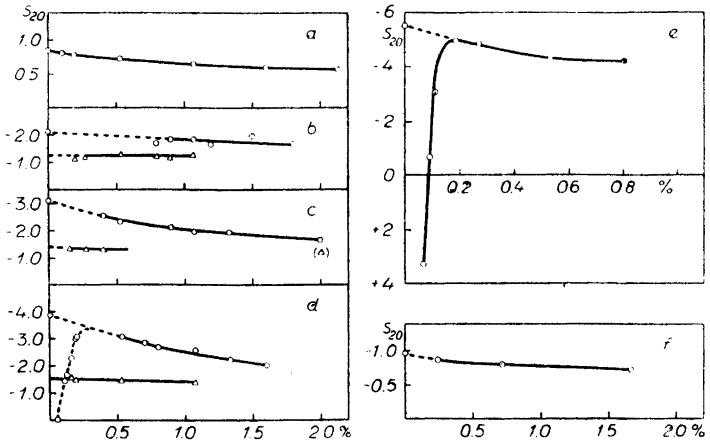

Fig. 1. Sedimentation constant s_{20} vs. soap concentration.

a. K-myristate at μ = 0.7
b. » » » » = 0.9
c. » » » » = 1.0
d. » » » » = 1.1
e. » » » » = 1.3
f. K-laurate » » = 1.1

micelles under the assumption that the micelles do not change with variations in concentration. The extrapolated values then give the sedimentation constant which the micelles would have at any soap concentration if there were no interaction between them. In Fig. 4 s_{20} is plotted as a function of the soap concentration.

The sedimentation behaviour of the soap micelles is as follows. At the ionic strength $\mu = 0.7$ the sedimentation constant of potassium myristate increases with dilution within the whole concentration interval investigated. At $\mu = 0.9$ anomalies occur and two components appear within the interval $c = 0.8$—1.1 per cent. Below $c = 0.8$ per cent only the slower component is present. Its concentration dependence is very small. Whether both components exist above $c = 1.1$ per cent is uncertain. Due to the limited resolving power of the centrifuge, the small difference in the s-values and unfavourable density conditions a separation of the peaks may not be possible. At $\mu = 1.0$ the concentration dependence seems to increase slightly. A slower moving component appears at $c = 0.4$ per cent, and this is the only one present at lower concentrations. Whether this slower component also exists at higher concentrations cannot be judged because of the reasons given above. At $\mu = 1.1$ the two components are still present even at low concentrations.

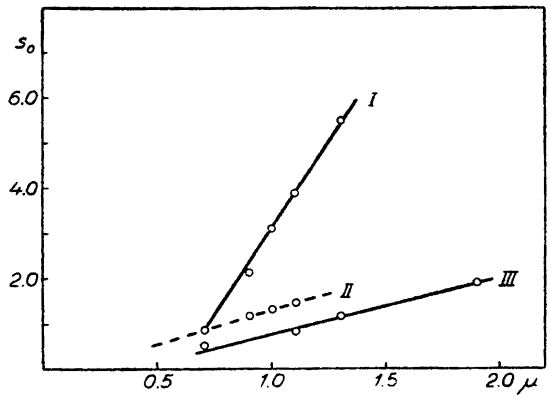

Fig. 5. Sedimentation constant vs. ionic strength.
I: K-myristate, faster component
II: » slower component
III: K-laurate ($s_{0,7\ \%}$)

That component which originally had the higher sedimentation constant has, however, at $c < 0.3$ per cent, an s-value which decreases rapidly with dilution, and at a very low concentration the sedimentation seems to change direction. The slower component is still present at $c = 1.1$ per cent and may persist even at higher values. Its concentration dependence is very low. At $\mu = 1.3$ only one peak seems to exist. A tendency to separate can be observed but the s-values were not reproducible. At high concentrations salting out effects made the measurements very difficult. The sedimentation constant starts to decrease at $c = 0.2$ per cent and is zero at $c = 0.1$ per cent. Then it increases again and the direction of sedimentation changes. This behaviour can only be explained as a change in the structure and partial spec. volume of the micelles.

The concentration dependence of potassium laurate is very small (Fig. 4 f). No separation into two peaks has been observed.

It is evident from Fig. 4 that the concentration dependence increases as the amount of electrolyte in the medium increases. Using Gralén's relation [21]

$$s(c) = s_0/(1 + k_s \cdot c) \tag{20}$$

where s_0 is the sedimentation constant at infinite dilution, the following k_s-values can be obtained

	μ	k_s
Potassium myristate	0.7	0.29
	1.0	0.44
	1.3	0.53
Potassium laurate	1.1	0.19

The change of s_0 with the ionic strength is given in Fig. 5. Curves I and II refer to potassium myristate (fast and slow component respectively), curve III refers to potassium laurate. The relationships are evidently linear but they are very difficult to interpret because the partial specific volume and also the density of the solution change with the ionic strength.

Evidently the increase in the s_0-values of the faster component of potassium myristate with increasing ionic strength indicates a pronounced enlargement of the micelles. This property of micelles has been shown earlier by Mattoon, Stearns and Harkins [19] and by Debye [20]. The degree of association of the slower component in potassium myristate seems to be almost independent of the amount of low molecular electrolytes present. The same is true for potassium laurate; here also s_0 changes very little with the ionic strength.

DIFFUSION MEASUREMENTS

The first diffusion measurements on soaps were published some twenty years ago. McBain and coworkers [22] studied the diffusion of potassium laurate, sodium oleate and some sulfonic acids in water. Jander and Weitendorf [23] introduced sodium chloride and sodium hydroxide as low molecular electrolytes in the diffusion of sodium laurate. They showed that the product of the diffusion constant and the viscosity of the medium were independent of the amount of low molecular electrolytes; this was in any case true at high concentrations of such electrolytes. Hartley and Runnicles [24] made measurements on cetylpyridinium chloride in the presence of sodium acetate. The low solubility of the soaps at ordinary temperatures made the measurements in a wider range of concentrations difficult. This obstacle was overcome by Lamm [17], who used an alkaline medium in his experiments on potassium salts of fatty acids. He showed that the diffusion constant of potassium myristate was about one tenth that of potassium laurate in the same medium. The measurements performed in this investigation are a continuation of Lamm's work. The main problem here was to study the diffusion constant of these soaps as a function of the ionic strength of the medium.

The experimental method developed by Lamm [15] has been used. From Wiener's equation [25], valid for an ideal diffusion, the diffusion constant can be calculated in several ways. Of these the area method (giving D_A) and the moment method (giving D_m) have been used. D_A and D_m have units of 10^{-7} cm^2/sec. All the measurements were made by the free diffusion between solutions differing in soap concentration by about 0.4 per cent. The levelling of the solutions was very difficult because of the small difference in density (about 0.0003—0.0005 g/cm^3). Some of the first measurements were made in a glass

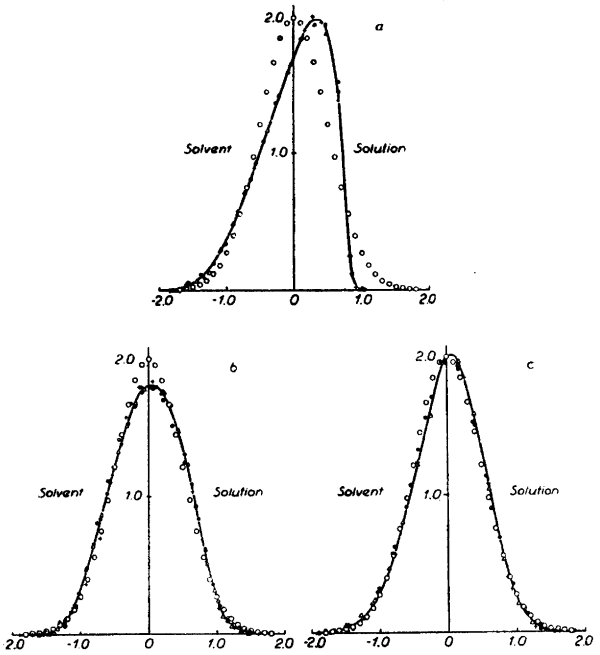

Fig. 6. Normal diffusion curves of soap solutions. Normal ideal curve (—O—O—).

a. 0.4%/0.1% K-laurate at $\mu = 1.9$
b. 1.2%/0.8% » » » $= 1.9$
c. 0.6%/0.2% K-myristate » » $= 0.9$

cell of the same type as that used by Lamm in which the boundary is moved in the tube until it has reached its correct position. This often has a blurring effect on the boundary resulting in diffusion diagrams with poorly defined base lines. Furthermore an error in the real starting time is introduced. Later a cell according to Claesson [26] was used. This cell gives a sharp boundary and also a correct starting time.

The results of the diffusion measurements are given in Table 3. In Fig. 6 the concentration gradient curves in normal coordinates are given for some of the diffusion experiments performed. A comparison is here made with the ideal (Gaussian) curve. In Fig. 7 the relation between D_A and the ionic strength is given. Table 4 gives the concentration dependence of D_A. All the diffusion constants refer to a temperature of 20° C and are corrected for the viscosity of the medium.

From Fig. 7 and Table 3 it is evident that the diffusion rate decreases with increasing ionic strength. This tendency is more pronounced for potassium

Table 3. Dependence of the diffusion constant on ionic strength.

μ	Medium	K-laurate		K-myristate	
		D_A	D_m	D_A	D_m
0.3	0.1 *M* K_2CO_3	—	—	14.0	—
0.5	0.2 *M* KBr + 0.1 *M* K_2CO_3	11.0	—	6.0	6.0
0.7	0.4 *M* —»—	—	—	3.6	3.6
0.9	0.6 *M* —»—	—	—	2.4	2.4
1.1	0.8 *M* —»—	6.0	5.9	1.3	1.3
1.3	1.0 *M* —»—	4.3	—	0.80	0.85
1.9	1.6 *M* —»—	2.7	—	—	—
1.1	1.0 *M* KCl + 0.1 *M* KOH	7.9	7.6	—	—
1.7	1.6 *M* KCl + 0.1 *M* KOH	5.9	6.4	—	—

myristate than for potassium laurate. Evidently the diffusion rate is also dependent on the particular anion of the electrolyte added. Thus in a medium containing potassium chloride and potassium hydroxide the diffusion constant of potassium laurate is considerably higher than in a medium consisting of potassium bromide and potassium carbonate at the same ionic strength. In regard to the dependence on the soap concentration, the diffusion constant of potassium laurate increases as its concentration decreases. The opposite is true for potassium myristate, except in the lowest concentration, where the diffusion constant rapidly increases. This behaviour seems to be in agreement with the results obtained in the sedimentation measurements; the numerical value of the sedimentation constant decreases at low concentrations. Fig. 6

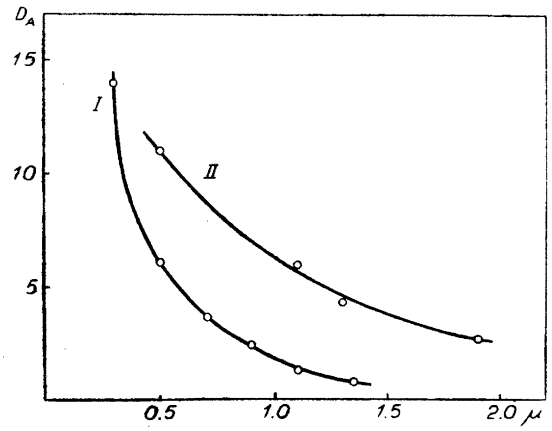

Fig. 7. Diffusion constant D_A vs. ionic strength.

I: K-myristate
II: K-laurate

Table 4. Dependence of D_A on concentration.

c per cent	D_A	
	K-laurate 1.6 M KBr + 0.1 M K_2CO_3	K-myristate 0.8 M KBr + 0.1 M K_2CO_3
1.2 /0.8	2.5	1.5
1.0 /0.6	—	1.4
0.8 /0.4	2.6	1.3
0.6 /0.2	2.7	1.3
0.4 /0.1	3.6	1.3
0.15/0	3.3	6.4

shows that in this concentration range the concentration gradient curve is skew. Thus the diffusion measurements also indicate a change in the structure of the micelles at low concentrations and high ionic strengths.

VISCOSITY MEASUREMENTS

The viscosities of the soap solutions were studied at a temperature of 30° C in an Ostwald viscometer with an effluent time for water of 206 sec. at this temperature. A correction for the influence of surface tension was applied according to Drucker [27]

$$\eta_{rel} = \eta_{rel\ obs} \frac{1 + K}{1 + K \cdot h} \tag{21}$$

where K is a constant of the viscometer which accounts for the change in hydrostatic pressure caused by the difference in the radius of the surface of the liquid in the upper and lower part of the viscometer respectively; h is the ratio between the capillary rise of the solution and the medium respectively. The correction factor had a highest value of about 1.006.

The results of the viscosity measurements are given in Figs. 8 and 9. From curves I—IV in Fig. 8 it is evident that the relative viscosity, η_{rel}, of the potassium myristate solutions increases rapidly with increasing ionic strength. The increase for potassium laurate is moderate (curves V and VI). The change in intrinsic viscosity, $\eta_{sp/c}$, is shown in Fig. 9. An almost linear relation between $\eta_{sp/c}$ and c seems to hold for potassium laurate even in a medium of high ionic strength. For potassium myristate the curves are convex toward the c-axis at μ-values lower than 0.9 and concave at higher μ-values. At

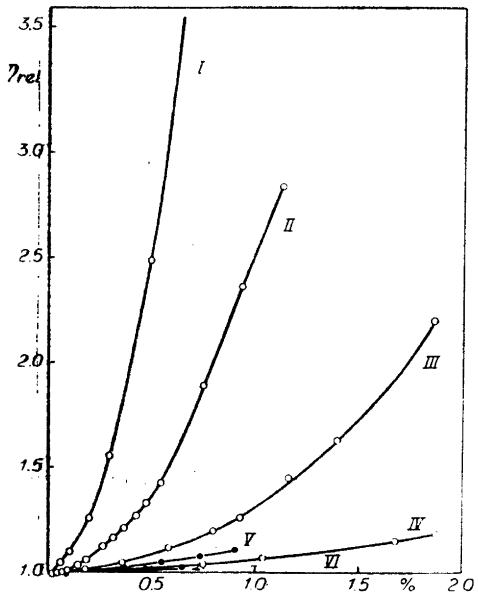

Fig. 8. Relative viscosity as a function of concentration.

I: K-myristate at μ = 1.3
II: » » » = 1.1
III: » » » = 0.9
IV: » » » = 0.7
V: K-laurate » » = 1.9
VI: » » » = 1.1

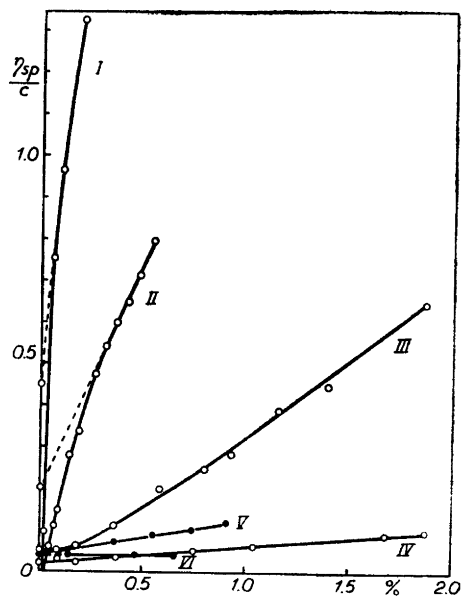

Fig. 9. Dependence of $\eta_{sp/c}$ upon concentration.

I: K-myristate at μ = 1.3
II: » » » = 1.1
III: » » » = 0.9
IV: » » » = 0.7
V: K-laurate » » = 1.9
VI: » » » = 1.1

$\mu = 0.9$ the concentration dependence is rather pronounced, with the slope of the curves increasing rapidly with increasing ionic strength. A constant slope seems to exist above a certain concentration: 0.3 per cent at $\mu = 1.1$ and 0.1 per cent at $\mu = 1.3$. These changes in η_{rel} and $\eta_{sp/c}$ seem to be in complete agreement with the results obtained in the sedimentation and diffusion measurements. In order to obtain $[\eta] = \lim_{c=o} \eta_{sp/c}$ of the "stable" micelles extrapolation has been made from the linear part of the curves in Fig. 9. The results are given in Table 5. For potassium myristate there is a considerable increase in $[\eta]$ with increasing ionic strength, whereas the $[\eta]$-values of potassium laurate seem to be constant.

Table 5. Dependence of intrinsic viscosity $[\eta]$ *on ionic strength.*

Soap	μ	$[\eta]$
K-myristate	1.3	0.45
»	1.1	0.20
»	0.9	0.05
»	0.7	0.02
K-laurate	1.9	0.04
»	1.1	0.04

SOLUBILITY OF *p*-DIMETHYL AMINOAZOBENZENE IN MICELLES

Kolthoff and Stricks [30] have shown that the solubility of *p*-dimethyl aminoazobenzene in soap solutions increases considerably above the critical concentration. The solubility is a linear function of the soap concentration. Some measurements were made in order to see the extent to which the two components in potassium myristate solutions could cause an anomalous shape of the solubility curve. The same technique as that of Kolthoff and Stricks was used.

For potassium myristate in a medium with $\mu = 1.1$ a change in the slope of the solubility curve occured at about 0.1 per cent of soap. At $\mu = 0.7$ the solubility curve is a straight line for all concentrations used. For potassium laurate at $\mu = 1.9$ the solubility increases at 0.04 per cent of soap. This concentration coincides with the critical concentration at this ionic strength. Above 0.04 per cent the solubility curve is a straight line.

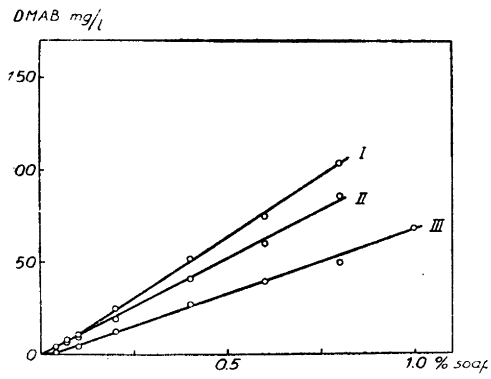

Fig. 10. Solubility of p-dimetyl aminoazobenzol (DMAB) mg/l.

I: K-myristate at $\mu = 1.1$
II: » » » $= 0.7$
III: K-laurate » » $= 1.9$

According to Ekwall [2] the saturation capacity of the micelles is defined by

$$\frac{mg/l \text{ dissolved substance}}{c_{tot} - c_{crit}}$$

where c_{tot} and c_{crit} are the total and critical soap concentrations respectively. From the data in Fig. 10 the following values have been calculated:

Potassium myristate:	
Kolthoff and Stricks	2820
$\mu = 0.7$	2650
$\mu = 1.1$ $c < 0.1$ per cent	2900
$c > 0.1$ per cent	3400
Potassium laurate:	
Kolthoff and Stricks	1480
$\mu = 1.9$	1750

The saturation capacity does not change greatly in different media. The value for potassium myristate at soap concentrations less than 0.1 per cent at $\mu = 1.1$ is about the same as the value given by Kolthoff and Stricks and the value obtained at $\mu = 0.7$. At soap concentrations above 0.1 per cent the saturation capacity is higher. This may indicate the existence of large micelles within this concentration range. According to Ekwall the saturation capacity increases above the critical concentration for the formation of roentgenographic micelles.

DEGREE OF ASSOCIATION AND SHAPE OF THE MICELLES IN DIFFERENT MEDIA

From sedimentation and diffusion data the molecular weight can be calculated according to Svedberg's formula

$$M = \frac{RTs}{D\,(1 - V\varrho)} \tag{23}$$

Using the values of s, D and $\overline{V}_{13}$ referring to the solvated micelles at 30° C and a soap concentration of 0.4 per cent, the molecular weights of the micelles given in Table 6 were obtained. The molecular weights, M_A, of the unsolvated micelles were then calculated from

$$M_A = M_c/(k + 1) \tag{24}$$

Table 6. "Molecular" weight and degree of association of K-myristate and K-laurate in various media.

Soap	Medium	μ	$1-\overline{V}_{13}\cdot\varrho$	M_c	M'_A	M_A	Degree of association
K-myristate	0.4 *M* KBr+0.1 *M* K_2CO_3	0.7	− 0.038	130.000± 15.000	150.000	48.000	180
»	0.6 » +0.1 »	0.9	− 0.049	400.000± 50.000	530.000	181.000	680
»	0.7 » +0.1 »	1.0	− 0.061	560.000± 65.000	620.000	270.000	1000
»	0.8 » +0.1 »	1.1	− 0.072	840.000± 95.000	870.000	430.000	1600
»	1.0 » +0.1 »	1.3	− 0.090	1.550.000±200.000	1.600.000	860.000	3200
K-laurate	0.8 » +0.1 »	1.1	− 0.058	60.000± 7.000	83.000	27.000	110
»	1.6 » +0.1 »	1.9	− 0.119	150.000± 20.000	170.000	87.000	360

where k is the degree of solvation. In Table 6 the degree of association is also given. For the calculation of the correct degree of association, correction for the solvation must be made. This is seen from a comparison of M_A' and M_A in Table 8. M_A' is calculated from (23) using $\overline{V}_1$ instead of $\overline{V}_{13}$ and differs quite considerably from M_A. The limits of experimental errors given for the molecular weights, M_c, are about ± 12 per cent. The error introduced by s and D has been estimated as ± 10 per cent, the error in $\overline{V}_{13}$ has been calculated to contribute about ± 2 per cent.

As is seen from Fig. 11 the degree of association of the potassium myristate micelles increases very rapidly with the ionic strength as soon as this exceeds about 0.7. For potassium laurate the degree of association is moderately

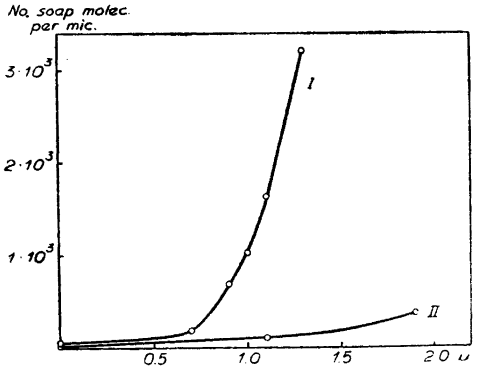

Fig. 11. Degree of association vs. ionic strength.

I: K-myristate
II: K-laurate.

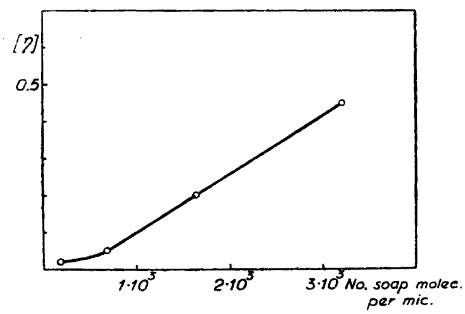

Fig. 12. Intrinsic viscosity [η] of K-myristate as function of degree of association.

dependent on the ionic strength even at high μ-values. The degree of associations corresponding to pure water ($\mu = 0$) have been taken from Mattoon, Stearn and Harkins [19]. Fig. 12 shows that the relation between intrinsic viscosity, $[\eta]$, and the number of soap molecules per micelle is linear except at low values of the degree of association.

The molar frictional coefficient, f_0, of an unsolvated spherical particle is given by Stokes's law

$$f_0 = 6\pi\eta N \left(\frac{3\ MV}{4\pi N} \right)^{1/3} \tag{25}$$

The molar frictional coefficient, f, of the actual molecule is given by

$$f = \frac{RT}{D} = \frac{M_c\,(1 - \bar{V}_{13}\,\varrho)}{s} \tag{26}$$

Assuming equation (25) to hold even for a solvated spherical molecule we get from equations (25) and (26):

$$\frac{f}{f_0} = \frac{1}{6\,\pi\eta} \left(\frac{RT}{DN} \right)^{2/3} \left(\frac{4\ (1 - \bar{V}_{13}\,\varrho)}{3\ \bar{V}_{13}\,s} \right)^{1/3} \tag{27}$$

The quantity f/f_0 gives a relative measure of the asymmetry of the micelle, the values calculated for potassium myristate and laurate are given in Table 7. Evidently the asymmetry is more pronounced the higher the ionic strength.

Table 7. The asymmetri factor f/f_0, calculated for K-myristate and K-laurate in various media.

Soap	Medium	f/f_0
K-myristate	0.4 *M* KBr + 0.1 *M* K_2CO_3	1.49
»	0.6 » + 0.1 »	1.60
»	0.7 » + 0.1 »	1.82
»	0.8 » + 0.1 »	2.22
»	1.0 » + 0.1 »	2.94
K-laurate	0.8 » + 0.1 »	1.18
»	1.6 » + 0.1 »	2.12

DISCUSSION

Among the existing ideas about the structure of the soap micelles, Hartley [8] represents the opinion that in dilute solutions the micelles are spherical and contain a number of soap molecules proportional to the square of the chain length. Only one type of micelle is present in the soap solution. Hess [31], Stauff [32], Philipoff [10] and Kiessig [6] discuss two different types of micelles. The small micelles are according to Stauff spherical and contain a comparatively small number of ions, for instance about 120 for a soap with 16 carbon atoms. An increase in concentration diminishes the hydration and dipoles are formed on the surface of the micelle. A further association to large disc-like micelles (Stauff gives dimensions of the order of magnitude of 250 × 2 500 × 2 500 Å) then eliminates these dipoles with liberation of energy. This arrangement is also favoured by the increasing repulsive forces between micelles in concentrated solutions [33]. Kiessig also regarded the large micelles as disc-like, consisting of about 10 double layers of soap molecules. Philipoff considered the small micelles as cylindrical and consisting of two parallel layers of molecules with the ionogenic groups directed outwards. Later Harkins [34] accepted this form both for the small and the large micelles. Addition of salt to the solution changes the diameter of the cylinder. According to Debye [20] the reason for this is that the coulomb interaction decreases and then the van der Waal forces cause increased association.

The results obtained in this investigation seem to be in agreement with the view of Harkins. At increasing salt concentrations the degree of association increases and judging from the f/f_0-values the diameter of the micelles also increases. Assuming the micelle to be cylindrical and the volume of a soap molecule to be 445 Å^3 (Stauff) we find that the diameter of potassium myristate micelles increases linearly from 46 Å to 194 Å when the ionic strength is changed from 0.7 to 1.3. The thickness is assumed to be constant 48 Å. In this model of the micelles the water of solvation forms a mantle around the associated soap molecules. Apparently there is no possibility of obtaining the very large roentgenographic micelles according to Stauff in these low concentrations by increasing the salt content of the solution. The observed anomalies at low concentrations and at high ionic strengths show that the ordered structure of the micelles disappears. The two components observed in the sedimentation of potassium myristate indicate some sort of transition region.

SUMMARY

Physico-chemical measurements have been made on micelles of potassium myristate and laurate in solutions containing potassium bromide and potas-

sium carbonate at various ionic strengths. In these media the degree of solvation, the partial specific volume, the sedimentation and diffusion constants have been determined. The viscosities of the solutions have been studied and also the ability of the micelles to dissolve *p*-dimethyl aminoazobenzene. From the experimental data the degree of association has been calculated. The size of the micelles increases with the ionic strength of the medium; this increase is very pronounced for potassium myristate and rather moderate for potassium laurate. The data obtained corroborate the belief that the micelles are cylindrical discs.

This investigation is part of a research program carried out at the request of the Government Commission on Industry.

The author wishes to acknowledge her indebtness to Professor The Svedberg for his very kind interest and for the many facilities which have been made available.

The author also wishes to thank Professor Ole Lamm for his interest in this work and for valuable discussions.

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Received December 4, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 126–139

Heat of Polymerization of Chloroprene

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Determinations of the heat of polymerization are of great importance in connection with the study of polymerization reactions. Several methods have been used for this purpose during recent years. In a series of papers Tong and Kenyon [1–5] have described an isothermal calorimeter and the use of this on monomers of the vinyl type. Goldfinger, Josefowitz and Mark [6] and Evans and Tyrrall [7] have used adiabatic calorimeters. Roberts, Walton and Jessup [8] have determined heats of polymerization from heats of combustion, a method used previously by many investigators. Most of the measurements have been done on monomers of the vinyl type and some on butadiene and isoprene. As far as the present authors know only one value of the heat of polymerization of chloroprene (2-chloro-1,3-butadiene) has been published. Thus Walter [9] for polymerization in sodium oleate emulsion at 30° C gives the value 15.1 ± 0.8 kcal/monomer unit. No details about the measurements are given.

In this investigation the heat of polymerization of chloroprene has been measured at different initiator concentrations. An isothermal calorimeter of a construction very similar to that of Tong and Kenyon [1, 2] has been used. The polymerization has been made in bulk. Measurements have also been made on methyl methacrylate and styrene mainly in order to check the calorimeter.

EXPERIMENTAL

Description of calorimeter

The main difficulty in constructing the calorimeter was to achieve sufficient stability for long time runs. The time of polymerization of chloroprene can be as long as 15—20 hours. The calorimeter is shown in Fig. 1. By means of a ground joint flask (A) is connected to a double walled, cylindrical part (B).

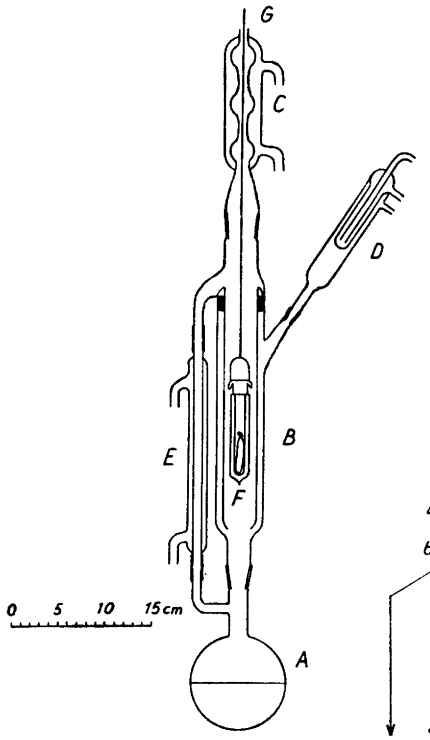

Fig. 1. Isothermal calorimeter.

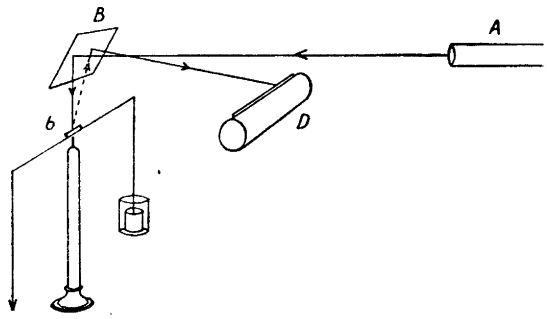

Fig. 2. Selfrecording arrangement.

To the outer jacket of (B) a condenser (D) is attached. The inner jacket is connected through a side tube and the condenser (E) to flask (A). At the opening for the side tube a trap is made in which the condensate from condenser (C) is caught. The inner jacket is fixed to the outer one by means of thin cork strips and gelatine acetic acid cement. Inside the inner jacket a dewar tube is suspended freely from one of the arms of a balance by means of a thin metal wire (G). This tube has a capacity of about 60 ml. For long polymerization runs it is necessary to diminish the heat losses from the liquid in the dewar tube as much as possible, for which reason the innerwalls are silverplated *. The reaction tubes containing monomer are placed in the dewar vessel. In order to save time the heat exchange liquid in (F) is heated to the boiling point by means of a small electric heater submerged in the liquid, or the tube (F) is filled with the boiling liquid.

* The dewar vessel was constructed by A. B. Lumalampan, Stockholm.

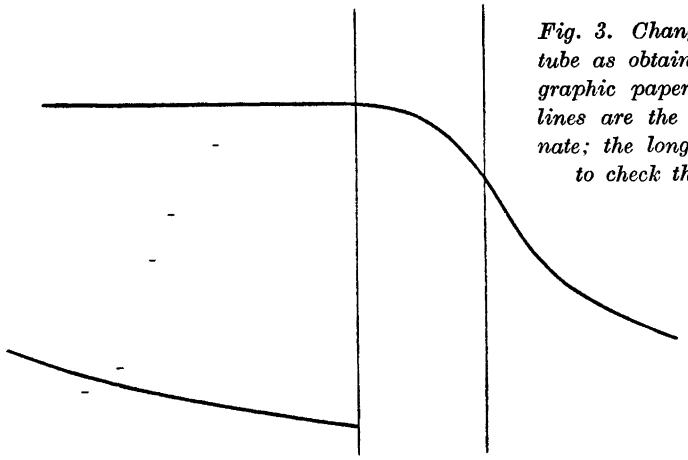

Fig. 3. Change in weight of the dewar tube as obtained directly on the photographic paper. The short, horizontal lines are the calibrations of the ordinate; the long, vertical lines are used to check the speed of the drum.

The selfrecording system shown in Fig. 2 is almost identical with that used by Tong and Kenyon [2]. The light beam from the thin filament of lamp (A) is reflected from mirror (B) to lens (b) (focal distance 1 m) attached to the midpoint of the beam of the balance. The lens is silverplated on the back side and hence reflects the light back to mirror (B) and to drum (D) which can be rotated at different speeds with a synchronous motor and a gear. A similar drum has been used by Andersson, Ställberg-Stenhagen and Stenhagen [10] and Claesson [11] for other purposes. By means of the lens (b) a sharp image of the filament is formed on the slit-shaped opening of the drum. A damping arrangement is attached to one of the arms of the balance. It consists of a glass cup, containing tare weights placed in a larger cup containing a zinc chloride solution of a suitable density. A thin layer of paraffin oil prevents evaporation of water from the zinc chloride solution. During a run a curve is obtained on photographic paper placed in the drum. The ordinate will be proportional to the change in weight of the dewar tube; the abscissa gives the time. In order to check the speed of rotation and to correct for shrinking of the photographic paper the slit is illuminated at definite time intervals. The ordinate is calibrated by means of known weights placed on the balance. An example of such a curve is given in Fig. 3. From these curves the relationship between the heat evolved during the polymerization and the time is easily obtained.

Purification of monomers

Chloroprene. This was obtained from large scale production in plant. There it had been washed with acid sodium sulfite to remove aldehydes, distil-

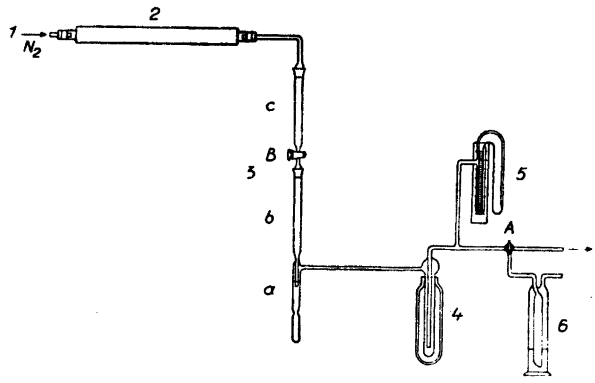

Fig. 4. Arrangement for filling the reaction tubes.

led in a column and stabilized with thiodiphenylamine. This chloroprene still contains water, peroxides and dissolved oxygen. For further purification it was stored some time over calcium chloride and then distilled at atmospheric pressure in a bead packed column with about eight theoretical plates. Some calcium carbonate was added in order to neutralize small amounts of hydrochloric acid split off during heating. The distillate was stabilized with thiodiphenylamine and stored over sodium sulfate. The quantity necessary for each run in the calorimeter was then distilled at atmospheric pressure in a wire-gauze column with about 35 theoretical plates. Carbon dioxide was passed through the column and the receiver was cooled with dry ice. All these precautions were taken in order to prevent, as far as possible, the formation of polymers and peroxides in the monomer.

Methyl methacrylate. This was distilled in the bead packed column at reduced pressure and then stored in a closed bottle at — 15° C.

Styrene. This was distilled in the bead packed column at reduced pressure and then immediately used.

Filling of the reaction tubes

Peroxides formed by reaction of the monomers and oxygen in the air influence the course of the polymerization. Therefore, special precautions were taken to prevent the monomers from being in contact with the air during the filling of the reaction tubes. The arrangement shown in Fig. 4 was used for this purpose. Nitrogen was introduced into the system at (1) and freed from oxygen in the copper oven (2), consisting of a glass tube filled to 50 cm of its

length with very small pieces of reduced copper. According to Meyer[12] such an oven is effective at a flow rate not exceeding 250 ml/min as long as it contains at least 30 cm of reduced copper beyond the part already oxidized. The flow rate used was 150 ml/min. It could be controlled by means of a gas washing bottle (6) partly filled with glycerol; the bottle could be attached to the system with a three-way stopcock (A). The tube (3b) is partly filled with sodium sulfate and, above this, with aluminum oxide (according to Brockman for chromatographic analysis). The contents were packed by means of a vibrator and held in position by plugs of cotton. The reaction tubes were attached to the lower part of the tube (3b) with a rubber tube in such a way that the ends of the glass tubes were in close contact. (4) is a cooling trap and (5) a manometer. The whole system can be attached at (A) to a vacuum pump.

The reaction tubes were made from test-tubes with an inner diameter of about 15 mm and a wall thickness of about 0.4 mm. The tubes were constricted in the middle; the open ends were smoothed. The sides of the bulb softened in a flame were pushed in with capillary tubes to increase the surface for better heat transference. At the same time the capillaries promote smooth boiling. The reaction tubes were made to contain about 5 g of monomer.

Parts (3a), (b) and (c) were carefully washed in a solution of sodium dichromate and sulfuric acid and then in water saturated with sulfur dioxide. After rinsing with alcohol and ether tube (3b) was filled with the adsorbents and the pieces (a), (b) and (c) were put together. Where ground joints were not used, the tubes were assembled as closely as possible by means of rubber vacuum tubes covered with picein. When the polymerization was to be performed with an initiator a weighed amount of this was placed in the reaction tube before it was attached. In order to remove the air from the system it was evacuated and filled with nitrogen. When this procedure had been repeated 5—10 times stopcocks (A) and (B) were closed. Monomer was then run into the tube (3c) and slowly sucked through the adsorbents in (3b) into the reaction tube, which was cooled in a mixture of dry ice and alcohol. After the filling was completed the reaction tube was sealed off at the constricted part and a small hook was formed.

Aluminum oxide was used to remove peroxides from the monomer. This method has previously been tried by Dasler and Bauer[13] on ether. They thus obtained an ether which was practically free from all peroxides. Experiments carried out on chloroprene have shown that the amount of peroxides could easily be reduced by about 98 per cent.

Treatment of the polymerizates

In order to calculate the heat of polymerization it is necessary to know the amount of polymer formed during the reaction.

The amount of polychloroprene was determined according to a method by Lewis and Mayo [14]. The polymerization mixture which at high degrees of conversion had a semi-solid consistency, was cut in small pieces and then allowed to swell in benzene. In a freeze drier the benzene and the unpolymerized chloroprene were removed. The polymerizate was slightly heated during the end of the procedure and a constant weight was obtained after about four days.

During the experiments with methyl methacrylate the method of Lewis and Mayo had not come to our attention. The polymerizate was dissolved in chloroform and some hydroquinone was added as a stabilizer. The polymer was then precipitated with methyl alcohol and dried in a vacuum oven at 50° C to constant weight. Evaporation of the supernatant showed that the precipitation was complete. — The polystyrene was treated in the same way.

Other chemical agents

The initiator used was recrystallized benzoyl peroxide (Benzoylperoxid reinst für wissenschaftliche Zwecken, Dr. Theodor Schuckardt, Görlitz, Deutschland).

The heat exchange media in the calorimeter were carbon tetrachloride (analytical grade; boiling point 76.8° C and heat of vaporization 46.4 cal/g) for methyl methacrylate and styrene, and chloroform (Baker's analyzed; boiling point 61.5° C and heat of vaporization 59.1 cal/g) for chloroprene.

RESULTS

The results of the measurements are given in Tables 1—3. The heat evolved as a function of time when 1 mole of the monomer polymerizes, is given in Figs. 5 and 7 for methyl methacrylate and chloroprene respectively. The first five experiments in Table 1 were made without any initiator. Experiments 1*, 2* and 4* were run with a monomer from which the peroxides had not been removed. In Fig. 5 the corresponding curves are marked with an asterisk. Evidently the removal of peroxides from the monomer is quite necessary in order to get reproducible results. The mean value of the heat of polymerization obtained in experiments 6—16 is 13.9 $\pm$ 0.3 kcal/monomer unit. From Fig. 6 where the heat of polymerization is plotted as a function of the amount

Table 1. Heats of polymerization of polymethyl methacrylate.

Experiment	Amount of initiator in per cent by weight	Degree of conversion in per cent	Heat of polymerization, $-\Delta H_p$, in kcal/monomer unit
1*	0	95.7	12.6
2*	0	94.7	13.4
3	0	21.9	12.9
4*	0	94.7	13.2
5	0	68.8	13.4
6	0.017	95.6	13.6
7	0.049	97.9	13.9
8	0.078	77.9	14.1
9	0.084	88.1	14.0
10	0.101	96.1	14.0
11	0.102	96.3	14.0
12	0.50	97.9	14.1
13	0.57	98.0	13.6
14	1.01	93.8	13.4
15	1.03	92.3	14.6
15	1.58	98.5	13.9

Mean value, 6—16: $-\Delta H_p^{349.8} = 13.9 \pm 0.3$

Table 2. Heats of polymerization of polystyrene.

Experiment	Amount of initiator in per cent by weight	Degree of conversion in per cent	Heat of polymerization, $-\Delta H_p$, in kcal/monomer unit
1	1.13	98.8	16.1
2	1.35	97.8	16.2
3	1.89	97.9	16.1

Mean value: $-\Delta H_p^{349.8} = 16.1 \pm 0.1$

of initiator, it is evident that no pronounced dependence on the initiator concentration exists. The spread of the values at higher concentrations can be ascribed to a vigorous boiling of the heat exchange medium causing weight losses by splashing. From the three experiments on styrene in Table 2 a mean value of the heat of polymerization of polystyrene of 16.1 ± 0.1 kcal/monomer unit is obtained. On chloroprene ten experiments were made with various amounts of initiator. From Fig. 8 it is evident that the heat of poly-

Table 3. Heats of polymerization of polychloroprene.

Experiment	Amount of initiator in per cent by weight	Degree of conversion in per cent	Heat of polymerization, $-\Delta H_p$, in kcal/monomer unit
1	0.08	90.8	15.6
2	0.38	95.4	16.7
3	0.74	98.7	17.1
4	0.88	99.2	17.1
5	1.69	97.7	17.2
6	1.92	100.0	17.3
7	2.19	98.3	17.5
8	2.90	98.5	18.1
9	3.18	98.4	18.1
10	3.43	98.4	18.1

Value extrapolated to zero concentration of initiator: $-\Delta H_p^{334.5} = 16.2 \pm 0.3$

merization shows a marked dependence on the initiator concentration. The value obtained by extrapolation to zero concentration is 16.2 ± 0.3 kcal/monomer unit.

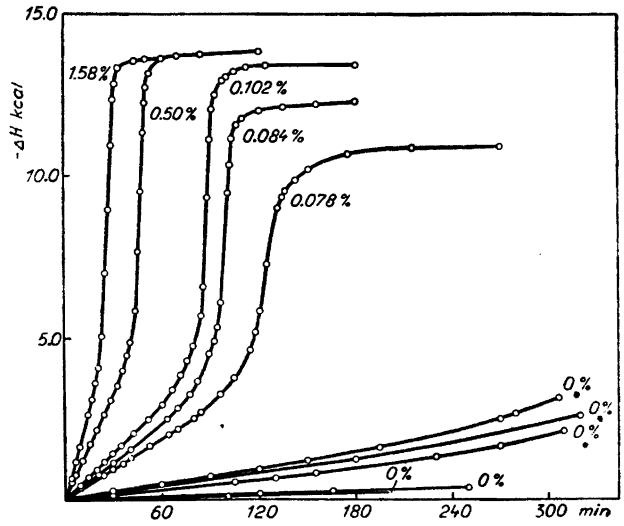

Fig. 5. Heat evolved vs. time when 1 mole of methyl methacrylate is polymerized at different concentrations of initiator.

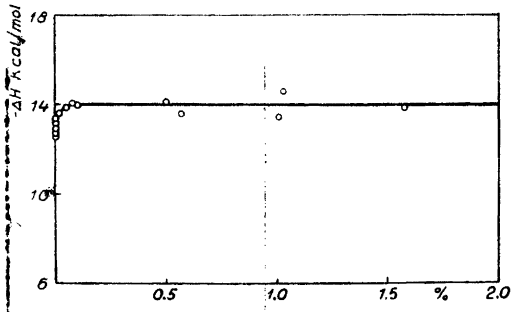

Fig. 6. Heat of polymerization of polynethyl methacrylate vs. amount of initiator.

In calculating the values of the heat of polymerization no corrections have been made for the amount of initiator contained in the macromolecules or for the heat evolved in the activation reaction. These corrections would be large, however, only in a case where a large amount of initiator was used. — Corrections should also be made for the integral heat of dilution. In this case, however, the degrees of conversion are high and judging from heats of swelling given, for instance, by Hock and Schmidt [15] and Gee [16], such a correction is probably less than 1 per cent.

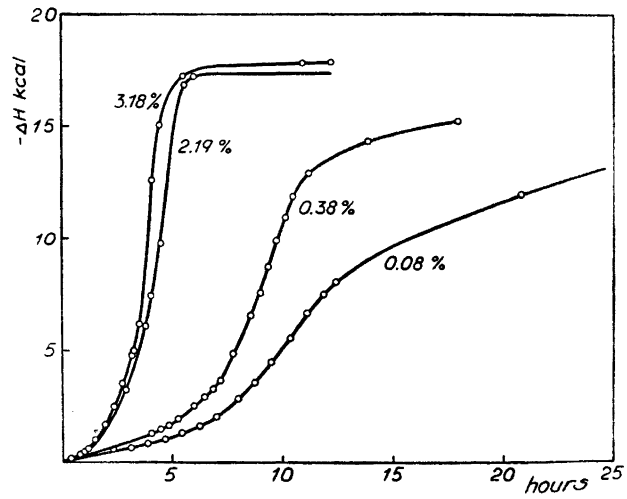

Fig. 7. Heat evolved vs. time when 1 mole of chloroprene is polymerized at different concentrations of initiator.

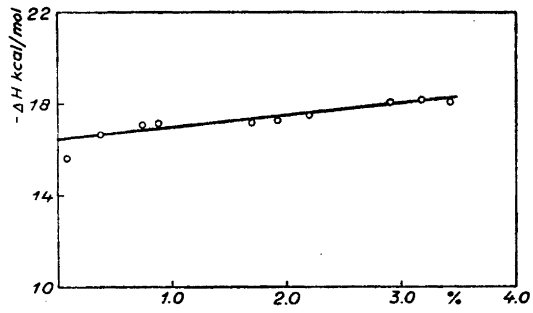

Fig. 8. Heat of polymerization of polychloroprene vs. amount of initiator.

DISCUSSION

As mentioned before the determinations of the heats of polymerization of polymethyl methacrylate and polystyrene were made in order to check the calorimeter. The values determined by other authors are given below:

Polymethyl methacrylate:

$-\Delta H_p$ in kcal/monomer unit	Method		
21.4	Heat of combustion;	ref.	(17)
7.9 ± 0.4	Adiabatic calorimeter;	ref.	(6)
12.9 ± 1.1	Adiabatic calorimeter;	ref.	(7)
13.0 ± 0.2	Isothermal calorimeter;	ref.	(1)
13.0 ± 0.2	Isothermal calorimeter;	ref.	(2)

Polystyrene:

$-\Delta H_p$ in kcal/monomer unit	Method		
10.6	Heat of combustion;	ref.	(18)
14.8	Heat of combustion;	ref.	(18)
21.9	Heat of combustion;	ref.	(19)
23	From the activation energy of polymerization and depolymerization;	ref.	(20)
15.0 ± 0.47	Adiabatic calorimeter;	ref.	(6)
16.68	Heat of combustion;	ref.	(8)
16.1 ± 0.2	Isothermal calorimeter;	ref.	(3)

The value obtained here for polymethyl methacrylate, $-\Delta H_p = 13.9 \pm 0.3$ kcal/monomer unit, is slightly higher then the values obtained by Tong and Kenyon [1, 2] but coincides, within the limits of experimental error, with the value given by Evans and Tyrrall [7]. Tong and Kenyon have not given any values for the degree of conversion. According to our experience this

seldom reaches 100 per cent. The mean degree of conversion from our experiments nos. 6—16 is 93.7 per cent. If a correction for the degree of conversion were applied to Tong and Kenyon's data the value 13.9 ± 0.2 would be obtained. We do not claim that this is the true explanation of the discrepancy. It is very striking, however, that the value obtained here for polystyrene, $-\Delta H_p = 16.1 \pm 0.1$, is in very good agreement with the value obtained by Tong and Kenyon [3]. In our investigation only three determinations have been made; the agreement between them is very good and there is no reason to believe that the values are wrong. — No judgement can be made from our data in Table 2 as to the dependence on the concentration of initiator, which was reported by Tong and Kenyon [3], because of the narrow range of concentrations studied in our experiments.

Some of the polymethyl methacrylates obtained in this investigation have been used by Claesson [21] for adsorption analysis experiments. The frequency curves obtained (Fig. 9) show several maxima as do the curves obtained by means of fractional precipitation on other samples of polymethyl methacrylate (Kinell [22]). The curves in Fig. 9 show that complete reproducibility of the polymerization reaction is difficult to achieve. Comparing curves (e) and (f) corresponding to the amounts of initiator of 0.101 and 0.102 per cent and the degrees of conversion of 96.1 and 96.3 per cent, it is evident that the course of the reaction has not been the same in both cases. Curves (c) and (d) are also different. They correspond to 0.078 and 0.084 per cent of initiator respectively. The discrepancy here is also evident from the curves in Fig. 5. Curves (a) and (b), corresponding to polymers formed from monomers containing only peroxides in the amounts present as impurities, are almost identical in shape; they are only displaced against each other. The experimental material presented does not permit any definite explanation of these discrepancies. It may be pointed out that the rate of the reaction will be so high especially at larger amounts of initiator that the heat evolved can not be conducted away rapidly enough; therefore a rise in temperature will occur and the reaction can not proceed under isothermal conditions. In such a case the size and shape of the reaction tubes play an important role.

The value obtained for the heat of polymerization of polychloroprene, $-\Delta H_p = 16.2 \pm 0.3$ kcal/monomer unit, is in fairly good agreement with the value, $-\Delta H_p = 15.1 \pm 0.8$ kcal/monomer unit, given by Walker [9]. The heat evolved is dependent on the amount of initiator (Fig. 8). The explanation of this can not yet be given. — Flory [23] has made a calculation of the heat of polymerization of different kinds of monomers. For dienes he finds a heat of polymerization of about 20 kcal/monomer unit. In general the experimental values are lower than the calculated ones. An explanation of this has been

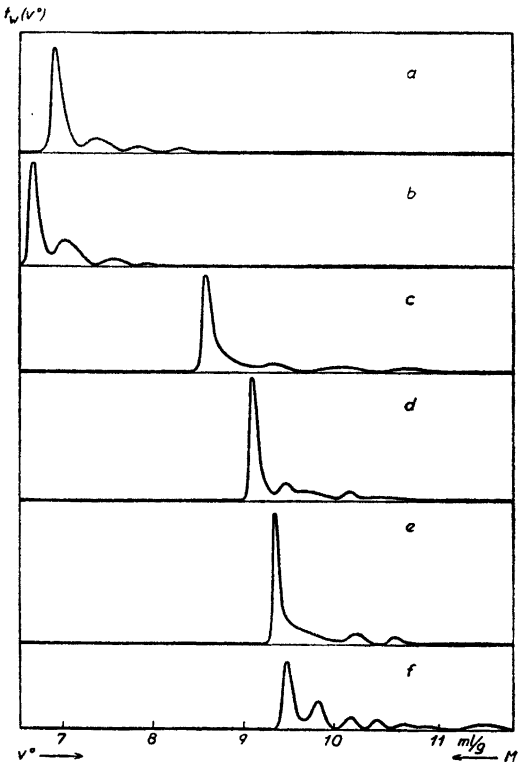

Fig. 9. Frequency curves of polymethyl methacrylates. v^0 is the specific retention volume and M the molecular weight. (From frontal analysis experiments on active carbon by Claesson [21]).

given by Flory [24] and Evans and Polanyi [25]. During polymerization the monomer units can arrange themselves in various ways. It is possible to get both "head-to-tail" and "head-to-head, tail-to-tail" arrangements. In some cases it has been possible to determine the actual arrangement. Thus polyisobutylen has a head-to-tail structure (Thomas *et al.* [26] and Fuller, Frosch and Pape [27]). In these molecules, however, there are very large steric hindrances which are responsible for the observed low heat of polymerization. In the case of polychloroprene roentgenographic measurements (Kenney [28], Garbsch and von Susich [29], Fuller [30], Birkett Clews [31] and Lindgren [32]) have shown that on stretching to 200—300 per cent elongation some of the molecules orient themselves to a crystalline structure which has a fibre period of 4.79 A. This value is in agreement with a *trans*-configuration of the crystallized polychloroprene (*cf.* Mark [33]). In a chain with this configuration built up from Fisher-Stuarts molecular models no steric hindrances can be located. A *cis*-configuration, however, seems to show such hindrances. On stretching only a comparatively small amount of the polymer will be oriented; therefore the possibility is not

excluded that a *cis*-configuration can predominate in the amorphous parts of the polymer. Furthermore some of the macromolecules may have been formed by 1,2-addition which gives a very compact structure compared to the 1,4-addition. All these facts make it probable that the heat of polymerization of polychloroprene has a value lower than about 20 kcal/monomer unit.

SUMMARY

An isothermal calorimeter for determination of heats of polymerization has been described. Measurements made on polymethyl methacrylate, polystyrene and polychloroprene formed by initiation with benzoyl peroxide from monomers which have been purified from peroxides by means of aluminum oxide, have given the following heats of polymerization:

Polymethyl methacrylate	$-\Delta H_p^{349.8}$	$= 13.9 \pm 0.3$	kcal/monomer	unit
Polystyrene	$-\Delta H_p^{349.8}$	$= 16.1 \pm 0.1$	»	»
Polychloroprene	$-\Delta H_p^{334.5}$	$= 16.2 \pm 0.3$	»	»

The heat of polymerization of polychloroprene shows a dependence on the amount of initiator. The reason for this can not yet be given.

This investigation is part of a program of research on synthetic rubber carried out at the request of the Government Commission of Industry.

We are most grateful to Prof. The Svedberg for his very kind interest in this work and for the many facilities placed at our disposal.

We also thank Prof. Stig Claesson for many interesting discussions and much valuable advice.

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Received December 4, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 140–145

Complex Formation in Dilute Aqueous Solutions of Cupric Chloride. Spectrophotometric Determination of Equilibria Involving Slight Complex Formation in Electrolytic Solution

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In a recent paper by the present author [1], the complex formation between cupric and sulphate ions in aqueous solutions was investigated spectrophotometrically. In that article a method for the determination of the thermodynamic complexity constant and the activity coefficient in systems of slight complexity was described. This method has since been further developed. In the following, the improved method is applied to the determination of the thermodynamic dissociation constant and the activity coefficient of the first cupric chloride complex.

EXPERIMENTAL

The light absorption measurements were carried as before out with a Beckman Quartz Spectrophotometer Model DU. 1 cm and 5 cm cells were used. The chemicals were of the best quality obtainable and were used without purification. Concerning further experimental details, the reader is referred to the previous papers by the present author [1, 2].

METHOD OF CALCULATION

In dilute aqueous solutions of cupric chloride, the complex ion $Cu(H_2O)_3Cl^+$ is practically the only one present [3]. The equilibrium expression, according to the law of mass action, is

$$f_{Cu^{++}} [Cu^{++}] f_{Cl^-}[Cl^-] / f_{CuCl^+} [CuCl^+] = K_0 \qquad (1)$$

which may be rewritten:

$$[Cu^{++}]\ [Cl^-] / [CuCl^+] = K/_0F = K \tag{2}$$

where

$$F = f_{Cu^{++}} f_{Cl^-} / f_{CuCl^+} \tag{3}$$

For the determination of the thermodynamic constant K_0 and the activity coefficient F, a series of dilute cupric chloride solutions with varying amounts of hydrochloric acid were prepared. At wavelengths near 300 mμ, the light absorption of hydrochloric acid solution is negligible and that of cupric ions is very small, but that of the complex is considerable. From Beer's law we obtain:

$$\varepsilon = E/d - \varepsilon_{Cu^{++}} [Cu^{++}] = \varepsilon\, [CuCl^+] \tag{4}$$

where E is the extinction, d the thickness of the absorbing layer in cm, $\varepsilon_{Cu^{++}}$ the molecular extinction coefficient of cupric ion and ε that of the complex. Inserting (4) into (2) gives:

$$\frac{(c_{Cu} - e/\varepsilon)x}{e/\varepsilon} = K_0/F = K \tag{5}$$

or

$$\varepsilon - K_0 e/F c_{Cu} x - e/c_{Cu} = 0 \tag{6}$$

where $x = [Cl^-]$ and c_{Cu} is the total copper concentration. The concentration of chloride ion is approximately the same as the stoichiometric concentration. After the equilibrium constant has been determined, however, it is possible to calculate the concentration of the complex from

$$[CuCl^+] = c_{Cu} c_{Cl} / (K + c_{Cu} + c_{Cl} - [CuCl^+]) \tag{7}$$

by the iterative method. The accurate chloride ion concentration is now obtained from

$$[Cl^-] = c_{Cl} - [CuCl^+] \tag{8}$$

The activity coefficient in equation (6) may be calculated from the Debye-Hückel equation

$$pF = \frac{2.04\ \sqrt{I}}{1 + a\ \sqrt{I}} - BI \tag{9}$$

where I is the ionic strength. The parameters a and B are unknown. We, therefore, assign them the values in alkalipechlorate solutions and after the molecular extinction coefficient ε has been calculated from equations (6) by the method of least squares, it is possible to calculate more accurate values for a and B by means of equation (5). This method of calculation is much less laborious than that presented in the previous paper by the present author[1].

The activity coefficient of the first cupric chloride complex, in alkali perchlorate solutions, may be determined as follows. In dilute cupric chloride solutions, with no alkali chloride or hydrochloric acid the concentration of the complex is very small compared to the total copper concentration. Therefore, we obtain from the law of mass action:

$$\frac{2\ c_{Cu}{}^2}{e/\varepsilon} = K_0/F \tag{10}$$

or

$$p(e/2c_{Cu}{}^2) = p(\varepsilon/K_0) + pF \tag{11}$$

By means of this equation it is possible to calculate the activity coefficient from the extinction of a series of cupric chloride solutions, where the ionic strength is varied by addition of alkali perchlorate. In addition the quotient ε/K_0 is obtained. For the determination of the activity coefficient in this case it is thus unnecessary to know either the molecular extinction coefficient or the thermodynamic dissociation constant. Although the method has been described with reference to a particular system, it can easily be adapted to any other such system.

RESULTS

In Table 1 a series of measurements in mixed solutions of cupric chloride and lithium perchlorate is presented. The values

$$\varepsilon/K_0 = 1625\ (\lambda = 272\ \text{m}\mu),\ a = 1.788 \text{ and } B = 0.212$$

were obtained from equation (11) by the method of least squares. The activity coefficient function may thus be represented by the equation:

$$Fp = \frac{2.04\ \sqrt{I}}{1 + 1.788\ \sqrt{I}} - 0.212\ I \tag{12}$$

This equation expresses very satisfactorily the data over the whole range of ionic strengths investigated. The maximum deviation is 0.006 and the average deviation 0.003. The error caused by the approximation $[Cu^{++}] = c_{Cu}$ is at most of the same order of magnitude as the average deviation.

The results in mixed solutions of cupric chloride and hydrochloric acid are recorded in Table 2. By using equation (6) the values

$$K_0 = 0.90 \text{ and } \varepsilon = 1478 \ (\lambda = 272 \text{ m}\mu)$$

Table 1. The activity coefficient of the first cupric chloride complex in lithium perchlorate solutions. $c_{Cu} = 0.01000$.

$\sqrt{I}$	e ($\lambda = 272$ mμ)	$p(e/2c^2) - p(\varepsilon/K_0)$	ΔpF (Deviation)
0.123	0.0544	0.1971	+ 0.0047
0.173	0.1750	0.2691	− 0.0058
0.222	0.1566	0.3173	− 0.0032
0.272	0.1430	0.3568	+ 0.0005
0.297	0.1368	0.3760	+ 0.0013
0.343	0.1268	0.4090	− 0.0004
0.383	0.1206	0.4308	+ 0.0018
0.453	0.1114	0.4652	+ 0.0017
0.513	0.1044	0.4934	− 0.0033
0.616	0.0990	0.5165	+ 0.0010
0.705	0.0960	0.5298	+ 0.0011
0.783	0.0960	0.5298	+ 0.0058
0.854	0.0960	0.5298	+ 0.0049
0.981	0.0980	0.5209	+ 0.0015
1.094	0.1020	0.5035	− 0.0023
1.128	0.1046	0.4926	+ 0.0003
1.196	0.1080	0.4787	− 0.0045
1.346	0.1224	0.4243	− 0.0024
1.537	0.1494	0.3378	− 0.0019
1.611	0.1644	0.2962	+ 0.0004
1.673	0.1794	0.2583	+ 0.0033

were obtained by the method of least squares. The maximum deviation of an individual value of the thermodynamic complexity constant is 0.03, and that of the molecular extinction coefficient 60. The average deviations are 0.01 and 20, respectively. The accuracy obtained is thus very satisfactory. In this case, the quotient of the molecular extinction coefficient and the thermodynamic dissociation constant is 1650, in satisfactory agreement with the above

Table 2. Determination of the thermodynamic dissociation constant and the activity coefficient of the first cupric chloride complex at 25° C. $\lambda = 272$ mμ.

$c_{Cu} \cdot 10^3$	x	e	$\sqrt{I}$	pF	K_0 ($\varepsilon = 1478$)	ε ($K_0 = 0.896$)
5.00	0.0100	0.0516	0.1225	0.2041	0.889	1490
10.00	0.0200	0.173	0.173	0.2670	0.929	1425
7.50	0.0402	0.238	0.219	0.3162	0.885	1497
5.83	0.0958	0.354	0.319	0.4008	0.889	1490
8.890	0.1300	0.658	0.373	0.4360	0.904	1466
3.333	0.259	0.419	0.512	0.5003	0.880	1502
2.500	0.341	0.377	0.587	0.5218	0.903	1468
2.000	0.509	0.417	0.715	0.5405	0.893	1483
1.667	0.675	0.438	0.823	0.5418	0.897	1477
1.229	0.763	0.357	0.875	0.5379	0.904	1468
1.304	0.880	0.438	0.939	0.5297	0.884	1494

independently determined value 1625. The activity coefficient in hydrochloric acid solutions may be expressed by the equation:

$$pF = \frac{2.04\ \sqrt{I}}{1 + 1.645\ \sqrt{I}} - 0.252\ I \qquad (13)$$

The previous data on cupric sulphate solutions were recalculated using the improved method described above. The results in lithium sulphate solution were

$$pK = 2.149 - \frac{4.05\ \sqrt{I}}{1 + 1.536\ \sqrt{I}} + 0.0576\ I \qquad (14)$$

The results are otherwise the same as before, except for the value of pK_0 which is 0.05 higher. This obviously arises from the uncertainty in the determination of the derivative "d log ε/d log $[SO_4]$" required in the old method.

In Fig. 1 the activity coefficient of the first cupric chloride complex is represented as a function of the ionic strength in lithium perchlorate solution.

DISCUSSION

The thermodynamic dissociation constant of $CuCl^+$ is, according to a recent investigation by J. Bjerrum [3], greater than or approximately equal to unity. This is thus in agreement with the value $K_0 = 0.90$ obtained in the present paper. The activity coefficient has not been previously determined.

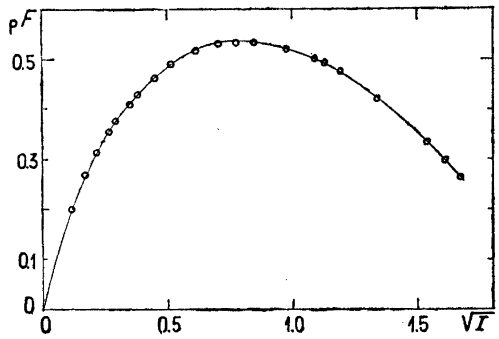

Fig. 1. Activity coefficient of the first cupric chloride complex in lithiumperchlorate solutions.

Some series of measurements were carried out at different wavelengths in the investigation of the effect of the higher complexes when hydrochloric acid is added. The measurements showed that the higher complexes do not interfere when the chloride ion concentration is smaller than 0.25. When the concentration of hydrochloric acid exceeds this value, the values of the activity coefficient obtained from the measurements, at different wavelengths, gradually begin to differ.

SUMMARY

Methods for the spectrophotometric determination of the thermodynamic complexity constants and the activity coefficient of complexes in systems of slight complexity are described.

The thermodynamic dissociation constant and the activity coefficient of the first cupric chloride complex are determined in hydrochloric acid and lithium perchlorate solutions. The Debye-Hückel equation expresses the results in lithium perchlorate solutions very accurately over wide ranges of ionic strength. In hydrochloric acid solutions the higher complexes begin to interfere when the concentration of hydrochloric acid is higher than 0.25.

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2. Näsänen, R. *Acta Chem. Scand.* 3 (1949) 1400.
3. *Cf.* Bjerrum, J. *Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd.* **22** (1946) no. 18, including a summary of the previous studies.

Received November 7, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 146 – 159

The Borides of Manganese

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This investigation is part of a study on binary alloys between transition elements and boron.

Only a few investigations of the manganese-boron system have been reported. Previous investigations [1-6] report the existence of borides of the composition MnB and MnB_2. MnB has been investigated by X-ray methods [7], but the results reported here are not consistent with these findings as will be discussed below.

GENERAL METHODS

The manganese, used as starting material, was purified by distillation in a high frequency vacuum induction furnace and had a purity of 99.6 %. The boron was prepared by reducing boron tribromide with hydrogen [8] and had a purity of 98—99 %. The alloys were prepared in evacuated silica tubes by sintering mixtures for 48—72 hours at 1 100—1 200 ° C. Single crystals of the intermediary phases were obtained by prolonged heating at this temperature usually for 7—10 days. Analyses of selected alloys were carried out according to the methods, given in[3].

The structure of the phases was determined by single crystal methods as described elsewhere [9]. The axes of the three orthorhombic phases were chosen so that the International tables could be used directly.

GENERAL SURVEY OF THE SYSTEM

Four intermediary phases, δ, ε, ζ, and η were observed. For preparations with boron content of more than about 70 atomic % some weak interferences were observed, which did not belong to the η -phase. It was not possible to decide whether these interferences belonged to a new phase or to impurities.

Single crystals were obtained from all the phases. They were metallic, hard and brittle and often had a reddish-brown colour.

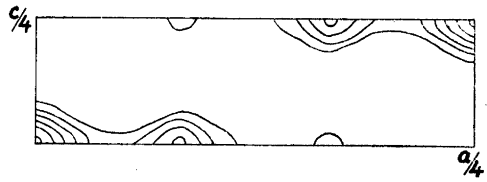

Fig. 1. δ-phase. Projection of the Patterson function on (010). ((400) and (004) are symmetry planes in the projection.)

The δ-phase probably had an extended homogeneity range with a boron content of about 20 atomic %. The ε-phase had the composition Mn_2B, the ζ-phase MnB and the η-phase Mn_3B_4.

The δ-phase

This phase appeared pure in photographs of material with a boron content of about 20 atomic %. Only very small single crystals were obtained. Laue photographs showed the Laue symmetry to be *mmm*. Rotation and Weissenberg photographs were taken around [010], using Mo-*K*-radiation. Accurate cell dimensions were obtained from powder photographs, giving the axes of the orthorhombic cell (for a boron content of 20 atomic %):

$$a = 14.53 \text{ Å}, \qquad b = 7.293 \text{ Å}, \qquad c = 4.209 \text{ Å}, \qquad V = 446.0 \text{ Å}^3.$$

The density found was 6.60, most closely corresponding to a cell content of 8 Mn_4B (calculated density 6.87).

Reflections $h\,k\,l$ were observed only for $h+k = 2n$ and $k+l = 2n$, $0\,k\,l$ only for $k = 2n$, $l = 2n$ and $k+l = 4n$, $h\,0\,l$ only for $h = 2n$, $l = 2n$ and $h+l = 4n$ and $h\,k\,0$ only for $h = 2n$, $k = 2n$ and $h+k = 4n$. The probable space group thus is *Fddd*.

Manganese positions. There are several possible ways of placing 32 metal atoms in *Fddd*. 16(*c*) + 16(*d*) may be excluded, because 642 and 624 are strong reflections. For the same reason a combination of (*c*) or (*d*) with (*e*), (*f*) or (*g*) is less probable. From space considerations (length of *c*-axis only 4.2 Å) (*g*) and the combination (*a*) + (*b*) are excluded. The projection of the Patterson function on the ac-plane *p*(*uw*) (Fig. 1) showed only one strong maximum on the *w*-axis, giving a vector with a length of *c*/2 in the [0 0 1] direction. If the metal atoms were situated in 32: (*h*), then only the value ¼ for the *z*-parameter would be possible. With this *z*-coordinate, however, only two vectors with a projected length less than *a*/2 in the [1 0 0] direction would exist, whereas the Patterson projection showed the existence of four such vectors. The remaining possible positions for the 32 atoms are thus

Table 1. δ-phase. Weissenberg photographs with Mo-K radiation. Comparison between observed and calculated $|F|^2$ values.

h k l	$\|F\|^2$ obs.	$\|F\|^2$ calc.	h k l	$\|F\|^2$ obs.	$\|F\|^2$ calc.	h k l	$\|F\|^2$ obs.	$\|F\|^2$ calc.
0 0 4	181	136	9 1 3	—	4	9 3 3	20	15
0 0 8	68	41	11 1 3	—	2	11 3 3	54	47
2 0 2	163	148	2 2 0	—	1	13 3 3	36	40
2 0 6	50	39	6 2 0	134	145	1 3 5	38	40
4 0 0	21	26	10 2 0	—	0	4 4 0	54	54
4 0 4	—	8	14 2 0	—	0	8 4 0	41	42
6 0 2	—	0	18 2 0	41	48	12 4 0	9	8
8 0 0	14	15	0 2 2	23	15	2 4 2	—	0
10 0 2	80	87	4 2 2	57	54	6 4 2	111	87
12 0 0	154	154	8 2 2	36	43	10 4 2	—	0
12 0 4	88	89	12 2 2	—	7	0 4 4	—	7
14 0 2	65	63	2 2 4	—	0	4 4 4	32	27
16 0 0	11	7	6 2 4	61	65	1 5 1	—	3
24 0 0	59	51	10 2 4	—	0	3 5 1	—	6
1 1 1	9	7	1 3 1	109	115	5 5 1	35	38
3 1 1	7	11	3 3 1	30	31	7 5 1	35	35
5 1 1	54	65	5 3 1	—	6	9 5 1	—	4
7 1 1	45	55	7 3 1	—	5	11 5 1	—	2
9 1 1	9	6	9 3 1	20	12	13 5 1	—	2
11 1 1	—	3	11 3 1	38	62	1 5 3	—	2
13 1 1	—	2	13 3 1	46	53	3 5 3	—	4
1 1 3	—	3	1 3 3	66	68	5 5 3	30	28
3 1 3	—	6	3 3 3	27	19	7 5 3	30	26
5 1 3	36	38	5 3 3	—	3	9 5 3	—	3
7 1 3	39	35	7 3 3	—	3	11 5 3	—	2

16(*e*) and 16(*f*). The possibility that the 32 atoms occupy two 16:(*f*) positions may further be excluded as vectors appear in $p(uw)$ with u-components different from $\frac{1}{2}$ and $\frac{1}{4}$. The Patterson function along the line $u\ 0\ 0$ showed maxima at $u = 0$, $u = 0.165$ and $u = 0.500$. Only one maximum thus appears in the interval $0 < u < \frac{1}{2}$ and for this maximum $u \neq \frac{1}{4}$. The possibility of placing the 32 atoms in two 16:(*e*) positions may thus be excluded and the remaining possibility is 16:(*e*) + 16:(*f*) for the manganese atoms.

(000; $0\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}\ 0\ \frac{1}{2}$; $\frac{1}{2}\ \frac{1}{2}\ 0$) +
16:(*e*) $x00$; $\bar{x}00$; $\frac{1}{4} + x\ \frac{1}{4}\ \frac{1}{4}$; $\frac{1}{4} - x\ \frac{1}{4}\ \frac{1}{4}$
16:(*f*) $0y0$, $0\bar{y}0$; $\frac{1}{4}\ \frac{1}{4} + y\ \frac{1}{4}$; $\frac{1}{4}\ \frac{1}{4} - y\ \frac{1}{4}$

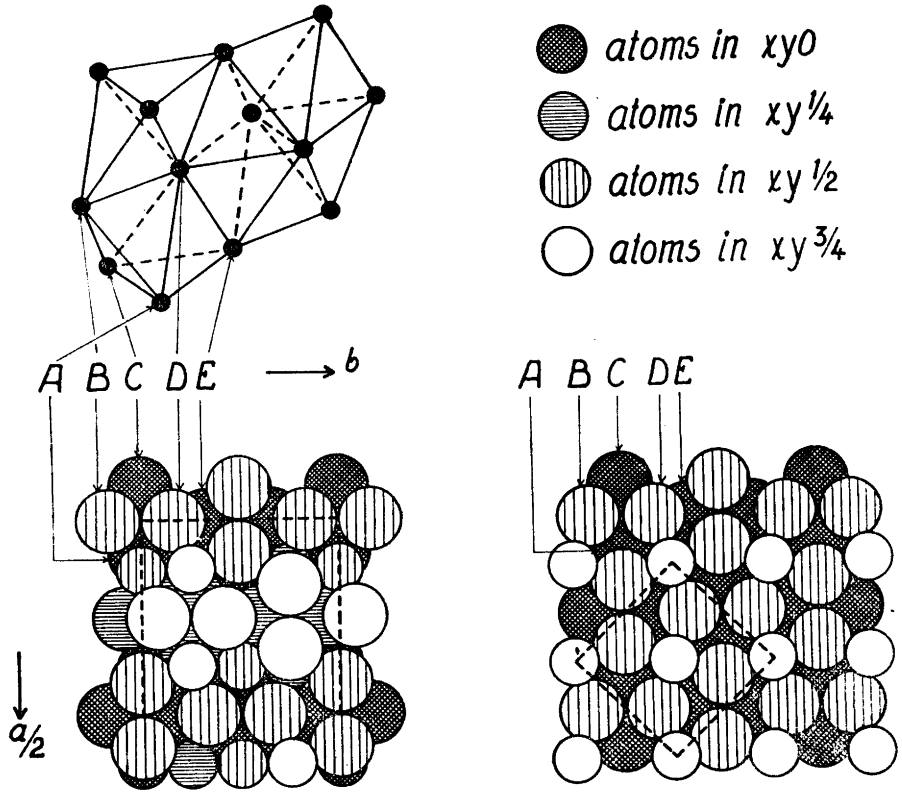

Fig. 2. δ- and ε-phases. Projections of the structures of the ideal δ-phase (left) and the ε-phase (right). The unit cells are indicated. Large circles represent manganese atoms, small circles boron atoms. The packing of the tetrahedra of manganese atoms in a sheet is shown separately.

The Patterson function along the line $0v0$ showed a maximum at $v = 0.35$ (of bad quality). Considering the maximum for $u = 0.165$ in P(u00) a probable pair of parameters thus is $x = 0.165/2$, $y = (1 - 0.35)/2$ (the pair $x = 0.165/2$, $y = 0.35/2$ must be excluded from space considerations). A more accurate determination of y was made from intensity calculations, giving $y = 0.333 \pm 0.004$. A comparison between observed and calculated $|F|^2$ values using these parameters is given in Table 1 (the influence of boron is neglected). The agreement is good. The maximum in P(u00) for $u = 0.500$ may be explained if the vectors with the component $a = \frac{1}{2}$, which are nearly parallel to the a-axis, are considered. The metal lattice may be regarded as built of parallel sheets of tetrahedra of mangenese atoms (Fig. 2). In the

tetrahedron ABCD, the edges AC and BD have a length of 2.41 and 2.44 Å resp. whereas the edges AB, BC, CD, and AD all have the length 2.71 Å. In the tetrahedron ACED, the two shorter edges AC and ED have a length of 2.41 and 2.43 Å resp. and the four longer edges each 2.71 Å. The two tetrahedra are thus nearly equal. Each metal atom in 16:(*f*) is surrounded by two neighbours at 2.43 Å one at 2.44 Å and six at 2.71 Å, all in the same tetrahedron sheet and two at 2.71 Å, one from each neighbour sheet. Each metal atom in 16:(*e*) is surrounded by one neighbour at 2.41 Å and six at 2.71 Å, all in the same tetrahedron sheet, and two at 2.43 Å and two at 2.71 Å, all in the same neighbouring sheet. The structure may be compared with the Me_2B-borides of the $CuAl_2$-type (*C* 16), to which the ε-phase Mn_2B also belongs. The metal lattice of these borides also consists of sheets of tetrahedra, as pointed out by Hägg [10]. The sheets are arranged in a different manner, forming two sheet systems at right angles. For Mn_2B each manganese atom is surrounded by one neighbour at 2.37 Å, two at 2.46 Å, four at 2.69 Å and four at 2.73 Å. The distances from a manganese atom to its metal neighbours thus are nearly the same for both lattice types.

Boron positions. The largest holes in the lattice are in the position 16:(*e*) with a value of the parameter $x = 0.375$. They are thus situated between adjacent sheets of manganese tetrahedra, forming strings in the [011] direction. An atom, placed in such a hole, would be surrounded by eight metal atoms, all at a distance of 2.19 Å from its centre. Additional holes of the lattice are situated in the tetrahedra of metal atoms. An atom, placed in such a hole, would be surrounded by four manganese atoms at a distance of 1.61 Å. The first type of holes thus has space for atoms with a radius of up to about 0.98 Å, compared with 0.40 Å for atoms in the last type of holes. The first possibility is thus the only possible for boron with its radius of about 0.87 Å. The metal lattice has space for 16 boron atoms per cell in these holes, giving an upper limit of 33.3 atomic % boron for the δ-phase. The reflections 840 and 931, which appear at nearly the same angle in the powder photographs, appeared at slightly varying positions in preparations with different boron contents. This indicates an extended homogeneity range. It was not possible to determine the limits, however, because of the small variations. The δ-phase appeared pure in preparations with a boron content of about 20 atomic %. About 50 % of the holes of the manganese lattice are thus occupied by boron atoms, and the number may possibly vary. The distance boron-boron, if two adjacent holes are occupied, is 2.11 Å.

The δ-phase, with a boron content of about 20 atomic %, thus crystallizes in ***Fddd***. The orthorhombic unit cell contains 32 manganese atoms, occupying the positions 16:(*e*) and 16:(*f*). The parameters are $x = 0.083$ and $y = 0.333$.

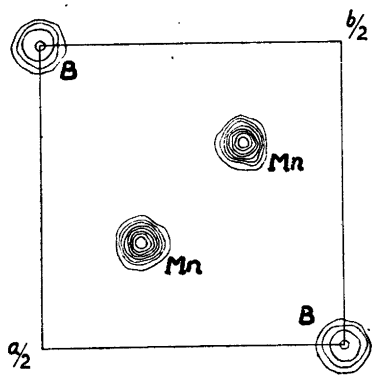

Fig. 3. ε-phase. Projection of the electron density on (001).

The boron atoms are placed in 16:(*e*) with $x = 0.375$. About 50 % of the boron sites are occupied by boron atoms.

The ε-phase

This phase was found homogeneous in preparations with a content of 33.3 atomic% boron. The hard and brittle crystals were often plate-like with a reddish-brown lustre. No displacements of the interferences were observed in the range between the δ- and ζ-phases, indicating a definite composition Me_2B. Laue photographs showed the Laue symmetry to be $4/mmm$. Rotation and Weissenberg photographs were taken around [001] using Mo-K radiation. Accurate cell dimensions were obtained from powder photographs, giving the axes of the tetragonal cell:

$$a = 5.148 \text{ Å}, \; c = 4.208 \text{ Å}, \; c/a = 0.817, \; V = 111.5 \text{ Å}^3.$$

The value of the length of the axes indicated an isomorphism between this boride and the borides of $CuAl_2$-type (C 16)[10–13], which was verified by further structure analysis. With a cell content of 4 Me_2B the calculated density is 7.19, in good agreement with the observed value 7.20. Reflections $h\,k\,l$ were observed only for $h + k + l = 2n$, $h\,k\,0$ for $h + k = 2n$, $h\,h\,l$ for $l = 2n$ and $0\,k\,l$ for $k = 2n$ and $l = 2n$, showing that space group $I4/mcm$ is possible. The metal atoms of the Me_2B-borides mentioned are situated in 8:(h) in $I4/mcm$. The section P($uw0$) of the Patterson space was investigated in order to determine the parameter x of 8:(h). The value $x = 0.163$ was obtained. This value is slightly less than the value $x = 0.167$ reported for Fe_2B[10]. The axial ratio, however, is slightly different. Each manganese atom is surrounded

Table 2. ε-phase. Weissenberg photographs with Mo-K radiation. Comparison between observed and calculated $|F|$-values.

$h\ k\ l$	$\|F\|$ obs.	$\|F\|$ calc.	$h\ k\ l$	$\|F\|$ obs.	$\|F\|$ calc.	$h\ k\ l$	$\|F\|$ obs.	$\|F\|$ calc.
2 0 0	45	55	9 1 4	31	28	6 3 3	—	1
2 0 2	51	63	10 1 1	24	29	7 3 0	44	47
2 0 4	34	33	10 1 3	21	28	7 3 2	35	35
4 0 0	44	54	2 2 0	32	33	7 3 4	37	40
4 0 2	57	62	2 2 2	14	14	8 3 1	—	1
4 0 4	39	55	2 2 4	20	21	8 3 3	—	1
6 0 0	65	85	3 2 1	—	8	9 3 0	44	46
6 0 2	54	69	3 2 3	—	7	9 3 2	51	53
6 0 4	71	68	4 2 0	36	32	9 3 4	44	41
8 0 0	—	14	4 2 2	20	18	10 3 1	—	3
8 0 2	—	23	4 2 4	21	25	10 3 3	—	3
10 0 0	29	31	5 2 1	64	69	11 3 0	—	14
10 0 2	40	39	5 2 3	49	61	11 3 2	—	6
10 0 4	20	29	5 2 5	41	49	12 3 1	—	0
12 0 0	37	43	6 2 0	29	29	12 3 3	—	0
12 0 2	35	34	6 2 2	32	38	4 4 0	41	35
12 0 4	37	41	6 2 4	24	23	4 4 2	23	22
1 1 0	35	33	7 2 1	45	45	4 4 4	24	28
1 1 2	38	43	7 2 3	38	41	5 4 1	51	56
1 1 4	16	19	7 2 5	33	35	5 4 3	49	50
2 1 1	73	98	8 2 0	18	13	5 4 5	38	42
2 1 3	65	76	8 2 2	—	3	6 4 0	36	35
2 1 5	59	57	9 2 1	—	11	6 4 2	37	43
3 1 0	70	69	9 2 3	—	10	6 4 4	29	29
3 1 2	42	48	10 2 0	—	20	7 4 1	42	37
3 1 4	41	48	10 2 2	—	11	7 4 3	35	34
4 1 1	60	68	11 2 1	44	38	7 4 5	31	30
4 1 3	51	59	11 2 3	40	36	8 4 0	—	14
4 1 5	43	46	11 2 5	29	34	8 4 2	—	6
5 1 0	—	11	12 2 0	—	14	9 4 1	—	9
5 1 2	22	22	12 2 2	—	21	9 4 3	—	9
6 1 1	—	10	3 3 0	75	94	10 4 0	19	22
6 1 3	—	9	3 3 2	79	97	10 4 2	—	15
7 1 0	—	18	3 3 4	50	70	11 4 1	35	33
7 1 2	26	27	4 3 1	—	6	11 4 3	26	32
8 1 1	47	49	4 3 3	—	5	11 4 5	18	30
8 1 3	46	46	5 3 0	40	36	12 4 0	21	18
8 1 5	34	40	5 3 2	23	23	12 4 2	24	25
9 1 0	26	32	5 3 4	24	28	12 4 4	13	17
9 1 2	26	22	6 3 1	—	1	5 5 0	—	9

by three neighbours at 2.37, 2.46 and 2.46 Å, four at 2.69 and four at 2.73 Å. To verify the position of the boron atoms, obtained from space considerations (4:(*a*) in *I*4/*mcm*), the projection of the electron density on (001) was calculated (Fig. 3). (The signs of the observed $|F|$-values could be calculated since the position of the heavy manganese atoms was determined from the Patterson section.) The boron atoms may thus be situated in 4:(*a*) or 4:(*c*). In 4:(*a*) the distance boron-manganese would be 2.20 Å, in 4:(*c*) 1.93 Å. The first possibility is thus the more probable one, giving a radius of the boron atom of 1.02 Å. In Table 2 a comparison between observed and calculated $|F|$-values for several reflections is given. The lattice (Fig. 2) is similar to that of the δ-phase and is discussed above.

The ε-phase, Mn_2B, thus is of the $CuAl_2$-type (*C* 16). The value of the parameter for the metal atoms is 0.163.

The ζ-phase

This phase crystallized homogeneously in preparations with a boron content of 50 atomic%. The interferences always appeared at fixed angles for preparations with boron content between the ε- and the η-phases. The homogeneity range thus is narrow. Rod-shaped single crystals with a reddish-brown lustre were obtained. Laue photographs showed the Laue symmetry to be *mmm*. Rotation and Weissenberg photographs were taken around [010] using Mo-*K* radiation. Accurate cell dimensions were obtained from powder photographs, giving the axes of the orthorhombic cell:

$$a = 5.560 \text{ Å}, \; b = 2.977 \text{ Å}, \; c = 4.145 \text{ Å}, \; V = 68.61 \text{ Å}^3.$$

The values of the length of the axes indicated isomorphism between this boride and the borides of FeB-type (*B* 27)[11], which was verified by further structure analysis.

With a cell content of 4 MnB the calculated density (6.37) most closely corresponds to the measured density (6.45).

Reflections 0 *k l* were observed only for $k + l = 2n$ and *h k* 0 only for $h = 2n$ showing that space group *Pnma* is possible. The further structure analysis showed the ζ-phase to be isomorphous with FeB and with the metal atoms in 4(*c*) of *Pnma*. Bjurström's values of the parameters, $x = 0.180$ and $z = 0.125$ were in accordance with the results from a Patterson function projection on (010). The only holes, large enorgh for boron atoms, have their centres in 4:(*c*) with parameters $x = 0.031$ and $z = 0.614$ (compared with

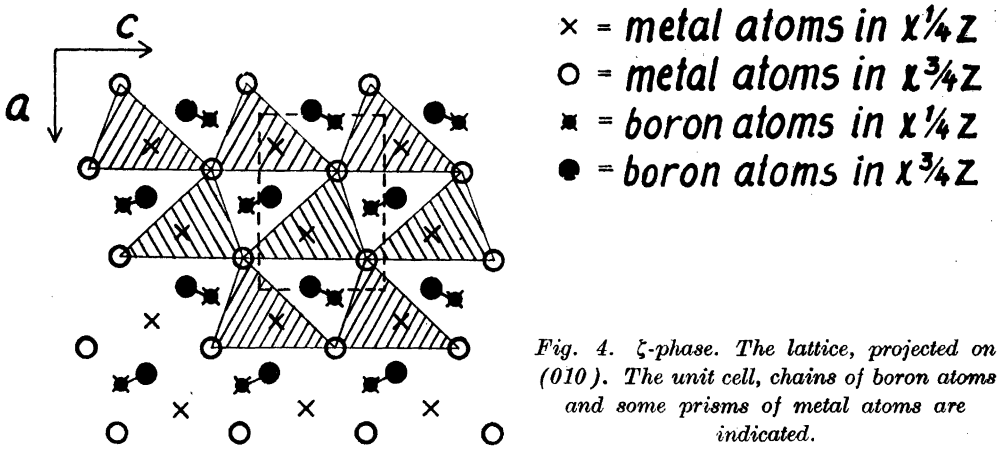

Fig. 4. ζ-phase. The lattice, projected on (010). The unit cell, chains of boron atoms and some prisms of metal atoms are indicated.

$x = 0.036$ and $z = 0.61$ for FeB). In Table 3 a comparison between observed and calculated $|F|$-values for several reflections is given. In Fig. 4 the lattice is projected on (010). The lattice may be regarded as consisting of trigonal prisms of iron atoms with boron chains in the channels between them. Each metal atom is surrounded by four metal neighbours at 2.67 Å, two at 2.70 Å, two at 2.97 Å and two at 2.98 Å. Each boron atom is surrounded by six metal atoms at about 2.19 Å and the boron-boron distance is 1.80 Å. In Fig. 4 the prisms are indicated in order to point out the similarity between this phase and the two types represented by CrB[9] and the δ-phase in the molybdenum-boron system[12]. Both these types may be regarded as consisting of trigonal metal prisms with boron chains in the channels. For CrB all chains are parallel, for the molybdenum boride they extend in two directions. The similarity between MnB and Cr_3C_2[14] may also be noted.

According to previous investigations[7], MnB is orthorhombic with axes 2.95 kX, 11.5 kX and 4.10 kX. As will be observed two of these reported axes are nearly the same as for the ζ-phase above, whereas the third is doubled. No signs of this doubling was observed in this investigation, however. The structure suggested is not at all in accordance with the results obtained for the ζ-phase or any other intermediary phase observed in this investigation. The shortest distance manganese-manganese reported, 2.3 Å, seems too small. The parameters for boron are only roughly indicated and it is not possible to discuss the proposed structure from the data given.

The ζ-phase, MnB, thus is of the FeB-type (*B* 27).

Table 3. ζ-phase, Weissenberg photographs with Mo-K radiation. Comparison between observed and calculated |F|-values.

h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.
2 0 0	29	37	6 1 0	23	25	2 2 0	25	28
4 0 0	—	5	8 1 0	19	17	4 2 0	—	4
6 0 0	35	38	10 1 0	22	22	6 2 0	33	33
8 0 0	32	32	0 1 1	35	42	8 2 0	25	30
10 0 0	—	7	1 1 1	40	43	1 2 1	11	11
0 0 4	54	51	2 1 1	29	33	2 2 1	28	26
0 0 8	30	28	3 1 1	11	10	3 2 1	33	37
1 0 1	17	15	4 1 1	12	9	4 2 1	27	33
2 0 1	34	34	5 1 1	23	23	5 2 1	19	22
3 0 1	47	46	6 1 1	25	24	1 2 2	52	46
4 0 1	39	40	7 1 1	20	19	3 2 2	—	8
5 0 1	24	25	1 1 2	31	32	5 2 2	15	17
1 0 2	60	60	3 1 2	38	44	7 2 2	28	36
3 0 2	—	10	5 1 2	29	37	1 2 3	—	11
5 0 2	22	20	7 1 2	—	3	2 2 3	24	22
7 0 2	39	40	9 1 2	24	22	3 2 3	30	30
1 0 3	17	13	0 1 3	27	30	4 2 3	32	30
2 0 3	28	25	1 1 3	28	32	0 2 4	52	45
3 0 3	30	35	2 1 3	28	27	2 2 4	15	20
4 0 3	33	34	3 1 3	—	10	4 2 4	—	3
5 0 3	22	22	4 1 3	—	8	6 2 4	26	27
6 0 3	—	9	5 1 3	19	18	1 2 5	—	6
7 0 3	—	2	6 1 3	20	21	2 2 5	—	18
8 0 3	—	3	7 1 3	—	20	3 2 5	24	26
9 0 3	—	13	8 1 3	21	19	4 2 5	19	23
10 0 3	22	20	2 1 4	34	35	1 2 6	24	28
2 0 4	23	23	4 1 4	32	33	1 2 7	—	7
4 0 4	—	3	0 1 5	27	25	0 2 8	29	27
6 0 4	32	29	1 1 5	27	23	2 3 0	35	35
8 0 4	30	27	2 1 5	—	18	4 3 0	36	33
1 0 5	—	7	3 1 5	—	9	6 3 0	22	21
2 0 5	17	20	4 1 5	—	4	8 3 0	19	14
3 0 5	30	28	5 1 5	22	17	0 3 1	28	27
4 0 5	23	25	1 1 6	—	17	1 3 1	31	27
1 0 6	32	31	3 1 6	31	26	2 3 1	25	22
3 0 6	—	6	5 1 6	22	24	3 3 1	—	10
5 0 6	—	13	0 1 7	—	16	4 3 1	—	7
7 0 6	27	29	1 1 7	—	18	5 3 1	21	17
2 1 0	41	51	2 1 7	—	16	6 3 1	20	19
4 1 0	38	44	2 1 8	17	20	7 3 1	19	17

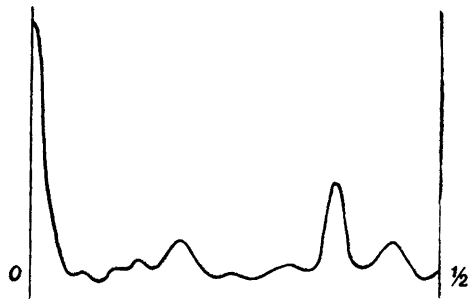

Fig. 5. η-phase. The Patterson function along the line 0v0.

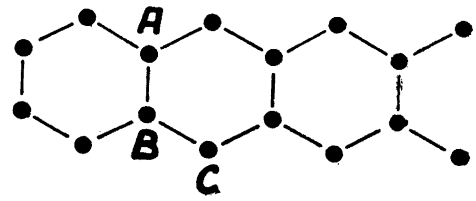

Fig. 6. η-phase. Double chain of boron atoms.

The η-phase

A fourth intermediary phase was obtained in a pure state with a content of 57 atomic% boron. It probably had the definite composition Mn_3B_4. Well defined single crystals were obtained, needle- or plate-shaped with a reddish-brown, metallic colour. Laue photographs showed the Laue symmetry to be *mmm*. Rotation- and Weissenberg photographs were taken around [001] and [100], using Mo-K radiation. Accurate cell dimensions were obtained from powder photographs, giving the axes of the orthorhombic cell:

$$a = 3.032 \text{ Å}, \; b = 12.86 \text{ Å}, \; c = 2.960 \text{ Å}, \; V = 115.4 \text{ Å}^3.$$

The density found was 5.90, in close agreement with the density calculated for 2 Mn_3B_4(5.99). Reflections $h\ k\ l$ only appeared for $h + k + l = 2n$, $0\ k\ l$ for $k + l = 2n$, $h\ 0\ l$ for $h + l = 2n$ and $h\ k\ 0$ for $h + k = 2n$. Probable space groups are thus *Immm*, *I*222, $I2_12_12_1$, and I*mm*. The length of the axes and the space group indicated isomorphism between this phase and the δ-phase in the tantalum-boron system [13]. This was verified by further structure analysis. The Patterson function was investigated along the line 0v0 (Fig. 5). In addition to the maximum at $v = 0$, three major peaks appeared, one strong at $v = 0.372$, due to a manganese-manganese vector and two at $v = 0.183$ and 0.442 resp., each due to two manganese-boron vectors (compare the δ-phase of the tantalum-boron system). The values of the parameters thus are $y = 0.186$ for manganese in 4:(g), $y = 0.369$ for boron in 4:(g) and $y = 0.442$ for boron in 4:(h). The corresponding values for the tantalum boride were $y = 0.180$ for tantalum and $y = 0.375$ and 0.445 for boron, the value for tantalum determined from powder line intensities and values for boron from space considerations. From space considerations the

Table 4. η-phase, Weissenberg photographs with Mo-K radiation. Comparison between observed and calculated |F| values.

h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.
2 0 0	95	100	1 5 0	85	97	2 9 3	35	31
4 0 0	54	63	3 5 0	65	65	0 10 0	49	53
6 0 0	47	46	0 5 1	24	33	2 10 0	41	45
1 0 1	53	50	2 5 1	18	26	4 10 0	38	32
3 0 1	29	33	1 5 2	62	75	1 10 1	—	13
0 0 2	105	98	3 5 2	60	58	0 10 2	41	44
2 0 2	72	81	5 5 2	46	41	2 10 2	34	39
4 0 2	56	60	0 5 3	—	22	4 10 2	28	29
6 0 2	47	43	0 6 0	70	77	1 11 0	59	77
1 0 3	29	33	2 6 0	61	60	3 11 0	54	60
0 0 4	70	64	4 6 0	40	40	5 11 0	42	44
0 0 6	50	44	1 6 1	25	18	0 11 1	24	31
1 1 0	59	51	0 6 2	50	59	2 11 1	16	27
3 1 0	31	29	2 6 2	49	51	1 11 2	54	68
1 1 2	32	36	4 6 2	41	36	3 11 2	56	54
3 1 2	32	27	1 6 3	—	12	5 11 2	30	42
0 1 3	—	14	1 7 0	—	10	0 11 3	20	24
1 2 1	76	70	0 7 1	47	48	0 12 0	—	19
3 2 1	48	47	2 7 1	43	39	1 12 1	—	24
5 2 1	33	30	4 7 1	30	27	0 12 2	—	16
0 2 2	—	5	1 7 2	—	7	1 13 0	—	11
1 2 3	59	57	0 7 3	36	33	0 13 1	49	54
3 2 3	51	44	2 7 3	32	29	2 13 1	46	48
1 3 0	26	28	4 7 3	28	23	4 13 1	34	35
3 3 0	—	18	0 8 0	26	28	1 13 2	—	10
0 3 1	71	96	2 8 0	29	23	0 13 3	51	42
2 3 1	61	73	1 8 1	56	78	2 13 3	36	38
4 3 1	42	48	3 8 1	38	57	0 14 0	—	11
6 3 1	23	33	5 8 1	32	40	1 14 1	45	49
1 3 2	19	21	0 8 2	25	23	3 14 1	31	37
3 3 2	14	16	2 8 2	19	20	5 14 1	23	28
0 3 3	57	61	1 8 3	51	57	0 14 2	—	10
2 3 3	49	54	3 8 3	41	47	1 14 3	39	39
4 3 3	38	40	5 8 3	30	35	3 14 3	29	34
0 4 0	22	29	1 9 0	—	4	0 15 0	—	16
2 4 0	22	19	0 9 1	47	48	1 15 1	—	22
1 4 1	37	39	2 9 1	41	40	0 16 0	51	66
0 4 2	17	19	4 9 1	31	29	2 16 0	43	58
2 4 2	17	16	1 9 2	—	3	4 16 0	42	47
1 4 3	25	27	0 9 3	41	35	1 16 1	—	25

values of the parameters of boron in Mn_3B_4 would be 0.371 and 0.443 in close agreement with the values obtained from P(0v0). In Table 4 a comparison between several observed and calculated $|F|$-values are given, using the parameters obtained for manganese from P(0v0) and for boron from space considerations. The agreement is good. The manganese atoms in 4:(g) are surrounded by four neighbours at 2.68 Å, two at 2.81, two at 2.96 and two at 3.03 Å. The atoms in 2:(c) are surrounded by four neighbours at 2.81 Å, two at 2.96 Å and two at 3.03 Å.

The boron atoms are connected to double chains, running through the metal lattice, with a distance boron-boron of 1.75 Å in the same half and 1.47 Å between different halves of a double chain.

If the value 0.87 Å, obtained from earlier investigations, is used as an approximate value for the radius of the boron atom, the distance 1.47 Å is less than $2r_B$ (1.74 Å). It thus seems as if adjacent boron atoms from different halves of a double chain (A and B in Fig. 6) have a tendency to form boron pairs with strong boron-boron bonds. For Ta_3B_4, the same tendency was observed (distances AB = 1.54 Å, BC = 1.85 Å). The metal atoms of Ta_3B_4, however, have a greater radius, making possible a more symmetrical arrangement of the boron atoms with distances AB = BC = $2r_B$.

The η-phase, Mn_3B_4, thus crystallizes in *Immm*, isomorphous with Ta_3B_4. The parameters are $y = 0.186$ for manganese and $y = 0.371$ and 0.443 for boron

GENERAL DISCUSSION

The system manganese-boron belongs to the group, discussed by Hägg [15, 16]. The value of the ratio r_B/r_{Me} is > 0.59 and the structures of the intermediary phases do not belong to the simple types. They are, however, all metallic. Especially for the phases with isolated boron atoms the structures are complicated. The system is similar to the other binary systems between transition metals and boron. The general tendency for the boron atoms to form chains or nets through the metal lattice is also found for this system. The δ- and ε-phases have isolated boron atoms, but with a tendency to form strings. The ζ-phase has boron chains and the η-phase double chains.

SUMMARY

The binary system manganese-boron has been investigated by X-ray methods. Four intermediary phases all with metallic properties have been found to exist and their structures have been determined. The δ-phase with a boron content of about 20 atomic% and probably with an extended homo-

geneity range, and the ε-phase Mn_2B with $CuAl_2$-structure (C 16), are closely related.

The ζ-phase, MnB, is of the FeB type (B 27) and the η-phase, Mn_3B_4, isomorphous to Ta_3B_4 [13].

As in the other binary systems between transition metals and boron there is a tendency for the boron atoms to form chains or nets.

The author wishes to thank Professor G. Hägg for his continued interest in this investigation and Mr. Lars-Henrik Andersson for valuable assistance with preparations and calculations. He is further indebted to the *Royal Swedish Academy of Science* for a grant from the Edlund fund and to the *Swedish State Council of Technical Research* for financial support.

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Received November 21, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 160–164

Investigations on the Binary Systems of Boron with Chromium, Columbium, Nickel, and Thorium, Including a Discussion of the Phase "TiB" in the Titanium-Boron System

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Preliminary investigations on the binary systems columbium-, nickel-, and thorium-boron have been carried out at this institute. The results, though incomplete, may be of interest in connection with other investigations on this subject, published by one of the authors [1–5]. These papers include an initial report on the chromium-boron system, a sequel to which is given here. The system titanium-boron has been investigated by Ehrlich [6], who proposed the existence of a phase "TiB" with zincblende structure (*B* 3). However, a critical study, given below, shows that the structure probably is that of a face-centred titanium lattice with boron in the octahedral interstices.

The alloys were prepared and investigated as reported elsewhere [1,3]. No analyses of the alloys were carried out; the composition of the intermediary phases was determined from the amount of boron in the starting mixtures.

THE CHROMIUM-BORON SYSTEM

A report on the phase analysis and structures of two (ζ and Θ) of the five intermediary phases has been published. Some additional data on the δ- and η-phases have since been obtained.

The δ-phase. This phase, with a boron content of about 33 atomic%, is orthorhombic or very nearly orthorhombic with axes:

$$a = 14.7 \text{ Å}, \; b = 7.34 \text{ Å}, \; c = 4.29 \text{ Å}, \; V = 463 \text{ Å}^3.$$

The intensities of a few reflections on the single crystal photographs were not consistent with Laue symmetry *mmm* but with *2/m*. The possibility of a monoclinic cell with β equal or nearly equal to 90° thus can not be excluded. The lengths of the axes are about the same as for the corresponding axes of the δ-phase in the manganese-boron system. The phases are not isomorphous, however. If the chromium boride is orthorhombic the probable space groups are *Abmm* or *Abm*, whereas the manganese boride belonged to *Fddd*.

The η-phase. A complete structure determination of this phase showed it to be isomorphous with Ta_3B_4 and Mn_3B_4, thus belonging to space group *Immm*. The axes of the orthorhombic unit cell were found to be:

$$a = 2.984 \text{ Å}, \; b = 13.02 \text{ Å}, \; c = 2.953 \text{ Å}, \; V = 114.7 \text{ Å}^3.$$

The chromium atoms are situated in 4:(g) + 2:(c) and the boron atoms in 4:(g) + 4:(h). A satisfactory agreement between observed and calculated $|F|$-values was obtained with parameter values of $y_{Cr} = 0.186$, the same value as for Mn_3B_4, and $y_B = 0.372$ (4:(g)) and 0.442 (4:(h)). The boron atoms thus form double chains with a tendency of boron pair formation (distance boron-boron in the same half of a double chain 1.74 Å, between nearest boron atoms in different halves 1.51 Å).

THE COLUMBIUM-BORON SYSTEM

The phase analysis showed the existence of probably six intermediary phases. At room temperature the phases β, γ (CbB), δ (Cb_3B_4), and ε (extended homogeneity range, ideal composition CbB_2) are stable. Two more phases, β' and β'', probably exist, both stable only at higher temperatures.

The β,β', and β''-phases. Complicated powder photographs were obtained from preparations with compositions between the α-(pure columbium) and the γ-phases. At about 10 atomic% boron, probably two phases exist, one (β) stable at room temperature and the other (β') at higher temperatures. It was impossible to obtain β or β' pure, but a comparison between specimens quenched at 1200° C and specimens prepared at 800° C indicated that β' had a non-centred cubic metal lattice with $a = 4.210$ Å. All reflections corresponding to this parameter were observed. It was impossible to decide, however, whether all these reflections belonged to β' or some to β and some to β'. In the range 25—35 atomic% boron a third system of interferences sometimes appeared in quenched specimens. This indicates the existence of a third phase β'', stable only at higher temperatures.

The γ-phase. This phase, with composition CbB, was found to be isomorphous with CrB and TaB. It is thus orthorhombic, belonging to space group *Cmcm*. The axes were found to be:

$$a = 3.298 \text{ Å}, \; b = 8.724 \text{ Å}, \; c = 3.166 \text{ Å}, \; V = 91.09 \text{ Å}^3.$$

Both columbium and boron are in 4:(*c*) and a satisfactory agreement between observed and calculated $p|F|^2$-values was obtained for $y_{Cb} = 0.146$, the same as for CrB and TaB. From space considerations y_B was found to be 0.444.

The δ-phase. This phase was found to be isomorphous with Ta_3B_4, Cr_3B_4 and Mn_3B_4, thus belonging to space group *Immm*. The axes of the orthorhombic unit cell are:

$$a = 3.305 \text{ Å}, \; b = 14.08 \text{ Å}, \; c = 3.137 \text{ Å}, \; V = 146.0 \text{ Å}^3.$$

Satisfactory agreement between observed and calculated $p|F|^2$-values was obtained with the metal atoms in 4:(*g*) + 2:(*c*) and the parameter $y_{Cb} = 0.180$, the same value as for Ta_3B_4. From space considerations the parameters for boron were found to be 0.376 (4:(*g*)) and 0.444 (4(*h*)).

The ε-phase. This phase was found to be of the AlB_2-type (*C* 32) with axes of the hexagonal cell (at the composition CbB_2):

$$a = 3.089 \text{ Å}, \; c = 3.303 \text{ Å}, \; c/a = 1.07.$$

An extended homogeneity range for this phase was noted, extending on both sides of the ideal composition CbB_2.

THE NICKEL-BORON SYSTEM

According to Giebelhausen [7], phases Ni_2B, Ni_3B_2, NiB, and Ni_2B_3(?) exist. Bjurström [8] has determined the structure of Ni_2B being of $CuAl_2$-type (*C* 16).

A brief phase analysis showed the existence of at least four intermediary phases, γ existing pure in preparations with 25—30 atomic% boron, δ(Ni_2B), ε at about 40 atomic% boron and ζ at about 50 atomic% boron. Intensity variations were also noted for preparations with a higher boron content than 60 atomic%, but it was not possible to decide whether they were caused by a new phase. It was not possible to interpret the powder photographs of the γ, ε or ζ-phases. Especially noteworthy is the existence of the intermediary phase γ with a boron content lower than that of Ni_2B. This phase has not been reported by previous investigators.

THE THORIUM-BORON SYSTEM

Solubility of boron in the face centred thorium lattice was observed. In addition to the phase ThB_6, previously known, a phase at about 50 atomic% boron was found, giving complicated powder photographs. It was not possible to interpret them, and attempts to prepare single crystals were not successful.

THE TITANIUM-BORON SYSTEM

According to Ehrlich [6] three intermediary phases exist, and solubility of boron in the α-titanium lattice is also noted. A superlattice closely related to the α-titanium lattice was found in the range $TiB_{0.1}$-$TiB_{0.8}$. Two other phases "TiB" and TiB_2 exist, TiB_2 being of the AlB_2-type (*C* 32). "TiB" is reported to have zincblende structure (*B* 3). In the authors' opinion, however, the boron atoms are situated in other positions than in the tetrahedral interstices of the titanium lattice, corresponding to the sulfur positions of ZnS.

If "TiB" has *B* 3 structure, the titanium atoms occupy the positions of the unit cubic cell ($a = 4.202$ Å) with coordinates $000, 0\frac{1}{2}\frac{1}{2}, \frac{1}{2}0\frac{1}{2}$, and $\frac{1}{2}\frac{1}{2}0$. These are the positions of a face-centred lattice. The distance between adjacent titanium atoms is 2.97 Å. If the atoms are assumed to be spherical and in contact, this gives a value of 1.49 Å for the radius of the titanium atom in close agreement with values given before. In this face-centred titanium lattice there are two types of holes, respectively tetrahedrally and octahedrally surrounded by titanium atoms. The tetrahedral interstices which would be occupied in a structure of the zincblende type have room for atoms with $r < 0.34$ Å, the octahedral for atoms with $r < 0.62$ Å. Boron, with its radius of 0.87 Å is too great for both types of interstices. It is most probable, however, that it occupies the largest (octahedral) interstices. As the main contribution to the intensities is given by titanium, it is not possible to decide between these two alternatives from the rough intensity data given, but a careful intensity study would perhaps permit a choice. Ehrlich's attempts to obtain "TiB" in a pure state were not successful, interferences from "TiB", α-titanium and TiB_2 always being obtained together. Ehrlich's explanations of this fact, too low a reaction temperature or a supposed transition TiB (zincblende) — TiB_2 (with incomplete networks) seem unlikely. The present authors think that the most probable explanation would be that "TiB", in which even the octahedral interstices are too small for boron atoms at room temperature, will be stable only at higher temperatures. On cooling, part of "TiB" is decomposed into the stable phases α-titanium and TiB_2 whereas another part is retained at room temperature in a metastable condition.

The bonds within "TiB" thus probably are of the same nature as in the other borides of the transition elements, and the titanium-boron system with its radius ratio r_B/r_{Ti} near 0.59 has an intermediate position between simple and complicated systems. This will be discussed in greater detail in a paper, soon appearing in this journal.

SUMMARY

Investigations on the binary systems chromium-, columbium-, nickel-, and thorium-boron are reported. The titanium-boron system is discussed, and it is shown that the phase "TiB", previously assumed to have zincblende structure, probably has a face-centred titanium lattice with boron in the octahedral interstices.

In the chromium-boron system, the structure of the η-phase has been determined. It has the composition Cr_3B_4 and is isomorphous with Ta_3B_4 and Mn_3B_4. Some data for the δ-phase are also given.

For the columbium-boron system, a phase analysis has been carried out. The structures of three of the intermediary phases, CbB, Cb_3B_1 and CbB_2 (with extended homogeneity range) have been determined. They are isomorphous with corresponding phases of the tantalum- boron system.

A brief phase analysis of the nickel-boron system showed the existence of a phase with lower boron content than Ni_2B, which has not been previously reported.

In the thorium-boron system a new phase, probably with a complicated structure, was found with a boron content of about 50 atomic%.

The authors wish to thank Professor G. Hägg for his continued interest in this investigation. Financial support has been obtained from the *Swedish State Council of Technical Research*, which is gratefully acknowledged.

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Received December 1, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 165–184

Utilization of Ion Exchangers in Analytical Chemistry. XV

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In previous papers [1-4] the great applicability of cation exchange columns as analytical tools in separating quantitatively the cations of a solution from its anions has been demonstrated. During the last few years many similar applications of this technique have been described [5-18]. Summaries of what has been done so far in the field of ion exchange have been published recently [19-21].

In the present paper some questions of practical importance for this technique will be discussed, namely the influence of the particle size of the resin and the acidity of the solution upon the adsorption process, and the influence of particle size, regenerant concentration and some other factors upon the regeneration process.

Apparatus

The equipment now generally used in the laboratory for analytical purposes is an apparatus similar to that shown in Fig. 1 (A). This type of apparatus is chosen because it is important that air never enter the ion exchanger filter bed, since this would cause channelling, which would result in partial inactivation of the filter.

However, in the experiments presented in this paper another construction of the ion exchange column was used, Fig. 1 (B). The upper glass wool plug must be well packed, so that by means of the capillary forces of the liquid the air is prevented from entering into the filter. The resistance of the plug to the streaming liquid is not great compared to the resistance of the rest of the column. However this construction is possible only for rather short columns. The exit opening must not be more than about 15 cm below the upper glass wool plug.

In order to keep the flow-rate constant and equal from time to time, independent of the different resistances of the filter beds, the solution in question has been dropped into the column by means of a dosage capillary, Fig. 1 (B). The flow-rates have never been so great, that liquid has accumulated in the container of the column.

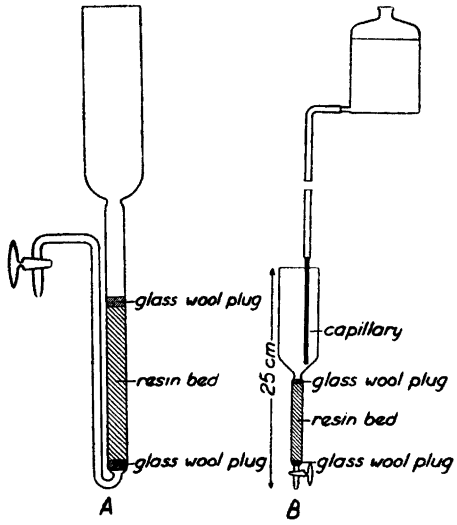


Fig. 1. Ion exchange columns.

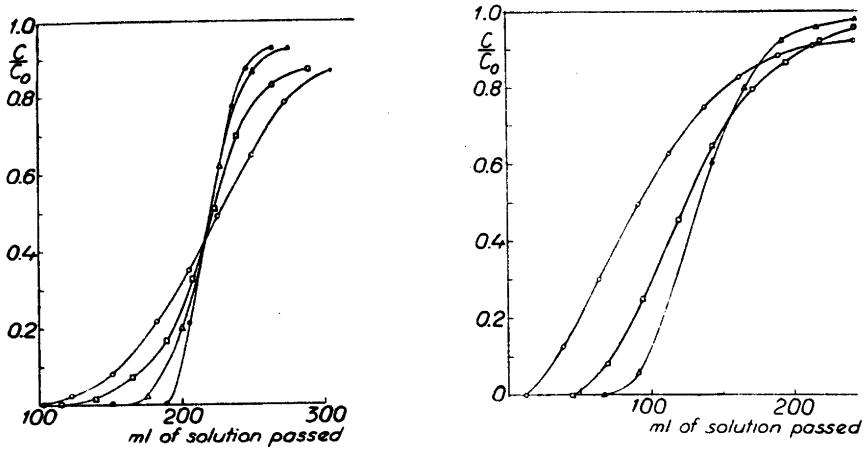
Except for a few preparatory experiments the dimensions of the filter beds have been: height 95 mm, inner diameter 12 mm. The free volume of the column (that is the part of the column below the upper glass wool plug which is occupied by the liquid phase) has been 8 ml. The ion exchange resin used throughout this work has been Wofatit KS (except for the preparatory experiments).

ADSORPTION PROCESS

Influence of the particle size

The theory of adsorption of ions in ion exchange columns is similar to the theory of adsorption of gases by gas adsorption filters. If, for example, a solution of KNO_3 of the concentration $C = C_0$ passes through a vertical column of an H^+-charged ion exchanger, and the flow-rate is so slow that equilibrium between the ions of the solution and the ion exchanger is always attained, one might expect a sharp boundary in the solution. Above this boundary the K^+-concentration C should be C_0, and below it C should be $= 0$. The conditions for this ideal case, of course, should be that no channels would occur in the column so that the solution could penetrate uniformly over the whole area, that the static equilibrium in all parts of the filter is obtained, and that the diffusion in the free liquid volume could be neglected [2, 3].

According to this theory the composition of the effluent would suddenly change from $C_{K^+} = 0$ to $C_{K^+} = C_0$, when a certain amount of the solution has passed through the column. However, in practice many factors interfere,



Figs. 2—3. Exchange isoplanes for Wofatit K resp. sulphite waste liquor resin. Solution: 0.037 M KNO_3.

○ *Particle size 2.0—1.0 mm*
□ » » *1.0—0.5* »
△ » » *0.5—0.25* »
● » » *< 0.25* »

so that the results deviate very much from the theoretically expected ones. When the function C/C_0 is plotted against the time or the volume of the effluent, an S-like curve, usually called an exchange isoplane, is always obtained [2, 3]. The reason why the isoplanes are S-like is that the ideal conditions mentioned above are not fulfilled. When commercial ion exchangers are used the chief reason is that the diffusion in the capillaries of the resin is slow [22–24] and therefore the static equilibrium is not attained. The diffusion in the capillaries being rate-determining as equilibrium in ion exchange is approached, the particle size of the resin must have a great importance upon the dynamic equilibrium occurring in a filter bed [4].

In the preparatory experiments presented in this paragraph two different ion exchangers have been investigated, namely Wofatit K and an ion exchanger made by condensing sulphite waste liquor with furfural [25]. The exchange isoplanes were determined for solutions of KNO_3 passing through columns of different particle size. The height of the columns was in this special case 135 mm and the diameter 10 mm. The ion exchanger was first regenerated with hydrochloric acid and washed with water. A 0.037 *M* solution of KNO_3 was then passed through the filter with a flow-rate of 10 ml/min. The effluent was collected in small fractions and the percent conver-

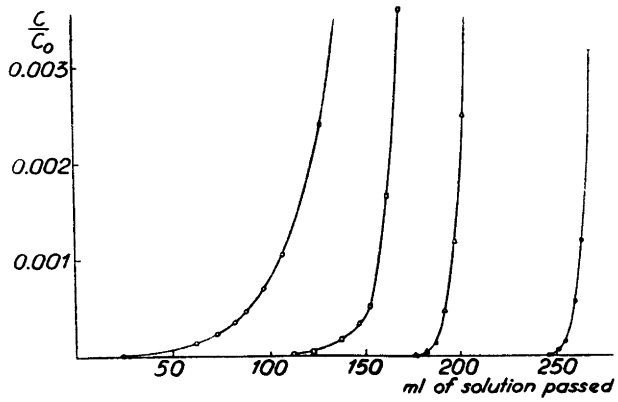


Fig. 4. Exchange isoplanes for Wofatit KS. Solution: 0.03 M $FeCl_3$.

○ *Particle size 1.0—0.6 mm*
□ » » *0.6—0.4* »
△ » » *0.4—0.2* »
● » » *0.2—0.1* »

sion was determined by titration with NaOH. The results are presented in Figs. 2 and 3, where C/C_0 is plotted against the volume of the solution passed through the filter columns.

For analytical purposes the lowest part of the curves are of the greatest interest. As a complement to the results shown, some determinations of these lower parts have been made with Wofatit KS and $FeCl_3$-solutions. In these experiments (and all the following) the dimensions of the columns were: height 95 mm and diameter 12 mm.

A freshly prepared solution of 0.03 *M* $FeCl_3$ was passed through the column at a constant flow-rate of 4.5 ml/min. The effluent was collected in fractions and the iron content determined colorimetrically with KSCN. The results are presented in Fig. 4, which shows only the lower parts of the exchange isoplanes. Especially in these experiments the great importance of using finely divided ion exchangers is demonstrated. The "break through capacity" of a column is very much increased as the size of the particles is diminished.

The results show that the greater the particle size, the more the curves deviate from the ideal shape. Where quantitative separation is desired, it is obviously of great importance to use relatively fine particles of the ion exchanger in the column.

When using ion exchangers for analytical purposes a particle size of 0.2—0.4 mm diameter is the most suitable, as far as the adsorption cycle is con-

cerned. Finer particles should be separated because they diminish the flow-rate too much for most purposes. Even a relatively small amount of finely divided exchanger would considerably raise the resistance of the filter bed to the streaming liquid. In most cases greater particles should be avoided, partly because of their disadvantageous influence on the exchange isoplane, but above all because of their detrimental effect on the regeneration of the filter, even if only a small amount is present (as will be shown below).

In certain cases, where the time of operation does not matter it may be convenient to diminish the particle size, and in other cases, where a high filtering velocity is desirable, it may be suitable to raise the lower limit of the particle size.

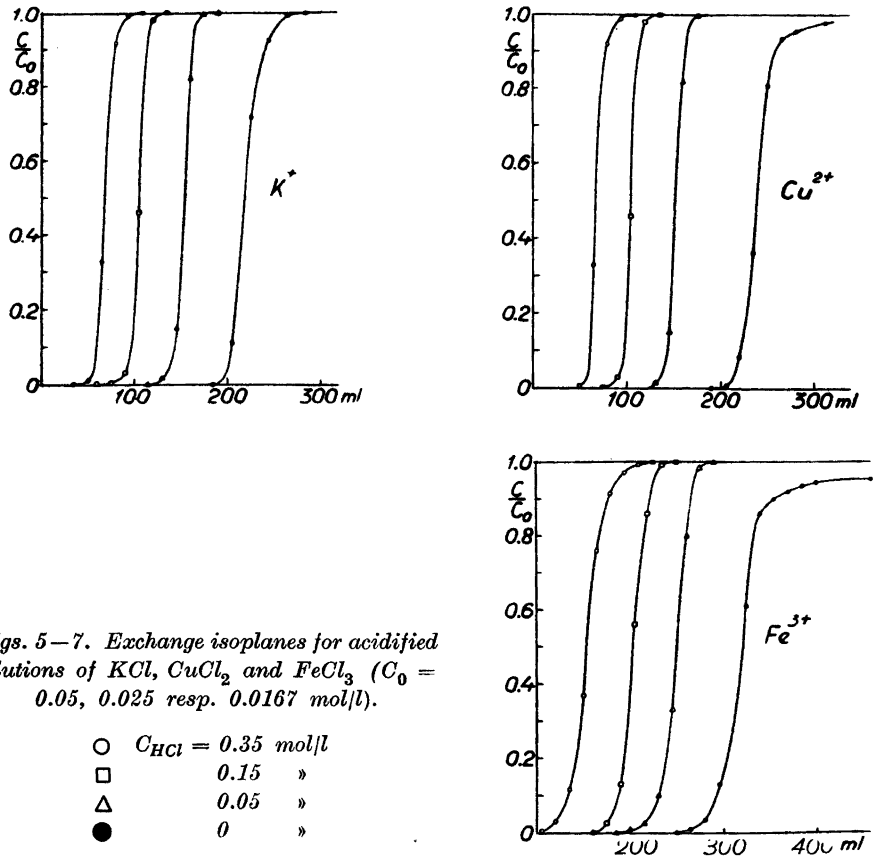
Effect of the acidity of the solution

In many cases the solution to be analyzed contains hydrogen ions beside the ions to be removed by the ion exchanger. As examples may be mentioned phosphate analyses where a low pH is necessary in order to prevent precipitation[1 V], or sulphite waste liquor[5]. The presence of hydrogen ions decreases the break through capacity for the other cations. Therefore the higher the acidity the greater is the filter volume needed. For routine analyses it is recommended to find out in every special case the amount of ion exchanger that is necessary to obtain quantitative conversion at the acidity in question.

In order to facilitate the estimation of the amount of ion exchanger needed (or the maximum acidity allowable) in different cases, experiments have been made with solutions of KCl, $CuCl_2$ and $FeCl_3$ containing varying amounts of HCl.

The solutions were 0.05 N with respect to the metal salt (*i. e.* 0.05 M, $\frac{0.05}{2}$ M and $\frac{0.05}{3}$ M resp.) and, besides, 0, 0.05, 0.15 and 0.35 M with respect to HCl. They were filtered through an H^+-charged column of Wofatit KS (0.2—0.4 mm), and the effluents were collected in small fractions that were analyzed. The KCl-solutions were evaporated to complete dryness and the remaining KCl was titrated with $AgNO_3$ according to Mohr. Cu^{2+} was determined iodometrically in a biphthalate buffer solution. Fe^{3+} was determined by titration with $KMnO_4$ after reduction with $SnCl_2$.

The results are shown in Figs. 5—7 which show the adsorption isoplanes. From these diagrams it is seen that a quantitative separation can be performed by filtering even very acid salt solutions through H^+-charged filters. However, the break through capacity of the filter is diminished. In Table 1 the break through capacities are tabulated for different capacities of the salt solutions. The break through capacity is arbitrarily chosen as the point where



Figs. 5—7. Exchange isoplanes for acidified solutions of KCl, $CuCl_2$ and $FeCl_3$ (C_0 = 0.05, 0.025 resp. 0.0167 mol/l).

○ C_{HCl} = 0.35 mol/l
□ 0.15 »
△ 0.05 »
● 0 »

$\frac{C}{C_0}$ reaches 0.001. Also the capacities for $\frac{C}{C_0} = 0.5$ are tabulated in this table. A correction has been made for the free volume of the filter, 8 ml, which has been deducted from the values observed in Figs. 5—7.

In Table 1 the break through capacity is expressed in terms of mval of the the metal cation. As is seen the capacity for the metal cations is considerably decreased when the acidity of the solution is raised. If instead the capacity is expressed in terms of the total amount of cations in the solution, *i. e.* mval cation plus hydrogen ion, the capacities shown in Table 2 are obtained. It is seen that when calculating the capacity in this way it is found to be greater for the acidified solution than for the pure salt solution.

From these experiments the following very crude rule can be formulated: The separation will be quantitative if the amount of metal cations and hydrogen ions together does not exceed the capacity of the filter for a pure salt

Table 1. Capacity of filter column (in terms of mval of cations, H^+ not included).

	Concn. of HCl (mol/l)	0	0.05	0.15	0.35
At $\frac{C}{C_0} = 0.50$	KCl	10.5	7.3	4.9	3.0
	$CuCl_2$	11.5	7.2	4.8	2.9
	$FeCl_3$	15.6	12.1	9.8	7.3
"Break through capacity" $\frac{C}{C_0} = 0.001$	KCl	8.8	5.4	2.9	1.6
	$CuCl_2$	9.5	5.3	3.3	1.8
	$FeCl_3$	12.1	9.1	7.6	4.8

solution. This rule may be of interest especially for the analyst not familiar with the ion exchange method. It may fail (Li^+, see below), but mostly it leads to overdimensioned filters.

The results are in accordance with previously published data. H^+ is known to have the least affinity for this kind of ion exchanger of all cations (except for Li^+ [2]). From this observation and from the Donnan equation governing the ion exchange equilibrium, it is also to be expected that the lower the acidity of the solution and the higher the valency of the metal cation, the greater is the total amount of metal cations that may be taken up by the filter without any leakage.

It may also be mentioned here that the amount of ion exchanger that is necessary for complete removal of polyvalent cations in presence of H^+ or other monovalent cations may be decreased (or the break through capacity of a filter may be increased) simply by diluting the solution with water [4].

Table 2. Capacity of filter column (in terms of mval of cations, H^+ included).

	Concn. of HCl (mol/l)	0	0.05	0.15	0.35
At $\frac{C}{C_0} = 0.50$	KCl	10.5	14.5	19.6	24.0
	$CuCl_2$	11.5	14.4	19.2	23.2
	$FeCl_3$	15.6	24.2	39.0	58.0
"Break through capacity" $\frac{C}{C_0} = 0.001$	KCl	8.8	10.8	11.6	12.8
	$CuCl_2$	9.5	10.6	13.2	14.4
	$FeCl_3$	12.1	18.2	30.4	38.0

REGENERATION PROCESS

For analytical purposes one generally works with H^+-charged cation exchangers, and the regeneration is made with an acid. Hydrochloric acid is generally chosen as a regenerant, because it has several advantages: it is a strong acid, it is cheap (even in very pure qualities), it is easy to evaporate and does not oxidize the ion exchanger (nitric acid does, even when rather dilute). Ordinarily other regenerants are used only if the system contains ions giving insoluble chlorides.

A systematic work on the regeneration of cation exchange columns for analytical purposes does not seem to have been published as yet. In the following, investigations concerning regeneration of ion exchange columns with hydrochloric acid will be described. The effect of the particle size of the ion exchanger, the concentration of the acid, the flow-rate of the acid and the time between adsorption and regeneration have been studied. As a complement, some experiments concerning the static ion exchange equilibria in the presence of relatively high concentrations of hydrochloric acid have been performed.

Effect of the particle size

In order to study the effect of the particle size on regeneration the exchanger was partially transformed into the Fe^{3+}-form (Fe^{3+}-charged) by passing 100 ml of a solution of 0.03 *M* $FeCl_3$ through the previously H^+-charged columns and washing afterwards with water. Then 5 *M* HCl was passed through the filter, the flow-rate being kept at 4.5 ml/min. The effluent was collected in fractions and evaporated. The iron content was determined by titration with $KMnO_4$ after reduction with $SnCl_2$.

The titrations of different fractions from one experiment have been made in the right order in a big Erlenmeyer flask without pouring out the successively collected liquid from time to time. In this way the accumulated titration errors have been diminished. The fractions corresponding to 95—100 % regeneration have been analyzed in a separate series (with smaller titration volumes) because of the greater requirements for accuracy. This method of titration has been used throughout this section (except for the experiments concerning static ion exchange equilibria).

The results are presented in Fig. 8, where the integral per cent regeneration of the columns is plotted against the volume of 5 *M* HCl used.

The experiments show, that the volume of acid necessary for the regeneration of the column increases very much when the particle size of the ion exchanger is increased. Using a smaller size means a saving of regeneration acid as well as of time. As mentioned in the preceding chapter, however, too small particle sizes should ordinarily be avoided. In all the following experiments we have used Wofatit KS with the particle size 0.2—0.4 mm, which seems to be most suitable for ordinary analytical purposes.

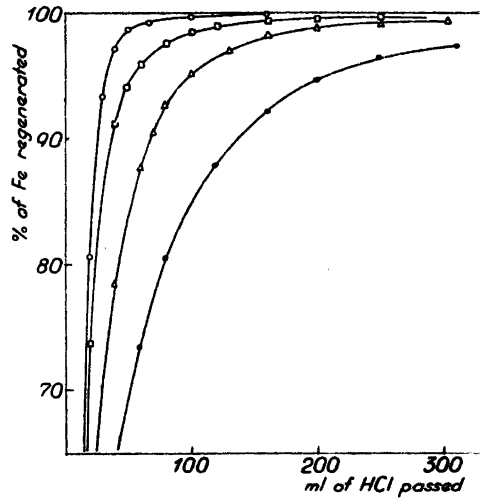


Fig. 8. Regeneration of Fe^{3+}-charged ion exchange column. Regenerant: 5 M HCl.

○ *Particle size 0.1—0.2 mm*
□ » » *0.2—0.4* »
△ » » *0.4—0.6* »
● » » *0.6—1.0* »

Effect of the concentration of the acid

Experiments have been made with cation exchangers partially transformed into the K^+-, Cu^{2+}-, Al^{3+}- and Fe^{3+}-form resp., which have been regenerated with hydrochloric acid of varying concentration.

100 ml of a 0.09 *N* salt solution (0.09 *M* KCl, 0.045 *M* $CuCl_2$, 0.03 *M* $AlCl_3$ or 0.03 *M* $FeCl_3$ respectively) was percolated through the column, and after washing with water the regeneration was started. Hydrochloric acid was passed through the column at a constant flow-rate. The effluent was collected in small fractions (5 or 10 ml, in certain cases, more), and after evaporation to dryness the fractions were analyzed. The experiments have been repeated with varying concentrations of the acid. The analyses were made as earlier described. Al^{3+} was precipitated and weighed as hydroxyquinolate. As a control, the precipitate in certain cases (small amounts) was dissolved and titrated with bromate.

Tables 3—8 contain the percentage of the originally adsorbed ions found in the eluate, tabulated as a function of the eluate volume and the concentration of the hydrochloric acid used. To these tables correspond curves similar to Fig. 8. They are, however, not reproduced here. With the help of these curves, it is easy to determine the volume of a certain regenerant that will be necessary to give a desired efficiency of regeneration. In Figs. 9—12 the volumes thus found are plotted as functions of the hydrochloric acid concentration at 90, 95, 98, 99, 99.5 and 99.8 % regeneration. The results correspond to the flow-rate 4.5 ml/min.

Table 3. Regeneration of K^+-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate 4.5 ml/min.

Concn. (mol/l):	1.09	2.17	3.12	4.41	5.47	6.61	7.75	9.88	12.3
Volume (ml)									
10	14.3	36.6	46.2	57.0	58.0	—	64.9	62.1	57.0
15	54.3	76.5	84.8	89.6	90.1	92.0	90.1	82.0	73.0
20	73.0	89.8	95.0	97.0	97.3	97.8	95.7	91.0	83.7
25	83.3	95.3	98.2	99.1	99.3	99.2	98.8	96.7	92.1
30	89.1	97.6	99.3	99.7	99.8	99.7	99.5	98.8	96.3
35	92.8	98.9	—	99.9	100	99.8	99.8	99.5	97.8
40	95.2	99.6	99.8	100		100	99.9	99.8	98.5
50	97.9	99.8	100				100	99.9	99.2
60	99.1	100						100	99.6
80	99.9								99.9
100	100								100

These diagrams show that for the cations studied the regeneration can be performed with a minimum volume of the regenerant if a certain optimum concentration of the acid is chosen. This optimum concentration is about 3 to 4 *M* HCl, and is about the same for all of the ions examined. The effect is greater at the greater flow-rate and less at the lower flow-rate, and it is most obvious in the case of Fe^{3+}. It is evident, that when using a too strong solu-

Table 4. Regeneration of Cu^{2+}-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate: 4.5 ml/min.

Concn. (mol/l):	1.09	2.17	3.12	4.41	5.47	6.61	9.88	12.3
Volume (ml)								
10	19.0	39.3	—	55.4	57.0	57.0	54.4	49.5
15	45.1	72.3	79.6	81.0	82.5	79.6	—	65.0
20	63.2	85.4	89.5	90.8	90.9	89.2	82.2	73.4
25	74.5	92.0	94.4	95.2	95.4	94.0	—	80.2
30	81.5	95.4	96.9	97.5	97.4	96.5	92.5	84.1
35	87.0	97.4	98.3	98.6	98.5	97.9	—	87.4
40	90.0	98.5	99.0	99.2	99.2	98.7	96.0	99.8
50	94.6	99.4	99.7	99.8	99.7	99.5	98.0	93.2
60	97.0	99.8	99.9	99.9	99.9	99.9	98.9	95.7
80	99.0	100	100	100	100	100	99.8	99.0

Table 5. Regeneration of Al^{3+}-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate: 4.5 ml/min.

Concn. (mol/l):	1.09	2.17	3.12	5.47	7.75	9.88
Volume (ml)						
20	49.2	66.6	73.7	73.8	69.9	65.2
30	—	78.0	84.7	84.7	81.3	76.0
40	70.1	86.8	90.3	90.2	87.5	82.6
60	80.7	93.3	95.4	95.3	93.7	90.0
80	86.7	96.4	97.6	97.6	96.4	93.9
100	90.6	98.0	98.6	98.5	97.8	96.0
150	95.9	99.4	99.5	99.5	99.2	98.2
200	98.1	99.7	99.8	99.7	99.5	99.0

tion of hydrochloric acid it will be practically impossible to elute Fe^{3+} quantitatively. This is a fact, which, if not observed, may cause considerable errors and discredit the use of ion exchange columns for analytical purposes.

A comparison between the results at the two flow-rates shows the influence of the flow-rate upon the regeneration, as will be shown more clearly by the experiments described below.

Table 6. Regeneration of Fe^{3+}-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate: 4.5 ml/min.

Concn. (mol/l):	1.09	2.17	3.12	4.41	5.47	6.61	7.75	9.88	12.3
Volume (ml)									
10	0.9	21.3	40.6	38.6	—	—	—	35.3	29.0
20	23.3	68.2	73.0	76.2	69.6	66.2	54.0	45.0	39.2
30	41.7	82.5	86.0	87.8	83.0	79.4	—	55.5	47.5
40	56.3	90.3	91.8	93.4	89.8	85.9	73.9	62.5	54.0
50	65.0	94.2	95.0	96.4	93.5	89.8	79.4	66.8	59.3
60	71.3	—	97.0	97.5	95.8	92.5	83.4	70.8	63.0
70	76.1	97.3	—	—	—	—	—	—	—
80	80.0	97.9	98.5	98.9	97.6	95.3	87.6	76.6	69.7
100	85.0	98.7	99.2	99.4	98.7	96.8	90.5	80.1	74.5
150	92.0	99.4	99.7	99.8	99.6	98.5	94.4	85.6	81.0
200	95.1	99.5	99.9	99.9	99.8	99.1	96.0	89.0	85.7
250							97.1	91.0	

Table 7. Regeneration of Cu^{2+}-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate: 0.8 ml/min.

Concn. (mol/l):	2.17	3.12	4.41	6.61	7.75	9.88	12.3
Volume (ml)							
10	55.0	71.2	85.5	85.6	85.7	85.0	85.2
15	95.8	99.6	99.7	99.4	99.6	99.3	97.8
20	99.7	100	100	100	100	99.9	99.4
25	100					100	99.8
30							99.9

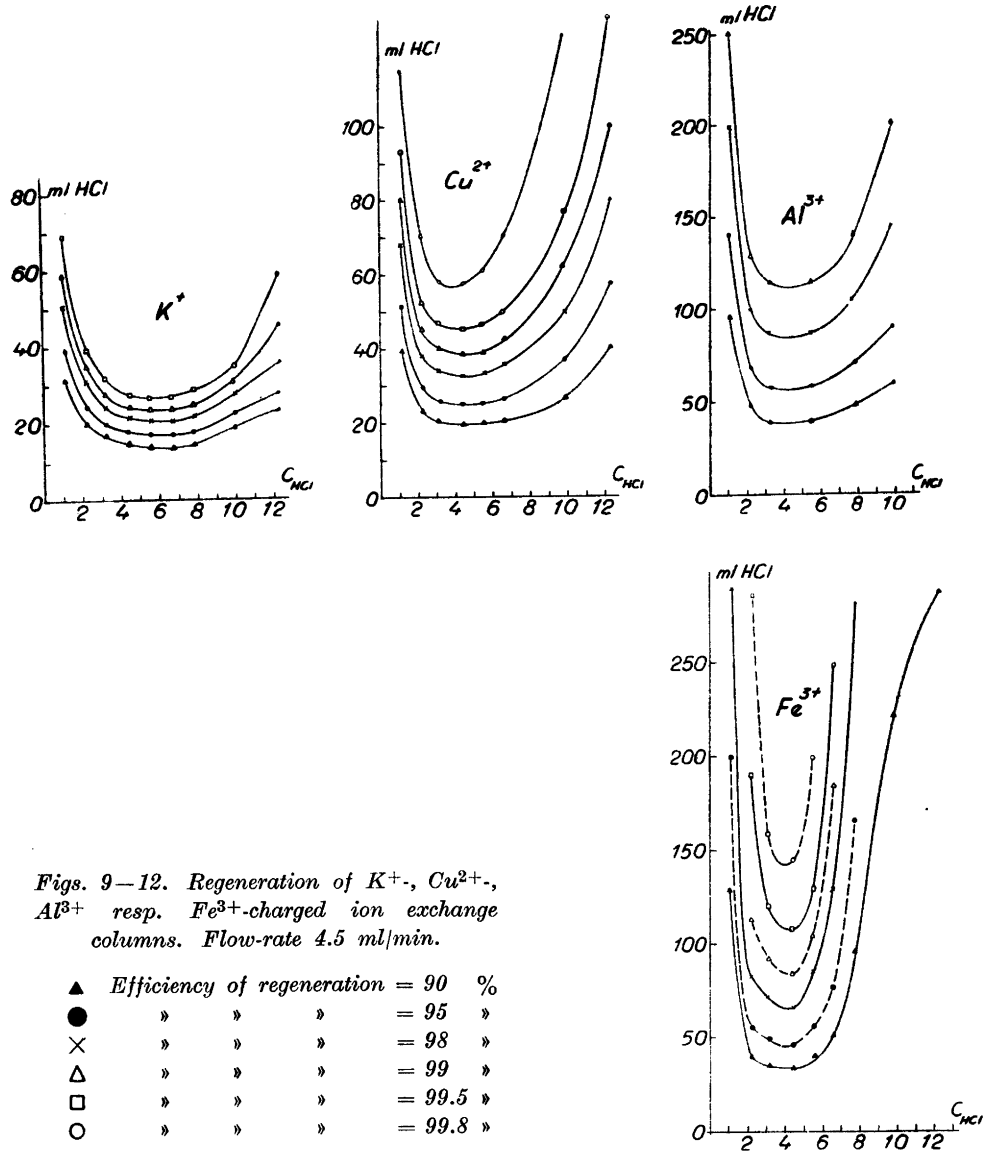
Table 8. Regeneration of Fe^{3+}-charged ion exchange column. The figures express the completeness (in %) of the regeneration as a function of the volume and concentration of the hydrochloric acid used. Flow-rate: 0.8 ml/min.

Concn. (mol/l):	2.17	3.12	4.41	6.61
Volume (ml)				
10	32.8	48.5	59.1	66.5
15	78.8	88.8	92.5	87.6
20	92.1	96.7	98.0	94.2
25	96.5	98.9	99.3	96.8
30	98.4	99.4	99.7	98.0
40	99.6	99.9	99.8	98.7
50	99.9	100	99.9	99.0
60	100		100	99.4
75				99.7
100				99.8

Effect of the flow-rate

These investigations have been performed with ion exchangers partially transformed into the Cu^{2+}- and Fe^{3+}-form resp., which have been regenerated with 3.3 *M* HCl at different flow-rates. Fractions were taken at one or two minute intervals, and the time was measured from the moment the acid began to flow out from the dosage capillary into the column. In Figs. 13 and 14 the regeneration efficiency is plotted as a function of the time required for regeneration.

The regeneration curves for the different flow-rates have nearly the same shape. The chief difference is their varying displacement along the time axis. This is due to the free volume of the filter, which was about 8 ml. It takes some



Figs. 9—12. Regeneration of K^+-, Cu^{2+}-, Al^{3+} resp. Fe^{3+}-charged ion exchange columns. Flow-rate 4.5 ml/min.

▲ *Efficiency of regeneration = 90 %*
● » » » = 95 »
× » » » = 98 »
△ » » » = 99 »
□ » » » = 99.5 »
○ » » » = 99.8 »

time before the regenerant has reached all parts of the filter column and the regeneration process is started all over the column, and this start time is different for the different flow-rates. If the curves are corrected with respect to this time they will all very nearly coincide.

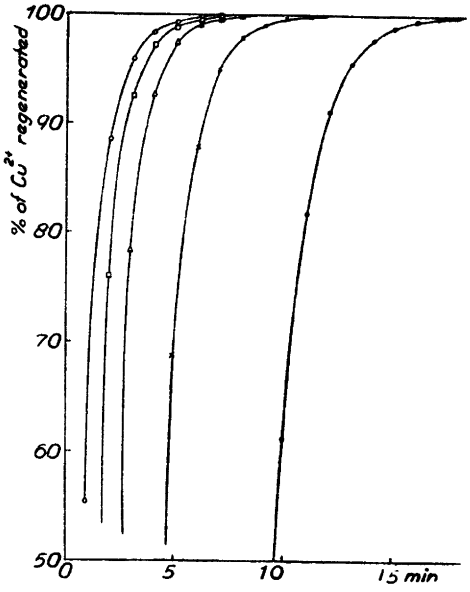


Fig. 13. Regeneration of Cu^{2+}-charged ion exchange column. Regenerant 3.3 M HCl.

○ *Flow-rate 11 ml/min*
□ » » *6.6* »
△ » » *4.0* »
× » » *2.2* »
● » » *0.95* »

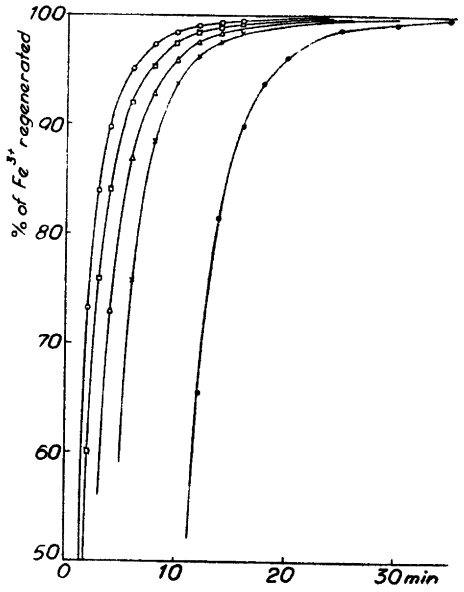


Fig. 14. Regeneration of Fe^{3+}-charged ion exchange column. Regenerant 3.3 M HCl.

○ *Flow-rate 13 ml/min*
□ » » *7.8* »
△ » » *4.0* »
× » » *2.2* »
● » » *0.95* »

The experiments show that, within wide limits, variation of the flow-rate of the regenerant has very little influence upon the speed of the regeneration. It is the time of the regeneration process that determines the efficiency, not the flow-rate nor the volume of acid used. For example the regeneration of the Fe^{3+}-charged filter takes about 30 minutes, independent of the flow-rate (except for very low flow-rates, of course). It is of no value to try to hasten the regeneration by increasing the flow-rate over a certain value. For example, from Fig. 14 it is seen that if the flow-rate of the regenerant is raised from 2.2 to 13 ml/min, the only effect will be an increase of the volume of the effluent from 70 to 350 ml. The efficiency of the regeneration will not be increased, nor will the result be achieved in a shorter time (except for the difference in start time: 4 min).

It may be mentioned that, for a study of the kinetics of the regeneration process, it would be better to let the regenerant pass through the column from

below. Avoiding convection streaming due to the greater specific gravity of the acid in this way, would make the conditions more ideal[4]. However the purpose of these investigations has been to examine the regeneration process under the conditions ordinarily used in laboratory work. When as small volumes as possible are desirable, back wash regeneration may be used even for analytical purposes.

Effect of time between adsorption and regeneration

In all the preceding experiments the regeneration was started within 15 to 30 minutes after the filter had been charged with the metal ion in question. However it was of interest to determine if the regeneration process would be slower if the filter was allowed to stand charged for a long time before the regeneration. In one experiment the filter was not regenerated until 20 days had passed from the charging with Fe^{3+}-ions. The regenerant was 3.3 *M* HCl and the flow-rate was 4.0 ml/min. The results are presented in Table 9 (experiment A). This table also contains the values from a corresponding experiment (B), in which the regeneration was started within 15 minutes after charging. As seen from the table the time between the adsorption and regeneration has only a slight influence upon the regeneration process.

Table 9. Regeneration of Fe^{3+}-charged ion exchange column.

Regeneration time (min)	Efficiency of regeneration (in %) A	B
2	17.9	19.0
3	57.9	56.0
4	74.1	72.4
6	87.5	87.0
8	93.3	92.9
10	95.4	96.0
12	97.7	97.5
14	98.5	98.4
16	99.0	99.1
20	99.5	99.4
25	99.7	99.7
30	99.8	99.8

Experiments concerning the static ion exchange equilibria

Earlier investigations with strongly swollen ion exchangers [3] have shown that the Donnan equation can be used for quantitative calculations of ion exchange equilibria. The measurements were carried out in dilute solutions of univalent cations. As the concentrations of the micellar solution were fairly low, it was possible to neglect the activity coefficients in the micellar as well as in the outside solution. Wofatit KS, however, like all slightly swelling ion exchangers now commercially available and used for industrial and analytical purposes, has a very high concentration of the micellar solution, and consequently the activity coefficients cannot be neglected. As no reliable means of calculating them exists, the exchange equilibrium must be studied empirically. A great number of measurements concerning dilute solutions have appeared. As far as the equilibria in concentrated solutions are concerned no systematic measurements have been published.

In order to elucidate the regeneration process, some measurements of static ion exchange equilibria will be described. The experiments were made with solutions of chlorides of K^+, Cu^{2+}, Al^{3+}, Cr^{3+} or Fe^{3+} respectively, and in each case with a large excess of hydrochloric acid. The composition of the solutions obviously corresponds to the conditions of regeneration of an ion exchange column with hydrochloric acid.

The experiments were made as follows:

A large amount of air dry ion exchanger in the hydrogen form was prepared and stored in a bottle with a close-fitting stopper. It had an exchange capacity of 2.39 mval/g as determined in separate experiments through charging until saturation with K^+-, Cu^{2+}- and Al^{3+}-ions and analyzing.

10.00 ml of a solution of KCl (or $CuCl_2$, $AlCl_3$ or $FeCl_3$ resp.), containing 4.78 mval of cations was pipetted out into each of nine 125 ml Erlenmeyer flasks with ground glass stoppers, and the solutions were evaporated to dryness. 2.00 g of air dry H^+-charged ion exchanger were then put into the dry flasks and 50.00 ml of hydrochloric acid in the concentrations shown in Table 10 were added with a pipet. The flasks were then closed and shaken several times a day, and after five days 25.00 ml of the liquid was pipetted out of each flask for analysis, which was made according to the methods previously described. The chromium experiments were made in a somewhat different way (see below). Chromium was determined iodometrically after oxidation to chromate with hot concentrated perchloric acid.

As mentioned above, each series of experiments comprised (as a rule) 9 different concentrations of the acid. Reference measurements have been made, firstly where 10.00 ml of the metal-salt solution in question has been analyzed for the metal directly, and secondly where 10.00 ml has been pipetted out in an Erlenmeyer flask and evaporated; 50.00 ml of hydrochloric acid (of the two concentrations 3 *M* and 10 *M*) has been added,

Table 10. Regeneration equilibrium.

Concn. of hydrochloric acid added (mol/l)	1.09	2.17	3.12	4.41	5.47	6.61	7.75	9.88	12.3
K^+	82.6	—	91.8	—	95.0	95.8	96.1	96.8	98.0
Cu^{2+}	85.8	97.0	99.2	99.6	99.6	99.8	99.7	99.7	99.6
Fe^{3+}	78.0	95.8	98.8	99.8	100.0	98.8	95.8	89.7	81.5
Al^{3+}	49.2	93.6	96.7	98.4	98.3	98.6	98.7	99.7	—
Cr^{3+}	69.4	89.4	95.6	98.5	100.1	99.7	99.9	100.0	100.1

and so on, completely corresponding to the main determinations except for the absence of the ion exchanger. These two ways of reference measurements have given results that very closely agree.

In the Al^{3+}-series no determinations were made with concentrated hydrochloric acid, because $AlCl_3$ is rather insoluble in it. In the Cr^{3+}-series it was found impossible to dissolve the evaporation residue in cold hydrochloric acid. Therefore these experiments were made so that 40.00 ml of hydrochloric acid of different concentrations and 10.00 ml of a solution of $CrCl_3$ in water (containing 4.78 mval) were added to an Erlenmeyer flask containing 2.00 g of the H^+-charged ion exchanger.

A summary of the results is presented in Table 10, where the percentage of metal cation found in the solution at equilibrium is given. As is seen from the measurements, Fe^{3+} deviates in behavior from the other ions and from what might be expected from the Donnan equation. According to this equation a higher concentration of the acid should perform a more complete regeneration, and that is also the case for the ions K^+, Cu^{2+}, Al^{3+} and Cr^{3+}.

As far as Fe^{3+} is concerned, the existence of a maximum value of the static regeneration equilibrium at a certain concentration of the acid seems to be difficult to explain from the facts collected here. The tendency of Fe^{3+} to form complexes with chloride ions should favour the regeneration at high acid concentration. Further experiments are needed in order to explain this anomaly.

It might seem preferable in these experiments to analyze the ion exchanger instead of the solution. This is not possible, however, as was shown in orientating experiments (not presented here). After separation of the ion exchanger from the bulk of the solution by means of a small glass filter funnel, the adhering solution must be washed away with distilled water. The very short period during which the concentration of ions in the acid solution around the ion exchanger is thus sinking towards zero is long enough to make possible a considerable rearrangement of ions.

Orientating experiments have also shown that the volume of water that is taken up by the ion exchanger in the swelling process has only a negligible influence upon the measurements. For 1 *M* acid the correction volume is found to be 0.50 ml, but at the higher concentrations which are of more interest in this connection the corrections are practically immeasurable. Other measurements have shown that the position of the equilibrium was exactly the same after 1 day as after 5 days and that the same results were obtained even when the equilibrium was approached from different sides (*i. e.* from H^+-charged and from Fe^{3+}-charged ion exchanger).

DISCUSSION

The experiments presented in this paper confirm the earlier known fact that the rate of adsorption of ions is mainly determined by the rate of diffusion in the capillaries of the particles of the ion exchanger. It is also evident that this diffusion process is the rate determining factor in the regeneration process. This will explain the great influence of the particle size upon the speed of adsorption and regeneration as well as it explains the fact that within wide limits it is the time of regeneration that is essential for efficiency and not the amount of acid used.

The experiments with varying concentrations of the acid in regenerating filter columns gave the somewhat unexpected result that the use of a moderately concentrated acid (3 to 4 *M* HCl) gave a maximum speed of regeneration. When using more concentrated acid the regeneration is slower, as is also the case when more dilute acid is used.

Earlier works on the swelling of ion exchangers, containing sulphonic acid groups [3] have shown that the swelling is greatest in pure water, and that the higher the concentration of the electrolytes in the solution, the more the ion exchangers shrink. The optimum effect now obtained in the regeneration experiments may be explained by the shrinkage of the ion exchanger due to the increasing concentration of the acid. This shrinkage is accompanied by a diminished diffusion velocity of the ions in the capillaries of the ion exchanger. The increasing viscosity of the more concentrated acids has an influence in the same direction. At a certain point these effects predominate over the favourable effects of increasing acid concentration which are observed at lower concentrations and which are generally due to a displacement of the ion exchange equilibrium. As far as Fe^{3+} is concerned, the static regeneration equilibrium explains the extremely pronounced optimum found in the dynamic regeneration curves.

One would perhaps believe that ions that have been kept adsorbed for a very long time would be difficult to elute from the resin because they would have had time to diffuse deep into very narrow and unapproachable capil-

laries. However any effect in this direction has not been observed for the resin examined.

From the present investigations it is possible to draw the following conclusions of how to regenerate an ion exchange column. The particle size should ordinarily be 0.2—0.4 mm. Greater and smaller particles should be removed. As regenerant should be used 3 to 4 *M* HCl. The regeneration may start by passing acid through the column at a fairly high flow-rate so that the acid will penetrate the whole filter column. Then the flow-rate is preferably diminished to low values — with the filter dimensions here used 1 to 2ml/min — until the regeneration is complete, which will take 5 to 30 minutes, depending upon the cation in question.

The flow-rates are more conveniently expressed in terms of ml per minute per ml of ion exchanger, this being applicable to filters of different dimensions. The recommended values of regeneration conditions then are: Start flow-rate about 1 ml per minute per ml of ion exchanger, followed by a flow-rate of about 0.1 to 0.2 ml per minute per ml of ion exchanger.

SUMMARY

Some questions of practical importance for the ion exchange technique described in earlier papers have been investigated. The effect of the particle size is shown, and an optimal size is recommended. The ion exchange is shown to be quantitative even for rather acid solutions. The conditions for regeneration of the ion exchange column with hydrochloric acid have been studied. A maximum speed of regeneration is obtained for a regenerant concentration of 3 to 4 mol/l and it is shown that the time of regeneration is ordinarily a more important factor than the amount of regenerant used. It is further shown to be especially important in the case of Fe^{3+} to follow the directions of regeneration given.

The authors wish to thank the Head of the Department, Professor O. Collenberg, for his great interest in this work. The *Statens Tekniska Forskningsråd* has supported the work financially, which support is gratefully acknowledged.

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Received October 5, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 185–199

Swelling and Heterogeneous Hydrolysis of Cotton Linters and Wood Pulp Fibers Related to Their Fine Structure

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The dual fine structure of all kinds of cellulose fibers, that is, the arrangement of the cellulose chain molecules in regions of high lateral order ("crystallites") and in regions where the lateral order is lower ("amorphous regions"), is today well established. There is a gradual transition from the symmetrical three-dimensional arrangement in the crystallites to an eventually highly disordered state in the amorphons regions. Hermans [1] has pictured the arrangement of the transition areas as shown in Fig. 1. Due to preferential cleavage of the crystallites along plane A_0, the leaflet structure shown will be typical for the transition of a crystallite into the disordered state.

Compared with the lenght of the cellulose molecule, the size of the regions exhibiting high lateral order is relatively small. In an undegraded native fiber, each cellulose chain will pass through a relatively high number of crystallites. The degree of order and the relative amount of transition regions are therefore of the greatest importance to the behavior of the fibers, especially in reactions which, at least in their initial state, involve little or no swelling of the fibers. There is no necessity to assume that the transition areas are similiar for all kinds of cellulose fibers. On the contrary, it seems reasonable to assume that the conditions prevailing when the cellulose chains

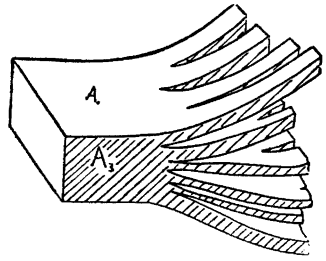

Fig. 1. Diagram of cellulose crystallite and its transition regions according to Hermans[1].

arrange themselves, *e.g.* the amount of noncellulosic materials present, may greatly influence the final arrangement and the extent of these regions.

During heterogeneous hydrolysis of cellulose fibers, the chains in the disordered areas are attacked first, and part of them are cleaved and brought into solution as glucose or small chain fragments. Based upon this principle, Nickerson [2] introduced a method where the loss in material is regarded as an expression for the percentage of "crystallinity" in a fiber. Similar procedures were applied later by several investigators [3–6]. It has, however, been suggested [7, 8] and recently conclusively shown [9] that, at least in regenerated fibers, the amount of the ordered areas increases upon hydrolysis. Besides the determination of loss of material, much interest has been focused on the constant average degree of polymerization (limit DP), which the hydrolyzed material assumes after a certain time of hydrolysis. This limit DP is thought to be similar to the original crystallite size.

Less attention has been directed to the rate of drop in DP during the hydrolysis process. One of the reasons may be that many of the investigators used fibers — refined and swelled cotton and wood pulps and regenerated fibers — where the drop in DP to a constant value takes place almost instantaneously under the applied conditions. It is, however, to be expected that with suitable conditions, the rate of decrease in the average DP will be greatly influenced by the arrangements in the disordered regions and should furnish valuable information.

Due to the "recrystallization", the limit DP will not be a direct measure of the length of the crystallites. It might, however, be expected to bear a close relationship to the length of the crystallites, and to that part of the transition areas and molecular fringes which, because of an appropriate arrangement, will rearrange into a closer packed system.

We have recently [10] shown that native cotton linters and wood pulp fibers behave quite differently during heterogeneous hydrolysis, both under mild and relatively severe conditions. Whereas the degradation of cotton linters proceeds rapidly and the hydrolyzed material almost instantly reaches a constant limit DP value, the degradation of wood pulp fibers is rather slow and the limit DP value is approached gradually. Fig. 2, taken from [10] illustrates clearly the contrast in the degradation of these two fibers during heterogeneous hydrolysis.

The present paper deals with further investigations on this difference between cotton linters and wood pulps. Pulp fibers, cooked to different average degrees of polymerization, and cotton linters are swelled in alkaline solutions of increasing swelling power. The fibers thus treated are hydrolyzed, and the rate of loss of material and the drop in DP, together with the

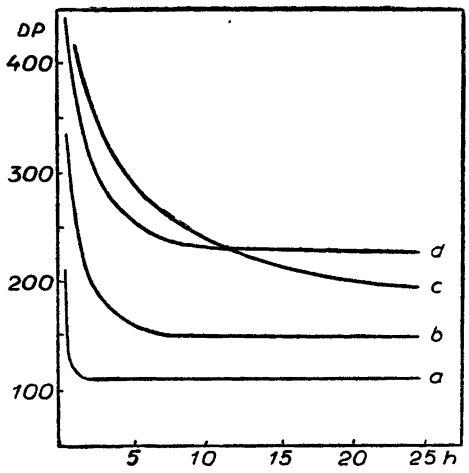

Fig. 2. Drop in DP during heterogeneous hydrolysis for a: Cotton linters, b: Rayon pulp, c: Unbleached rayon pulp, d: Extracted holocellulose, from (10).

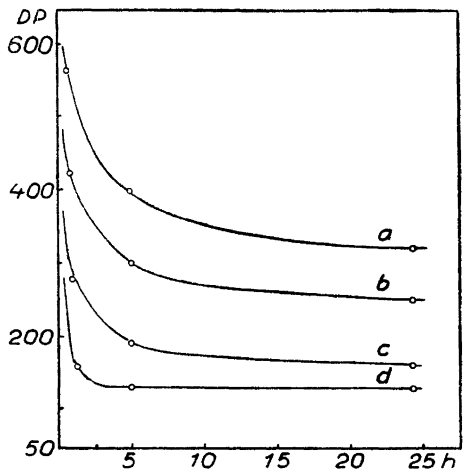

Fig. 3. Drop in DP during heterogeneous hydrolysis of a: High viscosity pulp. b: Medium viscosity pulp, c: Low viscosity pulp, d: Cotton linters (Italian),

limit DP values, are determined. The water absorption data for the swelled and hydrolyzed materials are also measured. A considerable dissimilarity is found to exist between the cotton linters and wood pulp fibers. This dissimilarity is considered in relation to the known fine structure of fibers, and can be explained by assuming a different arrangement of the transition and disordered areas in these two fibers.

EXPERIMENTAL

Materials

One sample of cotton linters was of Italian origin, carefully bleached but not further refined. The another sample was an acetate grade linters from Hercules Powder Co.*. The pulps were chlorine bleached sulfite spruce pulps made either in the laboratory or commercially. The following analytical data according to CCA standard methods may be regarded as average values:

α-Cellulose	87 —89	%
Pentosan	1.0 — 1.2	»
Lignin	0.3 — 0.6	»
Ash	0.08— 0.10	»

* We wish to express our gratitude to Dr. H. M. Spurlin, Hercules Powder, Co., Wilmington, Del. USA, who kindly furnished us with this material.

The average DP's varied as shown in the tables. All samples were extracted with alcohol — benzene (1 : 2) for 24 hrs, and air dried.

Methods

Swelling was accomplished with sodium hydroxyde solutions of 0 (water) — 2 — 4 — 6 — 8 and 10 per cent by weight at 3° C for 2 hrs. Under these conditions, ten per cent alkaline solution will cause intracrystalline swelling. The swelling solution (500 ml to 15 g of fibers) covered the fibers completely. The fibers were recovered by washing carefully with ice water several times, leaving in water overnight at room temperature, washing again with a large amount of water and finally with acetone, and then air dried. The loss of material caused by the swelling was found by determining the carbohydrates in the combined filtrate and wash-water. The values thus obtained checked very well with the recovered material. The swelling caused no decrease in the viscosity average DP, in fact a small increase was observed, due to dissolution of low-molecular materials.

Hydrolysis was carried out with 2.5 *N* H_2SO_4 in a thermostat regulated to 97° C (± 0.2° C). A special hydrolysis apparatus was used which allowed fresh acid to flow continuously through the material during the hydrolysis *. By carrying out the hydrolysis in this way no humidification or darkening of the material takes place. The amount of reducing sugar was determined at appropriate times as glucose in the hydrolyzate, *i. e.* in the acid leaving the apparatus. When used for further studies, the hydrolyzed material was washed with hot water, dilute ammonia and again with hot and cold water, finally with acetone, and dried under vacuum at 60° C for 12 hours. The yield determined in this way checked fairly well with the glucose determinations, the former usually being one to two per cent lower. The yield values in the tables refer to the glucose determinations.

The viscosity DP's were determined by converting to the nitrate and determining the intrinsic viscosity in acetone solution by the method described earlier [10], and in viscometers similar to those described by Wagner [11]. Correction was made for kinematic error. The intrinsic viscosities were converted to DP's using Staudinger's equation with $K_m = 10^{-3}$ (c in g/l). The water regain was determined at 55 % rel. humidity (over 40 weight per cent H_2SO_4 at 25° C) on materials dried in a vacuum oven at 60° C for 24 hrs, and over P_2O_5 at room temperature for several days.

EXPERIMENTAL RESULTS

The drop in DP during the hydrolysis of three pulps, cooked to varying viscosities, and the cotton linters is shown in Fig. 3. All samples were swelled in water only. The same difference as found earlier [10], in the behavior of cotton linters and wood pulp fibers is demonstrated here to an even greater extent. Already after 4—5 h, the DP of the linters reaches a constant limiting value. The decrease in DP of the wood pulps is again much slower, and the limit DP value is approached gradually and apparantly is not reached

* For informations regarding this apparatus we are indepted to Dr. W. E. Rosevear, E. I. du Pont de Nemours (through professor C. B. Purves, Mc Gill University, Montreal, Canada).

Table 1. Drop in degree of polymerization for wood pulp dependent upon time of hydrolysis and previous swelling.

Material	Swelled in NaOH Weight %	Degree of polymerization after hydrolysis in hours		
		1	5	24
High viscosity pulp (DP = 1950)	0	560	400	310
	2	510	330	270
	4	370	275	240
	6	200	170	160
	8	90	60	60
	10	70	60	60
Medium viscosity pulp (DP = 1300)	0	410	300	240
	2	380	275	230
	4	330	250	225
	6	220	180	170
	8	75	65	60
	10	60	55	55
Low viscosity pulp (DP = 850)	0	275	195	165
	2	265	190	160
	4	235	175	150
	6	140	125	115
	8	60	60	50—55
	10	55	50	45—50
Very low viscosity pulp (DP = 500)	0	255	190	160
	4	215	170	145
	6	95	85	85
	8	55	45	40—45
	10	50	45	40—45

even after 24 h. Fig. 4 (upper curve) indicates that, under the applied conditions, 30—40 h. are necessary before the hydrolysis curve levels off at an apparently constant value.

The limit DP of the pulps is apparently not constant but depends greatly upon the degree of cooking, decreasing as the viscosity of the pulp decreases. The limit DP of the high viscosity pulp is about 300 while that of the low viscosity pulp is only 160. All of the limit DP's of the unswelled pulps are therefore higher than the limit DP found for the cotton linters (130—140), but approach this value as the severity of the cooking increases. It should be noted that the slopes of the hydrolysis curves for the pulps remain nearly constant after about two hours, regardless of the degree of cooking.

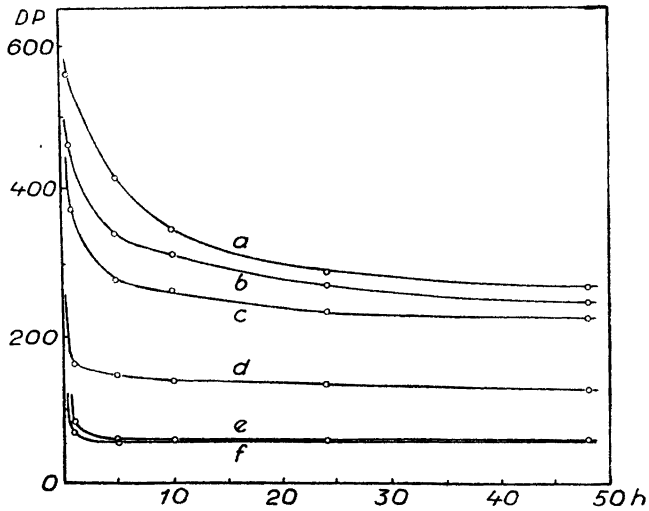

Fig. 4. Drop in DP during heterogeneous hydrolysis of a pulp swelled in a: Water, b: 2 % NaOH, c: 4 % NaOH, d: 6 % NaOH, e: 8 % NaOH, f: 10 % NaOH.

Table 1 shows the pronounced influence alkaline treatment exerts on the hydrolysis of the pulps. For one particular pulp, not included in Table 1 but later used for determinations of dissolved material during hydrolysis, similar data for a longer time of hydrolysis are plotted in Fig. 4. Even swelling in two and four per cent solutions increases the rate of degradation and results in a somewhat lower limit DP. Swelling in six per cent solution has a marked influence, not only upon the hydrolysis rate but also upon the limit DP value. When the strength of the swelling solution is raised to eight per cent, a sharp increase in the rate of hydrolysis occurs, and the limit DP value, which now is very low, is reached almost instantly. When swelled in a ten per cent alkaline solution, further change in the behavior of the pulp is very small. The limit DP value for all the pulps is almost the same after being swelled in eight or ten per cent alkaline solution, regardless of the original DP; whereas the limit DP's for samples swelled in the intermediate concentrations vary widely with the degree of cooking. As can be seen from Table 2, the alkaline swelling of the cotton linters do not show the same effect as described for the wood pulps. The rate of drop in DP changes very little and the limit DP hardly at all as the strength of the alkaline swelling solution is increased from zero to eight per cent. When the strength reaches ten per cent, the rate of decrease in DP is somewhat faster and the limit DP is lowered.

In Fig. 5 and Fig. 6, dissolved material is plotted against time of hydrolysis for one of the pulps and the acetate grade cotton linters, swelled in alkaline

Table 2. Drop in degree of polymerization for cotton linters dependent upon time of hydrolysis and previous swelling.

Material	Swelled in NaOH Weight %	Degree of polymerisation after hydrolysis in hours		
		1	5	24
Italian origin	0	160	140	140
	2	155	140	140
	4	155	140	140
	6	145	140	140
	8	145	140	140
	10	105	90	85
Acetate grade from Hercules Powder	0	155	140	140
	4	155	140	140
	6	155	140	140
	8	145	130	130
	10	85	80	80

solutions of increasing strength. For the pulp, the amount of material dissolved is seen to decrease at first as the strength of the alkaline solution is increased from zero to four per cent. For a material swelled in six per cent solution, the amount dissolved is somewhat higher than for the untreated material; and still higher for material treated with eight and ten per cent alkali. The increase is, however, rather small.

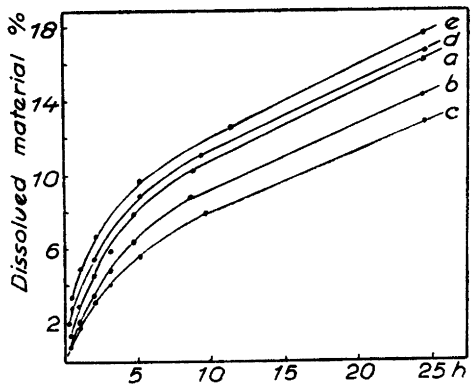

Fig. 5. Loss in material during heterogeneous hydrolysis of a pulp swelled in a: Water, b: 2 % NaOH, c: 4 % NaOH, d: 6 % NaOH, e: 8 and 10 % NaOH.

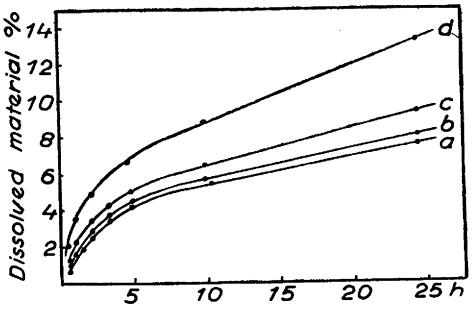

Fig. 6. Loss in material for cotton linters swelled in a: Water, b: 2, 4 and 6 % NaOH, c: 8 % NaOH, d: 10 % NaOH.

The decrease in the loss of material shown in the two and four per cent curves is caused by the dissolution of easily hydrolyzable material during the alkaline swelling. These losses amount to 2.5 and 5.8 per cent, respectively. The dissolution during the swelling explains also the small increase in hydrolyzed material represented by the six, eight and ten per cent curves. In these cases, the swelling losses were higher, amounting to 12 per cent for the first and 16—17 per cent for the last two samples. It is, however, remarkable that these samples, which behaved so differently when the effect of the hydrolysis was measured with regard to drop in DP's, are so similar in their yield curves. Table 3 demonstrates clearly that the amount of material dissolved during hydrolysis changes only slightly with increasing swelling, in spite of large decreases in DP.

Table 3. Degree of polymerization and loss in material after five hours of hydrolysis dependent upon degree of swelling.

Material	Swelled in NaOH Weight %	DP	Loss in material %
Cotton linters (Italian)	0	140	4.3
	2	140	4.1
	4	140	4.1
	6	140	4.1
	8	130	4.4
	10	90	6.2
High viscosity pulp	0	400	9.4
	2	330	7.5
	4	275	6.2
	6	170	6.6
	8	60	9.2
	10	60	9.6
Medium viscosity pulp	0	300	6.7
	2	275	5.5
	4	250	5.3
	6	180	5.6
	8	65	8.5
	10	55	8.9
Low viscosity pulp	0	195	6.4
	2	190	5.6
	4	175	5.1
	6	125	6.6
	8	60	8.9
	10	50	8.8

It should be noted that the slopes of the linear parts of the yield curves (Fig. 5) are almost alike for all pulp samples, and only a very small increase is observed for the eight and ten per cent curves. This shows that, after dissolution of the easily hydrolyzable part, the attack on the ordered parts is not appreciably influenced by the previous swelling.

For the cotton fibers (Fig. 6), very little change in the yield curves occurs up to swelling in six per cent solution. A small increase is observed for the eight per cent curve. The first notable increase in loss of material takes place, however, when the linters are swelled in the ten per cent solution. The slopes for the linear parts of the yield curves are seen to be alike up to eight per cent followed by a much steeper curve for the material swelled in a ten per cent solution. This shows that the attack on the more ordered parts of cotton linters is greatly increased when the alkaline solution is strong enough to cause intracrystalline swelling.

Table 4 shows the relative water regains for one of the linters and pulps for samples swelled but not hydrolyzed. The relative regains are obtained by choosing the regain of the water swelled sample as unity. The measured regains are given in column three. Due to dissolution of material during the swelling, these values do not give a correct picture of the increase in disordered areas caused by the swelling. Assuming that the amount which went into solution belonged to the highly disordered part and would have formed Cellulosehydrate II ($C_6H_{10}O_5 \times 1\ 1/2\ H_2O$) under the conditions employed, the values in column five are obtained. Based on these data, it is seen that for the cotton, swelling in up to six per cent alkali causes little change, swelling in an eight per cent solution increases the water regain somewhat, but the only large increase, from 1.12 to 1.56, is found when the linters are swelled in ten per cent lye. The pulp samples show a continuous increase from 1.0 to 1.10 when swelled in solutions from zero to four per cent. A sharper increase, bringing the regain to 1.27, is found for the sample swelled in six per cent lye, and a larger jump in the regains, from 1.27 to 1.70, is observed when an eight per cent solution is employed. No further increase occurs when the strength of the swelling solution is raised to ten per cent.

The water regains in column three should be an expression for the relative amounts of disordered material present in the swollen samples. It is surprising that the marked differences between the pulp samples in the rate of hydrolysis, expressed as drop in DP, are not reflected in their regains. No change in the regains occurs for samples swelled in lyes of zero, two, and four per cent, and only a relatively small increase is found for the six per cent sample, whereas the hydrolysis rate showed a marked difference for these samples (Fig. 4 and Table 1). A more pronounced increase in the water regains

Table 4. Relative water regains for cotton linters and wood pulp dependent upon degree of swelling.

Material	Swelled in NaOH Weight %	Measured water Regain *	Material dissolved %	Corrected water regain *
Cotton linters (Italian)	0	1.00	—	1.00
	2	1.00	0.6	1.01
	4	1.05	0.6	1.06
	6	1.05	0.6	1.06
	8	1.08	1.2	1.12
	10	1.44	2.2	1.55
Low viscosity pulp	0	1.00	—	1.00
	2	1.00	2.0	1.04
	4	1.00	3.7	1.10
	6	1.07	6.5	1.27
	8	1.36	12.0	1.70
	10	1.36	13.5	1.74

is noticed for the eight and ten per cent samples and is in accordance with the hydrolysis rate.

It is interesting to note that the water regain for the cotton linters has the most pronounced increase between the eight per cent sample (1.08) and the ten per cent sample (1.44), in accordance with earlier data [12]. For the pulp samples, this pronounced increase (from 1.07 to 1.36) takes place already for samples swelled in eight per sent NaOH, and no further increase is observed when ten per cent solution is employed.

Table 5 shows the absolute water regains for cotton linters and two pulp samples prior to hydrolysis, and the minimum water regains obtained during the hydrolysis. The minimum values were reached after one hour of hydrolysis. It is seen that the regains of the wood pulp, before as well as after hydrolysis, are always higher than those of the linters. The regains for the hydrolyzed pulp samples are seen to decrease with swelling solutions of concentrations up to six per cent, after which a sharp increase occurs. The regain for the hydrolyzed cotton remains constant up to the ten per cent sample, which shows a much higher regain.

* Water regain for material swelled in water chosen as unity.

Table 5. Absolute water regains for cotton linters and pulps before and after hydrolysis, dependent upon degree of swelling.

Materials	Swelled in NaOH Weight %	Water regain prior to hydrolysis %	Minimum water regain after hydrolysis
Cotton linters (Italian)	0	4.9	4.3
	2	4.9	4.2
	4	5.1	4.3
	6	5.1	4.3
	8	5.3	4.2
	10	7.2	5.0
Medium viscosity pulp	0	6.4	5.7
	2	6.4	5.7
	4	6.4	5.6
	6	6.7	5.3
	8	7.8	5.8
	10	7.7	5.8
Low viscosity pulp	0	5.9	5.4
	2	5.8	5.3
	4	5.8	5.2
	6	6.2	5.1
	8	7.8	5.9
	10	7.7	6.0

DISCUSSION

The explanation for the differences exhibited by the cotton linters and wood pulp fibers during heterogeneous hydrolysis must be sought in divergences in their fine structures. The recent works of Rånby[13] and Rånby and Ribi[14] have made it plausible to assume that the size of the regions exhibiting high lateral order do not vary much between native cotton and wood pulp fibers, being in the order of 120—140 glucose anhydride units. The difficulty in obtaining this low limit DP for the pulp fibers in contrast to the linters (Table 1), shows that the supermolecular arrangement between the crystallites cannot be the same in these two fibers.

The intercrystalline arrangement in the linters obviously allows the acid to penetrate without difficulty and cleave the cellulose chains into fragments, the size of which is not affected by prolonged hydrolysis nor by intercrystalline swelling solutions. The penetration of the acid into the intercrystalline por-

tions of the pulp fibers is, however, clearly hindered, and the cellulose chains are cleaved at fewer places than are the chains in linters. The size of the fragments formed is apparently not constant but depends upon the severity of the cooking conditions and even more upon the degree of swelling the pulp may have undergone.

In Fig. 7 a and b, proposed arrangements for the fine structures of linters and wood pulp fibers are schematically pictured. These are in agreement with the outline above and will be seen to explain the experimental observations.

The leaflet transition regions in the linters are supposed to be relatively short and rapidly develop into molecular fringes (Fig. 7 a). The chains in the loose molecular fringes will be easly available for acid attack and this explains the rapid drop in DP during hydrolysis. The fragments formed will, perhaps after some rearrangements, mainly consist of single crystallites. Intercrystalline swelling will have little influence upon the limit DP because the transition regions soon reach a high degree of order, which is not effected by swelling agents of this type. It will be recalled (Table 1) that swelling in alkaline solutions from zero to eight per cent did not influence either the rate of hydrolysis or the limit DP. It was only when the solution caused intracrystalline swelling (10% NaOH) and was transformed to Cellulose II that the limit DP showed some decrease.

For the wood pulp fibres it is proposed that the leaflet, perhaps mainly of a two-dimensional, arrangement regarded as typical for the transition areas, partially extends throughout the intercrystalline regions (Fig. 7 b). The crystallites would thus be interconnected by molecular arrangements exhibiting a certain degree of order, as well as by single cellulose chains. Such an intercrystalline arrangement would be partly resistant towards acidic agents under not too severe conditions. The hydrolysis product would therefore consist not only of single crystallites but perhaps mainly of two or several crystalline regions joined through one or more acid resistant leaflet structures. In agreement with this it was found that the limit DP of native fibers which had not been exposed to too severe cooking conditions (the two first samples in Table 1) was much larger than that corresponding to the crystallite size according to Rånby and Ribi.

The intercrystalline arrangements discussed above would be susceptible to swelling solutions and might be expected to be destroyed by solutions of relatively low swelling power. It was also found (Table 1 and Fig. 4) that the rate of hydrolysis increased and the limit DP decreased as the swelling power of the alkaline solution was raised. The gradual decrease in limit DP with increasing swelling power indicates that the proposed intercrystalline

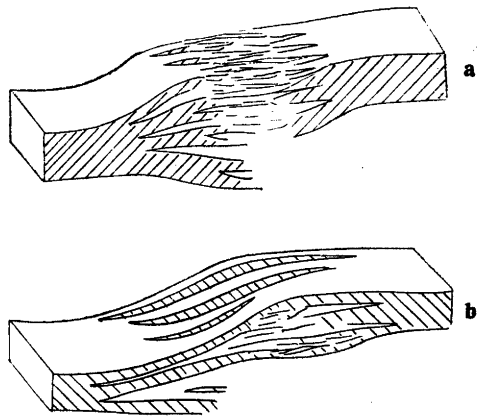

Fig. 7. Proposed arrangements for the intercrystalline structure in a: Cotton linters, b: Wood pulp fibers.

arrangements are destroyed successively and thus exhibit a wide range of orderliness.

It might be mentioned at this stage, that the material used by Rånby and Ribi [14], as representative of a pulp fiber and designated "wood pulp", was a refined sulfite spruce pulp sample *, whose hydrolysis behavior was found in this laboratory to lie somewhere between the values given by four and six per cent samples of the "Low viscosity pulp" in Table 1. On hydrolysis such a material might be expected to yield a relatively large amount of individual crystallites, or more correctly secondary crystalline fragments, which are found to have a limit DP very close to that of cotton linters, namely 110—140 for the former, against 130—140 for the latter. The limit DP value for the six per cent sample of the "Very low viscosity pulp" (Table 1) is, however, seen to be lower (80—85) than the DP of the crystallite proposed by Rånby and Ribi, and suggests that the size of the regions of high lateral order in wood pulps is smaller than those in cotton linters.

It must be regarded as significant that the lowest limit DP values, as well as the highest water regains (Table 4), in case of the pulps, were reached for samples swelled in an eight per cent NaOH solution, whereas a ten per cent solution was necessary for the linters. This indicates that for the pulps the transition from Cellulose I to Cellulose II occurs at conditions which are considered to cause intercrystalline swelling only [15]. This suggests that there might exist a difference also in the orderliness or in the form of the regions exhibiting high lateral order in wood pulp and cotton fibers. It will be recalled that the linear part of the yield curves were steeper for the pulp than

* Private Communication from Mr Rånby.

for the linters (Figs. 5 and 6), indicating that the crystalline regions are more susceptible for attack in pulps than in linters. Table 5 showed that the water regains after hydrolysis were higher for the pulps than for the linters, showing that in the former, in spite of similarity in crystallinity of these two fibers [16], more hydroxyls are available for absorption of water; that is, the ordered regions in pulp fibers seem to have a larger surface than the crystallites in cotton linters. It might therefore be supposed that the crystalline regions in wood pulp fibers are flatter, more ribbonlike, than those regions in cotton linters.

The proposed fine structural differences between wood pulp and cotton linters are naturally outlined only very schematically. Further investigations of both a chemical and physical nature are necessary before a more detailed picture can be presented. It might not be unreasonable to assume that other pure cellulose fibers, *e.g.* cotton and ramie, have fine structures similar to cotton linters, whereas all wood pulp fibers have fine structures more similar to that proposed for the spruce pulp fibers.

SUMMARY

Comparative hydrolysis has been carried out on cotton linters and wood pulp fibers. Whereas linters are rapidly degraded to a constant limit DP, the degradation of wood pulps is much slower and a limit DP is reached only after prolonged hydrolysis. The limit DP depends greatly upon the severity of cooking.

Swelling in alkaline solutions from zero to eight per cent has no effect on the degradation of linters. A somewhat lower DP is obtained for samples swelled in ten per cent solution. The effect of a similar swelling on pulps is remarkable. Even swelling in alkaline solutions of low concentrations exerts a pronounced influence upon the degradation. A fast degradation to a low limit DP is obtained for samples swelled in eight per cent alkaline solution. No further change is observed when the concentration is raised to ten per cent.

For cotton a significant increase in dissolved material during hydrolysis and in water regains occurs when samples are swelled in ten per cent alkali. Only a small increase was found in the yield curves for the pulps and the large increase in the water regains took place already for samples swelled in eight per cent alkali.

It is supposed that these dissimilarities between cotton linters and wood pulp fibers are due to differences in their fine structures. Arrangements are proposed, assuming looser molecular packing in the disordered regions and rather short transition areas for the linters, and a somewhat higher order in

the disordered regions and relatively extended transitions regions for the pulp fibers. In the pulp fibers the crystallites are proposed to be somewhat shorter and flatter than in linters.

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Received November 11, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 200–204

Tetrachloro-Cyclohexanone and Tetrachloro-Cyclohexanol Obtained from Cyclohexanol

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When cyclohexanol is chlorinated in sunlight or strong artificial light considerable quantities of a colourless solid substance of m.p. 84° C are deposited which, according to analysis, has the composition $C_6H_6Cl_4O$. Some crystals of the same substance have been obtained also when cyclohexanone was chlorinated under corresponding conditions. In the latter case, however, the chlorination usually leads to a breaking of the carbon ring and the formation of hexenal and (when water is present) caproic acid and adipic acid.

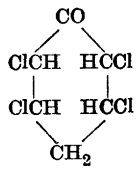
The chemical properties of this new compound (I) are rather interesting. Although the results of our investigation leave no doubt as to the correctness of the assumption that it is, in fact, a tetrachloro-cyclohexanone, the chemical reactivity of the keto group is strongly sterically hindered and it does not react with the ordinary keto-group reagents like hydroxylamine, phenylhydrazine or semicarbazide. In this respect it resembles chloranil, and surpasses the lower substituted quinones.

The reduction of the carbonyl group is possible, however, when aluminium isopropylate is used. This reaction takes place rather slowly, but the acetone formed in the course of it clearly proves the presence of a keto group in the molecule. When the reaction is finished, a colourless crystalline substance (II) melting at 62° C and having a composition corresponding to the formula $C_6H_8Cl_4O$, may be isolated from the reaction mixture.

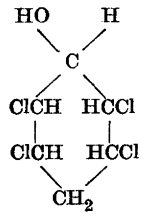
The alcoholic character of this new substance (II) is not easily demonstrated, as no derivatives could be obtained by the action of phenylisocyanate, diazomethane, or acid chlorides. A series of experiments were also carried out with the aim of replacing the hydroxy group by chlorine, but without any success. A strong steric hindrance must therefore be present in the reduction product as well.

A further interesting property of the ketone (I) is its behavior towards alkalies. Cold, dilute solutions of barium hydroxide easily brings the substance into solution, four equivalents of alkali thereby being neutralized. From this solution the entire content of chlorine is immediately precipitated with silver nitrate after the solution has been acidified with nitric acid. If, on the other hand, the solution of the ketone in barium hydroxide is heated with an excess of the alkali more barium hydroxide is neutralized and a crystalline precipitate is formed. We have not, so far, examined the precipitate thus obtained. The products obtained by cold hydrolysis using dilute alkaline solutions (ammonia, potassium hydroxide or barium hydroxide) appear to be rather complex, and we have not tried to isolate any of its components.

If the substance I is heated to 260—270 ° C hydrogen chloride is evolved and 2,6-dichlorophenol is formed. This seems to indicate that two of the chlorine atoms of the tetrachloro-cyclohexanone are linked to carbon atoms adjacent to the carbonyl group. We have started an electron diffraction investigation of the vapour of this substance, using the rotating sector method and from this we hope to be able to get more precise information concerning the positions of the chlorine atoms in the molecule. So far the results obtained seem to confirm that the structural formula I, based on the chemical properties of the substance, is the correct one.



I



II

It seems rather probable that our substance I corresponds to the tetrabromocyclohexanone of m.p. 119° C prepared by Boudroux and Taboury [1]. The properties of this substance are indeed strikingly similar to those of substance I. It is easily hydrolysed by alkalies, splits off hydrogen bromide when heated, thus giving 2,6-dibromophenol [2], and fails to react with the ordinary keto-group reagents.

We have prepared the bromine compound, as described in Boudroux and Taboury's paper, and compared the crystals of this substance with those of our substance I. The results of the analysis, which E. Wang Lund was kind enough to carry out, are summarized in Table 1.

Table 1. Crystallographic characteristics of the two tetrahalogen-cyclohexanones.

$C_6H_6OCl_4$	$C_6H_6OBr_4$
Orthorhombic with 4 molecules in the unit cell	Orthorhombic with 4 molecules in the unit cell
$a = 8.79$ Å	$a = 10.9$ Å
$b = 16.4$ Å	$b = 14.7$ Å
$c = 6.23$ Å	$c = 6.28$ Å

Although the unit cells are very similar and the ratio of the molecule volumes approximately such as would be anticipated for analogous bodies, we still hesitate in expressing the view that they are strictly isomorphous because one of the lattice constants is actually *smaller* in the bromine compound than in the chlorine compound. The development of the crystals is also different, the chlorine compound usually forming needles, the bromine compound plates.

EXPERIMENTAL PART

Tetrachloro-cyclohexanone (I)

Bubbles of gaseous chlorine were led through a water-cooled glass flask containing cyclohexanol directly exposed to sunlight or artificially illuminated. Hydrogen chloride was evolved, and soon considerable quantities of colourless crystals were formed. After separation from the mother liquid and washing with carbon tetrachloride, the substance was recrystallized from carbon tetrachloride or ligroin. The needle-shaped crystals thus obtained were well developed and melted at 84° C.

$C_6H_6Cl_4O$	Calc.	C 30.54	H 2.56	Cl 60.11	Mol. wt. 236	
	Obs.	» 29.68	» 2.61	» 59.50	» » 235	(benzene solution)

Tetrachloro-cyclohexanol (II)

10 g of I were treated with aluminium isopropylate, according to Lund [3]. Traces of acetone are present in the distillate from the beginning of the experiment, but the reaction is very slow. After 50—60 hours no further formation of acetone could be detected. The isopropyl alcohol was evaporated, *in vacuo*, and the remaining solid was treated with dilute sulphuric acid. The tetrachloro-cyclohexanol formed during the reaction was steam distillated and extracted from the distillate with ethyl ether. The ether solution was dried with anhydrous sodium sulphate and evaporated, and the remaining nearly colourless oil left to crystallize. After two recrystallizations from methanol, about 7 g of the new substance were obtained forming thick needles of m. p. 62° C. The alcohol is more sensitive to high temperatures than the corresponding ketone, and even at room

temperature it cannot be kept for any lenght of time without marked decomposition. It is more resistent towards alkalies, however, than the ketone.

$C_6H_8Cl_4O$	Calc.	C 30.28	H 3.39	Cl 59.60	Mol. wt. 238
	Obs.	» 30.46	» 3.40	» 60.21	» » 236 (benzene solution)

Hydrolysis of I

Cold, dilute sodium hydroxide solution was employed, and the remaining small excess of alkali titrated with 0.1 *N* hydrochloric acid. The hydrolysis of 0.1244 g of I required 20.85 ml of 0.1 *N* sodium hydroxide corresponding to 59.5 % chlorine.

The hydrolysis of 0.1522 g of I with 0.1 *N* potassium hydroxide yielded 0.3661 g of silver chloride:

Calc. for four atoms of chlorine	Cl 60,11
Obs.	» 59.50

The hydrolysis products were also extracted with ethyl ether, the ether solution dried with anhydrous sodium sulphate, and the solvent evaporated. A colourless viscous liquid was obtained which contains unsaturated substances since it adds on bromine. On standing or when heated, the liquid takes on a dark colour.

If the hydrolysis is performed with an excess of aqueous barium hydroxide in the absence of carbon dioxide, and the solution is heated to the boiling point, more of the base is neutralized and a white precipitate is formed.

2,6-Dichlorophenol from I

Tetrachloro-cyclohexanone was heated in a spherical flask fitted with a vertical reflux condensor. The temperature of the condensor was kept at 85—90° C. When heated on the sand bath, at 260—270° C, a vigorous evolution of hydrogen chloride took place. When this had subsided, the substance remaining in the flask was allowed to distil: at 217° C almost the entire contents of the flask distilled over. The distillate had the sharp odour of the chloro-phenols and gave a violet colour with ferric chloride. The crystalline mass was recrystallized from petroleum ether giving colourless needles of 2,5-dichlorophenol of m. p. 67° C.

Monochloro-cyclohexanol

After removing substance I, some ethanol was added to the liquid chlorination product of cyclohexanol and the mixture kept at a temperature a little above 0° C for some time. New crystals were then deposited which after recrystallization from ethanol-water had a melting point of 121—122° C. This substance shows a marked association tendency in benzene solution: the cryoscopical molecular weight increasing markedly with the concentration.

g substance per 20 g benzene	0.5066	0.2418	0.1173
Mol. wt. observed	157	148	146

$C_6H_{10}OHCl$	Cl 26.34	H (hydroxy-) 0.75	Mol. wt. 135	
Obs.	» 25.93	» » 0.69		

SUMMARY

By chlorination of cyclohexanol a tetrachlorocyclohexanone and a monochlorocyclohexanol were formed. The reduction of the former with aluminium isopropylate led to a tetraclorocyclohexanol. Both the ketone and the tetrachloroalcohol exhibit strong steric hindrance to reactions with their functional groups. When treated with cold, dilute alkalies all chlorine atoms in tetrachlorocyclohexanone were split off, and on heating the hydrolysed products, additional barium hydroxide was neutralized. Some physical examinations of the tetrachlorocyclohexanone have been carried out.

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Received November 25, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 205–208

Short Communications

A New Colour Reaction on Copper and Certain Carbonyl Compounds

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During an investigation of oxalylhydrazide [1] it was found that a number of its condensation products with aldehydes and ketones give a very sensitive blue colour reaction with copper salts. Several hydrazones not earlier mentioned in the literature were prepared and tested. The hydrazones of simple aliphatic aldehydes (formaldehyde, acetaldehyde, propionic aldehyde, *n*-butyric aldehyde and 2-ethylbutyric aldehyde) and ketones (acetone, methyl-ethyl ketone, methyl-iso-butyl ketone, methyl-*n*-amyl ketone) and of the cyclic ketones cyclopentanone and cyclohexanone give the reaction distinctly. With other hydrazones, for instance those of benzaldehyde and furfural, no reaction of the same kind could be obtained. For reagent purposes the bis-cyclohexanone-oxalyldihydrazone, $C_6H_{10} : N \cdot NH \cdot CO \cdot CO \cdot NH \cdot N : C_6H_{10}$, seems to be the best one.

A solution of this compound in dilute alcohol gives a blue coloration with a copper salt in faintly alkaline solution. If the reaction is carried out in an ordinary test-tube, copper can be detected even at a dilution of 1 : 40 000 000. Ammonium salts and free hydrazide promote the reaction. By means of this reagent the presence of copper in filter paper and in commercial distilled water can easily be shown. The blue colour is rather stable and it therefore seems to be possible to use the reaction for quantitative determinations, too.

Because the hydrazide itself gives a *green* colour reaction of moderate sensitivity with copper salts and the formation of hydrazones takes place very easily in most cases, the reaction can obviously also be used as a test for those carbonyl compounds, the hydrazones of which give the blue colour reaction with copper salts. Thus the presence of such compounds in several kinds of vegetable materials was easily shown; further in a number of commercial solvents, even in those of analytical grade. It must, however, be pointed out that the reaction is not a general one for carbonyl compounds.

Concerning the nature of the blue solution nothing can be stated at the present moment. Several facts, however, suggest that the complex-forming compound is not the dihydrazone itself but a monohydrazone, like $R : N \cdot NH \cdot CO \cdot CO \cdot NH \cdot NH_2$, which is formed by the hydrolyses of the dihydrazone. More experimental work is needed on this point.

The author wishes to express his thanks to Swedish Natural Science Research Council for financial support and to Mrs. Greta Lundquister for assistance in the experimental work.

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Received Februar 7, 1950.

Über den oxydativen Abbau von Alkohollignin

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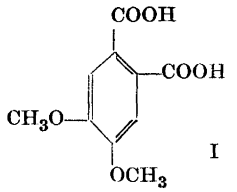
Die energische Oxydation von methyliertem Lignin bezw. Holz mit Kaliumpermanganat liefert nach Freudenberg, Meister und Flickinger [1] nur Veratrumsäure in geringer Ausbeute. Wird dagegen Lignin oder Holzmehl zunächst mit starkem Alkali erhitzt, dann methyliert und oxydiert, so erhält man abgesehen von der einer Nebenreaktion entstammenden Dehydrodiveratrumsäure ungefähr 20 % Veratrumsäure und 6—10 % Isohemipinsäure. Auch beim entsprechenden Abbau aller anderen Ligninpräparate, die durch Aufschluss von Holz oder isoliertem Lignin mit Bisulfit, Thioglykolsäure, Methanol und Chlorwasserstoff, Eisessig und Magnesiumchlorid sowie mit Kalium in flüssigem Ammoniak erhalten wurden, fanden Freudenberg und Mitarb.[1, 2] bisher nur Veratrumsäure (2,5—6 %) und Isohemipinsäure (1,5—3 %).

Bei Untersuchungen über die Alkoholyse des Lignins, über welche später berichtet wird, wurde nun erneut ein Alkohollignin mit Permanganat oxydiert, das aus dem Holz durch 8-stündiges Kochen mit 3-proz. äthylalkoholischer Salzsäure ausgelöst und dann mit Alkali und Dimethylsulfat methyliert war. Bei dieser Oxydation wurden neben 9,2 % Veratrumsäure, 0,5 % Dehydrodiveratrumsäure und 0,95 % Isohemipinsäure nun auch noch 1,3 % Metahemipinsäure (I) und ungefähr 1,5 % Bernsteinsäure isoliert.

Weitere Abbauprodukte konnten bisher nicht in reiner Form gewonnen werden. Die Metahemipinsäure wurde als solche, sowie als Dimethylester (Schmp. 88°) mit einem synthetisch dargestellten Vergleichspräparat [3] identifiziert.



I

Mit Metahemipinsäure wurde nun zum ersten Mal aus einem Ligninpräparat ein Abbauprodukt isoliert, dessen Entstehung mit den Freudenberg'schen Ligninformeln [4] nicht in Einklang zu bringen ist. Es erhebt sich daher die äusserst wichtige Frage, ob im nativen Lignin bereits diese Konfiguration vorgebildet ist, welche nach Methylierung und Oxydation Metahemipinsäure liefert oder ob diese Konfiguration einer Sekundärreaktion bei der Isolierung des Alkohollignins ihre Entstehung verdankt.

In späteren ausführlichen Mitteilungen wird über Versuche berichtet, Metahemipinsäure auch aus anderen Ligninpräparaten zu isolieren oder deren Entstehung infolge von Sekundärreaktionen aufzuklären. Sollte Metahemipinsäure auch aus anderen Ligninpräparaten gebildet werden, so ist zweifellos eine Abänderung der bisherigen Vorstellungen über die Konstitution des Lignins notwendig, welche sich der Verfasser ausdrücklich vorbehält.

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Eingegangen am 23. Januar 1950.

The Oxidation of Benzene by Means of Hydrogen Peroxide Catalyzed by Ferric Ions

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The oxidation of benzene by hydrogen peroxide and ferrous salt is mentioned by several authors, latest by Merz and Waters[1] who obtained beside phenols also diphenyl as oxidation products. Merz and Waters are of the opinion that the oxidizing effect in hydrogen peroxyd-ferrous salt solutions is due to the presence of free hydroxyl radicals.

In an earlier paper [2] I have shown that the decomposition of hydrogen peroxide catalyzed by ferric salts can be explained by the reaction mechanism:

$$Fe^{+++} + HO_2^- \rightleftharpoons FeOH^{++} + O \quad (1)$$
$$O + HO_2^- \rightarrow O_2 + OH^- \quad (2)$$

or:

$$Fe^{+++} + HO_2^- \rightleftharpoons FeOOH^{++} \quad (1a)$$
$$FeOOH^{++} \rightleftharpoons FeOH^{++} + O \quad (1b)$$
$$O + HO_2^- \rightarrow O_2 + OH^- \quad (2)$$

From this mechanism it would be expected that solutions of hydrogen peroxide containing ferric salt, due to the free oxygen atoms also would be able to oxidize many substances, which I have proved to be the case.

The following is a description of a qualitative experiment, of which, in 1948, I carried out several others with similar results.

500 ml of a saturated solution of benzene in water which was 0.003 *M* with regard to ferric nitrate and 0.01 *M* with regard to nitric acid and to which was added 5 g 30 % hydrogen peroxide (Perhydrol Merck) was allowed to stand at room temperature. In the course of about thirty minutes the colour of the solution changed through reddish to dark brown, after which it turned more and more pale and finally became yellow. When the brown colour began to fade the hydrogen peroxide began to decompose with evolution of oxygen. From time to time a 10 ml sample was tested for phenols by bromine water.

The behaviour of the reacting mixture is shown in the following table:

Minutes after mixing	Colour of the solution	Reaction with bromine
10	Reddish	Weak opalescence
20	»	White turbidity
30	Brownish	» precipitate
35	Dark brown	» »
40 *	Lighter brown	» »
50	» »	No »
60	Yellow	» »

The white precipitate formed by addition of bromine was isolated and identified as tribromophenol by its melting point. From the final solution oxalic acid could be isolated and identified as such by its equivalent weight and by the melting point of its derivative with benzylthiocarbamide. Furthermore it was found that the oxygen formed contained considerable amounts of carbon dioxide.

The oxidation process can be explained in the following way:

In the first stage oxygen atoms formed by reaction (1) in the above scheme oxidize benzene to phenol; thus reaction (2) is prevented and no free oxygen is formed. In the later stages phenol is oxidized to dark coloured compounds, probably quinones or quinhydrones which are finally decomposed to carbon dioxide and oxalic acid. When most of the oxidizable substances have disappeared, the evolution of oxygen according to reaction (2) commences.

Merz and Waters have, in their experiments, observed the formation of diphenyl in small yields. I have not tested my solu-

* Evolution of oxygen commences.

Use of Different Alkali Carbonates in the Methylation of Acetylacetone

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In 1894, Claisen [1] reported that Fette had prepared methylacetylacetone by alkylation of acetylacetone with methyl iodide and potassium carbonate in alcohol or ether solution, but he did not give any detailed procedure.

In recent years this method has been of some importance in preparing alkylated β-ketoesters [2,3]. In these cases ketones have been used as solvents.

Fette [1,4] stated that potassium derivatives of β-diketones were markedly more reactive than the sodium derivatives. In order to confirm this and to work out a practical method for the synthesis of alkylated β-diketones, a kinetic study of the methylation of acetylacetone with the aid of methyl iodide and an alkali carbonate has been carried out.

The reaction appears to be of the first order and the reaction velocity is directly proportional to the concentration of methyl iodide. The monomethylation process proceeds about ten times as fast as that of the dimethylation. The monomethylation reaction constant with potassium carbonate is of the order of 1.8×10^{-3} min^{-1} at 769 mm and reflux temperature.

The reaction constants with the other alkali carbonates are: *sodium* carbonate about a fiftieth, *rubidium* carbonate seven times, and *cesium* carbonate ten times that with *potassium* carbonate. This very great difference is perhaps due to the solubility of the carbonate and the acetylacetonate in the reaction medium.

The use of rubidium and cesium carbonate in alkylation processes is therefore very advantageous when the reaction proceeds slowly with potassium carbonate. The disadvantage of the relative high costs of these carbonates can be overcome as they can be readily recovered.

Experimental. Long necked, thin walled, round bottomed micro flasks were made from 15 cm lengths of Pyrex tubing with an external diameter of 6 mm and an internal diameter of 3 mm. The diameters of the completed flasks were 14 mm.

Pyrex wool and a carbonate (23 mg of sodium, 30 mg of potassium, 50 mg of rubidium, or 70 mg of cesium carbonate) were placed in a number of these micro flasks. Small jackets, for reflux condensers, were placed on the necks of the flasks, and the necks were scratched immediately under the condensers. 0.200 ml of an acetone solution, containing 10.00 mg of acetylacetone and 40.000 mg of methyl iodide, were added to each flask with cooling in an ice bath. The mixture was then refluxed by immersing the flasks in a bath at 65°, while alcohol at — 15° was circulated through the condensers. At fixed intervals the neck of a flask was broken, the flask washed, wipped off with a piece of filtering paper and then broken. The contents were washed into an Erlenmeyer flask with 2 per cent acetic acid and the iodide ions formed titrated with silver nitrate using eosine as indicator.

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Received Februar 10, 1950.

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tions for this substance, but it should be noted, that it could be formed by oxygen atoms by a side reaction leading to phenyl- and hydroxyl-radicals or by a ternary reaction between one oxygen atom and two benzene molecules.

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Received February 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 209–227

The Borides of Some Transition Elements

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In 1945, the author started an investigation on the borides of the transition elements. During the years 1945—1949 a great number of binary systems between transition elements and boron have been studied. The investigations have been reported in this journal [1–6] and include the metals chromium, manganese, nickel, zirconium, columbium, molybdenum, tantalum, tungsten, and thorium. They have mainly been concentrated on phase analyses and structure determinations of the intermediary phases. In the present paper a survey of the results will be given, including a discussion of the structures. Some boride structures reported by other investigators are included in the discussion.

EARLIER INVESTIGATIONS

The earlier investigations on borides often are of questionable value, mainly because of the unsatisfactory methods of preparation, the impurity of commercial boron used as starting material, and the great difficulties of isolating and studying the intermediary phases by classical chemical methods. References to these previous works are found elsewhere [7–8]. Some investigations by means of X-ray methods are reported for the iron-, cobalt-, nickel-, and titanium-boron systems [9–12], on the borides of composition MeB_2 [13–15] and MeB_6 [16–19] and on some miscellaneous borides [20–23]. Some of the results have been critically discussed by the present author [1–6].

METHODS OF PREPARATION AND INVESTIGATION, INCLUDING A METHOD FOR PREPARING BORON OF HIGH PURITY

Earlier investigators have used different methods for preparing metal borides, *e. g.* electrolysis of fused salt baths, containing oxides of metal and boron, reduction of oxide mixtures with carbon, and direct synthesis from the

elements. Of these methods, the last seemed to be the most accurate and was used in these investigations. The main problems connected with this method are the great difficulties of getting pure starting materials and of reaching the high temperatures under controlled conditions, which are necessary for the syntheses. The transition metals used were either commercially obtainable in a pure state or could easily be purified. Commercial boron, however, has a content of only 65—80 % of boron. Several methods have been used in order to get pure boron; references are found elsewhere [24]. The methods described only give very small amounts. In order to obtain greater amounts, a new method for preparing boron with a purity of 98—99 % was developed by the author [24]. It consists essentially of the vapour phase reduction of boron tribromide with hydrogen in a silica tube at 750—800° C.

The borides were prepared by sintering or melting the constituents together. This was either done in evacuated silica tubes at 1 100—1 200° C or in vacuum furnaces at 1 200—2 000° C. Two different vacuum furnaces were used, a resistance furnace with a graphite tube as heating element or a high frequency induction furnace. Both were constructed for this investigation and are described elsewhere [25-26].

The usual method of investigation was to heat mixtures of metal and boron in different proportions. Phase analyses of the products were carried out by X-ray methods, and selected alloys were chemically analyzed. The structures of the pure phases were determined by powder or by single crystal methods. More details about the X-ray work are found in the reference papers [1-6].

STRUCTURES OF THE INTERMEDIARY BORIDE PHASES

A general tendency of the boron atoms to form chains, nets, or three-dimensional frameworks with increasing boron content of the systems has been found. The structures will be discussed on the basis of the boron arrangement.

Radius of the boron atom. From phases with small metal atoms, in which a metal-boron or boron-boron contact probably exists, the radius of the boron atom was found to be 0.86—0.88 Å. 0.87 Å may be accepted as a mean value, in close agreement with 0.86 Å, reported for boron in CaB_6 [19], which seems to be the most reliable value from earlier experiments. Distances less than 2×0.86 Å between part of the boron atoms have been observed only for one type of boride phases, the borides with double chains of boron atoms (Table 6). This shortening probably results from the formation of double bonds.

Solubility of boron in the undistorted metal lattice. Solubility of boron was observed in thorium, α-zirconium and in tantalum at higher temperatures.

Table 1. Me_2B-borides of the $CuAl_2$-type.

Metal	r_B/r_{Me}*	a (Å)	c (Å)	c/a	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)
Ta	0.60	5.778	4.864	0.84	2.73	2.47	2.43
W	0.62	5.564	4.740	0.85	2.68	2.38	2.37
Mo	0.63	5.543	4.735	0.85	2.67	2.37	2.37
Mn	0.69	5.148	4.208	0.82	2.37	2.20	2.10
Fe	0.69	5.109	4.249	0.83	2.41	2.18	2.12
Co	0.69	5.016	4.220	0.84	2.37	2.15	2.11
Ni	0.70	4.990	4.245	0.85	2.36	2.14	2.12

* For calculating the radius ratio the values $r_B = 0.87$ Å and r_{Me} = the value for twelvefold coordination of the metal, given by Pauling [30], have been used. The interatomic distances given in the tables contain a few small corrections compared with the original papers.

According to Ehrlich [12], boron is also soluble in α-titanium. For the other metals investigated no solubility was observed.

Borides with isolated boron atoms. To this group belongs a great number of borides of the $CuAl_2$-type (C 16) as well as the closely related δ-phase in the manganese-boron system (Tables 1 and 2). Both types consist of sheets of tetrahedra of metal atoms with boron atoms in the holes between these sheets (Fig. 1). Each boron atom is surrounded by eight metal neighbours at equal distances. For the borides of the $CuAl_2$-type, the radius of the holes varies for different metals between the limits 0.96 Å for nickel to 1.11 Å for tantalum, the boride of which was stable only at lower temperatures and for which the boron content was impossible to determine. For the other structure type with isolated boron atoms (δ Mn-B) the corresponding value 0.98 Å was found. In all the borides with isolated boron atoms, the holes thus are appreciably larger than the boron atoms, if these are assumed to have the radius 0.87 Å. A larger radius for boron in borides with isolated boron atoms than in other borides may thus be possible, but the great variation in the values indicates that the boron atoms are more loosely accomodated in these lattice types than in the other types.

Table 2. δ-phase in the Mn-B system.

Metal	r_B/r_{Me}	a (Å)	b (Å)	c (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)
Mn	0.69	14.53	7.293	4.209	2.41	2.19	2.11

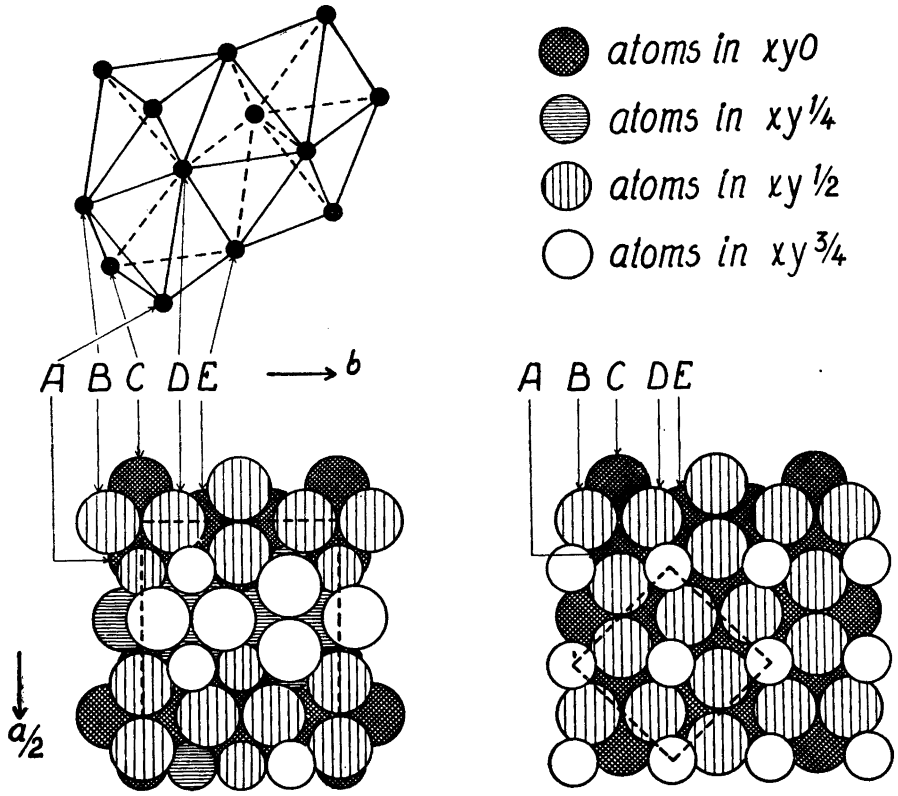

Fig. 1. Borides with isolated boron atoms. (Large circles represent metal atoms, small circles boron atoms.)
Left: δ-phase in the Mn-B system.
Right: Me_2B-borides of the $CuAl_2$-type.

Geometrically, the boron atoms are arranged in strings through the metal lattice. Thus for instance in the borides of the $CuAl_2$-type the boron-boron distance within a string is $c/2$ giving values of the boron-boron distance within the limits 2.11 Å (for cobalt) to 2.43 Å (for tantalum). The distances between nearest boron atoms in different strings is considerably larger (*e. g.* 3.55 for cobalt, 4.09 for tantalum). Also the geometrical arrangement of the boron atoms in the δ-phase in the manganese-boron system is that of strings. The boron-boron distance in a string is 2.11 Å, whereas the distance between nearest boron atoms in different strings is 3.63 Å. The boron atoms in these phases have been regarded as not forming any boron-boron bonds, but the string formation may indicate a tendency for boron-boron bond formation.

Table 3. MeB-borides of the FeB-type.

Metal	r_B/r_{Me}	a (Å)	b (Å)	c (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	Angle between the boron bonds
Mn	0.69	5.560	2.977	4.145	2.67	2.19	1.80	112°
Fe	0.69	5.506	2.952	4.061	2.63	2.15	1.80	110°
Co	0.69	5.253	3.043	3.956	2.60	2.12	1.86	110°

Param. x_B and z_B 0.031 and 0.620 (FeB), 0.037 and 0.625 (CoB). Bjurström's values slightly changed [9].

Table 4. MeB-borides of the MoB-type.

Metal	r_B/r_{Me}	a (Å)	c (Å)	c/a	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	Angle between the boron bonds
W	0.62	3.115	16.93	5.44	2.86	2.23	1.74	127°
Mo *	0.63	3.110	16.95	5.45	2.86	2.23	1.74	127°

* The axes of MoB in the original paper [1] have been given with the values a = 3.105, c = 16.97 Å. These, however, are the values at the lower limit of the homogeneity range (48.8 atomic % boron). The values, given in Table 4, are those obtained for a boride with 50 atomic % boron.

Table 5. MeB-borides of the CrB-type.

Metal	r_B/r_{Me}	a (Å)	b (Å)	c (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	Angle between the boron bonds
Ta	0.60	3.276	8.669	3.157	2.90	2.40	1.86	116°
Cb	0.60	3.298	8.724	3.166	2.92	2.42	1.86	117°
Cr	0.69	2.969	7.858	2.932	2.65	2.19	1.74	115°

Borides with boron chains. Three structure types with the boron atoms forming zig-zag chains have been observed (Tables 3—5). They are closely related to each other. The metal lattices may be regarded as consisting of trigonal prisms connected in different ways, for instance as in Fig. 2. Channels appear through which boron chains are extended in the lattices. Each boron atom is situated in the centre of a trigonal prism of metal atoms and in contact with six metal atoms with their centres at the corners of the prism. A seventh metal atom is situated at a slightly larger distance outside the prism. Each boron atom is also in contact with two other boron atoms.

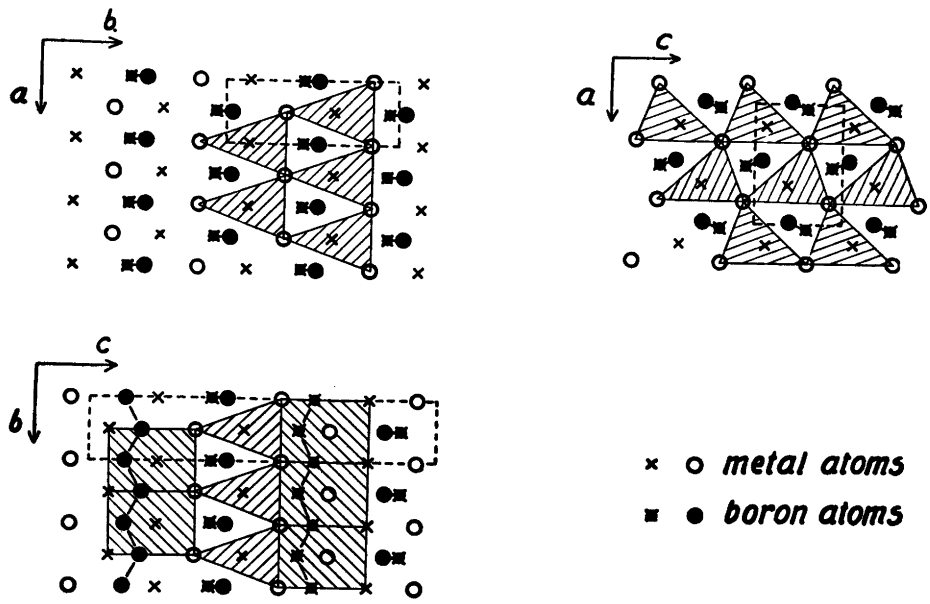

Fig. 2. Projections of boride structures with boron chains. (Circles and crosses are situated at levels 1/2 apart.)
Above left: CrB-type.
Below left: MoB-type.
Right: FeB-type.

The borides of the FeB- and CrB-types seemed to have the fixed composition MeB, whereas those of the MoB-type showed an extended homogeneity range, extending slightly on both sides of the ideal composition MeB (48.8< "MoB"<51.5 atomic % boron, 48.0<"WB"<50.5 atomic % boron). For both these borides, the *a*-axes increase and the *c*-axes decrease with increasing boron content.

There are several possibilities for the explanation of the extended homogeneity ranges. A possibility for a phase with a higher boron content than 50 atomic % would be a lattice in which some of the metal atoms are missing or their positions occupied by boron. Such a lattice is less probable for these borides, however, as a decrease of both *a*- and *c*-axes with increasing boron content would result.

Metal atoms may also occupy positions in the channels, positions which are normally occupied by boron. A phase with such a lattice would exist only for a lower boron content than 50 atomic %, but this alternative may be excluded as the *a*-axis of such a hypothetical lattice would decrease with increasing boron content of the phase.

A third alternative, which already has been suggested by the present author[1], is a variation of the number of boron atoms in the channels. Such a variation would probably be reflected in a variation of the axes of the unit cell in accordance with the observations (this will be clear if the boron-metal and metal-metal contacts of the lattice are considered, Fig. 2). In an ideal MoB-lattice with a boron content of 50 atomic %, all the channels of the metal lattice must contain boron atoms. They are situated in the largest interstices of the channels, and each such interstice is occupied by a boron atom. For a boride with a lower boron content than 50 atomic %, the number of boron atoms per unit length in the channels will be lower than the number of interstices. This may be caused by vacancies in the places occupied by boron atoms at 50 atomic %. This leaves fragments of boron chains with their boron atoms still in the largest holes of the channels. Another possibility is the formation of extended chains, the angle between the boron bonds thus being increased and the boron atoms still being in contact through the whole channel. For such a chain, the largest interstices of the channel and the boron atoms of the chain would no longer be in phase, and the boron atoms would be forced to occupy interstices, which for an ideal phase are too small for a boron atom (the smallest holes of the channels having space for atoms with a radius of only 0.77 Å, compared with 0.87 Å for the largest). The result would be a lattice deformation of the metal, probably periodic.

For a phase with a higher boron content than 50 atomic %, a chain with a greater number of boron atoms per unit length than the number of "ideal" interstices and a uniform distribution of boron atoms over the whole chain is the only probable possibility. Also in this case a periodically expanded metal lattice is to be expected. The angle between the boron-boron bonds in the "compressed" chain would be less than the value for an "ideal" chain with its boron atoms in the largest interstices of the channels.

It is to be noted that the angles between the boron-boron bonds for the chains in the borides of the MoB-type are larger than the corresponding angles for the other borides with boron chains. The tendency for the boron-boron bonds to form a lower angle than in the borides of the MoB-type may be an explanation of the extension of the homogeneity ranges for this type of borides, an extension which has not been observed for the other borides with chains. This tendency would only account for the extension above 50 atomic %, however.

There is finally also a possibility that all the boron atoms are never situated in the largest interstices of the channels but they form chain fragments with a lower bonding angle than that corresponding to the boron atoms situated in the interstices. These chains would thus successively add more

Table 6. Me_3B_4 borides.

Metal	r_B/r_{Me}	a (Å)	b (Å)	c (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å) (in pair)	Distance B-B (Å) (in chain)
Ta	0.60	3.29	14.0	3.13	2.97	2.40	1.54	1.85
Cb	0.60	3.305	14.08	3.137	2.98	2.40	1.58	1.84
Mn	0.69	3.032	12.86	2.960	2.68	2.24	1.47	1.75
Cr	0.69	2.984	13.02	2.953	2.68	2.23	1.51	1.74

boron atoms over the homogeneity range. This possibility is less probable, as the composition MeB seems to be of special importance for the borides with chains.

A deformation of the metal lattice would probably influence the powder photographs in a visible way. A reinspection of these showed that for the molybdenum-boron phase a slight variation in the relative intensities for some reflections existed, if powder photographs from specimens with low and high boron content were compared (*e. g.* 2 1 3 compared with 1 1 10,2 1 5 compared with 2 0 8). The variations were too small to permit any accurate determinations or to draw any conclusions concerning the origin of these variations.

It is unquestionable, however, that for the borides of the MoB-type the number of boron atoms in the channels of the metal lattice varies. But it has not been possible to explain why the borides of the FeB- and CrB-types do not also show any extended homogeneity ranges or where in the channels the boron atoms are situated, when the boron content of the phase differs from 50 atomic %.

Borides with double chains (fragments of nets). In the borides of composition Me_3B_4 (Table 6), the boron atoms form double chains. The double chains may also be regarded as fragments of hexagonal boron nets, and these borides thus have an intermediate position between borides with boron chains and borides with simple boron nets (Fig. 3). The boron-boron distance in the

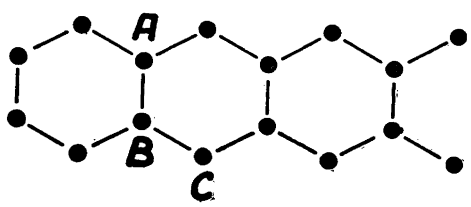

Fig. 3. Double chain of boron atoms.

Table 7. MeB_2-borides of the AlB_2-type.

Metal	r_B/r_{Me}	a (Å)	c (Å)	c/a	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	$\frac{a_{Me}}{a_{Cr}}$	$\frac{c_{Me}}{c_{Cr}}$
(Al)	0.61	3.00	3.25	1.08	3.00	2.38	1.73	1.010	1.060
Zr	0.54	3.169	3.530	1.11	3.17	2.54	1.83	1.067	1.151
Ti	0.59	3.028	3.228	1.07	3.03	2.38	1.75	1.020	1.053
Ta	0.60	3.078	3.265	1.06	3.08	2.41	1.78	1.037	1.065
Cb	0.60	3.089	3.303	1.07	3.09	2.43	1.78	1.040	1.077
V	0.65	2.998	3.057	1.02	3.00	2.31	1.73	1.010	0.997
Cr	0.69	2.969	3.066	1.03	2.97	2.30	1.71	—	—

Table 8. Me_2B_5-borides.

Metal	r_B/r_{Me}	a (Å)	c (Å)	c/a	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)
W	0.62	2.982	13.87	4.65	2.98	2.32	1.72
		3.011	20.93				
Mo	0.63	($r=7.190$, $\alpha=24°\,10'$)		6.95	3.01	2.32	1.74

same half of a double chain (BC) was found to be about 2 r_B, whereas the distance between adjacent boron atoms in different halves always was found to be shorter, about 1.50 Å. This indicates a formation of boron pairs in which the boron atoms probably are connected by double bonds. (The double bond radius for boron is 0.76 Å according to Pauling.) The boron atoms of type "C" are surrounded by six+one metal atoms and two boron atoms in the

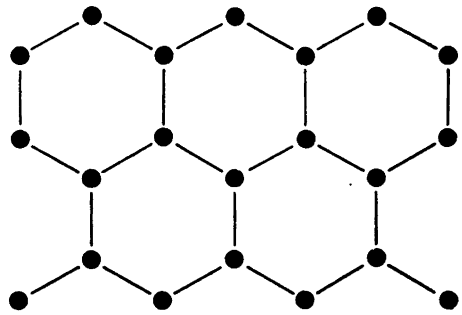

Fig. 4. Hexagonal net (H) of boron atoms.

same way as in the borides with boron chains. The boron atoms of type "B" are surrounded by six metal atoms at the same distance, situated at the corners of a trigonal prism, two boron atoms at the usual boron-boron distance and one boron atom at a shorter distance. All these borides had the fixed composition Me_3B_4; no extension of the homogeneity ranges was observed.

Borides with two-dimensional nets. The next step in the connection of the boron atoms, the formation of hexagonal boron nets (Fig. 4), was observed for several phases. Most of these borides belong to the AlB_2-type (*C* 32, Table 7). In the molybdenum- and tungsten-boron systems, there are phases with the ideal composition Me_2B_5, having more complicated structures (Table 8). The metal lattices of all these phases are closely related, and may be regarded as consisting of close-packed metal sheets (A, B, C) arranged in different ways. In analogy to the usual notation for hexagonal and cubic close-packings (A B A B and A B C A B C resp.), the sequence of metal sheets will be A A B B A A B B for the W_2B_5-type, A A B B C C A A B B C C for the Mo_2B_5-type, whereas the metal lattice for the AlB_2-type is simple hexagonal, A A A. Boron atoms are situated in the interstices between these metal sheets, either forming plane nets (H) with hexagonal meshes or slightly puckered sheets (K) formed by nets of the same kind as H but with additional boron atoms in the centres of some of the meshes. The phases may be described as sequences AHAHAH for the AlB_2-type, AHAKBHBKAHA for the W_2B_5-type, and AHAKBHBKCHCKAHA for the Mo_2B_5-type. For "CbB_2" and "TaB_2" of the AlB_2-type, extended homogeneity ranges were observed. They were extended on both sides of the ideal composition MeB_2, showing the existence of incomplete boron nets at lower boron contents than 66.7 atomic % and complete nets with additional boron atoms in the centres of the hexagonal meshes at higher boron contents [4]. A shortening of the boron-boron distance was observed when additional boron atoms were taken up in the meshes of the boron nets (H). A boron atom in a complete net (H) is surrounded by six metal atoms at equal distances situated at the corners of a trigonal prism with the boron atom in the centre, and three boron neighbours at the usual boron-boron distance.

Borides with three-dimensional boron frameworks. A great number of borides with composition MeB_6 have been reported, the metals also including some which are not transition elements [16–19] (Table 9). They are all isomorphous, and the metal atoms form a simple cubic lattice. X-ray studies on CaB_6(*D* 2_1) have shown that all the boron atoms form a three-dimensional framework with the metal atoms in the interstices. The boron atoms form octahedra. Each boron atom has five boron neighbours at the same distance 1.72 Å, four in the same octahedron and one in a neighbouring. It also has four metal

Table 9. MeB_6-borides of the CaB_6-type.

Metal	r_B/r_{Me}	a (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	$(Me\text{-}B)_{obs}/(r_B + r_{Me})_{calc}$
(Ba)	0.39	4.29	4.29	3.16	1.78	1.02
(Sr)	0.40	4.20	4.20	3.09	1.74	1.03
(Ca)	0.44	4.153	4.15	3.06	1.72	1.08
Yb	0.45	4.14	4.14	3.05	1.71	1.09
La	0.47	4.153	4.15	3.06	1.72	1.12
Ce	0.48	4.137	4.14	3.05	1.71	1.13
Nd	0.48	4.126	4.13	3.04	1.71	1.13
Pr	0.48	4.129	4.13	3.04	1.71	1.13
Gd	0.48	4.13	4.13	3.04	1.71	1.14
Th	0.48	4.16	4.16	3.07	1.72	1.15
Y	0.48	4.08	4.08	3.01	1.69	1.13
Er	0.50	4.110	4.11	3.03	1.70	1.16

neighbours situated so that the four metal atoms and the boron atom form a tetragonal pyramid with boron at the apex and the four metal atoms forming the square base. The boron-metal distance, however, is considerably greater than the sum of the normal atomic radii, e. g. for cerium 3.05 compared with $r_B + r_{Ce} = 2.69$ and for thorium 3.07 compared with 2.67. This may in part depend on the high coordination number of the metal atoms, each metal atom being surrounded by twenty four boron neighbours and six metal neighbours. For those metals which are large enough to expand the boron framework (*e. g.* barium and strontium) the ratio $(r_B + r_{Me})_{obs}/(r_B + r_{Me})_{calc}$ is nearly constant (Table 9), indicating that the increase of the metal radius owing to the high coordination is about 2 % compared with Pauling's values for twelvefold coordination. For the other metals with smaller radii an increase of this ratio is noted, indicating that the boron atoms are in contact, forming a rather stable boron framework with metal atoms in the interstices. The metal-boron bonds are probably rather weak for the smaller metal atoms.

Table 10. Borides of the UB_{12}-type.

Metal	r_B/r_{Me}	a (Å)	Shortest distance Me-Me (Å)	Shortest distance Me-B (Å)	Shortest distance B-B (Å)	$(Me\text{-}B)_{obs}/(r_B + r_{Me})_{calc}$
U	0.57	7.473	5.28	2.79	1.76	1.17

Recently the structure of UB_{12}[23] has been reported (Table 10). The structure, closely related to that of the MeB_6 borides mentioned, is that of a three-dimensional boron framework with uranium atoms in the holes. Each boron atom has five boron neighbours at a distance of about 0.88 Å, and the value of the distance boron-uranium is greater than $r_B + r_U$ as for the MeB_6-borides.

Finally it is of interest to note that these borides, which are close to the boron side of the systems, follow Hume-Rothery's (8-N)-rule, which would give five neighbours for boron.

GENERAL DISCUSSION

The structure types described include all those borides of transition elements of which the structures have been determined. It is to be observed that with the methods used, usually only the positions of the metal atoms have been directly determined. The positions of the boron atoms have been determined from space considerations, assuming that boron atoms occupy the largest holes of the metal lattice. For some phases in the chromium- and manganese-boron systems it has been possible by Fourier methods to obtain boron maxima by studying suitable projections of the electron density. The locations of these maxima have always been in accordance with the results from space considerations and support the results for isomorphous borides of heavier metals.

The existence of boron-boron bonds. A general tendency for the boron atoms to form an increasing number of boron-boron contacts with increasing boron content of the phases has been found. These contacts may be only a consequence of a convenient space arrangement of the atoms or they may involve boron-boron bonds, probably of a covalent type. The experimental evidence clearly shows that the contacts are of bonding character. The fact that boron chains appear in three different types of metal lattices is itself a strong support for the existence of real bonds within the chains. If the axes of the orthorhombic unit cells of the isomorphous borides TaB and CrB are compared (Table 5), the ratios a_{Ta}/a_{Cr} and b_{Ta}/b_{Cr} are 1.103, whereas the ratio c_{Ta}/c_{Cr} is only 1.077. Comparing CbB and CrB the corresponding values are $a_{Cb}/a_{Cr} = 1.111$, $b_{Cb}/b_{Cr} = 1.110$ but $c_{Cb}/c_{Cr} = 1.080$. The lattice is thus expanding less in the direction of the c-axis, *i. e.* the direction of the boron chains, than in the other directions when going from the smaller chromium to the larger tantalum and columbium atoms. For the Me_3B_4-borides with double chains of boron atoms (Table 6), the ratios are $a_{Cb}/a_{Mn} = 1.090$, $b_{Cb}/b_{Mn} = 1.095$ and $c_{Cb}/c_{Mn} = 1.060$, the double chains all being extended in the c-direction. The variation of the parameter for the metal atoms, which occurs when going from

the smaller atoms of chromium and manganese to the larger atoms of columbium and tantalum ($y_{Cr} = y_{Mn} = 0.186$, $y_{Cb} = y_{Ta} = 0.180$), also verifies the existence of boron-boron bonds. The distance within the boron pair (A—B, Fig. 3) only increases from 1.47 to 1.58 Å for this variation of parameter, instead of increasing to 1.69 Å, as would occur if the parameter remained constant 0.186 (comparing manganese and columbium).

Similar effects are visible for the nets. In Table 7, the ratios a_{Me}/a_{Cr} and c_{Me}/c_{Cr} have been calculated for the different borides MeB_2 of the AlB_2-type. With increasing radius of the metal atom, the lattice is expanding more in the direction of the c-axis than in the direction of the a-axis (the only exception is for VB_2, the axial lengths of which have been taken from literature [15]). The boron nets are all arranged normal to the c-axis, making an expansion in the c-direction easier than in the a-direction when the radius of the metal atom increases. As has already been pointed out elsewhere by the author [4], a decrease of the boron-boron distance is noted (observed by the decrease of the distances between certain metal atoms) when additional boron atoms are taken up in the holes of an hexagonal boron net. The decrease is caused by the forces between the boron atoms of the rings and the additional atoms in the ring centres.

For the three-dimensional boron frameworks, as has already been pointed out, the increased boron-metal distance, compared with the normal radius sum, is an indication of a rigid boron framework.

The existence of boron-boron bonds has thus indirectly been verified. It has not been possible to determine directly the electron density within these boron bonds. This in part results from the low scattering power of boron compared with that of the metals, and in part from the effects of termination of the Fourier series, caused by the small number of reflections obtainable from these unit cells which are usually small and highly symmetrical.

Analogies between borides and hydrides, carbides and nitrides. Hydrides, borides, carbides and nitrides of the transition elements form a group of phases with characteristic properties, as was pointed out by Hägg [27–28]. These compounds have metallic properties. If the ratio of the radius of the non-metal (r_X) to the radius of the metal (r_{Me}) is less than 0.59, the small non-metallic element is accomodated in a suitably sized hole in the simple metal lattice. The metal lattice belongs to one of the four types face-centred cubic, body-centred cubic, hexagonal close-packed or simple hexagonal. If r_X/r_{Me} is greater than 0.59 the structures are more complicated. Hägg's conclusions were mainly based on observations of hydrides, carbides and nitrides, few borides being known at that time. The present investigations have shown that as long as boron appears isolated in the metal lattice the borides are

analogous to hydrides, carbides and nitrides. Solid solutions of boron in the expanded lattices of the pure metals have been observed for thorium ($r_B/r_{Th} = 0.48$) zirconium ($r_B/r_{Zr} = 0.54$), titanium ($r_B/r_{Ti} = 0.59$), and tantalum ($r_B/r_{Ta} = 0.60$). For the other metals, all having a ratio greater than 0.59, no solubility has been observed by the present author or is reported in the literature. The systems with tantalum, columbium ($r_B/r_{Cb} = 0.60$) and titanium with a ratio near the critical limit are of special interest. They all seem to have an intermediate position between simple and complicated systems. This is shown by the fact, that simple phases exist at higher temperatures but are transformed to more complicated phases at room temperature. Also for the borides a critical radius ratio thus seems to exist with a value of about 0.59—0.60. The existence of a critical ratio indicates that a simple metal lattice can not be expanded beyond a certain limit. This would imply that the critical limit is also related in some way to the limit of solid solubility in the lattice of the pure metal. The experimental evidence is insufficient, however, to make any conclusions possible. Further experiments are also necessary in order to determine whether the type of metal lattice has any influence on the critical limit r_X/r_{Me}.

The boron atoms, as has been shown, have a marked tendency to form covalent boron-boron bonds. For borides in which the boron atoms are bonded in this way, the limit 0.59 seems to be of less importance. Simple hexagonal metal lattices are thus observed in the borides of AlB_2-type for radius ratios between 0.54 (for zirconium) and 0.69 (for chromium). The dominating factor seems to be the hexagonal boron nets, which easily fit into a simple hexagonal metal lattice. The simple cubic metal lattice, observed for the MeB_6-borides, is probably also only a result of a convenient space arrangement, isolated metal atoms being accomodated in a boron framework.

The stability of the hydrides, carbides, and nitrides decreases with increasing atomic number of the transition elements within a period. Data on the stability of the borides have not been obtained and many of them are apparently inert and stable at higher temperatures. A comparison of the boron contents of the phases of the elements of a period richest in boron shows, however, that the same tendency also seems to exist for the borides. In the first transition period the phases richest in boron have a boron content (in atomic %) of at least 67 for titanium and vanadium, 67 for chromium, 57 for manganese, 50 for iron, cobalt, and probably nickel. Attempts to prepare borides of copper have been hitherto unsuccessful. For the second period, this tendency is less obvious (67 for zirconium and about 70 for columbium and molybdenum) but for the third the same tendency is visible again (86 for lanthanum and some lanthanides, 72 for tantalum and 68 for tungsten).

Finally, it has been confirmed that the borides have metallic properties in analogy with the other members of this group. The results thus verify that the borides of the transition elements may be placed in the same group as the hydrides, carbides and nitrides, but the existence of boron-boron bonds is of importance and must be specially considered.

Application of Pauling's theory. A general theory for the metallic state has been given by Pauling [29-31]. According to this theory, the metallic bond is of the same nature as the covalent bond, but weaker. It thus applies the theory of the electron-pair bond, *dsp*-hybridisation of "pure" *d*, *s*, and *p*-orbitals and resonance. A method for calculation of bond numbers for different bonds and of the valency of different atoms is given, using the interatomic distances. The present author has tried to use this theory for the borides, but without success. If for instance the bond number for the metal-boron bond is calculated for the isomorphous Me_2B-borides, the following values are obtained: 0.28 for tantalum, 0.34 for molybdenum and tungsten, 0.42 for manganese, 0.44 for iron, 0.49 for cobalt, 0.48 for nickel. This increase in bond number may be an indication of an increasing strength of the metal-boron bond, but it may also merely be a consequence of the method of calculation. Although Ta_2B, the boride giving the smallest bond number, is not stable at higher temperatures contrary to the other borides of this group, the difference in bond numbers seems too high. As all these compounds are isomorphous and thus probably energetically similar, it seems rather incredible that such a great variation in bond number for the same metal-boron bond exists. According to a rule, given by Pauling in [31], the bond numbers should have a great tendency to form small integer ratios, *e. g.* 1/2, 1/3, 2/3, 1/4 *etc.* No such tendency is found for the bond numbers discussed, however, the numbers increasing continuously when going from tantalum to cobalt.

Pauling has also used the theory on FeB [31] for which he assumes that electrons are transferred from the iron lattice to the boron to a maximum extent of about one electron per metal atom. The present author has studied all the borides with chain structures in an analogous way. Also for these borides there is a disagreement between isomorphous compounds, and the results can only be interpreted if one assumes that electrons are transferred from the metal lattice to the boron chains. Such an electron transfer is rather difficult to correlate with the properties of the borides with chain structures. All of these borides undoubtedly are metallic and the metallic properties indicate, contrary to Pauling's calculations, that electrons are transferred to the metal lattice from the boron chains and that these borides may be regarded as expanded metal lattices with boron chains in the interstices. These additiona

electrons of the metal lattice compensate for the loss in "metallic" properties, which would be a result of the increased metal-metal distance.

The formation of boron chains with covalent boron-boron bonds and an angle between two adjacent bonds of roughly about 120° is also difficult to explain, if electrons were transferred to the chains from the surrounding metal lattice. The electron configuration of a boron atom is $1s^2 2s^2 2p^1$, thus with three electrons in the second quantum level. If electrons are transferred to the boron chains, which appears according to Pauling, each boron atom would get between three and four electrons available for bond formation. With nearly four electrons per atom, however, a tendency for boron-boron double bonds would probably be visible. Neither the boron-boron distance observed nor the angle of about 120° between the bonds formed are compatible with this view. A double bond is considerably shorter than the distances observed, and the angle between two double bonds formed by the same boron ion probably would be 180°, by analogy with carbon. On the contrary a loss of electrons is more in accordance with the existence of covalent bonds within the chains. If in the ideal case one electron per boron atom is lost to the metal lattice, two electrons would be left, which is exactly the number necessary for each boron to form two covalent single bonds. It is thus more probable that a chain of the kind existing would be stabilized by a loss of electrons from the boron than by a transfer of electrons to the boron.

It is also of interest to note that Engel has put forth a theory for the electron distribution in metal lattices [32] which is in accordance with the above assumption. According to this theory, a body-centred metal lattice is characterized by an electron concentration of one $(s + p)$-electron per metal atom, an hexagonal close-packed lattice by two such electrons and a cubic close-packed lattice by three. Regarding the metal lattice of the borides of CrB-type, an obvious relationship between this lattice and an hexagonal close-packed lattice is noted (Fig. 5), although the former is very deformed. The metal lattices of the other two boride types with chains (MoB- and FeB-types), are similar to the CrB-lattice. If the present author's opinion of the electron transfer in these borides is correct, in the ideal case one electron per boron atom would be transferred from the boron chains to the metal lattice and the electron concentration of the metal would increase by one electron per metal atom. The borides with boron chains are formed by the metals chromium, columbium, tantalum, molybdenum, tungsten, manganese, iron and cobalt. All these metals at room temperature crystallize with cubic body-centred lattices with the exception of manganese and cobalt. Manganese, however, has a lattice not so different from the body-centred arrangement whereas cobalt is known in an hexagonal close-packed as well as a cubic close-packed modification. By

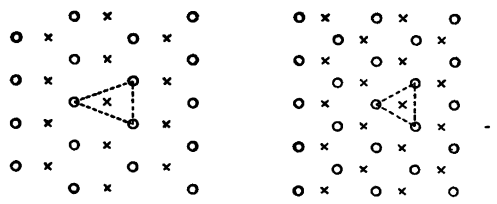

Fig. 5. Metal lattice of CrB (left) projected on the ab-plane, compared with an hexagonal close-packed metal lattice (right).

electron transfer from the boron chains in these borides, the electron concentration of the metal lattice increases. The borides of metals with a body-centred structure thus will have a tendency to possess an hexagonal close-packed metal lattice in accordance with the experimental evidence. Cobalt boride is the only exception, but it is to be noted that the axial ratio for CoB is considerably different from that of the isomorphous MnB and FeB ($a:b:c$ = 1.87 : 1 : 1.39 for MnB, 1.87 : 1 : 1.38 for FeB but 1.73 : 1 : 1.30 for CoB). Titanium and zirconium, which both crystallize in hexagonal close-packed lattices at room temperature, do not give any boride phases with boron chains. Nor have such phases been observed for copper or nickel with cubic close-packed lattices, in accordance with the view expressed above.

It is also of interest to compare these borides with Cr_3C_2[33] ($D\ 5_{10}$), the only known carbide with carbon chains. The lattice of this carbide is in many respects related to that of FeB, and it has zig-zag shaped carbon chains with a carbon-carbon distance of the order of magnitude of a single bond. Such a chain would in the ideal case be stabilized by a loss of two electrons per carbon atom to the metal lattice. The content of non-metallic element thus should be lower than the 50 atomic % found for boron, if the electron concentration of the metal lattices is of the same order of magnitude, in accordance with the experimental evidence.

Finally an electron transfer from the chains to the metal lattice would also account for the similarities in physical properties between these borides and the true interstitial compounds, where an electron transfer in the direction mentioned in some instances has been experimentally verified. The case of positive hydrogen in metal hydride lattices is wellknown. An electron transfer from the interstitial atoms to the metal lattice has also been suggested to occur in the ε and ζ carbonitrides of iron [34]. The effective atomic radius of carbon is less than that of nitrogen in these compounds. Since carbon replaces nitrogen within wide limits, without any marked structural change, each carbon atom is assumed to lose the same number of electrons (or the same fraction of an electron) as a nitrogen atom. The relative loss of electrons would thus be

greater for carbon than for nitrogen and might account for the smaller effective radius of carbon.

The experimental evidence thus indicates, according to the present author's opinion, that in the borides with boron chains there is an electron transfer from the chains to the metal lattice, contrary to Pauling's theory. There is a possibility that all these borides are to be regarded as chain analogs to the true interstitial compounds with isolated interstitial atoms. As the tendency for the chains to lose electrons is greater than for isolated atoms, the importance of Hägg's radius ratio is less in the former case. For the borides with double chains and nets, all or nearly all of the electrons of boron are engaged in the formation of boron-boron bonds, and the electron transfer boron-metal is probably small. The simple metal lattices observed for these borides are merely a result of a convenient space arrangement by which the metal-metal coordination still remains high. They have metallic properties, but Hägg's radius ratio is of no importance.

SUMMARY

A comprehensive discussion of the structural work on the borides of the transition elements is given, including tables of axial lengths and interatomic distances. The structures are discussed with respect to the arrangement of the boron atoms.

The boron atoms appear isolated, in chains, in double chains, in nets or in three-dimensional boron frameworks. The crystallographic evidence clearly shows that the boron-boron contacts are of bonding character.

Similarities between borides and hydrides, carbides, and nitrides exist, but Hägg's critical radius ratio is valid only if the boron atoms appear isolated in the metal lattices.

Pauling's theory for the metallic state seems not to be applicable to the borides. For the borides with chains, for instance, according to Pauling an electron transfer from the metal to the boron chains occurs. The present author thinks, according to the experimental evidence, that an electron transfer in the opposite direction is more probable.

The present investigation of the metallic borides has been carried out at the Institute of Chemistry, University of Uppsala, during the years 1945–1949. I here wish to express my gratitude to my inspiring teacher, Professor G. Hägg, for suggesting the work, for valuable discussions and for never failing support. I am also very grateful to my colleagues at the Institute for stimulating discussions and good fellowship. My thanks are due to several students for valuable help, to Mrs. M. Englund and Mr. R. Nordlund for

preparing the figures in this and preceeding papers and to Mr. S. Wahlgren for technical assistance. This investigation has been supported continously by the *Swedish State Council of Technical Research*, which support is gratefully acknowledged.

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Received January 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 228–238

Studies on the Sulphonation of Lignin. I *

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The fundamental reaction of the normal sulphite process is the sulphonation of lignin with formation of soluble lignin sulphonic acids. The pioneer work of Hägglund showed that a solid, insoluble lignin sulphonic acid is first formed [1]. Delignification takes place in later stages of the cook due to a hydrolytic process accompanied by further sulphonation. In this way, lignin sulphonic acids are obtained containing about one sulphonic acid group per two methoxyl groups.

So-called strong sulphite pulp still contains a considerable amount of solid, insoluble lignin sulphonic acids, generally in the form of calcium salts.

Kullgren found that the corresponding free acids, when heated with water or alcohols, are converted into water soluble acids [2]. This process is obviously due to a hydrolytic fission of linkages connecting neighbouring lignin molecules or lignin and carbohydrate molecules (in the strong pulp). The insolubility of the solid lignin sulphonic acid must be due to the presence of these large molecular complexes.

By sulphonating wood with strong solutions of sodium sulphites, possessing great buffer capacities and varying pH, Hägglund and Johnson were able to obtain low sulphonated wood containing far more solid, insoluble lignin sulphonic acids than strong pulp [3]. When subjected to the Kullgren process, they furnished large amounts of soluble low sulphonated lignin sulphonic acids. By variation of the pH of the sulphonation liquor, and stepwise performance of the Kullgren process, it is possible to obtain low sulphonated lignin sulphonic acids possessing varying contents of sulphonic acid groups. An additional advantage of this method is that the sulphonic acids are obtained in a state of high "purity". The largest portion of these acids can be preci-

* Part 46 on the Chemistry of the Sulphite Process by E. Hägglund and co-workers.

pitated with 4,4'-bis-dimethylamino-diphenylmethane. "Low sulphonated lignin sulphonic acids" obtained in this way have been employed in this investigation and in various other recent studies in these laboratories [4].

When heated with ordinary sulphite cooking acid, the low sulphonated lignin sulphonic acids are rapidly converted into more highly sulphonated products. Ultimately, acids containing more sulphonic acid groups are formed than those generally obtained from ordinary technical waste liquors. This is apparently due to the mild conditions under which the acids have been prepared. It is possible to carry out the sulphonation stepwise. The ultraviolet absorption spectra of low, medium, and high sulphonated materials, with relation to the methoxyl content, have been found to be almost identical [4]. This shows that no double bonds which are conjugated with aromatic nuclei are formed or disappear during the sulphonation. No fundamental changes in the lignin structure appear to take place during this sulphonation, and it may be concluded that the low sulphonated lignin sulphonic acids contain non-sulphonated lignin elements of probably the same general structure as the original lignin in the wood. Hence, in these low sulphonated lignin sulphonic acids we apparently possess almost ideal materials for the investigation of the later stages of the sulphonation of lignin.

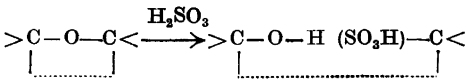
The nature of the groups responsible for the sulphonation reaction has been the subject of much speculation. Omitting the less probable suggestions, it is sufficient to mention only two.

Based on extensive analytical material, Holmberg concluded that the sulphonation essentially involves a substitution of sulphonic acid groups for hydroxyl groups of outstanding reactivity [5]. In α-phenethyl alcohol he found a suitable model substance [6]: $C_6H_5—CH(OH)—CH_3 \xrightarrow{H_2SO_3} C_6H_5CH(SO_3H)CH_3$. The yield and rate of reaction were, however, not very satisfactory. This may explain, at least partly, why Holmberg's idea has not been very generally accepted. In a recent review, v. Wacek, for instance, states that substitution of benzyl alcoholic hydroxyl groups can only account for a fraction of the total sulphonation of lignin [7].

Recently one of the present authors showed that more lignin-like analogues of Holmberg's α-phenethyl alcohol, *e. g.* vanillyl alcohol or veratryl alcohol or their homologues, undergo very facile conversions into the corresponding sulphonic acids [8], and the investigations were extended to complex ethers of the same type [9]. Hence the above mentioned objections to Holmberg's view are no longer valid. If this theory were correct, the sulphonation of lignin would be accompanied by a loss of hydroxyl groups.

Based on various considerations and model experiments, Freudenberg has

suggested a different mechanism. He assumes that the sulphonation of lignin is due to the fission of a heterocyclic ring followed by sulphonation [10]:



As a matter of fact, Freudenberg's view is related to that of Holmberg, since he assumes that the ring is of a benzyl ether type. Holmberg has shown that such ethers are cleaved by thioglycolic acid in a similar way [11].

If Freudenberg's theory were correct, the sulphonation of lignin should be accompanied by the liberation of one hydroxyl group for every sulphonic acid group introduced.

In several papers and reviews, Freudenberg points out that both types of reactions may occur simultaneously. However, he obviously considers the ring opening reaction to predominate, and in calculating the lignin composition from, for example, lignin sulphonic acids, he "prefers to subtract H_2SO_3" while Holmberg subtracts SO_2 [12].

It was originally supposed that a phenolic hydroxyl group is formed when the ring is opened. Later Freudenberg, Lautsch, and Piazolo obtained results which were difficult to reconcile with this assumption [13]. Instead they suggested that a tetrahydrofurane ring, of the type occurring in pinoresinol, is cleaved. However, pinoresinol and its alkyl ethers have not been found to react with the formation of sulphonic acids under the conditions of the normal sulphite cook [14].

The authors have attempted to settle the question of the appearance or disappearance of hydroxyl groups when sulphonic acid groups are introduced into the lignin molecules, by following analytically the changes in hydroxyl content when low sulphonated lignin sulphonic acids are progressively sulphonated. It is clear that from such studies nothing can be said about the mechanism of the introduction of the sulphonic acid groups already present in the low sulphonated lignin sulphonic acids employed. The experimental results described below cover about fifty per cent of the reactive groups in lignin, and this may perhaps indicate that the conclusions may be extended to include, at least, most of the active groups in the lignin molecules.

MATERIAL

The low sulphonated lignin sulphonic acids were prepared according to a method previously described [4]. They represent about twenty five per cent of the total lignin of the wood. Their sulphur content was approximately one sulphur atom per three C_{10}-elements (= three methoxyl groups), and they could be sulphonated to about one sulphur

atom per 1.4 C_{10}-elements. Part of the sulphur in these high sulphonated preparations is "loosely bound" — the amount of "alkali stable" sulphur being approximately one sulphur atom per 1.5 lignin elements. The recovery of lignin after resulphonations, based on methoxyl estimations, was about 60—75 per cent (compare Table 4).

HYDROXYL ESTIMATIONS

The acetylation method was selected for the estimations of the hydroxyl content of the various sulphonic acids.

The first problem was to find derivatives of lignin sulphonic acid suitable for acetylation. Sodium or barium lignin sulphonates could not be properly acetylated, mainly due to their insolubility in the acetylation mixture. The free lignin sulphonic acids are very unstable in the dry state. It was therefore considered advisable to avoid the use of desiccated acids.

Finally, it was found that the pyridine salts of the lignin sulphonic acids were fairly well suited for the purpose. Even in this case, however, it was difficult to obtain homogeneous reaction mixtures, and it was found necessary to repeat the acetylations three times in order to secure a constant acetoxyl content. Later investigations showed that this difficulty could be overcome by vigorous mechanical stirring of the reaction mixture. In this way some preparations were obtained containing slightly more acetoxyl groups than the corresponding ones obtained by repeated acetylations, but the difference is unsignificant (compare Tables 1 a and b).

First the well known acetylation method of Verley-Bölsing was tried. Due to various reasons, *e. g.* the difficulty to obtain perfectly dry starting material, the retention of acetic acid within the gelatinous acetylation product, and, especially in the case of high sulphonated lignins, the loss of some sulphur during the acetylation, this method had to be abandoned. It was also attempted to measure the increase in equivalent weight, caused by the acetylation, by means of potentiometric titrations, but this method also failed for similar reasons.

Finally, it was found that the isolated acetylated pyridinium salts could be analysed according to Clark[15] with reproducible results. The preparations were deacetylated with alkali and the acetic acid recovered by steam distillation of the acidified solution. Some sulphur dioxide was simultaneously formed and had to be taken into consideration.

RESULTS AND DISCUSSION

Table 1 gives the composition of the barium salts of four different lignin sulphonic acids (A, B, C, and D). The last three were obtained by successive sulphonation of acid A.

A Ac, B Ac, C Ac, and D Ac are the corresponding acetylated pyridinium salts.

Table 1. Analyses of barium salts of the lignin sulphonic acids A, B, C, and D, and of the pyridinium salts of the corresponding acetylated lignin sulphonic acids (A Ac, B Ac, C Ac, and D Ac).

Ba-salt of	A		B		C		D	
Pyridinium salt		A Ac		B Ac		C Ac		D Ac
%								
CH_3O	13.06	10.96 (10.5)[1]	12.73	10.25 (10.9)[1]	11.58	10.18 (9.7)[1]	9.75	9.34 (8.4)[1]
S	4.19	3.07	5.73	3.54	5.74	4.18	8.24	6.12
Ba	9.2	—	10.9	—	13.1	—	19.8	—
N	—	1.22	—	1.47	—	1.94	—	2.50
C	53.3	57.9	51.2	58.6	47.6	57.7	40.2	52.6
H	4.79	5.32	4.54	5.27	4.28	5.15	3.62	4.84
CH_3COO	—	26.6	—	23.9	—	21.0	—	15.4
S (loosely bound)	0.1	—	—	—	0.18	—	0.68	—
$\frac{S}{OCH_3}$	$\frac{1}{3.3}=0.30$[2]	$\frac{1}{3.7}=0.27$	$\frac{1}{2.3}=0.44$	$\frac{1}{3.0}=0.33$	$\frac{1}{2.2}=0.46$[2]	$\frac{1}{2.5}=0.40$	$\frac{1}{1.3}=0.75$[2]	$\frac{1}{1.6}=0.63$
$\frac{Ba}{2S}$	$\frac{1}{0.98}=1.02$		$\frac{1}{1.13}=0.89$		$\frac{1}{0.94}=1.07$		$\frac{1}{0.89}=1.12$	
$\frac{N}{S}$		$\frac{1}{1.10}=0.91$		$\frac{1}{1.05}=0.95$		$\frac{1}{0.94}=1.06$		$\frac{1}{1.07}=0.93$
$\frac{CH_3COO}{OCH_3}$		1.28		1.23		1.08		0.87
$\frac{S+CH_3COO}{OCH_3}$		1.55		1.56		1.48		1.50

[1] Calculated value based on the methoxyl and barium content of the original salt and the acetoxyl content of the acetylated pyridinium salt. The formation of a small amount of sulphur dioxide during the acetylation was ignored.

[2] "Loosely bound" sulphur is subtracted.

Table 1 a. Control acetylations of the pyridinium salt of preparation A with strong mechanical stirring. Analyses of pyridinium salts.

Time of acetylation. Hours	3	6	9	3	6	9	3	6	9	3	6	9
Per cent acetic anhydride in the acetylation mixture	10			50			10			50		
Temperature	100°			100°			50°			50°		
OCH_3 per cent	10.35	10.33	10.30	10.43	10.30	10.17	10.41	10.37	10.42	10.57	10.51	10.41
CH_3COO per cent	25.0	26.2	26.1	24.9	25.5	25.8	16.2	20.6	20.7	15.1	16.9	20.9
$\frac{CH_3COO}{OCH_3}$	1.27	1.33	1.33	1.25	1.30	1.33	0.82	1.04	1.04	0.75	0.85	1.06

Table 1 b. Control acetylations of the pyridinium salt of preparation D with strong mechanical stirring. Analyses of pyridinium salts.

Time of acetylation Hours	6	9	6
Per cent acetic anhydride in the acetylation mixture	10	10	50
Temperature	100°	100°	100°
OCH_3 per cent	9.01	9.02	8.99
CH_3COO per cent	16.0	17.0	16.6
$\frac{CH_3COO}{OCH_3}$	0.93	0.99	0.97

From Table 1 (last line) it can be seen that the number of sulphonic acid groups plus hydroxyl groups per methoxyl group is practically constant (1.48—1.55) in the various sulphonic acids. Under the assumption that no obscuring rearrangements occur during the sulphonation process (this assumption appears to be probable in view of the constancy of the ultraviolet absorption) this result shows that the sulphonation is mainly due to a substitution of a hydroxyl group by a sulphonic acid group.

The average, 1.52, of the quotients of the last line in Table 1 also represents the number of hydroxyl groups per methoxyl in the "original lignin" (S = nil), if it is assumed that no extrusion of water took place when the low sulphonated acid A was formed.

When comparing the quotients sulphur : methoxyl in Table 1, it is seen that the acetylation of each preparation is accompanied by the expulsion of a certain amount of sulphur. The amount is not particularly great, but large enough to exceed the amount of "loosely bound sulphur" present in the preparations. About 0.1 sulphur atom is expelled per methoxyl group in all cases except for the preparation with the lowest degree of sulphonation, from which only very little sulphur is split off.

In Table 2, the atomic composition of the lignin sulphonic acids is given on the customary C_9-basis. The values are calculated from the analyses given in Table 1. (OH calculated from the acetylated pyridinium salts, assuming that no methoxyl groups disappear on acetylation; other calculations based on the corresponding barium salts.) In Table 2 (a) it has been assumed that the sulphonic acid groups expelled during the acetylation have been exchanged for acetoxyl groups, and in (b) that no other groups were introduced when the sulphonic acid groups were extruded.

Table 2. Atomic composition of lignin sulphonic acids.

Sulphonic acid	C	H a	H b	OCH_3	—O— a	—O— b	OH a	OH b	SO_3H
A	9	6.6	6.6	0.94	1.0	1.0	1.17	1.20	0.28
B	9	6.6	6.5	0.96	0.7	0.6	1.06	1.17	0.41
C	9	6.9	6.8	0.94	1.4	1.3	0.94	1.01	0.44
D	9	7.2	7.1	0.93	1.3	1.2	0.71	0.81	0.77

By substituting OH for SO_3H and calculating the average atomic composition of the "lignin" corresponding to the acids A—D in Table 2 (a), the following is obtained:

$$C_9H_{6.8}(OCH_3)_{0.94}\ O_{1.1}OH_{1.45}$$

Table 2 (b) gives in the same way:

$$C_9H_{6.8}(OCH_3)_{0.94}O_{1.0}OH_{1.53}$$

In an interesting study of the stepwise delignification of spruce wood with sulphite cooking acid at 70°, which has been briefly referred to above, Freudenberg, Lautsch, and Piazolo obtained a series of lignin sulphonic acids of increasing sulphur content. The corresponding free acids were desiccated at 55° and the hydroxyl contents estimated according to the method of Verley-Bölsing. From the analytical results recorded, the following average composition of these acids (OH instead of SO_3H) has been calculated:

$$C_9H_{7.0}(OCH_3)_{0.91}O_{0.76}(OH)_{1.58}$$

This formula agrees tolerably well with those of our preparations from low sulphonated lignin sulphonic acids.

It is of interest to compare these figures for the "lignin" from lignin sulphonic acids, with those for "lignin" obtained from various thiol acid lignins obtained by Holmberg in 1942 [5]. (Holmberg gives no figures for hydroxyl.) He finds the following average composition:

$$C_9H_{8.91}(OCH_3)_{0.92}O_{2.85}$$

The corresponding figures calculated from Table 2 (a or b) are:

$$C_9H_{8.2}(OCH_3)_{0.94}O_{2.5}$$

This composition agrees fairly well with that of Holmberg's preparations, if it is assumed that the "lignin" from the lignin sulphonic acids contains approximately 0.3 H_2O per C_9-unit less than that from Holmberg's thiol acid lignins.

Peniston and McCarthy dialyzed technical waste sulphite liquor and obtained preparations of the following compositions [16]:

$$C_9H_{7.2}(OCH_3)_{0.94}O_{2.75}$$

$$C_9H_{7.0}(OCH_3)_{0.95}O_{2.84}$$

The composition of these lignins differs considerably from those reported above — the hydrogen figures being remarkably low in spite of the high oxygen content.

We have also calculated the quotients sulphur : methoxyl and hydroxyl : methoxyl as well as (sulphur + hydroxyl) : methoxyl in the various sulphonic acids of Freudenberg and his collaborators and the result is shown in Table 3.

Table 3. Relations of sulphur, hydroxyl, and sum of sulphur and hydroxyl to methoxyl in a series of lignin sulphonic acids of increasing sulphur content prepared by Freudenberg, Lautsch, and Piazolo.

Sulphonic acid preparations	1	2	3	4	5	6	7	8
S/OCH_3	0.51	0.47	0.48	0.56	0.70	0.84	0.96	1.50
OH/OCH_3	1.25	1.30	1.18	1.11	1.09	0.99	0.88	0.95
$(S + OH)/OCH_3$	1.76	1.77	1.66	1.67	1.79	1.83	1.84	2.45

The sum of sulphonic acid and hydroxyl groups is constant also in this series of sulphonic acids. Only the last preparation (8) is distinctly different. This material, however, was obtained in a minute yield and represents only about one per cent of the total lignin of the wood. The results of Freudenberg, Lautsch, and Piazolo are in accordance with the view that lignin is sulphonated essentially by substitution of hydroxyl groups by sulphonic acid groups.

EXPERIMENTAL

Resulphonation of low sulphonated lignin sulphonic acids

A sample of the barium salt of A, (Table 1) corresponding to 4.4 g dry barium salt, was dissolved in water and the solution passed through a cation exchanger ("Wofatit K") saturated with hydrogen ions. Calcium carbonate (1.5 g) was added to the filtrate, and

the volume made up to 100 ml. Sulphur dioxide was passed through the solution until the total amount of sulphur dioxide was about 5 per cent. The solution was heated in a sealed tube at 135° for various lengths of time. The sulphonic acid preparations B, C, and D (Table 1) were obtained in this way from A by heating 1 ¼ hours (B), 2 hours (C), and 10 hours (D).

The tubes were opened, nitrogen passed through the solutions until almost all free sulphur dioxide had been driven off, the calcium ions exchanged for hydrogen ions by means of passage through Wofatit K, nitrogen again passed through the solution to remove sulphur dioxide, and finally barium carbonate added to neutralize the solution. This was filtered, concentrated in a vacuum to about 50 ml, refiltered or centrifuged from a small amount of barium sulphite, and the clear solution poured into five volumes of alcohol. The precipitated barium lignin sulphonates were collected by centrifuging, washed several times with alcohol, and finally with ether. For analysis the samples were dried at 80° in a vacuum over P_2O_5 until constant weight was reached (about seven days). The recovery of lignin based on methoxyl estimations was somewhat varying — generally about 70 per cent. (For examples compare Table 4.)

Table 4. Yield of high sulphonated lignin sulphonic acids on sulphonation of low sulphonated lignin sulphonic acids.

Low sulphonated lignin sulphonic acid. Ba-salt		Cooking acid		Time of heating at 135°, hours	High sulphonated lignin sulphonic acid. Ba-salt		
g	S/OCH_3	ml	% SO_2		Yield g	S/OCH_3	% yield based on OCH_3
4.4	0.37	100	5.2	1.5	2.8	0.46	63
4.4	0.37	100	6.8	3.0	3.8	0.64	76
4.4	0.37	100	7.0	6.0	3.3	0.62	66
4.1	0.31	100	7.6	0.75	3.6	0.37	75
4.2	0.31	100	8.0	10.0	3.5	0.82	66

Preparation of pyridinium salts of acetylated lignin sulphonic acids

By repeated acetylation (Table 1): Water solutions of the barium salts of the acids A—D were passed through hydrogen ion saturated Wofatit K and the filtrates neutralized with pyridine. The solutions were evaporated over sulphuric acid in vacuum desiccators. The dry material was powdered, and ten times its weight of acetylation mixture (10 per cent solution of acetic anhydride in pyridine) was added. After 24 hours at room temperature (for swelling), the mixture was heated for three hours in boiling water in a flask fitted with a calcium chloride tube. Ether was then added and the precipitated, acetylated pyridinium salt was powdered under ether, carefully washed with ether, collected, and dried to constant weight in a vacuum desiccator over sulphuric acid. The drying required a long time (about three weeks). In order to obtain completely acetylated products the acetylation procedure was repeated 3—4 times.

By acetylation with stirring (Tables 1 a and 1 b.): 0.5 g of the pyridinium salts of the lignin sulphonic acids A and D were added to acetylation mixtures (10 ml) containing pyridine and acetic anhydride in the proportions 1 : 1, resp. 9 : 1. The mixtures were heated at 50 or 100° for varying times with efficient mechanical stirring. The low sulphonated material (A) dissolved completely in the acetylation mixture. The highest sulphonated preparation (D) failed to yield a perfectly clear solution. The acetylated products were isolated by precipitation with ether.

Estimations of acetoxyl groups

The method of Clark was employed [15]. The saponification, however, was carried out with 1 N aqueous, instead of alcoholic, potassium hydroxide, because the preparations were more soluble in water than in alcohol. Some sulphur dioxide passed over together with the acetic acid. The distillate was therefore titrated with 0.03 N barium hydroxide to get the sum of acetic and sulphurous acid. Some hydrogen peroxide was then added and the water evaporated. The barium sulphate formed was collected by filtration and weighed.

The correction for sulphur dioxide was equal to about 1—2 % acetoxyl groups.

SUMMARY

Low sulphonated lignin sulphonic acids have been subjected to stepwise sulphonation with ordinary sulphite cooking acid. By acetylation of the pyridinium salts of the various lignin sulphonic acids obtained and complete analysis of the preparations, it has been found that for each sulphonic acid group introduced into the low sulphonated lignin sulphonic acid about one hydroxyl group disappears.

It is concluded that, at least in the later stages of the sulphonation of lignin, the sulphonation involves the substitution of hydroxyl groups of outstanding reactivity. The experiments cover approximately fifty per cent of the groups which become sulphonated.

The expenses for this investigation were defrayed partly by grants from *Statens Tekniska Forskningsråd* and partly by economic support from *Cellulosaindustriens Centrallaboratorium*, Stockholm. The studies were commenced at that laboratory, and we wish to express our sincere gratitude to Professor E. Hägglund for his interest in this work, which was undertaken as the result of discussions with him.

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Received November 12, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 239–244

The Splitting with Silver Salts of the Cysteine-porphyrin Bonds in Cytochrome c

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When acid acetone is added to hemoglobin, myoglobin, catalases, horse radish peroxidase, cytochrome a and b, the prosthetic group is released from the protein part, which precipitates, while the prosthetic group remains in the dissolved state. In the cases of hemoglobin [1] and horse radish peroxidase [1] this fact has been used for studies of the linkages between protein and prosthetic group. If the same treatment be applied to cytochrome c the whole molecule is precipitated without fission, and thus its porphyrin-protein linkages are in this respect more stable. The thio-ether bridges from the side chains 2 and 4 of the porphyrin skeleton to the protein [2] are very likely not affected by acid acetone. Starting with the assumption that, if these linkages could be broken, it should be possible to split cytochrome c into free protein and prosthetic group and to open possibilities to carry out further studies on the heme-linked groups, we have been searching for a tool for that splitting.

Holmberg [3] used mercuric chloride to release thioglycolic acid from S-methylthioglycolic acid. Because of the oxidizing power of the mercuric ions, the acid deteriorated. Peters and Wakelin [4] reported the splitting of thio-ethers with silver salts under mild conditions.

In preliminary experiments, we found the ability to break the cysteine-porphyrin bonds in cytochrome c to be common to several metals. We tried silver, mercurous, mercuric, lead, cupric, cadmium, ferric, calcium, and magnesium salts, all of which except the three last mentioned liberated the prosthetic group so that it was extractable with ether in the presence of sufficient quantities of acetic acid or remained in the liquid phase upon the addition of an excess of acid acetone. We have used silver salts to avoid undesirable oxidation and double salt formation.

After splitting of cytochrome c at faintly acid reaction the acetone precipitated protein was soluble in water to give a slightly straw-coloured, clear solution of pH 4—5. An increase in pH to about 7 gave a slight opalescence, which grew to a thick precipitate at pH 9, the precipitation being reversed by acidification. If the excess of silver had been previously removed by dialysis, the precipitate formed at pH 9 redissolved at higher pH. The nitroprusside reaction was negative at room temperature and alkaline reaction, but turned to be positive immediately upon the addition of cyanide.

Since the reaction is carried out in an acid medium, the cytochrome c, completely oxidized, shows the absorption band at 610—640 mμ. This band does not alter during the reaction. After the addition of acid acetone and centrifugation, the acetone solution of the iron-porphyrin shows a band of the »hemin» type with a maximum at 625 mμ. The iron-porphyrin from cytochrome c shows the same solubility properties as most other hemins. Of a special interest is, however, its high solubility in alcohols, decreasing with increasing number of carbon atoms in the alcohols up to *n*-octanol (no higher alcohols tried). The iron-porphyrin could be crystallized from aquous *n*-butanol and glacial acetic acid. During the isotope investigations at this institute to be reported elsewhere, use was made of this high solubility, which permits a solution of the hemin from cytochrome c in *n*-butanol to be washed with 5 % hydrochloric acid or water for the removal of inorganic and ferritin iron.

The velocity of the splitting has been studied under some different conditions. In the first experiments we used silver nitrate, but to avoid oxidation due to the nitric acid the sulfate was preferred for the final modification. In all cases the progress of the splitting, measured in terms of ether-acetic acid extractable porphyrin bound iron or light absorption of the aceton solution, was found to follow closely curves for first — order reactions. The velocity constants were obtained by plotting log $a/(a-x)$ versus time and the slopes of the straight lines determined with the method of least squares, the velocity constants then being equal to $2.303 \times \frac{\mathrm{dlog}\ a/(a-x)}{\mathrm{d}t}$, where a is the total reacting amount and x the amount reacting in the time t.

The results are shown in Figs. 1—3, and details are given in the experimental part. The indices of k refer to the number of the experiment as given in the experimental part.

It is outside the scope of this paper to interpret kinetically these results and elucidate the reaction mechanism. The binding of silver ions by carboxyl groups and by enolized peptide bonds may be negligible at these pH values [5],

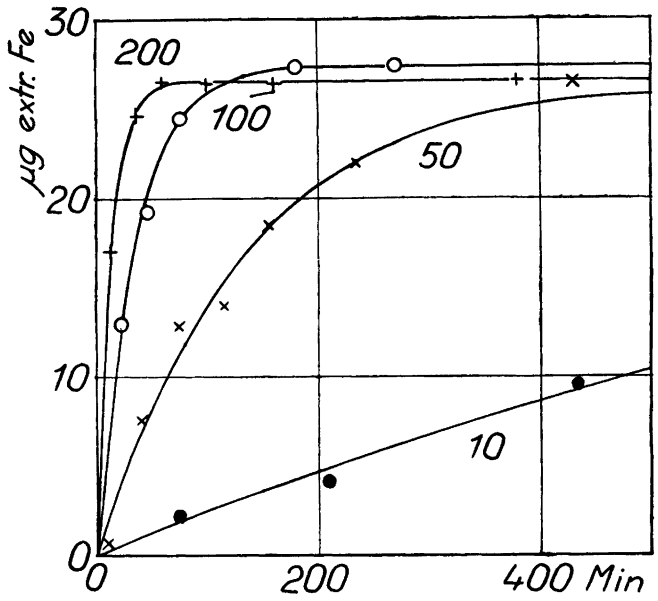

Fig. 1. Influence of ratio silver salt/cytochrome on the reaction velocity. $k_1 = 7.68 \times 10^{-2}\ min^{-1}$. $k_2 = 3.08 \times 10^{-2}\ min^{-1}$. $k_3 = 7.53 \times 10^{-3}\ min^{-1}$. $k_4 = 9.6 \times 10^{-4}\ min^{-1}$.

but the behaviour of the four residual sulphur atoms in cytochrome c, not linked to the porphyrin, is still unknown. In experiments 2, 5, and 6 the progress was followed simultaneously to the iron determinations by diluting samples with the buffers used in the experiments and determining the positions and optical densities of the Soret bands. The velocity constants obtained in this way were about 50 % higher than those from the iron determinations. In experiments 5 and 6 the top of the band migrated from 408 to 391—92 mµ, whereas it in experiment 2 was consistently found at 391 mµ.

We have not determined the rate of disappearence of silver ions from the solutions.

From experiments 2 and 7 the heat of activation was calculated according to the Arrhenius formula as 19 700 cal./mole, from experiments 8 and 9 as 19 700 cal./mole, and from experiments 9 and 10 as 16 400 cal./mole.

EXPERIMENTAL PART

Two different preparations of cow heart cytochrome c were used for the experiments reported in this paper, both of which had been obtained according to Keilin and Hartree [6], and both of purity 0.95 (0.41 % iron).

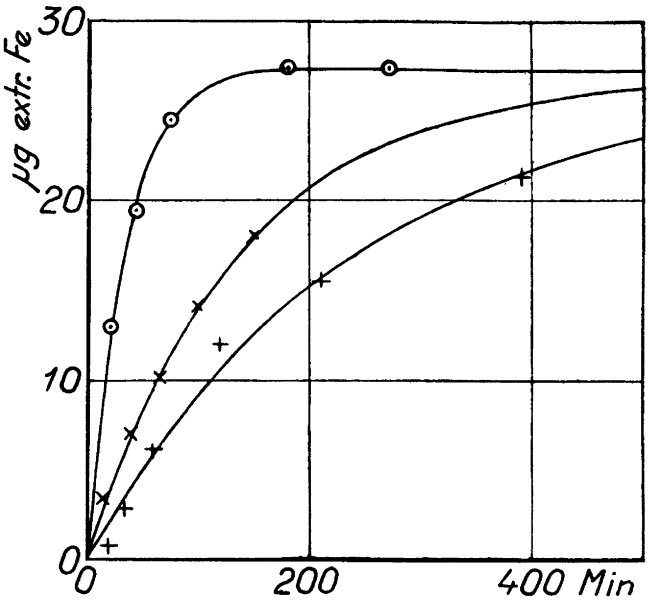

Fig. 2. Influence of acidity on the reaction velocity.
+ × ⊙ refer to expts. 5, 6, and 2 respectively.
k_5 = 4.08 × 10^{-3} min^{-1}. k_6 = 7.35 × 10^{-3} min^{-1}.
k_2 = 3.08 × 10^{-2} min^{-1}.

Influence of the ratio silver salt/cytochrome on the reaction velocity (Fig. 1.)

Expt. 1. 3.92 ml 1.37 × 10^{-3} *M* cytochrome c + 1.074 ml 1.00 *M* silver nitrate + 5.00 ml glacial acetic acid (200 moles of silver nitrate/mole cytochrome). Temp. + 40° C. After certain times samples of 1.00 ml were withdrawn and extracted with 3 × 3 ml ether-glacial acetic acid 3 : 1, the combined extracts were then analyzed for iron. The splitting was carried out in a 40 ml closed vessel, protected against light.

Expt. 2. Cytochrome and glacial acetic acid as above, but with 0.536 ml silver nitrate + 0.54 ml distilled water. Extraction was carried out as above. Silver nitrate/cytochrome = 100.

Expt. 3. As above, but 0.268 ml silver nitrate + 0.81 ml distilled water. Silver nitrate / cytochrome = 50.

Expt. 4. As above, but 0.054 ml silver nitrate + 1.03 ml distilled water. Silver nitrate / cytochrome = 10.

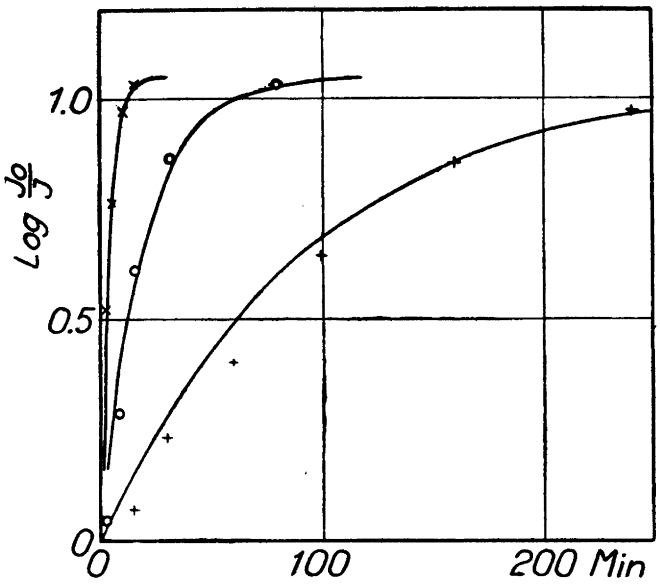

Fig. 3. Influence of temperature on the reaction velocity.
$k_8 = 2.79 \times 10^{-1}$ *min*$^{-1}$ *(80° C, ×).* $k_9 = 5.16 \times 10^{-2}$ *min*$^{-1}$ *(60° C, ○).*
$k_{10} = 1.06 \times 10^{-2}$ *min*$^{-1}$ *(40° C, +).*

Influence of acidity on the reaction velocity (Fig. 2)

Expt. 5. 3.92 ml 1.37×10^{-3} *M* cytochrome + 0.536 ml 1.00 *M* silver nitrate + 0.54 ml distilled water + 5.00 ml acetate buffer of pH 4.6, ionic strength 0.1.

Expt. 6. As above, but with acetate buffer of pH 4.0, ionic strength 0.1. Compare experiment 2.

Influence of temperature on the reaction velocity

Expt. 7. As expt. 2, but temperature + 20° C. $k_7 = 3.52 \times 10^{-3}$ min^{-1} Compare experiment 2.

From these experiments we decided to use the following mixture for the standardised procedure. To 1 ml of the cytochrome solution should be added 0.2 ml glacial acetic acid and 1 ml of the silver salt solution. As mentioned above, the sulfate was substituted for the nitrate. Our silver sulfate solution contained 800 mg/100 ml ($M = 2.57 \times 10^{-2}$).

Some experiments on the influence of heat with the standardised reaction mixture and another preparation of cytochrome were then made. In 10 ml centrifuge tubes were taken 0.50 ml 9.09×10^{-4} *M* cytochrome solution, 0.10 ml glacial acetic acid, and 0.50 ml silver sulfate solution. The tubes were closed, immersed in a thermostat, removed after different times, cooled in tap water, and the contents precipitated with 10 ml acid acetone (10 ml 5 *N* sulfuric acid to 500 ml acetone). The protein residues were washed with 2 ml acid acetone. The light absorption at the wavelength for maximal absorption in the Soret band region (determined in each case and consistently found to be 392 mμ) of the combined extracts was measured after their dilution to 75 ml with acid acetone (Fig. 3).

In the following papers we will describe the analyses of the split products and a micromethod for the determination of cytochrome in tissues.

SUMMARY

In confirmation of the thio-ether configuration of cytochrome c we have found, that salts of some heavy metals release the prosthetic group from the protein part, so that the former can be extracted with ether-acetic acid or remains in the dissolved state upon the addition of an excess of acid acetone. Velocity constants for some given reaction conditions have been determined. This splitting seems to be useful in experiments, where the iron-porphyrin of cytochrome c has to be isolated, *e. g.* for isotope work.

The author's thanks are due to *Stiftelsen Thérèse och Johan Anderssons minne* for a grant.

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Received January 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 245–250

On the Autoxidation of Linoleic Acid in Aqueous Colloidal Solution

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The autoxidation of the unsaturated fatty acids has in recent years been extensively studied in a number of laboratories. The results have been reviewed by Farmer [1], Bergström and Holman [2], Swern, Scanlan and Knight [3] and Lundberg [4].

This work has mainly been concerned with the early phases of the autoxidation at lower temperatures and, in order to minimize the influence of the carboxyl group, esters have generally been used as such or diluted with hydrocarbon solvents. In several cases it has been found that the main primary autoxidation product contains a hydroperoxidic group attached to a carbon atom in α-position to a double bond.

In the case of linoleic acid and similar compounds with methylene interrupted double bonds, it has further been found that an absorption band at 232 mμ appears that is caused by the conjugation of the double bonds [5–9]. In the earlier stages of the autoxidation the absorption band increases parallel with the oxygen uptake and hydroperoxide formation. When the reaction is continued the autoxidation of this conjugated system leads to concurrent extensive polymerisation which generally occurs when substances containing conjugated double bonds autoxidize. The reaction product also becomes progressively more complex as the autoxidation proceeds due to secondary reactions of the hydroperoxidic group, resulting in the formation of hydroxylic, epoxidic, carbonylic, carboxylic groups etc.

The free radical mechanism that has been proposed by Farmer for the hydroperoxide formation and the double bond conjugation seems to have been well established through kinetic studies in some cases [10]. However, very little is known about the later stages of the autoxidation when the hydroperoxides decompose in various ways and the second molecule of oxygen is absorbed.

The spectral changes and the products formed in the enzymatic oxidation of linoleic acid and other similar compounds in aqueous media with soy bean lipoxidase has been investigated by Bergström [11,13], Holman [12,14] and others.

In this very rapid reaction the same spectral changes as in the ordinary autoxidation were found to occur and the same hydroxystearic acids were isolated after hydrogenation of the reaction products. With the pure crystalline enzyme at low temperatures (0—5°) the increase in absorption at 232 mμ corresponded to the formation of one pair of conjugated double bonds for every oxygen molecule absorbed [13,14]. In the ordinary autoxidation at 20—50° temperatures the absorption only reaches at most two thirds of this value. In the enzymatic oxidation, however, extinction values in this range were also observed when the reaction was run at higher temperatures (37°).

The action of the enzyme lipoxidase is apparently limited to the uptake of one mole oxygen per mole linoleate whereas in the autoxidation of methyl linoleate there is no sharp break in the oxygen uptake that continues even after the uptake of more than two moles oxygen per mole fatty acid ester (Franke [15]).

We were interested to know how the aqueous suspension of sodium linoleate behaved in this respect and to compare the autoxidation in aqueous media with the enzymatic oxidation under similar conditions. The autoxidation in an aqueous system also seems of interest from a biochemical point of view.

The rate of oxygen uptake in the earlier phases of the autoxidation of sodium linoleate in phosphate buffers has been investigated by Smith and Stotz [16]. They found the rate depending on the amount of copper ions in the solution and also investigated the influence of various antioxidants forming complexes with copper ions. These authors or earlier workers in this field do not seem to have followed the ultraviolet spectrum during the autoxidation of linoleate in aqueous media nor the autoxidation to the final stages.

RESULTS

We have followed the autoxidation of sodium linoleate in a system similar to that used for the lipoxidase work referred to above. The sodium salt of 2 mg linoleic acid in 1.5 ml redistilled water containing borate buffer pH 9 was shaken in Warburg vessels at 37° in an atmosphere of air. The oxygen uptake curves shown in Fig. 1 show the influence of copper ions at different concentrations. Without any addition of copper salts an induction time of more than 24 hours was observed and this time was progressively shortened as the concentration of copper ions increased. At the same time the maximum speed of oxygen

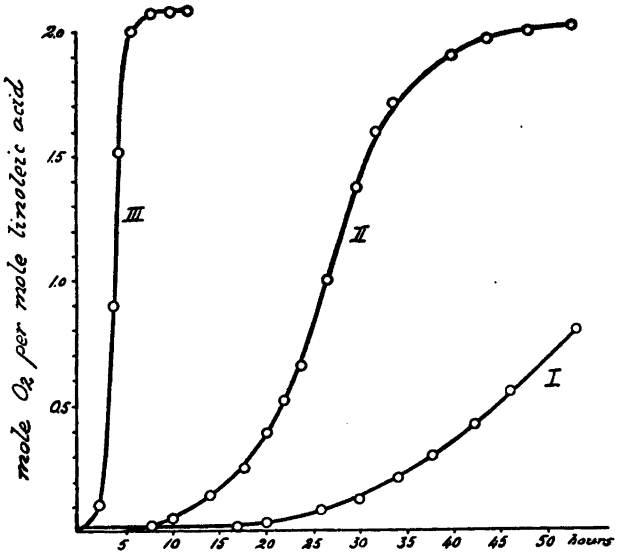


Fig. 1. Influence of cupric ions on the rate of oxygen uptake of sodium linoleate in aqueous colloidal solution. I. No addition of $CuCl_2$. II. 10^{-6} M $CuCl_2$. III. 10^{-5} M $CuCl_2$.

uptake increased. The more or less S-shaped curves always levelled off when 2 moles of oxygen per mole fatty acid had been taken up.

In similar runs in 10^{-5} molar $CuCl_2$ solution the reaction was stopped at different levels. A sample was taken out and diluted with ethanol as described in the experimental part and the ultraviolet absorption spectrum determined. In Fig. 2 the molar extinction of the linoleate at 232 mμ from a number of different runs are plotted against the oxygen uptake.

If the extinction is calculated per mole oxygen absorbed the values of the earlier parts of the curve are in the range $\varepsilon = 15$—$20\,000$. These parts thus resemble the corresponding curve for the autoxidation of methyl linoleate when shaken as such in an oxygen-containing atmosphere at this temperature and also the earlier phases of the enzymatic oxidation at this temperature although the speed is very much slower ($>$ 100 times) in the nonenzymatic reaction.

As the oxydation proceeded (Fig. 2) the ultraviolet absorption increases at a progressively slower rate. It then leveled off when approximately one mole of oxygen had been absorbed, and then gradually decreased so that when the oxygen uptake had ceased at two moles the absorption band at 232 mμ had almost disappeared.

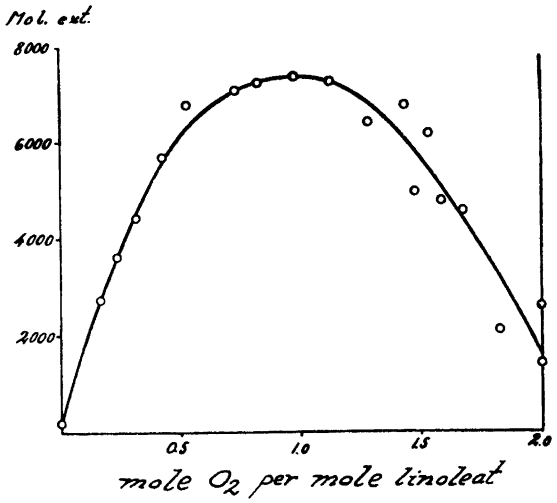


Fig. 2. Molar extinction at 232 mμ of linoleate at different stages of the autoxidation in aqueous medium.

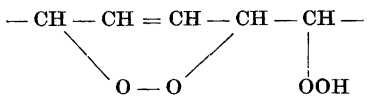
That a similar decrease of the ultraviolet absorption at 232 mμ occurs when ethyl linoleate is oxidized as such appears probable from the figures in a paper by Holman [21], where the spectrum has been determined on autoxidizing linoleate containing carotene. Recently Allen, Jackson and Kummerow [22] have published similar observations.

The peroxide content was also determined on aliquots with the ferric thiocyanate method of Balls *et al.* [17]. The curve showing the peroxide content at various oxidation levels roughly had the same shape as the curve of the ultraviolet absorption. The peroxide content reached a maximum when about one mole oxygen per mole fatty acids had been absorbed and then decreased as the oxygen uptake proceeded. The content, however, was not reduced to such a low level as the ultraviolet absorption but about 0.5—1 mol of peroxidic groups remained. However, this method is known to give too high values [18].

In a preparative run 2 g linoleic acid were oxidized under similar conditions until two moles oxygen per mole fatty acid had been absorbed. The reaction products were extracted from the aqueous phase after careful acidification with hydrochloric acid. The ether residue was a light mobile oil with a strong odor indicating that some chain splitting had taken place.

The dispersion in micellar form in the aqueous medium thus seems to have inhibited the extensive polymerization that occurs when the undiluted acid or ester autoxidizes (*cf.* Treibs [19]). The sharp break in the oxygen uptake curve after the uptake of two moles of oxygen also indicates that the product of the autoxidation might be of a fairly reproducible composition.

It appears possible that monomeric peroxides of the general structure I are formed by autoxidation of the primary hydroperoxide with conjugated



double bonds. This method might be of value to obtain material to determine the structure of the compound of linoleate with two moles of oxygen, a compound normally difficult to obtain due to the marked polymerization in the ordinary autoxidation [19].

Further discussions as to the structure of the primary and secondary reaction products and the reaction mechanism must await further chemical work. These preliminary results are being published because the work must be discontinued for the present.

EXPERIMENTAL

Autoxidation of sodium linoleate. To 100 mg linoleic acid was added 0.37 ml 1.0 *N* NaOH. The resulting gel was stirred until homogeneous and distilled water was then added drop by drop with vigorous stirring. The solution was then made up to 50 ml in a volumetric flask with 0.1 *M* sodium borate buffer pH 9. One ml of this solution and 0.5 ml of the cupric chloride solution was added in the main compartment of the Warburg vessel. These autoxidations were made in air at 37° with 100 oscillations per minute.

The ultraviolet absorption was determined with a Beckman ultraviolet spectrophotometer on suitable dilutions of the reaction mixture with alcohol. Peroxide determinations were made according to Balls, Axelrod and Kies [17]. The determinations were made at 535 mμ in a Coleman spectrophotometer. Linoleic acid was prepared by the method of McCutcheon [20].

SUMMARY

The autoxidation of sodium linoleate in aqueous solution under conditions similar to those of the lipoxidase standardization has been investigated.

The rate of oxygen uptake is dependent on the concentration of cupric ions. The maximum oxygen uptake is two moles per mole linoleate. The absorption at 232 mμ first increases and then decreases to low values.

The reaction product with two moles of oxygen does not show any signs of polymerization.

This work is part of an investigation supported by *Statens naturvetenskapliga forskningsråd.*

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Received December 22, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 251–259

The Crystal Structure of the Epsilon Isomer of 1,2,3,4,5,6-Hexachloro*cyclo*hexane

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The determination of the structure of this isomer of hexachloro*cyclo*hexane is a part of the investigations of the *cyclo*hexane derivatives, which are being carried out at the University of Oslo by O. Hassel and co-workers. The crystal structure and the configuration of the molecule have in this case been found directly from the X-ray data without any a priori assumptions about the mutual arrangement of the atoms.

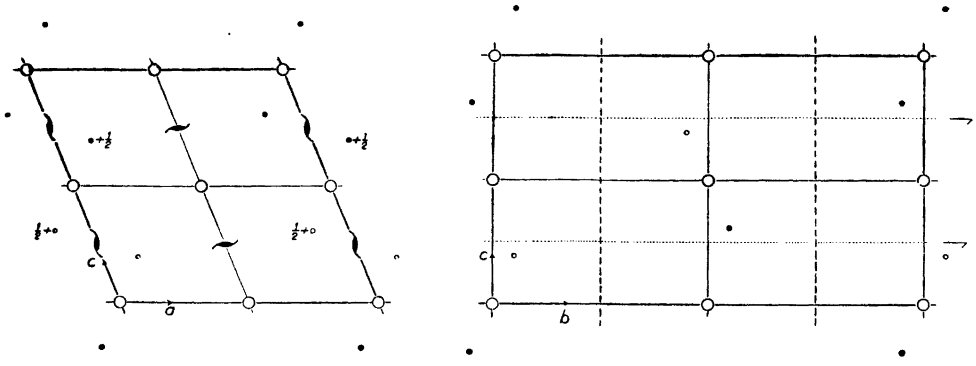
EXPERIMENTAL

The isolation of the epsilon isomer and crystallographic data for it have been described by Kauer, Du Vall and Alquist[1]. The crystal system is monoclinic, and the unit cell contains two molecules. By means of rotation and Weissenberg photographs the dimensions of the cell were found to be $a = 7.02$ A, $b = 11.79$ A, $c = 6.80$ A and $\beta = 112°$. The data given by Kauer *et al.*[1] are $a = 6.94$ A, $b = 11.66$ A, $c = 6.77$ A, $\beta = 111°$. The space group is $C_{2h}^5 - P2_1/c$. The dipole moment of the molecule is said to be zero, a result which was confirmed by measurements carried out in Oslo[2].

In order to determine the intensities, two complete sets of Weissenberg photographs were taken, the first set by rotating the crystal about the a-axis, the second one by rotation about the b-axis. Unfiltered Cu-radiation was used, and the intensities, about 500 in all, were measured on an arbitrary scale by a visual comparison between the $K\alpha_1$, the $K\alpha_2$, the $K\alpha_1 + K\alpha_2$ and the $K\beta_1$ spots.

DETERMINATION OF THE PARAMETERS OF THE CHLORINE ATOMS

Figs. 1 a and b show the 4-fold position of the atoms ($x, y, z; \bar{x}, \bar{y}, \bar{z}; \bar{x}, \frac{1}{2} + y, \frac{1}{2} - z; x, \frac{1}{2} - y, \frac{1}{2} + z$) and the symmetry elements in the unit cell of



Figs. 1 a and b: Symmetry elements and equivalent positions in space group

$C_{2h}^5 - P2_1/c$

O *centre of symmetry*

⌇ , — — — → *2-fold screw axis*

— — — — — *glide plane*

O. ● *general positions of the atoms.* O *and* ● *signify* + *y and* − *y respectively in Fig. 1 a,* + *x and* − *x in Fig. 1 b.*

the space group $C_{2h}^5 - P2_1/c$. As the crystal has a 2-fold screw axis parallel to the *b*-axis, the Patterson-Harker section $P(u, \frac{1}{2}, w)$ may be used to determine the projection of the atoms along this axis. The vector distance from the origin to a maximum in the synthesis $P(u, \frac{1}{2}, w)$ gives twice the vector distance of the corresponding atom from the screw axis. Further the presence of a glide plane gives the possibility to determine the projection of the atoms parallel the *ac*-plane on to the *b*-axis, as the distance of a maximum from the origin in the section $P(0, v, \frac{1}{2})$ gives twice the distance of the corresponding atom from the glide plane.

Figs. 2 and 3 show the sections $P(0,v, \frac{1}{2})$ and $P(u, \frac{1}{2}, w)$ respectively. We may only expect to find the 3 "space group" peaks (also named Harker peaks) [3] due to the chlorine atoms. Fig. 2 in fact gives 3 well separated peaks. The positions correspond to:

$$2y_1 = 0.376 \qquad 2y_2 = 0.260 \qquad 2y_3 = 0.142$$

In Fig. 3, however, 6 peaks appear. Probably the 3 maxima labelled A, B and C correspond to "space group" peaks. First they are the highest ones and next they all lie close to the *a*-axis. The fact that all the maxima in the Patterson projections $P(u, w)$ and $P(v, w)$ (Figs. 4 and 5) have approximately

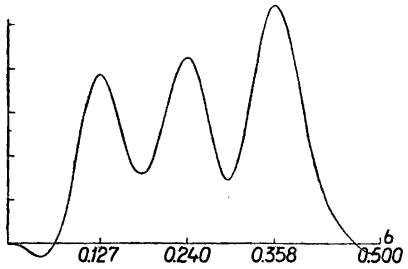


Fig. 2. Patterson synthesis $P(0,v,\frac{1}{2})$. The ordinates are given on an arbitrary scale. The same scale is used in all Patterson sections. The contours in the 2-dim. syntheses correspond to $P(u, v, w) = 1$, 2, 3, — — — —

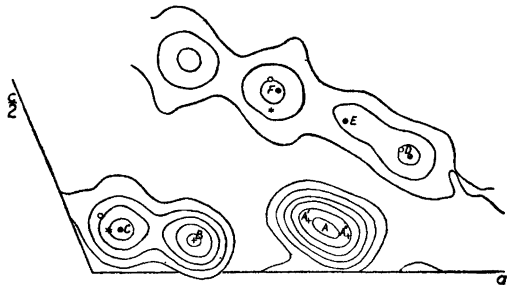


Fig. 3. Patterson synthesis $P(u, \frac{1}{2}, w)$. + denote chlorine space group peaks, ○ carbon space group peaks. ✳ and ● denote peaks arising from vector distances between not symmetry-equivalent chlorine atoms and vector distances between chlorine and carbon atoms respectively, having v-values in the interval 0.5 ± 0.06.

the w-values 0 and 1/2 seems to indicate either that the "space group" peaks in Fig. 3 all lie close to the a-axis (giving z-values $\pm$ 1/4), or are all near the line $w = 1/2$ (giving $z = 0$). The peaks labelled D, E and F in the same figure must in that case be due to Cl—Cl or Cl—C distances, where the vector separations parallel to b are close enough to 1/2 for the peaks to appear in the distribution $P(u, \frac{1}{2}, w)$.

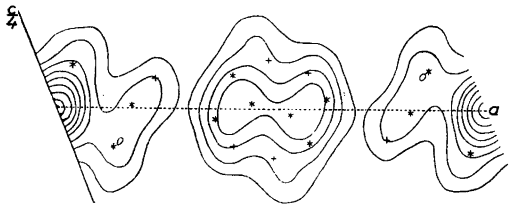


Fig. 4. Patterson projection on (010). Contours are drawn at equal intervals on an arbitrary scale. + and ✳ denote the system of vector peaks arising from the chlorine atoms, the frequency of the distances ✳ being twice the distances +.

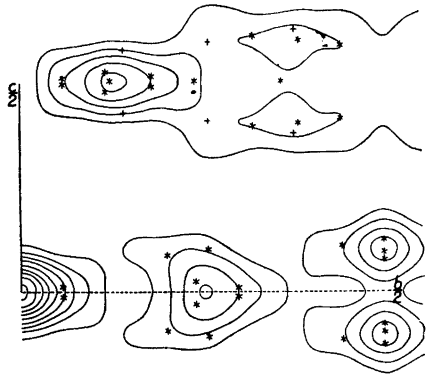
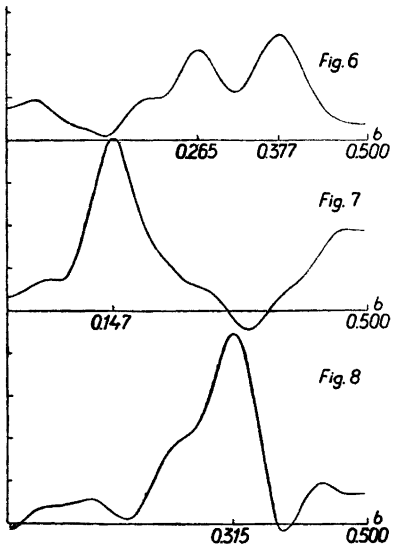


Fig. 5. Patterson projection on (100). The contours and the crosses have the same meaning as in Fig. 4.



Figs. 6—8. Fig. 6 represents the Patterson synthesis $P(2x_A, v, 2z_A)$. Fig. 7 the synthesis $P(2x_B, v, 2z_B)$ and Fig. 8 the synthesis $P(2x_C, v, 2z_C)$.

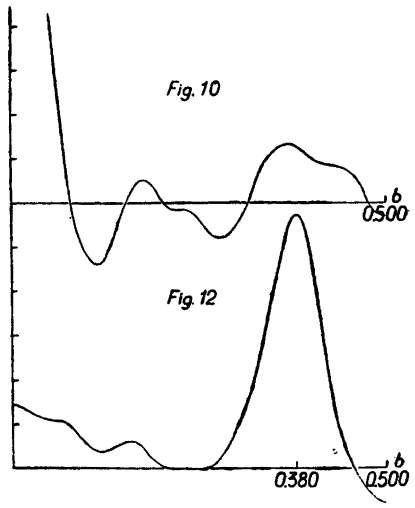


Fig. 10 and 12. Fig. 10 represents the Patterson synthesis $P(0, v, 0)$, Fig. 12 the synthesis $P(x_{B_2}-x_{A_1}, v, z_{B_2}-z_{A_1})$.

If x_1, y_1, z_1 and $\bar{x}_1$, $\bar{y}_1$, $\bar{z}_1$ indicate the coordinates of two centrosymmetrical atoms, then the section $P(2x_1, v, 2z_1)$ must have a maximum in $v = 2y_1$. As a further check, the distribution in the sections $P(2x_A, v, 2z_A)$, $P(2x_B, v, 2z_B)$ and $P(2x_C, v, 2z_C)$ were calculated (A,B and C refer to the same letters in Fig. 3). The results are shown in Figs. 6, 7, and 8 respectively. The maxima in Fig. 6 have the positions $v = 0.265$ and $v = 0.377$. The maximum labelled A in Fig. 3 therefore consists of two overlapping "space group" peaks. The maximum at $v = 0.147$ in Fig. 7 confirms the assumption that B also forms a "space group" peak, whereas the big peak at $v = 0.315$ in Fig. 8 must be due to a vector distance between atoms which are not symmetry-equivalent.

Based upon the above discussion the possible xz-values of the chlorine atoms must be as indicated in Fig. 9. This projection is also named the implication diagram[2]. In transferring the synthesis $P(u, \frac{1}{2}, w)$ to the implication diagram, a certain ambiguity is involved, since it is quite impossible to determine whether the peak labelled B in Fig. 3 implies the positions B_1, B_2, B_3, B_4 or B_1', B_2', B_3', B_4', in Fig. 9, for example. To eliminate this ambiguity and thus obtain the real projection of the chlorine atoms, the following procedure has been adopted: The projection of 4 symmetry-equivalent atoms may of

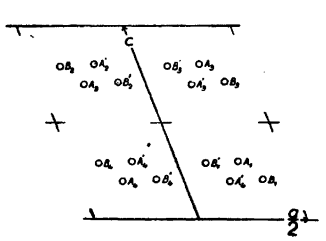


Fig. 9. Implication diagram for the chlorine atoms on (010). (Possible projections of the chlorine atoms on (010).)

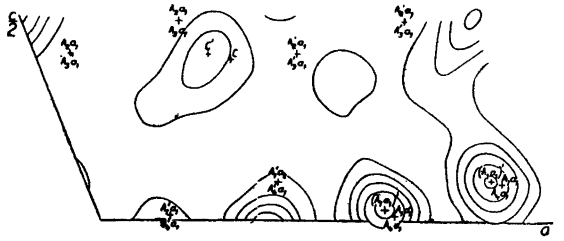


Fig. 13. Patterson synthesis $P(u, 1/4, w)$.

course be arbitrary chosen, as only the mutual arrangement of the atoms is of importance. For instance the projections labelled B_1, B_2, B_3 and B_4 in Fig. 9 may be chosen. If in the projection of the other 8 chlorine atoms pairs coincide, then the section $P(0, v, 0)$ must show a maximum. Fig. 10, representing the synthesis $P(0, v, 0)$ shows no maximum big enough to explain a Cl—Cl distance different from zero. (In this special section such a maximum should have a value 4 times a maximum in Figs. 6 and 7, or twice a maximum in Figs. 2 and 12.) However, the distribution in the section $P(x_{A1} - x'_{A3}, v, z_{A1} - z'_{A3})$ approximately given by $P(2x_C, v, 2z_C)$ in Fig. 8 [$P(x_{A1} - x'_{A3}, v, z_{A1} - z'_{A3}) = P(0.077, v, 0.608)$ and $P(2x_C, v, 2z_C) = P(0.107, v, 0.609)$] exhibits a maximum (at $v = 0.315$). The real projection of the 12 chlorine atoms is therefore given by A_1, A_2, A_3, A_4, A'_1, A'_2, A'_3, A'_4, B_1, B_2, B_3, B_4, in Fig. 9. The Patterson projection $P(u, w)$ (Fig. 4) also confirms this result, as there is a very good correspondance between the positions of calculated and experimental peaks.

The synthesis $P(0, v, \frac{1}{2})$ (Fig. 2) gave the y-parameters of the chlorine atoms. In this case there is no ambiguity, and the implication diagram (Fig. 11) is therefore equal to the projection of the atoms along the ac-plane on to the b-axis.

The relation between the xz-values and y-values can be determined in several ways. From Fig. 7 we know that there is a relation between the projections labelled B_1, B_2, B_3, B_4 (Fig. 9) and b_1, b_2, b_3, b_4 (Fig. 11). One combi-

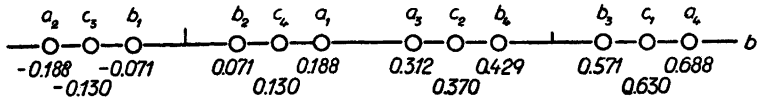


Fig. 11. Projection of the chlorine atoms on the b-axis.

nation may be made arbitrarily, *e.g.* B_1b_1, and then the further relations B_2b_2, B_3b_3 and B_4b_4 follow automatically. A combination of B_1 with b_2, b_3 or b_4 would have given the same arrangement, the only difference being a translation of the origin to one of the other seven non-equivalent centres of symmetry in the unit cell. The y-value corresponding to the projection A_1 (Fig. 9) for instance, can be determined by means of the section $P(x_{B3} - x_{A1}, v, z_{B3} - z_{A1})$ This synthesis (Fig. 12) has a maximum at $v = \pm 0.380$, giving $y_{A1} = 0.191$ or $y_{A1} = -0.049$. The first value corresponds to the projection a_1 in Fig. 11 ($y_{a1} = 0.188$), and we therefore have the relations A_1a_1, A_2a_2, A_3a_3, A_4a_4. Utilizing the approximations $P(u, y_{a1} - y_{b1}, w) \approx P(u, 1/4, w)$ and $P(u, y_{b4} - y_{a1}, w) \approx P(u, 1/4, w)$, the distribution in the section $P(u, 1/4, w)$ (Fig. 13) confirms the result just obtained. Crosses in Fig. 13 denote the position of the 8 peaks calculated when assuming the relations A_1a_1, A_2a_1, a.s.o. The ambiguity is due to the position of the special plane $v = 1/4$ bisecting the distances between the two symmetry planes $v = 0$ and $v = 1/2$.

The relation between the projections A_1', A_2', A_3', A_4' (Fig. 9) and c_1, c_2, c_3, c_4 (Fig. 11) can easily be found from Fig. 8, as we have the approximation $P(x_{A1} - x_{A3}', v, z_{A1} - z_{A3}') \approx P(2x_C, v, 2z_C)$.

The maxima at ± 0.315 gives $y_{A3}' = -0.127$ or $y_{A3}' = 0.503$. Within the limits of error, the value $y_{A3}' = -0.127$, is equal to y_{C3} in Fig. 11 ($y_{C3} = -0.130$), and we therefore find the remaining relations $A_1'c_1$, $A_2'c_2$, $A_3'c_3$ and A_4c_4.

As a final confirmation of the correctness of the relations found between the xz-and the y-values, the system of vector peaks arising from the chlorine atoms are marked in the Patterson projection $P(v, w)$ (Fig. 5). The agreement between the positions of calculated and experimental peaks is very satisfactory.

The direct determination of the positions of the chlorine atoms has now been completed. The overlapping of the two "space group" peaks in Fig. 3, however, gives rise to an uncertainty in the determination of the correspond-

Table 1. The parameters of the atoms expressed as fractions of the corresponding lattice translation.

		x	y	z
Cl_1	(Cl_{B1b1})	0.369	−0.071	0.212
Cl_2	(Cl_{A1a1})	0.270	0.188	0.312
Cl_3	($Cl_{A4'c4}$)	−0.190	0.130	0.298
C_1		0.206	0.013	0.005
C_2		0.089	0.115	0.095
C_3		−0.035	0.041	0.177

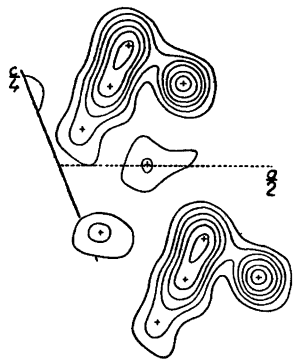


Fig. 14. Electron density projection along b-axis on (010). Contours are drawn at intervals of 5 electrons per A^2.

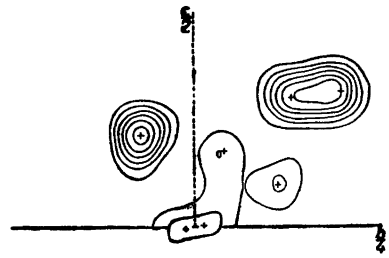


Fig. 15. Electron density projections along a-axis on (100). Contours are drawn at intervals of 5 electrons per A^2.

ing x, z-values. To obtain more correct values, the peaks labelled A_1a_1 and C in Fig. 13 were utilized. The last peak is due to the vector distance $(2x_{A4}', 2y_{A4}', 2z_{A4}') \approx (2x_{A4}', 1/4, 2z_{A4}')$. The resulting parameters are given in Table 1, and the corrected positions of the "space group" peaks in Fig. 3 are labelled A′ and A″, the corrected positions of the peaks in Fig. 13 $(A_1a_1)'$ and C″. The probable error in the parameters may be about $\pm$ 0.03 A.

DETERMINATION OF THE POSITION OF THE CARBON ATOMS

Fig. 14 shows a Fourier projection along the a-axis, Fig. 15 a Fourier projection along the b-axis. The signs of the observed $F(hkl)$ values were obtained by calculating the structure factors for the chlorine atoms only. After the positions of the carbon atoms have been fixed, a re-calculation of the structure factors for the chlorine and the carbon atoms shows that only

Table 2. Intramolecular distances given in Ångström units (see Figs. 16 and 17).

$Cl_1 - Cl_2$	3.27 (3.21)	$C_1 - C_2$	1.71 (1.54)
$Cl_2 - Cl_3$	3.26 (3.21)	$C_2 - C_3$	1.48 (1.54)
$Cl_3 - Cl_4$	3.28 (3.21)	$C_3 - C_4$	1.50 (1.54)
$Cl_1 - Cl_3$	4.80 (4.71)	$C_1 - C_4$	2.89 (2.95)
$Cl_2 - Cl_4$	4.76 (4.71)	$C_2 - C_5$	3.06 (2.95)
$Cl_3 - Cl_5$	5.47 (5.44)	$C_3 - C_6$	2.79 (2.95)
$Cl_1 - Cl_4$	5.17 (5.02)	$C_1 - Cl_1$	1.75 (1.79)
$Cl_2 - Cl_5$	6.32 (6.32)	$C_2 - Cl_2$	1.76 (1.79)
$Cl_3 - Cl_6$	6.39 (6.32)	$C_3 - Cl_3$	1.90 (1.79)

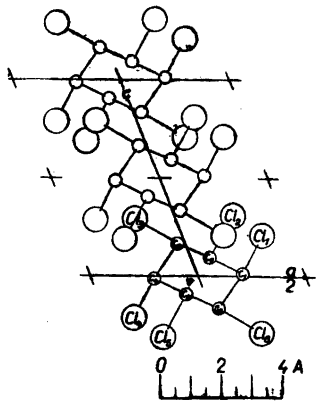


Fig. 16. Arrangement of atoms in the b-axis projection.

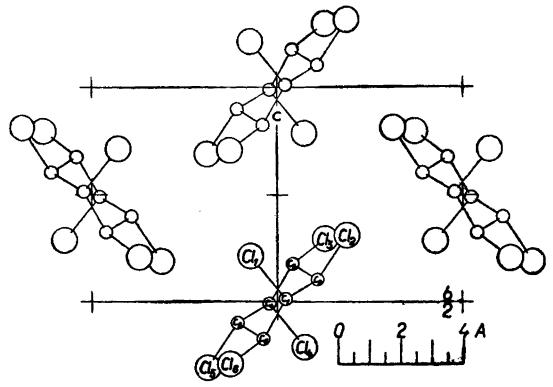


Fig. 17. Arrangement of atoms in the a-axis projection.

4 out of 95 reflections used, would have the wrong sign, and these, having very small F-values, would have a negligible effect on the position of the centres of the peaks. In Figs. 14 and 15, both the chlorine as well as the carbon atoms appear clearly. For two of the chlorine atoms, the resolution in both projections is so poor that the parameters cannot be determined. Within the assumed limits of error, the values $x = 0.366$, $z = 0.214$ an d$y = -0.072$, $z = 0.211$ for the third chlorine atom agree with the parameters already found. The parameters of the carbon atoms are given in Table 1. Owing to overlapping, diffraction effects and errors in the intensity measurements, great accuracy in the parameters cannot be expected. The errors may possibly be $\pm$ 0.1 A.

It is now possible to explain the extra peaks appearing in the various sections of $P(u, v, w)$. However, consideration will be confined to recording the positions of the "space group" peaks calculated for the carbon atoms in Fig. 3, and also in the same figure the peaks due to Cl—C distances having vector separations parallel to the b-axis in the interval $v = 0.5 \pm 0.06$.

DESCRIPTION OF THE STRUCTURE

Figs. 16 and 17 give the projections of the structure along the a- and b-axis. The smallest calculated distances between two chlorine atoms belonging to different molecules, are 3.74 A, 3.76 A and 3.89 A. In the molecule the carbon atoms form a puckered sixmembered ring, the chlorine atoms being in the

positions $\varepsilon\varkappa\varkappa\varepsilon\varkappa\varkappa$. The same result was previously obtained by Bastiansen, Ellefsen and Hassel[4]. Using the electron diffraction sector method, they showed that the molecule had this configuration in the vapour state. Table 2 gives the calculated intramolecular distances derived from the parameters in Table 1. The corresponding calculated distances based on a model of the molecule where the C—C distance is 1.54 A, the Cl—C distance 1.79 A and the valency angle equal to 109.5°, are added in brackets in the same table. Unfortunately, the inaccuracy in the parameters of the carbon atoms makes it impossible to attach any importance to the deviation between the theoretical and experimental C—C and C—Cl distances. However, the average distance of 3.27 A as compared with 3.21 A calculated between two chlorine atoms and especially the comparatively great difference of 0.15 A between the theoretical and experimental value for the 1ε, 4ε chlorine distance, seem to indicate a certain deformation of the molecule.

SUMMARY

The crystal structure of the epsilon isomer of 1,2,3,4,5,6-hexachloro-*cyclo*hexane has been determined from X-ray data only. The parameters of the chlorine atoms were derived from one- and two-dim. Patterson syntheses. Two-dim. Fourier projections were used to determine the coordinates of the carbon atoms. The investigations show that the carbon atoms in the molecule form a puckered six-membered ring, the chlorine atoms being in the positions $\varepsilon\varkappa\varkappa\varepsilon\varkappa\varkappa$.

In conclusion I want to express gratitude to Dr. L. K. Frevel, Midland, Mich., U.S.A. for placing the substance at our disposal and to Prof. O. Hassel in Oslo for suggesting the problem and for helpful criticism of the text of this paper. I also wish to thank Prof. G. Hägg in Uppsala for enabling me to carry out the experimental part of the investigations during a visit to his laboratory.

LITTERATUR

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Received November 14, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 260–267

Electrophoretic and Titrimetric Measurements on Bacterial Flagella

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By recent investigations [1-3] it has been demonstrated that bacterial flagella in many respects show the properties of a simple protein. The N-content is 16.0—16.4 %, 14 amino acids have been found, but no nucleic acids, fatty material or carbohydrates, except as impurities. The flagella are reversibly precipitable with ammonium sulphate. Therefore it was judged worthwhile to investigate to some extent the electrochemical properties of the flagella in order to find out whether they also behave like proteins in this respect or whether characteristic deviations could be found.

EXPERIMENTAL METHODS

For the electrophoretic experiments the Tiselius apparatus [4] was used together with the Philpot-Svensson optical system [5]. The ionic strength of the buffers was 0.05—0.20. Mostly solutions of an ionic strength of 0.10 were used, composed of 0.05 *M* NaCl and buffering substance. No appreciable boundary anomalies appeared with this system. In the range of pH 4—5.4 acetate buffers were used, between 5.6 and 7.5, veronal-acetate (Michaelis buffers) or phosphate, between 8 and 9.3, veronal and above 9.3, glycine buffers.

In order to obtain acid-base titration curves of the flagellar protein, it was found necessary to use a method which needed only about 15 mg of the substance to be investigated on account of the scarcity of the flagellar material. Therefore, the titration solutions were added in steps to the same protein solution, the pH of which was continously read. The flagellar solution (10—20 mg in 4 ml of CO_2 free distilled water) was poured into a beaker and here also the glass and the calomel electrode of a direct reading pH meter (Radiometer's autojonometer, accuracy 0.02 pH units) were placed

together with a glass rod as a stirrer. For the titration micropipettes of the micrometer-syringe type were used. For the titration through the range of pH 2.5 — 11 0.2—0.4 ml of 0.1 *N* solutions were needed. The accuracy of the delivery of the acid and base solutions was about 0.5—1 %. — The titrations were performed with 0.1 *N* KCl present in the protein solution. By using 0.1 *N* acid and base the ionic strength of the reaction mixture was maintained approximately constant.

It was shown by the full reversibility of the curves on back titration that no significant amounts of CO_2 were absorbed in the alkaline region during the titration. (The solution was kept alkaline only for a few minutes.) In addition the pH-values of the protein solutions showed no drift with time.

In order to obtain values of the free H^+ and OH^- ions present in the reaction mixtures, titration curves were made with only 0.1 *N* KCl. The values obtained were in good agreement with the theoretically expected ones and those obtained by other authors [6,7].

In order to test the adopted method titrations with ovalbumin were performed. Close accordance was obtained with the results published by Cannan *et al.* Duplicates gave a difference of 2 % in the total acid base binding capacity of the protein between pH 3.0 and 10.8.

RESULTS AND DISCUSSION

Electrophoresis

The flagella investigated, from *Proteus vulgaris* and *Bacillus subtilis*, prepared in a highly purified state by methods described previously [2] migrate in the electrophoresis tube with only one, very sharp boundary. Even preparations, shown by chemical methods [2] to be to a certain degree inhomogeneous give rise to only one peak, showing the same mobility as the purest possible preparations. Only rather crude preparations migrate inhomogeneously. Therefore, electrophoresis does not seem to be very sensitive as a criterion of purity in this case.

At constant ionic strength reproducible values of the mobility of the flagella have been obtained. Fig. 1 shows the results obtained with an ionic strength of 0.10 at pH values between 5 and 9.5 with flagella from *Proteus vulgaris*. Below pH 5 and above pH 9.5 the flagella disintegrate and therefore no measurements could be performed here. Phosphate buffers have been avoided (Michaelis veronal-acetate buffers were used instead) since the divalent phosphate ions have been found to have a decided effect on the mobilities [8]. This is in accordance with our own experiences (*cf.* Table 1). The mobility curve

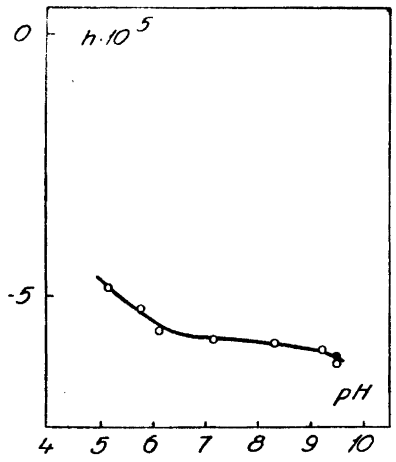

Fig. 1. Electrophoretic mobility curve for bacterial flagella (Dark dot) = Bac. subtilis. (Open rings) = Proteus vulgaris.

is of the same kind as those given by most proteins. On account of the instability of the flagella their isoelectric point can not be established.

As has been reported previously [1] bacterial flagella are converted into particles with fairly homogeneous molecular weight of about 40000 on hydrolysis at pH 3—4 at 20°. As these particles seem to undergo further hydrolysis when dialysed [1] electrophoretical measurements meet with serious difficulties. Quite reproducible values of the mobility of the particles mentioned have not been obtained. However, the mobility is always lower than that of the whole flagella (but still anodic above pH 5) and only one component has been observed in runs at pH 6.8 and 9.5. At pH 5.1, however, a tendency of separation into different components has been observed, but here the whole material seems to be electrophoretically inhomogeneous, probably on account of decomposition phenomena (*cf.* above).

At pH 4.2 the decomposed flagella showed cathodic migration.

The influence on the mobility of the undisintegrated flagella by the ionic strength and by different ion species is shown in Table 1.

The strong dependence of the mobility on the ionic strength and on the kinds of the ions present in the buffer is evident. However, the mobility of haemoglobin [9] and egg albumin [10] at different ionic strengths also show changes of the same order of magnitude as is demonstrated above.

As is shown by the figures, the bivalent ions Mg^{++} and Ca^{++} causes a somewhat greater depressing effect on the mobility than the monovalent Na-ion (at the same total ionic strength). However, the same sign of the mobility is obtained and no recharging of the flagella is obtained as has been shown to be the case with myosin [11].

Table 1.

Buffer	Total ionic strength	pH	Mobility $\times 10^5$	Kind of flagella
Phosphate	0.05	6.80	— 9.35	*Proteus*
»	0.10	6.80	— 7.11	»
+ 0.05 *M* NaCl	0.10	6.80	— 6.35	»
+ 0.05 *M* »	0.10	6.80	— 6.40	*Bac. subtilis*
+ 0.15 *M* »	0.20	6.80	— 4.54	*Proteus*
+ 0.05 *M* Na_2SO_4	0.20	6.80	— 4.92	»
+ 0.025 *M* $MgCl_2$	0.125	6.80	— 4.64	»
+ 0.05 *M* »	0.20	6.80	— 3.64	»
Glycine				»
+ 0.05 *M* $CaCl_2$	0.20	9.05	— 4.01	
Glycine	0.10	9.50	— 6.14	*Bac. subtilis*
+ 0.05 *M* NaCl	0.10	9.50	— 6.27	*Proteus*

As is shown by the figures given, no significant differences in the mobilities of the flagella from *Proteus vulgaris* and *Bacillus subtilis*, respectively, has been observed.

Titration curves

The acid base binding capacity of many proteins have been investigated (for references see Cannan *et.al.* [6]). Mostly, in the diagrams published a quantity h, expressing the equivalents of acid bound to (h positive) or dissociated from (h negative) one gram or one mol of the protein, is plotted against pH. In this case h is expressed in eq./g since no molecular weight of the flagella or a subunit of them is exactly known.

h is generally defined to be zero at the isoionic point of the protein in question. The isoionic point, at moderate ionic strengths not far away from the isoelectric point, may be determined from pH measurements on solutions containing only the protein ions. Such solutions can be prepared by exhaustive electrodialysis. Since the flagella are destroyed by this procedure and have been shown to move in electrophoresis at all pH:es within their stability range, no isoionic point can be determined in this case. Flagellar solutions, prepared by repeated ultracentrifugations from distilled water and final redissolving in CO_2 free water, show a pH between 6.4—7.4. Therefore pH 7.0, somewhat arbitrarily, has been chosen as a zeropoint for the quantity h.

Titration measurements on three highly purified preparations of flagella from *Proteus vulgaris* have given consistent values of the total acid base binding capacity between 3.0 and 10.5 within 4 %. Fig. 2 shows such a titration curve. First NaOH has been added to the flagellar solution (open rings on the

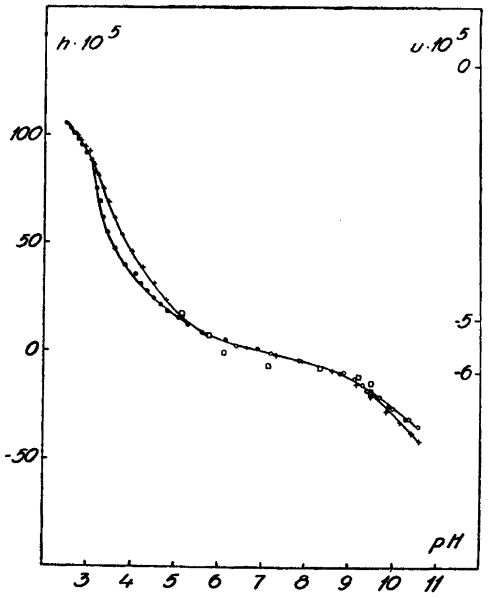

Fig. 2. Titration curve of bacterial flagella (Proteus vulgaris). Electrophoretic data are also included (squares). For details see the text.

curve). The pH is hereby raised from pH 6.5 to 10.6. Then HCl is added (dark dots) and pH falls from 10.6 to 2.45. Once more NaOH is added (crosses) and pH rises to 10.7.

Complete reversibility of the acid base binding capacity of the flagella within the range of their stability is shown by the curve. However, when titration is performed between pH 5.5 and 2.5 and back again, different curves are obtained. Under the experimental conditions prevailing, the disintegration of the flagella into smaller particles occurs between pH 4.5 and 3.5 (no sharp critical point has been observed as yet). The discrepancies of the titration curves in this region therefore are most easily explained by assuming that part of carboxyl anions in the undisintegrated flagella are not accessible to the acid for sterical reasons (if chemical bonds /*e.g.* peptide bonds/, were affected, the branches of the titration curve would not coincide above pH 5.5). After this disintegration has taken place, the consumption of acid will be abnormally high, more titrable groups being set free. This explains the very steep part of the titration curve of the flagella between pH 3.5 and 3 (dark dots). In accordance with this view, solutions of already decomposed flagella give fully reversible titration curves in this pH region.

Also in the pH range above 9 the disintegrated flagella show a higher buffering capacity than the undecomposed ones, indicating that more basic groups have been made accessible in the smaller molecules formed.

It has been shown [12] that essentially the acidic groups of aspartic and glutamic acid (pK about 4), the imidazole and α-amino groups possibly present (pK 7—8) and the ε-amino groups of the lysine are responsible for the buffering capacity of the proteins in the pH range of 2—11. The titration curve of the bacterial flagella shows the expected zones of high buffering capacity between 3 and 4 and above 9, as do most other proteins [6, 7] which is in accordance with the considerations above and with the amino acid composition of the flagella [2]. Since very little histidine has been shown to be present in the flagella it is somewhat difficult to explain the buffering capacity between pH 6 and 9. It may be caused by an unusually high content of free α-amino groups or by some unknown component, or by impurities. Also in myosin and in β-lactoglobulin an unexplained buffering capacity in this region has been found [13].

By extending the measurements to pH 1.5—2 and using high protein and salt concentration in the reaction mixtures in order to obtain highest possible accuracy a limiting value in the titration curves can be obtained, indicating the total positive groups present in the protein molecule [6]. However, for such determinations the necessary amounts of flagellar material have not been available.

A few measurements have been performed at other ionic strengths than 0.10. The deviations found have been of the same kind as those found with other proteins [6].

COMPARISON BETWEEN THE TITRATION CURVE AND ELECTROPHORETIC DATA

As has been shown previously [8, 11] the titration and mobility curves of a protein can be brought to coincide if a proportionality factor between h and u (mobility) is used and if h is put to zero at the isoelectric instead of the isoionic point, *i.e.* the h curve is shifted along the h and u-axis.

As no isoelectric or isoionic point of the flagella is known (*cf.* above) the shifting of the mobility curve along the u-axis must made in another way. Let k be the proportionality factor between h and u, L the constant used for the shifting of the mobility curve. We get the condition for the best coincidence between the mobility and titration curves:

$$\Sigma\,(k \cdot u_i + L - h_i)^2 = \min.$$

where $\Sigma i = n$ is the number of electrophoretic experiments.

By differentiation and solving the equations obtained, k and L are determined. The result is plotted in Fig. 2. As can be seen, an approximative

agreement is obtained between the titrimetric and electrophoretic measurements. In any case the deviations found are not considerably greater than those found by Longsworth in a similar investigation on ovalbumin.[8]

As is shown by the titration curve the charge of the flagella before and after disintegration at pH 3—4 must be supposed to be the same in the range of pH 5.5 to 9 as the different parts of the curve here coincide. As has been mentioned the electrophoretic mobility is lower after disintegration. However, this does not imply a contradiction since after the disintegration the frictional force per unit mass of protein may have augmented considerably.

SUMMARY

Electrophoretic measurements on bacterial flagella have given mobility curves characteristic for a protein. Below pH 5 and above pH 9.5 no electrophoretical measurements could be performed on account of the instability of the flagella.

Flagella from the species *Proteus vulgaris* and *Bacillus subtilis* have shown the same mobility.

The influence of ionic strength on the mobility is marked. Specific ion effects have been observed, but no reversing of the charge has been shown to be the case as with myosin.

Acid base titration curves on the flagella have the characteristic features of such curves obtained from other proteins. Comparison between curves obtained before and after the disintegration of the flagella at pH 3—4 leads one to suppose that for sterical reasons not all ionizable groups in the undecomposed flagella are accessible to the acid and base solutions used in the titration.

Between pH 5 to 9.5 the titration and mobility curves can be brought to coincide approximately.

The author is very much indebted to Prof. A. Tiselius for kind interest in this work and for valuable criticism. The author also wishes to thank Drs H. Svensson and I. Brattsten for helpful discussions and suggestions.

This investigation is part of a research program on bacterial flagella, which is financially supported by the Swedish Natural Science Research Council.

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Received December 20, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 268–276

Investigations on Bacterial Flagella

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The bacterial flagella have already been extensively studied from the morphological and immunological points of view. So far, staining methods and serological tests largely have been used. The results of these studies will not be discussed here, especially as a short summary has already been given [1] and any textbook of bacteriology will give further information. It may only be mentioned that the flagella are generally assumed to be the actively motile organs of the bacteria, which, however, is difficult to prove by direct observation on account of the minute dimensions of the individual flagella (thickness about 120 Å [2]). — Furthermore, the flagella show antigenic properties of a rich differentiation.

The aim of the present investigations [1–8] has been to gain information about the chemical nature and the detailed structure of the flagella and also, if possible, some knowledge about their functioning. On account of their dimensions (*cf.* above) mostly methods employed for investigations of macromolecules have been used, together with analytical methods, morphological studies and serological tests. — As far as the author knows, only one paper concerning the chemical nature of the flagella has been published earlier [9].

As has been mentioned above, the type of flagellation is not quite the same for different species. For practical reasons the investigations have been limited to a few representative types. Furthermore, as the investigations were performed at an institute not equipped with facilities for handling pathogenic material, most of the investigations were carried out on harmless bacteria. As types for such bacteria two commonly occurring and easily cultivatable bacteria, *Proteus vulgaris* and *Bacillus subtilis* were chosen. They show the very common peritrichate flagellation, *i. e.* the flagella are attached all over the surface of the cell. A few observations have been made on *Salmonella paratyphi* B (the preparative work was in this case performed at the State Bacteriological Laboratory [10]).

PREPARATIVE METHODS

The success of the purification of the flagella and the obtainable yield depends above all on the conditions during the cultivation of the bacteria. As has been shown [1] a cultivation temperature of 13—15° C is essential for the purity, and the harvesting of the bacteria has to take place shortly after the logarithmic growth phase (generally 3—5 days at the temperature chosen) in order to avoid poor yields. After harvesting the bacteria with saline solution and detaching the flagella from the bacterial bodies by shaking in an ordinary shaking machine (2—3 beats a second for half an hour) the bacterial bodies are brought down with an ordinary laboratory centrifuge (about 3000 r. p. m.). The supernatant, containing the flagella, is then centrifuged at 50 000 g (27 000 r. p. m.) in the Beam's air-driven ultracentrifuge. The flagella are deposited as pellets and resuspended. Further purification involves a series of centrifugations at moderate and high speed (19 000, 27 000 r. p. m. respectively) in order to remove impurities [1]. Purification is also effected by salting out [4]. Repeated centrifugations or repeated salting out does not improve the purity of the preparations markedly. Also extractions with ether or petrol ether has no appreciable effect.

Some alterations in the cultivation and the purification process referred to above, however, have been made during the course of the work. Instead of petri dishes, aluminum plates, 50 × 50 cm, have been used for the cultivation. Furthermore, instead of the tedious ultrafiltration, the flagella are now concentrated by salting out with ammonium sulphate from the saline solution obtained after harvesting the bacteria and centrifuging down the bacterial bodies. The flagella are then resuspended in a smaller volume before the centrifugations in the Beam's centrifuge.

Cultivation of the bacteria on cellophane membranes, moistened in broth and placed on the surface of the nutrient agar has also been tried and found rather convenient.

The mentioned modifications in the cultivation of the bacteria and in the preparative process has proved to give flagellar material of the same degree of purity and posessing equal properties in other respects as the preparations earlier investigated.

CHEMICAL AND PHYSICOCHEMICAL INVESTIGATIONS AS TESTS OF PURITY OF THE OBTAINED FLAGELLAR PREPARATIONS

The obtained preparations form homogeneous, almost colourless pellets after centrifuging down in the preparative ultracentrifuge at 27 000 r. p. m. When resuspended in distilled water or dilute salt solutions or by simple swelling in the same mediums stable solutions or suspensions are formed.

No difficulty generally is encountered when the purity of an inorganic compound is to be defined. Serious difficulties, however, arise with high molecular substances, as proteins associated with other substances (*e. g.* the complex serum albumin-bilirubin [11]). Such difficulties are of course still more apparent in the case of the extremely „high molecular" particles of viruses. With regard to their dimensions the flagella may be compared with such particles.

In the author's opinion, the best way to proceed in this case is to try to get preparations of as reproducible properties as possible and then by further studies investigate these properties and try to find out to what extent the conception of a "pure substance" can be applied in the actual case.

As to the bacterial flagella, the most sensitive tests of reproducibility of the preparations have been simple chemical analyses, especially of the nitrogen and phosphorus content, carbohydrate analyses and investigations of the ultraviolet absorption. The results of such analyses have been published earlier [1,4]; however, a summary will be given here.

With preparations obtained from *Proteus vulgaris*, cultivated at 13—15° C, and salted out, the following figures have been obtained:

% N: 16.3—16.5 (3 determ.)
% P: 0.03—0.05 (4 »)
% carbohydrate: less than 0.2
% possibly fatty material: about 0.7
% tyrosin-N/total N: 0.81—0.84

The reproducibility must be regarded as good.

Flagella from *Bacillus subtilis*, prepared in the same manner as described above for *Proteus vulgaris*, have given the following values:

% N: 15.8—16.0
% P: as for *Proteus*
% possibly fatty material: as for *Proteus*
% carbohydrate: 1—2 %
% tyrosin-N/total N: 0.57—0.59

Preparations from bacterial cultures incubated at higher temperature or not submitted to salting out [1] have given less reproducible values (*e. g.* prep. from 30° cultures gave N-values of about 14 %, 0.2—0.5 % P. and so on).

Paper chromatograms of hydrolysates of different preparations have shown a constant qualitative amino acid composition of the flagella, and only small differences are found in preparations from *Proteus* and *Bac. subtilis* [4].

Analyses of the ash contents and some further elements occuring in the flagella from *Proteus vulgaris* have been performed. — The ash contents were determined by incineration at 500°, the metals sodium, potassium and calcium spectrometrically. A colorimetric method was used for the estimation of magnesium [12*]. For the determination of chlorine and total sulphur, the fla-

* For the spectrometric determinations I am indebted to Mr. A. Emanuelsson of the Agricultural Institute, Ultuna. The estimations of organic S were performed by Dr. W. Kirsten, Institute of Medical Chemistry, Uppsala.

gellar solution was evaporated with magnesium nitrate and the residue was incinerated at 500°. The ashes were dissolved in nitric acid. Chlorine and sulphur were approximately estimated from the turbidity of the test solution after adding $AgNO_3$ and $BaCl_2$ (comparison with known solutions); the accuracy was judged to be about 10 %.

The following analytical figures were obtained.

% ash: 1.0
% Na: 0.29
% K: 0.08
% Ca: 0.04
% Mg: 0.04
% Cl: about 0.07
% total S: about 0.2
% organic S (+ S in SO_4-ions bound to protein): 0.20

Less pure flagellar preparations have given somewhat higher values.

The related analytical figures give strong evidence that the obtained flagellar preparations, especially from *Proteus vulgaris*, are composed of protein of constant composition. Special tests after disintegration of the flagella [1, p. 880] makes it plausible that this protein makes up at least 95 % of the whole preparation. Since by investigations in the electron microscope [1] practically only flagellar threads are found in the preparations, this protein is obviously identical with the flagella. (Theoretically a protein, not detectable in the electron micrographs, *e. g.* with a particle size considerably smaller than that of the flagella (*cf.* below) could be present in the preparations. But then this protein would be bound either to have chemical properties not discernable from those of the flagella, or to be present in constant amount in relation to the flagella component. Both alternatives seem rather improbable.) — As the flagella are of uniform thickness and of quite the same appearance as before being detached from the bacterial bodies, it may be justified to talk of flagellar preparations of 95 % purity. Serological tests [4] also show no change in the properties of the flagella in this respect before and after the purification process.

Other criteria of homogeneity have been tried, but proved less sensitive than those already mentioned. Electrophoresis reveals inhomogeneity with certainty only in rather crude preparations [7]. Also in electron micrographs considerable amounts of material, especially of a size at the limit of the resolution power of the microscope, may easily be confused with the back-ground structure, which may make one overestimate the degree of purity of the preparation.

FURTHER PHYSICOCHEMICAL INVESTIGATIONS

Decomposition of the flagella in acid medium. The irreversible decomposition of the bacterial flagella by lowering the pH to 3—4 has been described [3,4]. Particles of a sedimentation constant of 2—2.5 S are obtained with flagella from the three species *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella paratyphi* B. The sedimentation diagram indicates a rather homogeneous size of the particles. From the determination of the diffusion constant (upper limit 5—5.5 $\times$ 10^{-7} cm^2/sec.) and the partial specific volume (0.72—0.73) a lower limit of the molecular weight of 40 000 can be calculated for the flagellar fragments.

The cause of the decomposition phenomenon is not clear. However, it may be noted that in this region the flagellar fragments show isoelectric properties [7].

Electrophoretic and titrimetric measurements [7]. As to the acid-base binding capacity and the electrophoretic mobility, the flagella show characteristic protein properties, with the exception that some of the ionizable groups may not be accessible to the acid or base for steric reasons.

X-Ray investigations [2,8]. These investigations have shown that, on the basis of the X-ray reflections formed by the flagellar fibers, the flagella belong to the great family of elastic, fibrous proteins called the keratin-myosin-epidermis-fibrinogen group, showing the α-keratin pattern in the native state. (The other group of fibrous protein is the collagen group.) In addition, the flagella can be stretched into the so called β-form at room temperature, a property also shown by keratin and myosin. (The β-pattern is also shown by most denatured fibrillar and globular proteins.)

Therefore, at least from the X-ray point of view, the flagella may be regarded as primitive hairs or muscle fibers. The X-ray diagrams suggest a structural simplification of the flagellar fibers as compared with *e. g.* the hair keratins.

No essential difference has been found between the diagrams formed by flagella from *Proteus vulgaris* and *Bac. subtilis*.

X-Ray investigations on wet and dry flagella [8] show that the only difference is a sharpening of some reflections in the wet diagrams. Therefore, the water does not alter the arrangement of the fibrillar structure and the protein seems to be present as comparatively invariant anhydrous units, as has been found to be the case with haemoglobin [13]. The swelling of the flagella in a moist atmosphere or by direct wetting is undoubtedly caused by water forming layers of variable thickness between the protein units (*cf.* haemoglobin again).

MORPHOLOGICAL STUDIES

It has already been mentioned, that electron micrographs show the same appearance of the flagella before and after the purification process. However, the purified flagella appear somewhat shorter, on account of breakage. The micrographs published so far [1] have shown a somewhat granular appearance of the flagella. As was suggested, this appearance depends upon the crystallization process occurring in the pure gold used in the shadowing technique. When a mixture of gold and manganin (1 : 1) is used, the shadowed micrographs show flagella with a smooth surface.

By comparing the flagella with tobacco mosaic virus particles the thickness of the former has been determined to about 120 Å [2].

When the flagella are salted out from distilled water with ammonium sulphate a precipitate is obtained, consisting of spirals of a quite regular period along the spiral axis. This period has been found different for the investigated bacterial species, 2.0 μ for *Proteus vulgaris* and 2.5 μ for *Bacillus subtilis* [5].

The flagella aggregate themselves into regular, wavy structures when solutions of flagella are dried on glass slides [6]. The wavy period is slightly shorter than the spiral one.

Observations of living bacteria with dark field microscopy show that the bacteria often carry a spiral-formed tail, undoubtedly formed by individual flagella twisted together into a bundle [14–16]. The spirals are drawn out when the bacteria are in lively movement as compared to resting or slowly moving organisms. Reichert [14] gives the value 2 μ for the spiral period of the tails of resting *Proteus* bacteria (and 2.5 μ for *Salmonella*). This is quite the same value as found for the salted out spirals (*cf.* above). Somewhat puzzling is that Reichert claims the spirals to be right handed, whereas the present author always has found left handed spirals with the characteristic 2 μ period when investigating living cultures or salted out spirals. (The investigations have included 10 strains of *Proteus vulgaris* with different flagellar antigens, and have been performed with phase contrast and dark field microscopy.) As Reichert gives no photographical material it is difficult to decide whether a misinterpretation of the microscopic material is plausible or whether bacterial strains with right as well as with left handed flagellar spirals exist.

In any case, the formation of spirals of a definite spiral period is a characteristic feature of bacterial flagella. These spirals are observed in bacterial cultures on living organisms or as discarded tails [15] and in preparation of pure flagella when salting out is applied. The period of the spirals is modified when the bacteria are rapidly moving.

At least for a definite bacterial species, all spirals are wound in the same way. This proves that the spiral arrangement must depend on the molecular structure of the flagella themselves [6].

The fact that only relatively pure flagellar preparations comparatively free from foreign structural elements form spirals when salted out forms an analogy to the classical crystallization process and may thus be used as a test of the homogeneity of the preparations. However, the chemical tests mentioned above are at least as sensitive.

SOME REMARKS REGARDING THE FUNCTIONING OF THE FLAGELLA

As has been mentioned above, the flagella are generally believed to be the motile organs of the bacteria. Using dark field microscopy, Pijper has criticized this view and has argued that the flagella are only mucous threads peeled off from the surface of the bacterium [17]. This view has again been criticized [18-20].

The present investigation has not been undertaken with the aim of deciding between these two views. Combined chemical, physiological and morphological studies may solve the problem. However, Pijper is obviously wrong when he assumes the flagella of *Proteus vulgaris*, *Salmonella paratyphi* and *Bacillus subtilis* (these species form his essential investigation field) to be mucous threads, since the present investigations clearly show their protein character.

As compared to the contractile muscle protein, actomyosin and its components, myosin and actin, it may be noted that the flagella do not contain any sulphydryl groups (negative cysteine analyses) and that their qualitative amino acid composition is rather different, compared to myosin [*Cf* 4,21]. — Sulphydryl groups play an important role in the formation of the actomyosin complex [22].

An actively motile organ obviously must be furnished with a source of energy. As judged from the analyses, energy rich materials, *e. g.* adenosine triphosphate or carbohydrates, are not present in the flagella; however, such compounds may be weakly bound to the surface of the flagella when the latter are still attached to the bacterial body. They are not necessarily present in amounts big enough to influence the thickness of the flagella and are easily washed away during the purification. In this connection it may be mentioned that myosin and actomyosin can be prepared in a state containing very little phosphorylated compounds [23,24].

In any case, physicochemical and morphological studies related above strongly suggest a very specialized and elaborate structure of the flagella, indicating the nature of the organs, to be well suited to serve a special function in the bacterial organisms. It is of great interest that at the same time the

flagella can be characterized in the same manner as large protein molecules and virus particles (*e. g.* salting out from stable water solutions into ordered aggregates, electrophoresis, preparative ultracentrifugation (*cf.* above).

ACKNOWLEDGEMENT

To my teacher, Professor Arne Tiselius, I wish to express my sincere gratitude. He introduced me to scientific biochemical work and to this field of investigation. Throughout the work he has shown a most stimulating interest, in the form of valuable discussions, criticism and help in various ways.

I am very much indebted to Professor Sven Gard. His great experience in the actual problems has been of the utmost value for the success of the work.

I also wish to thank very much Professor W. T. Astbury, who suggested the X-ray investigations, for facilities put at my disposal at the Dept. of Biomolecular Structure of the University of Leeds, and for valuable and interesting discussions.

Part of the investigations have been performed with the equipment of the Institute of Physical Chemistry, Uppsala. For these facilities I am very much indebted to the former Head of the Institute, Professor The Svedberg and to Professor Stig Claesson.

To the staffs of the Institutes of Biochemistry and Physical Chemistry I wish to express my sincere gratitude for discussions, criticism and help in various forms. In this connection I wish to thank my personal technical assistants whose very skilful help made this work possible.

Also from other Institutes I have received much help during the investigation. In this respect I want to express my indebtedness to the staffs of the State Bacteriological Laboratory, Stockholm, and of the Institutes of Physiological Botany and Histology, Uppsala.

The investigations have been financially supported by grants from The Swedish Natural Science Research Council.

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Received February 20, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 277–282

Preparation of Phenyl Thiohydantoins from Some Natural Amino Acids

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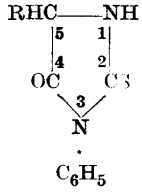
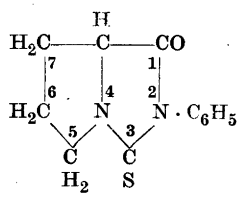
In the development of a method for determination of the amino acid sequence in peptides[1] it became necessary for reference purposes to prepare the 3-phenyl-2-thiohydantoin derivatives (I.) of the natural amino acids. Some of these are described in the literature. Aschan [2] was the first to report the preparation of phenyl thiohydantoins from amino acids. Later Brautlecht [3] described the analogous derivatives from an additional number of amino acids.



(I.)

$HOOC \cdot RHC \cdot NH \cdot CS \cdot NH \cdot C_6H_5$

(II.)



(III.)

(R = amino acid side chain)

In the present work the intermediate phenylthiocarbamyl (PTC) derivatives (II.) were obtained by allowing the amino acids to react with phenyl isothiocyanate in alkaline solution. It was found that some desulfuration of the PTC-derivative took place if the total amount of alkali required was added at the beginning of the reaction and if the temperature was too high. For this reason alkali was added continuously and the temperature was not allowed to exceed 40° C. The crude PTC-derivatives were not purified but transferred directly into the phenyl thiohydantoins by refluxing in acid.

EXPERIMENTAL

Preparation of phenylthiocarbamyl (PTC) amino acids

One hundredth of a mole of the amino acid was dissolved in a mixture of 25 ml of water and 25 ml of pyridine. The pH of the solution was adjusted to about 9 as shown by an indicator paper by the addition of *N* NaOH. The solution was heated to 40° C and kept at that temperature during the reaction. 2.4 ml of phenyl isothiocyanate was added with vigorous stirring. Small portions of *N* NaOH were added to keep the pH at about 9. The reaction was completed when the alkali consumption ceased. This as a rule required less than 30 minutes.

Pyridine and excess phenyl isothiocyanate were then removed by repeated extractions with equal volumes of benzene. Subsequently an amount of *N* HCl equivalent to the total addition of sodium hydroxide was added. This usually brought about precipitation of the PTC-amino acid and if necessary the mother liquor was concentrated to increase the yield. In the cases of arginine and histidine the PTC-derivatives were soluble at an acidic reaction. It was found that the PTC-arginine could be precipitated isoelectrically at pH ~ 7 and the PTC-histidine at pH 3.5.

Preparation of 3-phenyl-2-thiohydantoins

Unless otherwise stated for the particular hydantoin the following procedure was employed.

The PTC-amino acid was suspended or dissolved in 30 ml of *N* HCl and refluxed for two hours. The reaction mixture was then repeatedly concentrated to dryness *in vacuo* in order to remove hydrochloric acid.

The yield of hydantoin calculated on the amino acid was 80—90 %.

Recrystallizations were carried out from mixtures of glacial acetic acid and water. Preparations were dried *in vacuo* (1 mm Hg) over P_2O_5 and pellets of KOH for 48 hours prior to analyses.

Melting points were determined on a heating block (Fisher-Johns) and are uncorrected.

Optical rotations of the hydantoins were determined in absolute ethanol. A 0.5 dm tube was used. Owing to the limited solubility of the hydantoins the accuracy of these measurements is not higher than $\pm$ 2°.

Nitrogen analyses were carried out by the micro-Kjeldahl method. Sulfur analyses were done according to Zimmermann [4].

3-Phenyl-2-thiohydantoin. Prepared from glycine. M. p. 245—48° (d.). M. p. acc. to Brautlecht [3] 240—42° (d.).
Found: N, 14.53; S, 16.81.
Calc. for $C_9H_8ON_2S$: N, 14.58; S, 16.69.

5-Methyl-3-phenyl-2-thiohydantoin. Prepared from DL-alanine. M. p. 185°. Acc. to Brautlecht [3] 180—84°.
Found: N, 13.45; S, 15.36.
Calc. for $C_{10}H_{10}ON_2S$: N, 13.57; S, 15.53.

5-Ethyl-3-phenyl-2-thiohydantoin. Prepared from DL-α-amino-*n*-butyric acid. M. p. 192°. Acc. to Brautlecht[3] 190–92°.
Found: N, 12.68; S, 14.62.
Calc. for $C_{11}H_{12}ON_2S$: N, 12.72; S, 14.54.

5-Isopropyl-3-phenyl-2-thiohydantoin. Prepared from DL-valine. M. p. 206°. Acc. to Brautlecht[3] 206–08°.
Found: N, 11.94; S, 13.55.
Calc. for $C_{12}H_{14}ON_2S$: N, 11.97; S, 13.70.

5-Isobutyl-3-phenyl-2-thiohydantoin. Prepared from L-leucine. Recryst. from ethanol. M. p. 178°. Acc. to Brautlecht[3] 176–79°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 11.18; S, 12.83.
Calc. for $C_{13}H_{16}ON_2S$: N, 11.28; S, 12.91.

5-[Sec-butyl]-3-phenyl-2-thiohydantoin. Prepared from L-isoleucine. Recryst. from ethanol. M. p. 173–75°. $[\alpha]_D^{20} = +12°$ ($c = 1$ % $^W/_V$).
Found: N, 11.25; S, 12.81.
$C_{13}H_{16}ON_2S$ requires N, 11.28; S, 12.91.

5-[2-Methylmercaptoethyl]-3-phenyl-2-thiohydantoin. Prepared from DL-methionine. M. p. 132°.
Found: N, 10.53; S, 24.26.
$C_{12}H_{14}ON_2S_2$ requires N, 10.51; S, 24.05.

1-Oxy-2-phenyl-3-thio-1-imidazolidino-[1,5-a]-pyrrolidine. (Formula III.) Prepared from L-proline. M. p. 179°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 11.94; S, 13.62.
$C_{12}H_{12}ON_2S$ requires N, 12.07; S, 13.80.

1-Oxy-2-phenyl-3-thio-1-imidazolidino-[1,5-a]-6-hydroxy-pyrrolidine. Prepared from L-hydroxyproline. Recryst. from water. M. p. 145–48°.
Found: N, 11.35; S, 12.60.
$C_{12}H_{12}O_2N_2S$ requires N, 11.28; S, 12.88.

5-Benzyl-3-phenyl-2-thiohydantoin. Prepared from DL-phenylalanine. M. p. 187°. Acc. to Brautlecht[3] 187°.
Found: N, 10.07; S, 11.47.
Calc. for $C_{16}H_{14}ON_2S$: N, 9.93; S, 11.36.

5-p-Hydroxybenzyl-3-phenyl-2-thiohydantoin. Prepared from L-tyrosine. M. p. 216°. Acc. to Brautlecht[3] 214–16°. $[\alpha]_D^{20} = -15°$ ($c = 1$ % $^W/_V$).
Found: N, 9.35; S, 10.69.
Calc. for $C_{16}H_{14}O_2N_2S$: N, 9.39; S, 10.76.

5-[3'-Indolylmethyl]-3-phenyl-2-thiohydantoin. Prepared from DL-tryptophan. When an attempt was made to form the hydantoin in the ordinary way by refluxing the PTC-derivative with *N* HCl, a sticky, amorphous, dark brown product resulted. It was then

found that refluxing with glacial acetic acid for two hours was sufficient to bring about the reaction. The resulting product showed a yellow discoloration which could be removed by repeated crystallizations from mixtures of glacial acetic acid and water. On exposure to light the dry product slowly took on a reddish color. M. p. 177°.
Found: N, 13.11; S, 10.06.
$C_{18}H_{15}ON_3S$ requires N, 13.08; S, 9.97.

3-Phenyl-2-thio-5-hydantoinacetic acid. Prepared from DL-aspartic acid. Recryst. from ethanol-water. M. p. 229°. Acc. to Brautlecht [3] 233–34°.
Found: N, 11.16; S, 12.75.
Calc. for $C_{11}H_{10}O_3N_2S$: N, 11.19; S, 12.81.

3-Phenyl-2-thio-5-hydantoinacetamide. Prepared from DL-asparagine. Recryst. from ethanol-water. M. p. 234°. Acc. to Brautlecht [3] 234°.
Found: N, 16.75; S, 12.93.
Calc. for. $C_{11}H_{11}O_2N_3S$: N, 16.87; S, 12.86.

3-Phenyl-2-thio-5-hydantoinpropionic acid. Prepared from L-glutamic acid. Recryst. from ethanol-water. This compound crystallized with 1 mole of water which for removal required drying at 110°. M. p. 166–67°. Acc. to Brautlecht [3] 169–70°. $[\alpha]_D^{20} = 0$ ($c = 1$ % $^W/_V$).
Found: N, 10.52; S, 12.05.
Calc. for $C_{12}H_{12}O_3N_2S$: N, 10.60; S, 12.13.

5-[4-(β-Phenylthioureido)butyl]-3-phenyl-2-thiohydantoin. Prepared from L-lysine. Recryst. from ethanol-water. M. p. 162–64°. $[\alpha]_D^{20} = 0$ ($c = 0.5$ % $^W/_V$).
Found: N, 13.97; S, 15.95.
$C_{20}H_{22}ON_4S_2$ requires N, 14.07; S, 16.09.

5-(3-Guanidopropyl)-3-phenyl-2-thiohydantoin hydrochloride. Prepared from L-arginine. Recryst. from boiling water. M. p. 189°. $[\alpha]_D^{20} = 0$ ($c = 1$ % $^W/_V$).
Found: N, 21.41; S, 9.55.
$C_{13}H_{18}ON_5ClS$ requires N, 21.35; S, 9.77.

5-(4'-Imidazolylmethyl)-3-phenyl-2-thiohydantoin hydrochloride. Prepared from L-histidine monohydrochloride. Recryst. from abs. ethanol. M. p. 200–06° (d.). $[\alpha]_D^{20} = +6°$ ($c = 0.5$ % $^W/_V$).
Found: N, 18.10; S, 10.43.
$C_{13}H_{13}ON_4ClS$ requires N, 18.14; S, 10.38.

DISCUSSION

All the amino acids investigated with the exception of lysine formed 3-phenyl-2-thiohydantoin derivatives with their unmodified side chains occupying position 5 in the hydantoin ring. Lysine after reaction with phenyl isothiocyanate became substituted with phenylthiocarbamyl groups at both

the α-amino and ε-amino positions and the latter substituent was retained when the hydantoin was formed. The α-imino acids in consequence of their cyclic structures formed fused ring systems completely analogous with the hydantoins formed from the α-amino acids.

Attempts have also been made to prepare the phenyl thiohydantoin derivatives of cystine, serine and threonine. Only the threonine derivative formed a crystalline compound of definite composition. Elementary analyses of the threonine derivative showed a discrepancy from the expected composition indicating the loss of one molecule of water. During the formation of the hydantoin from cystine a considerable evolution of hydrogen sulfide was noticed. These facts taken in conjunction with earlier observations on the hydantoins and thiohydantoins of serine [5] and cystine [6,7] suggest that the elements of water or in the latter case hydrogen sulfide are split off from the side chains during the reaction.

The optical rotations were determined of those hydantoins which had been prepared from optically active amino acids. The specific rotations were generally low or nil demonstrating again the marked tendency for racemization during the formation of hydantoins [8,9]. The electron attracting phenyl group in position 3 of the ring appears to augment this tendency [10].

SUMMARY

The 3-phenyl-2-thiohydantoin derivatives of the following amino acids have been prepared: Glycine, alanine, α-amino-*n*-butyric acid, valine, leucine, *isoleucine*, *methionine*, *proline*, *hydroxyproline*, phenylalanine, tyrosine, *tryptophan*, aspartic acid, asparagine, glutamic acid, *arginine*, *lysine*, *histidine* *. Some of their properties are described.

The 3-phenyl-2-thiohydantoins display a marked tendency for racemization.

This investigation has been supported by grants from *Harald och Greta Jeanssons Stiftelse* and from *Stiftelsen Therese och Johan Anderssons minne.*

The technical assistance of Miss K. Diehl and Mr. J. Sjöquist is gratefully acknowledged.

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Received December 22, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 283–293

Method for Determination of the Amino Acid Sequence in Peptides

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The problem of determining the amino acid sequence in peptides has attracted the interest of several investigators in the past because of its bearing on protein structure. The subject has been reviewed by Fox [1]. Since then new approaches, employing partial hydrolysis, have been made by Consden, Gordon, Martin and Synge [2, 3] and by Sanger [4].*

The applicabilities of the available methods are, however, for practical or theoretical reasons restricted and there is a need for alternative methods.

In 1927 Bergmannn, Kann and Miekeley [5] described the following reaction:

$$C_6H_5 \cdot NH \cdot CO \cdot NH \cdot CHR' \cdot CO \cdot NH \cdot CHR'' \cdot COOH \rightarrow$$

(I)

$$C_6H_5 \cdot N\text{——}CO + H_2N \cdot CHR'' \cdot COOH$$

$$OC \quad NH$$

$$\diagdown\ C\ \diagup$$

$$\cdot$$

$$HR'$$

(II)

(R', R'' = amino acid side chains)

Refluxing with hydrochloric acid split off the phenyl hydantoin (II) from the phenylcarbamyl peptide (I). The fact that the resistance to acid cleavage was less than that expected for a peptide bond, indicating a labilizing effect of the phenylcarbamyl group, apparently escaped the authors' notice. Later

* Recently Levy [14] has described a new procedure for the removal of terminal groups from peptides as 2-thio-5-thiazolidones.

Abderhalden and Brockmann [6] took advantage of this effect in developing a method for the stepwise degradation of polypeptides. Concomittantly, however, to the desired cleavage other peptide bonds in the chain were also attacked to some extent. This circumstance obviously detracts from the usefulness of the procedure.

The present attempt [7] to employ the above reaction for the determination of peptide structure was guided by several considerations. Firstly, it was postulated that the ease of the reaction parallels the ease of ring closure to hydantoin and from that point of view the phenyl*thio*carbamyl derivatives should be preferable.

Secondly, the cleavage should take place exclusively at the peptide bond adjacent to the carbamyl group, other bonds remaining untouched. Since the hydantoin formation does not require the presence of water, whereas of course peptide linkages are split by hydrolysis or analogous reaction, it was decided to carry out the reaction in an anhydrous, inert solvent. It was found that nitromethane was ideal in this respect. Nitromethane saturated with hydrogen chloride brought about an almost instantaneous and quantitative cleavage of phenylthiocarbamyl (PTC) dipeptides into phenyl thiohydantoin and amino acid at room temperature. It could also be shown for a PTC-tripeptide that the cleavage took place exclusively at the peptide bond adjacent to the PTC-substituent.

Furthermore, since the naturally occurring peptides are usually obtained in small amounts a micromethod was desirable. This requirement almost precluded the identification of the hydantoin by melting point, elementary analyses *etc.* The difficulty was solved by hydrolysis of the hydantoin to the corresponding amino acid and identification of the latter by the paper strip method [8].

The first part of the experimental section describes the formation and mode of cleavage of some PTC-peptides and the second part deals with the application of these reactions in a procedure for stepwise degradation of polypeptides on a micro scale.

EXPERIMENTAL

The peptides used in this work were all synthetic. Leucyltriglycine and alanylleucylglycine were provided as generous gifts by Professor K. Linderstrøm-Lang and Professor G. Ågren, and the dipeptides were the commercial preparations of Hoffmann-La Roche, Basel.

I. Preparation and cleavage of phenylthiocarbamyl (PTC) peptides

Nitrogen determinations were made by the micro-Kjeldahl procedure. Melting points were determined on a heating block (Fisher-Johns) and are uncorrected. Prior to analysis the preparations were dried for 48 hours *in vacuo* (1 mm Hg) over P_2O_5 and pellets of KOH.

Preparation and cleavage of PTC-alanylglycine. DL-alanylglycine (0.29 g) was dissolved in 10 ml pyridine-water (1 : 1). The solution was warmed to 40° and *N* NaOH was added to about pH 9 (glass electrode) which pH was maintained throughout the reaction by the addition in small portions of *N* NaOH. 0.48 ml phenyl isothiocyanate was added with vigorous stirring. The reaction was complete within half an hour. The total amount of *N* NaOH added was 2.4 ml. Pyridine and excess phenyl isothiocyanate were removed by repeated extractions with equal volumes of benzene. On the addition of *N* HCl to pH 3 *phenylthiocarbamyl-alanylglycine* separated as an oil which solidified on standing over night in the icebox. Yield 0.44 g. The material was then recrystallized from ethanol-water. M. p. 155°.

Found: N, 15.00. $C_{12}H_{15}O_3N_3S$ requires N, 14.96.

PTC-alanylglycine (0.28 g) was dissolved in 20 ml anhydrous nitromethane saturated with hydrogen chloride (for preparation see p. 290). Glycine hydrochloride started to crystallize immediately and was filtered off after 15 minutes. Yield 89 %. The material was then recrystallized from ethanol-water.

Found: N, 18.93. Calc. 18.66.

Paper chromatography using pyridine-amyl alcohol [9] showed identity with glycine. The nitromethane solution was evaporated to dryness at reduced pressure. The residue was taken up in a small volume of hot glacial acetic acid and a minor, insoluble residue filtered off. On addition of water to the glacial acetic acid solution and cooling the preparation crystallized. The material was recrystallized from absolute ethanol. M. p. 184—85°. Mixed m. p. with an authentic sample of 5-methyl-3-phenyl-2-thiohydantoin [10] 184—85°.

Preparation and cleavage of PTC-leucylglycine. DL-leucylglycine (0.38 g) was treated as described for DL-alanylglycine. The yield of *phenylthiocarbamyl-leucylglycine* was 0.52 g. The material was then recrystallized from ethanol-water. M. p. 147—48°.

Found: N, 13.07. $C_{15}H_{21}O_3N_3S$ requires N, 13.01.

PTC-leucylglycine (0.34 g) was dissolved in 20 ml nitromethane-HCl. Glycine hydrochloride immediately started to separate and was filtered off after 15 minutes. Yield 93 %. It was then recrystallized from ethanol-water.

Found: N, 18.87. Calc. 18.66.

Paper chromatography using pyridine-amyl alcohol demonstrated identity with glycine. The nitromethane solution was evaporated *in vacuo* to dryness and the residue extracted with a small volume of hot glacial acetic acid. On addition of water and cooling in the ice box crystals appeared, which were recrystallized from ethanol-water. M. p. 177—78°. The mixed m. p. with an authentic sample of 5-isobutyl-3-phenyl-2-thiohydantoin [10] was 178°.

Preparation and cleavage of PTC-leucyltyrosine. L-leucyl-L-tyrosine (0.60 g) was treated as described for DL-alanylglycine. The yield of *phenylthiocarbamyl-leucyltyrosine* was 0.72 g. It was then recrystallized from ethanol-water. M. p. 225—30° with decomposition.

Found: N, 9.60. $C_{22}H_{27}O_4N_3S$ requires N, 9.78.

PTC-leucyltyrosine (0.43 g) was dissolved in 20 ml nitromethane-HCl. Crystallization of tyrosine hydrochloride started immediately. The crystals were filtered off after 15 minutes. Yield 98 %. Recrystallization was carried out by dissolving the crystals in the smallest possible volume of water and neutralizing with sodium hydroxide.

Found: N, 7.68. Calc. 7.73.

Paper chromatography using pyridine-amyl alcohol showed identity with tyrosine. The nitromethane solution was evaporated *in vacuo* to dryness and the residue extracted with a small volume of hot glacial acetic acid. Crystallization occurred on addition of water and cooling in the ice box. The material was then recrystallized from ethanol-water. M. p. 176—77°. The mixed m. p. with an authentic sample of 5-isobutyl-3-phenyl-2-thiohydantoin [10] was 176—77°.

Preparation and cleavage of PTC-glycyltryptophan. Glycyl-L-tryptophan (0.52 g) was treated as described for DL-alanylglycine. The yield of *phenylthiocarbamyl-glycyltryptophan* was 0.71 g. Attempts to crystallize from organic solvent yielded only oils. The preparation was then dissolved in the minimum amount of 0.5 *N* NaOH from which it crystallized on the gradual addition of *N* HCl. M. p. 120—23°.

Found: N, 13.75. $C_{20}H_{20}O_3N_4S$ requires N, 14.14.

PTC-glycyltryptophan (0.40 g) was dissolved in 20 ml nitromethane-HCl. Tryptophan hydrochloride began to crystallize within a few seconds. After 15 minutes the crystals were filtered off. Yield 98 %. It was then recrystallized from ethanol-water.

Found: N, 13.83. Calc. 13.71.

Paper chromatography using pyridine-amyl alcohol showed identity with tryptophan.

The nitromethane solution was evaporated to dryness *in vacuo*. The dark brown residue was taken up in a small volume of hot ethanol and an insoluble residue filtered off. Addition of water to the alcoholic solution brought about precipitation. The material was recrystallized from ethanol. A brownish impurity was, however, very difficult to separate from the preparation. The crystals were then sublimed and resublimed at 150° in a vacuum of 0.05 mm Hg which resulted in a slightly yellowish product. M. p. 240—46° with decomposition. An authentic sample of 3-phenyl-2-thiohydantoin melted at 245—48° with decomposition [10].

Found: N, 14.40. Calc. for $C_9H_8ON_2S$: N, 14.58.

Preparation and cleavage of PTC-alanylleucylglycine. DL-alanyl-DL-leucylglycine (0.13 g) was treated as described for DL-alanylglycine except that only half the amounts of reagents were used. The yield of *phenylthiocarbamylalanylleucylglycine* was 0.16 g. The preparation resisted all attempts at crystallization. It was purified through repeated precipitations with water from its solution in glacial acetic acid.

Found: N, 13.96. $C_{18}H_{26}O_4N_4S$ requires N, 14.22.

PTC-alanylleucylglycine (40 mg) was suspended under anhydrous conditions in 2 ml nitromethane-HCl at 40° by means of a magnetic stirring device (p. 290). After 15 minutes nitromethane and hydrogen chloride were removed by careful evaporation *in vacuo* at room temperature. To the dry residue were added with stirring 1 ml of water and then dilute NaOH to pH 9. The solution was again evaporated to near dryness *in vacuo* at room temperature. The residue was extracted with glacial acetic acid and of this solution aliquots were taken for determination of total nitrogen and amino nitrogen according to Van Slyke with the modifications introduced by Kendrick and Hanke [11].

Found: N, 5.50 mg.; amino-N, 1.35 mg; amino-N/total N, 0.246. The calculated quotient for the cleavage of one peptide bond is 0.25 making the actual cleavage 98 % of the calculated.

PTC-alanylleucylglycine (40 mg) was treated with 2 ml nitromethane-HCl as described in the preceding section. After the removal of nitromethane and hydrogen chloride the residue was thoroughly extracted with 1 ml of water. Paper chromatography using pyridine-amyl alcohol was performed on the aqueous solution. 0.02 ml of the solution was applied to the paper alongside authentic samples of leucylglycine, leucine and glycine. From the test sample was obtained only one spot with the same R_F-value as that of leucylglycine ($R_F = 0.43$) and no trace of leucine ($R_F = 0.58$) or glycine ($R_F = 0.19$). Had the treatment of the PTC-peptide with nitromethane-HCl caused a cleavage to 1 % of the peptide bond between leucine and glycine, there should have been present 2.5 γ of leucine and 1.5 γ of glycine in the applied test sample.

Actual experiments showed that it was easily possible to demonstrate the presence of these amounts on a paper chromatogram. Consequently an eventual cleavage of the bond between leucine and glycine must have been less than 1 %.

II. Procedure for the stepwise degradation of polypeptides

Preparation of the PTC-peptide

The method was worked out for amounts of peptides corresponding to 1/10 millimole. A mixture of equal volumes of pyridine and water was found to be a good solvent for both peptides and phenyl isothiocyanate. The rate of the reaction is within limits higher the higher the pH. A pH of 8.6 was found to give a conveniently rapid reaction and this pH was maintained throughout the reaction by the addition of known amounts of alkali.

Since the alkali consumption is proportional to the amount of PTC-peptide formed, the course of the reaction could be conveniently followed and this was done for a number of amino acids and peptides. The rate varied somewhat but was consistently higher for di- and tripeptides than for amino acids (Fig. 1), the probable explanation being that the *a*-amino group reacts in its uncharged form and that at pH 8.6 this group is less dissociated in the peptides than in the amino acids [12].

Method.

The reaction is carried out in a special tube (Fig. 2) which is immersed in a water bath at 40°. One tenth of a millimole of the peptide is dissolved in 2 ml pyridine-water (1 : 1). The solvent should contain 3 mg bromothymol blue per 100 ml. By the addition of alkali the color is adjusted to that corresponding to pH 8.6. For comparison another tube is placed alongside containing solvent and indicator the pH of which is adjusted to 8.6 by means of a glass electrode. To the solution of the peptide is added 70 μl of phenyl isothiocyanate. The reaction mixture is vigorously stirred with a mechanical stirrer in order to keep the solution saturated in respect to phenyl isothiocyanate. The pH is maintained at 8.6 by addition of *N* sodium hydroxide from a microburette.

After completion of the reaction pyridine and excess phenyl isothiocyanate are removed by repeated extractions with equal volumes of benzene in the same tube as that used for the reaction.

The aqueous solution of the sodium salt of the PTC-peptide (1 ml) is then evaporated to complete dryness by a lyophilizing procedure. The solution is

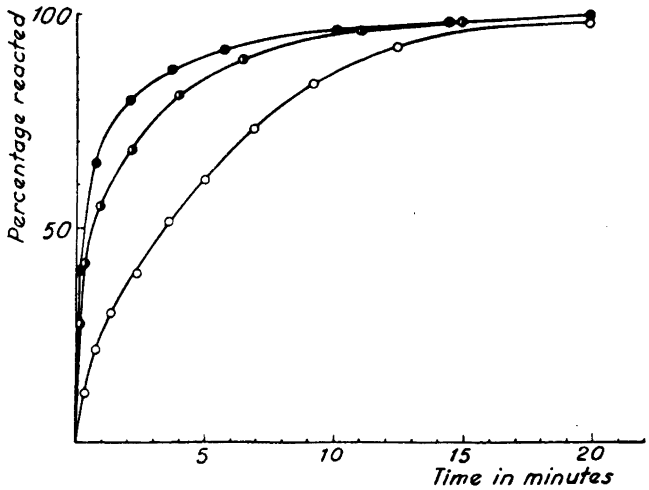

Fig. 1. Course of the reaction between phenyl isothiocyanate and a) leucine (—○—○—), b) leucylglycine (—◑—◑—), and c) leucylglycylglycine (—●—●—) in pyridine-water. pH 8.6. Temperature 40°.

first frozen to about — 15° and then a container with phosphorous pentoxide (Fig. 2) is attached to the tube and the system connected to a good vacuum oil pump. The drying operation generally requires 5—6 hours. On the repeated passage of the peptide through the degradation cycle sodium chloride will gradually accumulate making it difficult to keep the preparation frozen during the lyophilizing procedure. The dry PTC-peptide is then freed from the salt by extraction with glacial acetic acid which leaves the salt as an insoluble residue. The acetic acid is subsequently removed by evaporation *in vacuo*.

Cleavage of the PTC-peptide

The reaction is carried out in anhydrous nitromethane-HCl. The main rate limiting factor appears to be the solubility of the PTC-peptide in nitromethane. For a number of dipeptide derivatives the solubility has been found to be high and the cleavage in then practically instantaneous at room temperature, whereas the tri- and tetrapeptide derivatives were less soluble and as a consequence more time was required for the reaction to reach completeness. In order to increase the speed and to insure complete reaction, the PTC-peptide is finely powdered and suspended in the medium.

The reaction products separate spontaneously owing to the fact that the phenyl thiohydantoins are sufficiently soluble in the medium to stay in solu-

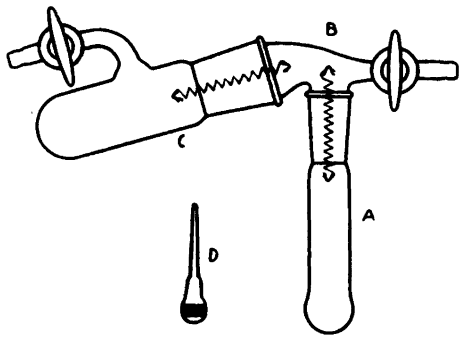

Fig. 2. Assembled apparatus. A, reaction tube; B, midpiece; C, container for drying agent; D, stirrer with sealed in magnet. The apparatus is made of Pyrex glass. (1/4.)

tion, whereas the residual amino acid or peptide (as hydrochloride) is more or less insoluble and therefore precipitates. The phenyl thiohydantoin, however, is always contaminated with a small fraction of the residual peptide. This circumstance has not caused any difficulty in the identification of the hydantoin and the objection is rather the loss of material which eventnally may become considerable after many repetitions of the procedure.

Method.

The anhydrous solution of hydrogen chloride in nitromethane is prepared in the following way. Nitromethane is dried first over calcium sulfate and then over phosphorous pentoxide. It is then distilled at atmospheric pressure and the fraction boiling at 101—02° used. This fraction is saturated with dry hydrogen chloride. The hydrogen chloride is prepared by letting conc. sulfuric acid drip into conc. hydrochloric acid. The gas is passed in a finely divided state through conc. sulfuric acid and then through the nitromethane. The nitromethane rapidly takes up 3—4 % w/w of hydrogen chloride. The solution is stored with protection against atmospheric moisture.

In the tube with the dry PTC-peptide is placed a small magnetic stirrer sealed in glass (Fig. 2) and then 2 ml of nitromethane-HCl is added with protections against atmospheric moisture. The tube is closed, immersed in a water bath at 40° and the contents are thoroughly stirred so that all lumps are broken up and a fine suspension is formed. The time required for the reaction to reach completion depends on the nature of the particular PTC-derivative (p. 289).

At the end of the reaction the insoluble material is filtered off and the filtrate transferred to a thickwalled test tube. This operation is conveniently carried out with the aid of an Emich filter stick to which is sealed a capillary siphon.

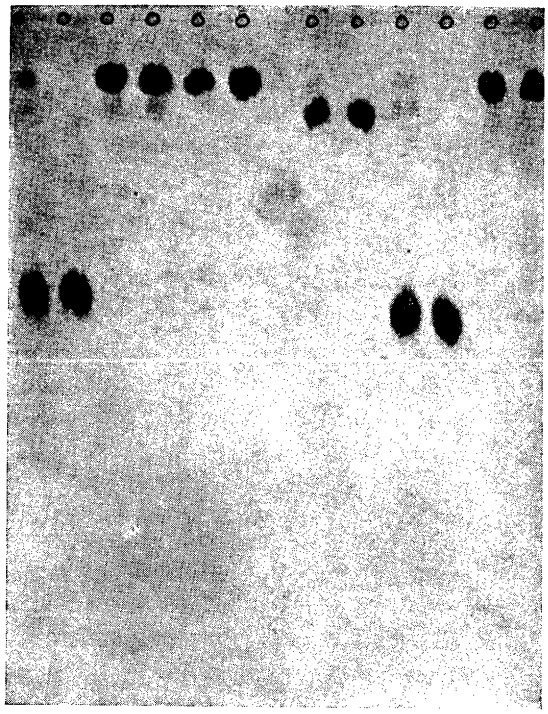

Fig. 3. Paper strip identification of amino acids obtained by stepwise degradation of leucylglycylglycylglycine (left) and alanylleucylglycine (right). Solvent: Pyridine-amyl alcohol. The samples are applied in the order from left to right: 1st amino acid, leucine, 2nd amino acid, 3rd amino acid, 4th amino acid, glycine and 1st amino acid, alanine, 2nd amino acid, leucine, 3rd amino acid, glycine.

The insoluble residue left in the reaction tube is washed repeatedly with small volumes of dry ether in order to remove excess hydrogen chloride. The sample is then ready for renewed treatment with phenyl isothiocyanate.

The filtrate is evaporated to dryness on the steam bath in a stream of nitrogen.

Identification of the removed amino acid

The phenyl thiohydantoin is subjected to alkaline hydrolysis and the resulting amino acid identified by means of the paper strip method [8,9]. Phenyl thiohydandoin derivatives of a large variety of amino acids [10] were hydrolysed in this way, and it was found that with few exceptions the original amino acid

could be recovered *. The exceptions were arginine and asparagine. The arginine derivative gave rise to two ninhydrin positive compounds of which one was ornithine [13]. The other was not identified **. The asparagine derivative yielded aspartic acid. The hydrolysate of the tryptophan derivative showed in the chromatogram two ninhydrin positive spots of which the more intense spot corresponded to tryptophan itself.

Method.

To the residue after evaporation of the nitromethane is added 2 ml 0.25 *N* barium hydroxide and the tube is sealed. Hydrolysis is carried out at 140° for 48 hours. The tube is then opened and the barium is precipitated as carbonate by passing a stream of carbon dioxide through the solution.

It is not necessary to remove the precipitate of barium carbonate prior to the application to the filter paper but it is advisable to heat the sample shortly in order to dissolve the amino acids with low solubility in water.

Degradation of alanylleucylglycin

The directions given above were followed. The amount of tripeptide used was 26 mg. For the cleavage of the PTC-peptides 15 minutes were sufficient. The glycine remaining after the second cleavage was identified directly. The paper strip identifications of the amino acids are shown in Fig. 3.

Degradation of leucylglycylglycylglycine

The procedure described was followed. The amount of tetrapeptide used was 30 mg. The last amino acid was also transformed to its PTC-derivative, treated with nitromethane-HCl and hydrolysed in the ordinary way. The paper strip identification of the amino acids can be seen in Fig. 3.

DISCUSSION

The applicability of the method can only be briefly discussed in view of the limited number of instances in which it has been employed.

It is obvious from the very nature of the procedure that the presence in the peptide of a free α-amino or α-imino group is a prerequisite for its applicability.

* Phenyl thiohydantoins have been prepared from all the amino acids commonly occuring in proteins with the exception of serine, threonine, cystine and glutamine [10]. The behavior of the hydantoin derivatives from the latter amino acids is therefore not known.

** Immediately after the treatment with ninhydrin the colour of this spot was yellow but later on it turned purple.

Furthermore only molecules exclusively built up of peptide bonds between α-amino (α-imino) and α-carboxyl groups can be expected to lend themselves to a complete degradation.

It is possible that some of the amino acids will behave differently from those investigated. Of particular interest in this respect are the amino acids serine, threonine and cystine because in these cases there is a tendency for other reactions to occur concomittantly with the hydantoin formation [10]. This question is being investigated.

In an accompanying paper [10] it has been shown that optical activity is more or less completely lost on the formation of phenyl thiohydantoins from optically active amino acids. Whether or not racemisation will occur under the different conditions of the degradation procedure is of obvious interest and will be investigated.

SUMMARY

It has been shown that phenylthiocarbamyl peptides are easily cleavable into 3-phenyl-2-thiohydantoins and peptides with one less amino acid residue.

This reaction has been employed in the development of a micromethod for determination of the amino acid sequence of peptides. The details of the method are given and its applications to a tripeptide and a tetrapeptide are described.

The applicability of the method is briefly discussed.

This investigation has been supported by grants from *Harald och Greta Jeanssons stiftelse* and from *Statens medicinska forskningsråd.*

The technical assistance of Miss K. Diehl and Mr. B. Sandell is gratefully acknowledged.

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Received December 22, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 294–299

Preparation of Furoic Acid from Ribo Nucleic Acid

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Methods have recently been worked out in this laboratory for the preparation of the nitrogenous components of ribo nucleic acid (PNA) with a view to applying them to tracer studies [1–4]. Ribose, the carbohydrate of PNA has, however, not so far been included in the isotopic investigations. The main reason for this has been the lack of a suitable method for the preparation of this compound from small amounts of the PNA. The purpose of this paper is to present such a method and at the same time to describe its application to a problem, where glycine marked in the carboxyl group with C^{14} has been used as a precursor for the polynucleotides.

It seemed that the crystallisation of ribose from amounts of as little as utmost 200 mg of PNA would prove very difficult. Because of this it was decided to transform the ribose into furoic acid (pyromuic acid), a compound which is relatively easy to characterize and to obtain in pure form. This was achieved by preparing furfural from the ribose containing material by steam distillation in the presence of strong mineral acid, oxidation of the furfural to furoic acid with ammoniacal silver oxide, and purification of the acid by sublimation *in vacuo*.

EXPERIMENTAL

Preparation of PNA. The preparation of the polynucleotides and then separation into desoxyribo nucleic acid and PNA was carried out according to Hammarsten [1].

Preparation of furfural. Furfural was first prepared from the PNA. As usually occurs when furfural is prepared from PNA the aldehyde is formed mainly from the ribose bound to the purines and not from that bound to the pyrimidines. For the conversion of pentoses to furfural different authors have used distillation with anhydrous zinc chloride, trichloro acetic acid, phosphoric acid and strong mineral acids [5–7]. The best yields have

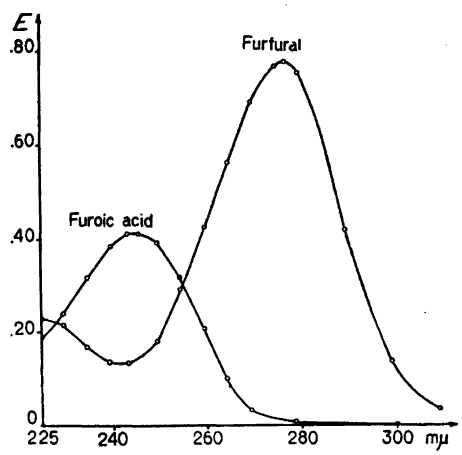

Fig. 1. Absorption curve in ultraviolet of water solutions of furfural and furoic acid.

usually been obtained with sulphuric and hydrochloric acids. Of the two hydrochloric acid gives a somewhat better yield (about 20 % more than does sulphuric acid). It is, however, very important for the oxidation of the furfural that the furfural solution after distillation should not contain any chloride ions. This could never be achieved when hydrochloric acid was used not even if the solution was twice redistilled with steam.

For this reason sulphuric acid was chosen for the preparation of furfural. 100—200 mg PNA were dissolved in 75 ml 9 N sulphuric acid. The solution was subjected to slow steam distillation for about 3 hours, the velocity of the distillation being about 30—40 ml per hour.

At the beginning of the investigation the distillation was considered finished, when a drop of the distillate gave no colour when applied to an anilin acetate paper [7]. Later the control of the distillation was carried out by taking advantage of the characteristic absorption in the ultra violet of the furan ring [5]. The absorption (Fig 1) was read in the Beckman photometer against water at the absorption maximum (277 mμ). For calculating the molar extinction coefficient a standard solution was prepared by twice redistilling *in vacuo* a preparation of furfural obtained from Eastman Kodak. This product had a specific gravity of 1.1581 at 20° and a boiling point of 159° C (1.1598 and 161.7° respectively for authentic material). The molar extinction coefficient was 14.800 at 277 mμ.

By following the absorption of the distillate it was possible to follow the course of the experiment (Table 1).

Oxidation of furfural. The formation of furoic acid from furfural has been carried out in various ways [9]. Only one method, however, that of oxidation

Table 1. Distillation of furfural from 250 mg PNA followed by means of lightabsorption in the ultraviolet.

Time minutes	E_{277}	Furfural obtained mg
0—30	111.5	18.1
30— 50	56.0	9.1
50— 70	15.5	2.5
70—100	26.8	4.3
100—150	6.8	1.0

of the pentose with silver oxide seemed likely to afford reasonable good yields on the mg scale. This method has even been used for quantitative estimation of furfural [10].

Slightly more than the amount of silver oxide theoretically necessary was freshly precipitated from a *N* solution of silver nitrate by the addition of 2 *N* sodium hydroxide. The precipitate was centrifuged off and washed with 2 × 5 ml of water. Afterwards it was dissolved in the smallest possible amount of 3 *N* ammonia and added to the furfural solution (about 100 ml). The oxidation was allowed to proceed for one minute at 100°. The solution was then evaporated to dryness in vacuo in order to remove all the ammonia. About 5 ml of hot water were added to the dry residue and the resulting suspension was centrifuged. The sediment was extracted with 2 × 5 ml hot water and the supernatants were combined. In order to decompose the silver furoic acid an excess of warm 0.1 *N* HCl was added. The silver chloride was centrifuged off and washed twice with hot water. The combined solutions were again evaporated to dryness in vacuo. The dry residue was extracted several times with ether, transferred to a small dish and the solution evaporated to dryness. In this way the furoic acid was collected on as small a surface as possible. The solid material was scraped of the walls of the dish and transferred with a small spatula to the sublimation apparatus (Fig. 2). The last traces of the furoic acid were transferred to the apparatus with the aid of a small amount of ether, which was evaporated before sublimation. This process which is described below was employed as the final stage of purification.

Furoic acid like furfural has a very characteristic absorption in the ultra violet [8]. The absorption curve (Fig. 1) and the molar extinction coefficient were determined on a synthetic specimen, which was prepared according to Wilson [11]. It had a melting point of 130—131° (132—133° for authentic material), an absorption maximum at 245 mμ and a molar extinction coefficient of

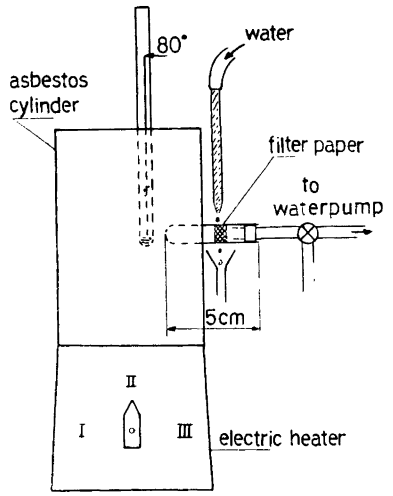

Fig. 2. Apparatus for microsublimation.

11.100. Furoic acid shows practically no absorption at 277 mμ which is the absorption maximum for furfural. Whereas at 245 mμ furoic acids has an absorption maximum and furfural a minimum (Fig. 1).

This makes it possible to follow the oxidation of furfural to furoic acid by measuring the ultra violet absorption of the solution during the reaction (Table 2).

Purification of furoic acid. A small test tube containing the crude product was immersed to half its height in an air bath of about 80°, and evacuated by a water pump. The upper part of the tube was surrounded by a strip of filter paper on which water was allowed to drip continuously. In this way the furoic

Table 2. The oxidation of furfural to Ag-furoic acid followed by measurement of absorption in the ultra violet. The quotient $\frac{E_{245}}{E_{260}}$ *served as measure of the course of the oxidation. The quotient of a standard of Ag-furoic acid was 2.13.*

Time	E_{245}	E_{260}	E_{277}	$\frac{E_{245}}{E_{260}}$
0	0.110	0.368	0.610	0.33
10 sec	0.147	0.325	0.570	0.45
20 »	0.410	0.245	0.110	1.57
40 »	0.460	0.225	0.025	2.04
1 min	0.450	0.210	0.015	2.14
3 »	0.460	0.220	0.012	2.09
4 hours	0.410	0.194	0.002	2.11

Table 3. The radioactivity per minute of about 15 mg $BaCO_3$ obtained from the various substances.

Material	Spec. activity, counts	Dilution of glycine carboxyl
Glycine administered	15 000	2
Mixed Polynucleotides	90	330
Furoic acid (ribose)	78	380
'Protein'	42	720

acid was sublimed on to a narrow area under the filter paper. The sublimation was allowed to proceed for 12 hours. At the end of this time the tube was cut and the acid collected. The melting point of the white and crystalline product varied from 129 to 131 degrees.

The over all yield of the whole procedure from PNA to furoic acid was 40—50 % calculated on the assumption of a tetranucleotide.

Experiments with $CH_2(NH_2) \cdot C^{14}OOH$. The present method has been tried on polynucleotides of chicken liver obtained during an experiment by Hammarsten *et al.*[12]. In this work one chicken received intraperitoneally a total of 3 g labelled glycine, divided into 4 doses at 6 hourly intervals. The animal was sacrified 12 hours after the last injection and the liver dried immediately with alcohol and ether.

The polynucleotides were prepared according to Hammarsten [1] and a 'protein' fraction was obtained by extraction of the dry organ powder with hot trichloro acetic acid as described by Schneider [13]. The ribose of the PNA was prepared as described above. The carbon from all samples was converted to $BaCO_3$ and the activity measured in a Geiger-Müller counter. The results are summarized in Table 3.

The higher level of C^{14} in PNA ribose compared with that in 'protein' indicates that there is a rather significant synthesis of PNA-ribose from glycine. On the other hand the isotope content of the mixed polynucleotides is somewhat higher than that of ribose. It seems best to postpone a more detailed discussion until other precursors for PNA-ribose have been investigated.

SUMMARY

A method for the isolation of furoic acid from small amounts of ribo nucleic acid is described.

This method has been used in an isotopic experiment, where glycine labelled with C^{14} in the COOH group has been used as precursor for ribo nucleic acid.

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Received November 3, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 300–306

The Fading of Crystal Violet in Alkaline Solution

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When an aqueous solution of crystal violet ($(CH_3)_2N \cdot C_6H_4)_3C \cdot Cl$, is made alkaline, the colour gradually fades, but reappears on acidification. At very small hydroxyl ion concentrations a distinct violet colour remains indicating that the reaction does not go to completion, an equilibrium between the dye and the reaction product obviously being established.

This fading reaction is common to a great number of triphenyl methan dyes, amino compounds as well as hydroxy compounds (*e. g.* the phthaleins). The reaction taking place is the same in all instances and may be written:

$$\rangle C^+ + OH^- \rightleftharpoons \rangle C \cdot OH$$

As the absorption maximum of crystal violet in the visible region of light is found in the yellow-green part of the spectrum the fading reaction is very convenient for spectrophotometric rate measurements.

Since Brønsted [1] advanced his theory concerning the salt effect many chemists have contributed to the problem of ionic reactions, both theoretically and experimentally. It is generally assumed that the velocity constant at small concentrations of the reactants and small ionic strengths is given by [2,4]:

$$\ln k = \ln k_0 - \frac{z_A z_B e^2 N}{\varepsilon R T} \cdot \frac{1}{r_A + r_B} + \frac{z_A z_B e^2 N}{\varepsilon R T} \cdot \frac{\varkappa}{1 + a\varkappa} \qquad (1)$$

where k_0 is the molar rate constant extrapolated to $\varepsilon = \infty$, z_A and z_B the valences of the reacting ions, e the electronic charge, N Avogadro's number, ε the dielectric constant, R the gas constant, T the absolute temperature, r_A and r_B the radii of the ions, a the distance of closest approach, and the Debye $\varkappa$ is given by

$$\varkappa = \sqrt{\frac{8\pi e^2 N}{1000\, \varepsilon k T}} \cdot \sqrt{I}$$

k being Boltzmann's constant and I the ionic strength. (The use of symbols employed in this article is that recommended by the "Commission on physico-chemical symbols and co-ordination of scientific terminologies" of the "International union of chemistry".)

Equation (1) is an approximation, and we may further neglect the term $a\varkappa = 3.25 \cdot 10^7 \cdot a\sqrt{I}$ at $T = 300°$ and $\varepsilon = 80$; $a\varkappa$ is thus of the same order of magnitude as $\sqrt{I}$, and we get the limiting law valid for small ionic strengths:

$$\ln k = \ln k_{0,\varepsilon} + \sqrt{\frac{8\pi}{1000}} \cdot \frac{z_A z_B e^3 N^2}{(\varepsilon R T)^{\frac{3}{2}}} \cdot \sqrt{I} \qquad (2)$$

Omitting the term $a\varkappa$ means that the dimensions of the ions are regarded negligible [3].

For the fading of crystal violet the dependence of $\ln k$ on $\sqrt{I}$ is linear until $\sqrt{I} = 0.15$, the slope of the curve being in accordance with the calculated value within about 5 %. The reaction was studied in water at 25° C with the addition of sodium chloride, potassium sulphate and barium chloride as neutral salts. It is easily shown that the stoichiometric scheme above is also the kinetic equation of the reaction. Using hydroxyl ion concentrations 100 to 1000 times as great as the dye concentration, the logarithms of the extinctions plotted against the time give straight lines. This fact indicates that the reaction velocity is proportional to the dye concentration, and furthermore that the equilibrium concentration of the coloured form of the dye is very small. This is also demonstrated by measuring the equilibrium extinction, which in all cases was a few per cent of the initial extinction. On account of the smallness of the final extinction it was impossible to determine the equilibrium constant with any accuracy; it is however of the order of magnitude $2 \cdot 10^4$ at room temperature. When measured in solutions with different hydroxyl ion concentrations but identical and constant ionic strength (in casu 1.0 M NaCl) as suggested by Brønsted [1, p. 205 f.] — the velocity constants proved to be proportional to the hydroxyl ion concentration.

In order to check the Arrhenius equation

$$\log k = B - \frac{\Delta H}{2.3\, R\, T} \qquad (3)$$

the reaction was studied at 45°, 35°, 25° and 15° C. In La Mer's treatment of the problem [4] he has derived the following equations for the dependence of ΔH and B on $\sqrt{I}$ at 25° C:

$$\frac{\Delta H - \Delta H_0}{2.3\, R\, T} = 0.51\, z_A z_B \sqrt{I} \quad \text{and} \quad B - B_0 = 1.52\, z_A z_B \sqrt{I} \qquad (4)$$

At 30° C, the middle of the interval examined, the constants are found to be 0.59 and 1.59 respectively. The equations of La Mer are identical with those derived by Moelwyn-Hughes [5]. The variation of ΔH and B with the ionic strength only show a poor agreement with the theory, probably on account of the difficulty of keeping hydroxyl ion concentrations of the order of magnitude 10^{-3} M sufficiently constant in unbuffered solutions.

EXPERIMENTAL

Crystal violet was synthesised from 4,4'bis(dimethylamino) benzophenone, dimethylaniline and phosphorus oxychloride [6] and recrystallised 4 times from water. On drying in the air the product could not be obtained with a definite amount of water of crystallisation; but after drying for two weeks in a vacuum dessicator with phosphorus pentoxide the product was anhydrous and on analysis gave the following results:

$C_{25}H_{30}N_3Cl$ (408)	Calc.	N 10.30	Cl 8.69
	Found	» 10.16, 10.27	» 8.82, 8.70

Nitrogen was determined by the micro-Dumas method according to Pregl, and chlorine was determined by semi-micro Volhard titration in the filtrate after precipitation of the carbinol with chlorine free sodium hydroxide and corrected for the solubility of silver chloride in the combined filtrate and wash water. A 10^{-4} molar stock solution was made by weighing the correct amount and dissolving in a calibrated flask with carbon dioxide free water. This solution as well as all the others used were kept protected against the carbon dioxide in the atmosphere.

Sodium hydroxide, carbonate free, was prepared from 16—18 normal NaOH (Merck zur Analyse) which was filtered from the insoluble sodium carbonate through a sintered glass filter with protection against the carbon dioxide in the air. The stock solution was made up from carbon dioxide free water to about 0.02 N and kept in a paraffin lined bottle provided with a buret and soda lime tubes. It was standardised against hydrochloric acid, which in turn was standardised against borax (Kahlbaum zur Analyse).

Barium hydroxide (Kahlbaum z. A.) was dissolved in water, filtered, standardised, and preserved like sodium hydroxide.

Sodium chloride, *potassium sulphate* and *barium chloride* (Kahlbaum z. A.) were accurately weighed, dissolved in carbon dioxide free water in calibrated flasks.

Water. It was found, that a carbon dioxide free sample could be prepared by bubbling air, which had passed a washing bottle with diluted sulphuric acid and a large tower with soda lime through a 6 l pyrex flask with distilled water from the laboratory still at a rate of two bubbles per second for 24 hours. The pH of the water used was between 6.5 and 7.5 measured with a glass electrode. By controlling the pH after two months' storage it was found, that the flask, which for some years had been used for storing water, gave off no alkali to the water.

The spectrophotometer used was a Hilger Nutting spectrophotometer with the absorption tubes placed in a metal box, through which water from the thermostat was circulated. The temperature was constant within $\pm$ 0.01° C, and the thermometer used was controlled against a standard thermometer.

All the volumetric instruments were calibrated by weighing out with water.

Procedure. The various ingredients for a run except the dye solution were measured into a 100 ml measuring flask, which was then filled up with water until about 5 ml below the mark and placed in the thermostat. When temperature equilibrium was reached the requisite amount of crystal violet solution, as a rule 4 ml, was added so as to make the solution $4 \cdot 10^{-6}$ molar in respect to the dye. Simultaneously the stop watch was started. The solution was made up to 100 ml with water and again placed in the thermostat. By siphoning the solution into the absorption tube under protection against the atmospheric carbon dioxide the tube was rinsed five times with the solution and finally filled. The comparison tube was constantly filled with distilled water. Now the thermostat box with the absorption tubes was placed in position and the pointolite lamp lighted. The time elapsed after the start of the stop watch amounted to about 6 minutes. The extinction D was measured at intervals of 1, or in the slower experiments 2, 3, or 5 minutes at 586 mμ wavelength (absorption maximum of crystal violet). In a series of experiments with identical composition the solution was constantly irradiated in some of them, while in the others it was screened from the light except in the 10 second periods of measuring. The differences in the velocity constants lay within the experimental error, so it is concluded, that the light does not influence the reaction.

METHOD OF CALCULATION

According to Christiansen [7] the uncertainty of the rate constants is smallest, when calculated from observations made between 20 and 60 % conversion, and in most cases the reaction is only followed in this interval. In some cases the measurements were continued to a higher degree of conversion, and it was found that the points were still distributed at random about the same straight line, but the deviations were larger. Only in a few of the slow experiments the plot showed a faint curvature indicating a decrease in the rate constant. The slope of the plot of log D against t, and the intersection with the ordinate axis determining k and log D_0 in the equation

$$\log D = \log D_0 - kt$$

were calculated by means of the method of least squares, using the Gaussian normal equations

$$n \log D_0 - k \Sigma t = \Sigma \log D$$
$$\log D_0 \Sigma t - k \Sigma t^2 = \Sigma t \log D$$

In solving the equations it is a great facilitation, that when the t values constitute an arithmetical progression with the difference 1, the determinant $n\Sigma t^2 - (\Sigma t)^2$, as can be easily shown, is equal to $\frac{1}{12}(n^2 - 1)\, n^2$, where n is the number of observations. It should be noted, that the k thus calculated is not the true velocity constant but differs from it by the factor 2.30259, and it will be designated k' in the following.

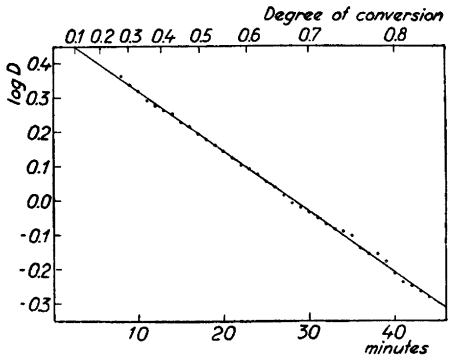

Fig. 1. 0.004 M NaOH, 0.025 M NaCl and 3.5 10^{-6} M crystal violet at 25° C.

RESULTS

Fig. 1 shows the plot of a single measurement at 25° C. The straight line is drawn according to the values log $D_0 = 0.493$ and $k' = 0.01750$ calculated by the above method including all observations. At the top of the diagram is marked the degree of conversion. When calculated from the first 16 observations ($0.2 < \alpha < 0.6$) $\log D_0$ is found to be $= 0.495$ and $k' = 0.01763$; the mean error of the $\log D$ values is 0.0047. In the same way the last 21 observations ($\alpha > 0.6$) give $\log D_0 = 0.493$ and $k' = 0.01748$, and the mean error 0.0084.

Table 1 shows the dependence of the velocity constant on the hydroxyl ion concentration in a series of measurements in 1 *M* sodium chloride.

Fig. 2 shows a plot of log ($k'/[OH^-]$) against $\sqrt{I}$. The straight line has the theoretical slope 1.018. It is seen, that the limiting law holds untill $\sqrt{I} \sim 0.15$. There is obviously no difference between the effect of univalent and divalent ions.

Table 1. k' in 1 M NaCl at 25° C as a function of [OH⁻].

$[OH^-]$	k'	$\frac{k'}{[OH^-]}$	Deviation
0.002	0.0044	2.200	− 0.118
0.003	0.0059	1.967	+ 0.115
0.004	0.0086	2.148	− 0.066
0.005	0.0106	2.120	− 0.038
0.006	0.0126	2.100	− 0.018
0.007	0.0145	2.071	+ 0.011
0.008	0.0169	2.113	− 0.031
0.010	0.0208	2.080	+ 0.002
0.012	0.0236	1.967	+ 0.115
0.016	0.0329	2.056	+ 0.026
Mean:		2.082	0.073 ~ 3.5 %

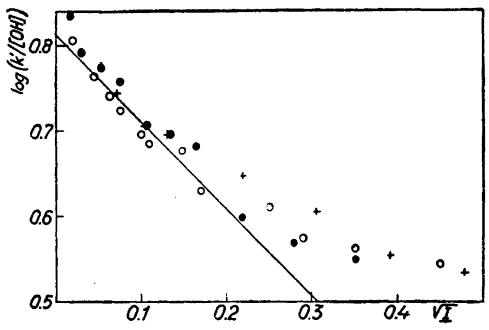

Fig. 2. Measurements at 25° C. Open circles: NaOH with and without NaCl; filled circles: $Ba(OH)_2$ with and without $BaCl_2$; crosses: NaOH with K_2SO_4.

Table 2 comprises the logarithms of the velocity constants measured at different temperatures and ionic strengths; each value is mean of 2—4 measurements. The table includes the terms C and A calculated by the method of least squares from the equation

$$\log \frac{k'}{[OH^-]} = C + A\sqrt{I}$$

which is identical with equation (2). In the last column are stated the theoretical values of A.

Table 2. Log (k'/[OH^-]) as a function of temperature and ionic strength.

$\sqrt{I}$ =	0.0316	0.0707	0.1000	C	A	A (theor.)
15° C	0.386	0.343	0.312	0.420	− 1.09	− 1.00
25° C	0.773	0.731	0.695	0.810	− 1.14	− 1.02
35° C	1.128	1.085	1.063	1.156	− 0.96	− 1.04
45° C	1.498	1.471	1.423	1.539	− 1.00	− 1.06

In Fig. 3 is shown a plot of log (k'/[OH^-]) against $1/T$ at different ionic strengths including the extrapolated values at $\sqrt{I} = 0$ (the column headed C in Table 2). The straight lines representing the equation

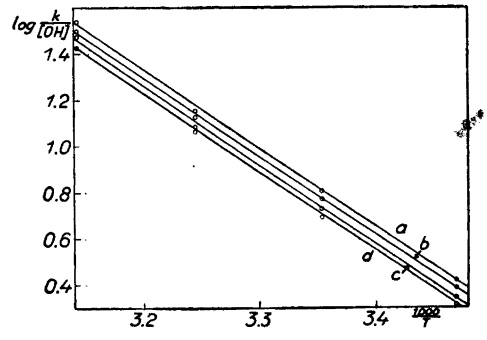

Fig. 3. Relation between log (k'/[OH^-]) and 1/T. $\sqrt{I}$ is for curve a 0.000, b 0.0316, c 0.0707, and d 0.1000.

Table 3. B' and ΔH as functions of the ionic strength.

$\sqrt{I}$	B'	ΔH
0.0000	12.20	15530 cal
0.0316	12.13	15490 —
0.0707	12.23	15680 —
0.1000	12.08	15530 —

$$\log \frac{k'}{[OH^-]} = B' - \frac{\Delta H}{2.3\ RT}$$

are calculated by the method of least squares, and in succession from above correspond to $\sqrt{I} = 0.000$, 0.0316, 0.0707 and 0.1000.

The values of B' and ΔH calculated for these lines are shown in Table 3. They obviously do not satisfy equation (4) p. 301, in which $z_A z_B = -1$ must be inserted for this reaction.

SUMMARY

The reaction between crystal violet and hydroxyl ions in pure water and dilute salt solutions has been studied at 15°, 25°, 35°, and 45° C. It is found that the salt effect is in accordance with Brønsteds theory, provided that the ionic strength is less than 0.02. The velocity constants found satisfy the Arrhenius equation in the interval examined, but the accuracy of the measurements is insufficient to verify the relation between the Arrhenius constants and the ionic strength as derived by La Mer.

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Received December 8, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 307–316

Die Methoden zur Bestimmung der Selbstdiffusion in festen Stoffen

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Die Bestimmung der Konstante der Selbstdiffusion in festen Stoffen hat Interesse aus folgenden Gründen:

Die Abhängigkeit der Diffusionskonstante von der Temperatur hat die Form

$$D = D_0 \cdot \exp\ (-E/RT)$$

wobei beim Vorliegen reiner Gitterdiffusion die Energiegrösse E in Beziehung zu gitterenergetischen Grössen gebracht werden kann [1]. Der Faktor D_0 ist ebenfalls wichtig, da auch er in Zusammenhang mit gittertheoretischen Grössen steht und zur Entscheidung über den Charakter der jeweils vorliegenden Fehlordnung beitragen kann [2]. Schliesslich ist die Kenntnis der Selbstdiffusionskonstante nötig für quantitative Ansätze zur Berechnung der Geschwindigkeit von Festkörperreaktionen, bei denen im allgemeinen die Diffusion als zeitbestimmende Teilreaktion auftritt [1].

Während früher die Bestimmung der Selbstdiffusion nur indirekt möglich war, können die in der letzten Zeit mit Hilfe radioaktiver Indikatoren durchgeführten Bestimmungen als direkt [4] angesehen werden, wenn man von dem Isotopieeffekt absieht, der indessen im allgemeinen zu klein ist, um auf die oben genannten Betrachtungen einwirken zu können [5].

Aus allen diesen Gründen sind im hiesigen Institut eine Reihe Selbstdiffusionsbestimmungen unter Verwendung verschiedener Methoden im Gange. Während über die experimentellen Ergebnisse demnächst berichtet werden soll, seien schon hier die zur Zeit existierenden und erprobten Methoden zusammengestellt.

DIE METHODEN

Zunächst die Methoden, die ohne Verwendung radioaktiver Isotope auskommen.

1. Bestimmung mit Hilfe von Indikatorsubstanzen

Als Indikatorsubstanz bezeichnet man eine solche Substanz, die der zu untersuchenden chemisch möglichst ähnlich ist; so ist z. B. CuJ als Indikatorsubstanz für AgJ verwendet worden.

Man ermittelt für das Indikatorion die »spezifische Ionenbeweglichkeit» im Mischkristall AgJ/CuJ, d. h. den Anteil der Kupferionen an der Leitfähigkeit des Mischkristalls, dividiert durch ihren Molenbruch [6]. Ermittelt man auch ihre Diffusionskonstante in AgJ, (etwa nach der Methode von Stefan Kawalki), so muss dieser letzte Wert offenbar mit dem Quotienten der spezifischen Leitfähigkeit des reinen AgJ und der oben definierten »spezifischen Ionenbeweglichkeit» der Kupferionen in CuJ/AgJ multipliziert werden, um die Selbstdiffusionskonstante des Ag in AgJ zu ergeben [7].

2. Bestimmung aus der Ionenleitfähigkeit

Die als Nernst-Einsteinsche Formel bekannte Beziehung zwischen Diffusionskonstante und Beweglichkeit liefert durch den Vergleich mit dem aus der spezifischen Leitfähigkeit erhaltenen Ausdruck für die Beweglichkeit

$$D = \frac{n \cdot \varkappa}{F \cdot c \cdot z \cdot e} kT$$

wobei D die Diffusionskonstante, n die Überführungszahl, $\varkappa$ die spezifische Leitfähigkeit, k die Boltzmannkonstante, T die absolute Temperatur, c die Konzentration in Äquival./cm^3, z die Valenz des betrachteten Ions, F die Faradaykonstante und e die Elementarladung bedeuten.

Die strengere Theorie dieser Beziehung und die Grenze ihrer Verwendbarkeit finden sich in der Arbeit von Wagner [8]. (Bei den im allgemeinen untersuchten Substanzen und Temperaturen ist der Proportionalitätsfaktor zwischen Diffusionskonstante und spezifischer Leitfähigkeit von der Grössenordnung 10^{-5}, wenn erstere in cm^2/sek, die letztere in Ohm^{-1}cm^{-1} gemessen wird.)

Bei den folgenden beiden Methoden werden zwar radioaktive Indikatoren verwendet, die Selbstdiffusionskonstante wird aber nur indirekt ermittelt.

3. Bestimmung aus der Geschwindigkeit beim Isotopenaustausch

Wenn bei einer Austauschreaktion zwischen zwei Phasen, die das gleiche Element enthalten und von denen die eine radioaktiv indiziert ist, die Diffusion in der anderen Phase als langsamste Teilreaktion zeitbestimmend ist, lässt sich die Selbstdiffusionskonstante in dieser Endphase bestimmen [9].

Sollte der eigentliche Austausch zeitbestimmend sein, so lässt sich für die Diffusion in der Endphase zumindest ein Minimalwert angeben. So hat Zimen [10] für die Selbstdiffusionskonstante der Silberionen in einer AgBr-Photoschicht bei Zimmertemperatur als unteren Grenzwert $D = 2 \cdot 10^{-12} \mathrm{cm}^2/\mathrm{sec}$ gefunden.

4. Bestimmung aus dem Diffusionsanteil des Emaniervermögens

Bei der Analyse der Hahnschen Emaniermethode gelangten Flügge und Zimen [11] zu einer Abschätzung der Selbstdiffusionskonstante.

Auf einem anderen Weg geht Jagitsch [12] vor, der aus der Übereinstimmung des Temperaturinkrementes der elektrischen Leitfähigkeit einiger fester Salze mit dem Temperaturinkrement des Diffusionsanteiles ihres Emaniervermögens auf einen Zusammenhang zwischen dieser letzten Grösse und der Selbstdiffusion schloss und sie einander direkt proportional setzt.

Wir gehen nun zu den radioaktiven Messmethoden über, wobei wir zwei Hauptgruppen unterscheiden können: Bei der einen (5—7) wird die durch Diffusion eintretende Verteilung *des Radioindikators nach* Zerlegung *des Diffusionsmediums untersucht. Dies könnte prinzipiell auch bei Verwendung stabiler Indikatorisotope und massenspektrographischer Analyse geschehen. (Auf einen ähnlichen Fall der Bestimmung einer Selbstdiffusionskonstante wies A. Klemm hin [13].) Bei der zweiten Gruppe (8—11) ist eine Zerlegung nicht nötig, sondern die* Verteilung *wird unter Zuhilfenahme der* Absorption *durch direkte Messung der Strahlung bestimmt.*

5. Die Methode von Stefan und Kawalki

Das Prinzip der Methode ist alt und oft auf Fremddiffusionsuntersuchungen angewendet worden. Im radioaktiven Versuch werden 3 inaktive Tabletten von bestimmter Dicke auf eine gleich dicke indizierte Tablette gepresst und nach der Diffusion wieder getrennt. Die nun vorliegende Verteilung der

Aktivität wird bestimmt. Die daraus resultierende Diffusionskonstante ist den in der Fachliteratur oft wiedergegebenen Stefan-Kawalkischen Tabellen zu entnehmen.

6. Die Ausbreitung einer dünnen Schicht

Die aktive Substanz wird in einer sehr dünnen Schicht auf eine Tablette des zu untersuchenden Stoffes aufgebracht. Nach der Diffusion wird die Tablette in möglichst dünne Fraktionen senkrecht zur »Diffusionsrichtung» zerlegt und die radioaktive Konzentration dieser Fraktionen bestimmt. Der Konzentrationsverlauf bei erwähnten Anfangsbedingungen ist bekanntlich:

$$C = \frac{1}{\sqrt{\pi Dt}} \exp\left(-\frac{x^2}{4\,Dt}\right)$$

Trägt man also ln C als Funktion von x^2 auf, so erhält man eine gerade Linie, deren Neigung $-1/4\,Dt$ ist. So mass Banks [14] die Selbstdiffusionskonstante in metallischem Zink zwischen 10^{-8} und 10^{-9} cm²/sec. Bei noch feinerer Zerlegung dürfte man so noch 1—2 Zehnerpotenzen geringere Diffusionskonstanten messen können.

7. Bestimmung durch Übergang bei idealem Kontakt

Zwei Tabletten, von denen die eine aktiv und die andere inaktiv ist, werden mit absolut ebenen und glatten Oberflächen aufeinander gepresst und unter Druck gehalten, worauf sie auf Diffusionstemperatur gebracht werden [15]. Die Diffusion der radioaktiven Substanz geschieht in Richtung auf die inaktive Tablette, die nach erfolgter Diffusion wieder abgenommen und auf ihre Radioaktivität hin untersucht werden kann. Die Vollständigkeit des Kontaktes kann durch gleichzeitige Messung der Leitfähigkeit kontrolliert werden.

Für die überdiffundierte Menge gilt:

$$S = q\, C_0 \sqrt{\frac{Dt}{\pi}}$$

woraus die Diffusionskonstante zu errechnen ist, da die Kontaktfläche q und die Konzentration C_0 bekannt sind. Mit der Methode sind Diffusionskonstanten zwischen 10^{-12} und 10^{-15} cm²/sec bei verschiedenen Bleisalzen gemessen worden [16]. Theoretisch gesehen ist der Anwendbarkeitsbereich der Methode

beliebig gross; in der Praxis ist die geringste damit messbare Diffusionskonstante durch die Notwendigkeit des eben nur bei einer bestimmten Temperatur ausreichend guten Kontaktes gegeben; die grösste damit messbare Diffusionskonstante durch die bei hohen Temperaturen eintretende Rekristallisation, die eine Trennung ohne Schädigung der Tabletten verhindert.

8. Bestimmung auf Grund der Absorption von α-Strahlung

Anfangsbedingungen wie bei 6, die Schicht ist α-strahlend. Es wird die Ionisation, die durch ein senkrecht von der Tablettenoberfläche ausgehendes Strahlenbündel hervorgerufen wird, vor und nach der Diffusion gemessen [17]. Den Wert vor der Diffusion setzt man = 1. Für den Konzentrationsverlauf nach der Diffusion gilt der unter 6 erwähnte Ausdruck, das heisst, dass

$$\int_0^{a-b} \frac{1}{\sqrt{\pi\, Dt}} \exp\ \left(-\frac{x^2}{4\, Dt}\right) dx$$

α-Strahlen ins Elektroskop gelangen, wobei a die Reichweite der betrachteten α-Strahlen in dem untersuchten Medium und b eine der durchdrungenen Luftschicht (und eventuell einer das Elektroskop verschliessenden Folie) äquivalente Strecke in eben diesem Medium ist. Da im Elektroskop aber nicht die Anzahl der Strahlen, sondern der durch sie hervorgerufene Ionisationsstrom gemessen wird, muss die Änderung der Ionisationsfähigkeit der α-Strahlen mit der durchdrungenen Materieschicht korrigiert werden. Es gilt für die Verminderung der Ionisationswirkung eine Funktion $I = \varphi(x)$, also für die gemessene Aktivität:

$$A = \int_0^{a-b} \frac{1}{\sqrt{\pi\, Dt}} (1 - \varphi(x)) \exp\ \left(\frac{-x^2}{4\, Dt}\right) dx$$

Führt man $\xi = \dfrac{a-b}{2\sqrt{Dt}}$ ein, so gilt:

$$A = \psi(\xi) - \frac{1}{\sqrt{\pi\, Dt}} \int_0^{a-b} \varphi(x) \exp\ \left(-\frac{x^2}{4\, Dt}\right) dx,$$ wobei ψ das Fehlerintegral bedeutet.

Die Auswertung geschieht graphisch.

Für die Ableitung der Formeln in 8 und 9, wie auch der übrigen Diffusionsformeln, vgl. den Artikel von Fürth [18]. Hevesy und Seith [17] massen mit dieser Methode Diffusionskonstanten von 10^{-9}—10^{-10}cm²/sec.

9. Bestimmung auf Grund der Absorption von Rückstosstrahlung

Diese Methode ist dadurch möglich, dass die Folgeprodukte von α-Strahlern einen Rückstoss geringer Reichweite erfahren und selbst auf Grund ihrer eigenen Radioaktivität bestimmt werden können, nachdem sie auf einer elektrisch geladenen Platte gesammelt worden sind. Diese Rückstossausbeute verringert sich offenbar mit fortschreitender Diffusion einer sehr dünnen Schicht aktiver Substanz, die auf eine Tablette des zu untersuchenden Stoffes aufgebracht war. Für die Anzahl der nach erfolgter Diffusion die Tablette verlassenden Rückstossatome gilt ein Ansatz wie in 8. Allerdings liegt in diesem Falle kein annähernd paralleles Bündel vor, sondern alle die Tablette verlassenden Rückstossteilchen werden auf Grund ihres ionisierten Zustandes auf die Auffangplatte gezogen. Nun verlässt aber die Tablette von den Rückstosstrahlen, die von der Tiefe x ausgehen, nur ein Anteil, der sich zur Gesamtstrahlung verhält wie die Oberfläche einer Kugelhaube von der Höhe a—x zur Oberfläche einer Halbkugel mit dem Radius a. (a ist die Reichweite in dem untersuchten Stoff). Dieses ergibt einen Korrektionsfaktor 1—x/a; also wird

$$A = \int_0^a \frac{1}{\sqrt{\pi\, Dt}} \left(1 - \frac{x}{a}\right) \exp\left(-\frac{x^2}{4\, Dt}\right) dx$$

Dieses führt schliesslich zu:

$$A = \psi(\xi) - \frac{1}{\xi\sqrt{\pi}} (1 - e^{-\xi^2}) \text{ mit } \xi = \frac{a}{2\sqrt{Dt}}$$

Die Diffusionskonstante entnimmt man aus der graphischen Darstellung. Eine solche sei wiedergegeben in Fig. 1 [16]. Mit der Rückstossmethode massen Hevesy und Seith [17] Diffusionskonstanten zwischen 10^{-14} und 10^{-17} cm²/sec.

10. Methode der aktiven Teiltablette

Diese Methode (vgl. 5) wurde zuerst von Zimen und Hillert [19,20] angewendet. Auf eine inaktive Tablette wird eine aktive Schicht von der Dicke l aufgepresst (erst zu 1/4, später zu 1/2 der Gesamtdicke h gewählt) und im Laufe der Diffusion die Aktivitätsabnahme auf des aktiven sowie Aktivitäts-

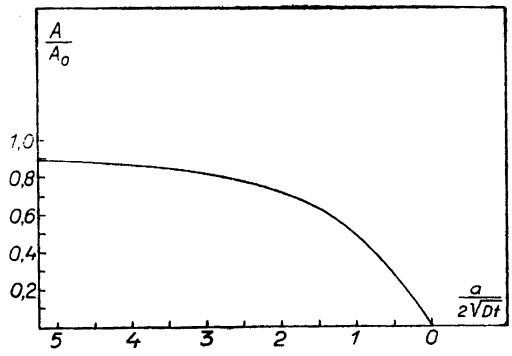


Fig. 1. Graphische Darstellung zur Ermittlung der Diffusionskonstante D (bei bekannter Versuchszeit t und Rückstossreichweite A) aus der Änderung der relativen Aktivität A/A_0 bei der Rückstossmethode.

zunahme auf der inaktiven Seite bestimmt. Unter Verwendung der Reihenentwicklung von Stefan-Kawalki und der allgemeinen Absorptionsformel für β-Strahlung:

$$A = \int_0^l C\ e^{-\mu x}\ dx$$

ergibt sich schliesslich für die Aktivitätsabnahme:

$$A_1 = C_0 \left[\frac{1}{\mu} (1 - e^{-\mu l}) \frac{h}{l} + \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{1}{n} \exp\ (-\ (\frac{n\pi}{l})^2\ Dt)\ \sin\ \frac{\pi n h}{l} \right.$$

$$\left. \frac{1 - e^{-\mu l} \cos\ \pi n}{\mu \left[1 + (\frac{n\pi}{\mu l})^2 \right]} \right]$$

und für die Aktivitätszunahme eine entsprechender Ausdruck.

Es genügt im allgemeinen, den Ausdruck für $n = 1$ und $n = 3$ auszuwerten. Die Diffusionskonstante entnimmt man auch hier einer graphischen Darstellung. Eine solche, die wir bei der Untersuchung von Kalziumsalzen verwendeten [21], wobei Diffusionskonstanten zwischen 10^{-7} und 10^{-9} cm²/sec gemessen wurden, sei in Fig. 2 wiedergegeben. Sie gilt für das Produkt $\mu l = 29$. (μ ist der Absorptionskoeffizient in cm^{-1}).

11. Methode der aktiven dünnen Schicht

Wie in 6, 8 und 9 wird eine dünne radioaktive Schicht durch Diffusion zur Ausbreitung gebracht.

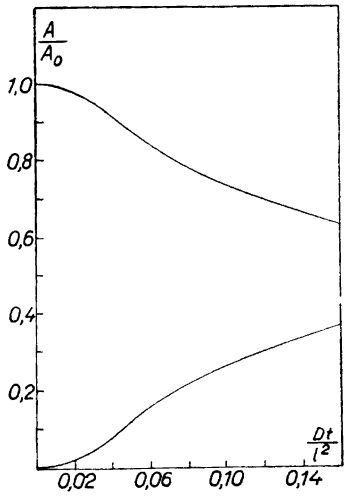


Fig. 2. Graphische Darstellung zur Ermittlung der Diffusionskonstante D (bei bekannter Versuchszeit t und Tablettendicke 1) aus der Änderung der relativen Aktivität A/A_0 bei der Methode der aktiven Teiltablette.

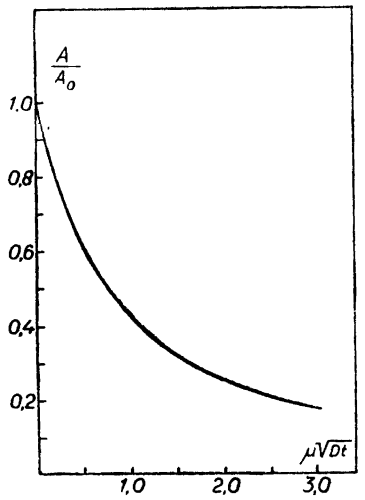


Fig. 3. Graphische Darstellung zur Ermittlung der Diffusionskonstante D (bei bekannter Versuchszeit t und bekanntem Absorptionskoeffizienten μ) aus der Änderung der relativen Aktivität A/A_0 bei der Diffusion einer aktiven dünnen Schicht.

Wie dort gilt für die Konzentrationsverteilung der Aktivität Q:

$$C = \frac{Q}{\sqrt{\pi\, Dt}} \exp\left(-\frac{x^2}{4\, Dt}\right)$$

Zusammen mit der Absorptionsformel

$$A = \int_0^\infty C\ e^{-\mu x}\ dx$$

ergibt sich schliesslich für die gemessene Aktivität nach der Diffusion:

$$A = A_0\ e^{\mu^2 Dt}\left[1 - \psi\left(\mu\sqrt{Dt}\right)\right]$$

Diesen Ausdruck haben drei verschiedene Autoren unabhängig voneinander angegeben [22–24]. Die Auswertung geschieht mit Hilfe der graphischen Darstellung

$$\frac{A}{A_0} = f\left(\mu\sqrt{Dt}\right)$$

die in Fig. 3 gezeigt wird. Mit dieser Methode wurden z. B. bei der Selbstdiffusion von Zn in ZnO Werte zwischen 10^{-8} und 10^{-11}cm²/sec gemessen [21].

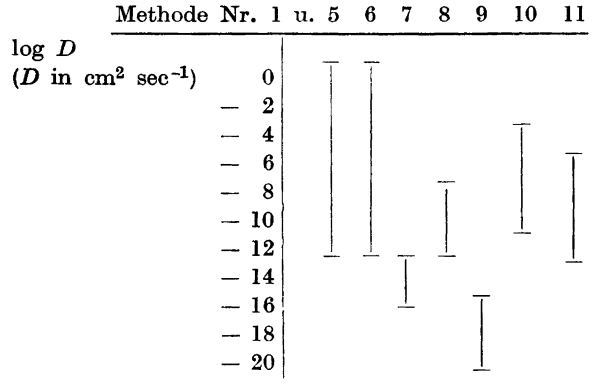
Will man ausserdem die Konzentrationsverteilung und damit die Konstanz des Diffusionskoeffizienten kontrollieren, so kann man auf der aktiven Seite der Tablette dünne Schichten abschmirgeln und jedesmal die Aktivität der Resttablette messen [23], die sich zur ursprünglichen Aktivität verhält wie

$$\frac{1-\psi\ (y_1)}{1-\psi\ (y_0)}$$

wobei x die abgeschmirgelte Schicht und $y = \dfrac{x}{2\sqrt{Dt}} + \mu\sqrt{Dt}$ ist.

Zum Schluss werde die grössenordnungsmässige Empfindlichkeit einiger der hier besprochenen Methoden miteinander verglichen. (Ein solcher Vergleich ist natürlich etwas willkürlich, da z. B. die Strahlungshärten der radioaktiven Indikatoren von Fall zu Fall beträchtlich variieren können und die Anwendung aktiver dünner Schichten bei höheren Temperaturen durch eintretende Verdampfung beschränkt wird.) Rechnet man mit den üblichen Werten und setzt für die Dauer des Diffusionsversuches als Grenzen 10^3 und 10^6 Sekunden, so erhält man folgendes Bild.

Man sieht, dass bei ein und dem gleichen System in verschiedenen Temperaturbereichen die Anwendung verschiedener Methoden möglich ist [16,18,20] und dass in jedem Bereich mindestens eine Methode zur Messung der für die Festkörperforschung wichtigen Selbstdiffusionskonstanten existiert. Dies den auf diesem Gebiet arbeitenden Fachkollegen in übersichtlicher Weise darzubieten ist der Zweck dieser Mitteilung.



ZUSAMMENFASSUNG

Eine zusammenfassende Betrachtung der Methoden zur Bestimmung des Selbstdiffusion in festen Stoffen wird gegeben, wobei die Anwendungsmöglichkeiten der verschiedenen Methoden miteinander verglichen werden. —

Wir danken Prof. J. A. Hedvall und Doz. K. E. Zimen für ihr Interesse an dieser Arbeit, ferner *Statens Tekniska Forskningsråd* und *Nobelstiftelsen* für die Bereitstellung von Mitteln.

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Eingegangen am 24. November 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 317–333

The Urea Denaturation of Fibrinogen

I. Kinetic Aspects

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Because of its property of increasing the solubility of proteins, urea is a reagent often employed by biochemists. It has been shown [1] that fibrin, the insoluble protein resulting from the action of thrombin on fibrinogen, is also soluble in urea solutions. Several recent investigations, [2,3] making use of this solubility, have dealt with the chemical and physicochemical differences between fibrin and its precursor.

The advantage of working in homogeneous medium is obvious; but a question which arises immediately when using urea in experiments with proteins is whether or not it has a denaturing effect on the protein in question. Meisner and Wöhlisch [4] considered fibrinogen resistant to the denaturing effect of urea, and even thought to avoid its spontaneous denaturation by storing it at 0° C in solutions of 30 per cent urea. Diebold and Jühling [5], continuing investigations along this line, found that urea denatured fibrinogen, but at 4° C, the denaturation was extremely slow. With higher temperatures the rate of denaturation was found considerably increased. Since Diebold and Jühling report qualitative, rather than quantitative data, and since the results of investigations on fibrinogen and fibrin in the presence of urea are difficult to interpret without a clear picture of its denaturing effect under different conditions, it seemed desirable to investigate the problem more thoroughly.

The urea denaturation of proteins has been subjected to much study, but very few data are presented on the kinetics of the process. Hopkins [6] showed that the urea denaturation of egg albumin has a negative differential rate-temperature coefficient. The same observation has been made recently in an investigation of the urea denaturation of β-lactoglobulin [7]. As pointed out by Clark [8], the negative temperature coefficient may be the result of two opposite actions of urea on the egg albumin molecules: (a) denaturation,

which renders the protein insoluble, and (b) splitting of the denatured molecules into soluble products. Usually the rate of denaturation is determined on the basis of the appearance of insoluble protein. If the tenperature coefficient of the second reaction is greater — both being positive — the resulting differential rate-temperature coefficient should be negative. The second effect is apparent only at fairly high urea concentrations. At lower urea concentrations than those employed by Hopkins, Clark [9] found a positive temperature coefficient of the denaturation of egg albumin.

Although the experiments along this line are not conclusive, the reaction seems to be of the first order in the case of chorion gonadotropin [10], with a positive temperature coefficient[11]. Lauffer[12], in his very extensive study on the denaturation of tobacco mosaic virus protein by urea, also found the reaction to be of the first order, but the velocity constant was a linear function of the reciprocal of the initial virus concentration. The temperature coefficient was negative below 23° C and positive above this temperature. He made the assumption that the urea denaturation consists of two or more parallel simultaneous reactions, one having a negative differential rate-temperature coefficient. As Hopkins did in the case of egg albumin denaturation, Lauffer supposed the formation of an urea-tobacco mosaic virus complex, and the subsequent denaturation of this.

There is complete lack of data on the reaction type of urea denaturation of other proteins. The investigation here reported was undertaken also with the idea of contributing to the data on the kinetics of urea denaturation of proteins.

EXPERIMENTAL

Materials

Fibrinogen was prepared from oxalated bovine plasma. Four liters of blood were mixed with 1 liter of 2 per cent sodium oxalate solution and the cells removed by centrifugation. The plasma was cooled to 0° C and the fibrinogen precipitated by adding ice-cold, saturated $(NH_4)_2\,SO_4$ solution to 0.24 saturation. The precipitate was centrifuged, and redissolved in 500 ml physiological saline. The insoluble residue, mainly red corpuscles, was centrifuged down. The clear solution was diluted with saline to 3 000 ml and the fibrinogen precipitated again by adding saturated $(NH_4)_2\,SO_4$ solution to 0.24 saturation. The resulting precipitate was centrifuged, washed with ice-cold distilled water, and dissolved in 200 ml of physiological saline solution. All the above operations were performed at a temperature as close as possible to 0° C. The fibrinogen solutions were slightly opalescent.

The method described above was followed in order to obtain quickly, through few steps, material pure enough for the kinetic study of the denaturation. This was attained by precipitating the second time from a rather big volume. The fibrinogen is very sensitive to repeated precipitations. In order to obtain a preparation as close to the native state as possible, further purification by reprecipitations and the removal of $(NH_4)_2 SO_4$ by dialysis were omitted. The fibrinogen preparations used contained, therefore, a small amount of $(NH_4)_2 SO_4$.

The concentration and purity of preparations were determined by clotting an aliquot of the fibrinogen with thrombin, and then washing, drying, and weighing the resulting clot. From another aliquot the total protein concentration was determined by means of precipitation with 20 per cent trichloracetic acid. The resulting precipitate was washed, dried, and weighed. The solutions contained 30 to 40 mg fibrinogen per milliliter and their purity ranged from 85 to 95 per cent.

All the other substances used were analytical reagents.

Methods

The denaturation is manifested by a loss of or a change in the specific properties of the protein. The most apparent of these changes is that of solubility. The denatured protein becomes insoluble in a solvent in which it was soluble in the native state. The estimation from time to time of the protein fraction which became insoluble is the easiest and most commonly used method for the determination of the denaturation rate of a protein.

Conditions under which the denatured protein is completely insoluble, while the solubility of the native protein is unaffected, must be carefully chosen, if an effective separation is to be secured. The urea denatures the protein, but in moderate and high concentrations it keeps the denatured product in solution. Therefore the urea should be removed by dialysis or its concentration lowered by dilution until it is not in sufficient amount to keep the denatured protein in solution. By either of these procedures the denatured protein precipitates, provided the pH of the solution is suitable. The precipitation is optimal at the isoelectric point and the denatured protein is more or less soluble at moderate alkaline or acidic pH.

In the preliminary investigations the precipitation of the denatured fibrinogen was effected by dialysing out the urea against 0.9 per cent NaCl solution the pH of which was brought to approximately 7 with a few drops of $N/1$ NaOH. After complete removal of the urea, the pH of the solution was adjusted to 6.3 with phosphate buffer; and the precipitate formed during the dialysis

and by the addition of the buffer solution was centrifuged down. The unaffected fibrinogen remained in the supernatant liquid, was clotted by thrombin, and its amount estimated. The fraction of the fibrinogen originally present recovered as fibrin clot gave a measure of the extent of denaturation. In these experiments the pH of the solution was not the most favorable for the separation of the denatured fibrinogen. Presumably a fraction of the denatured fibrinogen remained in solution after adjusting the pH at 6.3, and it was thereafter incorporated in the clot formed by the native fibrinogen. The extent of denaturation is, therefore, somewhat greater than determined by the above method.

The method of dialysis is not applicable to kinetic studies. Since urea also exerts its action during the dialysis the reaction time is uncertain. For this reason the method of precipitation by dilution was adopted. The buffered fibrinogen solution was kept in a constant temperature water bath, and sufficient time was allowed for reaching the temperature equilibrium; urea solution at the same temperature was then added, and the solution thoroughly mixed. During the experiment the solution was stirred continuously. At definite time intervals after mixing the fibrinogen solution with urea, samples of 5 ml were withdrawn and diluted in centrifuge tubes with 50 ml of M/10 phosphate buffer of pH 5.9. The pH of the final mixture was 6.05. The denatured fibrinogen precipitated in the early stage of the reaction as a fine fluffy precipitate, later as long filaments clumped together. To assure complete separation of the denatured material, the solutions were allowed to stay three hours at room temperature, then centrifuged. It was easy with the aid of a spatula to collect the sticky precipitate in a compact pellet. The precipitate was washed with physiological saline solution, then two times with distilled water, dried at 105° C, and weighed.

Attempts have been made to separate the denatured from the native fibrinogen by salting out. The salting out curves of the two substances were determined and are shown in Fig. 1. Denatured fibrinogen, which precipitated completely by dilution with buffer of pH 5.9, was only partially precipitated by dilution with neutral $(NH_4)_2SO_4$ solutions up to 0.15 saturation. Ammonium sulfate had, at low concentrations, a salting in effect, followed at higher concentrations by a salting out effect on the denatured fibrinogen. As Fig. 1 shows, the most favorable salt concentration for the separation of native and denatured fibrinogen is approximately 0.15 $(NH_4)_2SO_4$ saturation, but even in this case about 10 per cent of the denatured fibrinogen may remain in solution.

The above experiments made us prefer separation of denatured fibrinogen by dilution with buffer of pH 5.9, rather than by precipitating it by salting out.

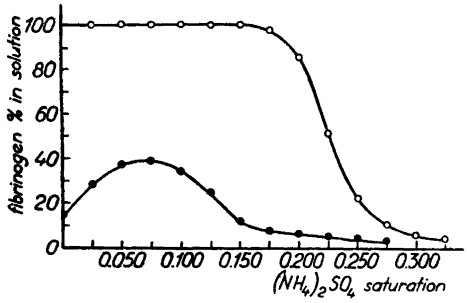

Fig. 1. Salting out curves of native and urea denatured fibrinogen. 100 per cent corresponds to 1.47 mg protein per milliliter.
○ *native fibrinogen.*
● *denat. fibrinogen.*

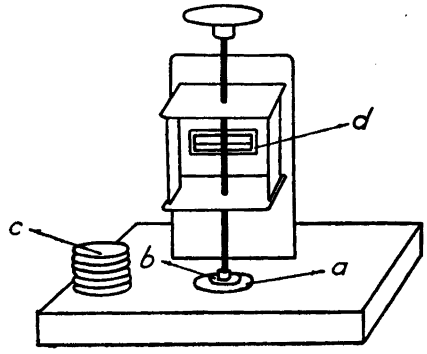

Fig. 2. Apparatus for the determination of the compressibility of fibrin gels. For explanation see text.

Difficulties were encountered when attempts were made to determine the urea denaturation of fibrin, and none of the above methods was found practicable. Fibrin dissolved in urea was denatured to various extents. When the urea was removed by dialysis or the solution diluted with buffers of different pH, all the fibrin precipitated out, denatured as well as unaffected.

When the urea was dialysed out against a buffer of pH 7.6 — a procedure which favored the formation of a 'fine type' gel [13] — the fibrin solutions which were exposed to the effect of urea at different pH, temperature, etc., yielded gels with quite different physical properties. The gels, where denaturation was expected according to the results obtained by fibrinogen, were opaque and soft, while those where presumably denaturation did not occur were transparent and elastic. The tensile strength of 'coarse type' gels, obtained by dialysing out the urea at pH 6.3, was lowered in those in which denaturation occured. It is probable from the above results that the mechanical properties of a fibrin gel are proportional to the amount of undenatured fibrin present and are not influenced by the inclusion of denatured fibrin. On the basis of this assumption, the extent of denaturation of fibrin has been determined by measuring the compressibility of the gels formed after the removal of the urea.

A small apparatus, shown in Fig. 2, was constructed to make possible the quantitative determination of the compressibility. The fibrin solutions, after undergoing the action of the urea at different conditions, were brought to about pH 7.6, poured into cellophane tubes, and then dialysed at 0° C for

48 hours against M/10 phosphate buffer of pH 7.6. After dialysis, the tubes contained cylindrical gels of 18 mm diameter. Small discs, of 10 mm height, were cut from them with the aid of a knife constructed from two shaving blades, fixed parallel at a distance of 10 mm from each other. The gel disc was cautiously transferred into the cylindrical hole of the apparatus, *a*, which had the same diameter as the gel, and so placed that the cut surface was horizontal. Compression was exerted by a round plate, *b*, of 14 mm diameter, which could be loaded with loads of different weight, *c*. The compression of the gel was followed by projecting on a centimeter scale the image of a hair fixed perpendicular to the axis in the frame *d*. With an enlargement of 1 : 20, it was possible to determine easily a deformation of 0.05 mm. The position of the image on the scale was observed, and the gel then loaded with 10 g every 30 seconds. The loading was continued at a a rate of 10 g per 30 seconds up to 50 g and then decreased in the same manner. The results were reproducible with different pieces cut off from the same gel.

Results

A. *The reaction type of the urea denaturation of fibrinogen.* Fibrinogen solutions of 15 to 40 mg per milliliter concentration were used. Twenty milliliters of fibrinogen solution were mixed with 5 ml. *M*/1 sodium phosphate buffer of pH 7 and 25 ml of 60 per cent urea solution at 30° C in the manner already described. Thus the experiments were performed in the presence of rather high salt concentrations, the final ionic strength being approximately 0.34. In such high salt concentration the small individual variations in the $(NH_4)_2\ SO_4$ content of different fibrinogen preparations had no significance. By using high buffer concentration the adjustment to the desired pH was assured and a possible shift of pH during the denaturation process avoided.

Fig. 3 shows the results obtained with three different fibrinogen concentrations : 8.0, 12.6, and 16.2 mg fibrinogen per milliliter in the final mixture. The amount of denatured fibrinogen is plotted against the time of standing in contact with 30 per cent urea. All points corresponding to the three different concentrations are apparently on the same curve. Plotting the logarithm of the concentration of unaffected fibrinogen against the reaction time, as done in Fig. 4, gave a straight line. In Fig. 3 the smooth curve corresponds to the straight line of the logarithmic graph. The experimental points do not deviate more than 1 to 3 per cent from this ideal curve. Above 90 per cent denaturation this error — common to a gravimetrical protein determination — causes pronounced scattering of the experimental points on the logarithmic graph. On the basis of the above findings the reaction must be considered

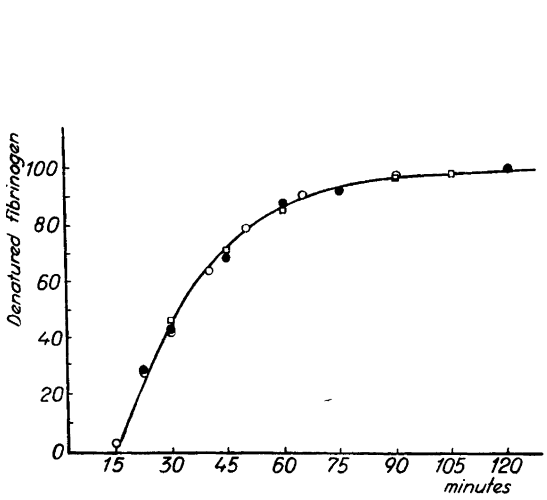

Fig. 3. Urea denaturation of fibrinogen. 30 per cent urea concentration, 30° C, pH 7.
○ *16.2 mg fibrinogen/ml*
● *12.6 mg fibrinogen/ml*
□ *8.0 mg fibrinogen/ml*

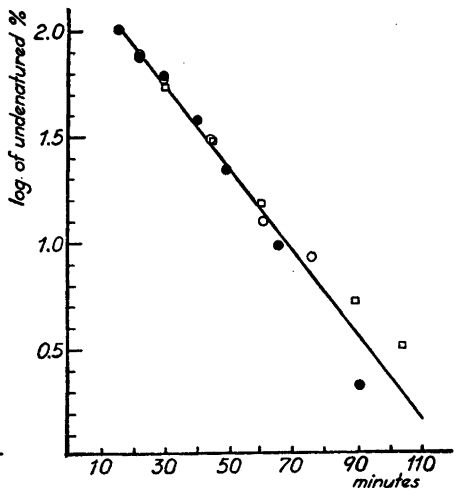

Fig. 4. Logarithm of undenatured fibrinogen plotted against time. Same experiments as in Fig. 3.

as one of the first order. In all the experiments, performed at various temperatures, different urea concentrations, and different pH, these conditions were fullfiled.

As can be seen in Figs. 3 and 4 in the first 15 minutes the amount of precipitated denatured fibrinogen was very small, if any. The data suggest an inductive period, after which the reaction started as a first order one. To explain this, it is supposed that the urea denaturation of fibrinogen consists of two distinct processes. The primary process is a definite chemical reaction of the first order; the result of this is fibrinogen molecules already denatured, but still possessing a considerable degree of hydration and, consequently, a rather high solubility. They are not precipitated at pH 6, but only after a secondary action of urea, which diminishes their hydration and renders them insoluble at the pH in question. The secondary alteration has the same velocity for all the molecules affected during the primary process, which means that the precipitation of denatured fibrinogen will follow the course of the primary change, but with a shift in time equal to the duration of the secondary changes. The change in the solubility of denatured fibrinogen is more apparent at a pH close to the isoelectric point, and becomes less pronounced at a pH distant from it. In other words, a very short secondary action of the urea

will suffice to render the molecules insoluble at a pH near the isoelectric point, while at a higher pH the secondary action will be longer the more alkaline is the pH of the diluting solution.

To test the correctness of this assumption, fibrinogen was denatured at pH 7 and 30° C in contact with 30 per cent urea. The reaction was followed as usual by diluting samples at different time intervals, but using for this purpose three buffers of different pH : 5.5, 5.9, and 6.4. Fig. 5 presents the results in logarithmic plot. As was expected, the inductive periods are increased proportionally with the pH of the diluting solution, but the straight lines in all cases have equal slopes, showing that the velocity constants are the same with only the precipitation of the denatured product suffering a retardation proportional to the pH of the diluting buffer.

Findings similar to these are reported by Hopkins [6] in connection with the urea denaturation of serum proteins. This author found that the nitroprusside test was positive very soon after the addition of urea, but the denatured serum proteins began to precipitate on dialysis or dilution only after some hours of previous standing in contact with urea. He concluded: 'It would seem as though these proteins undergo with readiness the chemical changes which in all cases are associated with denaturation, but the colloid particles of the product are more resistant than those of egg albumin to the dehydration which characterizes the change from lyophil to the lyophobe condition.'

The solubility of the denatured product would offer another possible explanation of the inductive period. In this case the precipitation would start only after the saturation of the solution with the denatured fibrinogen. It is very easy to convince one's self of the wrongness of this assumption. Indeed, the amount of finally precipitated denatured fibrinogen was always equal to or very near by 100 per cent of the fibrinogen originally present. In the case of a solubility of the denatured fibrinogen the precipitate at the end of the denaturation process would be obviously less than 100 per cent; and the reaction would apparently slown down.

Fig. 5 shows also the curve obtained by precipitating the denatured fibrinogen with 0.15 saturated $(NH_4)_2SO_4$ solution. It was already mentioned that the denatured product is slightly soluble in this solution even after a prolonged action of urea. Accordingly, it can be seen that the reaction is apparently slowed down and the amount of precipitated fibrinogen did not reach 100 per cent of the originally present.

Thus the assumption of a primary process, followed by secondary physicochemical changes in the state of denatured molecules seems to be justified. A further proof of this concept will be presented in section II of this paper.

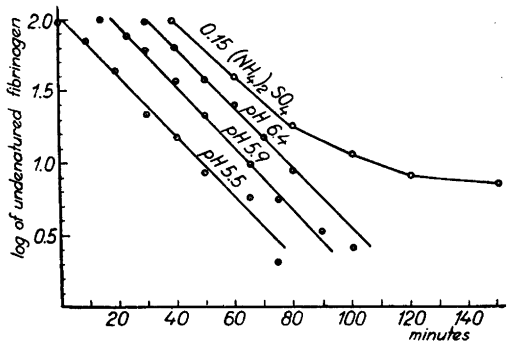

Fig. 5. The urea denaturation of fibrinogen followed by diluting samples with buffers of different pH and 0.15 saturated $(NH_4)_2SO_4$ solution. Denaturation at 30° C, pH 7, and 30 per cent urea concentration.

From the results described above it seemed probable that a buffer which has a pH equal to the isoelectric point of fibrinogen would be the most effective for precipitating denatured fibrinogen in the earliest stage of denaturation. Such a buffer can be used to dilute fibrinogen solutions which have stood in contact with 30 per cent urea, because the urea concentration after dilution is still sufficiently high to avoid the isoelectric precipitation of the native fibrinogen; but is not applicable to the dilution of solutions with lower urea concentrations where the native fibrinogen will precipitate also. This was the reason why the precipitation of denatured fibrinogen with $M/10$ phosphate buffer of pH 5.9 was utilised throughout this work.

The secondary process seems to be affected in the same way as the primary by temperature and urea concentration. The slower the denaturation process, the longer the inductive period; the quicker the denaturation, the shorter the period.

B. *Effect of temperature and urea concentration on the denaturation rate.* In order to investigate the effect of the urea concentration on the reaction rate, 20 ml fibrinogen solution of 30 mg per milliliter concentration was mixed with 5 ml $M/1$ sodium phosphate buffer of pH 7 and 25 ml of urea solution of, respectively, 20, 40, and 60 per cent concentration. Thus the final urea concentrations were 10, 20, and 30 per cent. The mixtures were kept at different temperatures and the reaction rate determined. In all the cases an inductive period was observable, the significance of which has already been discussed. The velocity constants were calculated from the slope of the straight lines obtained by plotting the logarithm of the concentration of unaffected fibrinogen against the time of standing in contact with urea. In each case at least two independent experiments were made, and the given values correspond to the

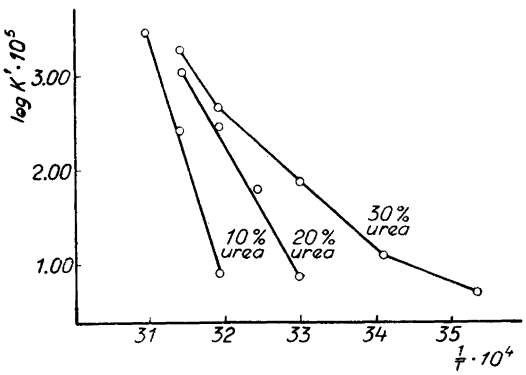

Fig. 6. The Arrhenius plot of the velocity constants of the urea denaturation of fibrinogen.

straight lines which best fitted all the experimental points. The results are summarised in Table 1.

It is evident that an increase in temperature brought about an increase of the reaction rate in all instances. The differential rate-temperature coefficient of the denaturation process is thus always positive — a result in contrast with the findings on egg albumin, β-lactoglobulin, and tobacco mosaic virus protein. Fig. 6 shows the Arrhenius plot of the results; *i. e.*, the logarithm of the velocity constants plotted against the reciprocal of the absolute temperature. It is apparent that in the presence of 10 and 20 per cent urea in the investigated temperature range the Arrhenius law is valid, the logarithm of

Table 1. Velocity constants and half reaction times of the urea denaturation of fibrinogen at different temperatures and urea concentrations. pH 7.

t (°C)	Urea conc.	k'	Half reaction seconds
40	10 %	7.85×10^{-5}	8 833
45	10 %	3.15×10^{-3}	220
50	10 %	1.35×10^{-2}	51
30	20 %	7.20×10^{-5}	9 625
35	20 %	6.19×10^{-4}	1 119
40	20 %	2.86×10^{-3}	242
45	20 %	1.06×10^{-2}	65
10	30 %	5.03×10^{-5}	13 750
20	30 %	1.26×10^{-4}	5 500
30	30 %	7.50×10^{-4}	924
40	30 %	4.56×10^{-3}	152
45	30 %	1.83×10^{-2}	37

the velocity constant being a linear function of the reciprocal of the absolute temperature. The considerable temperature coefficient of the reaction rendered difficult the covering of a larger temperature range than 10 to 15° C. At 30 per cent urea concentration, it was possible to investigate the reaction rate over a wider range. Between 20 and 40° C, the Arrhenius plot gave a straight line, but above and below this temperature there was a marked deviation from this line. It is possible that a similar departure would also be detectable in the presence of 10 and 20 per cent urea, if the determinations could have been extended over a larger temperature range.

The heat, the free energy and the entropy change of activation were calculated using the equations derived by Eyring and Stearn [14, 15] from the theory of absolute reaction rates:

$$\Delta H^{\ddagger} = RT^2 \frac{\mathrm{d} \ln k'}{\mathrm{d}\, T} - RT \tag{1}$$

$$\Delta F^{\ddagger} = -RT \ln k' + RT \ln \frac{kT}{h} \tag{2}$$

$$\Delta S^{\ddagger} = \frac{\Delta H^{\ddagger} - \Delta F^{\ddagger}}{T} \tag{3}$$

It can be seen that the heat of activation has approximately the same value as the classical energy of activation given by the well known Arrhenius relation:

$$E = RT^2 \frac{\mathrm{d} \ln k'}{\mathrm{d}\, T} \tag{4}$$

From equations (1) and (4),

$$\Delta H^{\ddagger} = E - RT \tag{5}$$

The energies of activation were calculated from the slopes of the Arrhenius plot and then substituted in equation (5) to obtain the heats of activation.

The values calculated are listed in Table 2. It is apparent that the heat of activation of the denaturation process is lowered to a great extent by urea. Considering only the segment between 20, and 40° C in the presence of 30 per cent urea, the heats of activation are, respectively, 120 000, 63 000, and 32 000 calories for 10, 20 and 30 per cent urea concentration. Thus, as while the urea concentration is increased arithmetically, the heat of activation decreases geometrically. At constant temperature the entropy of activation

Table 2. The heat, the free energy, and the entropy change of activation by the urea denaturation of fibrinogen at different temperatures and urea concentrations. pH 7.

t (°C)	Urea conc.	$\Delta H^{\ddagger}$	$\Delta F^{\ddagger}$	$\Delta S^{\ddagger}$
40	10 %	120 200	24 200	306.7
40	20 %	63 000	22 000	130.9
40	30 %	31 900	20 700	35.7
10	30 %	14 600	22 100	−26.5
30	30 %	31 900	22 075	32.4
45	30 %	54 300	21 100	104.4

is also considerably lowered by the increase of the urea concentration, whereas at constant urea concentration the rise of the temperature increases the entropy of activation.

The results may be explained in terms of the protein denaturation theory of Mirsky and Pauling [16]. These authors suppose that the activation process involves the breaking of hydrogen bonds. The great increase in entropy is the result of the increase in freedom of the groups liberated by this process. The liberated groups are unable to form new hydrogen bonds with water, but urea has a much greater tendency than water to from such bonds. It is probable that this tendency even exceeds that of the groups linked in the protein molecules; urea being thus able to break the hydrogen groups between these groups. The binding of urea molecules is an exothermic reaction, the heat liberated compensating in part the heat expenditure of the activation process. At the same time the freedom of the groups which bound urea is decreased, the result being a drop in the entropy change of the activation process. At high urea concentrations and low temperature the drop of the entropy caused by the binding of urea molecules, may exceed the entropy increase of the activation process, the result being a negative entropy of activation.

The free energy change of the activation was only moderately decreased by the presence of urea, because the heat and the entropy of activation were lowered in such a way as to compensate each other.

The urea-protein complex formation is exotherm; the number of bound urea molecules is, therefore, decreased when the temperature rises. A consequence of the decrease of the number of bound urea molecules with increasing temperature is the increase of the heat and of the entropy of activation. This can be actually observed on the data obtained in the presence of 30 per cent

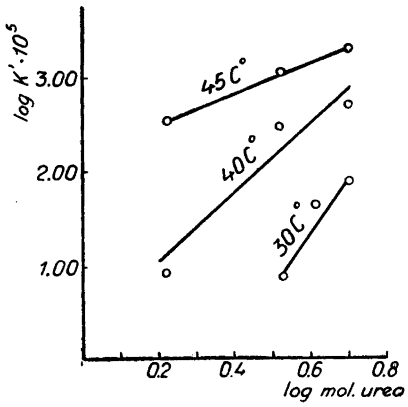

Fig. 7. The logarithm of the velocity constants plotted against the logarithm of the urea concentration.

urea (see Table 2). At high temperature it is evident that the number of bound urea molecules will be equal to zero, the heat of activation approaching thus a value equal with that found in the absence of urea. Fig. 6 clearly shows this tendency.

At constant temperature the increase of urea concentration brings about an increase in the velocity constant. This is easily explained by an increase in the number of bound urea molecules. According to Lauffer's derivations [12], the velocity constant at constant temperature is proportional to a power of the urea concentration. Thus, when log k' is plotted against log urea concentration, a straight line should result. The lines corresponding to different temperatures — according to the theory — are the steeper, the lower is the temperature. This straight line relation has been verified by Lauffer on tobacco mosaic virus and the change in the slope at different temperatures was also observed.

Fig. 7 shows a similar plot of the data obtained on fibrinogen. At 45° C, a straight line was obtained, but a 40° C, the data fitted less satisfactorily with a straight line relation. In spite of the incompleteness of data, it can be seen that the lines become much steeper when the temperature decreases. The slopes are equal with the average number of urea molecules bound, which are respectively 4.0, 3.2, and 1.4 at 30, 40, and 45° C. In the case of tobacco mosaic virus, Lauffer found 8.1 at 0° and 5.7 at 45° C. Comparing the results obtained with the two proteins, it is apparent that at the same temperature (45° C) fibrinogen bound less urea than the virus and that the rise of temperature dissociates the fibrinogen complex more rapidly.

C. *The effect of pH on the denaturation rate.* The determination of the reaction rate was conducted as in the preceding experiments. Twenty milliliters of fibrinogen solution containing 30 mg per milliliter, was mixed with 5 ml of buffer of different pH. Molal acetate buffers of pH 4.6 and 5.3, $M/1$ phosphate buffers of pH 5.9, 6.5, 7.0, and 7.6, and a borate buffer of pH 8.6 — equal volumes of $M/1$ boric acid and $M/4$ borax — were used. The solutions have the same molal concentration, but very different ionic strength. In spite of this, the results seem coherent, which shows that at such high values the variation in ionic strength has little effect. The buffered fibrinogen was mixed with 25 ml of 60 per cent urea solution. The pH of the solution was not altered significantly by the addition of urea. The mixture was kept at 20° C, then samples of 5 ml were diluted with 50 ml of buffer solution which composition was calculated in each case to give, after mixing, a final pH of 6.05. The amount of precipitate was determined in the described manner. In Table 3 are summarised the velocity constants obtained. The reaction rate was nearly constant between pH 7 and 8.6, whereas lowering the pH below 7 increased considerably the velocity constant. Below pH 7 the reaction rate was proportional with the 0.4 power of the hydrogen ion concentration. When, in this range, log k' was plotted against pH, a straight line was obtained, with the slope —0.4 (see Fig. 8) According to Steinhardt[17], this demonstrates that the activation of the molecules is accompanied by the binding of 0.4 proton per mol. of fibrinogen. It is hard to believe, however, that the above figure has this significance, since it is too small to have such a pronounced effect on the state of the molecule. Most probably the hydrogen ions have a catalytic effect.

In early orienting experiments, the effect of pH on the denaturation of fibrinogen by urea was studied by a method of dialysis. Four milliliters of

Table 3. Velocity constants and half reaction times of the urea denaturation of fibrinogen at different pH.

Urea conc.	t (°C)	pH	k'	half reaction (seconds)
30 %	20	4.6	1.10×10^{-3}	630
30 %	20	5.3	6.37×10^{-4}	1 087
30 %	20	5.9	3.88×10^{-4}	1 786
30 %	20	6.5	2.18×10^{-4}	3 178
30 %	20	7.0	1.26×10^{-4}	5 500
30 %	20	7.6	1.38×10^{-4}	5 021
30 %	20	8.6	1.39×10^{-4}	4 941

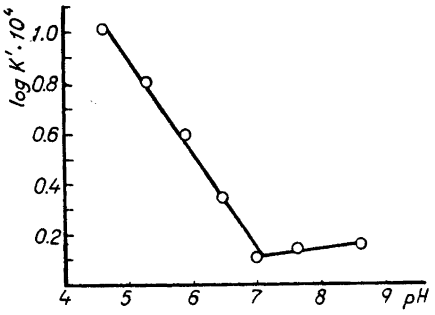

Fig. 8. The logarithm of the velocity constants plotted against pH. 30 per cent urea concentration, 20° C.

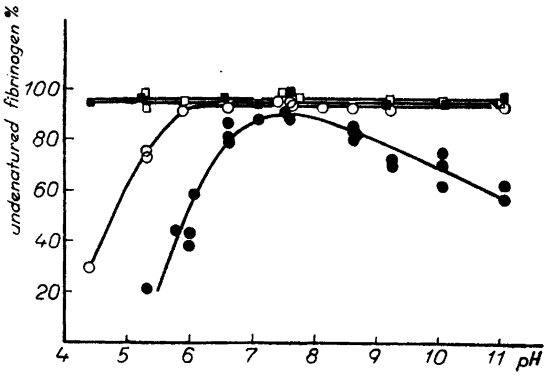

Fig. 9. The urea denaturation of fibrinogen at different urea concentrations and pH. Time of standing in contact with urea: 24 hours. 0° C.

□ *0* % *urea*
■ *10* » »
○ *20* » »
● *30* » »

fibrinogen solution of 25 mg per milliliter concentration were mixed with 1 ml *M*/1 buffers of different pH, and 5 ml of urea solution at 0° C. Urea solutions of 20, 40, and 60 per cent were used. The mixtures stood 24 hours at 0° C; their pH was then brought to approximately 7 with a few drops of *M*/1 NaOH or *M*/1 HCl; the solutions were then dialysed, the precipitated fibrinogen centrifuged down, and the unaeffected fibrinogen clotted by thrombin.

The results are shown in Fig. 9. It can be seen that at 10 per cent urea concentration the amount of fibrinogen recovered as fibrin clot is equal, over the whole pH range studied, to the amount obtained in controls containing physiological saline solution instead of urea. The same is true for 20 per cent urea concentration above pH 6.0. Below this pH, the amount of denatured fibrinogen increases rapidly with the lowering of pH. At 30 per cent urea, the maximal stability is approximately at pH 7.6 and the amount of unaffected fibrinogen falls on either side of this point. According to Wu and Yang [17] the pH of maximal stability of egg albumin against urea denaturation is 7, the two proteins being similar in this respect.

The results obtained by the study of the influence of pH on the velocity constants of denaturation are in accord with the above findings. The pronounced accelerating effect of the acidic reaction and the relatively small change

brought about by the increase of pH from 7 to 8.6 in the presence of 30 per cent urea can be seen here also.

D. *The urea denaturation of fibrin.* Fifty milliliters of fibrinogen of 40 mg per milliliter concentration were clotted by adding 1 ml of thrombin solution. The clot was allowed to stay at room temperature for three hours, then cooled to 0° C, and dissolved by adding 15 g of solid urea. The fibrin solution obtained was divided in three equal portions and calculated amounts of distilled water and 60 per cent urea solution added in order to have after addition of buffer a final protein concentration of 13.3 mg per milliliter, and a final urea concentration of, respectively, 10, 20, and 30 per cent. Three sets of tubes were prepared with the solutions of different urea concentrations, each tube containing 9 ml fibrin solution and 1 ml of $M/1$ buffer of different pH. The mixtures were kept at 0° C for 24 hours, then neutralised, and dialysed against $M/10$ phosphate buffer of pH 7.6, as described in the section on methods.

Fig. 10 shows the maximal compression obtained with load of 50 g by the gels which were obtained from fibrin solutions treated with 10, 20, and 30 per cent urea at different pH. The compressibility of the gels obtained from the fibrin solutions with 10 per cent urea was constant over the whole pH range studied. With 20 per cent urea constant compressibility was observed above pH 6.0, but below this pH the gels softened proportionally with the increase in acidity. Thirty per cent urea concentration had a marked effect below pH 7 and above pH 9; between pH 7 and 9, the mechanical properties of the fibrin gels were very alike.

A comparison of the curves with those in Fig. 9, which represent the denaturation of fibrinogen in similar conditions, reveals a great similarity between the resistance of the two proteins against urea denaturation. The above findings suggest that the urea denaturation of fibrin does not differ from that of fibrinogen.

SUMMARY

1. The urea denaturation of fibrinogen is a reaction of the first order. The primary reaction is followed by secondary changes characterized by a gradual decrease of the solubility of the denatured product.

2. The denaturation process has a positive differential rate-temperature coefficient. The Arrhenius law is valid over small temperature intervals only.

3. Increase of urea concentration increases the reaction rate and decreases the activation energy and entropy of the process. The free energy change of the activation process is lowered only moderately by urea.

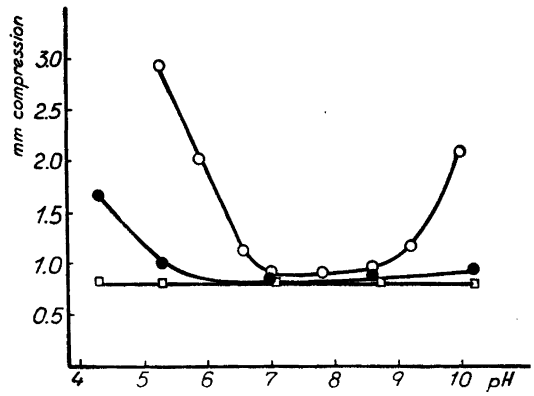

Fig. 10. Compression of fibrin gels obtained with load of 50 gm.
○ *30 % urea*
● *20 » »*
□ *10 » »*

4. The reaction rate is increased by lowering the pH below 7, in the presence of 30 per cent urea. Between pH 7 and 8.6, the reaction rate is nearly constant.

5. The urea denaturation of fibrin does not differ from that of fibrinogen.

The author wishes to acknowledge his indebtedness to Professor Hugo Theorell for inviting him to work in his laboratories, and for his very kind interest throughout the work.

He also wishes to express his tanks for a grant from The Institute for Muscle Research, New York.

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Received September 9, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 334 – 343

The Urea Denaturation of Fibrinogen

II. Physicochemical Changes

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The physicochemical properties of a number of proteins are considerably altered by urea and other related substances. Fibrinogen exposed to urea undergoes remarkable changes. In the presence of 30 per cent urea the non-Newtonian character of the viscous flow of fibrinogen solutions is greatly reduced [1] and the double refraction of flow disappears [2]. Meissner and Wöhlisch [3] showed that the opacity and the ability of fibrinogen solutions to form threads (Spinnbarkeit) is greatly diminished by urea. These effects follow almost immediately the addition of urea, and are readily interpreted by supposing a fragmentation of the rod-shaped fibrinogen molecules. The dissociating effect of urea is well known also in the case of other proteins, such as hemoglobin [4,5], myosin [6,7], tobacco mosaic virus [8,9], *etc.* In some of the cases this dissociation is reversible.

Prolonged action of concentrated urea solutions brings about changes connected with the denaturation of the molecules. Hopkins [10] found that the viscosity of egg albumin and serum protein solutions was increased and that finally gelation occured in the presence of approximately 60 per cent urea. Neurath and his co-workers [11,13] investigated in more details the viscosity and diffusibility changes of egg albumin and of serum proteins in urea solutions. They concluded that the increase of viscosity and the decrease of diffusibility may be explained by an unfolding of the protein molecules during the process of denaturation. An increase of the viscosity and opacity of fibrinogen solutions in urea was found earlier by Diebold and Jühling [14]. They correctly interpreted these findings as signs of denaturation.

In the course of investigations on the kinetics of the urea denaturation of fibrinogen, reported in section I of this paper, similar observations were made. The main object of the experiments to be described in the present section is

the investigation of these physicochemical changes and their correlation with the denaturation process.

EXPERIMENTAL

Materials

Fibrinogen was prepared by the method described in the first section of this paper. Throughout this work solutions containing 30 mg fibrinogen per milliliter were used.

All the other reagents used were of analytical purity.

Methods

The amount of denatured fibrinogen was estimated by precipitation, the urea-fibrinogen mixture being diluted with 10 volumes of $M/10$ phosphate buffer of pH 5.9. The details of the method have already been given in the preceding paper.

The change in opacity of urea-fibrinogen solutions was determined in a Beckman spectrophotometer at two wave length: 350 and 600 mμ. Twenty milliliters of fibrinogen solution were mixed with 5 ml of $M/1$ phosphate buffer of pH 7; were kept in a water bath until the desired temperature was reached; and were then mixed with 25 ml urea solution at the same temperature. The mixture stood in the constant temperature water bath. From time to time an aliquot was withdrawn, and its transmission coefficient determined immediately. When it became difficult to handle the solution owing to its high viscosity, it was left in the cell of the spectrophotometer, and placed with this in the water bath.

Viscosity determinations were made with an Ostwald viscosimeter, which had an outflow time of 32.9 seconds with distilled water at 35° C. The viscosimeter was placed in a constant temperature water bath. Four milliliters of fibrinogen solution mixed with 1 ml of $M/1$ phosphate buffer of pH 7 were placed in the water bath and, after the temperature equilibration, 5 ml of urea solution of the same temperature were added. At the moment of mixing, a stopwatch was started and 7 ml of the solution were withdrawn and transferred into the viscosimeter. The viscosity of the solution was determined from time to time. The increase of viscosity was rather quick in comparison with the outflow time of the viscosimeter. With the assumption of a linear increase of viscosity during single determinations, the viscosity value corresponds to the viscosity of the solution at the middle time of determination. The viscosity data were plotted in consequence against the time obtained by adding half of the outflow time to the moment of start of the determination.

Electrophoretic determinations were made with the Tiselius apparatus. Ten milliliters of fibrinogen solution were mixed with 1 ml of $M/1$ buffer of pH 7, and 3.3 g of solid urea were added. The mixture was kept at 35° C for 20 minutes, which treatment sufficed to denature almost completely the fibrinogen. Ten milliliters of an urea-buffer mixture were added, and the solution dialysed at 4° C for 16 hours against 2 000 ml of the same urea-buffer mixture. Borate buffer of pH 8.6 and phosphate buffer of pH 7 were used, each of 0.1 ionic strength and containing 20 per cent urea. After dialysis the solution was filled up to 25 ml with the urea-buffer mixture, and then subjected to electrophoresis.

The electrophoretic behavior of denatured fibrinogen was compared with that of the native. Ten milliliters of the fibrinogen solution were mixed with 10 ml of urea-buffer mixture and dialysed under the same conditions as already described. Experiments were also performed with mixtures of native and denatured fibrinogen in urea-buffer solutions.

Before and after electrophoresis the fibrinogen solutions were tested by dilution with 5.9 phosphate buffer. There was no precipitation or turbidity by the native fibrinogen, showing that the fibrinogen had not suffered any alteration during the experiment, while the denatured was completely precipitated.

The mobilities were determined by runs of four hours, with a potential gradient of approximately 8 volts/cm. The mixtures of native and denatured fibrinogen were subjected to electrophoresis for 12 hours, with a potential gradient of 5 volts/cm.

Results

A. Increase of turbidity. In the presence of 10 and 20 per cent urea the increase of turbidity of the solution followed the denaturation process, but with an inductive period even longer than that occuring when the denatured fibrinogen was precipitated by dilution with a buffer of pH 5.9 (see first section of paper). The effect was studied in the temperature range of 35 to 50° C. The final turbidity was higher in the presence of 10 per cent urea than in the presence of 20 per cent. The velocity of the increase of turbidity was influenced by the temperature in the same fashion as the denaturation itself. It was greater if the temperature was higher, and at the same temperature was greater in 20 than in 10 per cent urea.

At 30 per cent urea concentration and 40° C, there was no change in the light transmission of fibrinogen solutions over a period of 90 minutes, although 15 minutes under these conditions are sufficient to denature the fibrinogen completely.

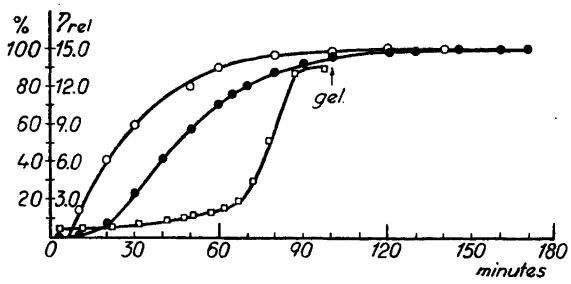

Fig. 1. The amount of denatured fibrinogen, the opacity, and the viscosity of the solution plotted against time. 12 mg fibrinogen per milliliter, 20 per cent urea, 35° C, pH 7.
○ % *denatured fibrinogen*
● » *increase of opacity*
□ *relative viscosity*

If the solution has no true light absorption, as in our case at 350 and 600 mμ, the intensity of the transmitted light (I) is given by the relation: $I = I_o - I_{sc} - I_r$, where I_o is the intensity of the primary beam, while I_{sc} and I_r are, respectively, the light scattered in all directions except that of the primary beam, and the light reflected on the surfaces of the traversed media. Since in the Beckman spectrophotometer a blank cell is used, I_r is eliminated automatically and the transmission coefficient, $\frac{I}{I_o}$, determined enables the calculation of the relative intensity of scattered light by the relation: $\frac{I_{sc}}{I_o} = 1 - \frac{I}{I_o}$ (see Nanninga *l. c.*).

From the figures calculated with this relation, the initial scattering of the solution was substracted and the differences expressed as the percentage of the finally reached constant value of the scattering.

In Fig. 1 is plotted the increase of the scattering of a fibrinogen solution in 20 per cent urea at 35° C calculated from the transmission coefficients at 350 mμ in the above described manner.

According to Rayleigh's relation — when the other factors involved are constant — the relative intensity of the scattered light, $\frac{I_{sc}}{I_o}$, is proportional to the number of scattering particles. The determination of $\frac{I_{sc}}{I_o}$ thus makes it possible to estimate the concentration of the scattering substance. Several authors have indeed attempted a quantitative protein estimation on the basis

of the turbidity obtained by various precipitating agents [15], [16]. Korányi and Hatz [17] found the protein concentration to be proportional to the relative intensity of the scattered light: while Looney and Walsh [16], and Nanninga [18] demonstrated the proportionality between protein concentration and relative light absorption $(\frac{I_o - I}{I_o})$. These findings are in complete accord with the above derivation.

Thus the values of light scattering presented in Fig. 1 may be considered a measure of the concentration of fibrinogen molecules at a certain stage of denaturation, *viz.*, at a stage which is characterized by an increased light scattering power. The logarithmic plot of the concentration of fibrinogen molecules which had not attained this stage, calculated from the scattering, $\log (100—100 \frac{I_{sc}}{I_o})$, is shown in Fig. 2. The calculated points fall close to a straight line and the velocity constant obtained from the slope of the line is 6.4×10^{-4} — a finding in good agreement with the 6.2×10^{-4} calculated from the data obtained by the precipitation method (see first section of paper).

These findings seem to support the conclusion arrived at in the first part of these investigations, *i. e.*, the existence of a primary process of the denaturation, followed by secondary physicochemical alterations. Most probably the increase of the light scattering is caused by a dehydratation of the particles, which follows the primary denaturation process. The result of this is a sharpening of the refractive index gradient on the surface of the particles, which increases the light scattering.

Further analysis of the opacity can still give information about the physicochemical changes involved in the denaturation process. Heller and Vassy [19] showed that the Tyndal spectra, *i. e.*, the variation of the intensity of scattered light with wave length, enables the determination of the size of the scattering darticles. The wave length exponent in the equation: $k = c \,.\, \lambda^{-n}$, where k is the absorption coefficient, c a constant, and λ the wave length, is a function of the particle size. If the largest diameter of the scattering particles is smaller than 1/10 of λ, n is equal to 4, but decreases very much if the particle size grows over this value. Ferry and Morrison [20] applied this principle to the analysis of the structure of fibrin gels, but they determined, instead of the wave length exponent, the ratio of the absorption coefficients at two different wave lengths. The ratio $\frac{k_{350\ m\mu}}{k_{600\ m\mu}}$ is equal to 8.6, if the wave length exponent is 4 and decreases if n is less than 4. Fig. 3 shows this ratio plotted against time, the fibrinogen solution used being kept at 35° C in the presence of 20

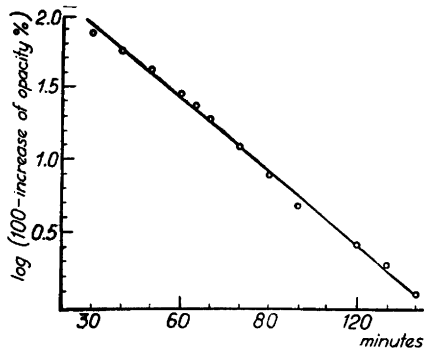

Fig. 2. Log (100 — % increase of opacity) plotted against time. Same experiment as in Fig. 1.

per cent urea. It can be seen that the ratio first increases, showing a decrease of the particle size — an effect which may be connected with the disintegration of fibrinogen by urea. Later the decrease of the ratio indicates a coarsening of the system, possibly brought about by the unfolding of the molecules and their association in bundles.

B. Increase of viscosity. The viscosity of fibrinogen in 10 and 20 per cent urea solutions increases very much and, if the protein concentration is high enough, the solution gelifies. The effect was investigated in the temperature range of 35 to 50° C. The increase of viscosity is enhanced by increase of temperature, and at the same temperature is quicker in 20 than in 10 per cent urea solution. Fig. 1 shows also the increase in viscosity of a fibrinogen solution in 20 per cent urea at 35° C. The increase of viscosity is characterised by a period of slight increase, after which the curve of increase rises sharply.

In the presence of 30 per cent urea at 40° C there is only an insignificant rise of the viscosity of fibrinogen solutions.

The changes in viscosity and opacity seem to be correlated. In the presence of 30 per cent urea both are absent, whereas at lower urea concentrations the increase of opacity is always followed by an increase of the viscosity.

The gels formed by the denatured fibrinogen in the presence of urea are very loose; if shaken they are easily fragmented. If the urea is washed out, they become elastic and very opaque; if concentrated urea solution or solid urea are added to a gel formed in the presence of 10 or 20 per cent urea, the gel dissolves. The resulting solution has a low viscosity, but the opacity does not disappear completely. If fibrinogen in 30 per cent urea solution is completely denatured and then diluted with distilled water to 20 per cent urea concentration or below, in the first moment after dilution it does not show any change, but after a few minutes becomes turbid, and finally gelifies.

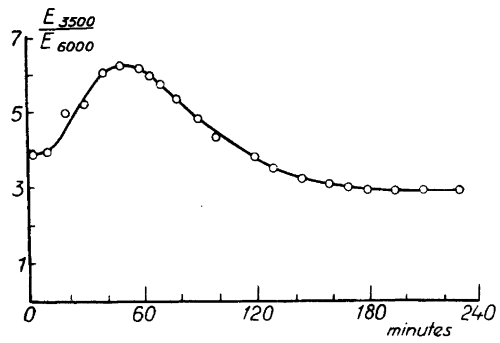

Fig. 3. The ratio of the absorption coefficients at 350 and 600 mμ plotted against time. 12 mg. fibrinogen per milliliter, 20 per cent urea, pH 7, 35° C.

Fibrinogen thus behaves differently from egg albumin, serum globulin, and serum albumin, which gelify even in urea solutions as concentrated as 60 per cent [10]. The denatured fibrinogen is soluble at urea concentrations higher than 30 per cent.

C. Electrophoretic investigations. The urea denatured fibrinogen in 20 per cent urea solutions moved as a single component with a sharp boundary. At pH 7 and 8.6 the mobility of denatured fibrinogen did not differ from that of the native, both being in 20 per cent urea solution. In order to detect small mobility differences, mixtures of native and denatured fibrinogen were subjected to electrophoresis. Only a single boundary was observed, showing that the mobilities at these pH-s were the same for both the native and the denatured fibrinogen. The viscosity of denatured fibrinogen was approximately double that of the native. The results indicate that the denaturation does not affect the net charge of fibrinogen in 20 per cent urea, and that the increase of viscosity by denaturation is without effect on the mobility of the protein.

Several authors also found denatured proteins to be homogeneous in electrophoretic experiments; but the mobilities differed from that of the native proteins. Pedersen [21] investigated the electrophoretic mobility of heat denatured serum albumin. In spite of the very accentuated heterogenity in the ultracentrifuge, the material was electrophoretically homogeneous. The pH-mobility curves of the native and denatured protein were almost parallel, but the denatured serum albumin had an isoelectric point 0.2—0.5 more alkaline than the native. Sharp, Cooper, and Neurath [22] also found urea-denatured horse pseudoglobulin, after reversal of the denaturation, to be electrophoretically homogeneous, with a higher mobility above and a lower below the isoelectric point than that of the native protein.

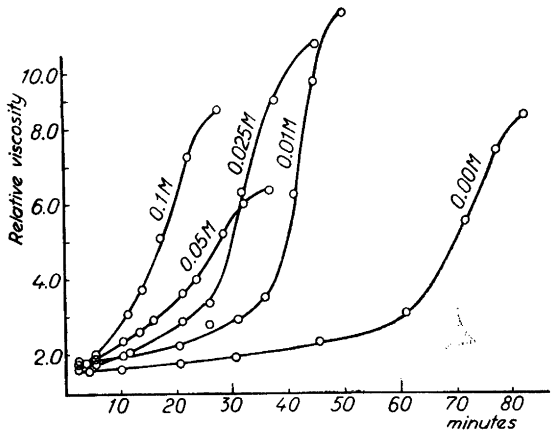

Fig. 4. The rise of viscosity of fibrinogen solutions in 20 per cent urea, in the presence of different concentrations of sodium caprylate. 12 mg fibrinogen per milliliter, 35° C, pH 7.6.

D. Effect of sodium caprylate on the urea denaturation of fibrinogen. Luck and his co-workers [23,26] investigated in great detail the preventive action of large organic anions against urea and heat denaturation of proteins. Duggan and Luck [23] tested the protective effect of a large number of organic anions against the viscosity rise of serum albumin solutions in concentrated urea. One of the most powerful stabilizers found was the caprylate anion. These authors are inclined to believe that the investigated substances protected the serum albumin molecules against the denaturation itself, and not merely prevented their aggregation.

It seemed interesting to try whether or not the caprylate anion has also a protective effect against the urea denaturation of fibrinogen. Dialysing experiments were performed, as described in section C of preceding paper. The fibrinogen was kept for 24 hours at 0° C in the presence of 30 per cent urea and 0.04 molar sodium caprylate; then the urea dialysed out. The pH of the solutions varied from 6.6 to 11.0. The above caprylate concentration proved to be very effective in preventing the urea denaturation of serum albumin [23,26]. No protective effect, however, was found in experiments with fibrinogen; on the contrary the caprylate accelerated the denaturation. The amount of unaffected fibrinogen was 45 to 80 per cent less in the set with caprylate, than in a control which did not contain it. The differences were the more accentuated, the more alkaline was the pH.

The effect of caprylate was tested also in kinetic experiments. The experimental procedure was the same as already described in section I of this paper. The denaturation in the presence of 0.01 molar sodium caprylate and 30 per

cent urea at pH 7 and 30° C proved to be strictly of the first order, with $k = 1.3 \times 10^{-3}$, whereas in the same conditions in the absence of caprylate $k = 7.5 \times 10^{-4}$. Thus the caprylate brought about a 1.73 -fold increase of the reaction velocity.

It may be that caprylate is not able to prevent the urea denaturation of fibrinogen, but, owing to its hydrotropic character, it may prevent the aggregation of the denatured particles and thus the increase of viscosity. In order to test this assumption, viscosity determinations were made in the manner described, in the presence of different concentrations of caprylate and 20 per cent urea at pH 7.6 and 35° C. A higher pH was used to prevent the precipitation of caprylate. As it can be seen from the results presented in Fig. 4, the caprylate is ineffective as a protective agent; even more, the increase of viscosity is the quicker, the higher is the caprylate concentration. Also, after a lapse of time, the solutions gelified in the presence of caprylate.

The caprylate has, thus, an effect on the urea denaturation of fibrinogen opposite from its effect on serum albumin. An intermediate position is occupied by egg albumin, caprylate having little or no effect on its urea denaturation [24].

The results clearly indicate that even where an agent has apparently the same effect — the increase of the viscosity of a protein solution — the mechanism which leads to this result may be quite different for different proteins. The efficiency of an inhibitor in one case and the inefficiency or even the opposite accelerator effect in other cases may be explained in this way.

SUMMARY

1. The urea denaturation of fibrinogen is accompanied by an increase of the opacity and viscosity of the solutions, if the urea concentration is below 20 per cent. At 30 per cent urea concentration, neither the opacity, nor the viscosity change.

2. In 20 per cent urea solution the urea-denatured fibrinogen is electrophoretically homogeneous and its mobility does not differ from that of native fibrinogen.

3. Sodium caprylate has no preventive action against the urea denaturation of fibrinogen; on the contrary, it accelerates the denaturation.

The author wishes to acknowledge his indebtedness to Professor Hugo Theorell for inviting him to work in his laboratories, and for his very kind interest throughout the work.

He also wishes to express his thanks for a grant from The Institute for Muscle Research, New York.

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Received September 9, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 344–350

Properties of Fibrin Dissolved in Urea Solutions

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Several investigators of the late 19th century reported the solubility of fibrin clots in urea solutions.[1,2] Wöhlisch and his co-workers, however, could not confirm this finding;[3,4]; they found the fibrin insoluble at room temperature in concentrated urea solutions, though when heated to 100° C it dissolved easily.

The problem has considerable importance, because the protein gels and coagula — heat-denatured egg albumin, tobacco mosaic virus, *etc.* —, where the particles are bound by weak secondary forces, are all soluble in urea solutions. Insolubility may thus be an indicator of stronger primary chemical bonds between the particles.

It seems that the negative results of Wöhlisch *et al.* have been accepted without further proof by most of the authors. Indeed, Ferry in his recent rewiev on protein gels[5] considered the insolubility of fibrin in urea solutions as an argument in favor of the theory of polymerization of fibrinogen through co-valent bonds to form the fibrin gel.

Loránd[6], reinvestigating the problem, found fibrin resulting from the action of thrombin upon pure fibrinogen to be readily soluble in concentrated urea solutions. On the contrary, fibrinogen clotted by thrombin in the presence of serum and calcium ions, as well as the clot obtained by the recalcination of oxalated plasma, was insoluble. It is possible that the failure to dissolve fibrin reported by the authors cited above was due to contamination of their fibrinogen preparation with the serum factor and calcium. Wöhlisch and his associates used fibrinogen prepared by salting out with NaCl. It is known that the NaCl precipitation of fibrinogen is less sharp than that by $(NH_4)_2SO_4$[7], thus favoring the precipitation of other plasma proteins with the fibrinogen.

In the experiments which will be described, the solubility of fibrin in concentrated urea solutions was definitely confirmed and some of the properties of the solutions of fibrin in urea investigated.

EXPERIMENTAL

Materials

Fibrinogen was prepared from oxalated cattle plasma by salting out with $(NH_4)_2 SO_4$ at 0.24 saturation. The details of the method have already been described [8].

The reagents employed were of analytical purity. Impure urea preparations gave unsatisfactory results, the dissolution of fibrin being very slow and incomplete when they were used.

Fibrin solutions were prepared in the following way: 50 ml of solution containing 20 mg fibrinogen per milliliter were clotted by adding 1 ml of thrombin solution. Hoffmann la Roche's commercial thrombin preparation was used in the concentration of 2 mg per milliliter. After two hours, the clot was dissolved at 0° C by adding 15 g of solid urea. The dissolution was complete in about one hour. The solution was then filled up to 100 ml with distilled water. The water must be added cautiously, under continuous stirring: otherwise, the fibrin precipitates. This stock solution was stored at 0° C; and when used, was diluted with distilled water and 60 per cent urea solution for the preparation of solutions of different composition.

Methods

The viscosity of the solution was determined with an Ostwald viscosimeter at 0° C. At this temperature the distilled water had an outflow time of 23 seconds. Owing to the thixotropy of the solutions, the outflow time decreased in successive repetitions of the determination. A nearly constant value was reached finally, sometimes after 8 to 10 determinations, and this was considered as corresponding to the viscosity of the solution. The viscosities were calculated relative to that of distilled water at the same temperature.

The double refraction of flow was investigated qualitatively with the apparatus described by Gerendás [9].

Results

A 'coarse type' gel dissolves more easily in urea solutions than a 'fine type' gel. The coarse and fine qualifications of fibrin gels are in accord with the definitions of Ferry and Morrison [10]. More rapid dissolution was observed if the urea was added immediately after gelation than if it was added later. The following experiment serves to illustrate this: Samples of 10 ml of a solution containing 10 mg fibrinogen per milliliter were clotted by adding 1 ml of thrombin solution. In about 30 seconds the solution clotted. The clot was then broken with a glass rod, 3 g of solid urea were added, and the mixture continuously stirred. If the urea was added 30 seconds after the clotting, the dissolution was complete in about 2 minutes. After 5 minutes of waiting

it took 25 to 30 minutes, while after 15 minutes it required 40 to 50 minutes. It is apparent that the gelation of the solution does not mean the completeness of the reaction. The reaction continues for a long time, as shown by the growing resistance against the dissolving action of urea. Similarly Ferry and Morrison [19] showed a continued increase of the tensile strength and opacity of fibrin clots for a long time after the moment of gelation.

A. Reconstitution of the fibrin gel after removal of the urea. When the urea from a fibrin solution is dialysed out, the cellophane tube will be completely filled out by a fibrin gel, which has all the characteristics of the original gel. The pH of the fibrin solution in urea in the experiment here reported was brought to approximately 6.5. The solution was then dialysed against a $M/10$ phosphate buffer of the same pH. The result was an opaque coarse type gel. On the contrary, when a slightly alkaline fibrin solution was dialysed against $M/10$ phosphate buffer of pH 8, a fine type gel was formed.

The quality of the resulting gel is determined only by the pH during the dialysis and not by the type of the original fibrin gel. A fine type gel can be converted in this way into a coarse one and vice versa; and this transformation can be repeated at will.

In the interpretation of these findings the question arises wheter the urea reversed completely the fibrinogen-fibrin transformation or whether it merely split the gel into modified fibrinogen particles. The thrombin which served for the clotting of fibrinogen is present in the fibrin solution and its activity can be demonstrated after the urea has been removed. If the first assumption is the correct one, the reclotting would occur as a result of the action of thrombin on the reconstituted fibrinogen molecules; while if the second is the explanation, then the preformed particles would gelify without the intervention of thrombin with removal of the urea. It is easy to verify the latter assumption. Indeed a fibrin solution in 30 per cent urea gelifies instantaneously when it is diluted with four volumes of distilled water. The rapidity of the reaction demonstrates the presence of preformed particles.

B. Effect of pH and urea concentration on the gelation of fibrin solutions. The stock fibrin solution was mixed with suitable quantities of distilled water and 60 per cent urea solution in order to have after the addition of 1/10 volume of buffer a final protein cencentration of 5 mg per milliliter and urea concentrations of, respectively, 7.5, 10, 15, 20, and 30 per cent. To cover the pH range from 5.9 to 12.0 primary-secondary phosphate, borate, and secondary-tertiary phosphate buffers were used in $M/1$ concentration. The mixtures were kept at 0° C for 24 hours, then their viscosity determined at the same temperature.

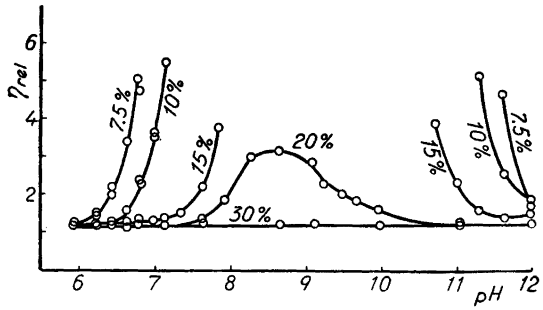

Fig. 1. The viscosity of fibrin dissolved in urea solutions at different urea concentrations and pH. 5 mg fibrin per milliliter, 0° C.

The results are summarised in Fig. 1. Over the entire pH range studied the viscosity of fibrin solutions in 30 per cent urea did not differ from that of fibrinogen solutions in similar conditions. These solutions had Newtonian viscosity and showed no double refraction of flow.

At 20 per cent urea concentration the viscosity rose at pH values more alkaline than 7.6 and reached a maximum at pH 8.6; it then decreased again to the original value. At still lower urea concentrations the increase of viscosity started at a pH the more acidic, the lower was the urea concentration. At 15 per cent urea concentration and below, the increase of viscosity finally led to a gelation of the solution. At considerably alkaline pH values, the solutions remained in the sol state. As it can be seen in Fig. 1, the pH range of gelation was widened by lowering the urea concentration.

In the regions of rising viscosity, the solutions became more and more thixotropic. The phenomenon was more accentuated in strong alkaline solutions on the redissolution side and, parallel with this, the viscosity showed a strongly anomalous character. The specific viscosity was no longer constant but rose with increase of the fibrin concentration; and, at the same time, a pronounced double refraction of flow appeared. With the highly viscous solution shaking the tube containing the solution was sufficient to obtain a strong and persistant double refraction. It is obvious that in this region what was determined was only an apparent viscosity resulting from the true viscosity and the structural rigidity of the solutions.

After the addition of buffer the viscosity did not instantaneously reach an end value. At high urea concentrations and at pH values far from the optimal value for gelation, the process requires several hours. This was the reason why the determinations were made after 24 hours, a time sufficient

to ensure stabilization of the viscosity. The increase of viscosity, however, was very rapid and gelation occured practically immediately after the addition of buffer at low urea concentrations and at the optimal pH for gelation *i. e.*, at pH 8.6.

DISCUSSION

As it has been shown, the fibrin clot resulting from pure fibrinogen readily dissolves in concentrated urea solutions. Neither the -S-S-, nor the -NH-CO-, nor any other co-valent bond immaginable between the chemical groups present in proteins can be broken by urea. This seems to justify the conclusion that by the formation of fibrin clots no co-valent bonds take part.

Urea affects the electrostatic forces between the charged groups by increasing the dielectric constant of the medium. Although this effect may have some importance, it is far from being the cause of the dissolution of fibrin. The dielectric increment of urea is not very high, dipolar ions like glycine for example, having a much greater effect on the dielectric constant. The dielectric constant of a 15 per cent urea solution is 93.5, while that of a 2 molar glycine solution is 125. A fibrin solution in 15 per cent urea, when diluted with 2 molar glycine solution, gelified exactly at the same degree of dilution as when it was diluted with distilled water, in spite of the fact that the dielectric constant of the medium was still further increased by dilution with the glycine solution.

Most probably the urea affects the hydrogen bonds of the protein molecules [11, 12]. Hydrogen bonds are formed between the —NH— and —CO— groups of adjacent polypeptid chains. The structural resemblance of urea and these groups makes possible a competition of the urea molecules for the same places as those by which the polypeptid chains are bound together. If this mechanism is really the cause of the dislocation of the protein particles, the hydrogen bonds must play a considerable role in the building of the fibrin gel. Mommaerts [13] has already arrived at a similar conclusion on the basis of the effect of urea on the clotting of fibrinogen.

Laki and Mommaerts [14] have shown that the gelation of fibrin is possible only above a certain pH. Fibrinogen incubated wirh thrombin at a pH below this value is transformed into modified particles, which gelify instantaneously if the pH is brought to a proper value. Lyons [15] showed the same phenomenon with fibrinogen incubated with thrombin at a pH more alkaline than 10. There is a pH zone where the fibrin gel can be formed. The dissolution of the gel occurs only at much lower and much higher pH values than the limits of this zone — a fact described by several authors [16, 17, 18, 19]. It is apparent from the experiments described in this paper that urea narrows

the pH stability range of the fibrin gel. At 20 per cent urea concentration, of the ability to form gels only some rise of viscosity was left; while at 30 per cent urea concentration, this capacity was completely anihilated. The role of the pH in the gelation process is still not clear; it is apparent only that the dissolving effect of urea is counterbalanced to some extent by the rise of pH in the zone more acidic than 8.6, and it is favored by the rise of pH in the zone more alkaline than 8.6.

The viscosimetric behavior of fibrin solutions in 30 per cent urea, as well as of those in lower urea concentrations in certain pH ranges, indicates that the particles, in respect to their shape and size, are identical with those of fibrinogen. The thrombin does not alter the shape and size of the fibrinogen molecules; it only modifies some of their physicochemical properties, increasing their tendency to form gels. Laki and Mommaerts [14] arrived at the same conclusion in an investigation of fibrinogen solutions which were incubated with thrombin at acidic pH.

Increase of viscosity and gelation were observed in the urea denaturation of fibrinogen. [20]. The possibility of denaturation must, therefore, be excluded in the experiments described in this paper. As reported in a previous paper [21], at 20 per cent and lower urea concentrations the urea has no perceptible denaturing action on fibrinogen and fibrin at 0° C in the pH range investigated. The viscosity changes, therefore, which were observable in fibrin solutions, could not have been caused by denaturation. The viscosity of the solutions once arrived at a constant value, did not change for a period as long as two weeks, and the viscosity of fibrinogen solutions under exactly the same conditions did not show any increase in four days. In the presence of 30 per cent urea the fibrin was more or less denatured, but this seemed to have no influence on the viscosity of the fibrin solutions. It has already been reported [20] that the denaturation of fibrinogen in 30 per cent urea also leaves unaffected the viscosity of the solution, fibrinogen and fibrin being similar in this respect.

SUMMARY

1. Fibrin clots are soluble in concentrated urea solutions.

2. When urea is dialysed out, the clot is reconstituted. The pH of the fibrin solution during the dialysis decides whether a coarse or a fine type gel will be formed.

3. The viscosity of fibrin in 30 per cent urea solution is normal and equal to that of fibrinogen in similar conditions. The pH has no influence on the viscosity. At 20 per cent urea concentration the viscosity is increased by increasing the pH up to 8.6. Further increase of pH again decreases the

viscosity. At still lower urea concentrations, the increase of pH leads to gelification; and at more alkaline pH to the redissolution of the gel.

4. The results are discussed.

The author wishes to acknowledge his indebtedness to Professor Hugo Theorell for inviting him to work in his laboratories, and for his very kind interest throughout the work.

He also wishes to express his thanks for a grant from The Institute for Muscle Research, New York.

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Received September 9, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 351 – 358

Electrophoretic Investigation of Fibrin and Fibrinogen Dissolved in Urea Solutions

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The solubility of fibrin in urea solutions makes it possible to investigate the electrophoretic mobility of this protein. Such fibrin solutions contain modified fibrinogen molecules, which, after removal of the urea reform the original fibrin gel [1,2]. Laki and Mommaerts have shown [3] that the clotting of fibrinogen occurs in two steps. The first one is the chemical change in the fibrinogen molecule brought about by the thrombin and the second, the transformation of the system into a gel. In urea solutions this second physicochemical process is reversed and it is very probable that the particles in solution are identical with those formed in the first phase of clotting.

If the action of thrombin involves some of the ionizing groups of fibrinogen, a study of the differences in electrophoretic mobilities between fibrinogen and fibrin may give some information about the nature of the process of clotting.

EXPERIMENTAL

Materials

Fibrinogen was prepared from oxalated bovine plasma as described in a previous paper [4]. The solutions contained 25 to 35 mg fibrinogen per milliliter and their purity ranged from 90 to 95 per cent.

The commercial thrombin preparation of Hoffmann-La Roche, Basel was used.

All the other substances used were reagents of analytical purity.

Methods

The fibrinogen solutions were diluted to contain 25 mg of fibrinogen per milliliter. Ten milliliters of fibrinogen solution were clotted by adding 1 ml of thrombin. The concentration of the thrombin solution was 1 mg per milliliter. Clotting occured in about one minute. The clot was allowed to stand at

room temperature for an hour for the completion of the thrombin action; was then broken with a glass rod, and dissolved at 0° C by adding 3 g of urea. The dissolution was complete in about an hour, after which 7 ml of urea-buffer solution were added. The composition of urea-buffer solution varied from experiment to experiment, as indicated later in text.

The fibrinogen solutions were prepared in exactly the same manner, with the exception that instead of thrombin 1 ml of physiological saline solution was added. Both solutions were dialysed for 24 hours against 2000 ml urea-buffer solution. The tube containing the bags and the buffer was rocked at + 4° C. After dialysis the protein solutions were diluted to 20 ml with the urea-buffer solution.

Electrophoretic mobility determinations were made with the Tiselius apparatus. The potential gradient varied in the different experiments between 5 and 10 volts/cm (voltage between 200 and 350 volts). In general the migration was followed for four hours, and readings were made every half hour. The migration per hour was determined graphically, and the arithmetical mean of the migrations of the ascending and descending boundaries was used for the calculation of the mobility.

The difference between the mobilities of fibrinogen and fibrin is small. To exclude the possibility that the observed differences were due to experimental error, a control run was made with a mixture of fibrinogen and fibrin at each pH studied. The solutions of the two proteins were prepared as described above. In order to minimize the possible action of thrombin on fibrinogen during the dialysis, the two solutions were mixed just before filling the electrophoresis apparatus, although the high urea concentration employed certainly inhibits completely the action of thrombin [5]. In these experiments the duration of the electrophoresis was 10 to 12 hours, and a lower potential gradient was used, about 5 volts/cm. The descending boundary was mostly blurred; in these experiments, therefore, only the migration of the ascending boundary was followed. The observed components were identified by means of the mobilities determined by runs with a single component.

The conductivity of the solutions was determined in the water bath of the electrophoresis assembly to an accuracy of $\pm$ 1 ohm. The temperature of the water bath was + 0.1° C. For the calculation of the potential gradient, the mean value of the conductivity of the protein and of the urea-buffer solution was used. When only the migration of the ascending boundary was followed, the conductivity of the urea-buffer solution served for the calculation of the potential gradient, as recommended by Svensson [6]. The pH of the solutions was controlled with the glass electrode.

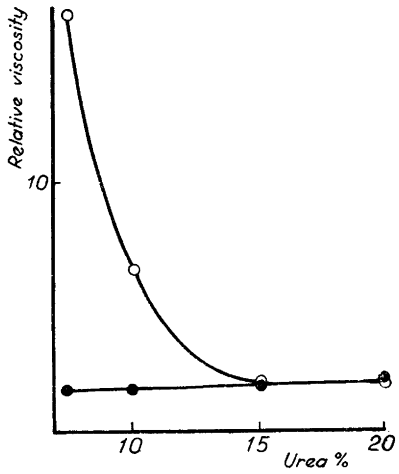

Fig. 1. The relative viscosity of fibrinogen and fibrin in urea solutions of different concentrations. 10 mg, protein per milliliter, 0.4 ionic strength, pH 6.8.

○ *fibrinogen*
● *fibrin*

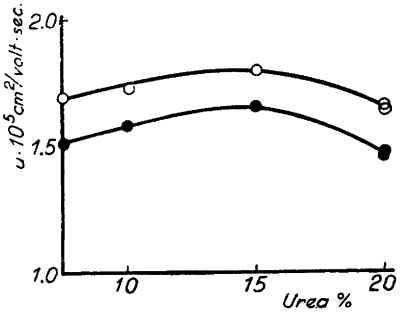

Fig. 2. The electrophoretic mobility of fibrinogen and fibrin in urea solutions of different concentrations. 10 mg. protein per milliliter, 0.4 ionic strength, pH 6.8.

○ *fibrinogen*
● *fibrin*

An Ostwald viscosimeter served for the viscosity determinations at 0° C. The reported viscosities are relative to the viscosity of distilled water at 0° C.

Results

In previous investigations it was found that between pH 5.9 and 11.0 urea solutions of 30 per cent concentration have a denaturing action on fibrinogen, while 20 per cent urea has not [7]. Therefore, in this pH range, the experiments were performed in 20 per cent urea solutions. Below pH 5.9 urea causes denaturation also in 20 per cent concentration. At pH 5.3 and 4.4, therefore, solutions of 10 per cent urea were used, which do not denature fibrinogen even at this acidic pH.

In 20 per cent urea solutions in the pH range from 7.6 to 11.0 the viscosities of fibrin solutions are higher than those of fibrinogen solutions of the same concentration. The difference is maximal at pH 8.6, where, at the protein and salt concentration used, the fibrin solution has a relative viscosity of 7.13; the fibrinogen, only 1.46. In spite of the fact that in 30 per cent urea solution these viscosity differences are absent, for the reason mentioned above, higher urea concentrations than 20 per cent were not used.

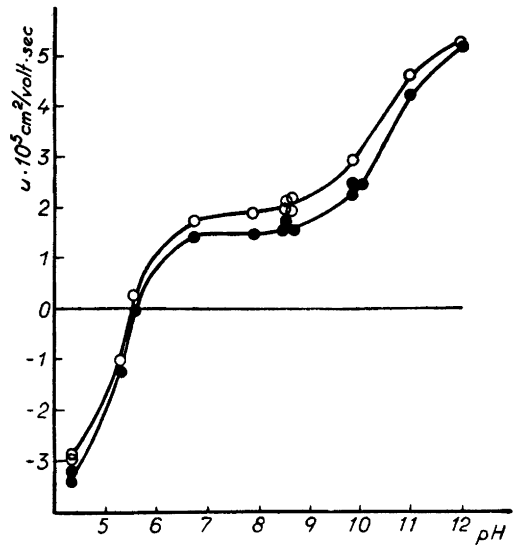

Fig. 3. The electrophoretic mobility of fibrinogen and fibrin plotted against pH. 10 mg protein per milliliter, 0.1 ionic strength, 10 respectively, 20 per cent urea.
○ *fibrinogen.*
● *fibrin.*

A. Influence of the urea concentration on the mobility of fibrinogen and fibrin. To determine to what extent the viscosity influences the electrophoretic mobilities, experiments were performed in which, at the same pH, ionic strength, and protein concentration, the concentration of urea was varied. With urea concentrations above 15 per cent at pH 6.8 in phosphate buffer of 0.4 ionic strength, the viscosities of fibrin and fibrinogen are equal. Lowering of the urea concentration from 15 to 7.5 per cent increases to a considerable degree the viscosity of fibrin solutions, whereas the viscosity of fibrinogen solutions is not affected by this change in the urea concentration.

The viscosity of the two proteins is plotted against the urea concentration in Fig. 1 and the electrophoretic mobilities of the same solutions are shown in Fig. 2. The mobility curves of fibrin and fibrinogen are parallel, in spite of the fact that the viscosity curves are strongly divergent. The mobility of fibrin in 7.5 per cent urea is the same as in 20 per cent urea, whereas the viscosity is ten times higher in the first case than in the second — 16.50 and 1.62. Both of the proteins possess a maximum mobility in 15 per cent urea solution.

B. Influence of ionic strength on the mobility of fibrinogen and fibrin in urea solutions. In the presence of 20 per cent urea, the ionic strength seems to have but little influence on the electrophoretic mobility of the two proteins. At pH 6.8 the ionic strength was increased from 0.1 to 0.4 by increasing the concentration of the phosphate buffer. The mobilities were the same over this

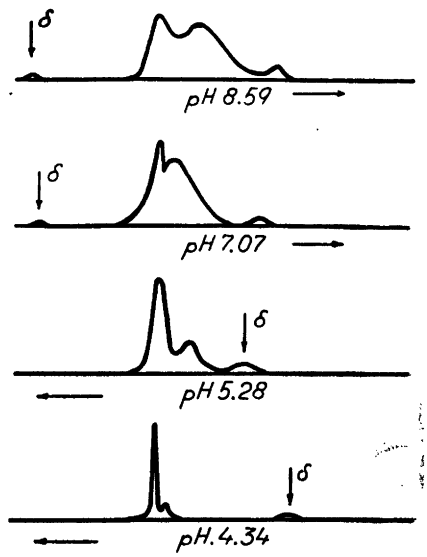

Fig. 4. Electrophoretic patterns of mixtures of fibrinogen and fibrin, (1 : 1), 10 mg. protein per milliliter, 0.1 ionic strength, 20, respectively 10 per cent urea, pH 8.59, 7.07, 5.28, 4.34; potential gradient 5 volts/ cm, duration of the experiment 12 hours.

ionic strength range. Constant mobilities were obtained also when, at pH 8.6 and 20 per cent urea concentration, the ionic strength was increased from 0.05 to 0.2 by increasing the concentration of the borate buffer, and even when it was increased up to 0.55 by adding NaCl to the buffer.

C. *Influence of pH on the mobility of fibrinogen and fibrin.* In all the determinations performed in order to determine the pH dependence of mobility the ionic strength of the buffers was 0.1. Acetate buffers were used from pH 4.4 to 5.3, phosphate buffers from 5.6 to 7.9, borate buffers from 8.6 to 10.0, and secondary-tertiary phosphate buffers from 11.0 to 12.0. The mobility of fibrinogen and fibrin as a function of pH is plotted in Fig. 3. It can be seen that the mobility curve of fibrin is always below that of fibrinogen; *i. e.*, in the region alkaline to the isoelectric point the fibrinogen is the faster, whereas in the region acidic to the isoelectric point the fibrin is the more rapid component. Between pH 5.3 and 6.8 the two curves are very close together, but at a more basic pH they separate, rejoining again at very alkaline values.

The mobilities determined in mixtures of fibrinogen and fibrin were the same as those determined in runs with a single component. The two components separate slowly, owing to the small difference in their mobility. In Fig. 4 can be seen the electrophoretic pattern of an 1 : 1 mixture of fibrinogen and fibrin at four different pH. At pH 4.4 and 5.3, the faster component is fibrin, whereas at pH 7.8 and 8.6 the fibrinogen is the faster component. In both upper patterns there can be seen a very small component faster than

fibrinogen and fibrin — a component which probably corresponds to the globulins co-precipitated with the fibrinogen.

In all the runs performed the fibrin has shown a much sharper boundary than the fibrinogen — a finding pictured in the patterns of Fig. 4, where the steeper, higher peaks correspond to fibrin and the broader to fibrinogen.

The isoelectric points of the two proteins in 20 per cent urea are very close together. Judging from the mobility curves, the fibrinogen has an isoelectric point of 5.5, while that of fibrin is somewhat more alkaline, at pH 5.6.

DISCUSSION

The difference in the electrophoretic mobility of fibrinogen and fibrin may be due either to a difference in their net charge, or to differences in the shape and size of the particles, or to both of these factors. Over a distinct pH range the high viscosity of fibrin in urea solutions, as compared with that of fibrinogen in similar conditions, clearly indicates a structural difference between these two protein solutions. The high viscosity of fibrin solutions in urea may be accounted for by (a) the structural rigidity of the solution, caused by large forces between the particles, and by (b) the polymerisation of the molecules in long rod-shaped particles.

In interpreting the electrophoretic results, the question should be answered whether or not the structure of the solution or the shape and size of the particles has an effect upon the electrophoretic mobility.

The structural rigidity of the medium seems to have little or no influence in the cases reported in the literature. It has been shown that the mobility of zinc particles and air bubles remained constant in a 1 per cent gelatin solution during the sol-gel transformation [8]. The movement of the zinc particles is only an indicator of the movement of the gelatin micelles themselves; thus, the setting of the sol seems to have no influence on the mobility of the gelatin micelles. The same is true for the electrophoretic migration of red blood corpuscles in a gelatin sol or gel. There are evidences that this unexpected result is caused by the thixotropy of gels. The gel structure is destroyed before the particle, and then afterwards reconstituted. With more concentrated or non-thixotropic gels this transformation cannot occur, and the particles are immobile [9].

The shape and size of microscopic particles has no influence on the electrophoretic mobility. Abramson and Michaelis [10] demonstrated this with particles coated with gelatin — particles varying widely in size, shape, and orientation. It seems that the only determining factor is the surface charge density of the particles, and the same seems to be true also in molecular dimensions. Astrup

and Brodersen [11] found that cellulose polysulfonic acids have the same mobility, irrespective of their degree of polymerisation. The aggregation of heat-denatured serum albumin particles had also no influence on their electorphoretic mobility. The material was polydisperse in the ultracentrifuge, as shown by Pedersen [12], but was perfectly homogeneous in electrophoretic experiments. It is apparent that the degree of polymerisation is irrelevant if the charge per unit of the polymer is the same.

Stenhagen and Teorell [13] investigated the concentration-mobility relation of thymonucleic acid. By increasing concentration the electrophoretic mobility was found constant, in spite of the remarkable increase of viscosity caused by the tendency of the long rod-shaped thymonucleic acid molecules to give thixotropic gels.

In the experiments described in section A. of this paper, the viscosity of fibrin solutions was enormously increased by decreasing the urea concentration; in spite of this, the electrophoretic mobility changed but little. The small change may be explained by the increase of the true viscosity of the solvent and by the change of the dielectric constant. That the changes are due to the difference in the composition of the solvent, and not to the increase of viscosity, is shown by the fact that the mobility of fibrinogen, whose viscosity is not altered by the lowering of the urea eoncentration, is perfectly parallel with that of fibrin.

There are no viscosity differences between fibrinogen and fibrin below pH 7.5 in 20 per cent urea solutions; and, in spite of this, the mobility differences are about the same as at pH 8.6, where the viscosity of fibrin is approximately five times higher than that of fibrinogen.

The conclusion seems to be justified that the electrophoretic mobility differences found between fibrinogen and fibrin in 20 per cent urea solutions are not due to morphological differences, but to differences in the net charge of the two proteins. The net charge of the fibrin particles is smaller above the isoelectric point and larger below this point than that of fibrinogen.

The isoelectric point of fibrinogen as determined in the experiments reported is in good agreement with the values found by other authors in the absence of urea. Stenhagen [14] found 5.4 as the isoelectric point of fibrinogen by the electrophoresis of human plasma. Seegers *et al.* [15] obtained 5.5 by the electrophoresis of isolated pure fibrinogen. It seems that the urea does not shift the isoelectric point.

The mobility of fibrinogen seems also to be unaffected by the presence of 20 per cent urea. Armstrong, Budka, and Morrison [16] gave $1.78—2.3 \times 10^{5}$ as the mobility of fibrinogen isolated by the alcohol fractionation method, when investigated at pH 8.6 in diethylbarbiturate buffer of 0.1 ionic strength.

Avery and Munro [17] found, in the same conditions, 2.18—2.37 $\times$ 10^5 with the fibrinogen prepared by salting out. Dole [18], using the same buffer, reported 2.14 $\pm$ 0.25 $\times$ 10^5 as the mobility of fibrinogen in unfractionated human plasma. The value of 1.95—2.08 $\times$ 10^5 determined in our experiments in borate buffer of pH 8.6 and 0.1 ionic strength is only slightly lower than the above values, and demonstrates that at least at this pH value the urea does not affect appreciably the electrophoretic mobility of fibrinogen.

SUMMARY

1. The viscosity differences between fibrin and fibrinogen in 20 per cent urea solutions are not the cause of the electrophoretic mobility differences.

2. The ionic strength has little influence on the electrophoretic mobility of fibrinogen and fibrin in the presence of 20 per cent urea.

3. Fibrin has a lower mobility above and a higher below its isoelectric point than fibrinogen.

4. The isoelectric point of fibrinogen in solutions of 20 per cent urea lies at pH 5.5; that of fibrin, at pH 5.6.

The author wishes to acknowledge his indebtedness to Professor Hugo Theorell for inviting him to work in his laboratories, and for his very kind interest throughout the work.

He also wishes to express his thanks for a grant from The Institute for Muscle Research, New York.

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Received September 9, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 359–363

The Influence of the Cation Composition on Anion-Equilibria in Molten Salt Mixtures

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In previous investigations [1] it has been shown that in reactions of molten salts the cation present can influence, to a remarkable degree, the equilibria in which formally only anions take part.

This effect is especially important in acid-base reactions of high polymeric acids.

In the following, a thermodynamic equation is derived for the equilibrium constant of an anion reaction in a melt with mixed cations, as a function of the equilibrium constants of the melts with single cations and the cation ratio. (This equation may, of course, be modified for equilibria between cations in melts with mixed anions.)

Let the anion equilibrium be of the type:

$$X^{(-)} \rightleftharpoons Y^{(-)}$$

(Examples: Anion equilibria:

$$S_2O_7^{2-}{}_{(melt)} \rightleftharpoons SO_4^{2-}{}_{(melt)} + SO_{3(gas)}$$
$$CO_3^{2-}{}_{(melt)} \rightleftharpoons O^{2-}{}_{(melt)} + CO_{2(gas)}$$
$$2Cl^-{}_{(melt)} + Br_{2(gas)} \rightleftharpoons 2Br^-{}_{(melt)} + Cl_{2(gas)}$$

Cation equilibria:

$$M_1{}^+{}_{(melt)} + M_{2(metal)} \rightleftharpoons M_2{}^+{}_{(melt)} + M_{1(metal)})$$

The equilibrium is established in a salt melt containing a mixture of the cations A and B (A, B), with mole fractions N_1 and N_2, together with the

mixture of the anions X and Y (X, Y). — The ratio $A : B = N_1 : N_2$ is an independent variable. The ratio X : Y, however, always has to correspond to the value given by the equilibrium (I).

In accordance with usual convention, we define the activity a and the activity coefficient γ by means of the chemical potential:

$$\begin{aligned} \mu &= \mu^0 + RT \ln a \\ &= \mu^0 + RT \ln N + RT \ln \gamma \end{aligned}$$

where μ_0 is the chemical potential of the pure component, and μ that of the component with mole fraction N in a mixture.

In the following we have to consider 5 binary mixtures. The symbols used for these, and the corresponding subscripts used for N, a, and γ of the components are shown in Table 1.

Table 1.

Components	System	Subscripts
1 AX + AY	A(X,Y)	X(A), Y(A)
2 BX + BY	B(X,Y)	X(B), Y(B)
3 AX + BX	(A,B)X	A(X), B(X)
4 AY + BY	(A,B)Y	A(Y), B(Y)
5 (A,B)X + (A,B)Y	(A,B)(X,Y)	X(AB), Y(AB)

Hence the equilibrium constant of the mixed system (A, B) (X, Y):

$$K = \frac{a_{Y(AB)}}{a_{X(AB)}} = \frac{N_{Y(AB)}}{N_{X(AB)}} \cdot \frac{\gamma_{Y(AB)}}{\gamma_{X(AB)}} = K' \cdot \frac{\gamma_{Y(AB)}}{\gamma_{X(AB}$$

has to be expressed as a function of the equilibrium constants of the two simple systems A(X,Y):

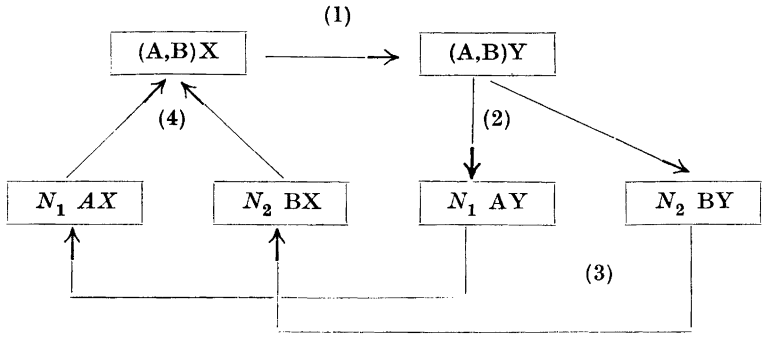
$$K_1 = \frac{a_{Y(A)}}{a_{X(A)}} = \frac{N_{Y(A)} \cdot \gamma_{Y(A)}}{N_{X(A)} \cdot \gamma_{X(A}} = K'_1 \cdot \frac{\gamma_{Y(A}}{\gamma_{X(A}}$$

and B(X,Y):

$$K_2 = \frac{a_{Y(B)}}{a_{X(B}} = \frac{N_{Y(B)} \cdot \gamma_{Y(B)}}{N_{X(B\ )} \cdot \gamma_{X(B)}} = K'_2 \cdot \frac{\gamma_{Y(B)}}{\gamma_{X(B}}$$

together with the ratio of $A : B = N_1 : N_2$ in the mixture.

The thermodynamic relation between K', K'_1 and K'_2 can be derived from the following cyclic process:



1) 1 mole (A,B)X is transformed reversibly into (A,B)Y:

$$\Delta\mu_1 = \mu_{(A,B)Y} - \mu_{(A,B)X}$$

$$= -RT \ln K = -RT \ln K' - RT \ln \frac{\gamma_{Y(AB)}}{\gamma_{X(AB)}}$$

2) 1 mole (A,B)Y is separated into N_1 mole AY and N_2 mole BY:

$$\Delta\mu_2 = N_1 \mu^0_{AY} + N_2 \mu^0_{BY} - \mu_{(A,B)Y}$$

$$= -RT (N_1 \ln a_{A(Y)} + N_2 \ln a_{B(Y)})$$

$$= -RT (N_1 \ln N_1 + N_2 \ln N_2) - RT (N_1 \ln \gamma_{A(Y)} + N_2 \ln \gamma_{B(Y)})$$

3) N_1 mole AY is transformed reversibly into AX, and N_2 mole BY into BX:

$$\Delta\mu_3 = N_1 (\mu^0_{AX} - \mu^0_{AY}) + N_2 (\mu^0_{BX} - \mu^0_{BY})$$

$$= N_1 \cdot RT \ln K_1 + N_2 \cdot RT \ln K_2$$

$$= N_1 \cdot RT \ln K'_1 + N_2 \cdot RT \ln K'_2$$

$$+ N_1 \cdot RT \ln \frac{\gamma_{Y(A)}}{\gamma_{X(A)}} + N_2 \cdot RT \ln \frac{\gamma_{Y(B)}}{\gamma_{X(B}}$$

4) N_1 mole AX and N_2 mole BX are mixed to form 1 mole (A,B)X:

$$\Delta\mu_4 = \mu_{(A,B)X} - (N_1 \cdot \mu^0_{AX} + N_2 \cdot \mu^0_{BX}) =$$

$$= RT\ (N_1 \ln N_1 + N_2 \ln N_2) + RT\ (N_1 \ln \gamma_{A(X)} + N_2 \ln \gamma_{B(X)})$$

For the total cycle, the sum of the changes in chemical potential is zero, hence it follows that:

$$\log K' = N_1 \log K'_1 + N_2 \log K'_2 \tag{1}$$

$$+ \left(N_1 \log \frac{\gamma_{Y(A)}}{\gamma_{X(A)}} + N_2 \log \frac{\gamma_{Y(B)}}{\gamma_{X(B)}} - \log \frac{\gamma_{Y(AB)}}{\gamma_{X(AB)}}\right)$$

$$+ (N_1 \log \gamma_{A(X)} + N_2 \log \gamma_{B(X)})$$

$$- (N_1 \log \gamma_{A(Y)} + N_2 \log \gamma_{B(Y)})$$

From this equation it can be seen that if all of the 5 binary mixtures behaved ideally, then log K would be a linear function of the cation composition of the melt.

The expression: $\log K = N_1 \log K_1 + N_2 \log K_2$ will ordinarily be valid as a first approximation.

The additional terms in the expression for log K' contain activity coefficients corresponding to deviations from ideal solutions in the 5 binary mixtures. All these coefficients can, at least in principle, be determined independently (*e. g.* from the melting diagrams).

It is important, however, that the activity terms ordinarily tend to cancel each other.

This will be the case for the second term if the deviations from ideal mixtures in the three mixtures A(X,Y), B(X,Y), and (A,B) (X,Y) are of the same sign. The term is insignificant if the deviations of the (A,B) (X,Y) system are equal to the average of the deviations of the two systems A(X,Y) and B(X,Y).

In the same way, the third and fourth terms will affect log K' in opposite directions if the deviations from ideal solutions in the two systems (A,B)X and (A,B)Y are of the same sign. The linear relationship between log K' and N can therefore be expected to be approximately valid even in systems where the single binary mixtures show considerable deviations from ideality.

In a subsequent paper, formula (1) will be compared with experimental results on the thermic decomposition of dichromate into chromate, chromium trioxide, and oxygen in the presence of mixed cations.

Our investigations have been carried out with aid of grants from *Statsminister Gunnar Knudsen og hustru Sophie f. Cappelens Familjelegat (Borgestad Legat IV)*, for which we wish to express our sincere gratitude.

SUMMARY

A thermodynamic expression is derived for the equilibrium constant of an anion equilibrium in a melt with mixed cations. As a first approximation:

$$\log K = N_1 \log K_1 + N_2 \log K_2$$

where K_1 and K_2 are the equilibrium constants for the melts with single cations and N_1 and N_2 are the mole fractions of the cations in the mixture. — The exact formula includes terms containing a number of activity coefficients, which are related to 5 simple binary mixtures.

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Received November 7, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 364 – 373

The Acidic and Basic Properties of Oxides

IV. The Thermal Decomposition of Dichromates

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In previous communications [1–3] dealing with the acid-base properties of oxides, the influence of the cations on the stability of the polyacids was discussed. It was stated, as a general rule, that the stability of the oxygen bridges decreases with both decreasing ionic radius and increasing polarizing power of the cation.

A particularly simple type of reaction which clearly demonstrates this rule is the thermal decomposition of pyrosulphates which consists of a breaking of the oxygen bridge of the pyrosulphate ion:

$$[O_3S - O - SO_3]^{2-}_{(m)} = SO^{2-}_{4(m)} + SO_{3(g)}$$

(m = melt)

In a earlier communication [2], an account is given of the influence of various cations and their mixtures on the decomposition equilibrium at temperatures where the pyrosulphate and the sulphate form a homogeneous melt.

In the following, we are dealing with corresponding investigations on the thermal decomposition of the dichromates, according to the equilibrium:

$$O_3Cr - O - CrO^{2-}_{3(m)} = CrO^{2-}_{4(m)} + 1/2\ Cr_2O_{3(s)} + 3/4\ O_{2(g)} \qquad (1a)$$

(m = melt)

This reaction offers an interesting opportunity to compare the cation effect on two very similar equilibrium systems. In addition, values of the equilibrium constants can be estimated with a considerably greater degree of accuracy — and thus the influence of the cation can be examined in more detail.

The data on the dichromate decomposition in the literature are rather scarce.

Stanley [4] repost that $Na_2Cr_2O_7$ splits off gaseous oxygen forming Na_2CrO_4 and Cr_2O_3 when the temperature is raised to 400° C.

Groschulf [5] found considerable decomposition when $K_2Cr_2O_7$ was heated to 1 000° C, but Hempel and Schubert [6] state that the decomposition starts at 500° C and is complete at 1 500° C.

The heat of reaction of the $K_2Cr_2O_7$-decomposition according to

$$K_2Cr_2O_{7(s)} = K_2CrO_{4(s)} + 1/2\ Cr_2O_{3(s)} + 3/4\ O_{2(g)} \tag{1b}$$

follows from the heats of formation of the components:

K_2CrO_4	— 333.4	kcal [7]
Cr_2O_3	— 269.1	» [8]
$K_2Cr_2O_7$	— 488.5	» [7]
$\Delta H°$	= 20.6	kcal

Data for the corresponding Na and Tl reactions are at present not available in the literature.

The following report is divided in to two parts. The first gives the results of the investigations on the decomposition of the pure K, Na, and Tl dichromates as a function of the temperature.

The second deals with the decomposition equilibria of mixed K-Na and K-Tl dichromates as a function of the composition.

I. THE DECOMPOSITION OF PURE DICHROMATES

A. Experiments

For the reaction (1a), we have:

$$K = \frac{a_{CrO_4^{2-}} \cdot p_{O_2}^{3/4}}{a_{Cr_2O_7^{2-}}} \tag{2a}$$

Values of:

$$K' = \frac{N_{CrO_4^{2-}} \cdot p_{O_2}^{3/4}}{N_{Cr_2O_7^{2-}}} \tag{2b}$$

(N = mole fraction) are found by determining the ratio $N_{CrO_4^{2-}}/N_{Cr_2O_7^{2-}}$ in melts in equilibrium with a known oxygen tension.

The composition of the melt was found by a simple gravimetric method. Starting with a weighed charge of dichromate + chromic oxide, the amount of oxygen expelled

during adjustment to equilibrium could be determined by weighing, after rapid cooling. During the experiments, pure O_2-gas or air of 1 atm was conveyed slowly upwards through a vertical furnace past a platinum crucible containing the weighed dichromate-chromic-oxide-charge. The arrangement was similar to that used in the pyrosulphate investigations.

The equilibrium was established in 2—6 hours in a pure O_2-atm. In an air atmosphere more time was required. By starting from the chromate side of the reaction, the melt consumed O_2, but the process was so slow that attempts to investigate the equilibrium from this side did not succeed.

The investigations in the Tl-system were complicated by the corrosive action of the melt on the platinum crucible.

In the experiments carried out at the highest temperatures [in the K-system 700°, in the Na-system and the K-Na-mixtures 660°], it was impossible to obtain absolutely constant weight. After a rather rapid decrease in weight during the first 2—3 hours, further heating resulted in a very small but continuous loss of weight, *e. g.* the weight as a function of time is shown for $Na_2Cr_2O_7$ at 662° in Table 1.

Table 1. The loss of weight of $Na_2Cr_2O_7$ at 662° C.

Starting with:

0.7716 g $Na_2Cr_2O_7$ + 0.0773 g Cr_2O_3

Time (min)	Weight (g)	dW/dt
		$g \cdot 10^{-4}$/min
0	0.8489	
90	0.8108	4.2
165	0.8101	0.09
225	0.8097	0.07
375	0.8089	0.05
Extrapolated ($t = 0$)	0.8110	

Assuming that the slow loss of weight at these high temperatures is due to evaporation of some kind, the values of K' were calculated by using corrected values for the loss of weight found by extrapolating to zero time.

The materials used were $K_2Cr_2O_7$ and $Na_2Cr_2O_7 \cdot 2\ H_2O$, "Baker's Analyzed". The sodium dichromate was dried at 170° C.

$Tl_2Cr_2O_7$ was prepared by precipitation of a Tl_2SO_4 solution with $K_2Cr_2O_7$ (in 2 *N* H_2SO_4). The analysis of the precipitate gave:

16.7 % Cr (theor. 16.64 %)

and

65.5 % Tl (theor. 65.43 %)

Cr_2O_3 was prepared by thermal decomposition of $(NH_4)_2Cr_2O_7$ (Merck reinst.).

It is of considerable importance in the following discussion that the Cr_2O_3 is practically insoluble in the dichromate-chromate melts. [The Na-K-meltphases which are completely soluble in water, gave no precipitate on addition of NH_3.]

In the Tl^I-dichromate-chromate melts there is a certain possibility of an oxidation reaction:

$$3\ Tl^I + 2\ Cr^{VI} = 3\ Tl^{III} + 2\ Cr^{III}$$

Attempts, however, to identify Tl^{III}, after dissolving the melt in diluted acid, was negative. The value of the red-ox-potential, in aqueous solutions containing chromate and dichromate ions in concentration of the same order of magnitude, also indicates that an oxidation of Tl^I will not take place to any appreciable extent.

Finally, it may be mentioned that it has been ascertained by X-ray examination that the higher Cr-oxides, already at temperatures below 400° C, are rapidly transformed in to the corund modification of Cr_2O_3. [In accordance with statements of Ryss and Siljanskaja[9].]

B. Discussion

The results of the equilibrium investigations are given in diagram 1.

A comparison of the experiments in oxygen and air atmospheres gives some information about the influence [compare (2b)] of the composition of the melt (*cf.* Table 3) on K'.

Table 3. K' as function of the chromate-dichromate ratio in the melt.

		$P_{O_2} = 1$ atm		$P_{O_2} = 0.21$ atm	
	$t°$ C	$N_{CrO_4^{2-}}$	log K	$N_{CrO_4^{2-}}$	log K
K	612	0.03	− 1.53	0.10	− 1.51
	650	0.04	− 1.35	0.16	− 1.33
Na	405	0.10	− 1.01	0.35	− 0.98
K : Na = 54 : 46	662	0.17	− 0.70	0.41	− 0.67

It is evident that the variation of log K' with the anion composition of the melt is very small. It scarcely exceeds the limit of experimental error.

The fact, however, that the values of log K', in all three systems, are found to change in the same direction, increasing with increasing chromate content, is an argument favouring the view that the observed variation actually corresponds to a change in the activity coefficient with the anion composition.

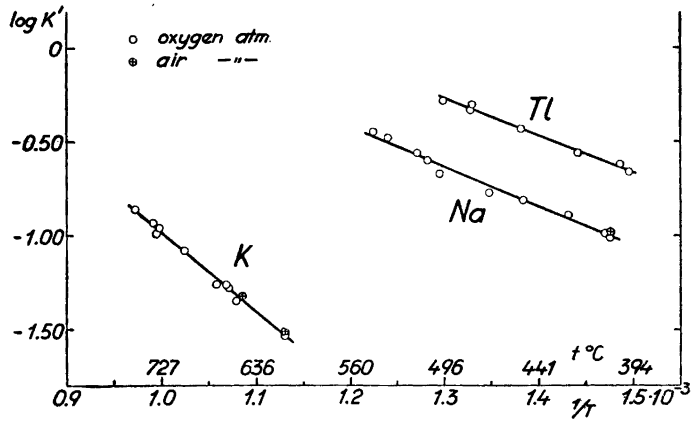

Fig. 1. The variation of log $K' = \log N_{CrO_4^{2-}}/N_{Cr_2O_7^{2-}}$ *with* $1/T$.

Writing

$$\frac{a_{CrO_4^{2-}}}{a_{Cr_2O_7^{2-}}} = \frac{N_{CrO_4^{2-}} \cdot \gamma_{CrO_4^{2-}}}{N_{Cr_2O_7^{2-}} \cdot \gamma_{Cr_2O_7^{2-}}} \tag{3}$$

(γ = activity coefficient)

and as a first approximation:

$$\log \gamma_{CrO_4^{2-}} = bN^2_{Cr_2O_7^{2-}}$$

$$\log \gamma_{Cr_2O_7^{2-}} = bN^2_{CrO_4^{2-}}$$

this gives a value of $b \leqq 0.1$, of the same order of magnitude both for the K-, Na- and the mixed K-Na-system.

It is evident that log K' can replace log K without appreciable error.

By plotting log K' against $\frac{1}{T}$ (Fig. 1) we get straight lines, within the limits of error, where:

$$-2.303\ R \frac{\mathrm{d} \log K'}{\mathrm{d}(\frac{1}{T})}$$

represents the following approximate ΔH-values for the dichromate decomposition:

K: 19.2 kcal ($t = 611$—$750°$ C)
Na: 9.6 kcal ($t = 400$—$550°$ C)
Tl: 9.0 kcal ($t = 400$—$500°$ C)

It should be noticed that the value of K' for the Na and Tl systems at 662° C (Fig. 2) seems to indicate an increase in ΔH with increasing temperature. The composition of the melt at these high temperature, however, is so substantially different from the experiments at the lower temperatures that effects caused by deviations from ideal solutions (which also will tend to increase the value of K') have to be considered.

The ΔH-value for the K-system agrees fairly well with the previous mentioned calorimetric value for the reaction (1 b), where:

$$\Delta H° = 20.6 \text{ kcal}$$

It is evident from diagram 1 that the stability of the oxygen bridge of the dichromate ion decreases in the sequence

K, Na, Tl

the same as found in the investigations of the thermal decomposition of the pyrosulphates. The values of the change in free energy of the latter reactions involve considerably larger limits of error, but nevertheless it seems possible to draw some conclusions from them.

The difference ΔF_K—ΔF_{Na} appears to be somewhat larger for the sulphate then for the chromate reaction (7.0>5.8 kcal at 660° C. Compare Fig. 1 with Fig. 1 in [3].) On the other hand, the difference ΔF_{Na}—ΔF_{Tl} is evidently larger in the chromate than in the sulphate system. It seems, therefore, that the polarizing effect of the cation is a more important factor for the stability of the Cr—O—Cr than for the S—O—S bridge.

II. THE THERMAL DECOMPOSITION OF MIXED DICHROMATES

The systems investigated were the mixtures of K-Na and K-Tl-dichromates. The experiments were carried out in the same way as for the pure systems except that the temperature was kept constant [662° C] for the whole range of cation compositions. The results are shown in Fig. 2, where log K' is plotted against the mole fraction of Na and Tl, respectively, in the melt.

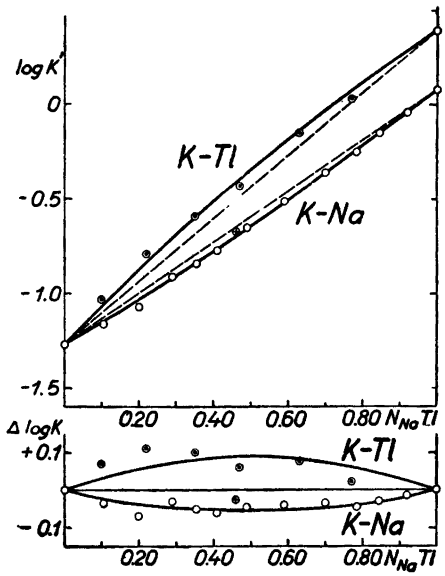

Fig. 2. The variation of log $K' = \log N_{CrO_4^{2-}}/N_{Cr_2O_7^{2-}}$ with the cation composition of the melt. (N = mole fraction.)

It is striking that log K', in the mixtures, does not differ very much from the straight line between the values of the pure components of the systems.

The K-Na mixtures show a small negative deviation, whereas the K-Tl-mixtures show a more appreciable positive deviation.

As a first approximation log K' can be calculated by the simple formula:

$$\log K' = N_1 \log K'_1 + N_2 \log K'_2 + b\, N_1 N_2$$

where N_1 and N_2 are the mole fractions of the cations Na, (Tl), and K in the melt, and b is a constant

$$b_{K-Na} = -0.20 \quad (t = 662^\circ\,C)$$
$$b_{K-Tl} = +0.35 \quad (t = 662^\circ\,C)$$

This approximation is in fairly good agreement with the experiments in the Na-K-system. In the K-Tl-system, however, it is evident that the deviations from linearity are not as symmetrical as represented by the simple term $b\, N_1 N_2$.

The thermodynamic relation between K', K'_1, and K'_2 (compare the foregoing paper) is the following:

$$\log K' = N_1 \log K'_1 + N_2 \log K'_2 \qquad (4)$$
$$+ (N_1 \log \gamma Y(A)/\gamma X(A) + N_2 \log \gamma Y(B)/\gamma X(B) - \log \gamma Y(AB)/\gamma X(AB))$$
$$+ (N_1 \log \gamma A(X) + N_2 \log \gamma B(X)) - (N_1 \log \gamma A(Y) + N_2 \log \gamma B(Y))$$

The activity coefficients correspond to deviations from idealty in the following 5 binary mixtures:

Components	Subscript of	
$Na_2Cr_2O_7 - Na_2CrO_4$	X(A),	Y(A)
$K_2Cr_2O_7 - K_2CrO_4$	X(B)	Y(B)
$Na_2CrO_4 - K_2CrO_4$	A(Y),	B(Y)
$Na_2Cr_2O_7 - K_2Cr_2O_7$	A(X),	B(X)
$(Na,K)_2Cr_2O_7 - (K,Na)_2CrO_4$	X(A,B),	Y(A,B)

[In the Tl-K-system, Na has to be replaced by Tl.]

The deviation from linearity of $\log K' = f(N)$ evidently is caused by the activity coefficient including terms.

As already pointed out (p. 368), the three $\gamma Y/\gamma X$ quotients [corresponding to the (Na), (K), and (Na,K) systems] seem to be comparatively small.

In addition, they are of opposite signs in eq. 4.

It therefore appears as if cancelling the second term causes no great error.

There also is reason to believe that this condition will be fulfilled in the Tl-K mixtures. Hence, the deviations from linearity in the first order must be connected with the γ_A and γ_B including terms.

Regarding the importance of the γ_A terms compared with the γ_B terms it is at present difficult to decide which are the most important.

There should be some reason to believe that the $\gamma_{A(Y)}$ and $\gamma_{B(Y)}$ (chromate) terms are more important than the $\gamma_{A(X)}$ and $\gamma_{B(X)}$ (dichromate) terms.

The larger size of the dichromate ion makes the average distance of the cations larger. Secondly, the oxygen atoms of the chromate ion are less polarized by the central chromium ion, and therefore are more exposed to attraction from the cations in the melt. If this is true the term $[N \log \gamma_{A(Y)} + N_2 \log \gamma_{B(Y)}]$ is chiefly responsible for the deviation of $\log K'$ from the straight line between $\log K'_1$ and $\log K'_2$.

The empirical formula is derived by putting

$$\log \gamma_{A(Y)} = b\, N^2_{B(Y)} \text{ and } \log \gamma_{B(Y)} = b\, N^2_{A(Y}$$

According to this assumption the negative deviation of log K' from linearity in the Na-K-melts corresponds to a *positive* deviation from Raoults law in the Na-K-chromate mixtures, and, in the same way, the positive deviation of log K' in the Tl-K-melts results from a *negative* deviation from Raoults law in the Tl-K-chromate mixtures. It seems reasonable that this negative deviation may be connected with a tendency to complex formation between Tl^+ and CrO_4^{2-}. This may also, perhaps, explain the unsymmetrical deviations observed on this system. The effect of a complex formation is expected to be more important in the Tl-poor than in the Tl-rich range of the system.

On the other hand there is also some evidence for the opiniom that a salt mixture of a noble gas ion and a transition element ion shows less negative deviation from ideality than corresponding mixtures of noble gas ions of the same size (compare the silver-alkali bromide melts investigated of Hildebrand and Salstrom [10])

In our systems the conditions would be analogous if the γ_X (dichromate) terms were the dominating in the expression of log K'.

SUMMARY

The equilibrium between CrO_4^{2-}-$Cr_2O_7^{2-}$ melts and O_2-gas shows that the stability of the dichromates on thermal decomposition, according to the equation:

$$Cr_2O_{7(m)}^{2-} = CrO_{4(m)}^{2-} + 1/2\ Cr_2O_{3(s)} + 3/4\ O_{2(g)}$$

[m = melt, s = solid, g = gas]

increases in the order Tl, Na, K.

It appears that the stability of the oxygen bridge Cr—O—Cr depends on the size and the polarizing power of the cation.

The results are compared with those obtained in the $S_2O_7^{2-}$-SO_4^{2-}-system.

The equilibrium constant:

$$K' = \frac{N_{CrO_4^{2-}} \cdot p_{O_2}{}^{3/4}}{N_{Cr_2O_7^{2-}}}$$

[N = mole fraction in the melt, p = gas pressure]

is determined at two different O_2-tensions, and the activity coefficients in the melts are discussed.

Equilibrium investigations were made in mixtures of K-Na resp. K-Tl-dichromates by varying the cation composition at a constant temperature. The variation of log K' is found to be expressed by the equation

$$\log K' = N'_1 \log K'_1 + N_2 \log K'_2 + b N_1 N_2$$

where N_1 and N_2 are the mole fractions of Na(Tl) and K, and K'_1, K'_2 are the equilibrium constants in the melts with single cations.

The equation is verified by thermodynamic calculations. The activity coefficients in the melts are discussed.

Our investigations have been carried out with aid of grants from *Statsminister Gunnar Knudsen og hustru Sophie f. Cappelens Familjelegat (Borgestad Legat IV)* for which we wish to express our sincere gratitude.

The experiments were carried out in the laboratories of Institutt for uorganisk kjemi, Norges Tekniske Högskole. We wish to express our gratitude to the director Professor, Dr M. Ræder.

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Received November 7, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 374 – 385

Copper Compounds of Acetylene

I. Acetylene Compounds with Cuprous Chloride

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The first to report a compound of the type n CuCl · C_2H_2 were Hofmann and Küspert [1], who found when bubbling acetylene through a solution of cupric chloride in absolute ethyl alcohol that a compound of composition 6CuCl · C_2H_2 had formed. Chavastelon [2] synthetized a compound 2CuCl · C_2H_2 and determined the vapour pressure at different temperatures.

Berthelot [3] investigated the absorption of acetylene in an acid solution of cuprous chloride and assumed from the results the existence of 3CuCl · C_2H_2 and 5CuCl · C_2H_2.

Manchot, Winthers and Oltrogge [4] made further absorption measurements, found from analyses the presence of 2CuCl · C_2H_2 and assumed the compound CuCl · C_2H_2 to exist, though no analysis was undertaken.

Gilliland, Bliss and Kip [5] repeated Chavastelon's vapour pressure measurements but found much higher pressures.

The purpose of my investigation was to determine the range of existence for these compounds with regard to temperature and pressure of acetylene and to determine the crystal structures of the compounds by X-ray analysis.

They were synthetized by methods given by the various authors, but my results did not always agree with theirs. Only two compounds, 3CuCl · C_2H_2 and 2CuCl · C_2H_2, could be reproduced.

When measuring the vapour pressures of these compounds, a tensiometrical method was first used. Cuprous chloride and an alcoholic solution of hydrogen chloride in a small bulb, also containing acetylene and connected to a mercury manometer, were vigorously shaken in a thermostat. When the compound 3CuCl · C_2H_2 was produced, the acetylene pressure decreased towards a limit. In a new experiment 3CuCl · C_2H_2 was treated like the cuprous chloride in the former, but at a pressure below the previously found limit. The ace-

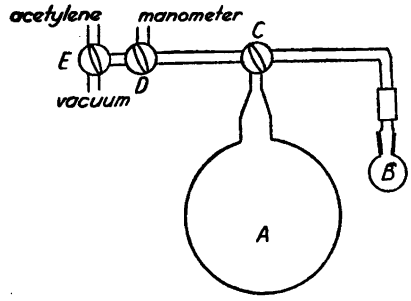

Fig. 1. Synthesis apparatus.

A. Gas container.
B. Reaction vessel.
C, D, E. Valves.

tylene compound decomposed, and the pressure increased to a limit differing from the former. Though the experiments continued for several days, no reproducible results could be reached by this method.

Instead the compounds were synthesized under controlled pressure of acetylene, and analysed immediately. A solution of hydrogen chloride in ethyl alcohol was used as reaction medium instead of aqueous solution, because the compounds could then be dried more quickly, and the disintegration diminished.

The X-ray determinations were undertaken when the range of existence had been determined for the compounds.

VAPOUR PRESSURE MEASUREMENTS

Experimental

Materials. The cuprous chloride used was of pro analysi quality. It was purified by solving in strong hydrochloric acid, the solution diluted with water and filtered. After washing with alcohol and ether, it was dried and stored in an evacuated exsiccator.

The acetylene was supplied by AGA Co., Lidingö, in a tank with their usual porous filling, but without acetone. Control analyses showed the gas containing 99 % acetylene, $\sim$ 1 % nitrogen, and traces of other gases.

The alcoholic solution of hydrogen chloride was prepared by mixing 1 part of conc. hydrochloric acid and 100 parts of ethyl alcohol of 99 % strength.

Synthesis apparatus. The apparatus constructed for the purpose is shown in Fig. 1. A small bulb B, containing 10 ml, was the reaction vessel. It was removable from the rest of the apparatus by a ground joint. The larger bulb A of 1000 ml was the gas container, and was connected to the reaction bulb, a manometer, a vacuum pump, and the acetylene storage by the valves C, D and E.

The apparatus was immersed in a thermostat, and the stirring during the synthesis achieved by vigorously shaking the small bulb, which was connected flexibly to the apparatus by a short rubber tube. This was glued to the glass and held by clamps. The ground joint was held by springs and sealed by picein.

The manometer was of mercury U-type with a steel scale. The pressure readings were corrected to 0° C of mercury, and reduced by the vapour pressure of ethyl alcohol at the temperature given.

All temperatures were measured on a mercury thermometer divided in 0.1° C.

When starting a synthesis, 0.5 g cuprous chloride and 5 ml hydrochloric alcohol was transferred to the reaction bulb. This was attached to the apparatus, and the joint carefully sealed. The system was evacuated, washed twice with acetylene, and finally filled to appropriate pressure, which had to be corrected a few times during the beginning of the synthesis. After 24—48 hours the reaction was complete. The small bulb was released, cooled to 0° C, and the contents pressed through a glass filter by acetylene of 2 atm. and immediately analysed. It was found by experiments, that when washing the compounds on the glass filter with alcohol and ether, saturated with acetylene, before analysing the losses of acetylene were larger than when analysing the compounds immediately.

Analyses. Acetylene was determined by a method given by Vestin [6]. The addition compound was decomposed by a solution of potassium cyanide, the gas evolved bubbled through 0.6 *N* solutions of silver nitrate, where the following reaction occured:

$$C_2H_2 + 3AgNO_3 = C_2Ag_2 \cdot AgNO_3 + 2HNO_3$$

The acid developed was titrated with 0.05 *N* solution of sodium hydroxide, using chlorphenol-red as indicator. The accuracy is approximately 1 per cent.

Copper was determined by electrolysis of the hot, diluted cyanide solution, using platinum electrodes, which were both weighed before and after the experiment. The accuracy was found better than 0.5 per cent.

Results

When possible, an estimation of the vapour pressure by tensiometrical measurements was made before starting the controlled syntheses. These were performed at 10, 15, 20 and 25° C at pressures up to 2 atm.

A study of the composition changes at different pressures was made, the limit pressure of two compounds being found by stepwise narrowing the pressure interval in which a change of composition occured. When the interval was a few mm mercury, the measurements were discontinued. Further narrowing is of little value, due to uncertaincies in the other experimental conditions. To avoid these as much as possible, the last intervals was determined by simultaneous synthesis at the higher and lower pressures.

The experimental results are found in Tables 1, 2, 3, and 4, where n is the number of moles acetylene per mole cuprous chloride.

Table 1. Syntheses from cuprous chloride at 10° C.

Temp. °C	Time h	p mm Hg	mM C_2H_2	mM Cu	n
10.02	24	178	0	—	0
10.03	22	188	0	—	0
10.02	24	192	0.472	3.06	0.16
10.15	24	198	0.436	1.65	0.27
10.00	24	397	0.323	0.973	0.33
10.00	24	400	0.444	1.42	0.31
10.00	24	403	0.546	1.24	0.44
10.00	18	407	1.78	2.43	0.44
10.00	18	411	0.578	1.24	0.47
10.03	16	415	0.985	2.12	0.47
10.02	16	415	0.985	2.12	0.45

Table 2. Syntheses from cuprous chloride at 15° C.

Temp. °C	Time h	p mm Hg	mM C_2H_2	mM Cu	n
15.02	16	260	0	—	0
15.02	16	263	0.281	1.03	0.28
15.11	24	577	0.963	2.88	0.33
15.10	22	591	1.06	3.47	0.33
15.08	40	594	1.30	3.62	0.28
15.10	26	598	0.742	1.69	0.44
15.06	48	642	0.530	1.12	0.48
15.09	24	790	0.677	1.48	0.46
15.09	24	825	1.46	3.14	0.47
14.87	40	1090	0.487	1.03	0.47
15.05	20	1181	1.18	2.68	0.44
14.94	30	1274	0.372	0.764	0.49
15.02	40	1496	0.630	1.29	0.49

Table 3. Syntheses from cuprous chloride at 20° C.

Temp. °C	Time h	p mm Hg	mM C_2H_2	mM Cu	n
19.98	22	350	0	—	0
19.97	16	352	0	—	0
19.97	17	353	0.206	0.716	0.29
19.99	22	355	0.518	1.58	0.33
19.99	19	357	0.531	1.74	0.31
19.98	28	364	0.515	1.55	0.33
19.94	48	749	0.521	1.62	0.32
19.89	24	752	0.469	1.42	0.33
19.89	24	757	0.504	1.09	0.46
19.90	24	762	1.558	3.38	0.46
19.90	24	764	0.511	1.06	0.48
19.90	24	765	0.720	1.58	0.46
19.84	18	774	0.540	1.11	0.49

Table 4. Syntheses from cuprous chloride at 25° C.

Temp. °C	Time h	p mm Hg	mM C_2H_2	mM Cu	n
25.02	24	256	0	—	0
25.05	24	373	0	—	0
25.03	24	400	0	—	0
25.05	16	440	0	—	0
25.04	24	460	0	—	0
25.02	24	470	0	—	0
25.02	24	473	0.434	1.45	0.31
25.05	22	484	1.31	3.92	0.33
25.05	24	496	0.525	1.69	0.31
25.03	24	517	1.24	4.04	0.31
25.03	24	540	0.780	2.69	0.30
25.05	24	693	1.27	4.51	0.28
25.05	24	790	0.225	0.705	0.32
25.01	24	820	0.598	2.00	0.30
25.01	24	860	0.482	1.71	0.28
25.04	21	874	0.455	1.52	0.30
25.04	22	930	0.652	2.12	0.31
25.03	15	952	0.498	1.52	0.33
25.08	24	961	0.729	2.31	0.32
25.00	20	988	0.650	2.06	0.32
25.05	22	1003	0.536	1.73	0.31
25.05	22	1007	0.710	1.56	0.46
25.05	14	1020	0.582	1.27	0.46
25.03	13	1180	1.01	2.21	0.46
25.05	20	1250	0.785	1.79	0.44
25.00	48	1490	0.623	1.32	0.47

As is seen from the tables, the number of moles acetylene per mole of copper averages to 0.31 and 0.47 instead of 0.33 and 0.50, as would be expected for the compounds $3CuCl \cdot C_2H_2$ and $2CuCl \cdot C_2H_2$. This discrepancy can be explained:

1. by losses of acetylene due to disintegration between syntheses and analyses, though the period was only a few seconds.
2. by incomplete reactions.

The moist compounds were found to decompose very fast, but when carefully dried they were comparatively stable. Precautions were taken to decrease the losses of acetylene between synthesis and analysis to a minimum.

To investigate the second possibility, series of experiments were performed to determine the composition at different times during the synthesis under the same conditions as in earlier experiments. Tables 5 and 6 show the results.

Table 5. Studies of the time required to synthesize $3CuCl \cdot C_2H_2$ at 25° C.

p mm Hg	Time h	mM C_2H_2	mM Cu	n
490	8	0.575	2.48	0.23
490	16	0.503	1.53	0.33
490	24	0.526	1.69	0.31
490	36	0.691	2.26	0.31

Table 6. Studies of the time required to synthesize $2CuCl \cdot C_2H_2$ at 25° C.

p mm Hg	Time h	mM C_2H_2	mM Cu	n
1 500	10	0.671	1.78	0.38
1 500	20	0.934	2.02	0.46
1 500	30	0.785	1.64	0.48
1 500	40	0.630	1.29	0.49

It was sometimes observed that the reaction was inhibited for a long time, even 12 hours, before it started and could then be almost completed in a few more hours. The reason could not be found.

In order to make sure that the compositions were reproducible and the equilibrium real, a few reactions were performed, starting from materials richer in acetylene than the product, *e. g.* $3CuCl \cdot C_2H_2$ from $2CuCl \cdot C_2H_2$. The results are given in Table 7.

The contents of Tables 1, 2, 3 and 4 show the existence of only two compounds, $3CuCl \cdot C_2H_2$ and $2CuCl \cdot C_2H_2$, in the pressure range up to 2 atm. at

Table 7. Decomposition of $2CuCl \cdot C_2H_2$ at 25° C.

Time h	p mm Hg	mM C_2H_2	mM Cu	n
18	363	0.078	2.25	0.03
22	700	0.795	2.32	0.34

temperatures between 10 and 25° C. The sudden pressure steps between these compounds show that the addition compounds of cuprous chloride and acetylene have distinct compositions and are not berthollides with continuously varying compositions. The vapour pressures are given in Table 8.

Table 8. Vapour Pressures of acetylene-cuprous chloride compounds.

Compound	Temp. °C	p mm Hg
$3CuCl \cdot C_2H_2$	10.02	190
»	15.02	262
»	19.97	353
»	25.02	472
$2CuCl \cdot C_2H_2$	10.00	402
»	15.09	596
»	19.89	755
»	25.05	1005

The enthalpy change $\Delta H°$ of the decompositions:

$$3CuCl \cdot C_2H_2 = 3CuCl + C_2H_2 \quad (1)$$
$$2CuCl \cdot C_2H_2 = 2\ 3CuCl \cdot C_2H_2 + C_2H_2 \quad (2)$$

have been calculated by plotting the logarithms of the vapour pressures found against $1/T$. The slope of the curve is $-0.43\ \Delta H°/R$. The points lie close to two straight lines, the divergences are less than 3 per cent of the pressure values.

Calculations were also made of the free energy or enchresy [7] change $\Delta G°$ and the entropy change $\Delta S°$. The results are given in Table 9.

Table 9. Thermodynamical constants at 25° C.

Reaction	p atm.	$\Delta G°$ cal mole	$\Delta H°$ cal mole	$\Delta S°$ cal mole · degree
$3CuCl \cdot C_2H_2 = 3CuCl + C_2H_2$	0.621	277	10 200	34
$3\ 2CuCl \cdot C_2H_2 = 2\ 3CuCl \cdot C_2H_2 + C_2H_2$	1.32	771	10 200	32

DISCUSSION

In relation to earlier investigations my experiments show:

1. In the range of temperature and pressure investigated only two compounds $3CuCl \cdot C_2H_2$ and $2CuCl \cdot C_2H_2$ exist. The compounds $6CuCl \cdot C_2H_2$[1] and $5CuCl \cdot C_2H_2$[3] are probably mixtures. The compound $CuCl \cdot C_2H_2$[4] has not been found in this range.

Syntheses at — 30° C and an acetylene pressure of 750 mm Hg. have resulted in a compound $CuCl \cdot C_2H_2$, which will be subjected to further investigation.

2. The vapour pressures of $2CuCl \cdot C_2H_2$ were found to be much higher than those found by Gilliland and co-workers [5] and even much higher than those Chavastelon [2] reported.

The former used a tensiometrical method, withdrawing acetylene and measuring the limit pressure. The vapour pressures of $2CuCl \cdot C_2H_2$ they give are about 65 per cent lower than those I have observed and about 25 per cent lower than mine for $3CuCl \cdot C_2H_2$. The differences decrease somewhat at higher temperatures.

My experiencies of the tensiometrical method of measuring the vapour pressures are that in this case it is not possible to reach the equilibrium pressure at given temperature from higher or lower pressures in reasonable time, owing to the low reaction rate. Nor is it possible to detect the existence of two compounds by this method.

My conclusions are that due to the deficiencies of the method they used, Gilliland and co-workers happened to measure the vapour pressures of $3CuCl \cdot C_2H_2$ instead of $2CuCl \cdot C_2H_2$ and that these readings are too low, because after withdrawing acetylene the pressure reaches equilibrium only after a very long time.

Chavastelon does not report how he measured the vapour pressures.

X-RAY INVESTIGATIONS

These were characterised by the efforts to overcome difficulties in preparing a stable powder for the X-ray analyses and to record diffraction lines of lowest intensities.

Experimental

A holocylindrical powder spectrograph of 19 cm diameter was used. X-ray radiation was produced by a discharge tube with a copper target at 46 kV and 6 mA. No monochromator was used. Ilford Red Seal X-ray Film, ex-

posures of 36—48 hours, and maximum energy developer were employed to obtain photograms, recording as many diffraction lines as possible with the comparatively feeble source of radiation.

Thin Pyrex glass tubes were drawn to capillaries 0.5 mm in diameter and with walls 0.01—0.02 mm thick. They were broken into two pieces, the synthesized suspension of acetylene — cuprous chloride poured through the wider opening of the tube and then pressed down through the capillary by acetylene of 2 atm. Some crystals blocked the capillary, and the liquid could be pressed out. The crystals were dried in the flowing gas, and the capillary, having been cooled on the middle, was sealed at both ends in a small flame.

Comparative exposures of cuprous chloride were performed in a similar way.

Results

No discrepancy could be found between photograms of $3CuCl \cdot C_2H_2$ and $2CuCl \cdot C_2H_2$. The only difference detected between these and photograms of cuprous chloride is they very weak lines 100 and 110 found in the former. The line 110 is doubtful. Both lines have, however, been found at repeated experiments. In all, two exposures of each acetylene compound and cuprous chloride were made, using newly prepared powders. Unfortunately the lines are too weak to appear on a photometer curve.

The intensities have been calculated assuming a cuprous chloride structure with acetylene interstitially placed in the hollow spaces of the pattern. The actual intensities of the lines 100 and 110, caused by the presence of acetylene, must be less than the corresponding intensities, if the reflection waves from all carbon atoms were of the same phase. These latter intensities can, however, be calculated without knowing the positions of the carbon atoms in the pattern, so it is possible to give an upper limit for the intensities of the lines 100 and 110 in comparison with the other lines in the photogram.

To make sure that acetylene did not escape from the powder during exposure, control analyses were made, but the small amount of sample makes the quantitative determination precarious. That at least 60 per cent of the acetylene was left is however beyond doubt.

The vapour pressures of the compounds do not exceed 760 mm. Hg at 18° C. Thermal decomposition is therefore unlikely. Disintegration due to the X-ray radiation is, however, possible and can not be prevented.

Table 10. Powder photogram of nCuCl, C_2H_2.

Cu-K radiation.

	$\sin^2\Theta$ calc.	$\sin^2\Theta$ obs.	I obs.	I calc.
100	0.0202	0.0206	v w	0.4
110	0406	0412	v v w	0.4
β111	0495	0501		
111	0606	0617	v st	10
β200	0660	0662		
200	0808	0802	v	0.6
β220	1321	1320		
220	1617	1616	v st	9
β311	1816	1815		
311	2223	2235	st	7
222	2425	2421	v w	0.2
β400	2642	2645		
β331	3137	3146		
400	3234	3242	m^-	2
331	3840	3839	m	3
β422	3962	3971		
420	4042	—	—	0.8
β511 / β333	4458	4452		
422	4850	4852	m^+	5
511 / 333	5457	5459	m	3
β531	5779	5775		
440	6467	6452	m^-	2
β620	6604	6601		
531	7074	7075	m^+	5
442 / 600	7276	—	—	0.4
620	8084	8088	m	4
533	8690	8689	m^-	4
622	8924	—	—	1
444	9701	9701	m^-	3

The cube edge a_0 is calculated to 5.406 Å.

DISCUSSION OF THE CRYSTAL STRUCTURE

On the presumption that the experimental results are correct, the crystal pattern of the addition compounds must be that of cuprous chloride with the acetylene molecules interstitially placed in the hollow spaces of the pattern. The contribution of acetylene to the lines in the photograms would then be

reflexes extinguished for the space group of cuprous chloride, T_d^2, and changed intensities for the others.

The scattering factor of carbon, f_C, decreases comparatively much faster at increasing sin Θ than f_{Cl} and f_{Cu}. Owing to geometrical configurations of the camera, the reflections are spread out over a larger surface at increasing Θ up to 45°. Perhaps it is the weakening of the "acetylene diffraction lines" by these two factors that makes it impossible to record more than the first two lines, 100 and 110.

To draw any conclusions as to how the acetylene molecules are placed in the pattern is naturally impossible under these circumstances. For cuprous chloride the structure is that of zinkblende, the atoms placed in the following way:

Space group T_d^2—$F\bar{4}3m$
4 Cu at 4(a): 000; $0\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}0\frac{1}{2}$; $\frac{1}{2}\frac{1}{2}0$.
4 Cl at 4(c): $\frac{1}{4}\frac{1}{4}\frac{1}{4}$; $\frac{1}{4}\frac{3}{4}\frac{3}{4}$; $\frac{3}{4}\frac{1}{4}\frac{3}{4}$; $\frac{3}{4}\frac{3}{4}\frac{1}{4}$.

Centres of the hollow spaces should be 4(b): $\frac{1}{2}\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}00$; $0\frac{1}{2}0$; $00\frac{1}{2}$ and 4(d): $\frac{3}{4}\frac{3}{4}\frac{3}{4}$; $\frac{3}{4}\frac{1}{4}\frac{1}{4}$; $\frac{1}{4}\frac{3}{4}\frac{1}{4}$; $\frac{1}{4}\frac{1}{4}\frac{3}{4}$.

The shape of the hollow spaces accessible to acetylene is uncertain. From the vapour pressure investigations the conclusion must be drawn that the acetylene molecules do not move i tunnels through the pattern, as gas molecules can, in for example zeolites, because the compounds would then be berthollides and their compositions vary continually with the acetylene pressure.

Perhaps other methods or investigations of similar compounds will answer these questions.

SUMMARY

In the range of 10—25° C and pressures up to two atm. only two compounds, $3CuCl \cdot C_2H_2$ and $2CuCl \cdot C_2H_2$, are formed from acetylene and cuprous chloride in acid solution. Their vapour pressures were determined at 10, 15, 20, and 25° C. $\Delta H°$, $\Delta G°$ and $\Delta S°$ of the decompositions of the compounds were calculated for 25° C.

X-ray powder photograms of the compounds differed by only one, possibly two, new lines from that of cuprous chloride. The acetylene molecules are probably placed interstitially in the pattern of cuprous chloride.

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Received December 6, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 386 – 389

An Apparent Relationship between Molecular Weights and Quantum Yields for the Inactivation of Enzymes and Viruses

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Recently in a review article [1] the quantum yields for the inactivation of a number of enzymes, proteins and viruses have been tabulated. It was stated that quantum yields are, roughly speaking, smaller the larger the molecular weight, but that no exact inverse proportionality was apparent. Since this writing a few revised molecular weights have been published [2]. The question has therefore been reexamined. In Fig. 1 there is plotted the logarithm of the quantum yields (Φ) against the logarithm of the respective molecular weights (M). It will be seen that the data reveals a trend which conforms to an equation of the type

$$\Phi = \frac{Q}{M^n} \tag{1}$$

where Q and n are constants. Now the quantum yield for inactivation may be defined as

$$\Phi = \frac{c_0 f}{ME} \tag{2}$$

where c_0 is initial concentration in grams of native protein per cubic centimeter, and f is the fraction of the material inactivated during the absorption of E einsteins per cubic centimeter (by the native protein). It will be seen that M bears an inverse relationship to Φ but since f is an unknown function of M, E, structure and composition, (nor is ΦM a constant) our photochemical results, expressible by equation (1) have no implicit explanation within the definition of the quantum yield.

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The problem can perhaps be considered geometrically. Our treatments will be quite elementary since further refinements do not seem justified at this time. Let us assume that in the primary process of inactivation a peptide bond adjacent to a chromophore is broken with a quantum efficiency ϕ (a constant). We further assume that if such a bond breaks on the surface of a protein molecule the quantum efficiency for the bond is the same, whereas if such a bond is ruptured inside a macromolecule the efficiency will be virtually zero because the protein framework holds the ion or free-radical chain ends so close together that recombination is preferred. There is equal probability for absorption by a given type chromophore irrespective of its position in the molecule (reference 1, page 96). Successful splitting of a weak bond will mean inactivation. The fraction of the total aromatic residues with adjacent CONH linkages at the surface will be (assuming spheres as a model of diameter d, as a first approximation)

$$\frac{\text{Volume of surface residues of thicknes } \delta}{\text{Volume of molecule } V} \cong \frac{6\,\delta}{d} \cong \frac{k}{M^{1/3}} \tag{3}$$

taking the volumes of protein molecules to be proportional to their respective molecular weights. For the overall quantum yield we can then write

$$\Phi \alpha \frac{k\phi}{M^{1/3}} \alpha \frac{Q}{M^{1/3}} \tag{4}$$

The resulting value of n is $1/3$ whereas the experimental value is about twice this. On the other hand, consider that a molecule has some sensitive volume of crossectional area, a. The sensitive volume is taken as the same size in all the proteins. The probability of hitting a in a spherical protein molecule placed in a beam of parallel light will be proportional to a/A where A is the cross sectional area of V, if the molecule is almost opaque to λ 2537 Å. The quantum yields is now

$$\Phi = \frac{k\,a\,\phi}{A} \cong \frac{k'\phi\,a}{M^{1/3}} \cong \frac{Q}{M^{1/3}} \tag{5}$$

which is of the correct form (compare (1)). Since only about 10 % of the residues in a protein are aromatic this is a very poor approximation for low molecular weight substances, however.*

* The possibility that $\Phi = \phi\,\varepsilon a\,[a]\,/(\varepsilon a\,[a] + \varepsilon p\,[p]) \cong \frac{Q}{M}$ (where εa, εp and $[a]$, $[p]$ are the molecular extinction coefficients and moler concentrations of sensitive volume and protein respectively) must also be disposed of since n is not unity. Here $\varepsilon a\,[a]/\,(\varepsilon a\,[a] + \varepsilon p\,[p])$ gives the fraction of absorbed light absorbed by the sensitive volume. In view of the small spread of M for the low molecular weight proteins and the scattering of points, the applicability of this approximation in the region of low M can not be excluded at present, however. Equation (5) may tend to be most applicable at very high molecular weights.

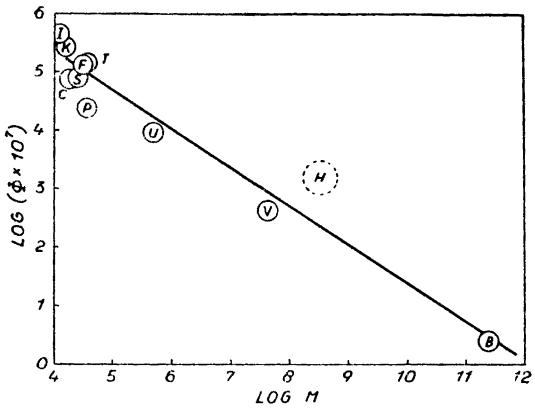

Fig. 1. A plot of the logarithms of quantum yields (2 537 Å) against the logarithms of molecular weights (reference 1, p. 158).

B = *B. coli (2699 Å), R. W. G. Wyckoff, J. Gen. Phys.* **15** *(1932) 351.*
C = *Chymotrypsin*
F = *Ficin*
H = *T_2 Bacteriophage; M estimated from electron micrograph and partial specific volume, A. R. Taylor, J. Biol. Chem.* **165** *(1946) 271.*
I = *Insulin*
K = *Ribonuclease*
P = *Pepsin*
S = *Soybean trypsin-inhibitor*
T = *Trypsin*
U = *Urease*
V = *Tobacco mosaic virus*

In Fig. 1 the best line through the experimental points with a slope of $-2/3$ has been drawn. It will be noted that a bacterium, a bacteriophage and a virus are also near this line.

The idea that the living cell has a sensitive spot is not new. It has been concluded by several authors that the life of each cell depends on a special molecule of this cell and that any injury to this molecule is spread over the whole cell. Conversely, we can also postulate, in view of our experimental curve, that the cell behaves like a single protein molecule.[3]

It is also of interest to estimate the possible size of the sensitive volume. If we associate the sensitive volume with some particular peptide bond and adjacent chromophore we can calculate from the curve the value of Φ for $M = 100$ (the number 100 is close to the average amino acid residue weight in a protein). The corresponding value of Φ is 0.51. It is surprising indeed to note that Rideal and Mitchell[4] report a value of 0.5 for the quantum efficiency of splitting peptide bonds in gliadin protein monolayers.

Finally, is there evidence that there is only one sensitive volume or area on an enzyme molecule? We tentatively identify this volume element with part of the functional group since it is the loss of enzymatic or hormonal activity which we measure. Trypsin loses its activity when combined with one molecule of inhibitor[5]. Chymotrypsin loses its activity after combining with one molecule of diisopropylfluorophosphate[6]. Both facts support the idea of a single functional group in these at least. Incidentally the tyrosyl residue is one of the strong chromophores in proteins and its presence is

known to be required for the activity of chymotrypsin, pepsin, insulin and tobacco mosaic virus, and doubtless others [7].

Whether or not an enzyme can be inactivated by the breakage of a bond not near the functional group or whether or not an enzyme can be inactivated without undergoing other changes associated with denaturation are related questions for which there is a paucity of data [1]. Work is in progress to help clarify these points.

SUMMARY

The apparent relationship between the quantum yields, Φ, for inactivation of enzymes, viruses and related proteins and *B. coli* and the corresponding molecular weights, M, is $\Phi = Q\ M^{-2/3}$ where Q is a constant. No satisfactory derivation of this relationship has been found although some possibilities have been examined.

The evidence can be interpreted to mean that each molecule has a single sensitive volume, possibly related to the active center in enzymes, which undergoes photolysis during inactivation. The larger the molecule the smaller is the fraction of the absorbed light which is absorbed by the sensitive volume and hence the lower the quantum yield for inactivation.

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Received November 22, 1949.

ACTA CHEMICA CSANDINAVICA 4 (1950) 390–398

Short Communications

Constituents of Pine Heartwood XVIII *. A Note on Cryptostrobin, an Isomer of Strobopinin from the Heartwood of *Pinus strobus* L.

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The heartwood of *Pinus strobus* (Weymouth Pine) has been investigated previously [1]. Among several other crystalline substances of the flavone or flavanone type, strobopinin, a substance with the composition $C_{16}H_{14}O_4$ and a m. p. of 225–227° (uncorr.), was isolated from this heartwood. The structure has not been determined, but it is believed to be a C-methyldihydroxyflavanone. Strobopinin is optically active, $[\alpha]_D^{20.5} -61°$ (in methanol) [1].

In the course of continued studies on the extraneous constituents of *P. strobus*, strobopinin was isolated from the acetone extract. From the mother liquors of strobopinin, a new compound was isolated in a pure state, after several recrystallisations. It formed colourless needles from dilute acetic acid, m. p. 202–203° (uncorr.), $[\alpha]_D^{20} -33°$ (methanol, $c = 1.1$). The analyses agree with the formula $C_{16}H_{14}O_4$. Thus, the new compound is an isomer of strobopinin. The presence of one C-methyl group and the absence of methoxyl groups was indicated by the usual methods of analysis.

The name cryptostrobin is proposed for the hitherto overlooked compound. As yet it is not certain whether cryptostrobin really occurs in the heartwood. It may have been formed by a rearrangement of strobopinin under the influence of alkali during the isolation. However, its optical activity makes this assumption less probable. Work on the determination of the structures of both substances is now in progress.

Experimental. The heartwood of *P. strobus* (3 kg) was extracted, as usual, with ether and then with acetone. The extracts were separated into fractions by the method described in Part IX [2]. The 0.2 % sodium hydroxide fraction of the acetone extract was acidified and shaken with ether. The ether solution was dried and the solvent evaporated. The remaining resinous product was dissolved in methanol, leaving an insoluble residue, consisting of crude chrysin. The methanol solution, on concentration, yielded a semicrystalline product which was triturated with benzene. The insoluble material yielded pure strobopinin after some recrystallisations from dilute acetic acid. The benzene solution was shaken with saturated sodium carbonate solution. Between the benzene and the aqueous phase a brown oily layer was formed, which was separated and acidified with dilute sulphuric acid. The emulsion obtained was shaken with ether, and the ether dried and concentrated. The residue was treated with methanol, leaving an insoluble semicrystalline product which

* XVII. *Acta Chem. Scand.* 4 (1950) 55.

Epi-pinoresinol *

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In the naturally ocurring glucosides phillyrin and forsythin, the aglycon is a lignan, related to pinoresinol. The empirical composition of phillygenol and forsythigenol is that of a pinoresinol monomethyl ether. It has recently been shown by Gripenberg that phillygenol monomethyl ether is identical to *epi*-pinoresinol dimethyl ether [1], the latter compound being the asymmetrical isomeride of pinoresinol dimethyl ether [2]. Forsythigenol is probably identical to phillygenol [3]. Synthetic experiments, instituted at these laboratories, were directed towards the synthesis of the above mentioned glucosides. The glucosides of the easily available pinoresinol, however, could not be obtained in a crystalline state, and further experiments were therefore abandoned. During these investigations, *epi*-pinoresinol was prepared. Since this substance has some interest as a potential natural product, a short description of its preparation and properties would appear to be warrented.

Epi-pinoresinol diacetate. Pinoresinol (10 g) was dissolved in a mixture of acetic acid (100 ml) and sulphuric acid (1 ml). After 40 hours the solution was poured into water (1 l), and the precipitated phenol mixture collected and acetylated with acetic anhydride (30 ml) and pyridine (20 ml). The crystalline acetates obtained were fractionated by recrystallization from ethanol. After repeated recrystallizations, a fraction of pure epi-pinoresinol (0.5 g), m. p. 151.5—152.5° and $[\alpha]_D^{20}$ + 90° (chloroform $c = 2$), was obtained. Further recrystallizations did not change these values. The other fractions, which consisted mainly of unreacted pinoresinol diacetate, were retreated with sulfuric acid in acetic acid solution, after

* *Also XIV contribution on the Constitution of resin phenols and their biogenetic relations.* Part XIII, this journal **3** (1949) 982.

was recrystallised three times from toluene and finally from 60 % acetic acid. After several recrystallisations, the m. p. remained constant, 202—203° uncorr. From the mother liquors, less pure crystals could be isolated. Total yield, 2.4 g. Colour reactions: Hot nitric acid, reddish-brown; cold sulphuric acid, yellow; ferric chloride, brownish-violet; diazotised benzidine, orange-yellow.

$C_{16}H_{14}O_4$ (270.3)

Calc.	C 71.09	H 5.22	C—CH_3	5.56
Found	» 71.13	» 5.22	»	4.62
			OCH_3	nil

The yield of acetic acid, as determined by the Kuhn-Roth method [3], corresponds to 83 % of the calculated value, which is in agreement with results obtained with similar compounds [4]. Thus, strobopinin yielded 4.69 % C—CH_3 (84 %) and methyl phloroglucinol 8.47 % (80 %).

This work has been financially supported by *Fonden för Skoglig Forskning*, for which we wish to express our appreciation. One of us (J. C. A-N) wishes to express his gratitude for a Spanish Government Scolarship, which enabled him to take part in the work.

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Received January 20, 1950.

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Received January 20, 1950.

Some Derivatives of 4-Dimethylamino-3,5-dinitrobenzoic Acid

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For separation of colourless substances it may sometimes be convenient to use chromatographic adsorption of coloured derivatives of these substances. Thus some alcohols have been separated as azobenzene carboxylates, aldehydes in form of their dinitrophenylhydrazones etc.

We have tried the azide and chloride of 4-dimethylamino-3,5-dinitrobenzoic acid (chrysanisic acid) as reagents for alcohols and amines, with which they give intensely coloured and well crystallized derivatives. These were, however, not especially suited for chromatographic separation, so the investigation has been discontinued and the purpose of this paper is only to describe some new compounds prepared in the course of the work.

4-Dimethylamino-3,5-dinitrobenzoic acid. Ten grams of 4-chlorobenzoic acid were dissolved in 200 g of conc. sulphuric acid and at a temperature of 80°, 33 g of potassium nitrate were added gradually. The temperature rose to 120—140° and was kept there for one and a half hour. The cooled solution was poured upon ice and the crystals of 4-chloro-3,5-dinitrobenzoic acid filtered by suction. After one crystallization from alcohol the melting point was 152°. Yield 98 %. The melting point of the pure compound is 158°, but it was found, that the over-all yield of 4-dimethylamino-3,5-dinitrobenzoic acid was larger, if no further purification of the chloro acid was performed. If desired, however, the acid may be purified via its sodium salt, which is sparingly soluble in cold sodium hydroxide solution.

A suspension of 15 g of 4-chloro-3,5-dinitrobenzoic acid in 60 ml of 33 % dimethylamine in alcohol was heated in a closed vessel at 100° for 4 hours. The solution was then evaporated to a small volume. By addition of hydrochloric acid yellow crystals of 4-dimethylamino-3,5-dinitrobenzoic acid separated. Yield 14 g, with a melting point of 210—220°. The crude product was dissolved in the minimum amount of hot alcohol and the solution filtered. By cooling large orange-yellow crystals separated. Yield 11 g + 1 g from the mother liquor, *i. e.* 77 %. M. p. 248°.

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which further amounts of the *epi*-form could be isolated.

$C_{24}H_{26}O_8$ (442.5)
Calc. OCH_3 14.0 Found OCH_3 14.0

Epi-pinoresinol. *Epi*-pinoresinol diacetate (1.0 g) was dissolved in hot ethanol (25 ml) and potassium hydroxide (0.5 g) in water (0.5 ml) was added. The potassium salt precipitated at once. After cooling, it was collected on a filter, washed with ethanol, acetone and ether, dissolved in a small amount of water, and precipitated with acetic acid. The *epi*-pinoresinol (0.6 g) was recrystallized twice from ethanol. M. p. 140.5—141.5°. $[\alpha]_D^{20}$ + 126° (Chloroform, $c = 2$).

$C_{20}H_{22}O_6$ (358.4)
Calc. OCH_3 17.3 Found OCH_3 17.2

A small amount of *epi*-pinoresinol was methylated with dimethyl sulfate and alkali. The dimethyl ether was isolated and recrystallized from ethanol. The melting point of the substance was 129—130°, alone or as admixture of pure *epi*-pinoresinol dimethyl ether, kindly supplied by Dr. J. Gripenberg.

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Received March 6, 1950.

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We have tried the azide and chloride of 4-dimethylamino-3,5-dinitrobenzoic acid (chrysanisic acid) as reagents for alcohols and amines, with which they give intensely coloured and well crystallized derivatives. These were, however, not especially suited for chromatographic separation, so the investigation has been discontinued and the purpose of this paper is only to describe some new compounds prepared in the course of the work.

4-Dimethylamino-3,5-dinitrobenzoic acid. Ten grams of 4-chlorobenzoic acid were dissolved in 200 g of conc. sulphuric acid and at a temperature of 80°, 33 g of potassium nitrate were added gradually. The temperature rose to 120—140° and was kept there for one and a half hour. The cooled solution was poured upon ice and the crystals of 4-chloro-3,5-dinitrobenzoic acid filtered by suction. After one crystallization from alcohol the melting point was 152°. Yield 98 %. The melting point of the pure compound is 158°, but it was found, that the over-all yield of 4-dimethylamino-3,5-dinitrobenzoic acid was larger, if no further purification of the chloro acid was performed. If desired, however, the acid may be purified via its sodium salt, which is sparingly soluble in cold sodium hydroxide solution.

A suspension of 15 g of 4-chloro-3,5-dinitrobenzoic acid in 60 ml of 33 % dimethylamine in alcohol was heated in a closed vessel at 100° for 4 hours. The solution was then evaporated to a small volume. By addition of hydrochloric acid yellow crystals of 4-dimethylamino-3,5-dinitrobenzoic acid separated. Yield 14 g, with a melting point of 210—220°. The crude product was dissolved in the minimum amount of hot alcohol and the solution filtered. By cooling large orange-yellow crystals separated. Yield 11 g + 1 g from the mother liquor, *i. e.* 77 %. M. p. 248°.

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which further amounts of the *epi*-form could be isolated.

$C_{24}H_{26}O_8$ (442.5)
Calc. OCH_3 14.0 Found OCH_3 14.0

Epi-pinoresinol. *Epi*-pinoresinol diacetate (1.0 g) was dissolved in hot ethanol (25 ml) and potassium hydroxide (0.5 g) in water (0.5 ml) was added. The potassium salt precipitated at once. After cooling, it was collected on a filter, washed with ethanol, acetone and ether, dissolved in a small amount of water, and precipitated with acetic acid. The *epi*-pinoresinol (0.6 g) was recrystallized twice from ethanol. M. p. 140.5—141.5°. $[\alpha]_D^{20}$ + 126° (Chloroform, $c = 2$).

$C_{20}H_{22}O_6$ (358.4)
Calc. OCH_3 17.3 Found OCH_3 17.2

A small amount of *epi*-pinoresinol was methylated with dimethyl sulfate and alkali. The dimethyl ether was isolated and recrystallized from ethanol. The melting point of the substance was 129—130°, alone or as admixture of pure *epi*-pinoresinol dimethyl ether, kindly supplied by Dr. J. Gripenberg.

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3. Sosa, A. *Bull. soc. chim. biol.* **39** (1947) 918.

Received March 6, 1950.

4-Dimethylamino-3,5-dinitrobenzoyl chloride. A solution of 5 g of 4-dimethylamino-3,5-dinitrobenzoic acid and 5 ml of thionyl chloride in 25 ml of dry benzene was refluxed for half an hour. By cooling the acid chloride crystallized in large yellow plates. By concentration of the mother liquor a second crop was obtained. Yield 80 %.

After recrystallization from benzene + petroleum ether the m. p. was 118—119°.

$C_9H_8O_5N_3Cl$ (273.6)

Calc.	N 15.36	Cl 12.96
Found	» 15.62	» 12.93

4-Dimethylamino-3,5-dinitrobenzoylazide. Sodium azide (1.5 g) was added to a cold solution of the acid chloride (5 g) in acetic acid and the solution was heated at 50° for 15 minutes. The azide crystallized on cooling, and was recrystallized from acetic acid. Yield 66 %. M. p. 125°.

$C_9H_8O_5N_6$ (276.2)

Calc.	N 30.42
Found	» 29.82

N,N' -bis-(4-dimethylamino-3,5-dinitro-phenyl)-urea. A mixture of 0.5 g of the azide, 0.5 ml of water and 5 ml of xylene was refluxed for one hour. On cooling crystals separated, which were filtered and recrystallized from dilute alcohol. Dark violet-red crystals. M. p. 231°.

$C_{17}H_{18}O_9N_8$

Calc.	N 23.43
Found	» 23.29

Methyl 4-dimethylamino-3,5-dinitrophenyl-carbamate. A mixture of 0.5 g of the azide, 1 ml of dry methanol and 5 ml of xylene was refluxed for 3 hours. The xylene was removed in vacuo and the residue recrystallized from a mixture of benzene and petroleum ether. Orange-red crystals. Melting point 175—176°.

$C_{10}H_{12}O_6N_4$ (284.3)

Calc.	N 19.76
Found	» 19.85

Ethyl 4-dimethylamino-3,5-dinitriphenyl-carbamate. Prepared in the same way as the methyl urethane. Orange-red crystals. Melting point 91—92°.

$C_{11}H_{14}O_6N_4$ (298.3)

Calc.	N 18.74
Found	» 18.72

The two urethanes may be separated by chromatographic adsorption from benzene-petroleum ether solution on aluminium oxide, the methyl derivative forming the upper zone.

Amides of 4-dimethylamino-3,5-dinitrobenzoic acid. The amides of aliphatic amines were prepared by dissolving the acid chloride and the amine in pyridine and refluxing for 10—15 minutes. The amides separated by addition of water and hydrochloric acid and were recrystallized from benzene. The methyl- and ethylamide were also prepared by addition of the acid chloride to cold ethanolic solutions of the amine. By this method, however, the ethyl ester is easily formed. The aromatic amides were prepared by refluxing a solution of the acid chloride and the amine in benzene and were recrystallized from ethanol. All the amides crystallize in yellow to orange needles.

			N % calc.	N % found	M. p.
Methylamide	$C_{10}H_{12}O_5N_4$	(268.2)	20.90	21.11	203°
Ethylamide	$C_{11}H_{14}O_5N_4$	(282.2)	19.86	20.42	148°
iso-Propylamide	$C_{12}H_{16}O_5N_4$	(296.3)	18.91	18.77	162°
n-Butylamide	$C_{13}H_{18}O_5N_4$	(310.3)	18.05	17.87	86°
Anilide	$C_{15}H_{14}O_5N_4$	(330.3)	16.96	17.46	248°
o-Toluidide	$C_{16}H_{16}O_5N_4$	(344.3)	16.27	16.19	213°
p-Toluidide	$C_{16}H_{16}O_5N_4$	(344.3)	16.27	16.10	234°

Received March 16, 1950.

A Note on "Carrier Displacement" Chromatography

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If a mixture of dissolved substances is displaced through an adsorption column by a substance with an adsorption affinity higher than for any of the substances to be separated, a "procession" of adjoining zones of characteristic concentrations is set up, each zone representing a pure component. The width of each zone measured as the volume it occupies in the effluent is proportional to the amount of substance in that zone. This procedure, which was first described by one of us [1], has since been applied to a number of separations for analytical and preparative purposes with amino acids, peptides, fatty acids, carbohydrates etc. Its chief advantage depends upon the possibility of avoiding the effects of »tailing» which makes chromatographic separations based upon adsorption extremely difficult for substances with curved isotherms, which normally characterize strong adsorption. For the same reason the method has also been used in separations on ionic exchange columns [2, 3] and in partition chromatography on buffered columns of high capacity, where the distribution isotherm is no longer linear [4]. The fact that the zones do not spread out after the stationary concentration has been reached, makes it possible to use very large columns and thus to effect difficult separations. A detailed theory shows that the plate efficiency of the method is very high [4]. One disadvantage of the method, however, has been that the zones are in close contact, and thus there is some difficulty in cutting the fractions properly in the effluent, particularly for narrow zones (small quantities). It has been suggested by one of the authors [5] that this difficulty might be overcome by interposing between the zones to be separated a number of substances of intermediate adsorption affinities, which would form part of the "procession" and afterwards could be removed by evaporation or extraction. It was also mentioned by Synge and Tiselius [6] that some phenomena observed in the successive elution of adsorption columns might be interpreted as resulting from this sort of displacement, and the analogy to the separation of closely related substances by distillation with "carriers" was pointed out.

We have recently applied such a procedure to separations of amino acids and peptides with results which seem very promising. As a "carrier system" we have used a mixture of homologous alcohols, all in aqueous solution. Thus in one experiment 0.2 mg methionine + 0.2 mg of leucyl-glycyl-glycine + 0.2 mg of phenylalanine were dissolved in a mixture of 8 ml 1 % isoamyl alcohol (boil. point 128°—132°) and 2.5 ml 1 % *n*-butyl alcohol, both in 0.1 *N* HCl. The mixture was displaced with a solution of 1 % *n*-amylalcohol in 0.1 *N* HCl through a column containing 7.8 ml of a mixture of equal parts of active charcoal (Carboraffin Supra) and Super-Cel. The resulting diagram, as obtained by successive interferometric observations in the effluent, shows three sharp boundaries at 33.9 ml, 49.2 ml and 68.0 ml separating the three alcohols. Subsequent investigation by paper chromatography showed that all methionine had collected at the first boundary, all leucyl-glycyl-glycine at the second and all the phenylalanine at the third and that there were zones of 12 ml resp. 14 ml of medium devoid of amino acids or peptides in between.

In displacement chromatography the substances appear in the order of their adsorption affinity, and thus this order should not depend on amounts or concentrations as long as the adsorption isotherms do not intersect. Consequently a given substance will always tend to collect

against one definite boundary, namely the one which separates zones of carrier substances of slightly higher and slightly lower affinity. As an illustration the following cases studied so far, using homogloous alcohols as carriers, may be quoted.

Valine: *tert.* butanol/water
Leucine: *sec.* butanol/*tert.* butanol
Methionine: *sec.* butanol/*tert.* butanol
Leucyl-glycyl-glycine: *iso*amyl/*n*-butylalcohol
Phenylalanine: *n*-amyl/*iso*-amylalcohol
Glycyl-tryptophan: benzylalcohol/*n*-amylalcohol

The procedure, for which we suggest the name »carrier displacement chromatography» has several advantages. The zones are sharp and of appreciable concentration, and the degree of separation can be predicted from the known behaviour of the carrier system. The spacing between the zones can be varied at will, by chosing suitable amounts of carriers and varying their proportions, provided that the adsorption column is large enough to give complete separation in the carrier system. Obviously it should be possible to apply the same principle to ionic exchange and displacement partition columns.

This type of separation can easily be demonstrated by a very simple arrangement. If the outlet of a small column (for example about 1 ml of a mixture of charcoal and Super-Cel 1 : 5) is allowed to touch the centre of a large circular filter paper between two glass plates, and an experiment similar to that described above is carried out (with correspondingly smaller volume), the filter paper will take up the effluent. After drying and spraying with ninhydrin, it will show a number of concentric rings corresponding to the amino acids and peptides present at the alcohol boundaries.

The work is continued with particular attention to peptide separations. A more detailed report will appear elsewhere.

The Use of Ethyl α-Thienylcyanoacetate in the Synthesis of Substituted α-Thienylacetic Acids

KURT PETTERSSON

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The preparation of substituted thienylacetic acids has been a rather cumbersome problem. This can be illustrated by the synthesis of benzylthienylacetic acid, which has been obtained by the following steps [1]: Ethyl α-thienylglyoxylate was prepared from thiophene and ethyl oxalyl chloride. The free acid was prepared and converted to benzylthienylhydroxyacetic acid by means of benzylmagnesium chloride. This acid was reduced with stannous chloride yielding benzylthienylacetic acid.

The same acid has now been prepared from ethyl α-thienylcyanoacetate by benzylation with the aid of anhydrous potassium carbonate and benzyl chloride [2]. The ethyl benzylthienylcyanoacetate was then converted to benzylthienylacetic acid. The method is very useful for preparing other substituted thienylacetic acids, especially if the corresponding halogen compound cannot be converted to the Grignard compound.

We are indebted to the Swedish Natural Science Research Council for financial support.

1. Tiselius, A. *Arkiv Kemi* **A 16** (1943) no. 18.
2. Partridge, S. M., and Westall, R. G. *Biochem. J.* **44** (1949) 418.
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6. Synge, R. L. M., and Tiselius, A. *Acta Chem. Scand.* **3** (1949) 231.

Received March 20, 1950

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Received March 20, 1950

Ethyl α-thienylcyanoacetate was prepared from thienylacetonitrile with the aid of sodium amide and diethylcarbonate by a general method [3]. Thienylacetonitrile has been prepared by Blicke and Zienty [4], who refluxed thenyl chloride with sodium cyanide dissolved in dilute alcohol. Using this method, however, I have obtained a considerable quantity of ethyl thenyl ether. This complication is to be expected as benzyl bromide, when refluxed with dilute alcohol, yields ethyl benzyl ether [5]. The complication can be avoided by using acetone as solvent instead of alcohol.

Thienylacetonitrile. In a 500 ml, three-necked roundbottom flask, fitted with a reflux condenser, a mercury-sealed stirrer and a dropping funnel were placed 9.0 g (0.18 moles) of sodium cyanide dissolved in 100 ml of water and 100 ml of acetone. The mixture was refluxed and stirred on an oil bath. 21.0 g (0.16 moles) of thenyl chloride dissolved in 50 ml of acetone was added during one hour and the mixture refluxed with stirring for three hours. The acetone was removed by distillation and the nitrile was extracted from the residue with ether. The ether solution was dried with anhydrous sodium sulphate, the ether removed by distillation and the residue fractionated *in vacuo*. The fraction boiling at 110—113°/ 13 mm was collecte das pure nitrile. Yield 14.0 g(70 %).

Ethyl α-thienylcyanoacetate was prepared from 0.11 moles of sodium amide, 12.3 g (0.10 moles) of thienylacetonitrile and 15.4 g (0.13 moles) of diethyl carbonate according to the general process [3]. Yield 8.0 g (41 %) of ethyl α-thienylcyanoacetate boiling at 156—160°/14 mm.

Analysis:

$C_9H_9O_2NS$	C	H	N	S
Calc.	55.36	4.65	7.17	16.42
Found	55.57	4.54	7.20	16.42

Ethyl α-thienylbenzylcyanoacetate. 3.0 g (0.015 moles) of ethyl α-thienylcyanoacetate, 4 g (0.03 moles) of benzyl chloride, 4.5 g (0.033 moles) of anhydrous potassium carbonate and 30 ml of acetone were refluxed on an oil bath for 12 hours [2]. The acetone was removed by distillation and the residue treated with water and extracted with ether. After drying with anhydrous sodium sulphate, the ether was removed by distillation and the residue fractionated in vacuo. The fraction boiling at 206—210°/13 mm was collected as pure ethyl thienylbenzylcyanoacetate. Yield 2.6 g (60 %).

α-Thienylbenzylacetic acid. 2.6 g (0.009 moles) of ethyl thienylbenzylcyanoacetate was treated for ten minutes on a water bath with 1.1 g (0.02 moles) of potassium hydroxide dissolved in 10 ml of alcohol. The precipitated potassium carbonate [6] was removed by filtration and washed with alcohol. To the filtrate was added a solution of 1.0 g (0.018 moles) of potassium hydroxide dissolved in 10 ml of water and the mixture was refluxed until no more ammonia escaped (12 hours). The alcohol was removed by distillation. The residue was acidified with diluted hydrochloric acid, and the precipitated acid was extracted with ether. After removing the ether, 1.8 g (86 %) of thienylbenzylacetic acid was obtained. The crude acid was recrystallized from dilute formic acid and finally from petrol. Melting point 74.5—75.5°. A mixture of the acid obtained by this method and the acid obtained by the method described earlier [1] showed no melting point depression.

Equiv. wt. Calc. 231.3 Found 230.8

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Received February 24, 1950.

Hydrolysis of Methyl Methanetrisulfonate

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An aqueous solution of methyl methanetrisulfonate reacts instantly with bromine [1]. The reaction is so rapid that the colour of an azo indicator such as methyl orange does not disappear before the trisulfonic ester has been brominated. This behaviour of the ester towards bromine calls to mind strongly that of the trisulfonylmethanes. A newly prepared solution of the ester in water requires a quantity of nearly two atoms (one mole) of bromine per mole ester, but the bromine consumption decreases with the time. The reason probably is the hydrolysis of the ester during which the negative ion $CH(SO_2O)_3^{3-}$ is formed as the end product of consecutive stages. On the presumption that only the unhydrolyzed trisulfonic ester or its carbanion will react immediately with bromine, the concentration decrease of the ester can be followed by withdrawal of portions of its aqueous solution from time to time and direct titration with bromine in the presence of the indicator. It will be noted that the velocity of the reaction seems to increase with increasing hydronium ion concentration. Since the hydrolysis makes the solution still more acid, the reaction was studied in hydrochloric acid, where the hydronium ion concentration is approximately constant. Then the reaction is found to be pseudounimolecular.

It is of decided interest to note that the trisulfonic ester, in spite of its strongly acid properties, does not change the colour of ferric chloride in alcohol soluiton. The same behaviour is shown by tris-(ethylsulfonyl)-methane. As is well known, the ester $CH(COOC_2H_5)_3$ will give an obvious colour change under similar circumstances, thus indicating a certain enolization.

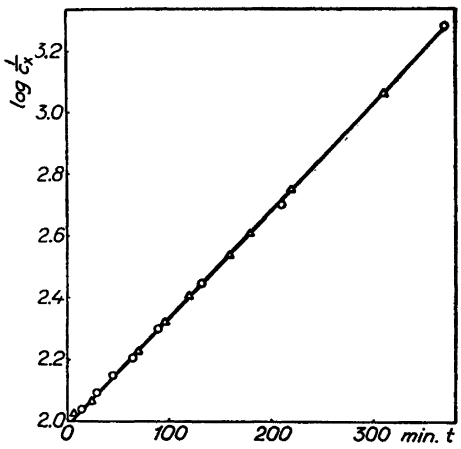

Fig. 1. Hydrolysis of methyl methanetrisulfonate in 0.1 M HCl. Two parallel series, both with $c_0 = 0.01$ M.

Experimental. The potassium methanetrisulfonate was prepared according to Backer [2]. Instead of transforming the potassium salt to the barium salt and treating it with sulfuric acid, the author directly obtained the trisulfonic acid by using a cation exchanger, Amberlite I R—100 H. A solution of potassium salt was percolated through a layer of the H^+-exchanger. The methyl methanetrisulfonate was prepared by the action of methyl iodide on the silver salt in benzene [1]. After boiling for ten hours 1.2 g methyl ester was obtained from 6.8 g silver salt. A solution of the trisulfonic ester ($c_0 = 0.01$ *M*) in 0.1 *M* hydrochloric acid was prepared and the reaction carried out at 25.0° C in a glass-stoppered flask from which samples were transferred with a four or five ml pipette into an Erlenmeyer flask. After addition of a few drops of 0.01 per cent solution of methyl orange the sample was immediately titrated with a solution of 0.5 *M* potassium bromide and 0.0025 *M* bromine until the indicator colour just disappeared. From this the actual ester concentration (c_x) can be calculated. The indicator blank was negligibly small. By

A Lithium Tungsten Bronze of Perovskite Type

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In a recent paper from this institute [1] the existence of a cubic lithium tungsten bronze of perovskite type was briefly mentioned. As the work on this subject must be postponed for some time, a few more of the details obtained so far will be given here.

The compound has been synthesized in two different ways: *a)* according to the method introduced by Brunner [2] by heating mixtures of lithium tungstate, tungsten trioxide, and tungsten dioxide *in vacuo* at about 850° C, and *b)* by cathodic reduction of fused acid lithium tungstate as was first done by Stavenhagen [3]. Attempts to prepare the compound by reducing acid lithium tungstate with hydrogen were not successful as inhomogeneous products were obtained. The products were purified by repeated alternate boiling with concentrated potassium carbonate solution, concentrated hydrochloric acid, and aqua regia and finally with a mixture of concentrated nitric acid and a small amount of hydrofluoric acid. In this way a dark blue or bluish violet crystalline powder was obtained by the first method, while the electrolytic reduction gave dark blue crystals of irregular shape. The samples were tested for purity by means of X-ray powder photographs and microscopic examination.

The powder photographs were taken in focusing cameras using Cu-K radiation. All observed reflections are consistent with a cubic unit cell of perovskite type. This lithium bronze is evidently isomorphous with the cubic sodium tungsten bronze, Na_xWO_3, described by Hägg [4]. The length of the cube edge, being about 3.72 Å, is slightly different for various preparations, probably due to the lithium content of the compound not being constant. The formula may thus be written Li_xWO_3. Values and limits of x cannot be given for the present for lack of analytical data. However, if the relation between colour and average valency of the molybdenum or tungsten atoms shown to exist for a great number of oxides and bronzes [1] is also valid for this lithium bronze, the blue colour might indicate an x value of 0.3 or 0.4 for the investigated preparations.

The authors are indebted to the *Swedish Natural Science Research Council* for financial support.

1. Magnéli, A. *Nova Acta Regiae Soc. Sci. Upsaliensis* [4] **14** (1950) no. 8.
2. Brunner, O. *Dissert.* Zürich (1903).
3. Stavenhagen, A. *Ber.* **32** (1899) 3064.
4. Hägg, G. *Z. physik. Chem.* **B 29** (1935) 192.

Received April 1, 1950.

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control experiments it was established that the generated methyl alcohol had no effect on the indicator blank. As an example, in Fig. 1, a curve for two parallel experiments is reproduced. The two series of points fall on the same straight line. If $k = \frac{2.303}{t} \log c_0/c_x$, the slope of the line gives a value of k of $8.04 \cdot 10^{-3}$. Another experiment ($c_0 = 0.01$ M) with 0.05 M hydrochloric acid at 25.0° C gives $k = 4.25 \cdot 10^{-3}$.

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Received April 4, 1950.

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Received April 1, 1950.

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control experiments it was established that the generated methyl alcohol had no effect on the indicator blank. As an example, in Fig. 1, a curve for two parallel experiments is reproduced. The two series of points fall on the same straight line. If $k = \frac{2.303}{t} \log c_0/c_x$, the slope of the line gives a value of k of $8.04 \cdot 10^{-3}$. Another experiment ($c_0 = 0.01$ M) with 0.05 M hydrochloric acid at 25.0° C gives $k = 4.25 \cdot 10^{-3}$.

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Received April 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 399 – 403

An Optical Arrangement for Getting Simultaneous Records of the Refractive Index and its Derivative for Stratified Solutions

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In a recent article [1], the author gave a survey over the different methods which have been used for recording the concentrations in cells with stratified solutions. These methods could be subdivided into two main groups, one of them being characterized by giving direct records of the concentration (by way of refractive index or light absorption), the other by giving records of the concentration derivative with respect to the position in the cell. In a comparison between the relative merits of the two groups of methods, it was found that both the concentration function and its derivative are very useful and that it is not possible to give one kind of record the absolute preference. On the contrary, the ideal method of observation should give records of both functions simultaneously.

To carry out such combined records in practice, it was said in the same article, would be easier if both records were based on the same optical principles. Since it did not seem possible to modify available derivative methods to give the integral function, it was instead tried to modify an integral method to give the derivative.

It has now been found, however, that a suitable combination between the inclined slit arrangement, which gives a record of the derivative, and a modification of the Rayleigh interferometer recently developed by Philpot and Cook [2] and the present author [1] can be carried out by very simple means if one starts from the optical system of the inclined slit method.

To make this clear, let us compare the optical systems of the inclined slit arrangement and the modified Rayleigh interferometer. Fig. 1 shows the optical components of the former arrangement and Fig. 2 those of the latter, both

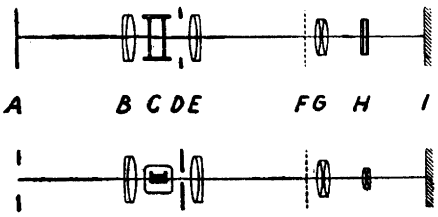
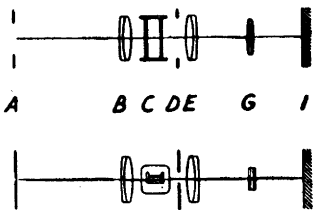


Fig. 1. The diagonal slit arrangement. Upper figure: elevation; Lower figure: plan.



Rig. 2. The interferometric arrangement according to Rayleigh and Philpot. Upper figure: elevation. Lower figure: plan.

systems being shown in plan and in elevation. In Fig. 1, A is a horizontal slit illuminated by light which is not necessarily monochromatic. The lenses B and E give an image of the slit in the plane F, where the inclined slit is situated in such a position that it crosses the image of A. Between the lenses, the light runs horizontally, one portion through and one beside the cell C. The diaphragm D has a lateral dimension equal to twice the breadth of the cell plus the thickness of the cell wall. The lenses G and H form together an astigmatic optical system with different focal powers in plan and in elevation. In plan, this lens system gives an image of the plane F, consequently also of the plane A, whereas in elevation the lenses give an image of the cell C. Thus, the vertical coordinate on the plate is related to the vertical coordinate in the cell, and the horizontal coordinate on the plate is related to the horizontal coordinates of the planes A and F.

In Fig. 2, the interferometric arrangement, A is a vertical slit illuminated by monochromatic light. The lenses B and E give an image of this slit on the plate I. Between the lenses, we have parallel light, one portion passing through and one beside the cell C. The diaphragm D has a lateral dimension equal to twice the breadth of the cell plus the thickness of the cell wall. The cylindrical lens G with a horizontal axis brings, in elevation, the cell C into focus on the plate I, while, in plan, it does not alter the focusing of the slit A.

A comparison between the two optical systems reveals that the following properties are common to both:

1) in plan, an image of the illumination slit is formed on the plate;
2) in elevation, an image of the cell is formed on the plate.

On the other hand, we find the following differences:

1) the inclined slit method requires a horizontal illumination slit, while the interferometric method requires a vertical slit;

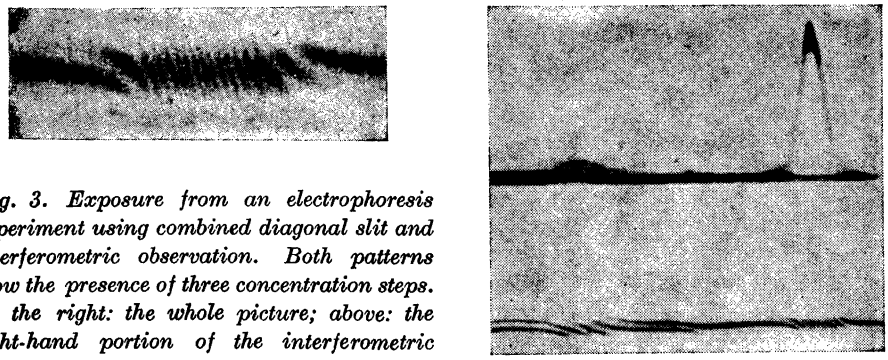


Fig. 3. Exposure from an electrophoresis experiment using combined diagonal slit and interferometric observation. Both patterns show the presence of three concentration steps. To the right: the whole picture; above: the right-hand portion of the interferometric pattern in greater magnification.

2) the inclined slit method requires a twofold focusing of the illumination slit, first to the plane of the inclined slit, then to the plate, whereas in the interferometric method a direct focusing from the illumination slit to the plate is possible;
3) the inclined slit method does not require monochromatic light, while the interferometric method does.

It is remarkable that, although the optical system of the inclined slit arrangement is unnecessarily complicated for the Rayleigh-Philpot-Cook interferometric arrangement, it is yet useful for it since it is characterized by a simultaneous focusing of the illumination slit and the cell, in plan and in elevation respectively. These properties are the necessary and sufficient conditions for interference. Consequently, when an inclined slit set-up is to be completed with an interferometric arrangement, nothing need be altered except the slits and the light source if this is not already monochromatic. *If the horizontal slit is replaced by a vertical slit, and if the inclined slit is removed, the result will be an interferometric arrangement according to Rayleigh and Philpot-Cook* *. This conclusion has been verified experimentally.

It follows from the above that simultaneous records of the refractive index and its derivative can be obtained by using one horizontal and one vertical slit in the plane A, Fig. 1, side by side. Since these slits are brought to focus on the plate when viewed from above, we will then get the two records side by side there. The inclined slits used hitherto, however, are not suitable in the combined procedure because their mechanical constructional details cut off the light from the vertical slit. It is necessary to construct a new diagonal slit which also carries a vertical slit in order to let the interfering light pencils through.

* Added in proof: This fact was discovered independently by Dr. G. Kegeles in June, 1949 (personal communication from Dr. L. G. Longsworth).

Double slits according to the above requirements have been constructed and placed in the planes A and F, Fig. 1, respectively, in ordinary electrophoresis experiments. Fig. 3 shows an exposure from such a run. Instead of measuring the areas of the peaks in the derivative diagram, one can count the number of fringes and measure the fraction of a fringe displacement within the same region in the interferometric pattern. On the other hand, the derivative pattern is more suitable and convenient for localizing the concentration steps and for resolving the different steps from each other. Since the cell magnification is the same in both patterns, it is very simple to pass from one to the other.

Fig. 3 is of considerable interest from the point of view of resolving power of the two methods since it offers a direct comparison and since it includes one refractive index gradient just on the border of the resolving power. The resolution of the diagonal slit method was studied theoretically by the author [3] with the result that a refractive index change of $18 \cdot 10^{-5}$ could still be measured with some precision in a 25 mm cell and with a wave-length of 5000 Å. It was pointed out, however, that this figure did not make full justice to the method chiefly because a refractive index change that can still be measured with some precision is far greater than the least perceptible change, the latter quantity generally being adopted as the definition of resolving power. Labhart and Staub [4] payed attention to this fact; nevertheless, they considered an interferometer more sensitive than the diagonal slit arrangement.

When a gradient curve obtained by the inclined slit method is compared with the corresponding interferometric pattern, one must not forget that the former is the derivative of the latter and that every differentiation is necessarily accompanied by a considerable loss of accuracy. Consequently, the two diagrams should not be compared until the interferometric pattern has been differentiated or the inclined slit pattern integrated. No doubt the interferometric pattern looks much more accurate, but it would be superficial immediately to conclude that it really is.

Hansen [5] concluded from purely theoretical reasoning that the resolving power of the *Schlieren* method, of which the diagonal slit method is a modification, is the same as that of interferometric methods. Taking practical considerations into account, however, he found the interferometric methods to be somewhat superior. Fig. 3 seems to strengthen this view. The very small refractive index gradient in the middle of the pattern gives a displacement of almost one fringe, which corresponds to a refractive index change in the cell of $1.8 \cdot 10^{-5}$. This gradient is just perceptible in the derivative pattern, hence the resolving power of the diagonal slit method is found to be roughly $2 \cdot 10^{-5}$ for the green mercury line and for a 25 mm cell. However, in the interfero-

metric pattern this gradient is more than just perceptible; it can still be measured with some precision since even fractions of fringes can be estimated. Thus the addition of an interferometric diagram to the inclined slit pattern not only increases the convenience of the evaluation. It also enhances the accuracy.*

SUMMARY

It has been shown that an inclined slit arrangement can be transformed into a self-recording interferometer according to Rayleigh and Philpot-Cook simply by replacing the horizontal illumination slit by a vertical slit and by removing the inclined slit. Accordingly, simultaneous records of the refractive index and its derivative can be obtained side by side with the same cell magnification by using vertical slits beside the horizontal and inclined slits. It has further been shown that the resolving power of the diagonal slit method is roughly $2 \cdot 10^{-5}$ for the green mercury line and for a 25 mm cell, which corresponds to the displacement of one fringe in the interferometric pattern. The possibility of measuring displacements smaller than one fringe increases the accuracy of refractive index measurements.

The author wishes to thank Professor The Svedberg for the encouragement he has given concerning this research program and Professor Arne Tiselius for the kind interest he has shown in the course of the work. The Swedish Technical Research Council gave financial support, which is gratefully acknowledged. The author is also indepted to *LKB-Produkter Fabriksaktiebolag*, Stockholm, which put its laboratories and the necessary mechanical constructions at my disposal.

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Received February 13, 1950.

* Similar conclusions are drawn by Dr. L. G. Longsworth in a recent investigation of the Philpot-Cook arrangement applied to electrophoresis (*Anal. Chem.*, in the press).

ACTA CHEMICA SCANDINAVICA 4 (1950) 404–415

The Prevalence of Unbranched Sulphur Chains in Polysulphides and Polythionic Compounds

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Divalent sulphur is apparently unable to add sulphur to any of its lone electron pairs to form branched groupings of the type $\rangle S\rightarrow S$ or $\rangle S=S$ (with coordinate or double sulphur-sulphur bonds). Thus, sulphur compounds such as disulphides, polysulphides and polythionic compounds are apparently all built up of unbranched, zigzag sulphur chains. The purpose of this article is to demonstrate that earlier arguments, on chemical basis, against the existence of unbranched sulphur chains in polythionic compounds and polysulphides are fallacious in view of recent work on the reactivity of these compounds. Furthermore, the purpose is to discuss the prevalence of unbranched sulphur chains on the basis of the following considerations.

Sulphur may be expected to add to an atom A to form a coordinate bond $\rangle A\rightarrow S$ or $\rangle\!\!\rangle A\rightarrow S$ only in cases where A is the less electronegative element, since such a bond implies that sulphur becomes the negative end of the polar bond. In the case where A is sulphur, a stable coordinate grouping $X_2A\rightarrow S$ or $X_3A\rightarrow S$ is accordingly probable only if the atoms or groups, X, bonded to A are able to decrease the effective electronegativity of A to a sufficient degree. Such cases do apparently not exist. In polythionic compounds X_2A where A is sulphur and the groups X are thio groups, A has in most cases a partial positive charge, as evidenced by the chemical reactions of the compounds. In such cases the groups X increase the effective electronegativity of A. Coordinate structures $X_2S\rightarrow S$ of the next higher polythionic compounds are therefore improbable.

A double bond $\rangle A=S$ or $\rangle\!\!\rangle A=S$ where A is sulphur, requires an expanded valency shell (beyond the octet) of A. Divalent sulphur is apparently unable to expand its valency shell for double bonding to additional sulphur atoms.

THE STRUCTURE AND REACTIVITY OF DISULPHIDES, POLYSULPHIDES AND POLYTHIONIC COMPOUNDS

In these compounds there are, generally, two structural possibilities, *viz.*, that of unbranched sulphur chains, and that of branched structures. The following compounds have been subjected to detailed structure investigation, by electron or X-ray diffraction methods: Rhombic sulphur [1], sulphur (S_8) vapour [2], disulphur dichloride [3], dihydrogen disulphide [4], dimethyl disulphide[4], di(*p*-bromophenyl) disulphide [5], dimethyl trisulphide [6], and di(2-iodoethyl) trisulphide [7,8]. The compounds are all built up of unbranched chains (8-membered unbranched rings in the case of rhombic sulphur and sulphur vapour). Lattice dimensions and space group are reported [7] for di(2-chloroethyl) trisulphide, and for two pentathionic compounds, *viz.*, monosulphur di(benzenethiosulphonate) and di(*p*-toluenethiosulphonate). A twofold axis of symmetry was found for these compounds, thus limiting the possibility of branched structures. Preliminary results [9] of an X-ray structure analysis of di(methanesulphonyl) disulphide, a tetrathionic compound, reveal an unbranched structure. Plastic or elastic sulphur consists of long, unbranched chains [10,11]. X-ray crystal studies of potassium trithionate [12], barium trisulphide [13], di(benzenesulphonyl) sulphide [7,14] and di(*p*-toluenesulphonyl) sulphide [7] are in accordance with unbranched chains; however, in these cases there is hardly any possibility for branched structures.

Dihydrogen disulphide and the dihydrogen polysulphides (tri, tetra, penta, hexa) are built up of unbranched sulphur chains, with terminal hydrosulphide groups, according to their Raman spectra [15,16]. The sensitivity of these sulphides towards basic substances is probably due to a base-catalysed prototropic change to unstable, branched structures [17]. Raman spectra of disulphur dimethoxide and *bis*(dimethylamide) indicate unbranched structures [18,19], and so do the Raman spectra of disulphur dichloride and dimethyl disulphide [20,21]. The ultraviolet absorption spectra of the following compounds provide strong evidence in favour of unbranched chains [22]: A series of organic disulphides, disulphur dichloride, diethyl tetrasulphide, diphenyl tetrasulphide, di(2-benzthiazolyl) tetrasulphide, polyethylene tetrasulphide, and di*cyclo*hexyl hexasulphide. For di(*n*-hexadecyl) trisulphide and tetrasulphide the ultraviolet absorption curves were not interpreted as giving definite results [23].

The X-ray emission spectra of the potassium polythionates are in accordance with unbranched chain formulae [24].

The chemical reactions of a series of polythionic compounds SX_2 show [25,26] that in those compounds a divalent sulphur atom forms a bridge between the thio sulphur atoms of two thio groups X. These considerations apply to the

following compounds, which, therefore, are built up of unbranched sulphur chains:

Monosulphur

di(thiocyanate)	$S(SCN)_2$
bis(dimethylthiophosphate)	$S(SPO(OCH_3)_2)_2$
di(ethanethiosulphonate)	$S(S_2O_2R)_2$
di(*p*-toluenethiosulphonate)	
di(thiosulphate)	$S(S_2O_3)_2^{--}$

The space group [7] of monosulphur di(*p*-toluenethiosulphonate) crystals is in accordance with this structure.

The investigations referred to above are thus unequivocally in favour of unbranched sulphur chains. Physico-chemical evidence reported in support of branched structures, such as the dipole moment of disulphur dichloride [27] and the parachor [28] and viscosity [29] of diethyl tetrasulphide, seems less conclusive.

The unbranched sulphur chain formulae for the polythionic acids were first proposed by Blomstrand [30] and Mendelejeff [31]. The arguments against these formulae are mostly of chemical nature. Thus, pentathionate and hexathionate in presence of small amounts of basic substances readily liberate sulphur, to give tetrathionate (for literature, see Ref. 26). So do organic polysulphides, to give disulphides [32–34]. Tetrathionate, pentathionate and hexathionate with sulphite give trithionate and thiosulphate; organic polysulphides [34–36] give disulphide and thiosulphate. The argument is [32,33,37–43] that the sulphur atoms which are thus easily removable, are bonded differently from the others, and that they therefore cannot be part of unbranched chains. The validity of this view, in the case of organic polysulphides, has recently been questioned by Farmer and Shipley [34] and Bloomfield [36], on the ground that diethyl tetrasulphide, as prepared from ethylthiol and disulphur dichloride, should have an unbranched structure, and yet it gives off two sulphur atoms to sulphite.

In view of recent work [25,44] on the chemistry of polythionic compounds the reactions with sulphite are, in fact, consistent with unbranched sulphur chain structures.

Thus, it was predicted some time ago [25], on the basis of the general reactivity of tetrathionate, pentathionate and hexathionate, that the reactions with sulphite are ionic displacements of thiosulphate by sulphite, as follows:

$$^{-}O_3S-S-S-S-S-SO_3^{-} + \overset{*}{S}O_3^{--} = {}^{-}O_3S-S-S-S-\overset{*}{S}O_3^{-} + S_2O_3^{--} \quad (1)$$

$$^{-}O_3S-S-S-S-SO_3^{-} + \overset{*}{S}O_3^{--} = {}^{-}O_3S-S-S-\overset{*}{S}O_3^{-} + S_2O_3^{--} \quad (2)$$

$$^{-}O_3S-S-S-SO_3^{-} + \overset{*}{S}O_3^{--} = {}^{-}O_3S-S-\overset{*}{S}O_3^{-} + S_2O_3^{--} \quad (3)$$

This theory has recently been confirmed by Christiansen and Drost-Hansen [45] by experiments with sulphite labelled with radioactive sulphur.

At the same time [25], the first steps of the analogous reactions with cyanide were formulated as ionic displacements of thiosulphate by cyanide. *E. g.*, hexathionate is assumed to react with excess cyanide in the following way:

$$^{-}O_3S-S-S-S-S-SO_3^{-} + CN^{-} = {}^{-}O_3S-S-S-S-CN + S_2O_3^{--} \quad (4)$$

$$^{-}O_3S-S-S-S-CN + \overset{*}{C}N^{-} = {}^{-}O_3S-S-S-\overset{*}{C}N + SCN^{-} \quad (5)$$

$$^{-}O_3S-S-S-CN + \overset{*}{C}N^{-} = {}^{-}O_3S-S-\overset{*}{C}N + SCN^{-} \quad (6)$$

$$^{-}O_3S-S-CN + 2\,OH^{-} = SO_4^{--} + SCN^{-} + H_2O \quad (7)$$

The catalytic decompositions of pentathionate and hexathionate into sulphur and tetrathionate are, likewise, compatible with unbranched sulphur chain formulae [25,26].

Now, the reactions of organic polysulphides with sulphite [34,36] and cyanide [46,47] probably take place by analogous ionic displacement mechanisms. It seems reasonable to assume that the first steps are ionic displacements of mercaptide by sulphite or cyanide. Thus, for tetrasulphides and sulphite:

$$RS-S-S-SR + SO_3^{--} = RS-S-S-SO_3^{-} + SR^{-} \quad (8)$$

$$RS-S-S-SO_3^{-} + \overset{*}{S}O_3^{--} = RS-S-\overset{*}{S}O_3^{-} + S_2O_3^{--} \quad (9)$$

$$RS-S-SO_3^{-} + SR^{-} = RS-SR + S_2O_3^{--} \quad (10)$$

The analogous mechanism for tetrasulphides and cyanide is:

$$RS-S-S-SR + CN^{-} = RS-S-S-CN + SR^{-} \quad (11)$$

$$RS-S-S-CN + \overset{*}{C}N^{-} = RS-S-\overset{*}{C}N + SCN^{-} \quad (12)$$

$$RS-S-CN + SR^{-} = RS-SR + SCN^{-} \quad (13)$$

In the last steps, the formed sulphenyl thiosulphate and thiocyanate react with the mercaptide, displaced in the first steps, to give disulphide. The electrophilic properties of the sulphenyl sulphur of sulphenyl thiosulphates and thiocyanates are well known [44,48].

Departures from this general scheme must be expected to occur depending upon the relative amounts of reagents present. Since sulphenyl thiosulphates and thiocyanates

react readily with sulphite and cyanide [44], the following reactions might, in presence of excess of the last ions, take the place of (10) and (13):

$$RS-S-SO_3^- + \overset{*}{S}O_3^{--} = RS-\overset{*}{S}O_3^- + S_2O_3^{--} \quad (14)$$

$$RS-S-CN + \overset{*}{C}N^- = RS-\overset{*}{C}N + SCN^- \quad (15)$$

If unsufficient amounts of sulphite and cyanide are employed, one might have, instead of (9), (10) and (12), (13):

$$RS-S-S-SO_3^- + SR^- = RS-S-SR + S_2O_3^{--} \quad (16)$$

$$RS-S-S-CN + SR^- = RS-S-SR + SCN^- \quad (17)$$

The trisulphides thus formed should react with sulphite and cyanide as follows:

$$RS-S-SR + SO_3^{--} = RS-S-SO_3^- + SR^- \quad (18)$$

$$RS-S-SR + CN^- = RS-S-CN + SR^- \quad (19)$$

with (10) or (14) and (13) or (15) as subsequent steps. According to the experiments reported by Farmer and Shipley [34] and Bloomfield [36], the reactions of tetrasulphides with sulphite in some cases do not proceed to the disulphide stage.

The first steps, (8), (11), (18) and (19), require some comments. Sulphite and cyanide have no effect on disulphides; thus, mercaptides displace the sulphite of sulphenyl sulphites [49], to give disulphides. These are displacements opposite to those of Eqs. (8), (11), (18) and (19). However, the course of the displacements, if only slightly reversible, evidently depends upon the possibilities for subsequent, more rapid reactions, like (9), (10) and (12), (13) in the present case; for disulphides, there are no such possibilities.

The above considerations apply to dialkyl and diaryl disulphides and polysulphides. Carbonic acid derivatives, such as dixanthyl, di(thiocarbamyl) and diaroyl disulphides, react with cyanide * to give thiocyanate and monosulphides [47, 50]. These reactions involve ionic displacements, as follows [25,51]:

$$(R_2NCS_2)_2 + CN^- = R_2NC(=S)-SCN + R_2NCS_2^- \quad (20)$$

$$R_2NC(=S)-SCN + R_2NCS_2^- = R_2NC(=S)-S-C(=S)NR_2 + SCN^- \quad (21)$$

The corresponding polysulphides, in presence of basic substances like alkalies and thiocarbonyl anions, liberate sulphur to give disulphides, not monosulphides [26, 52, 53]. The reactions of the disulphides with cyanide thus lend support to the hypothesis that corre-

* Preliminary experiments have shown that di(ethylxanthyl) and *bis*(diethylthiocarbamyl) disulphide react also with sulphite, to give one equivalent of thiosulphate.

sponding reactions of polysulphides are displacement reactions, and not base-catalysed liberations of sulphur, which afterwards reacts with cyanide to give thiocyanate.

Thus, the reactions with sulphite and cyanide, of compounds containing unbranched sulphur chains, are explainable on the basis of ionic displacement mechanisms. Arguments to the contrary, *i. e.*, that such compounds should not react readily with sulphite and cyanide, are untenable also from the fact that sulphur itself, which is built up of unbranched rings or chains, does so. This reasoning leads, from the established mechanism of the reactions of tetrathionate, pentathionate and hexathionate with sulphite, to the following theory concerning the mechanism of the reactions of sulphur with sulphite and cyanide.

The first step is the attachment of a sulphite or cyanide group to one end of a sulphur chain. This process might take place as a consequence of an ionic opening of the 8-membered ring of rhombic sulphur, *i. e.*, as an ionic displacement, by sulphite or cyanide, of electronegatively polarized sulphur from its electropositively polarized ring neighbour:

$$S_8 + SO_3^{--} = {}^-S-S-S-S-S-S-S-S-SO_3^- \qquad (22)$$

$$S_8 + CN^- = {}^-S-S-S-S-S-S-S-S-CN \qquad (23)$$

The next steps are successive ionic displacements of thiosulphate by sulphite, and of thiocyanate by cyanide:

$$^-S-S-..-S-S-SO_3^- + \overset{*}{S}O_3^{--} = {}^-S-S-..-S-\overset{*}{S}O_3^- + S_2O_3^{--} \qquad (24)$$

$$^-S-S-..-S-S-CN + \overset{*}{C}N^- = {}^-S-S-..-S-\overset{*}{C}N + SCN^- \qquad (25)$$

The last steps are, thus, the following:

$$^-S-S-SO_3^- + \overset{*}{S}O_3^{--} = S-\overset{*}{S}O_3^{--} + S_2O_3^- \qquad (26)$$

$$^-S-S-\overset{*}{C}N + CN^- = S-\overset{*}{C}N^- + SCN^- \qquad (27)$$

The sulphur chains bearing sulphite or cyanide groups (or thiosulphate or thiocyanate groups) at one end must, of course, be pictured as unstable intermediates only.

Also the reactions of sulphinates and of dialkylphosphites [54] with sulphur, to give thiosulphonates and dialkylthiophosphates, respectively, fit into this scheme.

COORDINATE AND DOUBLE SULPHUR-SULPHUR BONDS

The structural and chemical data thus pointing to the nonexistence of branched sulphur chains, one may inquire why such groupings are, apparently, unstable.

The sulphur-sulphur bond of a 'branch' may be coordinate, $\rangle S \rightarrow S$, or double, $\rangle S{=}S$. In a *coordinate bond*, both electrons of the shared pair are contributed by one of the atoms linked, the donor. The chemical evidence indicates that a sulphur atom acts as an acceptor for the formation of a coordinate bond only when the donor is less electronegative than sulphur. The same applies to selenium as an acceptor; in stable compounds the donor is always the more electropositive element. Consider as donors, the oxygen of ethers, R_2O, sulphur of sulphides, R_2S, nitrogen of amines, R_3N, and phosphorus of phosphines, R_3P. The electronegativity values, after Pauling [55], are: O, 3.5; N, 3.0; S, 2.5; Se, 2.4; P, 2.1. Whereas sulphoxides, R_2SO, selenoxides, R_2SeO, amine oxides, R_3NO, phosphine oxides, R_3PO, sulphides, R_3PS, and selenides, R_3PSe, are stable compounds, amine sulphides, R_3NS, selenides, R_3NSe, and analogous compounds like R_2OS, R_2OSe and R_2SSe are not known. Thus, sulphur and selenium do add to donors only which are less electronegative. In compounds with elements which are more electronegative, sulphur is always linked to (at least) two of these atoms. Examples are monosulphur and disulphur alkoxides, RO—S—OR and RO—S—S—OR, and amides, R_2N—S—NR_2 and R_2N—S—S—NR_2.

Turning to sulphur, the same element, as a donor for sulphur, it seems reasonable to predict, on the basis of the above evidence, that stable coordinate groupings $X_2S \rightarrow S$ are possible only if the atoms or groups, X, are able to reduce the effective electronegativity of the donor sulphur to a sufficient degree. Take the series:

$$\begin{array}{c} Cl-S-Cl \\ CH_3O-S-OCH_3 \\ (CH_3)_2N-S-N(CH_3)_2 \\ CH_3-S-CH_3 \end{array}$$

In the three first compounds the sulphur atom has a partial positive charge, due to the higher electronegativity of the atoms bonded to it, and in dimethyl sulphide it has a partial negative charge, from dipole measurements. Since positive charge increases and negative charge decreases the effective electronegativity of an atom [55], dimethyl sulphide should have the highest tendency to add sulphur to form a coordinate sulphur-sulphur bond. Apparently, it does not do so, and the chance for the other compounds to add sulphur to from such bonds should consequently be very small. Thus, the structure

$Cl_2S{\rightarrow}S$ discussed by Smyth *et al.* [27] to account for the dipole moment of disulphur dichloride, is improbable on this basis.

Next, consider the polythionic compounds:

$$NCS-S-SCN$$
$$(CH_3O)_2OPS-S-SPO(OCH_3)_2$$
$$C_2H_5SO_2S-S-SO_2SC_2H_5$$
$$p-CH_3C_6H_4SO_2S-S-SO_2SC_6H_4CH_3-p$$
$$^{-}O_3S_2-S-S_2O_3^{-}$$

In reactions with nucleophilic reagents, such as piperidine or thiocarbonyl anions, the middle sulphur atom of these compounds is the electrophilic part of the molecule [25], like the sulphur atom of monosulphur dichloride. This atom has therefore, presumably, a partial positive charge, there being resonance between covalent bonds and ionic bonds like:

$$NCS-\overset{+}{S}\ \overset{-}{S}CN \rightleftharpoons \overset{-}{N}CS\ \overset{++}{S}\ \overset{-}{S}CN \rightleftharpoons \overset{-}{N}CS\ \overset{+}{S}-SCN$$

The effective electronegativity of the middle sulphur atom of the above polythionic compounds is thus higher than that of electroneutral sulphur, and the structure $\rangle S{\rightarrow}S$ is therefore improbable in the next higher polythionic compounds.

The nonexistence of structures $R_2A{\rightarrow}S$ or $R_3A{\rightarrow}S$ where A is more electronegative than sulphur, is understandable from considerations of the nature of the coordinate bond. Such a bond implies that A impart a negative charge to sulphur and, itself, acquires a positive charge. The energy required to produce this dipole must oppose the energy of the bonds (electrostatic plus single covalent) thereby made possible, and, unless the first-named amount of energy is substantially lowered through a favourable electronegativity difference between sulphur and the donor, it is apt to reduce the strength of the resultant bond considerably. Even if the acceptor is more electronegative than the donor, as in amine oxides, the N—O bond is nearly of the length to be expected for a normal single covalent bond between the two elements, and is accordingly supposed to have nearly the same strength [56]. That is, there is no resultant electrostatic strengthening of the bond. Therefore, when the participants in a coordinate bond have the same electronegativity, it is not surprising that the bond is too weak for a stable compound to exist.

The analogous statement, that the stability of $\rangle S{\rightarrow}O$ bonds, as contrasted with $\rangle S{\rightarrow}S$ bonds, is attributable to the higher electronegativity of oxygen as compared with sulphur, has recently been made by Vogel-Högler [57] and Eucken and Wagner [58].

The argument concerning the necessity for the donor to be less electronegative than the acceptor is apparently not valid if the acceptor is the positive end of a dipole, like boron in the boron halides, especially if the donor, at the same time, is the negative end of a dipole. The elements of the sulphur group are unique in the sense that they, as atoms, are electroneutral and nonpolar, univalent acceptors.

As pointed out above, dimethyl sulphide should be more susceptible to add sulphur than the other compounds discussed, because the sulphide sulphur has a partial negative charge and therefore a smaller electronegativity than the sulphur of the remaining compounds. The same considerations apply to diaryl sulphides, and, furthermore, to the disulphides and polysulphides, since the organic groups may, likewise, induce a negative charge on one or more of the sulphur atoms of these compounds. Correspondingly, dialkyl and diaryl selenides should be the selenium compounds most susceptible to add selenium, since the selenium of the selenides is the negative end of the dipole, like the sulphur of the sulphides. It is perhaps significant that the only sulphur or selenium compounds reported, with two isomeric forms one of which may possibly have the structure $\rangle S\rightarrow S$ or $\rangle Se\rightarrow Se$, are di(β-naphtyl) diselenide [59] and diethyl pentasulphide [28].

A structure like $(CH_3)_2S\rightarrow S$, even if relatively stable with respect to dissociation into dimethyl sulphide and atomic sulphur, may still be unstable with respect to change into dimethyl sulphide and higher sulphur units such as S_2 and S_8 molecules. A slight dissociation into atomic sulphur:

$$(CH_3)_2S\rightarrow S \rightleftharpoons (CH_3)_2S + S$$

is apt to lead to complete breakdown of the disulphur compound, because of the rapid process $8\ S \rightarrow S_8$. The same argument applies to corresponding double bond structures.

A *double bond* $\rangle A{=}S$ where A is sulphur, requires an expanded valency shell, beyond the octet, of A.

There are indications [60, 61] that the divalent sulphur of thioacetals is able to expand its valency shell for hyperconjugation with the carbon already bonded to the sulphur atoms. In oxygen compounds, like thionyl chloride, sulphoxides, sulphones, and sulphur-oxy ions, sulphur does seem to form partial double bonds and thus to exceed the octet, judging from bond lengths [55, 62], dipole moments [56], and ultraviolet and infrared absorption spectra [63, 64]. Also, fluorine enables sulphur to exceed the octet, as in sulphur hexafluoride.

Sulphite and sulphinate ions, which both are derivatives of tetravalent sulphur, are apparently the only compounds capable of adding sulphur to give stable sulphur-sulphur compounds, where one of the sulphur atoms forms only one bond. It is a consequence of the hypothesis concerning the nonexistence of coordinate bonds $\rangle S\rightarrow S$ and $\rangle S\rightarrow S$ that the sulphur-sulphur bonds of thiosulphate and thiosulphonates are double bonds. That is so because the sulphite and sulphinate sulphur has, presumably, a positive charge, therefore a higher electronegativity than electroneutral sulphur, and thus cannot act as a donor for a coordinate bond with sulphur.

The Raman spectrum of thiosulphate contains no lines corresponding to a double sulphur-sulphur bond of normal strength [58]. However, if the sulphite sulphur really has a positive charge, there seems to be no other way in which to correlate the existence of thiosulphate with the nonexistence of branched sulphur chains, than to postulate double sulphur-sulphur bonds in thiosulphate.

Thus, the evidence indicates that sulphur expands its valency shell, for bonding to additional atoms, only when already bonded to strongly electronegative elements like oxygen and fluorine, or when the new bond is formed by such atoms. According to this view, the divalent sulphur compounds most susceptible to add sulphur to form double bonds $\rangle S{=}S$ should be monosulphur difluoride and dichloride, and monosulphur dialkoxides.

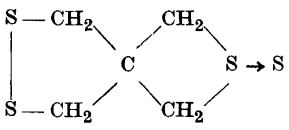
SUMMARY

The available physical and chemical evidence points to the nonexistence of branched structures $\rangle S{\rightarrow}S$ or $\rangle S{=}S$ in disulphides, polysulphides and polythionic compounds. The chemical reactivity of these compounds is consistent with formulae containing unbranched sulphur chains.

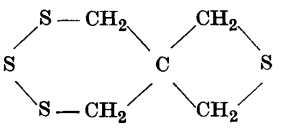
The nonexistence of branched structures is discussed in terms of the properties of coordinate and double sulphur-sulphur bonds.

A mechanism is suggested for the reactions of sulphur and of polysulphides with sulphite and cyanide.

Note added in proof: According to Backer and Evenhuis [65], tetrakis(bromomethyl) methane reacts with alkali disulphides and tetrasulphides to give organic sulphides, which are formulated by the authors as containing sulphur-sulphur bonds of the branched type. The proposed structure has recently been accepted by Challenger and Greenwood [66]. It may be noted, though, that the chemical evidence reported by Backer and Evenhuis in support of a branched structure, such as (I):



(I)



(II)

does equally well fit with the unbranched structure (II) not discussed by Backer and Evenhuis.

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Received December 13, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 416–421

Spectral and Oxidation Studies on Matricaria Ester (*n*-Decadiene-2,8-Diyne-4,6-oic Acid Methyl Ester)

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In 1941 Sørensen and Stene [1] reported the isolation of a highly unsaturated ester from the flowers of *Matricaria inodora L.* They found that the ester conformed to the empirical formula $C_{11}H_{10}O_2$, and that upon hydrogenation, it absorbed 6 moles of hydrogen to form methyl decanoate. In consequence of the identity of degradation products, the high molecular exaltation of the ester, and by analogy with the ester isolated from *Lachnophyllum gossypinum* by Wiljams, Smirnow and Goljmow [2], the matricaria ester was tentatively assigned the formula $CH_3\,CH = CH - C \equiv C - C \equiv C - CH = CHCOOCH_3$.

At the time the initial work was done, facilities for determination of ultraviolet absorption spectra were not available. It was believed that a study of the absorption characteristics of this highly unsaturated ester and its acid should be of help in elucidating its structure. It was with that end in mind that the present study was made. While the preparations were available additional studies were made concerning the oxidation of this highly unsaturated ester.

EXPERIMENTAL

The spectral measurements were made on freshly prepared samples shipped in vacuum ampules packed in solid carbon dioxide from the laboratory in Trondheim to Stockholm. Less than 36 hours elapsed between preparation and spectral examination with the Beckman spectrophotometer. All samples were recrystallized from pentane immediately before use. Oxidation studies were made upon 100 millimole samples under air at 37° in the Warburg apparatus, and samples were removed for spectral measurements at appropriate intervals of oxygen uptake. The procedure used has been described previously [3].

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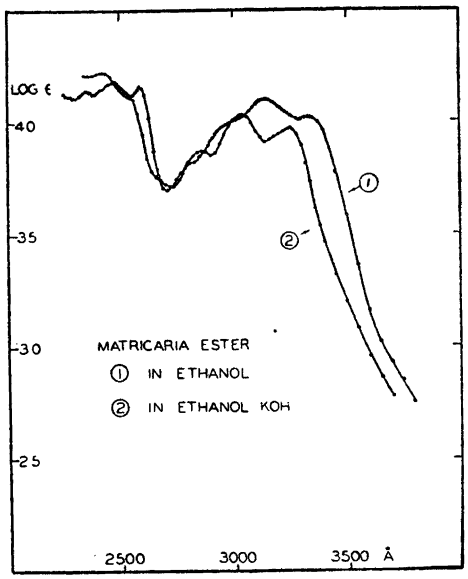

Fig. 1. Ultraviolet absorption spectra of matricaria ester. (1) in ethanol, (2) in alkaline ethanol.

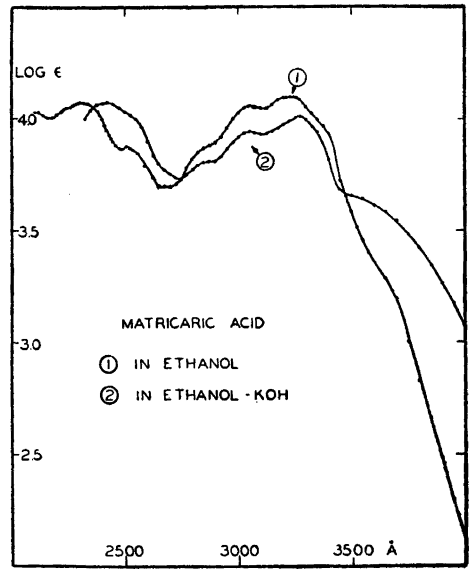

Fig. 2. Ultraviolet absorption spectra of matricaric acid. (1) in ethanol, (2) in alkaline ethanol.

RESULTS AND DISCUSSION

The ultraviolet absorption spectra of matricaria ester and related compounds exhibit very interesting and unusual properties. The spectra of matricaria ester in ethanol and alkaline ethanol, shown in Figure 1, have two families of absorption maxima. In alkaline solution these maxima lie at shorter wavelengths (see Table 1). The spectral shift as a consequence of alkaline treatment is more pronounced in the case of matricaric acid, whose spectra are shown in Fig. 2. A similar study was made with 3,8-dimethyldecadiene-2,8-diyne-4,6 (Fig. 3) which was prepared by C. M. Haug for comparison with matricaria ester. The spectra of this substance in ethanol and alkaline ethanol are almost identical, indicating that the shifts in spectra of the matricaria ester and acid are associated with the effect of alkali upon the ester and acid groups rather than upon the conjugated diene-diyne system.

The spectra of matricaria ester and 3,8-dimethyldecadiene-2,8-diyne-4,6 in non-polar solvents are shown in Fig. 4. Although the absorption maxima of the latter compound occur at lower wavelengths (see Table 1), the spectra show considerable similarity. Theoretical considerations, to be published separately, have led Sørensen to conclude that the two compounds have the

Table 1. Absorption maxima of matricaria ester, matricaric acid and 3,8-dimethyldecadiene-2,8-diyne-4,6.

Substance	*In ethanol*		*In ethanol-KOH*		*In hydrocarbon*	
	λ	ε	λ	ε	λ	ε
Matricaria ester	3330 Å	10 330	3250 Å	9 376	3360 Å	12 900
	3145	12 550	3040	8 147	3140	15 540
	2970 ~	9 680	2860	7 550	3010 ~	12 490
	2830 ~	6 730				
	2590	14 300			2590	23 060
	2470	15 230	2420	16 900	2460	25 380
	2340	13 770			2320	22 200
Matricaric acid	3600 ~	2 259	3600 ~	4 150		
	3400 ~	8 690				
	3250	12 560	3275	10 120		
	3050	11 400	3050	8 730		
	2875 ~	7 551	2875 ~	6 383		
	2500	7 780	2550 ~	9 728		
	2310	11 670	2425	11 770		
3,8-Dimethyldeca-	3070	10 590	3080	11 040	3085	11 120
diene-2,8-diyne-4,6	2890	13 370	2890	14 060	2900	14 450
	2730	9 530	2730	10 070	2740	10 220
	2590	5 530	2590	5 850	2600	5 875
	2310	26 310			2310	29 380
					2280	29 040
					2220	27 100

$\varepsilon = (\log_{10} I_0/I)/\text{cl}$, when c = mole per liter and l = 1 cm.
~ denotes inflection point.

same conjugated structure, $-CH=CH-C\equiv C-C\equiv C-CH=CH-$, and that the tentative formula is correct.

The autoxidation of matricaria ester was studied, using freshly recrystallized ester. The oxygen uptake, shown in Fig. 5, indicates that the reaction follows a diphasic course. The first phase, probably involving the addition a mole of oxygen, proceeded at 0.09 moles oxygen per mole ester per hour. The second phase continued at the rate of 0.024 M/M/hr until nearly one mole of oxygen was absorbed. The maximum rate of oxygen uptake of a nearly pure sample of methyl docosahexaenoate supplied by Dr. D. A. Sutton was found to be 0.12 M/M/hr. This ester contains the

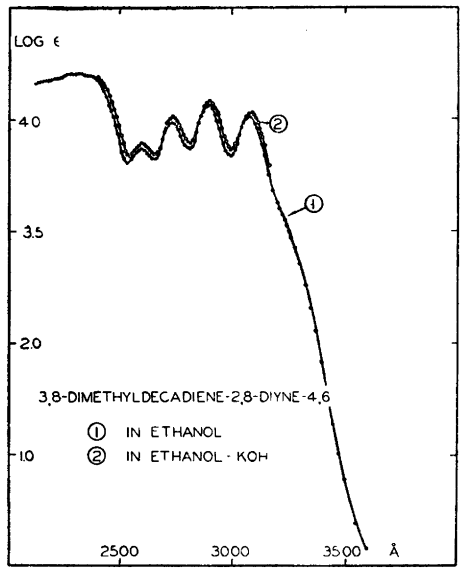

Fig. 3. Ultraviolet absorption spectra of 3,8-dimethyldecadiene-2,8-diyne-4,6. (1) in ethanol, (2) in alkaline ethanol.

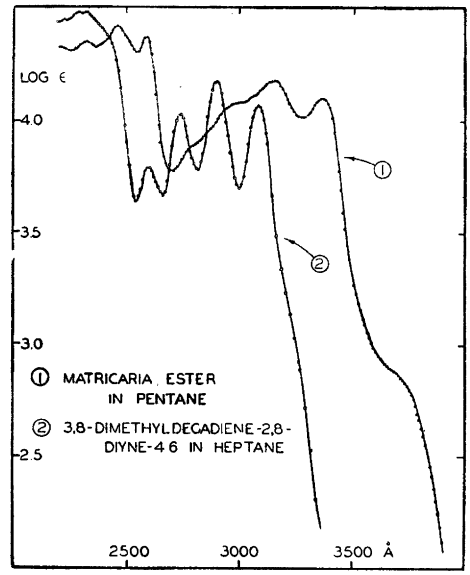

Fig. 4. Ultraviolet absorption spectra. (1) matricaria ester in pentane, (2) 3,8-dimethyl decadiene-2,8-diyne-4,6 in heptane.

same total unsaturation as does matricaria ester, but in the form of six isolated double bonds. The matricaria ester oxidizes at a rate comparable to that of linoleic acid [4].

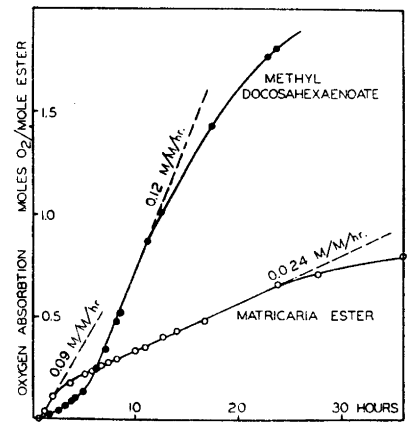

Fig. 5. The course of the oxidation of matricaria ester and methyldocosahexaenoate.

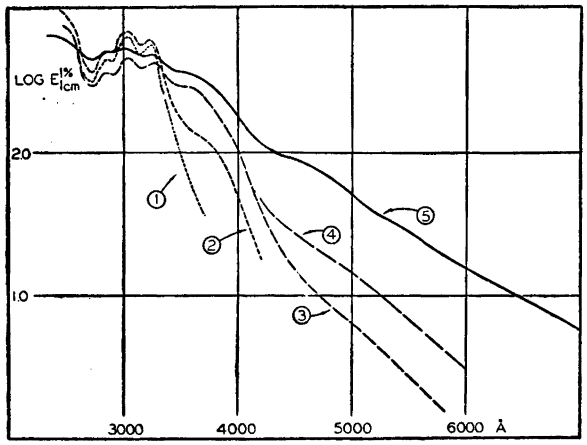

Fig. 6. Ultraviolet spectra of oxidized matricaria ester in alkaline ethanol. (1) fresh ester, (2) 0.08 M/M, (3) 0.1 M/M, (4) 0.26 M/M, (5) 0.80 M/M.

As the oxidation of the ester proceeds, the color of the sample progressively deepens, and the oxidized material loses its solubility in alcohol or ether. For this reason, the changes in the spectrum of the ester could not be followed during the oxidation. The oxidized ester, however, was soluble in alcoholic potassium hydroxide, permitting examination of the oxidized ester's spectrum in alkaline ethanol. Figure 6 shows the spectra of matricaria ester in alkaline solution during the course of the autoxidation. The effect of alkali upon the fresh ester is to move the absorption bands away from the visible, and eventually after some hours, to obliterate the fine structure. However, the spectra of oxidized matricaria ester in alkaline ethanol showed, not so much a depression of the principal bands, but a progressive increase in absorption toward the visible region. In this broad increase in absorption, a definite band is distinguishable at about 3550 Å. This band is the first to develop, the visible absorption increasing later. These two types of absorption, shown in Fig. 7, correspond to the phases of oxygen uptake mentioned previously. It appears that the 3550 Å chromophore is associated with the primary oxidation product.

SUMMARY

1. Ultraviolet absorption spectra of matricaria ester, matricaria acid and 3,8-dimethyldecadiene-2,8-diyne-4,6 are given for polar and non-polar solutions.

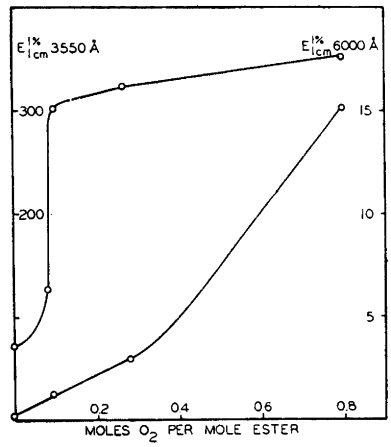

Fig. 7. Chromophore development during course of autoxidation of matricaria ester.

2. The similarity of absorption spectra of matricaria ester and 3,8-dimethyldecadiene-2,8-diyne-4,6 indicates that these contain similar conjugated unsaturated structures and that matricaria ester is *n*-decadiene-2,8-diyne-4,6-oic acid methyl ester.

3. Autoxidation of matricaria ester is extremely rapid and proceeds in two stages. A comparison of the rate of oxygen uptake of matricaria ester and methyldocosahexaenoate is made.

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Received November 9, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 422 – 434

Untersuchungen an künstlichen Peroxydasen

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Theorell und Mitarbeiter haben gezeigt, dass sich die Meerrettich-Peroxydase (Peroxydase II)[1] mit HCl in Aceton in der Kälte in prosthetische Gruppe (Protohämin) und Trägerprotein spalten lässt, sowie das eine erneute Zugabe von Protohämin und anderen Häminen zu einer Rekombination unter Änderung des sichtbaren Spektrums führt[2]. Die dabei entstehenden Verbindungen sollen hier „künstliche Peroxydasen" genannt werden („synthetic peroxidases" nach Theorell[3]).

Theorell und Paul[4] haben den Verlauf der sauren Spaltung der Meerrettich-Peroxydase an Hand der Änderung der Extinktion bei den Wellenlängen 490, 510, 530 und 550 mμ verfolgt und gefunden, dass dieser einer Reaktion I. Ordnung entspricht.

Das Ziel der vorliegenden Arbeit war nun, die Rekombination der freien Proteinkomponente mit verschiedenen Häminen messend zu verfolgen und die dabei gebildeten künstlichen Peroxydasen näher zu charakterisieren.

METHODEN

A. Ausgangsmaterialien

Zur Gewinnung des freien Proteins diente eine aus 200 kg Meerrettichwurzeln nach den Angaben von Theorell[5] gewonnene Peroxydasepräparation: Büschel feiner Nadeln von bis zu 16 μ Länge. Eigenschaften der elektrodialysierten Probe:

P. Z.*: 1020. N-Gehalt: 12,5 ± 0,3 %

$\frac{\varepsilon 402}{\varepsilon 280}$ = R. Z.** = 3,04. Hämingehalt: 1,36 %

Fe-Gehalt: 0.12 %

* P. Z. = "Purpurogallinzahl" [6]

** R. Z. = "Reinheitszahl" = $\frac{\Sigma_{402}}{\Sigma_{280}}$ bei pH 7.0.

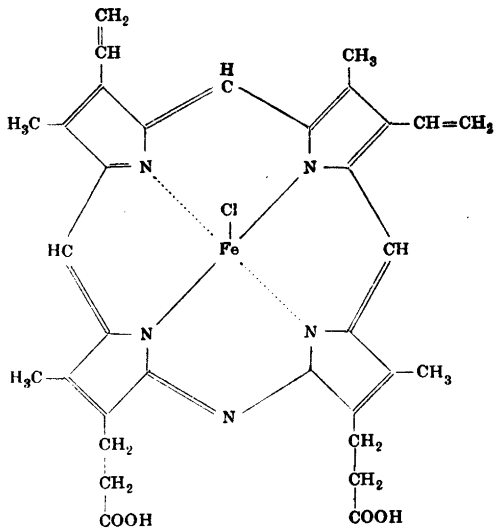

Abb. 1. Azahämin.

Die Spaltung wurde im Prinzip nach Theorell [2] durchgeführt, nur wurden die Bedingungen etwas schärfer gewählt: die dialysierte, etwa 0,5—1,5 %ige Peroxydase-Lösung wurde 2mal je 20 Min. bei 0° mit 0.1 % HCl-Aceton behandelt, jeweils kurz zentrifugiert und in 1%iger $NaHCO_3$-Lösung gelöst. Anschliessend wurde unmittelbar mit 1/15 *M* NaH_2PO_4-Lösung auf pH 7,1 gebracht und die Lösung im Kühlschrank verwahrt. Unter diesen Bedingungen wird zwar ein Teil des freien Proteins inaktiviert (die Aktivität nach erfolgter Rekombinatin liegt in der Regel zwischen 60 und 70 %), dafür aber eine fast quantitative Spaltung erzielt (98—99,8 %). Auf diese Weise kann in der Regel der Einfluss ungespaltener „Proto"-Peroxydase auf die Messungen vernachlässigt werden.

Als künstliche prosthetische Gruppen wurden folgende Hämine verwendet:

1. Protohämin. Aus Rinderblut hergestelltes krystallisiertes Hämin wurde nach einer von Fischer gegebenen Vorschrift [1] 2mal aus Eisessig umkrystallisiert.

2. Deuterohämin. Dasselbe Präparat wie bei Theorell und Mitarb.[1]

3. Mesohämin. Nach Zaleski [8] hergestellt und nach Fischer und Orth [7] krystallisiert.

4. Phäophorbid a-Hämin. Ebenfalls wie bei Theorell und Mitarb.[2]; das Hämin wurde nach Fischer und Bäumler [7] gewonnen und aus Chloroform-Petroläther umkrystallisiert.

5. Chlorin e_6-Hämin. Das Porphyrin wurde nach [3] synthetisiert und Eisen nach Zaleski [8] eingeführt.

6. Azahämin. Eine Probe dieser Substanz wurde uns in dankenswerter Weise von Dr. W. G. Rawlinson zur Verfügung gestellt. Es wurde nach Lemberg und Holden [10] hergestellt.

Zur Analyse wurde 2 Std. bei 30° im Hochvakuum getrocknet.

7,44 mg Substanz gaben 0,682 cm³ N_2 bei 24° und 745 mm Hg.

$C_{33}H_{31}O_4N_5FeCl$ (652.93)	ber.	N 10.73
	gef.	» 10.30

Formel siehe Abb. 1.

B. Herstellung der künstlichen Peroxydasen

Da eine präzise stöchiometrische Reaktion zwischen Protein und Hämin praktisch nicht durchführbar ist kamen 2 Möglichkeiten für die Herstellung der künstlichen Rekombinationsprodukte in Betracht: Überschuss an Protein oder Überschuss an Hämin. Da in der Literatur Angaben über die Empfindlichkeit des freien Proteins gegenüber Dialyse und Erwärmung vorlagen [1,2] wurde zuerst ein Proteinüberschuss verwendet. Die Dialyse vermochte aber nicht die freie Proteinkomponente durch Denaturierung oder auch Desaktivierung völlig zu eliminieren. Da die native Peroxydase sehr wärmebeständig ist [11,12] dachten wir, das „empfindliche" freie Protein durch Erwärmen zu denaturieren. Erstaunlicherweise gelang dies aber keineswegs. Systematische Versuche über die Wärmebeständigkeit von Peroxydase und freiem Protein wurden deshalb nötig, worüber unten berichtet wird.

Der andere Weg hingegen erwies sich als gangbar:

Die auf pH 7 gepufferte Lösung des Proteins wurde mit einem Überschuss der Lösung des betreffendens Hämins in 0,1 *N* NaOH (mit 1/15 *M* NaH_2PO_4 auf pH 7 gebracht) versetzt. Nach einem Tag wurde mit $(NH_4)_2SO_4$ gesättigt, wobei sowohl die gebildete Peroxydase wie auch der Überschuss an Hämin ausfielen. Nach dem Zentrifugieren wurde der Niederschlag in Wasser gelöst und gegen destilliertes Wasser gründlich dialysiert. Zum Dialysieren der sehr kleinen Volumina wurde ein geeignet geformtes, kurzes, beiderseits offenes Rohr an einem Ende mit einer Cellophanmembran verschlossen und in ein Becken mit destilliertem Wasser getaucht. Beim Abpipettieren kann der letzte Tropfen durch leichten Druck gesammelt und aufgesaugt werden. Nach einer Wiederholung der Fällung ging kein Hämin mehr in Lösung, die somit lediglich die künstliche Peroxydase enthielt. Das Spektrum dieser Lösung wurde nun bei pH 7,1 in 0,01 *M* Phosphatpuffer gemessen. Sodann wurde etwas festes KCN zugegeben und das Spektrum erneut ausgemessen. Die spezifische Absorption wurde auf Grund der Annahme gleicher Absorption bei

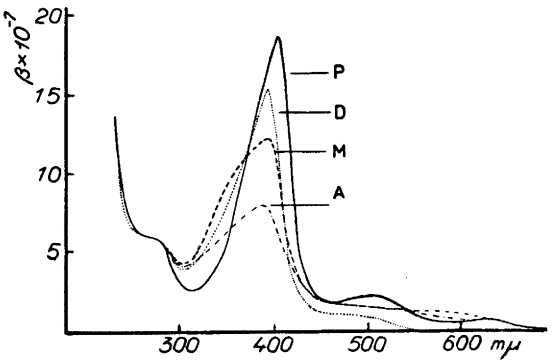

Abb. 2. Absorptionsspektra der künstlich hergestellten Peroxydasen.
P = Protohämin
D = Deuterohämin
M = Mesohämin
A = Azahämin

275 mμ für alle künstlichen Peroxydasen berechnet. Bei dieser Wellenlänge * liegt ein charakteristisches Plateau, wie aus Abb. 2 hervorgeht. Bewiesen ist die Annahme gleicher Absorption bei dieser Wellenlänge nicht, hingegen wird sie durch die relative Grösse der Extinktionszunahme bei der Rekombination gestützt.

C. Die Messung der Rekombination

An Hand der Spektren des reinen Hämins bei pH 7,1 einerseits und der aus demselben Hämin + Peroxydaseprotein entstandenen Verbindung andererseits wurde die Wellenlänge grösster Absorptionsdifferenz bestimmt. Die dabei für 5 Hämine gefundenen Werte sind mit anderen, meist neu bestimmten spektroskopischen Daten in Tabelle 1 zusammengestellt.

Tabelle 1.

Hämin-komponente	Freies Hämin		Künstliche Peroxydase		Peroxydase-CN-Komplex		Wellenlänge max.*** Differenz,
	ε_{max} mμ	$\beta \times 10^{-7}$	ε_{max} mμ	$\beta \times 10^{-7}$	ε_{max} mμ	$\beta \times 10^{-7}$	$\lambda \Delta \varepsilon = max$ mμ
Protohämin	362	10.6	402	18.7 **	423	20.2	406
Deuterohämin	385	11.2	393	15.3	—	—	402
Mesohämin	388	10.0	395	12.7	415	18.0	400
Azahämin	380	10.8	386	11.8	416	11.7	397
Phäophorbid a-Hämin	385	6.7	400	7.6	—	—	412

* Dem Gebiet der Absorptionsmaxima der cyclischen Aminosäuren.

** Nach Theorell [13].

*** Optimale Wellenlänge zur Verfolgung der Rekombination als Funktion der Zeit. Bei dieser Wellenlänge ist die Differenz der Absorption des betreffenden Hämins einerseits und der gebildeten Peroxydase andererseits am grössten.

Die Gültigkeit des Lambert-Beer'schen Gesetzes im praktisch verwendeten Konzentrationsbereich wurde mit Lösungen von Protohämin und Phäophorbid a-Hämin bekannter Konzentration geprüft und bestätigt.

Zur zeitlichen Verfolgung des Rekombinationsvorganges wurde das Spektrophotometer (Beckmann, Modell DU) auf die Wellenlänge mit $\lambda_{\Delta\varepsilon=\max}$ eingestellt und zwei Küvetten mit einem bestimmten Volumen der gleichen Häminlösung bekannter Konzentration und von pH 7,1 gefüllt *. Die eine der Küvetten wurde mit einem bestimmten Volumen Wasser versetzt, gemischt und als Blankwert eingestellt. Zur zweiten Kuvette wurde dasselbe Volumen einer auf pH 7,1 gepufferten Proteinlösung bekannter Konzentration gegeben, umgerührt, die Stoppuhr in Gang gesetzt und die Zunahme der Extinktion gemessen.

Die Messung der Aktivität der künstlichen Peroxydasen geschah mit Hilfe der Willstätter'schen Purpurogallinprobe [14] in der von Keilin und Mann [6] modifizierten Form. Die von zahlreichen Autoren, zuletzt Ettori[15], beobachtete Unzuverlässigkeit dieser Aktivitätsbestimmung wurde in hohem Grade von der Qualität des destillierten Wassers abhängig gefunden. Bei Verwendung von glasredestilliertem Wasser wurden die Resultate bedeutend reproduzierbarer. Bei der unten zu besprechenden Messung der Wärmedenaturierung wird nochmals hierauf eingegangen werden.

Prinzipiell wurde zur Bestimmung der Aktivität eines mit dem freien Protein zu kombinierenden Hämins folgendermassen verfahren: Die Restaktivität des freien Proteins (beruhend auf ungespaltenen Anteilen des ursprünglichen Enzyms) wurde gemessen. Nun wurden 2 gleiche Proben der Proteinlösung (beide von pH 7,1) mit einem Überschuss der in 0,1 *N* NaOH gelösten und mit Phosphat auf pH 7,1 gebrachten Lösung von Protohämin einerseits und dem zu untersuchenden Hämin andererseits versetzt. Nach 1 Tag Stehenlassen bei Zimmertemperatur wurden die Aktivitäten der beiden Lösungen gemessen.

RESULTATE

1. Aktivität

Für die peroxydatische Aktivität nach erfolgter Rekombination mit dem freien Protein wurden die folgenden Werte gefunden.

Die Abweichungen von den früher von Theorell [2] für Deutero- und Mesoperoxydase veröffentlichen Werten sind relativ gering. Die Aktivität des Aza-

* Diese Häminlösungen sind nur etwa 1 Tag bei 0° haltbar.

	Gemessen	Nach Theorell u. Mitarb.[2]
Deutero-Peroxydase	56 ± 2 %	62 %
Meso-Peroxydase	63 ± 2 »	53 »
Aza-Peroxydase	20 ± 2 »	—
Chlorin e_6-Peroxydase	0 %	0 »
Phäophorbid a-Peroxydase	0 »	0 »

hämins ist hier zum ersten Mal gemessen worden. Ob eine Verbindung „Phäophorbid a-Peroxydase" realiter existiert ist zweifelhaft, wie unten gezeigt wird.

2. Spektren

Aus den Spektren der Hämine und der zugehörigen Peroxydasen lassen sich folgende Tatsachen ablesen: Die Soretbande wird durch die Kupplung des Hämins mit dem freien Protein um 6 (Azahämin) bis 40 mμ (Protohämin) nach Rot verschoben. Gleichzeitig nimmt die spezifische Absorption am Gipfel der Bande um 9 (Azahämin) bis 77 % (Protohämin) zu. Die CN-Verbindungen (soweit gemessen) weisen ein um etwa 20 mμ nach Rot verschobenes Maximum auf und eine etwas erhöhte spez. Absorption. Die Spektren der künstlichen Peroxydasen ähneln denjenigen von natürlicher Meerrettich-Peroxydase (Abb. 2). Sie zeigen alle die typische Soretbande, wenn auch nie mit gleicher Intensität wie bei der Meerrettich-Peroxydase selbst. Typisch ist weiterhin das Minimum bei 300—320 mμ, sowie der oben erwähnte „Sattel" zwischen 260 und 280 mμ.

3. Rekombination

Der Verlauf der Rekombination wurde zunächst am Beispiel des in natürlicher Peroxydase eingebauten Protohämins studiert. Die Wellenlänge grösster Absorptionszunahme $\lambda_{\Delta\varepsilon=\max}$ liegt bei 406 mμ. Aus praktischen Gründen (Berechnung der Endkonzentration an Peroxydase u. a.) wurde meist bei 402 mμ gemessen. Die Kurven zeigen alle das für eine Reaktion I. Ordnung charakteristische Bild, wie aus Abb. 3 hervorgeht. In diesem typischen Beispiel wurde unter den folgenden Bedingungen gearbeitet: [Protein] = 7,6 μMol/l; [Protohämin] = 15 μMol/l; Temperatur 25°; λ = 402 mμ. Resultate: $\Delta\varepsilon$ = 0,306; Halbwertszeit der Rekombination 4,2 Minuten.

In weiteren Versuchen wurden die Konzentrationen des Proteins und des Hämins geändert. Die Protohämin-Konzentrationen lagen zwischen 3,2—22 μMol/l, diejenigen von Protein zwischen 7,6 und 20 μMol/l, und das Verhältnis

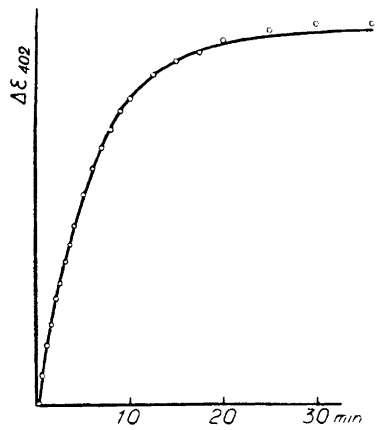

Abb. 3. Protohämin + Protein. t = 24°. HWZ = 4,2 Min.

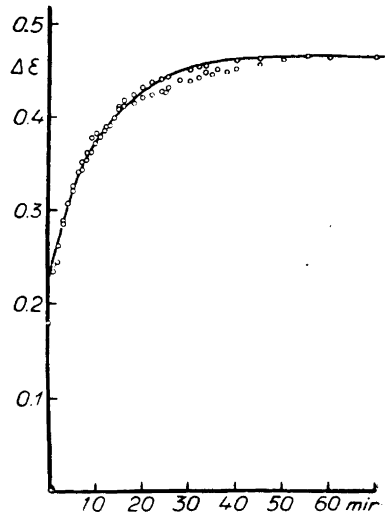

Abb. 4. Deuterohämin + Protein. t = 24°. HWZ = 8 Min.

$\frac{[\text{Hämin}]}{[\text{Protein}]}$ war = 0,16—2,8. Die Temperatur lag stets zwischen 20 und 26°. Die Resultate aller Versuche waren qualitativ die gleichen. Dagegen zeigte es sich, dass die Geschwindigkeit der Rekombination von Fall zu Fall sehr verschieden ist. Die Halbwertszeit schwankte in so weiten Grenzen wie 1,1—7,0 Minuten.

Rekombination des Proteins mit Mesohämin gab ebenfalls Kurven I. Ordnung mit Halbwertszeiten zwischen 2,4 und 8,5 Minuten. Deuterohämin gab eine Zeit für 50 % Rekombination von 3 bis 8 Minuten. Bei der Kombination mit Deuterohämin zeigte sich eine merkwürdige Erscheinung: Ungefähr ½ des erreichten Endwertes maximaler Absorptionszunahme wurde augenblicklich nach Zugabe des Hämins erreicht, jedenfalls vor der ersten Messung (30 Sekunden nach der Zugabe); der Rest der Absorptionszunahme erfolgte gemäss der normalen I. Ordnung-Reaktion, vgl. Abb. 4. Die Ursache der ersten, schnellen Reaktion soll in einer späteren Arbeit quantitativ untersucht werden.

Die Rekombination mit Azahämin wurde nur wenige Male ausgeführt. Die Extinktions-Zeit-Kurve eines typischen Experiments zeigt wieder den Verlauf einer Reaktion I. Ordnung mit einer Halbwertszeit von 2,6 Minuten.

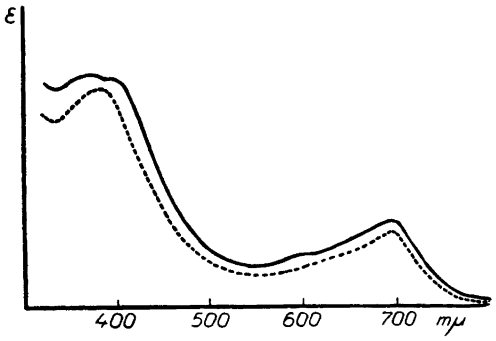

Abb. 5. Absorptionsspektra von
—— Phäophorbid a-Hämin + Protein
--- Phäophorbid a-Hämin allein

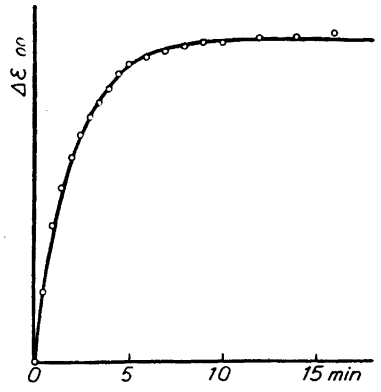

Abb. 6. Verlauf der Kombination zwischen Phäophorbid a-Hämin und Protein. t = 24°. HWZ = 1,2 Min.

4. Der Sonderfall des Phäophorbid a-Hämins

Theorell, Bergström und Åkeson[2] hatten schon festgestellt, dass der sichtbare Teil des Spektrums der meisten von ihnen untersuchten Hämine sich bei Zugabe von freiem Peroxydase-Protein verändert. Sie fanden im Prinzip 3 Gruppen von Häminen:

1. Solche, die mit dem freien Protein reagieren unter Farbänderung und Auftreten von Aktivität im Purpurogallintest. Hierher gehören: Protohämin, Deuterohämin und Mesohämin. Neu hinzugekommen ist nun auch Azahämin.

2. Solche, die mit dem freien Protein wohl Farbänderung, aber keine Aktivität geben, wie Chlorin e_6-Hämin, Phyllohämin, Pyrrohämin und Rhodohämin.

3. Solche, die weder Farbänderung noch Aktivität geben, nämlich Hämatohämin und Phäophorbid a-Hämin.

Es war nun von Interesse zu untersuchen, wie sich die Hämine der 3. Gruppe quantitativ gegenüber dem freien Protein verhalten. Die Reaktion des Hämatohämins soll in einem anderen Zusammenhang beschrieben werden. Das Phäophorbid a-Hämin wurde hier näher untersucht und zeigte folgendes Verhalten:

Wie Abb. 5 zeigt, verändert sich das Spektrum von Phäophorbid a-Hämin nur wenig durch die Reaktion mit der freien Proteinkomponente der Meerrettich-Peroxydase. Dass der Unterschied aber reel ist, zeigt die Abb. 6. Die Kurve zeigt den zeitlichen Verlauf der Rekombination, der die bekannte

Form der Reaktion I. Ordnung zeigt. Offenbar hat also eine Umsetzung stattgefunden.

Wie fest die Bindung zwischen Protein und Phäophorbid a-Hämin ist, wurde nun an Hand von Konkurrenzversuchen festgestellt. Dazu wurde folgendermassen verfahren: Eine Lösung des freien Proteins wurde zuerst mit einem leichten Überschuss an Protohämin versetzt und die Aktivität gemessen. Dies gab ein Mass für die Konzentration an aktivem Protein. Nun wurden 3 Proben der gleichen Proteinlösung abgemessen. Probe 1 wurde mit 0,8 Mol, Probe 2 mit 1,6 Mol Phäophorbid a-Hämin versetzt, Probe 3 unverändert gelassen. Nach 20 Stunden wurde die Aktivität gemessen, die, wie erwartet 0 % war. Die Proben 1—3 wurden in je 4 Teile geteilt und mit 0, 0,8, 1,6 und 5 Mol Protohämin versetzt. Die Aktivitätsmessung nach 24 Stunden gab innerhalb der Fehlergrenzen volle Rekombination. Vgl. Abb. 7.

Das Protohämin verband sich also mit dem Protein, als ob das Phäophorbid a-Hämin nicht vorhanden gewesen wäre.

DIE THERMOSTABILITÄT DER FREIEN PROTEINKOMPONENTE

Wie oben erwähnt, wurde versucht, einen Überschuss an freiem Protein durch Erwärmen zu desaktivieren oder denaturieren. Als sich zeigte, dass dies nicht mit der erwarteten Leichtigkeit gelang, wurde eine systematische Untersuchung der Stabilität des freien Proteins gegen Wärme notwendig. Die Versuche wurden gleichzeitig auch mit ungespaltener Peroxydaselösung durchgeführt.

Das verwendete Präparat war eine dialysierte Lösung krystallisierter Meerrettich-Peroxydase von der P. Z. 1020. Die ungespaltene Peroxydase und die freie Proteinkomponente wurden in etwa 0,02 *M* Phosphatpuffer von pH 7,1 gelöst. Die Konzentration betrug für die Peroxydase 0,20 μMol/l und für das Protein 0,17 μMol/l (auf aktives Protein berechnet). Die Proben wurden je 15 Minuten auf Temperaturen zwischen 40 und 100° erwärmt und anschliessend sofort unter fliessendem Wasser abgekühlt. Die Aktivität der Peroxydaselösungen wurde sofort gemessen, während die Proteinlösungen alle mit einem Überschuss der gleichen Protohäminlösung (pH 7,1) versetzt wurden. Die Aktivität dieser Lösungen wurde am folgenden Tage gemessen.

Bei der Ausführung dieser Versuchsreihe stiessen wir zunächst auf sehr grosse, unregelmässige Schwankungen der gemessenen Aktivitäten. Es wurde dann der Einfluss des destillierten Wassers untersucht und gefunden, dass bei Verwendung von aus Glas redestilliertem Wasser sowohl die Hitze-Denaturierung wie auch der Purpurogallintest reproduzierbar verliefen und ein vernünftiges Bild ergaben.

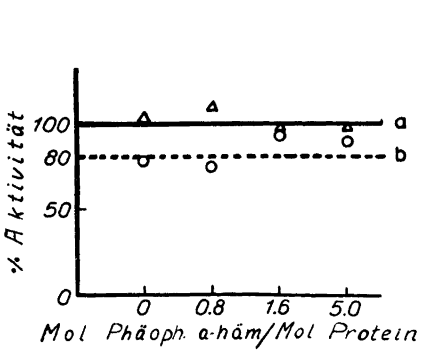

Abb. 7. Peroxydasealtivität nach Zugabe von Protohämin zum inaktiven Phäophorbid a-Hämin — Protein.
a. 1,6 Mol Protohämin △
b. 0,8 Mol » ○

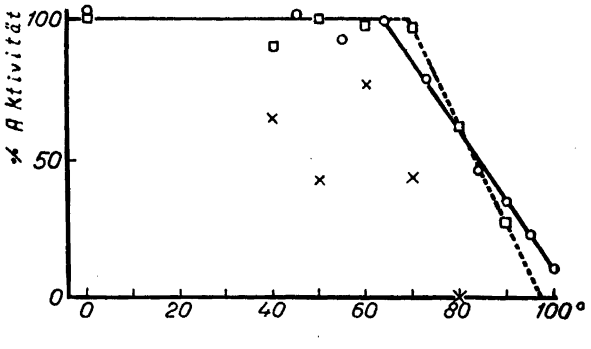

Abb. 8. Einfluss von Wärme auf
○ —— *Peroxydase (glasdest. Wasser)*
□ - - - *freie Protein komponente (glasdest. Wasser)*
× *Werte mit gewöhnlichem dest. Wasser*

Abb. 8 zeigt die erhaltenen Resultate: Die Meerrettich-Peroxydase ist bis 63° gegen 15 Minuten Erwärmung völlig beständig, die Aktivität fällt aber dann bei höherer Temperatur steil ab und erreicht den Nullpunkt bei etwas über 100°. Dies bestätigt die Messungen von Herrlinger und Kiermeier[12] an einem Präparat von der P. Z. 73, die fanden, dass die Aktivität bei Erwärmung auf über 60° schnell absinkt. Die Erscheinung der Aktivitätsregeneration wurde nicht von neuem untersucht, da dies schon ausserhalb des Rahmens dieser Arbeit lag.

Die freie Proteinkomponente zeigte erstaunlicherweise eine etwas höhere Schwelle, die Inaktivierung setzt erst bei 69° ein. Der Abfall ist dann aber steiler und erreicht den Nullpunkt bei etwa 98°. Einige Werte von Bestimmungen mit „gewöhnlichem" destilliertem Wasser sind zum Vergleich mit eingezeichnet.

DISKUSSION

Das Studium des zeitlichen Verlaufes von Rekombinationen von Häminen mit der freien Proteinkomponente von Meerrettich-Peroxydase zeigt, dass es sich dabei um eine im gewöhnlichen Spektrophotometer zu verfolgende Umsetzung handelt, die der Kurvenform nach eine Reaktion I. Ordnung darstellt. Dies steht in Übereinstimmung mit der Beobachtung von Theorell und Paul[4], dass auch die Spaltung der Peroxydase in wässriger HCl eine Reaktion I. Ordnung ist.

Zwei beobachtete Tatsachen bedürfen einer Erklärung: Bei der Rekombination mit Deuterohämin tritt zuerst ein „Sprung" in der Kurve auf, d. h. die erste Hälfte der Extinktionszunahme wurde in einer unter den gewählten Bedingungen unmessbar kurzen Zeit erreicht. Ob daraus geschlossen werden kann, dass sowohl eine ionogene, wie auch eine langsame „organische" Reaktion vor sich geht, wird sich erst nach einem genaueren Studium mit verfeinerten Messmethoden zeigen lassen. Unerklärt ist weiterhin, dass die Halbwertszeiten der Rekombination in weiten Grenzen schwanken, etwa zwischen 1 und 10 Minuten. Diese Schwankungen treten bei ein und demselben Hämin und identischen Proteinpräparationen auf. Sie sind nicht nachweisbar abhängig von der Konzentration der beiden Komponenten oder anderen bekannten messbaren Faktoren. Vorläufig kann für dieses Phänomen keine experimentell belegte Erklärung gegeben werden.

Die Beobachtungen am Phäophorbid a-Hämin führen zu folgenden Schlussfolgerungen: Die Tatsache, dass sich das sichtbare Spektrum des Phäophorbid a-Hämins, im Handspektroskop beobachtet, bei der Zugabe von freiem Protein nicht ändert [2], bedeutet noch nicht, dass keinerlei Reaktion stattfindet. Die Messungen zeigen, dass die Extinktion in einem messbaren Zeitraum gesetzmässig ansteigt. Dass die Bindung aber nicht an der für die aktiven Peroxydasen spezifischen Stelle des Proteins stattfindet, geht aus der glatten Kupplung der so erhaltenen Lösungen mit Protohämin hervor, die auf keinerlei Gleichgewichts- oder Verdrängungsreaktion schliessen lässt. Dass die Kombination Phäophorbid a-Hämin-Protein zu keinem peroxydatisch wirksamen Produkt führt, wurde bestätigt.

Eine Untersuchung der spektralen Änderungen im sichtbaren Spektralbereich bei der Rekombination dürfte in der Zukunft Aufschluss darüber geben, ob sich 2 oder auch mehrere verschiedene Rekombinationsreaktionen optisch voneinander trennen lassen.

Die Feststellung, dass die freie Proteinkomponente eine hohe Resistenz gegen Hitze zeigt, war recht überraschend. Es war früher festgestellt worden [2]: „In contradiction to peroxydase itself, the free protein is sometimes rather unstable. Even precipitation with ammonium sulfate and dialysis may sometimes denature it. However, it is stable for several days at + 4°C, if it is dissolved in bicarbonate after the precipitation with HCl-acetone, without any further treatment."

Die Resistenz gegen „Dialyse und Ammoniumsulfatfällung" scheint von der Qualität des Wassers abzuhängen, da bei Verwendung von reinem, glasredestilliertem Wasser eine Denaturierung nicht beobachtet wurde.

In welcher Weise „schlechtes" dest. Wasser auf die Erwärmungsversuche und den Purpurogallintest einwirkt, ist schwer zu sagen. Immerhin geht man

wohl nicht in der Vermutung fehl, dass Spurenmetalle die Reaktionen stören. Untersuchung des Destillationsrückstandes (50 cm^3) von 10 Liter gewöhnlichem dest. Wasser gab keine sichere positive Reaktion auf Cu und Zn (Na-diäthyldithiocarbaminat) und ebensowenig eine Fällung mit S'', NO_3' und Cl'. Bei der hohen Empfindlichkeit von Hämoproteiden gegen Metallionen ist aber eine Störung durch Mengen an Metallen möglich, die noch unter der Empfindlichkeitschwelle der durchgeführten Analysen liegen. Die Thermoresistenz des freien Proteins ist in aus reinem redestillierten Wasser bereiteten Pufferlösungen nach unseren Untersuchungen eine bedeutend höhere als auf Grund obigen Zitates vermutet worden war.

ZUSAMMENFASSUNG

Meerrettich-Peroxydase wurde mit HCl-Aceton in Hämin und freies Protein gespalten. Hierauf wurde mit verschiedenen synthetischen Häminen gekuppelt, wobei sich teils aktive, teils inaktive „künstliche Peroxydasen" bildeten. Die Kupplungsreaktion wurde spektrophotometrisch verfolgt; es wurde dabei gefunden, dass es sich um eine zeitlich messbare Reaktion, sehr wahrscheinlich I. Ordnung, handelt.

Verschiedene künstliche Peroxydasen wurden präparativ in genügenden Mengen hergestellt, um das Spektrum messen zu können. Folgende Aktivitäten wurden für die rekombinierten Peroxydasen gefunden:

Proto-Peroxydase	100 %	Aza-Peroxydase	20 %
Deutero-Peroxydase	56 »	Phäophorbid a-Peroxydase	0 %
Meso-Peroxydase	63 »	Chlorin e_6-Peroxydase	0 %

Mit Phäophorbid a-Hämin und Protohämin wurden Konkurrenzversuche angestellt, die zeigten, dass eine Umsetzung des freien Proteins mit Phäophorbid a-Hämin die Kupplung mit Protohämin in keiner Weise hindert.

Die Thermostabilität von Meerrettich-Peroxydase und der aus ihr gewonnenen Proteinkomponente wurde untersucht und gefunden, dass bei 15 Minuten Erwärmung Peroxydase bis 62°, dass freie Protein bis 69° praktisch voll aktiv bleiben. Die Gegenwart von Verunreinigungen, wahrscheinlich Spurenmetallen, im destillierten Wasser ist von grossem Einfluss auf die Messresultate, sowohl was die Desaktivierung selbst betrifft als auch im Purpurogallintest.

Der eine von uns (A.C.M.) ist der schweizerischen »Stiftung für Stipendien auf dem Gebiete der Chemie» zu grossem Dank verpflichtet, da diese Arbeit nur durch ein 2-jähriges Stipendium von seiten dieser Stiftung möglich wurde. A.C.M. dankt ferner den

zahlreichen Anregungen und Ratschlägen von seiten der Kollegen im hiesigen Institut, vor allem dem 1. Assistenten K. G. Paul.

Die Analyse wurde vom Mikroanalysenlaboratorium der *Medicinsk-kemiska Institution, Universitetet, Uppsala* (Leitung W. Kirsten) ausgeführt.

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Eingegangen am 31. Dezember 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 435–443

Bromination of Methyl Linoleate with N-Bromosuccinimide

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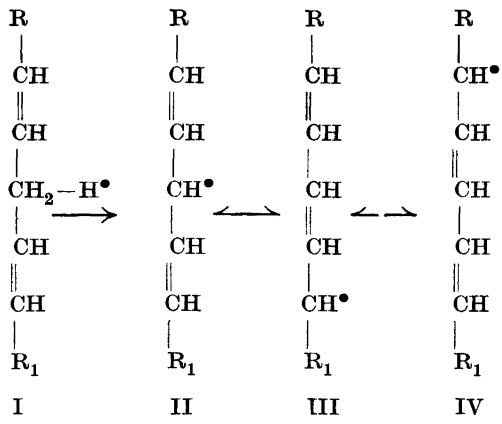
In connection with work on the autoxidation of methyl linoleate we thought it of interest to investigate the action of N-bromosuccinimide on this compound. Preliminary reports of this work have been published [1,2].

Ziegler *et al.*[3] mentioned that methyl oleate easily formed an unstable monobromide when refluxed 40 minutes in carbon tetrachloride with one mole N-bromosuccinimide. Recently Teeter [4] and also Sutton and Dutta [5] have investigated the bromination of some unsaturated fatty acids with this reagent. Their results will be discussed later.

The bromination of unsaturated compounds with N-bromoimides, which has become known as the Wohl-Ziegler reaction, shows several of the characteristics of a free radical reaction [8]. It is catalysed by ultraviolet light [6] and by added peroxides [7], and the point of attack generally is the same as that of other reagents that are believed to react through free radicals, *i. e.* benzenediazonium chloride (Waters *et al.* [8,9,10]; *cf.* also Karrer and Smid [7], Howton [17]). An extensive review has recently been published by Djerassi [11].

If the assumption that this bromination is a free radical reaction is correct, the bromination of methyl linoleate with bromosuccinimide would give rise to an intermediate radical II identical with that postulated to occur in the autoxidation of this type of unsaturated fatty acids according to Farmer's theory of autoxidation [12,13].

This radical is formed by the removal of a hydrogen atom from carbon atom 11 and can be assumed to be a resonance hybrid with the three contributing structures II, III and IV of which two have conjugated double bonds. The reaction of the radical with a bromine atom in N-bromosuccinimide would result in the formation of a mixture of bromides of which a certain proportion would have conjugated double bonds and consequently show the characteristic light absorption in the region of 232 mμ.



I II III IV

However, the allylic system with a bromine atom at carbon atom 11 must be expected to be so labile that a rearrangement leading to the conjugation of the double bonds could occur under the condition of the reaction (*cf.* Kharasch, Margolis and Mayo [20] and Bateman, Cunneen and Koch [14]). Thus the appearance of conjugated double bonds cannot be taken as an indication of a free radical mechanism in the same way as in the case of autoxidation. In the latter case, the hydroperoxides are more stable and less susceptible to this type of rearrangement.

RESULTS

When equimolecular amounts of methyl linoleate and N-bromosuccinimide were refluxed in dry carbon tetrachloride in ultraviolet light, the bromination reaction was complete after approximately ten minutes when practically all

Table 1.

U. V. irrad.	Time of boiling	Molar extinction				Bromine content *		Nitrogen content**
		Before evap.		After evap.		found	calc.	
		232 mμ	269 mμ	232 mμ	269 mμ			
With	10 min.	11.600	1.500	10.800	1.200	20.0—20.1 %	21.4 %	0
With	69 hrs	5.700	26.600	4.800	22.100	6.5— 6.7 »	—	0
Without	94 hrs 15 min.	4.800	25.000	4.400	21.700	7.3— 7.7 »	—	—

* Micro-Carius
** Micro-Kjeldahl

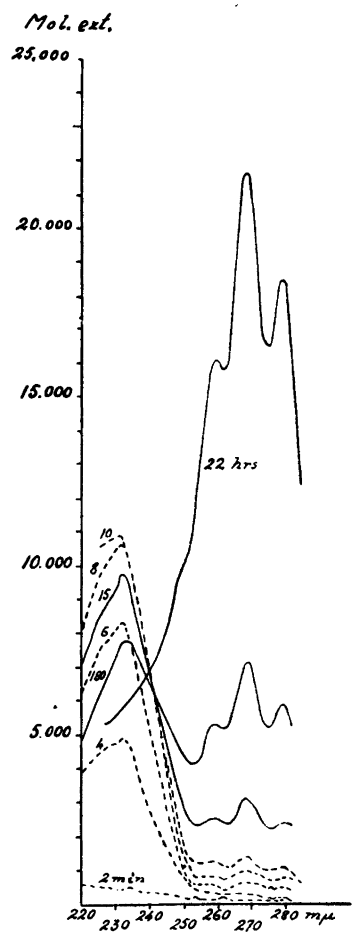


Fig. 1. Absorption spectra of methyl linoleate refluxed in carbon tetrachloride with one mole N-bromosuccinimide during times indicated.

bromine was bound in the fatty acid molecule (see Table 1). The ultraviolet absorption spectra of samples taken out after different times are shown in Fig. 1.

As the bromination proceeded a strong absorption band with a maximum at 232 mμ appeared and increased up to ten minutes when all N-bromosuccinimide had reacted. Three very weak maxima at 259, 269 and 279 were also present. If the reflux was stopped at this time, the spectrum of the reaction mixture stayed unchanged if stored in the cold. The results were similar if the reaction mixture was refluxed in an atmosphere of nitrogen or in air.

If the refluxing was continued, however, the absorption at 232 mμ decreased, whereas the low bands at 259, 269 and 279 increased (Fig. 1). After

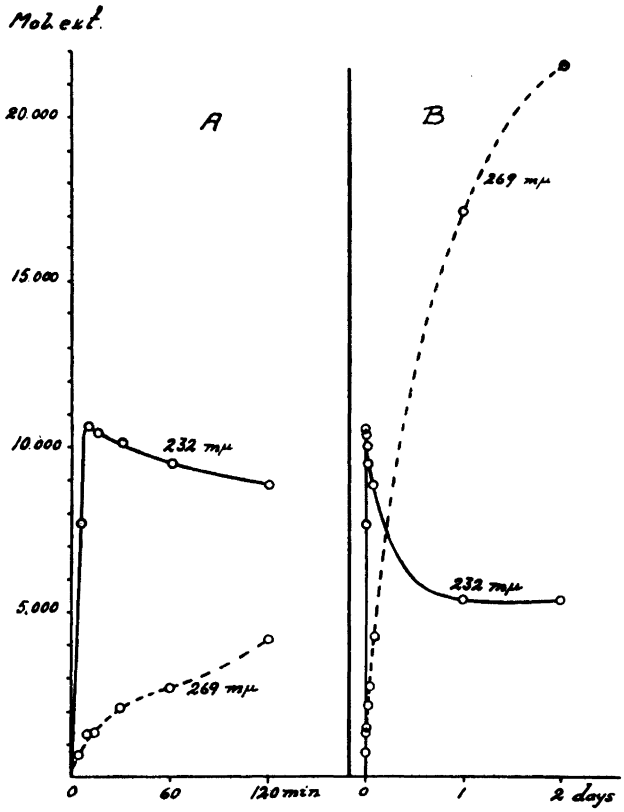


Fig. 2. Change of absorption at 232 mμ and 269 mμ with time of methyl linoleates when refluxed with N-bromosuccinimide. Illumination of the reaction mixture with ultraviolet light during the first fifteen minutes.

22 hours the former band could no longer be distinguished and only the latter absorption with three maxima characteristic for a conjugated triene was found. Simultaneously with these spectral changes during the prolonged reflux the main part of the bromine was progressively split off as hydrogen bromide from the brominated fatty acid. However, about 30 per cent remained even after very prolonged reflux and appears to be in a more stable position. The change of the absorption maxima at 232 and 269 mμ with time is shown in Fig. 2.

When the bromination was carried out under the same conditions as the experiment shown in Figs. 1 and 2 except that the illumination with U. V. light was omitted, the reaction was much slower although the curves followed the same general pattern (Fig. 3). All N-bromosuccinimide had reacted after

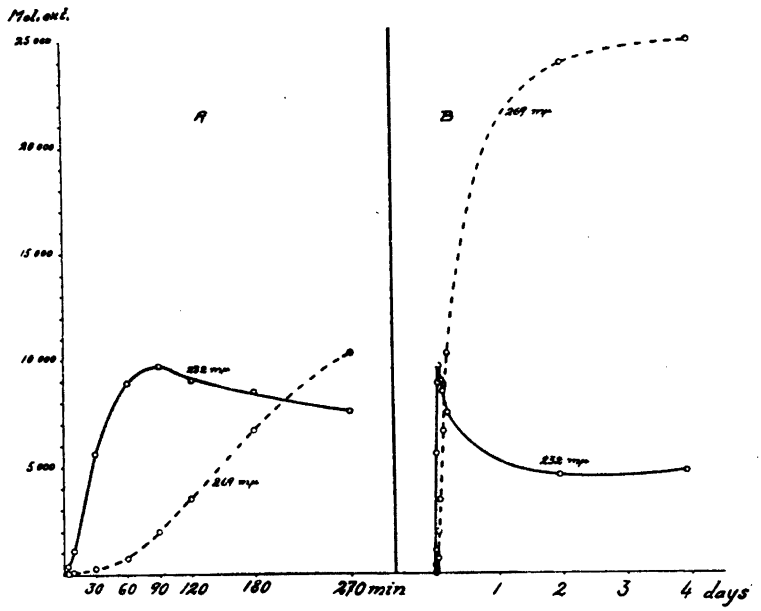


Fig. 3. Identical with fig. 2 except that the ultraviolet illumination was omitted.

about 90 minutes, whereas when the mixture was illuminated this reaction was completed in less than ten minutes.

The appearance of the band at 232 mμ in the initial bromination is very similar to the earlier phases of the autoxidation of methyl linoleate. In order to compare the two reactions, samples of linoleate were treated with different amounts of N-bromosuccinimide in U. V. light and the maximum absorption at 232 mμ recorded when all N-bromosuccinimide had reacted. The results of these experiments are plotted in Fig. 4. The earlier parts of the curve corresponds to a molar absorption of the reacted molecules of about 15, 000 whereas in the autoxidation at 37° generally about 20, 000 has been recorded. As the reaction proceeds, the molar absorption gradually falls off (Fig. 4). If one mole of conjugated diene is formed per molecule that has reacted, an absorption about 30 000 would be expected, as has also been recorded in the quick enzymatic oxidation of sodium linoleate with pure lipoxidase at 0° [15,16]. However, at higher temperatures and with longer reaction times the enzymatic as well as the ordinary autoxidation yields products with lower molar absorptions. This has been ascribed to the fact that only a certain part of the primary peroxide has conjugated double bonds and to the destruction of the newly formed conjugated system by further oxidation.

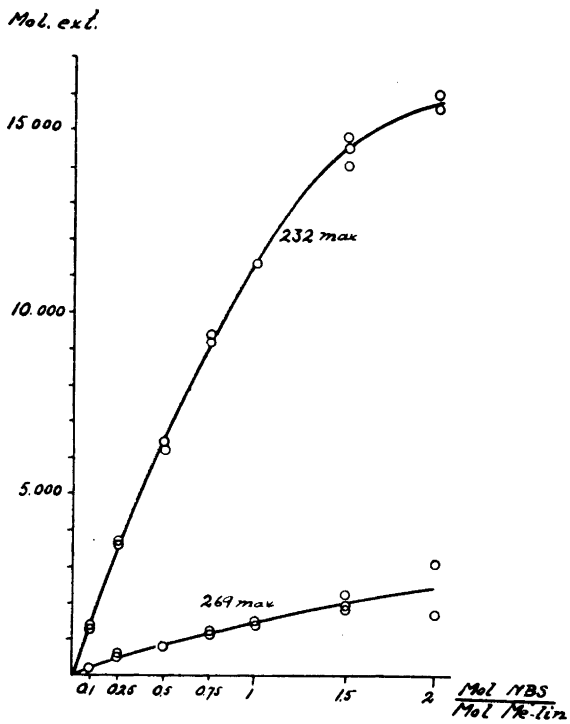


Fig. 4. Maximum absorption at 232 mμ of linoleate brominated with different amounts of N-bromosuccinimide.

In the case of bromination with N-bromosuccinimide it seems improbable that addition of the reagent to conjugated double bonds takes place as the reaction product was practically nitrogen free, as was also found by Teeter [4]. However, assuming that a free radical mechanism operates, the decreasing absorption might have several explanations. The progressively lower molar absorption at 232 mμ with more reagent might to some degree depend on bromination in the α-position to the newly formed conjugated system. Furthermore some 1 : 2 addition of bromine might have taken place as has recently been found by Howton [17] and others [18,19]. Some addition of secondarily formed hydrobromic acid might also take place [20].

Teeter [4] has tested the influence of various solvents and reaction times on the bromination. Dilution with carbon tetrachloride caused a progressively lower halogen transfer with 25 min. reaction time. 3.5 ml carbon tetrachloride with 10 g methyl esters of soy bean fat acids stirred at 70° reacted to 99 per cent after 40 minutes but as the bromine content was only 18.03 instead

of the calculated value of 21.4 %, some dehydrobromination may have taken place.

Teeter does not seem to have made any observation on the spectral properties of the reaction products. However, he reacted the bromides obtained with sodium methoxide and sodium cyanide to yield products which were apparently very complex mixtures.

Sutton and Dutta [5] recently investigated the bromination of a number of pure esters of unsaturated fatty acids with N-bromosuccinimide. They found that the bromide of oleate could be subjected to molecular distillation without decomposition, whereas corresponding products from linoleate and linolenate were less stable and decomposed under these conditions.

In our investigation we have used dilute solutions so that all N-bromosuccinimide was dissolved soon after the carbon tetrachloride began to boil and the succinimide formed also remained in solution until the reaction mixture had cooled to room temperature.

Sutton and Dutta, however, refluxed for example 8.4 g ethyl linoleate with 5.1 g N-bromosuccinimide in 50 ml carbon tetrachloride for 4 ½ hours with frequent shaking. The isolated product had a bromine content of 13.6 % (calc. for a monobromide 20.7 %) and an absorption corresponding to a content of 10.2 % triene.

They consider their results as indication that the bromination does not proceed via a free radical mechanism. In their experiments, reaction mixtures have been used more than 50 times more concentrated than ours. In our experiments a clear solution is obtained within 3—4 minutes after the reflux is started. In Sutton and Dutta's experiments a clear solution is probably never obtained and as they have not recorded the time required until all N-bromosuccinimide had reacted, or followed the change in the U. V. spectrum during the earlier stages of the reaction, it is difficult to compare our result with theirs. The secondary reactions might completely obscure the changes during the primary bromination.

The marked catalytic effect observed in our experiment by ultraviolet light, in accordance with earlier investigations, seems to indicate that the bromination under our conditions is a free radical reaction.

The observed spectral changes in the earliest stages during the actual bromination, showing that a considerable formation of conjugated double bonds takes place, do not necessarily imply that a free radical mechanism was causing these changes as is probable in the autoxidation. They could be explained by allylic rearrangements of the primary bromides [14,20]. The later changes are all caused by the secondary splitting off of hydrobromic acid and perhaps some addition of this acid. However, our results on the bromination

of linoleate with N-bromosuccinimide can all be explained if the primary bromination product is a free radical reaction as proposed by Waters [8], although they do not furnish conclusive proofs. Further work on the structure of the products and the kinetics of the reaction is needed to clarify this question.

EXPERIMENTAL

Methyl linoleate was prepared essentially according to McCutcheon [21], N-bromosuccinimide according to Bredt and Hof [22]. All brominations were done in dry, redistilled carbon tetrachloride. A mercury arc lamp (Orig. Hanau STU 500) was placed 43 cm from the reaction flask (Pyrex).

Example of bromination with one mole N-bromosuccinimide per mole linoleate:

165.6 mg N-bromosuccinimide, 100 ml carbon tetra chloride and 274.2 mg methyl linoleate was rapidly brought to boiling under reflux on a sand bath in ultraviolet light under an atmosphere of nitrogen or air. The reaction mixture became homogeneous after boiling 3—4 minutes. Samples of approx. 2 ml were withdrawn at different times and immediately cooled to room temperature in water. They were used for the starch-iodide reaction to follow the disappearance of the N-bromosuccinimide and for spectrophotometry. For the latter determination 1 ml was blown to dryness with CO_2, and this was repeated twice after the addition of 0.5 ml abs. alcohol to ensure that no carbon tetrachloride remained. This procedure takes at most 25—30 minutes and the sample is most of the time below 10° C. It was then immediately dissolved in ethanol and the spectrum examined at a suitable dilution in the Beckman spectrophotometer. The molar extinctions were calculated on the original linoleate content of the aliquot taken.

For determining the bromine content of the reaction products the bromination was carried out in the following way.

151.9 mg N-bromosuccinimide (100 %) + 75 ml CCl_4 + 250.4 mg methyl linoleate in 25 ml CCl_4 were boiled under reflux in ultraviolet light for 10 min. Then the mixture was cooled and 50 ml were withdrawn. The rest (50 ml) was boiled for 69 hours without illumination and treated as the first fraction.

The first fraction removed gave a negative starchiodide reaction. 1 ml was withdrawn for spectrophotometry. When the succinimide in the fraction had precipitated, the mixture was filtered through a sintered glass filter. The clear solution was evaporated in vacuo at about 35° C, Twice dissolved in a little cyclohexane and again taken to dryness. The substance was then dissolved in ether, washed with a buffer solution of pH 7 and then with water. The ether solution was dried with anhydrous sodium sulphate, filtered through a sintered glass filter and partially evaporated with the aid of a current of CO_2. The ether solution was made up to exactly 10 ml. 0.5 ml samples were taken for the following determinations.

a. Spectrophotometry.
b. Bromine determination (Carius-micro-method).
c. Nitrogen determination (Kjeldahl-micro-method).

The results are given in Table 1 (p. 436).

SUMMARY

The bromination of methyl linoleate with N-bromosuccinimide in refluxing carbon tetrachloride has been investigated.

Ultraviolet light strongly catalyzed the reaction.

During the bromination a strong absorption at 232 mμ appears due to conjugation of the double bonds. On prolonged reflux of the bromide hydrobromic acid is split off with triene formation.

The reaction mechanism is discussed.

This work has been supported by a grant from Svenska Oljeslageri AB, Göteborg.

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Received December 8, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 444–447

Constituents of Pine Heartwood

XIX. * The Heartwood of *Pinus pinea* L., *Pinus pinaster* Aiton, *Pinus halepensis* Mill., and *Pinus nigra* Arnold var. *calabrica* (Loudon) Schneider

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The present paper deals with an investigation of the heartwood of four pines belonging to the section *Diploxylon*, all of which are endemic to Southern Europe. The extractions were carried out according to the general scheme given in Part IX [1].

The heartwood of *Pinus pinea* (Stone Pine) gave a very high yield of ether extract (37 % of the heartwood). From the acetone extract, *l*-arabinose, pinocembrin, and pinosylvin monomethyl ether could be isolated.

The sample of *Pinus pinaster* (Maritime Pine) investigated was poorer in ether extractable compounds (10 % of the heartwood). One would expect a high content of resins, since *P. pinaster* is utilised for the commercial production of resins. *l*-Arabinose, pinobanksin, and pinocembrin were isolated from the acetone extract, but neither pinosylvin nor its monomethyl ether were found. This, of course, does not necessarily mean that these two substances are totally absent in the heartwood. Small quantities may easily escape detection, especially when they are accompanied by more or less resinous products. The heartwood of *P. pinaster* is known to show little resistance to decay, which is in accordance with the absence of significant quantities of pinosylvin phenols.

The heartwood of *Pinus halepensis* (Aleppo Pine) was extremely rich in resins. (Ether extract = 42 %.) The acetone extract contained a water-soluble fraction which reduced Fehling's solution, but no crystalline products could be isolated from it. The ether-soluble part of the acetone extract con-

* XVIII. *Acta Chem. Scand.* **4** (1950) 390.

tained pinosylvin and its monomethyl ether. However, no pinocembrin or pinobanksin could be isolated.

The heartwood of *Pinus nigra* var. *calabrica* (Corsican Pine = *P. laricio* Poiret) yielded 31 % of ether extract. *l*-Arabinose (in a crude state) and pinosylvin monomethyl ether were isolated from the acetone extract. The heartwood of the Austrian form of this species, *P. nigra forma austriaca* Aschers and Graebner, had been investigated previously [2]. It contained pinosylvin and its mono- and dimethyl ethers. Pinocembrin or pinobanksin have not been isolated from either of the two varieties.

The yields of the different substances (expressed in percent of air-dried heartwood) are listed in the table below.

Species	Ether extract	Membrane substances	*l*-Arabinose	Pinobanksin	Pinocembrin	Pinosylvin	Pinosylvin monomethyl ether
P. pinea	37	0.4	0.05	—	0.3	—	0.02
P. pinaster	10	0.3	0.3	0.02	0.08	—	—
P. halepensis ..	42	0.2	—	—	—	0.03	0.2
P. nigra var. *calabrica*	31	0.06	0.03	—	—	—	0.4

EXPERIMENTAL

The sample of *P. pinea* came from Valladolid, Spain, and the three other samples from Sierra de Cazorla, Jaén, Spain.

Pinus pinea

The heartwood (15 annual rings) gave a rather weak colour reaction with diazotised benzidine solution.

The wood (1.6 kg) was first cut into chips (2 × 4 × 30 mm) and digested with ether for 24 hours to remove most of the resins. The chips were then air-dried, milled, and extracted with ether and acetone, in the usual manner [1]. The milling of fresh wood proved impossible because of the high resin content. The total content of ether extract was 37 %. It was pale yellow and crystallised immediately.

The acetone extract was concentrated and treated with cold water (800 ml). The aqueous solution (= W) was decanted, and the sticky, insoluble residue was then shaken with ether (500 ml). A brown precipitate of insoluble »membrane substances» (5 g) separated from the ether solution, which was then extracted with saturated sodium bicarbonate (7 × 100 ml, extract = B), saturated sodium carbonate (5 × 100 ml,

extract = C), 0.3 % sodium hydroxide (5 × 100 ml, extract = H_1), and with 5 % sodium hydroxide (2 × 100 ml, extract = H_2).

W: The aqueous solution was concentrated to a brown syrup by vacuum distillation. This syrup was dissolved in hot ethanol, filtered, concentrated to a small volume, and cooled. Colourless crystals were formed, which melted at 155—157° *. No m. p. depression with *l*-arabinose. $[\alpha]_D^{20}$ + 105° ± 1° (equilibrium rotation in water, c = 2.4). Yield, 0.8 g.

B was acidified and extracted with ether. On concentration, the ether solution yielded a brown syrup which did not deposit any crystals.

C was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and decolourised by filtration through a small amount of aluminium oxide. The filtrate was evaporated to dryness and the residue recrystallised from benzene and from 50 % acetic acid, yielding colourless crystals, m. p. 192—193°, no m. p. depression with pinocembrin. $[\alpha]_D^{20}$ − 53° ± 1° (methanol, c = 3.3).

H_1 yielded an additional amount of pinocembrin which was combined with the material isolated from *C*. Total yield of pinocembrin 5 g.

H_2 was acidified and extracted with ether, and the ether solution dried and concentrated to a yellowish-brown crystalline residue. After recrystallisation from benzene and from 50 % acetic acid, it yielded 0.3 g of pinosylvin monomethyl ether, m. p. 118—119°.

Pinus pinaster

The colour reaction with diazotised benzidine was rather weak. 2.1 kg of heartwood were extracted. The extractions were carried out in the same way as described for *P. pinea*. The ether extract soon deposited crystals. The *W* fraction yielded 6 g of *l*-arabinose, m. p. 153—155°, $[\alpha]_D^{\xi C}$ + 104° ± 1° (equilibrium rotation in water, c = 2.3).

The *C* fraction deposited a crystalline yellow precipitate, which was separated, acidified, and recrystallised twice from toluene, yielding pinobanksin (0.33 g), m. p. 176—177°, $[\alpha]_D^{20}$ + 13° ± 1° (methanol, c = 3.9). From the sodium carbonate solution and from H_1, pinocembrin (1.6 g) was isolated. M. p. 193—194°, $[\alpha]_D^{20}$ − 49° ± 1° (methanol, c = 2.9).

The H_2 fraction yielded a very small amount of a sticky brown product, from which no crystalline substances could be isolated.

Pinus halepensis

The colour reaction with diazotised benzidine was of medium strength. 2.1 kg of heartwood were extracted. Like *P. pinea*, the wood had to be cut into chips and pre-extracted with ether before milling. The ether extract crystallised very soon. The *W* fraction yielded a small quantity of a brown syrup, but no crystalline sugars could be isolated from it. The water solution reduced Fehling's solution.

B and *C* also yielded resinous products, from which no crystalline substances could be isolated.

H_1 was acidified and extracted with ether. The ether extract was dried and concentrated, and the sticky brown residue vacuum-distilled. The distillate soon deposited crystals, which were recrystallised from benzene. Large, glistening, colourless leaflets,

* All melting points uncorrected.

m. p. 153—155°, were obtained. The m. p. was not depressed on admixture of pinosylvin. Yield, 0.7 g.

H_2 contained pinosylvin monomethyl ether, which was recrystallised from benzene. Yield, 3.6 g, m. p. 120—122°.

Pinus nigra var. *calabrica*

The heartwood gave a rather strong red colour reaction with diazotised benzidine solution. 1 kg of heartwood was cut into chips, pre-extracted with ether, milled, and extracted with ether and acetone in the usual manner. The ether extract (31 %) was pale yellow and deposited large crystals of resin acids.

l-Arabinose was isolated from the *W* fraction. Yield, 0.4 g, m. p. 153—155°, $[\alpha]_D^{20}$ + 101° ± 1° (equilibrium rotation in water, $c = 1.3$).

The *B*, *C* and H_1 fractions did not yield any crystalline products.

H_2 yielded 3.5 g of pinosylvin monomethyl ether, m. p. 118—119°.

SUMMARY

The heartwood constituents of four pines of Spanish origin have been investigated. The following substances were isolated.

From *Pinus pinea* L.: *l*-arabinose, pinocembrin, and pinosylvin monomethyl ether.

From *Pinus pinaster* Aiton: *l*-arabinose, pinobanksin, and pinocembrin.

From *Pinus halepensis* Mill.: pinosylvin and pinosylvin monomethyl ether.

From *Pinus nigra* var. *calabrica* Schneider: *l*-arabinose and pinosylvin monomethyl ether.

This work has been financially supported by *Fonden för Skoglig Forskning*, and one of us (J. C. A-N) wishes to express his gratitude for a Spanish Government Scolarship, which enabled him to take part in the work. For the supply of wood we are indebted to *Instituto Forestal de Investigaciones*, Madrid.

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Received January 26, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 448–455

Constituents of Pine Heartwood

XX. * Separation of Phenolic Heartwood Constituents by Paper Partition Chromatography

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In the previous papers of this series, the extraction of heartwood from different pine species and the isolation of crystalline phenolic compounds from the extracts have been described. The complete investigation of such extracts requires several kilograms of wood and is rather tedious and time-consuming. Also, one can never be sure that all crystalline substances occuring in the extracts have been detected. If there is a very small percentage of one substance along with an excess of syrupy resinous products, it can easily be overlooked. Therefore, it would be of great value to have access to a method which would enable a rapid and safe identification of all the different constituents of a heartwood extract from a small sample of wood. The method of paper partition chromatography, introduced by Consden, Gordon, and Martin [1], fulfils these requirements.

Some investigations concerning paper chromatography of flavones and their glycosides have been published recently. Bate-Smith separated antocyanin and flavone pigments from petal extracts [2], and later made a thorough study of the paper chromatography of antocyanins, flavones, and phenols [3–5]. Wender and co-workers also reported the successful separation of a few flavonoid pigments [6, 7].

The phenolic substances which have been isolated from pine heartwood are flavones (chrysin, tectochrysin), flavanones (pinocembrin, pinobanksin, pinostrobin, strobopinin, and cryptostrobin), and hydroxystilbenes (pinosylvin and its monomethyl ether). The paper-chromatographic separation of these substances in various solvents has been studied. The best results were obtained

* XIX. *Acta Chem. Scand.* 4 (1950) 444.

with a water-saturated mixture of equal volumes of benzene and ligroin, containing traces of methanol. This mixture will be referred to as the »standard solvent». Other solvents which gave rather good results were: chloroform-methanol-ligroin-water (2 : 1 : 7 : 5 vols., the supernatant layer used), ethyl ether-ligroin (1 : 5 vols., water-saturated), and carbon disulphide (water-saturated).

Since most of the substances mentioned above are colourless (chrysin and tectochrysin are yellow), they must be made visible by spraying the paper with some reagent after the chromatogram has been run. The best colouring reagent for these substances is a solution of bis-diazotised benzidine, as introduced by Koch and Krieg [8]. It has been used throughout this investigation for qualitative estimation of the phenol content of heartwood by direct staining. It forms red or yellow azo dyes with phenolic substances, and the spots on the filter paper are permanent and very intensely coloured (except for tectochrysin). The heartwood substances differ not only in R_F values and the colour of the spot, but also in the time required for the spot to become visible after spraying with benzidine reagent (See Table 1).

Table 1. Approximate R_F values at 20° in standard solvent and colour reactions with benzidine reagent. (Paper: Munktell OB.)

Substance	Structure	R_F	Colour of spot	The colour becomes visible
Pinosylvin	3,5-Dihydroxy-stilbene	0.05	Dark red	Immediately
Pinobanksin	3,5,7-Trihydroxyflavanone	0.14	Red	Within 0.5—1 min.
Chrysin	5,7-Dihydroxyflavone	0.17	Red	» 3—5 »
Pinocembrin	5,7-Dihydroxyflavanone	0.44	Red	» 1—3 »
Cryptostrobin [9]	Unknown, probably C-methyl dihydroxy-flavanones	0.48	Orange yellow	» 2—4 »
Strobopinin		0.65	yellow	» 1—3 »
Pinosylvin monomethyl ether	3-Hydroxy-5-methoxy-stilbene	0.71	Brick red	Immediately
Tectochrysin	5-Hydroxy-7-methoxy-flavone	0.91	Very pale yellow	Within 10 min.
Pinostrobin	5-Hydroxy-7-methoxy-flavanone	0.93	Orange red	» 7—10 »
Dihydropinosylvin	3,5-Dihydroxydibenzyl	0.11	Dark red	Immediately
3-Hydroxystilbene		0.78	Reddish violet	»
3-Hydroxydibenzyl		0.89	Bright yellow	»

The center of each spot is very difficult to define, the spots often being elliptical and their upper part more or less diffuse. Therefore, the distance from the starting point to the lower edge of the spot has been measured, and this distance divided by the distance travelled by the solvent front at the same time, is here defined as the R_F value.

The nine first substances in Table 1 have been isolated from pine heartwood extracts. For a comparison of R_F values, the three last substances, which are all structurally related to pinosylvin, have also been investigated. Dihydropinosylvin was not isolated in a crystalline state in the original communication by Erdtman. (See Part II [10]). It has recently been obtained in a crystalline state in this laboratory (m. p. 80—82°, private communication from Dr. B. Lindberg). 3-Hydroxydibenzyl has recently been isolated from subfossil Norwegian pine heartwood [11].

Removal or methylation of a hydroxyl group naturally lowers the water-solubility of the substance and thus increases the R_F value. This relation between structure and chromatographic behaviour was first observed by Bate-Smith [3,4]. (Compare also pinosylvin with its monomethyl ether and with 3-hydroxystilbene, chrysin with tectochrysin, and pinocembrin with pinostrobin, Table 1.) Another such relation is evident from the data given in Table 1. Hydrogenation of a double bond causes an increase in R_F, which is, however, smaller than that caused by removal or methylation of a hydroxyl group. (Compare pinosylvin with dihydropinosylvin, chrysin with pinocembrin, tectochrysin with pinostrobin, and 3-hydroxystilbene with 3-hydroxydibenzyl.)

If a mixture of all nine heartwood substances listed in Table 1 is chromatographed, some of the spots will appear too close to each other to make definite identification of each substance possible. Pinobanksin-chrysin, cryptostrobin-pinocembrin, strobopinin-pinosylvin monomethyl ether, and tectochrysin-pinostrobin are substance pairs which do not form two individual spots on the paper, since the differences in R are too small and the upper part of the spots are more or less trailing. Cryoptostrobin and strobopinin can sometimes be distinguished from their partners by the difference in colour reaction, but this is not possible when they occur in small quantities compared to the partners. A second difficulty lies in the identification of tectochrysin, which gives such a weak colour with benzidine reagent that the spot often escapes detection.

The heartwood extracts from pines belonging to the section *Diploxylon* (Hard Pines) do not seem to contain more than four of the phenolic heartwood constituents, namely pinosylvin and its monomethyl ether, pinocembrin, and pinobanksin. A one-dimensional chromatogram gives an excellent separation of this mixture. As follows from the R_F values, the pinosylvin spot comes out

just below the starting point, followed by the spots of pinobanksin, pinocembrin, and pinosylvin monomethyl ether at sufficient intervals. As the R_F values may vary within limits of a few percent, two chromatograms are always run on the same paper side by side, one containing the heartwood extract and the other a mixture of known substances.

Much greater difficulties arise if a mixture of all nine heartwood phenols is to be separated. As mentioned above, the tectochrysin spot is often difficult to observe. The safest identification of this flavone consists of observing the fluorescence of the paper in ultraviolet light. The flavones (chrysin and tectochrysin) give a yellowish-brown fluorescence, which is intensified by spraying with sodium carbonate solution, as indicated by Wender and Gage [6]. Pinosylvin and its monomethyl ether give a bluish-violet fluorescence in ultraviolet light which is independent of the treatment of the paper. Pinostrobin shows a very strong green fluorescence after treatment with sodium carbonate, which facilitates its identification. The colours of the other heartwood substances in ultraviolet light are of little value for their identification.

To achieve a better separation of the phenolic mixture, two-dimensional chromatograms were tried. By running the chromatogram with standard solvent in one direction and with chloroform-methanol-ligroin (2 : 1 : 7 + 5 water) in the other, chrysin can be distinguished from pinobanksin, cryptostrobin from pinocembrin, and strobopinin from pinosylvin monomethyl ether. Since the order of the R_F values is the same in all solvents hitherto tried, the spots do not move quite apart from each other, but the two substances either give different colour reactions (the two last-mentioned pairs), or require different time to become visible (pinobanksin-chrysin). Chrysin, being very sparingly soluble in both water and the organic solvent, causes trailing of the upper part of the spot and is thus distributed over a large area on a two-dimensional chromatogram. When the paper is sprayed with benzidine reagent, the pinosylvin and pinobanksin spots come out first, and after a few minutes they are surrounded by the pale red chrysin spot. Pinocembrin and pinosylvin monomethyl ether also give rather large and diffuse spots on the two-dimensional chromatogram. Fig. 1 shows a chromatogram containing all substances except tectochrysin.

A better separation of strobopinin from pinosylvin monomethyl ether is obtained by running the chromatogram with standard solvent in one direction and with carbon disulphide in the other. The two substances then form separate spots. In every other respect, however, carbon disulphide is inferior to the chloroform-ligroin-methanol mixture.

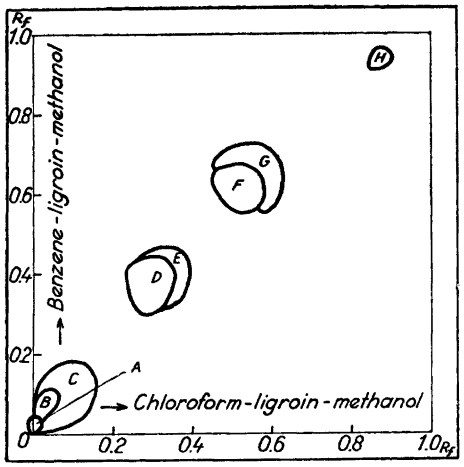

Fig. 1. Two-dimensional chromatogram of eight phenolic heartwood substances after spraying with benzidine reagent. A: Pinosylvin (dark red). B: Pinobanksin (red). C: Chrysin (pale red). D: Pinocembrin (red). E: Cryptostrobin (orange yellow). F: Strobopinin (yellow). G: Pinosylvin monomethyl ether (brick red). H: Pinostrobin (orange red).

Thus, the separation of all nine substances involves the following three operations:

1. A one-dimensional chromatogram with standard solvent, which gives good information as to the main constituents of the mixture. Some of them can be identified accurately (pinosylvin and its monomethyl ether, pinocembrin, and very often pinostrobin).

2. A one-dimensional chromatogram for identification of tectochrysin and pinostrobin. The best solvent for this separation is ethyl ether-ligroin (1 : 5), in which the R_F values are about 0.95 for pinostrobin and 0.8 for tectochrysin. The paper is observed in ultraviolet light before and after spraying with sodium carbonate solution. Chrysin, pinosylvin, and its monomethyl ether may also be identified by this method.

3. A two-dimensional chromatogram using as solvents the standard mixture and chloroform-methanol-ligroin-water (2 : 1 : 7 : 5) respectively, and bis-diazotised benzidine as developing reagent.

The chromatographic methods described above have been applied to heartwood extracts from a great number of pines. Most of the pines from the section *Diploxylon* contain the four substances pinosylvin, pinobanksin, pinocembrin, and pinosylvin monomethyl ether. Owing to the great sensitivity of the chromatographic method, the presence of all four substances can often be demonstrated even when only one or two of them have been isolated by classical methods. Pinosylvin seems to be the substance which is most easily overlooked in preparative work.

The chromatograms of heartwood extracts from pines belonging to the section *Haploxylon* are of far more complicated nature. They generally indicate the presence of all or most of the nine substances hitherto isolated, and, in addition, there are some spots which must be due to unknown substances.

Thus, the paper chromatography method will make possible a rapid and sensitive qualitative analysis of pine heartwood extracts without tedious fractionation by classical methods. It is also a valuable tool for discovering new components. A detailed report of the results obtained with heartwood extracts from numerous pines will be published later.

EXPERIMENTAL

One-dimensional chromatograms

The apparatus used for one-dimensional chromatograms was similar to that described by Wender and Gage [6]. A broad glass cylinder with a glass lid served as vapour chamber. An evaporating dish, standing on a framework of glass rods and metal wire, served as through. The paper strips, 3.5 × 42 cm (Munktell O or OB) were kept in place by a second evaporating dish of smaller dimensions, standing inside the first one. Two chromatograms were run on each paper, one of the unknown mixture and one of the reference mixture. Approximately 0.01—0.02 ml of an alcoholic solution of each mixture were applied as circular spots on the filter paper by means of a glass capillary. The spots were applied on a horisontal line 14 cm from one end of the paper strip at an interval of 1.8 cm. The most suitable concentration for the reference solution is 0.1 % with respect to each component. Too high concentrations cause too high R_F values and vigorous trailing of the spots. For a solution of unknown strength, several chromatograms with different dilutions must sometimes be run.

The paper strips were left hanging down from the dish (five to six strips at one time) in the closed cylinder for one to two hours to become saturated with the vapours of water and solvent, which were present in a liquid state on the bottom of the cylinder. The dish was then filled with solvent from a pipette by means of a small hole in the lid, which was normally covered by a piece of glass. The chromatogram was allowed to run till the solvent front had reached to 2—3 cm above the lower end of the strips. The strips were then taken out of the cylinder, the solvent front marked with a pencil line, and the solvent allowed to evaporate in the air. The time required for the standard solvent to travel the suitable distance (~ 35 cm) was 80—85 min. It travels 3—4 times faster than the butanol-acetic acid mixture used for chromatography of amino acids or sugars [1], when compared on the same type of paper.

The experiments were carried out in a room with the temperature thermostatically maintained at 20° ± 1°. Chromatograms run in an ordinary laboratory, without taking special care to maintain constant temperature, did not differ very much from those run in the thermostat room.

After drying in the air, the paper strips were dipped into bis-diazotised benzidine solution (composition, see below) and allowed to hang moistened with the solution for about two minutes. They were then carefully rinsed with cold water and hung up for drying at room temperature. The coloured spots continue to develop even during and

after rinsing. The rinsing is necessary to remove excess benzidine reagent, which would otherwise give the paper a brownish colour after some time.

The spots are best observed if the paper is made more transparent by painting with cellulose lacquer or better by soaking in molten paraffin.

For the identification of tectochrysin and pinostrobin, the ascending technique [12] was often preferred. The apparatus was similar to that described by Ma and Fontaine [13]. The paper sheet, 24 × 24 cm, allowed ten chromatograms to be run simultaneously. After drying, the paper was investigated under the quartz lamp and the visible spots marked with a pencil. It was then sprayed with 5 % sodium carbonate solution and again observed under the quartz lamp when still wet. The tectochrysin was now clearly visible by means of its yellowish-brown fluorescence, and the pinostrobin spot had a still stronger greenish colour.

Two-dimensional chromatograms

The two-dimensional chromatograms were run in the same apparatus as used for ascending one-dimensional chromatograms. A spot of the solution to be tested was applied near one corner of the paper. (Munktell OB, 24 × 24 cm). A steel rod through the central axis of the paper holder [13] prevented the paper from reaching the bottom of the glass cylinder. The cylinder contained enough solvent (both phases) to reach to a few cm below the lower edge of the paper. It was closed by a cork, and the paper left in the cylinder for one to two hours to attain equilibrium with solvent vapours. The chromatogram was started by siphoning an additional quantity of solvent (organic phase) into the cylinder, so that the lower edge of the paper reached into the solvent. For this purpose, a narrow glass tube was led through the cork to the bottom of the cylinder close to the wall. When the solvent front had mounted to a few cm from the upper edge of the paper, the paper holder was taken out of the cylinder, and the paper allowed to dry in the air. It was then inserted into the paper holder again at right angles to the direction previously used, and the chromatogram run with the second solvent. Finally, the paper was sprayed with the benzidine reagent and rinsed with water as described above.

Reagents

Standard solvent: Benzene (50 vols.), ligroin (b. p. 85—105°, 50 vols.), methanol (1 vol.) and water (50 vols.) were shaken in a separatory funnel, and the organic phase used as the solvent. The funnel was left standing in the thermostat room overnight before the solvent was used. New solvent must be prepared once a week.

Other solvents: A: Methanol (1 vol.), chloroform (2 vols.), ligroin (7 vols.), water (5 vols.).

B: Ethyl ether (1 vol.), ligroin (5 vols.), water (5 vols.).

Benzidine spraying reagent:[8] Benzidine (5 g) was stirred with conc. hydrochloric acid (14 ml) and the suspension dissolved in water (980 ml). A suitable amount of this solution was mixed with an equal volume of a 10 % sodium nitrite solution and the mixture stirred till it was clear and pale yellow. It must be used for spraying within 10 minutes after the mixing.

SUMMARY

It has been shown that all nine phenolic substances hitherto isolated from pine heartwood extracts can be separated and identified by paper partition chromatography.

With the aid of this method, a rapid and extremely sensitive qualitative analysis of pine heartwood extracts can be carried out with very small samples of wood.

The author is indebted to *Fonden för Skoglig Forskning* for financial support, to Mr. A. Misiorny for skilful experimental assistance, and to Dr E. C. Bate-Smith, Low Temperature Research Station, Cambridge, England, for sending me copies of his papers before they had appeared in print.

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Received February 17, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 456 – 461

The Iodine-Azide Reaction

III. The Catalytic Effect of the Pentathionate Ion

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The iodine-azide reaction was discovered by Raschig [1], who found that sodium thiosulphate and sulphide act as catalysts. The overall equation of the reaction is:

$$2\ N_3^- + I_2 \rightarrow 3\ N_2 + 2\ I^-$$

It is believed that only compounds which contain sulphide sulphur catalyze the reaction. Metz [2] has shown that tri-, tetra-, and pentathionate act as catalysts. The tetrathionate catalyzed reaction was investigated kinetically by Dodd and Griffith [3] and Hofman-Bang [4]. The rate of reaction was found to be directly proportional to the concentrations of tetrathionate and of azide ions, and independent of the concentration of iodine. It was shown [4] that kinetic experiments could be carried out in a very simple way by determining the time of consumption of a definite amount of iodine with starch as the indicator. A few introductory experiments with potassium pentathionate as the catalyst, showed that the rate of reaction, also in this case, was independent of the concentration of iodine, so that the same simple method could be used.

A reaction mechanism similar to that of the tetrathionate catalyzed iodine-azide reaction is proposed in analogy with that of the carbon disulphide catalyzed iodine-azide reaction [5,6]:

$$N_3^- + S_5O_6^{--} \rightarrow N_3S_5O_6^{---} \tag{2}$$

$$2\ N_3S_5O_6^{---} + I_2 \rightarrow (N_3S_5O_6)_2^{----} + 2\ I^- \tag{3}$$

$$(N_3S_5O_6)_2^{----} + 2\ N_3^- \rightarrow 2\ \overset{*}{N_3}S_5O_6^{---} + 3\ N_2 \tag{4}$$

$$\overset{*}{N_3}S_5O_6^{---} \rightarrow N_3^- + S_5O_6^{--} \tag{5}$$

where (2) is the rate determining reaction step, and (3), (4), and (5) are instantaneous. $N_3\overset{*}{S}_5O_6^{---}$ is assumed to be an activated ion which decomposes readily.

REAGENTS

Sodium azide: Sodium azide as previously described [4], was used. Potassium pentathionate, $K_2S_5O_6$, $1\frac{1}{2}$ H_2O: This salt was prepared according to Stamm, Seipold, and Goehring [7], and analyzed according to Kurtenacker [8] and Foss [9]. The prepared salt was found to be 98.2 % pure, and dissolved in water without any sulphur being seen. An impurity of approx. 2 % tetrathionate was in this case of no importance, because pentathionate turned out to be a much more powerful catalyst than tetrathionate.

The other reagents used were all of analytical grade.

EXPERIMENTAL PROCEDURE

(For further details see ref. 4.) Into a 300 ml Erlenmeyer flask was pipetted 30 ml of a solution, which was 4.013 *M* with respect to sodium nitrate and 0.1 *M* to potassium iodide, 0.2 ml 0.5 % starch solution, 10 ml 0.00978 *N* iodine in potassium iodide, and 10 ml 0.5 *M* sodium azide. The flask was placed in a water thermostat, and after the elapse of 15 minutes (for sake of temperature adjustment), 10 ml approx. 0.0002 *M* potassium pentathionate solution was added. The instant all the iodine present had been consumed, the blue colour of the experimental solution disappeared. When less than 10 ml iodine solution was used, water was added, so that the volume of the reacting solution was the same. Also for potassium pentathionate solution, water was used as substitute, whereas the substitute for sodium azide solution was 0.5 *M* sodium nitrate, so that the ionic strength did not change. The reacting solution was 2 *M* with respect to sodium nitrate. The purpose of the addition of potassium iodide is partly to prevent a considerable percentile change of the iodide ion concentration during the reaction, and partly to produce a sharp colour change.

EXPERIMENTAL RESULTS

The concentrations in the kinetic experiments were chosen so that the time of reaction would be only a few minutes. According to Kurtenacker *et al.* [10] pentathionate decomposes in aqueous solution to predominantly sulphur and tetrathionate. The rate of decomposition is fairly large at 25° C and is, within a pH-range of 9—5, independent of pH. In each experiment a fresh solution of potassium pentathionate was prepared by dissolving the salt

Table 1. Reaction between sodium azide and iodine at 25° C. Catalyst: Potassium pentathionate. In all experiments — besides the solutions mentioned in the table — were added 0.2 ml starch solution, and 30 ml solution, which was 4.013 M with respect to sodium nitrate and 0.1 M to potassium iodide. The concentrations of the stock solutions used were: 0.000193 M potassium pentathionate; 0.5 M sodium azide; 0.00978 N iodine, which was 0.02 M with respect to potassium iodide. The rate constants, k, were calculated according to equation (1).

Expt. no.		c	a	x	t Time in min	k
1	10 ml pentathionate 10 ml iodine 10 ml sodium azide	0.0000321	0.0831	0.00163	3.18	194
2	5 ml pentathionate 5 ml water 10 ml iodine 10 ml sodium azide	0.0000160	0.0831	0.00163	7.07	175
3	5 ml pentathionate 5 ml iodine 10 ml water 10 ml sodium azide	0.0000160	0.0831	0.00081	3.27	187
4	10 ml pentathionate 5 ml iodine 5 ml water 5 ml 0.5 M sodium nitrate 5 ml sodium azide	0.0000321	0.0415	0.00081	2.85	215
5	10 ml pentathionate 10 ml iodine 10 ml sodium azide	0.0000321	0.0831	0.00163	3.22	192

in water preheated to the experimental temperature, so that an experiment could be started about one minute later. In none of the experiments was any sulphur seen immediately after the iodine-starch colour had disappeared, but when the solution was allowed to stand for an additional 30 minutes, a slight opalescence was noticeable.

From the experimental results (see Table 1) it is seen that the rate of reaction is approx. proportional to the concentrations of potassium pentathionate and sodium azide, and independent of the concentration of iodine. The results are not really fine, but the reason is probably that pentathionate so easily

decomposes to tetrathionate and sulphur; it is, in this connection, worth noticing that the rate constant seems to increase with decreasing time of reaction. The rate constants, k, in Table 1, are calculated from the integrated rate expression:

$$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x} \tag{1}$$

where t is the time of reaction, c is the concentration of potassium pentathionate, a is the initial concentration of sodium azide, and $a-x$ is the concentration of sodium azide at the time t.

ENERGY OF ACTIVATION

The energy of activation of the pentathionate catalyzed iodine-azide reaction was determined by experiments analogous to those described above. The rate of reaction was determined at 20°, 25°, and 30° C. 30 ml solution, which was 4.013 M with respect to sodium nitrate and 0.1 M to potassium iodide, 10 ml 0.00978 N iodine, 10 ml 0.5 M sodium azide, 0.2 ml starch indicator, and 10 ml 0.000193 M potassium pentathionate were used in each experiment. In Table 2 are given the rate constants ($k_{exp.}$) calculated according to

Table 2. Energy of activation of the pentathionate catalyzed iodine-azide reaction. Concentrations in all experiments: $a = 0.0831$ M; $c = 0.0000321$ M; $x = 0.00163$ M. $k_{exp.}$ is the rate constant in each case calculated from equation (1). $k_{calc.}$ is calculated from the equation $\log k = H - \frac{A}{T}$, which is a straight line fitted to the experimental k and T values. The reacting solution was 2 M with respect to sodium nitrate.

Expt. no.	Temp. °C	t Time in min	$k_{exp.}$	$k_{calc.}$
1	20°	4.40	140	140
2	20°	4.45	139	
3	25°	3.22	192	192
4	25°	3.25	190	
5	30°	2.37	260	260
6	30°	2.37	260	

equation (1). Using the method of least squares, the numerical values of H and A were calculated according to the equation:

$$\log k_{exp.} = H - \frac{A}{T}$$

where T is the absolute temperature. The resulting expression is:

$$\log k = 10.337 - \frac{2401}{T}$$

By substituting the experimental temperature for T, is calculated $k_{calc.}$, which also is given in Table 2. The energy of activation was found to be:

$$A \times 4.571 = 2401 \times 4.571 = 10970 \text{ kcal/mole}$$

The energy of activation of the tetrathionate catalyzed iodine-azide reaction [4] is approx. 2000 kcal larger, which is also to be expected, as the tetrathionate reaction is ca. 30 times slower than the pentathionate reaction. The frequency exponents are nearly identical, resp. 10.34 and 10.47 with minutes as unit.

Although pentathionate does not seem to decompose much in the experiments carried out, it was nevertheless ascertained that a visible evolving of a gas took place, when rather concentrated solutions of sodium azide and potassium pentathionate were mixed. This reaction was definitely more rapid than the corresponding tetrathionate-azide reaction [4]. The gas was analyzed * according to Christiansen and Wulff [11]; the result was 98.4 % nitrogen, 0.7 % oxygen, and perhaps a trace of hydrogen. A buffered solution with pH approx. 5, and a solution of sodium azide and potassium pentathionate only, gave practically the same results. The small percentage of oxygen was probably due to contamination by atmospheric air. Consequently it is believed that pentathionate ions oxidize azide ions essentially to free nitrogen.

EXPERIMENTS WITH POTASSIUM HEXATHIONATE

Potassium hexathionate was prepared according to Weitz and Achterberg [12] and Stamm, Seipold and Goehring [7]. A few experiments with hexathionate as the catalyst were carried out in the same way as with pentathionate. The results were the same as those with pentathionate, and even the rate constant

* I am greatly indebted to Miss I. Wulff, who carried out the analysis.

was the same within a few percent. The reason presumably is that hexathionate decomposes almost instantaneously to pentathionate and sulphur in a slightly basic solution.

SUMMARY

Kinetic investigations were carried out on the iodine-azide reaction catalyzed by potassium pentathionate. It was a second order reaction with respect to azide and pentathionate ions. The rate of reaction was found to be independent of the concentration of iodine. This kinetic behaviour is analogous to that of the tetrathionate catalyzed iodine-azide reaction [3,4]. The energy of activation was determined to be 10970 kcal/mole in 2 *M* sodium nitrate solution.

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Received February 1, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 462–472

Über die Umsetzung von Formaldehyd mit Allylacetat *

I. Eine neue Synthese des 1,2,4-Butantriols und des 3-Oxytetrahydrofurans

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Der Aufbau des Butantriols-(1,2,4) aus einer Dreikohlenstoffkette gelang erstmalig Wagner [1], der auf metallorganischem Wege aus Allyljodid und Trioxymethylen in Gegenwart von Zink Allylcarbinol darstellte und dieses durch Permanganatoxydation in das Triol überführte. Pariselle [2] kondensierte Allylmagnesiumbromid mit Chlordimethyläther zum Methyläther des Allylcarbinols, der durch Bromaddition den Methyl-[$\gamma.\delta$-dibrom-*n*-butyl]-äther gab [3]. Durch Behandeln dieser Verbindung mit Bromwasserstoff gelangte er zum 1,2,4-Tribrombutan [4], das er über das Triacetat in das Butantriol-(1,2,4) umwandelte. Im Rahmen seiner schönen und umfassenden Studie erhielt er auch das 3-Oxytetrahydrofuran [5]. Durch Fichter und Herndl [6] wurde bei der Nitratmischelektrolyse von Adipaten in Verbindung mit einer reduzierenden Verseifung die Bildung von Butantriol-(1,2,4) beobachtet. All diese Verfahren sind präparativ unbequem und liefern unbefriedigende Ausbeuten. Es ist daher von praktischer Bedeutung, dass die Alkindiol-Synthese von Reppe [7] eine rationelle technische Darstellung des Butantriols-(1,2,4) und des 3-Oxytetrahydrofurans aus Acetylen ermöglicht. Aber auch diese Synthese führt über mehrere Stufen. Im folgenden sei ein Verfahren beschrieben, bei dem man, ausgehend vom Allylalkohol bzw. dessen Acetat, unter Ausnutzung der Olefinlücke in einem Arbeitsgange zum Butantriol-(1,2,4)-triacetat und 3-Acetoxytetrahydrofuran gelangt.

In einer früheren Mitteilung über die Umsetzung von Formaldehyd mit Äthylen [8] wurde kurz über die Reaktion zwischen Formaldehyd und Allyl-

* 5. Mitt. über Formaldehyd-Oletin-Reaktionen (1.–4. Mitt. *Z. Naturf.* **1** (1946) 448, 671, 676; **3b** (1948) 314.)

acetat berichtet, ohne dass in diesem Untersuchungsstadium sichere Angaben über die Natur der Reaktionsprodukte gemacht werden konnten. Die Mutmassung ging damals dahin, dass es sich bei der »niedrigsiedenden Verbindung» ($Sdp._{10}$ 63,2—63,5°) um 3-Acetoxytetrahydrofuran, bei der »hochsiedenden Verbindung» ($Sdp._{10 \cdot 5}$ 146—146,5°) um 3-Acetoxy-4-acetoxymethyltetrahydrofuran handeln könnte. — Wir haben die Untersuchung wieder aufgenommen, da uns die Reaktion einerseits unter dem Gesichtspunkt einer Synthese einfacher Desoxyzucker präparativ wichtig erschien. Andererseits hofften wir, in Verbindung mit einer kürzlich entwickelten Vorstellung über die mögliche Beteiligung der Formaldehyd-Olefin-Reaktion am stofflichen Aufbau der Pflanzen [9] das bisher vorliegende dürftige Material über den säurekatalysierten Formaldehydaufbau *funktioneller* Olefinderivate im Sinne von Modellversuchen erweitern zu können. Insbesondere war uns an der Feststellung gelegen, ob während der Reaktion nach erfolgtem »Glykolaufbau» aus den Primärprodukten durch Essigsäureabspaltung höhere Olefinderivate entstehen, die ihrerseits Zentren für einen erneuten Angriff des Formaldehyds darstellen und somit zu einem weiteren Anwachsen des Moleküls Veranlassung geben könnten. Bis vor kurzem waren nur Olefin-Kohlenwasserstoffe und einige halogenierte Olefine in saurem Medium mit Formaldehyd umgesetzt worden. 1946 wurde erstmalig der Aufbau eines Olefinesters, des Tetrahydrobenzylacetates zum Tetrahydrophthalan bzw. Hexahydrophtalanyl-acetat [10] beschrieben und die Umsetzung des Allylacetates [8] erwähnt.

Lässt man Allylacetat in Gegenwart von Eisessig und Schwefelsäure eine Zeitlang in der Siedehitze reagieren, so gelangt man zu einem schwarzbraunen Reaktionsgemisch. Dieses wurde, wie im experimentellen Teil näher ausgeführt, durch Destillation in verschiedene Fraktionen zerlegt und von jeder einzelnen die Verseifungszahl und Jodzahl bestimmt. Trägt man diese Kennzahlen in je ein Koordinatensystem als Abszisse und die mittleren Siedepunkte (oder ausgesprochenen Temperaturkonstanzpunkte) jeder Fraktion als Ordinate ein, so ergeben sich durch Verbindung der einzelnen Punkte zwei durch mehr oder minder scharfe Knickpunkte ausgezeichnete Kurven. Es leuchtet ein, dass Knickpunkte — manchmal aber auch durch vor- oder rückwärtige Verlängerung charakteristischer Kurvenzüge erhaltene Schnittpunkte — die Siedepunkte von Gemischen oder aber, bei genügendem Reinheitsgrad der einzelnen Fraktionen und bei einer grossen Anzahl ermittelter Kurvenpunkte, in mehr oder minder grober Annäherung Siedepunkte nebst den dazugehörigen Verseifungs- oder Jodzahlen von im Gemisch vorliegenden Verbindungen wiedergeben können. Entsprechende Überlegungen haben uns schon früher für Ausbeutebestimmungen von Estergemischen bei der Cyclohexen-Formaldehyd-Umsetzung gute Dienste erwiesen [11]. Auch im vorliegenden Falle haben

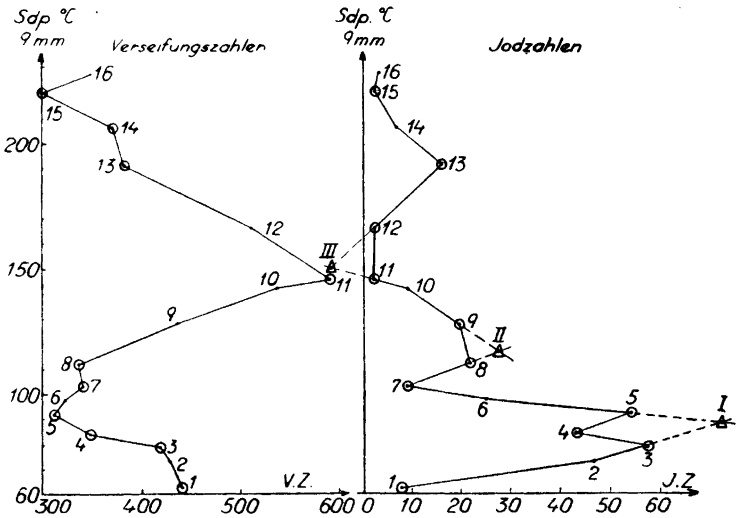


Abbildung 1. Allylacetat-Formaldehyd-Ansatz. Verseifungs- bzw. Jodzahlen in Abhängigkeit von der Siedetemperatur.

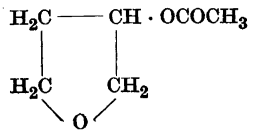
wir uns durch solche Kurven einen Überblick über die voraussichtliche Zahl der Reaktionsprodukte zu verschaffen gesucht. In der Abbildung 1 wurden für den im Versuchsteil beschriebenen Allylacetat-Formaldehyd-Ansatz die Kurven für die Verseifungs- und Jodzahlen nebeneinander gezeichnet in der Weise, dass man für die Abszissen beider Kurven die gleiche Länge wählte (300 V. Z.-Einheiten = 60 J. Z.-Einheiten). Die einzelnen Kurvenpunkte wurden fortlaufend nach Siedepunkten nummeriert, charakteristische Punkte mit einem Kreis umrandet, grob extrapolierte Kurvenstrecken (bei der J. Z.-Kurve) gestrichelt gezeichnet und die Schnittpunkte solcher Extrapolationsgeraden durch Dreiecke und römische Zahlen markiert.

Lässt man bei der Beurteilung der Kurven solche Punkte unberücksichtigt, die nur eine sehr schwache Abweichung von der Geraden bezeichnen und die möglicherweise durch kleine Versuchsfehler bedingt sind, so wären innerhalb des Siedeintervalls zwischen 60—150° C/9 mm (die höher siedenden Fraktionen sollen vorläufig nicht berücksichtigt werden) für die Verseifungszahlkurve die Punkte 1, 3, 4, 5, 7, 8 und 11, für die Jodzahlkurve die Punkte 1, 3, 4, 5, 7, 8, 9 und 11 als charakteristisch hervorzuheben. Nach den Aufbauprinzipien der Formaldehyd-Olefin-Reaktion können jedoch bei gegebenem Ausgangsmaterial im Hinblick auf die möglichen Reaktionsprodukte nur ganz bestimmte Verseifungs- bzw. Jodzahlen in Betracht kommen. Mögliche Reaktionsprodukte wären:

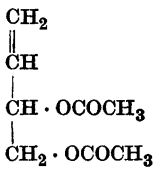
3-Acetoxytetrahydrofuran (I) V. Z. 431,2, J. Z. 0;
Buten-(1)-diol-(3,4)-diacetat (Erythroldiacetat) (II) V. Z. 651,6, J. Z. 147,5;
3-Acetoxymethyl-dihydrofuran (III) V. Z. 394,5, J. Z. 178,5;
Butantriol-2,4-methylenäther-1-monoacetat (IV) V. Z. 350,2, J. Z. 0;
3-Acetoxy-4-acetoxymethyl-tetrahydrofuran (V) V. Z. 554,9, J. Z. 0;
Butantriol-(1 , 2,4)-triacetat (VI) V. Z. 724,8, J. Z. 0.

In dieser Zusammenstellung wurden theoretisch denkbare Isomere nicht berücksichtigt.

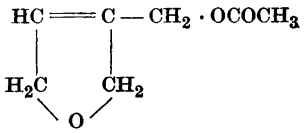
I



II



III



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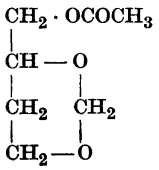
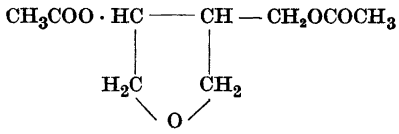
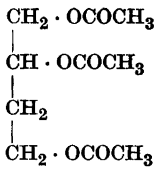


V



VI



Alle Kurvenpunkte, die bezüglich der Kennzahlen von den theoretisch möglichen bedeutende Abweichungen zeigen, können als solche Gemischen zukommende angesehen und daher ausser Acht gelassen werden. Zur Entscheidung der Frage, ob beispielsweise ein bestimmter Punkt der Verseifungszahlkurve einer Verbindung oder einem Gemisch entspricht, genügt meistens ein Blick auf die Jodzahlkurve und vice versa. Unter Zugrundelegung dieser Kriterien bezeichnen für die Verseifungszahlkurve von allen übrigen innerhalb des bezeichneten Siedeintervalls nur die Punkte 1,7 und 11 ziemlich reine Verbindungen. Die Verseifungszahlkurve würde übrigens im Anfangspunkt 1 einen deutlichen Knick aufweisen, wenn die sehr hohe Verseifungszahl der vorhergehenden, stark essigsäurehaltigen Fraktion angegeben worden wäre. Aus der zusammenhängenden Betrachtung der Verseifungs- und Jodzahlkurve zogen wir den vorläufigen Schluss, dass der Punkt 1 beider Kurven dem 3-Acetoxytetrahydrofuran (I), Punkt 7 dem Butantriol-2,4-methylenäther-1-monoacetat (IV) und Punkt 11 den Butantriol-(1,2,4)-triacetat (VI) zuzuordnen ist. Die Jodzahlkurve, die in keinem Punkte theoretisch mögliche Jod-

zahlen erreicht, zeigt an, dass in den Fraktionen, die den Punkten 3, 5, 8, 9 (bzw. I, II) und 13 entsprechen, Gemische mit ungesättigten Verbindungenvorliegen.

Nach diesen vorläufigen Ermittlungen haben wir uns bemüht, durch weitere Reinigung der einzelnen Fraktionen zu reinen Verbindungen zu gelangen und diese einwandfrei zu identifizieren. Hierbei zeigte sich, dass bezüglich der »niedrigsiedenden Verbindung» die ursprüngliche Vermutung zutrifft: Es handelt sich um das *3-Acetoxy-tetrahydrofuran*. Die Anwesenheit von Furanderivaten äusserte sich bereits in der zunächst überraschenden Feststellung, dass bei der Destillation des Reaktionsgemisches aus Allylacetat, Paraformaldehyd und konzentrierter Schwefelsäure das Destillat eine grüne Fichtenspanreaktion gab [10]. Destilliert man die reine »niedrigsiedende Verbindung» mit konzentrierter Schwefelsäure, so lässt sich im Destillat Furan durch die Fichtenspanreaktion nachweisen und die Anwesenheit von Dihydrofuran durch den Siedepunkt und die Entfärbung von Brom wahrscheinlich machen. Entsteht bei der Reaktion Furan, so ist ohnehin damit zu rechnen, dass dessen Bildung über die Zwischenstufe des Dihydrofurans verläuft. Offenbar wird aus dem Acetoxytetrahydrofuran zuerst Essigsäure abgespalten unter Bildung von Dihydrofuran, das dann durch die Schwefelsäure, die dabei in Schwefeldioxyd übergeht, zum Furan dehydriert wird. Die Überführung von 3-Acetoxy-tetrahydrofuran bzw. 3-Oxy-tetrahydrofuran in Furan liess sich auch mit Wasserstoffperoxyd und einer geringen Menge Schwefelsäure in Gegenwart einer Spur Eisen-II-sulfat erreichen. Diese im Reagenzglas ausführbare Reaktion scheint zum Nachweis hydrierter Furanderivate allgemeiner Anwendung fähig *. In beiden Fällen sind aber die entstehenden Furanmengen äusserst gering, so dass diesen Verfahren keine präparative, sondern nur analytische Bedeutung zukommt. Das 3-Acetoxytetrahydrofuran wurde durch Verseifen bzw. Umesterung in das 3-Oxytetrahydrofuran übergeführt, das in allen Eigenschaften mit den von Pariselle angegebenen Konstanten und in der Bildung eines bei 120° schmelzenden Phenylurethans übereinstimmt. Das 3-Acetoxytetrahydrofuran lieferte beim Kochen mit Essigsäureanhydrid in Gegenwart von Zinkchlorid Butantriol-(1,2,4)-triacetat.

Die aus dem Allylacetat-Ansatz erhaltenen Fraktionen 7.) $Sdp._9$ 100—106° und 8.) $Sdp._9$ 106—117° krystallisierten nach einiger Zeit. Nach mehrmaligen

* Zur Ausführung der Probe vermischt man im Reagenzglas etwa 0,1—0,2 ml der zu prüfenden Substanz mit etwas Wasser, gibt einige Tropfen Perhydrol, einige Tropfen konz. Schwefelsäure und ein Kryställchen Eisen-II-sulfat hinzu und erwärmt. Sollte die Reaktion zu heftig verlaufen, wird mit Wasser gekühlt. In die Reagenzglasöffnung wird dann ein mit Salzsäure befeuchteter Fichtenspan eingeklemmt, der sich in den aufsteigenden Dämpfen bei Anwesenheit partiell- oder perhydrierter Furanderivate grün färbt. Auch Tetrahydrofurfurylalkohol und Tetrahydrophthalan gaben positive Reaktion.

Umkrystallisieren aus Alkohol schmolz die farblose Substanz zwischen 43—44°. Zwecks Feststellung des genauen Siedepunktes wurde sie erneut destilliert und hatte dann den $Sdp._9$ 103—104°. Nach den ermittelten Kennzahlen, den elementaranalytischen Werten und dem Ergebnis der Molekulargewichtsbestimmung in Verbindung mit der Eigenschaft, beim Erwärmen mit Mineralsäuren Formaldehyd abzugeben, handelt es sich ohne Zweifel um den *2,4-Methylenäther des Butantriol-monoacetates.*

Die »hochsiedende Verbindung» erwies sich als *Butantriol-(1,2,4)-triacetat.* Die erneute Analyse führte zu der Summenformel $C_{10}H_{16}O_6$. Im Siedepunkt und in der Dichte stimmt die Verbindung mit den von Pariselle angegebenen Werten überein. Sie liess sich zum freien Butantriol-(1,2,4) umestern. Auch dieses zeigte befriedigende Übereinstimmung mit den von Pariselle bzw. von Fichter und Herndl bzgl. Siedepunkt, Brechungsindex, Dichte und Molrefraktion angegebenen Konstanten. Für das Trisphenylurethan (Schmp. 152—154°) konnten die Angaben dieser Autoren bestätigt werden. Das Butantriol-triacetat liess sich durch Destillation mit einer geringen Menge konzentrierter Schwefelsäure unter vermindertem Druck in ca. 40 % Ausbeute in das 3-Acetoxytetrahydrofuran überführen.

Zur Umsetzung des Allylacetates mit Formaldehyd wäre allgemein zu bemerken, dass es nicht erforderlich ist, das Allylacetat zuerst in Substanz darzustellen. In späteren Versuchen haben wir das Veresterungsgemisch aus annähernd äquimolekularen Mengen Allylalkohol und Essigsäureanhydrid direkt zur Umsetzung benutzt. In diesen Fällen wurde Allylalkohol mit einem geringen Überschuss an Essigsäureanhydrid auf 60° erwärmt. Die Reaktion schritt dann unter Selbsterwärmung weiter fort, wobei die Flüssigkeit zuletzt ins Sieden geriet (nötigenfalls kühlen!). Nach Abklingen der exothermen Reaktion wurde das Gemisch 1/2 Stunde gekocht und nach mässigem Erkalten in eine warme Lösung von Paraformaldehyd in Eisessig-Schwefelsäure eingetragen. Die Weiterbehandlung geschah wie im experimentellen Teil angegeben. Trotz Anwendung eines Überschusses an Formaldehyd erhielten wir bei allen Ansätzen stets beträchtliche Mengen unverändertes Allylacetat zurück, das dann in Nebenversuchen erneut mit Paraformaldehyd umgesetzt wurde. Die erzielten Ausbeuten an 3-Acetoxytetrahydrofuran und Butantriol-(1,2,4)-triacetat betrugen in Hauptversuchen durchschnittlich etwa je 15 % der Theorie, ohne Berücksichtigung der mittel- und hochsiedenden Fraktionen. Durch Anwendung etwas grösserer Schwefelsäuremengen lässt sich die Bildung der Methylenäther-Verbindung IV zugunsten der Bildung von Acetoxytetrahydrofuran und Triacetat weitgehend zurückdrängen [12]. — Über die Natur der bei der Umsetzung entstehenden ungesättigten Verbindungen können gegenwärtig noch keine sicheren Aussagen gemacht werden. Die Fraktionen

2.) bis 5.) ($Sdp._9$ 72—96°) könnten dem Siedepunkt nach möglicherweise 3-Acetoxymethyl-dihydrofuran (III) oder Erythrol-diacetat (II) enthalten. Eine nähere Untersuchung hierüber ist in Aussicht genommen. Zu der an sich bekannten Darstellung des Allylalkohols aus Glycerin und Ameisensäure haben wir einen Weg gewählt, der in der Literatur unseres Wissens nicht, oder jedenfalls nicht zusammenhängend, beschrieben worden ist. Es erschien uns daher am Platze, im experimentellen Teil eine kurze Beschreibung zu geben.

EXPERIMENTELLER TEIL

Allylalkohol

An einer 90 cm langen Raschigkolonne mit Destillieraufsatz und angeschlossenem Liebigkühler wurden 1890 g Glycerin mit 1000 ml 85 %iger technischer Ameisensäure im Ölbad langsam auf Siedetemperatur erhitzt. Bei einer Badtemperatur von 194° begann die Flüssigkeit zu destillieren. Die Destillationstemperatur stieg sofort auf 102°. Sie hielt sich hier bei langsam ansteigender Badtemperatur völlig konstant, um dann plötzlich auf 101° abzufallen. Das Destillat, das sich noch kurz vorher gegen eine Brom-Eisessig-Lösung als gesättigt erwies, begann nach dem Temperaturabfall sofort Bromlösung zu entfärben. Das gesättigte Destillat wurde verworfen und das ungesättigte für sich aufgefangen (813 g), wobei die Destillationstemperatur von 101° allmählich auf 35° zurückging. Durch Regelung der Badtemperatur sorge man dafür, dass die Destillationstemperatur nicht über 100° steigt. Erst gegen Schluss erhöhe man die Badtemperatur beträchtlich, um die Spaltung in Allylalkohol bzw. Allylformiat möglichst vollständig zu machen und diese aus dem Reaktionsgemisch völlig zu entfernen. — Nun wurden erneut 1000 ml Ameisensäure zugefügt und die Destillation fortgesetzt. Bei 104° destillierte zunächst wieder eine völlig gesättigte Flüssigkeit, die verworfen wurde. Sobald die Destillationstemperatur auf 100° gefallen war, ging ungesättigtes Destillat über (636 g), wobei die Destillationstemperatur wieder allmählich auf 20° zurück ging (Badtemp. stieg bis ca. 300°). Es wurden noch einmal 600 ml Ameisensäure zugegeben und die Destillation wie beschrieben fortgesetzt. Man erhielt so weitere 100 g ungesättigtes Destillat. Die Menge des Destillationsrückstandes betrug 220 g. Die ungesättigten Fraktionen wurden vereinigt und mit einer Lösung von 300 g Kaliumhydroxyd in 200 g Wasser 4 Stunden unter Rückflusskühlung verseift. Das Verseifungsgemisch destillierte man an der Raschigkolonne (konstant bei ca. 88°!), bis die Destillationstemperatur ziemlich schnell auf 98° stieg. Das Destillat versetzte man mit 100 g Kaliumhydroxyd, kochte 2 Std. unter Rückflusskühlung und destillierte erneut an der Raschigkolonne, wobei der Hauptanteil konstant bei 88—89°/752 mm überging. Zwecks Entwässerung wurde das Destillat unter Kühlung vorsichtig mit 900 g gebranntem Kalk versetzt (kräftige Reaktion!) und dann an der Kolonne destilliert. Man erhielt 871 g Allylalkohol vom $Sdp._{752}$ 92—93° (73 % der Theorie).

Umsetzung des Allylacetates mit Formaldehyd

I. *Hauptansatz:* Eine Mischung aus 250 g Paraformaldehyd, 800 ml Eisessig und 15 ml konz. Schwefelsäure wurde unter Rückflusskühlung bis zur völligen Auflösung des Paraformaldehyds erwärmt. Nach mässigem Abkühlen gab man durch den Kühler 594 g

Allylacetat hinzu und erhitzte die zunächst farblose Lösung 4 Std. zu schwachem Sieden, wobei sich diese allmählich verfärbte und die in der Flüssigkeit gemessene Temperatur von 103° auf 113,5° anstieg und hier konstant blieb. Das schwarzbraune Reaktionsgemisch versetzte man (zur Neutralisation der Schwefelsäure) mit 35 g wasserfreier Soda und destillierte zunächst bei gewöhnlichem Druck 1070 g einer farblosen Flüssigkeit (J. Z. 53,8) ab, die vorwiegend Essigsäure, daneben aber eine bedeutende Menge nicht umgesetztes Allylacetat und wahrscheinlich auch etwas Dihydrofuran enthielt (Destillat *A*). Führte man die gefundene Jodzahl nur auf die Anwesenheit von Allylacetat zurück, ergäbe sich für das Destillat ein Gehalt von 21,2 % = 227 g Allylacetat. Demnach hätten 61,8 % Allylacetat an der Reaktion teilgenommen. Dann wurden bei 15 mm Druck 525 g einer fast farblosen Flüssigkeit zwischen 59—215° abdestilliert (Destillat *B*). Als Rückstand hinterblieben etwa 50 ml eines dickflüssigen, mit Natriumsulfat vermischten Öles, das unberücksichtigt blieb. Das Destillat *B* wurde durch zweimalige Destillation an der Kolonne in folgende Fraktionen zerlegt:

Frakt. Nr.	Sdp. °C 9 mm	Mittl. Sdp. bzw. Konst. p. °C	Ausbeute g	V. Z.	J. Z.
1.	60—65—(72)*	62,5	36	442,5	7,9
2.	72—[73]*—79	73	8	429,3	46,5
3.	[79]—83	79	11	419,4	57,5
4.	83—84—(87)	84	10	347,6	43,0
5.	(87)—[91—92]—96	92	20	312,2	54,0
6.	96—100	98	18	324,3	14,9
7.	100—[103]—(106)	103	20	341,5	9,0
8.	106—117	112	5	336,5	21,6
9.	117—139	128	5	436,8	19,2
10.	139—144	142	8	537,4	8,7
11.	144—[145—147]	145,5	152	590,5	1,9
12.	147—184	166	15	510,4	2,1
13.	184—198	191	7	382,7	15,7
14.	198—214	206	30	372,1	6,5
15.	214—225	220	17	300,0	2,2
16.	225—230	227,5	15	369,4	2,7
17.	230—235	232,5	10	387,4	3,6
18.	Rückstand		32	358,3 (?)	7,7

* Es bedeuten eckige Klammern Temperatur-Konstanzpunkte, runde Klammern unbedeutende Mengen.

Die aus den Fraktionen 7 und 8 durch Absaugen gewonnene krystalline Substanz wurde mehrfach aus Alkohol umkrystallisiert. Schmp. 43—44°. $Sdp._9$ 103—104°, V. Z. 352,2, J. Z. 0, Molekulargewicht nach Beckmann (Benzol) gef. 156. Beim Erwärmen der Substanz mit einigen Tropfen Schwefelsäure tritt Geruch nach Formaldehyd auf. Es handelt sich um den *2,4-Methylenäther des Butantriol-1-monoacetates* (Theorie: V. Z. 350,2, J. Z. 0, Molgew. 160,2.

$C_7H_{12}O_4$ (160,2)	Ber.	C	52,48	H	7,55
	Gef.	»	52,41	»	7,06

Durch Feinfraktionierung des Hauptansatzes und weitere Aufarbeitung wurden folgende Substanzen erhalten:

3-Acetoxytetrahydrofuran. $Sdp._{10}$ 62°. Durch Verseifung mittels alkoholischer Kalilauge bzw. durch Umesterung mit methylalkohol. Salzsäure erhielt man daraus das *3-Oxytetrahydrofuran.* $Sdp._{16}$ 84—86°, farbl. Flüssigkeit,
$n_D^{19.1-} = 1{,}4431$, $D_4^{19.1} = 1{,}087$, Mol-Refr. 21,49*.

Pariselle gibt an: $Sdp._{13}$ 81—82°, $n_D^{20} = 1{,}4478$, $D_0 = 1{,}07$, Mol-Refr. 21,91 (ber. 21,61). Das *Phenylurethan* schmolz in Übereinstimmung mit den Angaben von Pariselle bei 120°. Amidzahl des Phenylurethans [13] gef. 264,5, ber. 270,8.

$C_{11}H_{13}O_3N$ (207,2)	Ber.	C	63,75	H	6,32	N	6,76
	Gef.	»	63,95	»	6,25	»	6,74

Butantriol-(1,2,4)-triacetat. $Sdp._{10}$ 146—147°, ziemlich dickfl. farbl. Öl, $D_4^{23.2} = 1{,}1375$, $n_D^{23.3} = 1{,}4421$. Pariselle gibt an: $Sdp._{11}$ 150°, $D_0 = 1{,}152$.

Frühere Analyse wurde wiederholt:

$C_{10}H_{16}O_6$ (232,2)	Ber.	C	51,72	H	6,93
	Gef.	»	51,92	»	7,14

Daraus durch Umesterung mit methylalkoholischer Salzsäure:

Butantriol-(1,2,4). $Sdp._{11}$ 167—168°, dickfl. Öl, $n_D^{19.6} = 1{,}4798$, $D_4^{19.6} = 1{,}1772$, Mol-Refr. 25,56 *. Pariselle gibt an: $Sdp._{13}$ 179°, $n_D^{20} = 1{,}47$, $D^{20} = 1{,}18$, Mol-Refr. 25,07 (ber. 25,07). Fichter und Herndl geben den $Sdp._{12}$ 172—174° an.

Trisphenylurethan Schmp. 152—154° (nach Pariselle Schmp. 149—152°, nach Fichter und Herndl Schmp. 153—155°).

$C_{25}H_{25}O_6N_3$ (463,5)	Ber.	C	64,78	H	5,44	N	9,07
	Gef.	»	64,68	»	5,34	»	9,25

II. *Nebenansatz:* Das Destillat *A* wurde zwecks Umsetzung des vorhandenen Allylacetates mit 50 g Paraformaldehyd (Unterschuss!) und 10 ml konz. Schwefelsäure 6 Std. unter Rückflusskühlung gekocht. Die schwarzbraune Flüssigkeit wurde nach Zugabe von 25 g wasserfreier Soda zunächst wieder bei gewöhnlichem Druck destilliert, wobei zwischen 100—119° 700 g Destillat (J. Z. 30,0) übergingen (Destillat *C*, Rückstand *D*). Destillat *C* wurde an der Raschigkolonne bei gewöhnl. Druck destilliert. Aufgefangen wurden ca. 130 ml zwischen 62—102° und ca. 200 ml vom Sdp. 102—108°. Beide Fraktionen wurden vereinigt, mit gesättigter Soda- und Natriumsulfatlösung in üblicher Weise gewaschen und die obere organische Schicht über wasserfreiem Natriumsulfat getrocknet. Die Destillation ergab: 1) 7 g vom Sdp. 61—80°, 2) 45 g vom Sdp. 80—104°. Die Fraktion 1) enthält neben Allylacetat vielleicht Dihydrofuran, während die Fraktion

* Für die Ausführung der phys.-chem. Messungen sind wir Herrn Dr. J. Hveding und Frl. Karen Mortensson-Egmund zu Dank verpflichtet.

2) vorwiegend aus ersterem besteht. Die Destillation des Rückstandes *D* führte zu folgenden Fraktionen: 1) Sdp.$_9$ 27—61°, 5 g, 2) Sdp.$_9$ 61—75°, 18 g, 3) Sdp.$_9$ 75—85°, 10 g 4) Sdp.$_9$ 85—95°, 24 g, 5) Sdp.$_9$ 95—142°, 10 g, 6) Sdp.$_9$ 142—148°, 55 g; 7) Rückstand ca. 10 ml rotes Öl. Die Fraktion 2) besteht im wesentlichen aus 3-Acetoxytetrahydrofuran, die Frakt. 6) im wesentlichen aus Butantriol-(1.2.4)-triacetat.

Überführung von Butantriol-(1,2,4)-triacetat in 3-Acetoxytetrahydrofuran

120 g Butantriol-triacetat (Sdp.$_{10}$ 146—147°) wurden mit 9 ml konz. Schwefelsäure (in drei Portionen) an der Widmerspirale unter vermindertem Druck destilliert. Man gewann 76 g farbloses Destillat vom Sdp.$_{10}$ 23—86° neben 35 g Rückstand (schwarze, blanke Krusten). Bei der Fraktionierung erhielt man 27 g einer farblosen Flüssigkeit, die zum grössten Teil konstant beim Sdp.$_9$ 59° überging. Ausbeute ca. 40 % der Theorie an Acetoxytetrahydrofuran.

Überführung von 3-Acetoxytetrahydrofuran in Butantriol-(1,2,4)-triacetat

155 g 3-Acetoxytetrahydrofuran (Sdp.$_9$ 56—58°) wurden mit 250 ml Essigsäureanhydrid und ca. 2 g Zinkchlorid 5 Std. unter Rückflusskühlung gekocht. Das dunkle Reaktionsgemisch destillierte man an der Widmerspirale, wobei zunächst eine beträchtliche Menge unverändertes Ausgangsmaterial, dann aber 122 g Butantriol-triacetat bei 144—145°/8 mm (V. Z. 714,8, Theorie 724,8) übergingen.

ZUSAMMENFASSUNG

Bei der Umsetzung von Formaldehyd mit Allylacetat in Eisessig-Schwefelsäure entstehen 3-Acetoxytetrahydrofuran, Butantriol-(1,2,4)-triacetat und Butantriol-2,4-methylenäther-1-monoacetat. Es wurde ferner gezeigt, dass sich bei der Reaktion höhersiedende ungesättigte Verbindungen bilden, die wahrscheinlich erneut mit Formaldehyd reagieren können und so als Zwischenglieder eines stufenweise erfolgenden, weitergehenden Molekülaufbaus anzusehen sind.

Mein wärmster Dank gebührt *Norges Teknisk-Naturvitenskapelige Forskningråd* und dem *Fridtjof Nansen-Fond* für finanzielle Unterstützung. Herzlich danken möchte ich auch den Herren Professoren E. Berner, H. Haraldsen und O. Hassel für die liebenswürdige Überlassung von Apparaturen und Chemikalien. Frl. Birgit Sanne bin ich für die Ausführung der Kennzahl-Analysen, Herrn stud.mag. scient. Gunnar Aksnes für gelegentliche geschickte Hilfe bei der Ausführung der Versuche verbunden.

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Eingegangen am 27. Januar 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 473 – 477

Über die Umsetzung von Formaldehyd mit Allylacetat *

II. Eine neue Synthese des Furans und Dihydrofurans

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Die meisten Darstellungsverfahren für Furan gehen bekanntlich vom Furfurol bzw. von der Brenzschleimsäure aus. Eine aufbauende Synthese mit dem Ziele der Furangewinnung ist unseres Wissens bisher nicht beschrieben worden. An aufbauenden Bildungsweisen finden sich in der Literatur folgende Angaben: Tschitschibabin [1] leitete einerseits ein Gemisch von Acetylen und Wasserdampf, andererseits Acetaldehyd über erhitztes Aluminiumoxyd und erhielt dabei, neben anderen Verbindungen, Furan bzw. dessen Homologe. Nach einem französischen Patent der Chemischen Fabrik Rhenania [2] entsteht beim Überleiten von Acetylen über einen erhitzten Raseneisenerz-Katalysator ein Öl, das Aldehyde, Ketone, Essigsäure und Homologe, Furan und dessen Derivate, usw. enthalten soll. Reppe [3] erwähnt in seiner Monographie die Entstehung von Furan als Nebenprodukt bei der Hydrierung von Butin-(2)-diol-(1,4) und bei der Dehydrierung von Buten-(2)-diol-(1,4). Durch Wasserabspaltung aus dem letzteren gelangte er auch zum 2,5-Dihydrofuran [3,4]. Valette [5] studierte die Wasserabspaltung aus Buten-(2)-diol-(1,4) näher und fand, dass dessen *trans*-Form ausschliesslich zum Crotonaldehyd führt, während die bei der Hydrierung von Butin-(2)-diol-(1,4) entstehende *cis*-Form neben 65 % Crotonaldehyd 35 % Dihydrofuran liefert. Von den wenigen aus der Literatur ersichtlichen Darstellungsweisen für Dihydrofuran sei nur diejenige von Pariselle [6] erwähnt, der dieses aus Dibrom-(1,2)-butanol-(4) durch Behandlung mit Kaliumhydroxyd über die Zwischenstufe des 3-Bromtetrahydrofurans gewann. Durch Addition von Brom an Dihydrofuran gelangte er zum 3,4-Dibromtetrahydrofuran [7], das er durch Behandeln mit Bromwasserstoff in das Erythrentetrabromid [8] überführte.

* 6. Mitt. über Formaldehyd-Olefin-Reaktionen.

Anlass zu der vorliegenden Untersuchung gab die Notwendigkeit der Beschaffung von Dihydrofuran, das vermutlich ein Nebenprodukt bei der Umsetzung des Allylacetates mit Formaldehyd [9] darstellt und das zur weiteren Aufklärung der Aufbaureaktion in grösserem Massstabe mit Formaldehyd umgesetzt werden sollte. Da uns die Reppesche Synthese, besonders nach den Erfahrungen Valettes, für die laboratoriumsmässige Darstellung nicht sonderlich geeignet erschien, lag es für uns nahe, das bei der erwähnten Allylacetat-Formaldehyd-Umsetzung anfallende 3-Acetoxytetrahydrofuran als Ausgangsmaterial heranzuziehen. Um dieses in möglichst grosser Ausbeute zu erhalten, veränderten wir, wie im experimentellen Teil beschrieben, die Versuchsbedingungen so, dass die Reaktion praktisch nur zum 3-Acetoxytetrahydrofuran führte, wobei allerdings erhebliche, durch Polymerisationsvorgänge bedingte Verluste nicht zu verhindern waren. Wegen dieser Polymerisationsvorgänge führte die katalytische Essigsäureabspaltung, die wir für die Darstellung von Dihydrofuran zuerst in Aussicht genommen hatten, und die, wie früher gezeigt wurde, beim Hexahydrophthalanylacetat [10] mit konzentrierter Schwefelsäure leicht gelang, beim 3-Acetoxytetrahydrofuran zu keinem befriedigenden Erfolg. Wir versuchten daher, die Essigsäureabspaltung auf thermischem Wege zu erreichen. Bei einer Kolonnentemperatur von etwa 570° erhielten wir aber — statt des erwarteten Dihydrofurans — *Furan*, das durch Diensynthese mit Maleinsäureanhydrid in das Δ^4-Dehydro-norcantharidin [11] übergeführt wurde. Dieses stimmte in allen Eigenschaften mit einem Vergleichspräparat überein. Durch Anwendung eines wirksamen Kühlaggregates konnte die Ausbeute an Furan beim Crockprozess auf etwa 85 % der Theorie gesteigert werden.

Bei der hohen Kolonnentemperatur ist die Reaktion offenbar über das beabsichtigte Ziel hinausgeschossen, indem neben der Essigsäureabspaltung Dehydrierung erfolgte. Als wir daraufhin bei sonst gleicher Versuchsanordnung die Kolonnentemperatur auf ca. 430° senkten, erhielten wir neben etwas Furan *Dihydrofuran* in etwa 70 % Ausbeute, bezogen auf umgesetztes Ausgangsmaterial. Das Dihydrofuran wurde zwecks Identifizierung auf dem von Pariselle angegebenen Wege in das 3,4-Dibromtetrahydrofuran und dieses in das Erythrentetrabromid übergeführt.

EXPERIMENTELLER TEIL

(Unter Mitarbeit von Gunnar Aksnes)

Darstellung des 3-Acetoxytetrahydrofurans

Eine Mischung aus 871 g Allylalkohol und 1603 g Essigsäureanhydrid wurde auf 60° erhitzt. Die Veresterung zum Allylacetat erfolgte unter kräftiger Selbsterwärmung, so dass gelegentlich gekühlt werden musste. Nach Beendigung der exothermen Reaktion

wurde 1/2 Stunde unter Rückflusskühlung gekocht. Nach mässigem Erkalten fügte man 665 g Paraformaldehyd und 40 ml konz. Schwefelsäure hinzu und erwärmte langsam auf 95°, bei welcher Temperatur eine kräftige Spontanreaktion eintrat, so dass das Erhitzen von aussen unterbrochen werden musste. Infolge der frei werdenden Reaktionswärme hielt sich das zunehmend dunkler werdende Gemisch im Sieden, wobei die in der Flüssigkeit gemessene Temperatur im Laufe von etwa 1/2 Stunde auf 108° anstieg. Das Reaktionsgemisch wurde nun 1/2 Stunde von aussen zum Sieden erhitzt. Dann destillierte man bei gewöhnlichem Druck 1570 g einer vorwiegend aus Essigsäure und Allylacetat bestehenden farblosen Flüssigkeit zwischen 90—114° ab (Destillat *A*, J. Z. 64,5). Der Rückstand wurde ohne vorherige Neutralisation der Schwefelsäure an der Widmerspirale bei 10 mm Druck destilliert, wobei 686 g Destillat zwischen 21—70° übergingen (Destillat *B*). Im Kolben hinterblieben 735 g eines schwarzen, festen Polymerisates. — Das Destillat *A* wurde mit 180 g Paraformaldehyd und 25 ml konz. Schwefelsäure 6 Stunden gekocht, wobei die Temperatur der siedenden Flüssigkeit von 88° auf 102° anstieg. Das schwarzbraune Reaktionsgemisch wurde wie vorstehend beschrieben behandelt, wobei man zunächst 1286 g »Essigsäure-Allylacetat-Mischung» zwischen 92—117° abdestillierte (J. Z. 32,8). Bei der Destillation des Rückstandes an der Widmerspirale erhielt man 217 g Destillat vom $Sdp._{10}$ 22—75° (Destillat *C*) und 270 g Rückstand (schwarzes, festes Polymerisat). Die Destillate *B* und *C* wurden vereinigt und 2mal an der Raschigkolonne destilliert. Man erhielt 305 g *reines* 3-Acetoxytetrahydrofuran vom $Sdp._8$ 58,5° = ca. 16 % der Theorie.

Überführung von 3-Acetoxytetrahydrofuran in Dihydrofuran und Furan

In der früher für die thermische Essigsäureabspaltung aus Trimethylenglykoldiacetat benutzten Apparatur [12] wurde 3-Acetoxytetrahydrofuran einmal bei einer Kolonnentemperatur von ca. 430° C, das andere Mal bei einer Kolonnentemperatur von ca. 570° im schwachen Stickstoffstrom bzw. in Stickstoffatmosphäre bei gewöhnlichem Druck destilliert. An Stelle der früher verwendeten *einen* Vorlage B wurden 4 hintereinander geschaltete Vorlagen benutzt, deren erste mit Eis-Kochsalz, die drei folgenden mit Kohlensäure-Aceton gekühlt waren. Bei richtiger Regelung der Badtemperatur und des Stickstoffstromes (die Zuleitung von Stickstoff kann ganz unterbrochen werden, sobald die Destillation richtig in Gang gekommen ist!) gelingt es leicht, die entstehenden Nebel im Kühlaggregat völlig zu verdichten. Bei einer Kolonnentemperatur von ca. 430° C entstand neben etwas Furan vorwiegend Dihydrofuran, bei einer Kolonnentemperatur von ca. 570° C fast nur Furan. Die Protokolle beider Versuche werden nachstehend wiedergegeben:

1. Kolonnentemperatur 420—430° C

Nachdem die Luft aus der Apparatur völlig durch Stickstoff vertrieben war, wurden 305 g 3-Acetoxytetrahydrofuran ($Sdp._8$ 58,5°) ohne Einleiten weiteren Stickstoffs im Verlaufe von 10 Stunden bei einer Badtemperatur von 185—192° destilliert. Die Destillationstemperatur bewegte sich dabei zwischen 60—120°. Erst als der Destillierkolben leer war, wurde die Apparatur mit Hilfe eines schwachen Stickstoffstromes durchgespült. Gesamtmenge des Destillates 295 g. Dieses wurde an der Widmerspirale bei gewöhnlichem Druck destilliert, wobei die Vorlage beim Übergehen der niedrigsiedenden Anteile mit

Kohlensäure-Aceton, später mit Eiswasser gekühlt wurde. Aufgefangen wurden folgende Fraktionen:

1.	Sdp.	21—[34]*—49°	25,5 g (Fichtensp.rk.stark positiv!)
2.	»	49—[62,5]—65°	54,3 g
3.	»	65—70°	19,3 g
4.	»	70—(105°)*	20,5 g
5.	»	105—(150°)	120,0 g
6.	»	150—176°	30,8 g
7.	Rückstand		19,6 g gelbe Flüssigkeit

* Es bedeuten eckige Klammern Temperatur-Konstanzpunkte, runde Klammern unbedeutende Mengen.

Nimmt man an, dass die Fraktion 1. überwiegend aus Furan, die Fraktionen 2.—4. überwiegend aus Dihydrofuran, die Fraktion 5. vorwiegend aus Essigsäure und die Fraktionen 6. und 7. aus unverändertem Ausgangsmaterial bestehen, so ergibt sich, bezogen auf 254,6 g umgesetztes Acetoxytetrahydrofuran, eine Ausbeute von 68,8 % Dihydrofuran und 19,1 % Furan.

3,4-Dibrom-tetrahydrofuran

11 g der vorstehend bezeichneten Fraktion 2. (Sdp. 49—[62,5]—65°) wurden in 10 ml Chloroform gelöst und unter Kühlung mit einer Lösung von 8 ml Brom in 15 ml Chloroform versetzt. Die Bromlösung wurde sofort entfärbt und fast vollständig verbraucht. Die Destillation ergab 26 g einer Flüssigkeit vom $Sdp._{10}$ 80—83°, die in Eis-Kochsalz-Mischung, wie von Pariselle für Dibrom-tetrahydrofuran angegeben, krystallisierte. $D_4^{20} = 2{,}0717$, $n_D^{21} = 1{,}5502$. Die in der Kälte erstarrte Substanz wurde bei 5° C auf Ton getrocknet. Schmp. 13—14° (Pariselle gibt 10—11° an).

1,2,3,4-Tetrabrombutan (Erythrentetrabromid)

Nach der Vorschrift von Grimaux und Cloez (*l. c.*) wurden 13 g Dibrom-tetrahydrofuran (vorstehend beschriebenes Präparat) mit 20 ml rauchender Bromwasserstoffsäure ($D = 1{,}7$) im Einschlussrohr 6 Stunden im Schiessofen auf 123° erhitzt. Das dunkelbraune Öl wurde im Vakuum destilliert. Aus dem fast farblosen Destillat (13 g, Sdp. 156°/10 mm), das beim Einstellen in eine Eis-Kochsalz-Mischung nicht krystallisierte, schieden sich nach Alkoholzusatz farblose Krystalle aus, die nach Umkrystallisieren aus Alkohol zwischen 117—118° schmolzen. Pariselle gibt für 1,2,3,4-Tetrabrombutan den gleichen Schmelzpunkt an.

$C_4H_6Br_4$ (373,8)	Ber.	C	12,85	H	1,62
	Gef.	»	13,16	»	1,72

2. Kolonnentemperatur 570—580°

50 g 3-Acetoxytetrahydrofuran wurden wie unter 1. beschrieben bei einer Kolonnentemperatur von 570—580° innerhalb von 4 Stunden destilliert. Die Badtemperatur bewegte sich hierbei zwischen 175—257°, die Destillationstemperatur zwischen 36—70°.

Gesamtmenge des Destillates 40,6 g. Dieses wurde an der Widmerspirale bei gewöhnlichem Druck destilliert (Vorlage gekühlt mit Kohlensäure-Aceton):

1.	Sdp.	(24)—[32°]	22,0 g Fichtenspanrkt. stark positiv. Fast reines Furan.
2.	»	32 —103°	1,5 g
3.	»	103 —105°	15,0 g
4.	Rückstand	ca. 5 ml Öl	

Δ^4-Dehydronorcantharidin

(3,6-Endoxo-Δ^4-tetrahydro-phthalsäureanhydrid)

8 g frisch destilliertes Maleinsäureanhydrid wurden nach Diels und Olsen in 5 ml Dioxan mit 10 g Furan (vorstehend erwähnte Fraktion 1.), Sdp. (24)—[32°]) umgesetzt. Man erhielt 8,5 g farblose Krystalle, die — mehrfach aus Aceton umkrystallisiert — im zugeschmolzenen Röhrchen bei 115—122° (unter Zers.) schmolzen. Mischprobe mit einem authentischen Präparat ebenso.

ZUSAMMENFASSUNG

Das aus Allylacetat und Formaldehyd leicht zugängliche 3-Acetoxytetrahydrofuran lässt sich durch Cracken — je nach der Höhe der angewendeten Temperatur — in guter Ausbeute wahlweise in Furan oder Dihydrofuran überführen.

Norges Teknisk-Naturvitenskapelige Forskningsråd und dem *Fridtjof Nansen-Fond* danke ich für finanzielle Unterstützung.

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Eingegangen am 27. Januar 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 478 – 486

Investigations on Metaphosphate of High Molecular Weight Isolated from *Aspergillus niger*

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In some previous articles we have shown the occurrence of enzymes breaking down metaphosphate of high molecular weight [1-4]. Such enzymes were found in moulds, yeasts and the bacterium *Proteus vulgaris*. In these enzyme experiments synthetic metaphosphates were used as substrates having molecular weights of more than one million. Thus an inorganic colloid was broken down by enzymatic means. As these microorganisms have enzymes which break down metaphosphates of high molecular weights one might perhaps expect to find compounds of similar character in these organisms.

In the latter years of the 19:th century investigations indicating the occurrence of metaphosphate in yeast were performed by Liebermann [5-7], Kossel [8] and Ascoli [9]. These earlier investigations were forgotten for a long time, until in 1936 MacFarlane [10] isolated a nucleic acid preparation of high phosphorus content from yeast. Extracting this substance with hydrochloric acid MacFarlane obtained inorganic metaphosphate. Later Wiame [11] also isolated from yeast such substances containing 17 per cent P. During the last few years the occurrence of metaphosphate in yeast has been further confirmed [12-15]. The metaphosphate in yeast seems to be of two kinds, one acid soluble and one acid insoluble [13,14]. The two forms seem to differ in their metabolic function. Metaphosphate has also been found in different mutants of *Neurospora* [16]. Inorganic metaphosphate has also been isolated from the mould *Aspergillus niger* by Mann in 1944 by extracting the mycelium with trichloro acetic acid [17].

In the previous literature no molecular weight determinations on the metaphosphate found in microorganisms are described. During our studies on the enzymatic breakdown of synthetic metaphosphates of very high molecular weights we raised the question if not at least a fraction of the naturally occurring

metaphosphate, for instance that found in *A. niger*, could be of colloidal nature [1]. However, if the mould contains metaphosphate of high molecular weight, it must be difficult to isolate this substance in an undegraded and water soluble form. The mould *A. niger* has an enzyme which very quickly breaks down the metaphosphate within the pH-interval 4—7. Besides, both at low and high pH-values the colloidal metaphosphate is a labile substance because of the high rate of the spontaneous breakdown. Therefore, when trying to isolate metaphosphate of high molecular weight from *A. niger* one must work out a suitable method for removing the enzyme and other organic substances without breaking down the metaphosphate.

Applying the following technique non-dialyzable substances of high molecular weights and of high phosphorus content were isolated [18,19]. *A. niger* was extracted at a slightly alkaline pH. The extract was then filtered through large amounts of active carbon in order to remove the enzyme and other organic material. Finally the solution was dialyzed in cellophane bags, evaporated to a small volume *in vacuo*, filtered through active carbon, dialyzed again and dried in vacuo in the frozen state. One of the preparations, isolated as sodium salt, contained 25 per cent P. The ash content was 85 per cent. The characteristic absorption of nucleic acids in ultraviolet light could not be observed. The sedimentation constants were 2.2 and 2.3 Svedberg units for 1.5 and 0.5 per cent solutions. Another of these sodium salts of high molecular weight isolated from *A. niger* contained 29.3 per cent P. The ash content was more than 90 per cent. The sedimentation constant was 2.3 S for a 0.8 per cent solution.

PREPARATION METHODS

For the following experiments we have used the same culture of *Aspergillus niger* as before. (*A. niger* v. Tiegh. no. 594 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council.) The mould was cultivated under sterile conditions in Petri dishes (diameter 20 cm) for one or two weeks at about 25° C on a medium of the following composition: 225 g pure saccharose, 75 g crude saccharose, 6 g K_2HPO_4, 12 g $NaNO_3$, 1 g $MgSO_4$, $7H_2O$ and 2700 ml water.

In the experiments reported in this article we have used the same preparation method as described in the two previous papers [18,19] and also some modifications of it. In the previous investigations blank determinations showed that the washed active carbon did not contain metaphosphate, but in order to be absolutely sure that no polymetaphosphate would be introduced in the preparations on the addition of active carbon, this was treated as follows:

The active carbon was kept in 0.3 *N* hydrochloric acid for some days, washed with distilled water and then with 5 per cent sodium or potassium carbonate solution and then with distilled water.

Four somewhat different preparation procedures have been used. (Preparations A—D.) A synthetic metaphosphate was also prepared in order to be used as a reference

substance. (Preparation E.) In the first preparation (A) the sodium salt was isolated as before; however, since sodium metaphosphate is a hygroscopic substance, in the later preparations (B—E) we have preferred to prepare instead potassium metaphosphate, less hygroscopic and easier to isolate.

Preparation A

1. Mould from 20—30 dishes cut in pieces.
2. Suspended in 900 ml 1 % sodium carbonate solution.
3. 15 min. at 80° C (in order to destroy the enzyme).
4. Ground in "Waring Blendor" with carbon and 300 ml 5 % sodium carbonate solution.
5. Filtered through filter paper.
6. Solution filtered through large amounts of active carbon.
7. pH of the filtrate adjusted to about 8.
8. Solution dialyzed in cellophane bags against distilled water for some days at 4° C.
9. Solution evaporated in vacuo at 30—40° C to about 50 ml.
10. Sodium chloride added to concentrated solution and solution filtered through active carbon.
11. pH of the filtrate adjusted to about 8 with sodium carbonate. Solution dialyzed against distilled water.
12. Solution dried *in vacuo* in the frozen state. Yield from 25 dishes: 50 mg, 29.4 % P. Theoretical value for $(NaPO_3)_n$, 30.4 % P.

Preparation B

The first twelve steps as described in A were used, but potassium salts were used instead of sodium salts.

Twelve crude preparations containing about 10—20 per cent P were taken together (in all 170 mg) and purified in the following way:

1. Crude products dissolved in 50 ml 0.2 % KCl solution.
2. pH adjusted to 8 with potassium carbonate.
3. Solution filtered through active carbon.
4. Filtrate dialyzed in nitrocellulose bag against distilled water at 4° C.
5. Solution dried *in vacuo* in the frozen state.

Yield from 309 dishes (Preparation B 2): 75 mg, 25.9 % P.
Theoretical value for $(KPO_3)_n$, 26.3 % P.
(Another more impure preparation (B 1) contained 24.4 % P.)

Preparation C

The first twelve steps as described in A, were used, but potassium salts were used instead of sodium salts and further the step no. 3 was changed from warming during 15 min. at 80° C to freezing at — 15° C for one day in order to destroy the cell walls.

Five crude preparations containing about 10—20 % P were taken together (in all 610 mg) and purified as follows:

1. Crude products dissolved in 15 ml water and pH adjusted to 9 with potassium carbonate.
2. 10 ml 30 % hydrogen peroxide added.

3. After 3 hours the solution was diluted to 50 ml with distilled water and filtered through active carbon.
4. Filtrate dialyzed in nitrocellulose bag against distilled water at + 4° C.
5. Solution dried *in vacuo* in the frozen state.
   Yield from 104 dishes: 190 mg, 26.6 % P.

Preparation D

1. Mould from 20—30 dishes cut in pieces.
2. Suspended in 900 ml water, pH adjusted to 9 with potassium carbonate.
3. 100 ml 30 % hydrogen peroxide added in portions.
4. After 1 hour the mixture was ground in "Waring Blendor".
5. 100 ml 30 % hydrogen peroxide added in portions.
6. After 1 day filtered through filter paper.
7. Solution filtered through large amounts of active carbon.
8. pH adjusted to 8—9 with potassium carbonate, 5 ml hydrogen peroxide added.
9. Solution dialyzed in nitrocellulose bags against distilled water for some days at 4° C.
10. Solution evaporated *in vacuo* at 30—40° C to about 50 ml.
11. Potassium chloride added to concentrated solution and solution filtered through active carbon.
12. pH adjusted to 8—9 with potassium carbonate.
13. Solution dialyzed against distilled water.
14. Solution dried *in vacuo* in the frozen state.
    Preparation D 1, yield from 17 dishes, 97 mg, 27.0 % P.

Preparations E and K, synthetic reference substances

For comparision with the naturally occurring metaphosphate of high molecular weight in *A. niger* a metaphosphate, synthetically obtained by heating NaH_2PO_4, was prepared in the following way.

15 g of a commercial sodium metaphosphate was dissolved in 300 ml 0.8 *M* KCl solution. The solution was dialyzed for some days in a bag of nitrocellulose against 0.2 *M* KCl solution at 4° C. Then the solution was dialyzed for several days against distilled water. The solution was then evaporated *in vacuo* to about 50 ml and then dried in vacuo in the frozen state. The substance obtained (E) contained 26.6 % P.

Another potassium metaphosphate of very high molecular weight (more than one million) was obtained by heating KH_2PO_4 at a temperature of 500° C. This preparation (K) contained 26.3 % P.

CHEMICAL INVESTIGATIONS

In Table 1 the data of the chemical analyses of the obtained preparations are shown. The samples used for phosphorus analyses and determinations of ash content were previously dried at 120° C, whereas the substances used for physico-chemical measurements were kept over phosphorus pentoxide at room temperature as we did not want to heat the substances before these investigations.

Table 1. Some chemical analyses of the preparations.

Preparation	P %	Ash %	N %	Carbohydrate %	Ca/P max. value for soluble complex
A (Na salt)	29.4	96			
B2	25.9	(99)	0.2	Traces	
C	26.6	98.4			1/3.4
D1	27.0	97.2		0.6	1/3.3
E Synth. ref. subst.	26.6	99.5			1/3.2
K Synth. subst.	26.3				1/3.0
$(KPO_3)_n$ Theoretical values	26.3	100	0.0	0.0	

The phosphorus analyses of the crude preparations were performed colorimetrically after hydrolysis with sulphuric acid according to Lowry and Lopez [20]. The phosphorus contents of the final products were determined gravimetrically following the ammonium molybdate method as described in Treadwell [21].

The ash contents were determined by heating samples of the substances in a small platinum crucible at $\sim 700°$ C.

The nitrogen was determined according to the micro Kjeldahl method. Such determinations have been made only when substances have been available also for this purpose, as amounts of 50—70 mg were used in order to get accurate values. A solution of one of the preparations (C) was also investigated in the Beckman spectrophotometer but the characteristic absorption of nucleic acids in ultraviolet light could not be observed. The carbohydrate content was approximately estimated by the orcinol method [22] using the polysaccharide dextran for comparison.

A sensitive criterion on polymetaphosphates is their ability to form soluble complexes with bivalent metal ions, *e. g.* calcium, until a certain atomic ratio Me/P is exceeded, at which point a remaining precipitate is formed. (For

Table 2. Some physico-chemical data of the preparations.

Preparation	Conc. %	s	D_A	Molecular weight
A	1.7 0.6	2.2	 19	4000
B1	0.7	2.4		
B2	2.0 0.7	(1.5)	 29	(2000)
C	1.7 0.9	2.6	 13	7000
D1	2.1 0.8	2.6	 15	6000
E Synth. ref. subst.	1.0 0.7	2.4	 11	8000

further details reference is made to the review by Karbe and Jander [23].) This test was performed by adding 0.05 *M* calcium chloride solution to 50 ml of a neutral 0.08 per cent solution of the substance to be investigated until the formation of a remaining turbidity. The values obtained from the two preparations from *A. niger* are rather consistent with that of the reference substance.

Table 3. Electrophoretic mobility of the preparations at pH 6.7.

Preparation	Conc. %	Mobility 10^5 cm^2/volt sec.
B2	0.25	11.4
C	0.25	10.8
D1	0.25	10.3
E (synth. ref. subst.)	0.25 0.50	10.9 12.0

PHYSICO-CHEMICAL INVESTIGATIONS

Some physico-chemical investigations were also performed on these preparations. The results are given in Table 2 and 3.

The sedimentation constants were determined in the ultracentrifuge according to Svedberg [24]. The determinations were made in a buffer containing 0.025 *M* Na_2HPO_4, 0.025 *M* NaH_2PO_4 and 0.20 *M* NaCl. The values obtained for the sedimentation constants, *s*, are given in Svedberg units and refer to a temperature of 20.0° C and pure water as a solvent. The diffusion measurements were carried out in a cell according to Claesson [25] using Lamm's scale method [26]. The solvent was the same phosphate buffer as that used in the sedimentation measurements. The diffusion constants, D_A, calculated according to »the area method» [26], is given in units of 10^{-7} cm^2/sec. and referred to pure water of 20.0 °C. The sedimentation and diffusion experiments showed that the preparations obtained were of a polymolecular nature. The molecular weights were calculated with the aid of Svedbergs formula [24], $M = RTs/D(1-V\varrho)$. The partial specific volume of the solute, *V*, was assumed to be 0.32. This value was obtained for a synthetic potassium metaphosphate of high molecular weight dissolved in 0.4 *M* NaCl solution. (The value of *V* will be discussed in a future paper.) On account of the difficulty in determining low sedimentation constants with accuracy and on account of the polymolecularity of the substances, the molecular weights calculated give the orders of magnitude rather than accurate values. However, there is no doubt that the isolated substances are compounds of rather high molecular weights. It seems probable that the molecular weights are dependent on the preparation technique. The preparation methods A and B including heating at 80° C yielded substances of lower molecular weights, indicating a breakdown during the preparation.

The electrophoretic mobilities were determined in the Tiselius apparatus [27] at a temperature of 0.5° C. The experiments were performed in the same buffer (pH 6.7) as the sedimentation and diffusion measurements. As expected the substances moved toward the anode. The mobilities were calculated from the descending boundaries. At a concentration of 0.25 per cent the mobilities of the substances isolated from *A. niger* are consistent with that of the synthetic polymetaphosphate.

DISCUSSION

The analytical data for these non-dialyzable substances isolated from *A. niger* show that they are of inorganic character containing only traces of organic material and that the phosphorus content is the same as that of pure metaphosphate. Further, they form soluble complexes with calcium ions. These complexes are precipitated at the same atomic ratio Ca/P as the syn-

thetic metaphosphate of about the same molecular weight. It may also be mentioned that these substances give the same metachromatic staining with »toluidine blue» as the syntetic reference substance. (These staining experiments performed by B. Sylvén will be published later.) The substances have also the same electrophoretic mobility as the synthetic metaphosphate used for comparison. The ultracentrifuge and diffusion measurements show that the isolated substances have high molecular weights. Therefore, it can be concluded from the experiments reported here that at least a fraction of the metaphosphate occurring in *A. niger* is of high molecular weight.

The approximate molecular weights given in Table 2 are average values for the polymolecular substances. Therefore, metaphosphate molecules of higher weights than these average values are also present in the isolated substances. It may also be mentioned that it is possible that the native metaphosphate in *A. niger* has higher molecular weight than the isolated substances on account of some breakdown during the preparation.

It is not yet definitely established whether the metaphosphate itself or perhaps an organic complex containing metaphosphate represents the compound originally present in the cells of the mould.

Metaphosphate has yet only been found in certain microorganisms and never in higher animals. It seems rather unlikely that colloidal metaphosphates should occur in animals. Preliminary investigations which were reported showed that only metaphosphates of low molecular weights *e. g.* sodium trimetaphosphate are broken down by extracts of liver and other organs [3].

The biological significance of metaphosphate of high molecular weight in microorganisms is not known yet. It seems probable that due to the energy-rich phosphate bonds in metaphosphate this substance supplies energy to important processes in moulds, yeasts and certain bacteria when it is broken down by enzymes. Metaphosphate may also be a suitable substance for the storage of phosphate in these microorganisms. Wiame's investigations indicate that the substance in yeast cells called volutin which can be detected by the metachromatic staining technique, contains metaphosphate [28,29,30]. The role of volutin in yeast has been studied by *e. g.* Lindegren [31] who is of the opinion that in yeast this volutin is essential for chromosome division.

SUMMARY

From the mould *Aspergillus niger* a water soluble, nondialyzable substance has been isolated which has been shown to be metaphosphate with a molecular weight of the order of magnitude 6 000—7 000, *i. e.* an inorganic substance of high molecular weight.

We are grateful for the valuable technical assistance rendered by Miss A. Karlsson and Miss I. Karlsson. The investigation was supported by a grant from *Statens Naturvetenskapliga Forskningsråd.*

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Received February 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 487 – 507

The Oxidation of Rubber Vulcanisates

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The slow, spontaneous deterioration in the tensile strength and elongation of rubber vulcanisates at room temperature, known as natural ageing is due principally to a reaction between the rubber and oxygen [1]. Although ageing is not identical with oxidation [2] the relation between these two phenomena provides a convenient mean of comparison.

It is generally accepted that an absorption of approximately one per cent by weight of oxygen corresponds to the complete destruction of a vulcanisate with regard to its mechanical properties [1].

The Arrhenius curve of the reaction can be determined by measurements in the range 50—150° C. Here the rate of oxidation will have a proper value allowing the completion of a run in a convenient amount of time. Using the obtained graph the rate of oxidation may be determined by extrapolation.

Index of ageing is here defined as the time required for the reaction of approximately 20 millimols of oxygen per mol C_5H_8 *i. e.* roughly one percent on the weight of the rubber. If a relationship can be derived between this index of ageing and the rate of ageing determined by mechanical means such data would be useful in calculating the resistance to ageing of a given vulcanisate.

Dufraisse [3] used a manometric technique in his fundamental study of the function of inhibitors in various autoxidation reactions. The conception of "inverse catalysis" as cited by Moureu and Dufraisse in theire exposition of the theory of antagonistic peroxides arose out of these investigation. Christiansen [4] showed that the reaction mechanism proposed was inadequate and proposed a chain reaction.

Dufraisse has constructed an apparatus which permits the simultaneous measurement of the rate of oxidation in 10—20 absorbers, and has shown the principal influence of a series of factors such as combined sulphur, time and temperature of vulcanisation, concentration of filler and inhibitor etc. Ordin-

arily the measurements are performed in comparison with a standard sample and the absolute values of the rates are not calculated [5,6].

According to a similar manometric method Williams and Neal [7] investigated acetone extracted vulcanisates, and stated a constant dp/dt corresponding to zero order reaction, which in turn was explained by the conception of a chain mechanism without attempting a quantitative formulation. Similar measurements were carried out by Morgan and Naunton [8], who confirmed the zero orden reaction for acetone extracted vulcanisates and concluded with the statement that the rates were in agreement with the Arrhenius equation. A chain reaction was described according to Bäckström [9], which gave quantitative expression of the kinetics.

The value of these manometric measurements has been somewhat reduced through an investigation by Carpenter [10]. Carpenter determined the rates in a constant pressure device and found that the rate of oxidation varied with the pressure of the oxygen in contradiction to the manometric findings, and further that the sample continued to oxidise at the same rate after reduction of the pressure of oxygen for a considerable time. This persistency of rate of oxidation is assumed as the possible explanation of the zero order reaction found by the manometric method.

Shelton and Winn [2] stated an autocatalytic reaction on vulcanisates of GR—S (the American buna rubber). The initial phase of the reaction could be described by the superposition of a first order reaction at active centres in the butadiene-styrene-structure and a zero order reaction with normal structures. The same kinetics were stated by Stafford [11] for irradiated rubber vulcanisates measured at constant pressure. On raw rubber le Bras [12] found more complicated variations of the rate of oxidation at constant pressure. Le Bras used a very interesting apparatus in which the oxygen was prepared by electrolysis, a method which is not free from danger as a trace of ozone might be expected in the electrolytic oxygen.

Unfortunately the determination and isolation of the peroxides in rubber is still an unsolved problem and in consequence the fundamental base for the kinetic treatment of the problems is lacking. The theory proposed by Bolland and Gee [13] for the autoxidation of esters of linoleic acid, however, seems to be a very attractive explanation which might be extended to the oxidation of rubber as mentioned by Mesrobian and Tobolsky [15]. According to Bolland and Gee the initiation is performed by free radicals formed by thermal decomposition of the peroxide and the termination by reactions between the radicals.

In a somewhat similar manner Cole and Field [14] explain the oxidation of GR—S where the free radical is formed through reaction of the diene complex with active oxygen. Termination by an inhibitor of the phenylnaphthylamine

type is effected by reaction between the primary peroxide and the imino-hydrogen atom in the inhibitor, whereby a new free radical with insufficient energy for chain propagation is formed.

In spite of the foregoing criticism of the manometric method it shows some indisputable merits, namely its simplicity and the ease with which multiple determinations are performed. In the following experimental part therefore, we will try to elucidate the possibilities of the method in respect of reproducibility of the results and for the empirical determination of the effects of anti-oxygens and of copper and manganese soaps.

PREPARATION OF THE SAMPLES

All compounds were mixed on an ordinary laboratory mill with all precautions. The raw rubber was cut from the interior of a selected bale of smoked sheets. Slabs were moulded in a cavity in iron moulds $200 \times 100 \times 2$ mm^3 between two hard, polished aluminum plates and immediately cooled in water after the removal from the press. The slabs were cut into about 10×20 mm^2 pieces and crumbed by 4—5 passages through tight rollers on the mill. The crumbs were further disintegrated in a small mill furnished with helical blades and the resulting powder sieved to pass 100 % through 30 mesh and 0 % through 60 mesh. The surface of the samples was approximately 200—300 cm^2/cm^3 and the value was roughly determined by counting all grains in 1—10 mg powder weighed on a microbalance and evenly spread on a piece of millimeter paper.

THE ABSORBERS

Preliminary experiments were carried out in a modified Dufraisse absorber made of glass. The absorber was filled with oxygen by flushing from a tank 10 min. This method brought about some difficulties in avoiding streaks in the grease used for the sealing of the ground glass joints, which was turned 180° to close the connection with the atmosphere through corresponding channels in the glass stopper and the absorber joint. Those difficulties are quite avoided in the absorber described below (Fig. 1).

The absorber is fitted with a very carefully ground joint 1 sealed with a proper amount of Cellogrease (Fisher Scientific Co.) on the upper third of the stopper. The reaction vessel *R* has a volume of 10 ml and the capillary is 500 mm with a bore of 0.8—1 mm and a volume of 0.3—0.4 ml. The capillary extends 15 mm into *R* and is covered by the perforated micro beaker *5*. In the mercury reservoir the capillary is fixed in a rubber stopper with a smooth bore, moistened with very little glycerol. The position of of the end of the

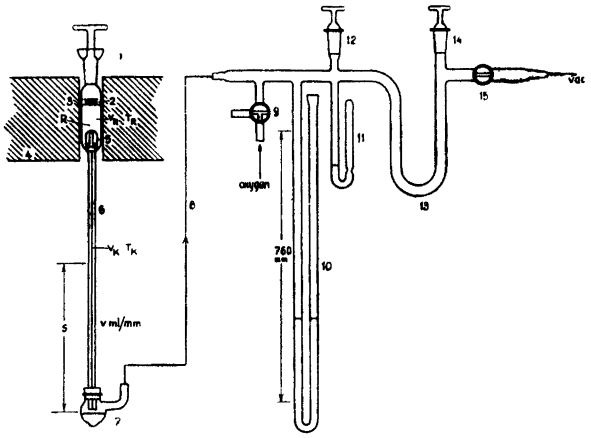

Fig. 1. Oxygen absorber.

capillary is adjusted to a few millimeters over the mercury surface. 2 g of rubber of the mentioned fineness are introduced into the absorber and 0.3—0.4 g ascarite is placed over the sample in a micro glass *2* to absorb water and carbon dioxide. The absorber is evacuated and filled by oxygen from the tank by means of the manometer *10—11* and the cocks *9—15*. This process is repeated 4—5 times, each time reaching a moderate vacuum about 2—5 mm Hg. Finally the absorber is lowered in the mercury, applying a slight pressure on the stopper *1*. At the last filling with oxygen one may stop at a pressure 40—70 mm below the barometric pressure, which forces the mercury to mount in the capillary, and the readings can take place immedeately after heat equilibrium is reached. Heating of the absorbers is performed in holes drilled in an electrically heated and automatically temperature controlled aluminiumblock *4* in which 10—20 parallel manometer can be placed. The temperature differential in the rubber sample is about 0.1—0.5° C using a closely controlled heating current.

Measurements at temperatures below 50° C are made in the absorber shown in Fig. 2. The capillary is 350 mm with a lumen of 2 mm. The filling with oxygen is performed by using the same method described above, with the auxiliary system placed in the ground glass joint g. The sealing liquid, ethyleneglycol, is stored in the funnel *h*, and the cock *i* is opened after the final addition of oxygen and the glycol flows in *e*, sealing the absorber. The funnel is then removed and the absorber placed in a water thermostat and is finally closed by means of the stopper with the cock *i*. By opening *i* and applying

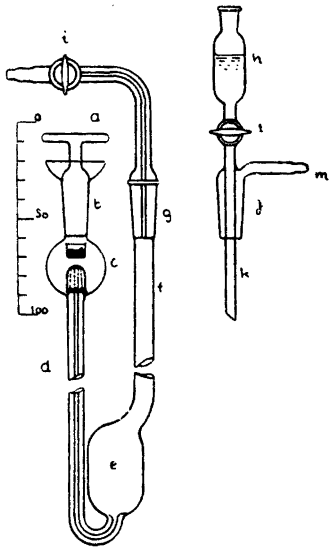

Fig. 2. Closed absorber.

gentle suction by mouth the glycol meniscus may be adjusted to any desired position in the capillary *d*. The rate is here measured with close temperature control and without influence of fluctuations of the barometric pressure. The vapor pressure of glycol is 2 mm at 35° C.

Most of the measurements were not conducted in absolute darkness but in very weak diffuse daylight.

CALCULATION OF THE RATE OF OXIDATION

With the symbols used in Fig. 1 and volume of the capillary w ml/mm the amount of oxygen in the absorber is given by:

$$\begin{aligned} O_2 &= \text{p. f. } (V_R \cdot \perp_R + V_K^* \perp_K) \text{ mols} \\ f &= 273.2/760 \cdot 22392 = 1.605.10^{-5} \\ V_K^* &= V_K - s.w \\ \perp &= 1000/T \end{aligned}$$

and the pressure in the absorber

$$p = B - s$$

where s is the height of the mercury column in the capillary, B the barometric pressure, V_K the total and V_K^* the volume corresponding to s of the capillary. By differentiation in respect to time h (hours) the rate is

$$v = \frac{dO_2}{dt} = -f \cdot \frac{dp}{dt}\left[V_R \perp_R + \perp_K (V_K + w\ (2p - B))\right] \qquad (I)$$

v is expressed in moles O_2 pro hour and g C_5H_8 omitting the constant 10^{-8} in all quotations of rates of oxidation.

The condition for zero order reaction is determined by integration of (I) keeping v constant:

$$\frac{K}{t \cdot f} = -Ap - Dp^2 + C \qquad (II)$$

where K denotes the constant value of v at temperature T and the constants of integration are

$$A = V_R \perp_R + \perp_K (V_K - wB)$$
$$B = w \cdot \perp_K$$
$$C = AB + DB^2$$

Consequently a straight line s—t relation corresponds to a slight decrease in v and opposite the s—t curve concording to a zero order reaction must be concave to the s-axis as the following calculation will show. Chosing $V_R = 9$ ml, $\perp_R = 3.7$, $\perp_K = 3.4$, $V_K = 0.4$ ml, $B = 760$ mm Hg, $w = 0.000\ 8$ ml/mm og dp/dt = const. = 10 mm Hg/h one have:

Table 1.

s mm Hg	v^s	t hours
0	591.6	0
100	583	9.89
200	574	19.63
300	565	29.22
400	556	38.67
500	547.9	47.97

v^s: rate of oxidation corresponding to s mm Hg

The column v^s is calculated from equation (I) and it is seen that the rate decreases 7 % calculated on v^o, which is insignificant compared with the normal accuracy of the method. The column t is calculated at $v = 591.6 = \text{const.}$ from equation (II):

$$368.6 \cdot t = -32.59 \cdot p + 0.0027\ p^2 + 26339$$

and the curvature of the s—t line becomes very little.

For the absorber Fig. 2 kept at constant temperature T the rate is calculated from:

$$v = f \cdot \perp \frac{dp}{dt}\left[V_0 + \frac{w}{b}\ (2\ p - B)\right] \qquad \text{(Approx.! III)}$$

where V_0 denotes the combined volume of absorber and capillary corresponding to $s = 0$ and b the ratio of the density of glycol at T° and mercury at 273° ($b = 7.91 \cdot 10^{-2}$ at 35° C.)

The readings of t, s, B and T_K are tabulated and the s—t curve plotted graphically.

RATE OF OXYDATION OF A STANDARD VULCANISATE

I. Without addition af antioxygen

As a typical fast oxidising vulcanisate a DPG-vulcanisate with the following formula was used throughout the investigation:

Smoked sheet	1 000	1 000
Sulphur	15	15
DPG	15	15
Zincoxide	100	100
Whiting	1 060	1 040
Carbon black	30	30
Antioxygen	nil	20

Vulcanisation unless otherwise stated 15 min/152° C
Vulcanisates without antioxygen are termed —
with » » » +

All the rates cited in the following tables are rates corresponding to $s = 0$: *the initial rate* computed from the s—t curve. In the Table 2 are collected some data concerning the purity of the fillers used:

Table 2.

Filler	Cu	% Mn	Fe
Danish whiting	0.0004	0.01	0.1
Winnofil (ICI)	0.0003	0.007	0.08
Zincoxide	0.005	nil	nil

Though all measurements on the — vulcanisates were conducted with great care with powders of approximately the same surface it will be seen from Table 3 that it is impossible to reproduce the value of v. The reproducibility in double or multiple runs using the same samples is well established at about $\pm$ 5—10 %, but changing to a new mix using exactly the same ingredient can yield results which will differ widely. This is the case for the three samples 4620C, 47161B and 4823 all with Danish whiting, of which 4620 is slow and 4823 fast oxidising and 47161 gives an Arrhenius line of a different slope. One must content oneself to locate an upper and lower line for the area in which the vulcanisate by chance will locate itself, and the limitiation is almost given by the $\perp$-log v-lines for 4620C and 4823. The purity of the whiting does not seriously affect the rates as shown by 4839 containing the pure precipitated stearic acid coated calcium carbonate from ICI (Winnofil) placed intermediately in the interval. The sample 4852 is made from a washed and vacuum dried smoked sheet from the interior of a bale. This process is expected to remove some of the natural antioxidants in the rubber, but 4852 on the contrary is slow oxidising at the lower limit of the interval.

All graphs of s-t are with great accuracy *strictly linear* up to 200—300 mm Hg at temperatures below 100° C and in some cases graphs with a slight increase in ds/dt occur *i. e.* almost zero order reaction. At higher temperatures (120—140° C) the curves initially start as straight lines but at 100—200 mm Hg the ds/dt value will rapidly decrease. Rates above 150 000 are not realised, not even with samples to which is added a considerable amount of copper stearate. Above 100° C the diffusion is becoming a controlling factor and it is difficult to prepare samples with surfaces exceeding about 300 cm^2/cm^3. Due to the lack of consistency it is impossible to determine the kinetic constants with a reasonable accuracy, but the energy of activation (mean value from Table 3) is round 21 kcal and the constant H about 15.6 (mol/mol.sec). Finally we can calculate a mean Arrhenius line given by

$$\log_{10} v = 8.52_3 - 4.63_0 \cdot \perp$$

Table 3. Initial rates of oxidation for vulcanisates without antioxygens.
mols O_2 per hour and g C_5H_8 ($\times 10^8$).

°C	No. of mix Rubber Filler surface	4620 C SS [1] whiting [2]	47161 B SS [1] whiting [2]	4823 SS [1] whiting [2] 186	4839 SS [1] Winnofil [3] 213	4852 washed SS whiting [2] 198	cm^2/cm^3
25		9.4					
27				22.3			
31.7		18.6					
33.0			27.7				
34.5 [4]					20.3	21.1	
40.7		37.0					
45.6			132				
49.3		120					
57.5			477				
59.5				671	603	485	
60.4		340					
69.4			1 680				
74.4				2 860			
79.5			3 800		1 940	1 640	
89.4				9 100			
100.4				18 500	11 400	6 900	
121.3				44 000			
	$\log_{10} v^\circ$ [5]	$7.809 - 4.436 \cdot \perp$	$9.844 - 5.016 \cdot \perp$	$8.371 - 4.504 \cdot \perp$	$8.874 - 4.778 \cdot \perp$	$7.711 - 4.414 \cdot \perp$	
	H [6] $^{mols}/_{mols \cdot sec}$	14.00	18.68	15.30	16.45	13.77	
	Q [6] kcol	20.4	23.1	20.7	22.0	20.3	

[1] Standard smoked sheets.
[2] Washed Danish whiting.
[3] Stearic acid coated, precipitated calcite from J. C. J.
[4] Measured in the closed absorber (Fig. 2).
[5] Derived by regression analysis.
[6] Arrhenius equation $\ln v^\circ = H - \frac{Q}{R \cdot T}$

which will be used as an average standard in discussing retardation by antioxygens and acceleration by copper and manganese soaps in the following measurements.

Table 4. Tensile properties.
Sample 47 · 161 B.

Addition %	Antioxygen parts	TB [1] kg/cm²	M_3 [2] kg/cm²	EB [3] %
nil	—	152	54	510
0.01 Cu [4]	—	157	57	510
0.05 Cu [4]	—	134	44	540
0.01 Mn [5]	—	132	38	520
0.05 Mn [5]	—	85	14	640
nil	2 [6]	163	55	520

[1] Tensile at break.
[2] Modulus at 300 % elongation.
[3] Elongation at break.
[4] As copper stearate.
[5] As manganese oleate.
[6] 2 part per 100 Smoked Sheets (Antioxygène A, Francolor).

II. Compounds with copper and manganese

To the base formula were added various amounts of copper stearate containing 9.4 per cent copper and manganese oleate with about 1 per cent manganese. The tensile and modulus of the vulcanisates is definitely reduced and the elongation increases as the figures in Table 4 will show.

The addition of 0.01 per cent of copper causes a little increase in tensile and modulus presumably through a cross-linking of oxygen. The chain scission is very clearly shown at the higher copper concentration and further in all cases with manganese. These samples age very fast at room temperature in the dark, and surface tackiness is developed in a few days, while the tackiness of copper containing specimens appears after some weeks. The *s-t graphs* are all very characteristic with *pronounced increase* in ds/dt in many cases after a straight line initial curve, recalling the well known autocatalytic process with a period of induction.

A set of samples with pale crepe instead of smoked sheets was typical in this respect as would be anticipated from the lower content of natural antioxygens in the crepe. The *s-t* graphs are shown in Fig. 3 and the rates tabulated in Table 5 which in addition shows the reproduction of duplicate determinations. The base — vulcanisat is very closely coinciding with 4823 (Table 3). The graph for 4620 H with 0.01 % copper proceeds as a straight line for

Table 5. Initial and final rates of oxidation Pale Crepe vulcanisates with Cu and no addition of antioxygen [1].
mols O_2 per hour and g C_5H_8 · ($\times 10^8$).

Sample	% Cu [2]	°C	initial rate $\sim s = 0$ [3]	final rate $\sim s = 500$
4620 G	nil	31.0	36.4 ± 1.6	
		49.4	253 ± 23	
		59.6	699 ± 21	
4620 H	0.01	31.0	88.7 ± 1.2	696 ± 1
		49.4	545 ± 15	
		59.6	1 270 ± 46	14 800 ± 5 500
4620 I	0.05	49.4	980 ± 24	
		59.6	3 900 ± 285	19 280 ± 125
4620 J	0.1	49.4	1 520 ± 90	
		59.6	5 000 ± 45	33 750 ± 1 650

[1] 2 mm slabs vulcanised 60 min at 142°C, surface of powder 280 cm^2/cm^3. All figures mean value of duplicate runs.
[2] As copper stearate.
[3] s in mm Hg.

the first 80 hours, then the slope is increasing at an even rate to 120 hours after which the increase becomes rapid. The figures in Table 5 show that the *final rates may exceed the initial rates by 5—6 units.*

The corresponding rates for smoked sheets are given in Table 6 and some typical s-t graphs shown in Fig. 4. The autocatalytic course is still evident

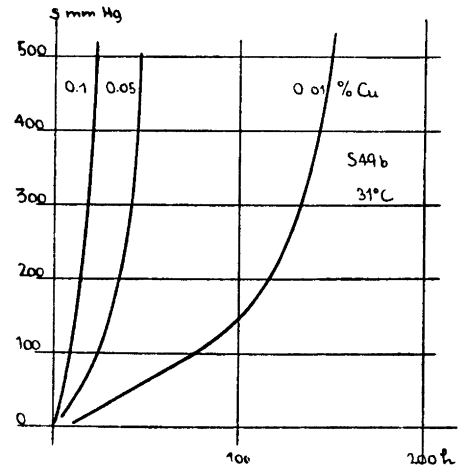

Fig. 3. s-t for Crepe (without antioxygen. + Cu).

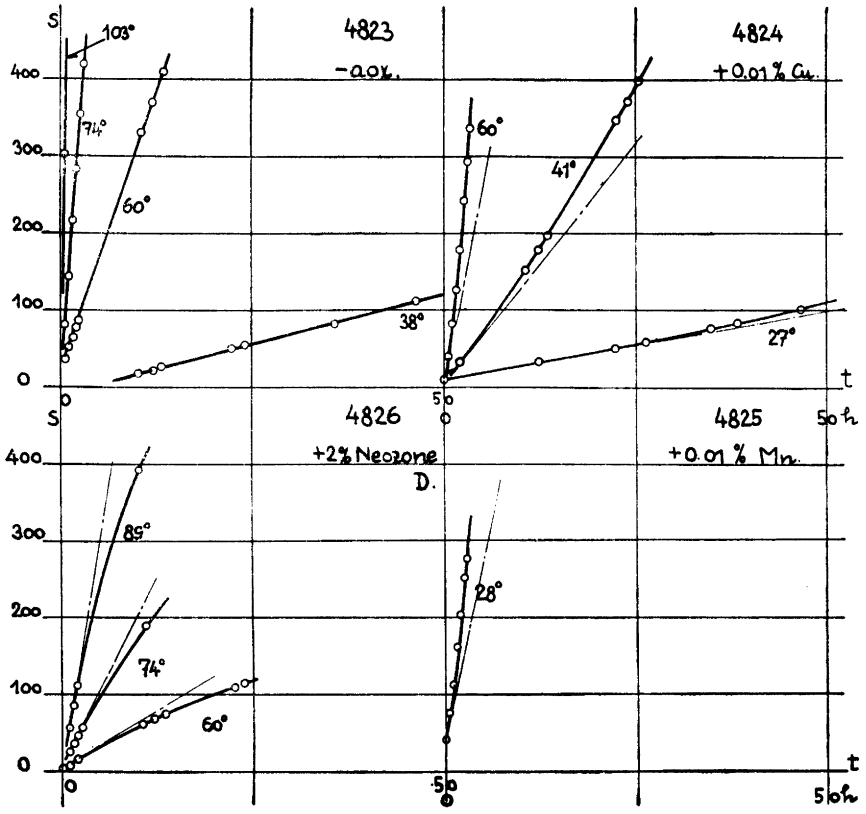

Fig. 4. s-t for smoked sheets ± with antioxygen, Mn and Cu as indicated. ± Mn and Cu.

but much less pronounced as in the case of the crepe. At the higher temperature the *s-t* graphs her have a point of inflexion, the reason for which is obscure as an inefficiency of diffusion might not be expected (Fig. 5).

The freshly prepared sample 4824 has constants very alike the base compound in accordance with the analogy in tensiles. If the sample is subject to ageing at room temperature the rate of oxidation increases very much as the sample of 48 178, aged 2 months, shows. The Arrhenius line in addition has much less slope.

(The sample 47 178 finally was measured at the time where the transition from a dry powder, to a very tacky sponge took place and during this transformation which lasted six days, the initial rate was increased from *2300 to 5300*, (at 28° C). Finally the sample formed a hard mass with an aromatic and sharp smell. The measurements show the possibility of an increase in rate

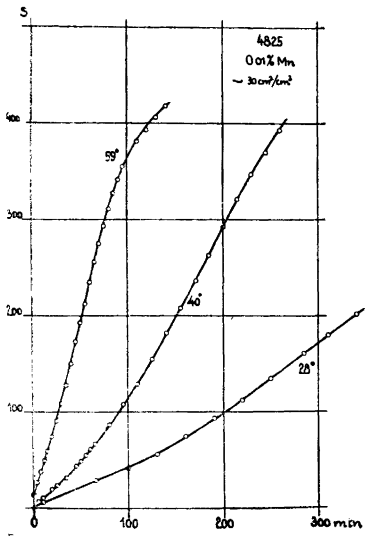

Fig. 5. s-t for smoked sheets + 0.01 Mn, 2 × 1 mm² slabs.

under conditions where a servere reduction of surface and diffusion must be assumed.)

Resuming we may stress that the *s-t curves show increasing values of ds/dt* and the magnitude of the rates are undefined depending upon the natural ageing of the sample. The acceleration may be judged as follows: with 0.01 % copper about 5 units for the fresh vulcanisate, about 200 units for aged vulcanisates and ranging towards 500 units for samples with 0.01 % manganese.

III. The effect of antioxygens

The graphs of the *s-t* values are all showing a *very pronounced decrease in ds/dt*, the shape of the curves resembles those cited of Carpenter [10] and Milligan and Shaw [17]. The order of reaction is in many cases approximately unity. Though the determination of the initial rate is not very well defined due to the geometry of the *s-t* curve the reproduction is very satisfying and all measurements of vulcanisates containing common antioxygens — as phenyl-β-naphthylamine (PBN) *e. g.* — are *concentrated at the same Arrhenius line* with good accuracy (A—A in Fig. 6).

The retardation is rather modest *e. g.* at 25° C about 2.6 units and at 70° C 3.4 units for phenyl-naphtylamines and aldolamines. For the more effective antioxygens of the phenylene-diamine aryl-substituted types the retardation may be judged to about 7 units at lower and intermediate temperatures.

Table 7 gives the date for various + vulcanisates.

Table 6. Initial rates of oxidation for vulcanisates with addition of copper and manganese without antioxygen.
mols O_2 per hour and g smoked sheets ($\times 10^8$).

No. of mix % addition condition of sample surface cm^2/cm^3	47 178 0.01 Cu [1] aged 2 mths 280	48 24 0.01 Cu [1] fresh 260	4825 0.01 Mn [2] fresh 30 [3]	4825 0.01 Mn [2] fresh 70 [4]
°C				
27.0		48.8		
28.0	2 300		2 060	
38.0		188		
38.9	5 400		3 000	
41.0				18 000
57.5	17 100	2 070		69 200
58.8			9 400	
69.4	39 400			
74.4		8 200		
$\log_{10} v^{\circ}$ [5]	$5.51-3.06 \cdot \perp$	$10.33-5.00 \cdot \perp$	$2.52-2.18 \cdot \perp$	
Q kcal	14.1	23.0	10.0	
H	8.71			

[1] As copper stearate.
[2] As manganese oleate.
[3] Cut with razor in 2×1 mm^2 slabs.
[4] Crumbed on the mill.
[5] By regression.

IV. Vulcanisates with both antioxygen and copper

The investigation has been only carried out in a preliminary scale. In Table 8 are quoted measurements on a series of vulcanisates, which were very difficult to disintegrate and consequently the surface became about 50 cm^2/cm^3. For the sake of brevity the table gives simply the ratio of the rates of the base vulcanisate and the vulcanisates with addition of copper and antioxygens. The symbol 2AAN/0.05 indicates 2 parts antioxygen pro 100 rubber (AAN = aldol-a-naphtylamine) and 0.05 % copper calculated on the whole mix.

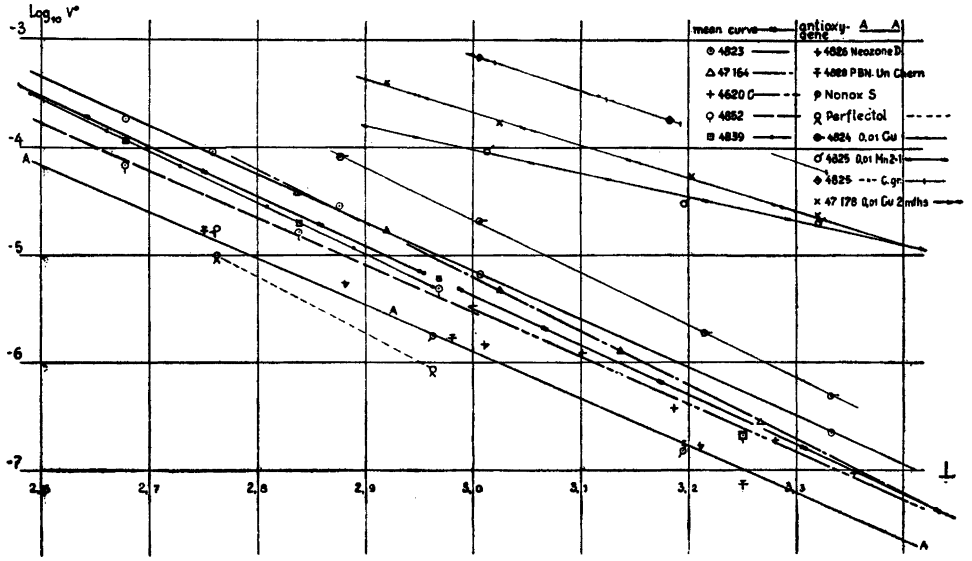

Fig. 6.

The retarding effect of the antioxygens is most pronounced at the high concentration of copper as indicated by the proportions Φ', and Φ'' in Table 8. An increase of the amount of antioxygen exceeding 2 parts per 100 rubber shows no effect as measured by the initial rate of oxygen absorption. PBN seems to give a somewhat greater protection against copper than does AAN, although the possibility of formation of complexes with copper has been stressed in case of AAN [18]. Jones and Craig [19] demonstrated the superiority of the aryl substituted phenylenediamines in combination with copper, a point of view which we are hoping to treat in a later communication. Due to the before stated instability of the copper containing vulcanisates the measurements cited in Table 8 are not quite consistent *e. g.* 2 parts PBN causes greater relative retardation than 4 parts in the samples with both 0.01 and 0.05 per cent copper.

APPENDIX

Some preliminary ageing tests

Several of the samples were aged in circulating air at 70, 95 and 130° C. The ageing tests were carried out using dumbbell specimens, cut from 2 mm thick slabs. The width of the narrow neck of the specimen was accurately

Table 7. Initial rates of oxidation for vulcanisates with 2 parts of antioxygens per 100 smoked sheets.
mols O_2 per hour and g C_5H_8. ($\times 10^8$)

No. of mix	47163	48 33	4826	4828	4829	4832
Antioxygen	Antioxygène A [1]	Perflectol [2]	Neozone D [3]	PBN [4]	Nonox S [5]	Agerite [6] White
°C:						
34.9				7.4		
38.0			17.1			
39.9				17.0	15.1	
59.5			147			
64.4		87		171	178	
68.2	263					
74.4			550			
79.8	530					
88.9		1 010			1 760	1 025
89.4			1 610			
90.6				1 700		
103.3			3 125			

Mean for 47163, 4826—28—29, $\log_{10} v^\circ = 7.11 - 4.33 \cdot \perp$, $Q = 19.9$, $H = 12.4$

[1] Phenyl-α-naphthylsamine ~ PAN.

[2] Blend of Flectol H, an acetone-aniline condensations product, and N—N′-diphenyl-*p*-phenylene diamine.

[3] Phenyl-β-naphthylamine ~ PBN.

[4] From United Chemicals, Praha.

[5] Unknown composition.

[6] N—N′-di-β-naphthyl-*p*-phenylenediamine.

5 mm. Tensile strenght (TB), elongation (EB) and modulus at 300 per cent elongation was determined. Measurements of the moduli showed complications and are consequently omitted.

The values of tensiles and elongation were plotted against the time in days and the slope of the zero tagent determined graphically. Calculating the slope as per cent of the zero value we are denoting these "decrease figures" as N_{TB} and N_{EB} defined by $N_x = -\frac{\partial x}{\partial t} \cdot \frac{100}{x}$

From the ageing curves was further determined the time elapsed for the reduction of the zero value to 50 per cent, which figures are termed as $t_{\frac{1}{2}TB}$ and $t_{\frac{1}{2}EB}$. Table 9 gives some data, measured at 70° C.

Table 8. Ratios of initial rates of oxidation for combinations of antioxygen and copper at 60° C and powders with a surface about 50 cm²/cm³.

Mix no.:	Aox.[2]/%Cu	$\Phi = \frac{v^{\circ}_{Aox/Cu}}{v^{\circ}_{\%}}$	$\Phi'^{1} = \frac{\Phi_L}{\Phi}$ (0.01 % Cu)	$\Phi'' = \frac{\Phi_M}{\Phi}^{1}$ (0.05 % Cu)
4620K	0 / 0	1.00		
» L	0 / 0.01	+ 5.8		
» M	0 / 0.05	+ 24		
» N	2PBN[3]/ 0	− 0.29		
» O	2AAN[4]/ 0	− 0.28		
» P	4PBN / 0	− 0.28		
» Q	4AAN / 0	− 0.32		
» R	2AAN / 0.01	+ 2.6	2.2	
» S	2AAN / 0.05	+ 6.6		3.6
» Y	4AAN / 0.01	+ 2.7	2.1	
» V	4AAN / 0.05	+ 6.0		4.0
» T	2PBN / 0.01	+ 1.5	3.9	
» U	2PBN / 0.05	+ 2.9		8.2
» Æ	4PBN / 0.01	+ 2.1	2.8	
» X	4PBN / 0.05	+ 4.7		5.1

[1] Φ' and Φ'' are a relative measure of the retarding power of the antioxygenes at a copper content of 0.01 and 0.05 respectively.

[2] Parts of antioxygen per 100 smoked sheets.

[3] PBN ~ phenyl-β-naphthylamine } from I. G. Farben.
[4] AAN ~ Aldol-α-naphthylamine }

\+ Denotes accelerations, − retardations.

In the column v_{70} the rate of oxidation is calculated from the average values of the Arrhenius lines. $\Phi^{v^{\circ}}$ denotes the ratio v_-/v_+ + and Φ_{Ag} is the corresponding ratio for N and $t_{\frac{1}{2}}$.

It is seen that the retardation of antioxygens is very consistent whether measured as ratios of rates of oxidation or as ratios of ageing using tensile. The elongation determines a lower retardation and the $t_{\frac{1}{2}}$ value a higher retardation. The ordinary antioxygens such as Flectol H and naphthylamines give a retardation of 3—4 units, while the more effective substituted *p*-phenylenediamines give a greater retardation about 6—7 units. The value for Agerite White must be discarded as it is expected that this antioxygen and Perflectol should be equal.

Table 9. Decrease figures from ageing tests at 70° C in circulating air.

Mix. no.	Antioxygen	N_{TB}	N_{EB}	$t_{\frac{1}{2}TB}$	$v°_{70°}$	$\Phi^{v°}_{70°}$	Φ_{Ag}		
							Φ_{TB}	Φ_{EB}	$\Phi_{t\frac{1}{2}TB}$
		% per day		days					
47 161 B	÷	9.9	0.71	11.2					
48 23	÷	9.7	1.30	5.2					
48 30	÷	6.4	0.69	10.3					
Average	÷ :	8.7	0.90	8.9	1 070				
47 162	PBN I. G. [1]	2.0	0.43	41					
47 165	Antioxygene MC [1]	1.5	0.11	50					
48 26	Neozone D [1]	3.4	0.54	34					
48 28	PBN [2]	4.0	0.62	34					
Average	+ PBN	2.7	0.43	40	310	− 3.4	− 3.2	− 2.1	− 4.4
47 163	Antioxygène A [3]	2.2	0.40	(50)			− 3.9	− 2.3	
47 164	» INC [4]	—	0.36	(54)				− 2.5	
48 29	Nonox S [5]	2.0	0.50	48			− 4.4	− 1.8	− 5.4
48 31	Flectol H [6]	2.6	(0.78)	(33)			− 3.3		
48 32	Agerite White [7]	2.4	0.42	(54)			− 3.6	− 2.1	
48 33	Perflectol [8]	1.4	0.34		160	− 6.3	− 6.2	− 2.6	
48 24	— with 0.01 %Cu	55	7.7	1	5 500	+ 5.1 (+ : accelerations) (— : retardations)	+ 6.3	+ 8.6	+ 8.9

$\Phi^{v°} = \frac{v°\div}{v°_{+}}$ or $\frac{v°\div/Cu}{v°\div}$; $\Phi_{Ag} = \frac{N\div}{N+}$ or $\frac{t_{\frac{1}{2}}+}{t_{\frac{1}{2}}\div}$ ÷: vulcanisates without antioxygen

+: » with »

[1] PBN.

[2] From United Chemicals, Praha.

[3] Phenyl-α-naphthylamine.

[4] Aldol-α-naphthylamine.

[5] Unknown composition.

[6] Acetone-aniline condensation product.

[7] N—N′-di-β-naphthyl-p-phenylenediamine.

[8] Blend of Flectol H and N—N′-diphenyl-p-phenylenediamine.

The effect of temperature is illustrated in Table 10 giving measurements at 70°, 95° and 130° C. The data show that the retarding effect decreases with increasing temperature. The coefficients of temperature are not consistent, being too low at 95°/130°, possibly due to insufficiency of diffusion.

Table 10. A. Retardations at 70°, 95° and 130° C.

°C	Mix. no.	antioxygen[1]	N_{TB} % pr. hour	$\Phi = \frac{N-}{N+}$	$v°$
70	4830	÷	0.266		1 070
	4831	Flectol H	0.108	2.5	310
	4833	Perflectol	0.058	4.6	160
95°	4830		3.7		8 900
	4831		2.7	1.4	2 240
	4833		1.1	3.4	
130°	4830		13.3		
	4831		6.4	2.1	
	4833		5.9	2.3	

Table 10. B. Temperature coefficients.

Mix. no.	$\Phi' = \frac{N_{T_1}}{N_{T_2}}$	
	95°/70°	130°/95°
4830	13.9	3.6
4831	25	2.4
4833	19	5.3
$v°_{\div}$	8.3	—
$v°_{+}$	7.2	—

[1] 2 parts per 100 smoked sheets.

Better figures would possibly result by the use of *normal rings* where the tensile properties are more strictly defined. For the reason of information the use of slabs however is very practical as little area of rubber samples is demanded for the performance of the tests.

Calculating the "index of ageing" from the rates of oxidation at 70° we find 1.2 days for the — -vulcanisate and 4.2 days for the +-vulcanisate *i. e.* about one tenth of the measured $t_{\frac{1}{2}TB}$-values, a quite reasonable magnitude taking into account the difference in surface.

When data from the course of the natural ageing are available the comparison of the N-values and the v-values at room temperature can be computed, by means of which we will obtain a final judgement of the significance of the ageing indices.

SUMMARY

An apparatus is described for the measurement of rate of the oxidation of rubber vulcanisates by means of a simple technique. Further formula for the calculation of the rate from pressure and time readings are derived *cf.* equations I and III.

For a diphenylguanidine vulcanisate some typical *s-t* graphs * are shown. If no antioxygen is added the *s-t* function becomes a straight line (in some cases with a slight increase of ds/dt) indicating approximately a zero order reaction. Duplicate runs agree very well ($\pm$ 5 %), but the reproduction of rates of samples from parallel mixes from the same base smoked sheets is very poor.

With addition of two parts of antioxygen per 100 smoked sheets the *s-t* graphs show a very pronounced decrease in slope and the order of reaction will be between zero and one. The reproduction is very good in all cases, and the measurements of mixes containing common antioxygens yields the same Arrhenius curve for the initial velocities.

Vulcanisates containing 0.01 per cent copper or manganese as soaps are very unstable. The *s-t* curve have an increasing slope in some cases after a straight line initial course. The rate is here a rather undefined property with a rapid increase with the age of the sample.

The rates of oxidation for powders of an approx. surface area about 300 cm^2/cm^2 are expressed in the following equations

without antioxygen	$\log_{10} v = 8.52 - 4.63 \cdot \perp$ **
with two parts of antioxygen	$\log_{10} v = 7.11 - 4.33 \cdot \perp$

(*Cf.* Tables 3, 5, 6, 7 and 8.)

Preliminary ageing test determined a factor for the retardation of the rates executed by ordinary antioxygens coninciding with the same property determined by the initial rates of oxidation.

Common antioxygens will retard the rate of oxidation 3—4 times and the aryl substituted phenylendiamines 6—7 times.

The acceleration of the rate of oxidation by copper and manganese soaps (especially in samples aged short time at room temperature in the dark) may amount to 200—500 times the velocity of the base vulcanisate. Calculated in molar concentrations the accelerating power of copper and manganese will be about 1 300 times greater than the retarding power of phenyl-β-naphthyl-amine.

* s is the height of the mercury (mm) at time t. The slope $ds/dt = -dp/dt$.

** $= 1000/T$.

Finally I wish to thank my firm A/S Nordiske Kabel- og Traadfabriker for the permission to the publication, and Prof. J. A. Christiansen and Messrs. J. S. Hunter and Dr. Morgan (Monsanto Chemicals Ltd.) for some very valuable help during the work.

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Received January 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 508 – 511

Paper Chromatography of the Amino-Acids in Horseradish Peroxidase

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In the course of studies concerning the attachment of the heme group to the protein part of horseradish peroxidase it was of importance to investigate which amino acids were present in the protein part. Theorell and Åkeson [1] in 1942 separated this protein after hydrolysis into acid, neutral and basic amino acids by an electro-dialytic micro-method. In the alkaline fraction they found histidine, arginine and lysine.

We used their technique and analysed the three fractions separately by paper chromatography using the ascending method introduced by Williams and Kirby [2]. Munktell OB filterpaper 48 × 48 cm was used, cut to a size of 45 × 45 cm and the solution of the sample was placed in one corner of the sheet, 5 cm from each edge, by means of a micropipette (ordinary type or the type described by Linderstrøm-Lang [3].) A Petri-dish with diluted ammonia was placed underneath the paper when applying acid solutions. The volumes ranged between 2 and 200 μl, but no more than 5 μl were put on the paper at the same time. A stream of compressed air was used to dry the paper after each application. The sheet was now formed into a cylinder and loosely sewn together by white twine. It was placed into a glass cylinder 50 cm high and 25 cm wide filled with a mixture of phenol-water 4 : 1 (4), some drops of 35 % ammonia and a few mg of 8-hydroxy-quinoline to prevent Cu-fronts on the paper. A beaker containing about 100 mg NaCN was placed on the bottom.* The glass cylinder was sealed with a 5 mm thick glass plate, tightened with "plastiline" and placed in a thermostat at 37° over night. At this temperature, the front of the solvent had reached the top of the paper after 10—12 hours, whereas at room temperature (24°) about 24 hours were needed.

* Complete saturation of the solvents is unnecessary in practice and may be even impracticable on account of eventual emulsification [5].

The sheet was cut up and dried by an air stream at room temperature. After cutting away a 5 cm wide strip at the top of the sheet (including the slightly colored front of the phenol) it was again sewn into a cylinder and turned 90°. The second run was made in a collidine-lutidine-water mixture 1 : 1 : 1 under the same conditions as the phenol run but without any additions to the solvent. After 10—12 hours the solvent had reached the top of the paper.

The sheet was dried as before and sprayed with a butanol solution saturated with water and containing 0.1 % ninhydrin and 1 % acetic acid [6]. The solution was applied with compressed air, using a sprayflask, made by "Kifa", Stockholm. After hanging in the thermostat at 37° for a few hours the paper distinctly showed the characteristic spots of the amino acids. Heating to 105° was unfavorable as Dent has already stated [7].

The analyses were first made with samples and mixtures of synthetic amino acids dissolved in 75 % EtOH (if necessary with addition of very small amount of conc. HCl) and the R_f-values measured. The following results were obtained:

Table 1. R_f-values of amino acids used.

Amino acid	Source	HCl µl/ml	$R_{f(37°)}$ in phenol	$R_{f(37°)}$ in collidine
1. D,L-Alanine	*	0	.55	.17
2. L-Arginine-HCl	Merck	0	.77	.01
3. D,L-Aspartic acid	Merck	30	.18	.17
4. L-Cysteic acid	**	0	.07	.30
5. D-Glutamic acid HCl	*	0	.30	.17
6. D,L-Glycine	Shering-Kahlbaum	0	.37	.12
7. L-Histidine-HCl	Hoffman-La Roche	15	.64	.15
8. L-Isoleucine	—»—	0	.71	.40
9. D-Leucine	Schuchardt	0	.71	.40
10. L-Lysine-HCl	Merck	0	.61	.01
11. D,L-Methionine	Eastman-Kodak Co.	0	.72	.41
12. D,L-Norleucine	*	0	.71	.40
13. L-Hydroxyproline	Schuchardt	0	.59	.18
14. D,L-Phenyl-alanine	*	0	.73	.47
15. L-Proline	*	0	.75	.20
16. D,L-Serine	Merck	20	.33	.13
17. D,L-Threonine	»	0	.47	.17
18. L-Tyrosine	Hoffman-La Roche	50	.55	.49
19. L-Valine		0	.69	.31

* Obtained by courtesy of Dr A. Wretlind, Karolinska Institutet, Stockholm.

** Prepared according to Gortner and Hoffman [8].

The solvents were purified in the following way:

1. *Lutidine.* A mixture of the 2,4- and 2-5-isomers with b. p. $155-160°_{760}$ was obtained from "Yorkshire Tar Distillers Ltd., Cleckheaton, Yorks". Further treatment proved to be unnecessary.

2. *s-Collidine.* The purified product was also perchased from "Yorkshire Tar Distillers Ltd". It was treated with bromine according to Consden *et al.*[9] and distilled *in vacuo*. B. P. 169—171°. The product was slightly yellow and darkened very slowly if at all.

3. *Water.* Redistilled in all-glass apparatus.

4. *Phenol.* U. S. P., Baker Co., was distilled *in vacuo* according to Draper and Pollard [10] but using Zn-powder instead of Al. B. P. 182°.

5. *n-Butanol.* The product obtained from "Kebo AB", Stockholm, was fractionated on a Widmer-column and the fraction boiling between 116—117° was used.

For the analysis of the horseradish peroxidase the following procedure was used:

1. 1 ml 1.4 % dialysed sample of the peroxidase, crystallized according to Theorell [11] and with a P. N. 1020, was hydrolyzed by refluxing with 2.95 ml 37 % HCl and 1.5 ml water (= 20 % solution) for 24 hours.

2. Humin substances were removed by filtration.

3. The hydrolysate was evaporated *in vacuo* after addition of two drops of 8 *N* H_2SO_4 to get rid of Cl^-.

4. Electrodialysis in the microapparatus of Theorell and Åkeson [1]. Each fraction was run twice.

5. Each fraction was evaporated to 0.1—0.4 ml *in vacuo*.

6. Small volumes corresponding to about 0.5 mg total content of amino acids were applied on the paper as described before.

For tryptophane analysis a sample of the peroxidase was hydrolysed in alkali and treated according to Steers and Sevag [12]. A 10 mg hydrolysate gave no color development, whereas 10 γ tryptophane (Merck) gave positive reaction.

The following results were obtained:

Table 2. Amino-acids found in horseradish peroxidase.

Anode (acid fraction)			Middle compartment (neutral fraction)			Cathode (alkaline fraction)		
Acid	Color	Present	Acid	Color	Present	Acid	Color	Present
asp.	purple	+	ala.	purple	+	arg.	dark purple	+
cys.	blue	+	gly.	purple	+	his.	grey purple	+
glu.	purple	+	hypro.	—	—	lys.	purple	+
			leu.	purple	+			
			met.	purple	+			
			phe.	grey purple	+			
			pro.	yellow	+			
			ser.	purple	+			
			thr.	purple	+			
			try.	—	—			
			tyr.	grey purple	+			
			val.	purple	+			

Unidentified spots were observed twice in the cathode fraction. One had R_f 0.67 in phenol and 0.30 in collidine, the other R_f 0.77 in phenol and 0.41 in collidine.

Abbreviations of the amino acids according to Brand and Edsall [13], cys = cysteic acid.

Work on the quantitative determination of the amino acids in horseradish peroxidase protein is being continued.

SUMMARY

A modification of the ascending method of paper chromatography according to Williams and Kirby [2] has been described. With the aid of this technique the amino acid composition of horseradish peroxidase was investigated. The usual amino acids were identified except tryptophan and hydroxyproline. Two unidentified spots were found in the cathode fraction of the electrodialyzate.

This work was aided by a grant from *Stiftelsen Therese och Johan Anderssons minne* for which the authors wish to thank.

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Received January 31, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 512 – 517

Chemical Investigations on *Pteropoda*

Isolation of a New Sterol, Pteropodasterol

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As far as we know species of the *Pteropoda* (order of the *Gastropoda*) have never before been investigated from a chemical point of view.* Through courtesy of "Fiskeridirektoratets Havforskningsinstitutt", Bergen, two species have now been collected for investigation, *viz.*, *Clione limacina* Phipps and *Limacina helicina* Phipps, the former being a naked, the latter a scaled pelagic snail.

The samples were collected the 28th July 1947 in Magdalena Bay on Svalbard by Konsulent Finn Devold to whom the author is very indepted for the material.

As shown in Table 1 the *L. helicina* contained some more water than *C. limacina*. The shell of *L. helicina* seems to consist of organic material. Compared with data from other molluscs given by Bergman [1], these pteropods seem to be among the less fatrich.

Table 1.

	Limacina helicina	*Clione limacina*
Dry weight (fat free)	9.68 g/100 g	6.88 g/100 g
Ash (525°)	3.07 g/100 g	2.71 g/100 g
Fat	0.72 g/100 g	0.68 g/100 g
Iodine number	166	167
Unsaponifiable matter	10.7 g/100 g fat	13.9 g/100 g fat
Sterol (as cholesterol)	62.1 g/100 g unsap.	40.8 g/100 g unsap.

* G. Rosenfeld (*Wiss. Meeresunters. Abt. Helgoland*, 5 (1904) 57) dealing with fat sources of the sea, found in *Limacina arctica* (= *L. helicina*) 7.3 g fat/100 g dry material with iodine number 164. 8.

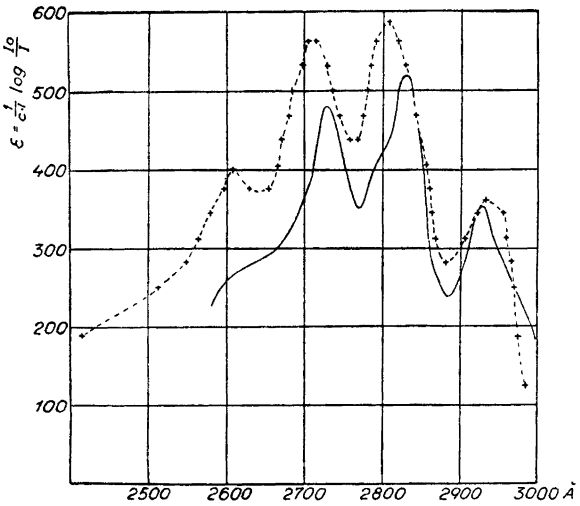

Fig. 1. Ultraviolet absorption spectra.
– – – Sterol from Clione limacina
——— 4 % ergosterol

The fats, extracted with alcohol and ether, were dark brown and viscous. The unsaponifiable matters were both semicrystalline solids with a bright yellow-red colour. Further examinations of the material were concentrated on the sterol fraction.

Till now cholesterol has been found in gastropods. These pteropods seem to be the first exception, since no cholesterol could be traced. For instance an etheral solution of the sterol fraction gave no precipitate on addition of bromine in acetic acid, and in no fractions any low melting acetate could be traced.

Molluscs are often found to contain more than one sterol. Therefore, different means were tried to separate the sterol fraction of *L. helicina* into different compounds. However, fractional crystallization and chromatography of the free sterol, its acetate and its 3,5-dinitrobenzoate always gave fractions with corresponding melting points and optical rotations the only contamination being small amounts of a sterol with conjugated double bonds.

Equally the sterol fraction of the *C. limacina* proved to contain only one sterol. Further the two sterols proved to be identical. The properties of this sterol indicate that it is a new compound which is later refered to as pteropodasterol.

Table 2.

Pteropodasterol	m. p. 132°	$[\alpha]_{20}^{D}$ — 40°
Pteropodasteryl acetate	— 136°	— — 47°
Pteropodasteryl 3,5-dinitrobenzoate	— 198.5°	— — 15°

The 3,5-dinitrobenzoate is one of the most suitable compounds for determination of the molecular formula. The analysis of this compound indicates the formula $C_{27}H_{46}O$ for the free sterol, but the range of melting points of the fractions analysed (m. p. 192°—198.5°) makes it probable that the samples were still impure. The physical constants of the hydrogenated sterol and its acetate were the same as those of stigmastanol as shown below.

Table 3.

Stigmastanol	Stanol		Stanyl acetate		
from	m. p.	$[\alpha]_{20}^{D}$	m. p.	$[\alpha]_{20}^{D}$	Ref.
Stigmasterol	135.4—36—37°	+ 24.8°	128—29.8°	+ 15.3°	2
»	135°	+ 28°	128°	+ 15.7°	3
Fucosterol	134.5—36°	+ 24.7°	128—30°	+ 15.1°	4
Spongillasterol	134—35°	+ 23.3°	129°	+ 11.5°	3
Pteropodasterol	134.5—35.5°	+ 22.3°	128—28.5°	+ 10.3°	

Pteropodasterol has been found to contain two double bonds. Hence the formula is $C_{29}H_{48}O$ and the carbon skeleton is the same as that of stigmasterol. The position of the double bonds has — in lack of material — not been investigated chemically. The methods of molecular rotation differences (M. R. D.) worked out by Barton [5,6], show that one double bond is in the 5,6-position and the other must be in the side chain. In Table 4 are given the molecular rotations of pteropodasterol and its M. R. D's according to Barton's nomenclatur, compared with those of related sterols.

The provitamine D content of the sterol fraction was determined by its UV absorption. In a sterol fraction, isolated from the unsaponifiable matter as the digitonin complex, and split according to Schønheimer and Dam [7], the provitamine content was found to be 4.5 %. By chromatography a fraction was obtained in the upper part of the column containing 10 % provitamine. Biological activity was not tested.

DISCUSSION

It is well known that vertebrates may synthezise their own sterols. Very little is known about the sterol metabolism of lower animals. Certain facts point to the possibility that lower animals may contain sterols of exogen

Table 4.

The M. R. D. according to Barton 1945 [2]

	$[M]_D^{20}$ sterol	$[M]_D^{20}$ acetate	$[M]_D^{20}$ 3,5-dinitrobenzoate	Δ^1	Δ^2
Chalinasterol	− 167	− 202		− 35	
Clionasterol	− 153	− 192	− 85	− 39	+ 68
Spongillasterol	− 174	− 219	− 109	− 45	+ 65
Poriferasterol	− 206	− 241	− 133	− 35	+ 73
Stigmasterol	− 202	− 241	− 133	− 39	+ 69
Pteropodasterol	− 165	− 214	− 91	− 49	+ 74

The M. R. D. according to Barton 1946 [3]

	$[M]_D^{20}$ sterol	$[M]_D^{20}$ acetate	Δ^1	Δ^2
Pteropodastanol	+ 94	+ 47		
Pteropodasterol	− 165	− 214	+ 259	+ 261
	found by Barton		$+ 251^{+10}_{-9}$	$+ 243^{+27}_{-18}$

origin, as stated by Bergmann [1]. All gastropods have been found to contain cholesterol, alone or together with another sterol. The occurence of a new sterol in two pelagic snails is worth notice. These pteropods are carnivorous, mainly feeding on *Calanus finmarchicus* and other crustaceans. Crustaceans are found to contain cholesterol. Thus the pteropods investigated are mainly feeding on a C_{27}-sterol whereas they contain a C_{29}-sterol. This leads to the conclusion that they contain no exogen sterol. These pteropods have their own metabolism since their organism seems to be able to exclude exogen sterol and synthezise its own one. Their metabolic product, however, is another one than that of other gastropods.

EXPERIMENTAL

Limacina helicina. The contents of a heat conserved glass jar were first extracted 4 times with ether, then an equal amount of ethanol was added, and the extraction with ether repeated 3 times more. The iodine number (175) of the fraction from the last three extractions, as determined by the micro method of Kaufmann [8], was higher than that of the first four ones (148). No fractionation effect could, however, be seen regarding the contents of unsaponifiable matter and of sterol. Half of the raw material was heat conserved, the rest was conserved with ammonium sulphate. No difference could be observed between the two parts of material preserved in these ways. The bulk of material was first denaturated and dried with ethanol, and then extracted 4 times with ether.

The fat was of a dark brown colour. This colour followed the fatty acids after saponification. Glycerol was determined as *iso*propyliodide [9] in a quantity corresponding to 82 % of the fatty acids calculated as triglycerides. Fat content 0.55—0.88 g/100 g wet material, fatfree residue 9.7 g/100 g wet material, and ash 3.07 g/100 g wet material. Unsaponifiable matter 10.7 g/100 g fat. The amount of sterol was determined as the digitonin complex and calculated as cholesterol; according to the work of Schønheimer and Dam [7] it was not found correct to calculate as pteropodasterol without special investigations. Found 62.1 g/100 g unsaponifiable matter.

Pteropodasteryl acetate. The bulk of unsaponifiable matter was extracted repeatedly with boiling methanol, and the sterol fraction recrystallized from methanol, m. p. 120.5 —22.5°. The crystals were dissolved in petroleum (b. r. 65—70°) and chromatographed. Different adsorbents were tried. Magnesium oxide was found to be the best one and was used in these experiments. The samples were brought on the column with petroleum and developed with petroleum-benzene 1 : 1. After crystallization from methanol the free sterol melted at 127—29.5°. The sterol was acetylated in boiling acetic anhydride. The acetate was crystallized twice from methanol and chromatographed. 8 fractions were collected and each fraction crystallized from methanol. All fractions melted in the range 131—34.5°, the m. p. of the highest melting fraction being 133—34.5°, $[\alpha]_D^{20}$ — 47.0° (41.1 mg in 3.14 ml, α_D — 1.23°). All optical rotations were measured in chloroform.

Pteropodasterol. The pure acetate was saponified. After recrystallization from methanol the free sterol was obtained, m. p. 130.5—32°, $[\alpha]_D^{20}$ — 39.9° (37.5 mg in 3.13 ml, α_D — 0.95°).

Pteropodasteryl 3,5-dinitrobenzoate. The free sterol was heated on a waterbath for 1 hour with a slight excess of 3,5-dinitrobenzoyl chloride in pyridine. The ester was crystallized from methanol and chromatographed, the highest melting fraction melting at 195.5—98.5°, $[\alpha]_D^{20}$ — 14.9° (45.2 mg in 3.12 ml, α_D — 0.43°).

Clione limacina. The material was treated in the same way as that of *L. helicina.* After extraction with alcohol and ether a dark brown, viscous fat was obtained in an amount of 0.68 g/100 g raw material, iodine number 167. The fatfree residue was dried in a vacuum, 6.88 g/100 g wet material, ash 2.71 g/100 g wet material. Unsaponifiable matter amounted to 13.9 g/100 g fat, and sterol content 40.8 g/100 g unsaponifiable matter (calculated as above).

Pteropodasteryl acetate. After some crystallizations from methanol, the sterol was chromatographed, acetylated and recrystallized from methanol, m. p. 134.5—36°, $[\alpha]_D^{20}$ — 47.9° (60.6 mg in 3.12 ml, α_D — 1.85°), and for another fraction prepared in the same way $[\alpha]_D^{20}$ — 47.1° (42.2 mg in 3.12 ml, α_D — 1.27°).

Pteropodasterol. The acetate was saponified, and the sterol crystallized from methanol, m. p. 131.5—32°, $[\alpha]_D^{20}$ — 40.4° (75.0 mg in 3.12 ml, α_D — 1.93°).

Iodine number of the free sterol has been determined according to the micro method of Kaufmann [8]. Dam [10] has found this method useful in the case of sterols.

	Iodine number	
Found for pteropodasterol	119	corresponding to 1.93 double bonds
Found for cholesterol	66.9	calculated 66

Pteropodasteryl 3,5-dinitrobenzoate. The free sterol was heated on a waterbath for 1 hour with a slight excess of 3,5-dinitrobenzoyl chloride in pyridine. The ester was re-

crystallized from methanol, added a little ether and chromatographed, m. p. 193—94.5°, $[\alpha]_D^{20}$ — 14.7° (34.6 mg in 3.12 ml, α_D — 0.32°).

Analysis of pteropodasteryl 3,5-dinitrobenzoate:

Found for *L. helicina*	C 70.13, 70.51	H 8.43, 8.34
» » *C. limacina*	» 70.35, 70.88, 70.37	» 8.42, 8.14, 8.46
Calculated for $C_{34}H_{48}O_6N_2$	» 70.34	» 8.29
» » $C_{35}H_{50}O_6N_2$	» 70.71	» 8.42

Mixed melting points of the acetates and the 3,5-dinitrobenzoates of the sterols from the two pteropods were 132—33.5° and 192.5—94.5° (turned clear 196°) respectively.

Pteropodastanyl acetate. 202.1 mg of the acetate was hydrogenated in acetic acid with a catalyst of palladium on barium sulphate. The acetate absorbed 20 ml hydrogen, corresponding to 1.93 double bonds. After recrystallization from methanol the hydrogenated substance showed m. p. 128—28.5°, $[\alpha]_D^{20}$ + 10.3° (66.45 mg in 3.12 ml, α_D + 0.46°).

Pteropodastanol. The free stanol was obtained after saponification of the saturated acetate and recrystallized from acetone, m. p. 134.5—35.5°, $[\alpha]_D^{20}$ + 22.3° (58.6 mg in 3.12 ml, α_D + 0.83°).

SUMMARY

The pteropods *Clione limacina* Phipps and *Limacina helicina* Phipps have been investigated. Both species have been found to contain a new sterol which is referred to as pteropodasterol.

The author wishes to express his sincere gratitude to the head of the laboratory, Prof. Dr. techn. N. A. Sørensen for his valuable advice and interest for this work.

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Received February 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 518 – 535

On Iodometric and Polarimetric Determination of Penicillin

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THE PRINCIPLE AND EARLIER FORM OF THE IODOMETRIC DETERMINATION

It was early found that certain products of inactivated penicillin, but not the pure active substance, show a marked consumption of iodine. Alicino [1] made use of this fact for the determination of penicillin. The principle is the following: Two identical samples are taken from a solution of penicillin. The one is inactivated with alkali (or penicillinase) and then neutralized with acid. The same amount of iodine is added to both samples and, after a time, they are titrated with thiosulphate. The difference in the consumption of iodine is a function of the amount of penicillin. Careful analytic descriptions have been given by, for example, Mundell, Fischbach and Eble [2]. In principle, the same iodometric determination is also included in the analytical descriptions of penicillin issued by the Food and Drug Administration, Washington, D. C. The methods, however, do not always give reproduceable values.

A new modification of the iodometric determination

Reagents. Sodium hydroxide 1 *N*, acid (hydrochloric or sulphuric acid) 1 *N*, buffer (0.3 *M* phthalate) pH 4.5, iodine solution 0.01 *N*, and standardized 0.01 *N* sodium thiosulphate.

The hydroxide and the acid need not be exactly normal, but they should, on the other hand, be equivalent to each other. A solution of 60 g potassium biphthalate and 80 ml 1.00 *N* sodium hydroxide diluted to 1 l is a suitable buffer, but any buffer with pH about 4.5, or, in most cases, one with pH between 4 and 5 or even pH 4—6, can be used if it has sufficient strength and does not react with other components in the reaction mixture. A iodine solution containing 12.7 g iodine and 20 g potassium iodide in 1 l

water is diluted 1 : 10 before use. The thiosulphate solution may be prepared by diluting 0.1 *N* sodium thiosulphate solution with boiled distilled water. The solution may be standardized after dilution and then frequently checked. Canbäck *et al.*[3] has stated that for certain reasons it is desirable to substitute an iodine solution which contains 1.27 g iodine and 8.3 g potassium iodide in one liter for the above-mentioned solution.

Procedure. From a *weakly* buffered (pH 5.5—7.5) or unbuffered water-solution, which contains about 2.5 mg sodium benzyl penicillin (approximately 4000 units; minimum 800, maximum 6000) per 5 ml or an equivalent amount of other penicillin, two samples, A and B, of 5 ml each are pipetted into 100 ml Erlenmeyer flasks with glass stoppers.

To A, add 5 ml buffer and 10 ml iodine. Close the flask immediately, keep it for 20 minutes in darkness at room-temperature, and then titrate with (v_a ml) thiosulphate.

To B, add 1 ml sodium hydroxide and, after 20 minutes in room-temperature, 5 ml buffer, 1 ml acid and 10 ml iodine. Close the flask immediately and keep it a further 20 minutes in darkness at room-temperature, and then titrate with (v_b ml) thiosulphate.

For the determination of procaine penicillin, a phthalate buffer, with pH 4.5, is satisfactory but one with pH 4.3 is to be prefered.

If the determination is carried out on an impure solution which has a high consumption of iodine at pH 4 to 5, not due to penicillin, and a strong buffer effect, the prescribed amount of iodine may be increased to 15 or 20 ml, and the amounts of hydroxide and acid to 2 or 3 ml. In this case it is also desirable to control the pH in the titrated solutions.

Calculations. If (v_a—v_b) is converted to 0.01000 *N* thiosulphate and divided by 100, the result gives the number of milligram equivalents of iodine, that have been consumed by the penicillin in the reaction-mixture. Concerning the conversion from equivalents of consumed iodine to mg or units of penicillin, see p. 528.

The effect of changes in the concentration of certain reagents, times, and temperatures

Pedersen [4] has systematically varied the alkali-concentration used by the destruction of the penicillin, the time for hydrolysis with the sodium hydroxide, the iodine-concentration, the time for the oxidation with iodine, the temperature, and the pH of the reactionmixture. In the oxidation with iodine, the pH is of the greatest importance, while moderate variations in the other factors have less effect. Pedersen recommends pH 5.8 to 6.3 in the reaction-mixture and Wild [4] a pH between 6 and 7.

Table 1. The effect of variations in the reaction times with alkali and iodine solution, in the amounts of these reagents, and in the amount of penicillin on the iodometric determination of sodium penicillin "Pc VI" (Table 3).

mg Na-benzyl-penicillin (Pc VI) in react.-mixture	Treatment with alkali for minutes	Treatment with iodine for minutes	Recovered penicillin (%) after treatment with: 1 ml 1 *N* sodium hydroxide and iodine solution			3 ml 1 *N* sodium hydroxide and iodine solution			Recovered penicillin %		
			10 ml	15 ml	20 ml	10 ml	15 ml	20 ml	average	ε	σ
0.5	20	20	103.1	100.5	100.5	100.4	99.7	103.7	101.3	± 0.7	± 1.7
1.6	20	20	101.1	102.1	101.8	103.1	102.0	101.8	102.0	± 0.3	± 0.7
2.5	20	20	100.8	98.0	100.2	103.2	99.6	99.2	100.2	± 0.7	± 1.8
2.5	40	20	100.3	100.0	99.2	103.6	99.2	100.0	100.4	± 0.7	± 1.7
2.5	40	40	101.1	101.5	103.5	101.1	103.5	102.8	102.3	± 0.5	± 1.2
2.5	20	40	99.2	101.1	101.9	100.3	100.3	101.9	100.8	± 0.4	± 1.1
3.5	20	20	99.8	101.3	103.1	101.7	102.0	99.7	101.3	± 0.5	± 1.3
Recovered % penicillin	average		100.8	100.6	101.5	101.9	100.9	101.3	101.2	± 0.3	± 0.8
	ε		± 0.5	± 0.5	± 0.6	± 0.5	± 0.6	± 0.6	± 0.2	—	—
	σ		± 1.2	± 1.3	± 1.6	± 1.4	± 1.6	± 1.7	± 0.5	—	—

A long series of determinations carried out according to Pedersen did not, however, give satisfactory results. This necessitated a careful investigation of the iodometric method.

The effect of increased amounts of sodium hydroxide and iodine and prolonged reaction times is, as may be seen from Table 1, not greater than the experimental error of single determinations. Moreover, the average 101.2 ± 0.2 %, obtained from all the values in Table 1, agrees well with the value 100.8 ± 0.2 % that has been obtained below for penicillin Pc VI. It is thus possible to vary the amount of sodium benzyl penicillin in the reaction-mixture between 0.5 and 3.5 mg, the amount of sodium hydroxide between 1 and 3 ml, the amount of iodine-potassium iodide solution between 10 and 20 ml, and the reaction time between 20 and 40 minutes without any significant effect upon the analytic results.

Thus, if the concentrations of iodine and potassium iodide used are in the same proportion, it is possible to increase them at least 50 % without the consumption of iodine being significantly changed. Canbäck *et al.*, who were informed of our results, found that an increase in the concentration of potassium iodide alone leads to an increase in the iodine consumption of the penicillin in the pH region immediately below 4. There is, however, no difficulty in keeping the concentration of potassium iodide constant. In all our experiments, this concentration has been maintained unchanged.

The iodometric penicillin determinations do not appear to be greatly affected by small temperature differences, but the values do show a tendency to increase with the tem-

Table 2. The effect of variations in temperature on the iodometric determination.

Added Pc VI mg	Temp. on treatment with iodine and alkali °C	Recovered amount of sodium penicillin %	
2.6	+ 10	97.4 97.4	97.4
2.6	+ 20	98.5 99.3	98.9
2.6	+ 30	99.3 101.2	100.3

perature. Accompanying treatments with iodine and sodium hydroxide were always carried out at the same temperature (Table 2). In normal determinations, a variation in the room-temperature, *e. g.* from + 15° to + 25° C, scarcely had any measurable effect.

Thus, none of the above-mentioned variables can cause any significant deviations in the iodometric determination of penicillin. Temperature, reaction times, and concentrations of reagents should, however, be kept as constant as practical working conditions permit.

The influence of pH

In the subsequent experiments the pH was varied in the iodine reaction-mixture. Acetate, citrate, phosphate, phthalate, and tartrate buffers (0.3 *M*) were used. The composition of the buffer solutions does not, however, play any rôle as long as they are sufficiently strong and do not react with other components in the reaction-mixture. In certain cases the pH of the solution showed slight displacements in relation to that of the buffer.

The investigation covers altogether eleven penicillin preparations. Four are ordinary commercial preparations of crystalline benzyl penicillin sodium, and seven are special preparations. In Figs. 1 and 2 the pH of the titrated solutions have been plotted as abscissae, while the number of equivalents of iodine that have been consumed per mole penicillin (if the sample is considered 100 % pure) have been taken as the ordinates.

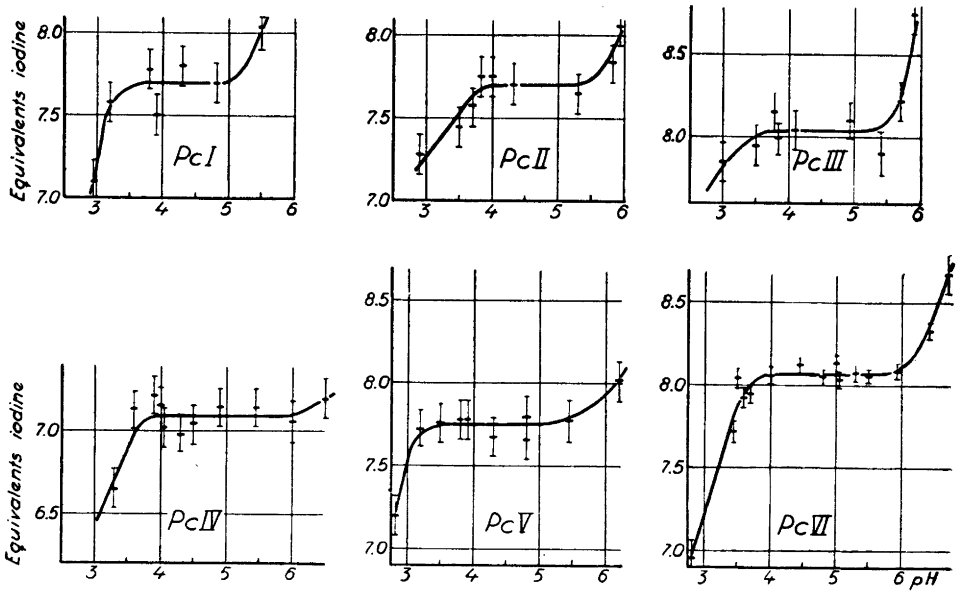

Fig. 1. The number of iodine equivalents consumed by 6 penicillin preparations are plotted against pH. The penicillins are described in Table 3. The errors in the determinations are represented by lines parallel with y-axis. Errors in pH max. ± 0.03.

Pc I: Eeach point represents one determination.
Pc II: Each point represents one determination.
Pc III: Each point represents one determination. except that at pH 3.85 which represents two.
Pc IV: Each point represents one determination.
Pc V: Each point represents one determination.
Pc VI: From the left (pH = 2.8) to the right (pH = 6.7) the points represent 1, 6, 4, 4, 10, 7, 5, 10, 4, 6, 4, 4, 8, 10, and 1 determinations, respectively.

The pH region where the curves run parallel with the pH axis is rather great, and a slight displacement in the pH is thus of subordinate importance. Where several determinations have been carried out at the same pH, the mean value and the corresponding standard error (ε) have been plotted. In those cases in which a certain value of a curve corresponds to only one determination, the mean value for σ (= 1.5 %) obtained in Table 3 has been introduced as the limits of error of the determination.

The curves of the preparations examined here are rather similar and do not agree with that given by Pedersen. The middle part of all curves is parallel with the pH axis in the region about pH 4—5, but the pH where the curves

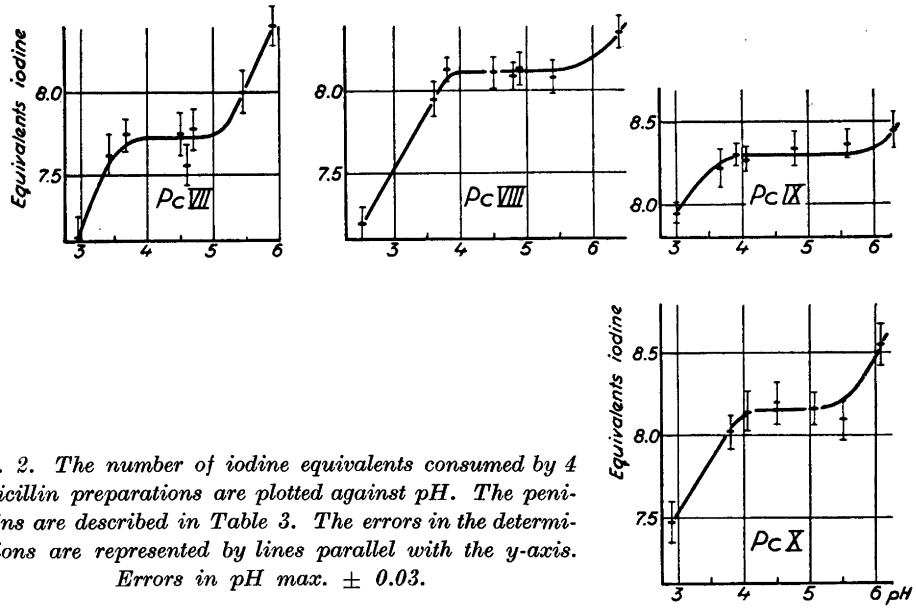

Fig. 2. The number of iodine equivalents consumed by 4 penicillin preparations are plotted against pH. The penicillins are described in Table 3. The errors in the determinations are represented by lines parallel with the y-axis. Errors in pH max. ± 0.03.

Pc VII: Each point represents one determination, except that at pH 3.7, which represents two.

Pc VIII: From the left (pH = 2.5) to the right (pH = 6.4) the points represent 2, 2, 4, 3, 3, 2, 2, and 2 determinations, respectively.

Pc IX: From the left (pH = 3.0) to the right (pH = 6.3) the points represent 4, 1, 6, 3, 2, 4, and 2 determinations, respectively.

Pc X: From the left (pH = 2.9) to the right (pH = 6.1) the points represent 1, 2, 1, 1, 2, 1, and 1 determinations, respectively.

begin to rise or fall are somewhat different for the penicillin preparations examined here.

In the pH range 4 to 5 or 6, where according to Brodersen [6] the penicillin keeps rather well for 20 minutes, constant values are obtained for the difference between the iodine consumption of the penicillin before and after the treatment with sodium hydroxide. At a lower pH, where the penicillin is quickly inactivated even in samples not treated with alkali, the difference in the consumption of iodine is less. At a higher pH (5 or 6—7), where, certainly, the penicillin is not disintegrated but where the presence of iodate may be conceived to give rise to a stronger oxidation, one obtains greater differences in the consumption of iodine.

For the iodometric determination of penicillin the pH in the iodine reaction-mixture should thus in ordinary cases be kept at about 4.5. In some cases, procaine seems to interfere with the determination at a pH higher than 4.6.

Table 3. Iodometric and polarimetric determination of certain penicillin preparations.

Penicillin preparation	Of the total penicillin content biolog. type determ. shows				Iodomet. determination					Polarimetric determination; content	Diff. in iodomet. and polarim. content	Remarks
	%G	%F	% H_2F	%K	pH-region	Number of determ.	Content %	Content ε	Content σ	%		
Pc I; cryst. sodium penicillin (Bristol)	99	1	—	—	3.8—4.8	4	96.2	±0.8	±1.6	95.1±0.6	+1.1	Ordinary commercial prep.
Pc II; cryst. sodium penicillin (Kärnbol.)	98	1.5	0.4	0.3	3.8—5.3	4	96.5	±0.8	±1.6	97.1±0.6	−0.6	» »
Pc III; cryst. sodium penicillin (Novo)	94	4	2	—	3.8—5.4	6	100.3	±0.6	±1.5	99.9±0.6	+0.4	» »
Pc IV; cryst. sodium penicillin (Astra)	97	2	0.7	0.3	3.8—5.4	7	88.7	±0.6	±1.5	89.4±0.6	−0.7	Less pure prep. included for comparison.
Pc V; cryst. sodium penicillin (Astra)	97	2	0.3	—	3.8—4.8	5	96.8	±0.7	±1.5	96.7±0.6	+0.1	Ordinary commercial prep.
Pc VI; cryst. sodium penicillin (Astra)	98.4	1.6	—	—	4.0—5.5	40	100.8	±0.2	±1.4	100.4±0.3	+0.4	Specially purified sodium benzylpenicillin
Pc VII; cryst. sodium penicillin (Kärnbol.)	99.5	0.5	—	—	3.7—4.7	5	96.5	±0.7	±1.6	97.3±0.6	−0.8	Specially purified sodium penicillin. Determination on remains in earlier opened flask (moisture?)
Pc VIII; benzylpenicillin di-isopropyletherate (Astra)	99	0.6	—	—	3.8—5.4	14	101.4	±0.3	±1.2	101.4±0.6	±0.0	The high content has for IX been verified by N_2 determ. which gave 102.7 %. Both propyletherates seem to lose ether on drying
Pc IX; benzylpenicillin di-isopropyletherate (Kärnbol.)	98	1.5	0.5	—	3.7—4.8	12	103.7	±0.5	±1.8	103.2±0.6	+0.5	
Pc X; N-ethylpiperidinpenicillin	98	2	—	—	4.1—5.5	5	102.0	±0.2	±0.6	98.8±0.6	+3.2	Precipitate obtained by G-determ.
PcXI; procaine penicillin (Astra)	95	4	1	—	4.2—4.6	8	99.8	±0.4	±1.2	99.5±0.6	+0.3	Ordinary commercial prep.
Total:						110	(1082.7)		—	(1078.8)	+3.9	
Average:						—	98.4±0.2		±1.4	98.1±0.2	+0.3	
Total Pc I to Pc IX and Pc XI						105	(980.7)		—	(980.0)	+0.7	
Average Pc I to Pc IX and Pc XI						—	98.1±0.2		±1.5	98.0±0.2	+0.1	

Table 4. Effect of the »blank iodine time» on the iodine consumption obtained at different pH.

Penicillin sodium "Pc V"					Penicillin sodium "Pc VI"					
pH in the reaction mixt. with iodine	Iodine consumption in equiv./mol. if the blank sample has been treated with iodine				pH in the reaction mixt. with iodine	Iodine consumption in equiv./mol. if the blank sample has been treated with iodine				
	0 min	5 min	10 min	20 min		0 min	5 min	10 min	20 min	30 min
2.3	8.31	7.85	7.28	6.82	2.5	8.53	8.38	8.07	6.80	6.58
3.2	7.83	7.76	7.76	7.72	3.3	8.40	8.23	7.98	7.63	7.82
3.9	7.79	7.84	7.81	7.79	4.0	8.08	8.05	7.86	8.02	8.02
4.3	7.73	7.73	7.75	7.68	4.4	8.00	7.96	7.78	7.92	7.88
4.8	7.80	7.72	7.72	7.67	5.0	8.11	8.16	8.20	8.11	8.08
5.5	7.73	7.69	7.67	7.63	5.5	8.16	8.08	8.09	8.10	8.05
6.2	8.14	7.99	8.06	7.99	6.4	8.45	8.39	8.34	8.40	8.27
Mean for the values corresponding to pH 3.2—5.5	7.78	7.75	7.74	7.70	Mean for the values corresponding to pH 4.0—5.5	8.09	8.06	7.98	8.06	8.01
ε	±0.02	±0.03	±0.02	±0.03	ε	±0.04	±0.04	±0.09	±0.04	±0.04
σ	±0.05	±0.07	±0.05	±0.06	σ	±0.07	±0.08	±0.20	±0.09	±0.08

The blank test in the iodometric determination

As has already been mentioned (p. 519), "blanks" (= A) have been carried out in such a way that the sample not treated with alkali has been allowed to react with iodine for as long a period as the sample treated with alkali, *i. e.* for 20 minutes. This is in opposition to earlier prescriptions [2, 4, 5], but agrees with general analytical practice, *i. e.* to treat the blank and the sample identically as far as possible. Within the pH range used, no destruction of the penicillin has been found to occur. If there were a measurable destruction, then the part of the pH curves now parallel with the pH axis should incline slightly upwards with rising pH; and no such tendency has been observed.

Investigations relating the change of the pH curve with the time of the iodine treatment of the blank have been carried out on the sodium benzyl penicillin preparations Pc V and Pc VI. The reaction time, referred to below

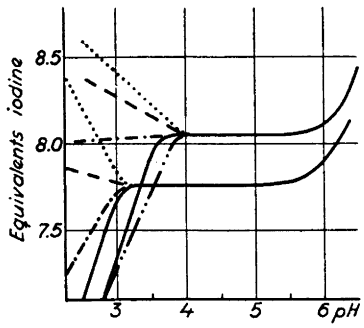

Fig. 3. Iodine equivalent curves for Pc V and VI from different "blank iodine times" (. . . . . 0 minutes, — — — — 5 minutes, — · — · — 10 minutes, ——— 20 minutes, and — · · — · · — 30 minutes).

as "blank iodine time", was varied from zero to 30 minutes, while the reaction time of the alkaliinactivated samples remained unchanged at 20 minutes. The values for the iodine equivalent obtained may be seen in Table 4. The "blank iodine time" does not play a very great rôle in the pH range 6.5—4 or 3. The mean values, which correspond to the part of the pH curve parallel with the pH axis, do, however, show a falling tendency with increased time. This tendency is confirmed by 12 parallel determinations carried out on Pc VI and 8 on Pc V, which were performed with both 5 and 20 minutes' "blank iodine time". The mean values give the contents 101.4 and 100.8, and 97.3 and 96.7 %, respectively. The differences between the corresponding values amount, however, to only about 1 %, and are within the experimental error of a single determination. At low pH (less than 3), on the other hand, one obtains considerable differences. Schematic curves for Pc V and VI can be graphically represented as in Fig. 3. The "blank iodine time" thus is not of too great importance when the iodometric determinations are carried out at a suitable pH. If, however, less pure samples are analysed, impurities which react slowly with iodine may be present. If in this case the "blank iodine time" is short, the impurities will not have time to react completely, and will be included in the determination as penicillin.

Experimental errors of the iodometric method

The limits of error in Table 1, and other such errors given for iodometric determinations in this paper, have been calculated throughout according to the usual formulae. The standard error of the average is thus denoted by ε and is calculated from $\varepsilon = \pm \sqrt{\frac{\Sigma \Delta^2}{n\,(n-1)}}$, and the standard deviation for a single determination is denoted by σ and calculated from $\sigma = \pm \sqrt{\frac{\Sigma \Delta^2}{(n-1)}}$.

The deviations in the iodometric determinations are considerably greater than the actual titration errors. Thus, ten determinations of Pc VI at pH 4.8 gave, for the mean value, a standard error (ε) of $\pm$ 0.5 %. The standard deviation (σ) for a single determination was $\pm$ 1.5 %. In all the other series, errors of approximately the same magnitude were obtained, even if the series included not only determinations carried out at exactly the same pH, but all those on the part of the pH curve running parallel with the pH axis. Examples of such values are given in Table 3. Thus, if single iodometric determinations are carried out on fairly pure substances, the deviation from the correct value in about 65 per cent of the cases should not exceed $\pm$ 1.5 % and in 95 per cent should not exceed $\pm$ 3 %.

The main cause of the great irregularities in iodometric determination of penicillin according to earlier prescriptions

Mundell, Fischbach, and Eble stated that the blank, *i. e.* the nonalkali inactivated sample, must be retitrated immediately, and should not be too acid (not below pH 3). The alkali inactivated sample, on the other hand, is purposely acidified to pH 2 to 3 with a slight excess of acid. Iodine is then added and the sample is titrated after 15 minutes. Experiments show that if the blank on pure sodium penicillin is titrated *immediately*, a pH displacement from 2 to 6 is of small importance. In consequence of the disintegrating of the penicillin, however, the iodine consumption increases rapidly in blanks whose pH is less than 3. In the determination according to Mundell *et al.* or the Food and Drug Administration Washington D. C., the pH is unsuitable because in this range the pH curve inclines very strongly. Compare the dotted parts of the curves in Fig. 3. The determination in the pH range 2—3 is therefore strongly affected even by variations of the pH and the time for which the iodine is allowed to react with the alkali-inactivated penicillin. The limits 2 to 3, which are given for pH, are too wide, and the way of adjusting pH without buffer is unsuitable. The rising pHcurve also explains the necessity of the low factor that is introduced. This factor is valid only for a point corresponding to a definitely established pH. As may be seen from Fig. 3, differences of 8 to 9 % may be obtained in the determination when the pH is changed from about 2 to 3. By carrying out the determination at a definitely established and more suitable pH (4—5), one can obtain 2 to 4 times better reproduceability. For some penicillin preparations (Figs. 1 and 2 Pc I, II, III, VI, VII, and X) the pH chosen by Pedersen is also rather unsuitable, because the pH curves begin to rise. At pH 5.8 and 6.3, which are the limit

values Pedersen indicates, determinations of Pc VI *e. g.* give the average values 101 and 104 % and single determinations of Pc VII at pH 5.8 and 6.3 give 10 % difference.

CONVERSION FACTOR

The part of the pHcurves parallel with the x-axis gives, as may be seen, y-values of about 8. This indicates that, after inactivation with alkali, one mole penicillin consumes about 8 equivalents of iodine. If we assume the value for pure penicillin to be exactly 8, the percent content of penicillin may be calculated. The difference of 1.00 ml between the consumption by the blank and the sample of 0.01 N thiosulphate then corresponds to 742 units of benzyl penicillin or 0.445 mg benzyl penicillin sodium, and 0.712 mg water-free benzyl penicillin procaine. The iodometric contents given in Table 3 have been calculated in this way from the mean values (with corresponding ε) for the determinations on the part of the curves parallel with the pHaxis.

POLARIMETRIC DETERMINATIONS

In order to confirm the correctness of the assumption that the equivalent weight of the penicillin on iodine reaction is one-eighth part of its molecular weight, the content of the preparation should also be determined according to some other method than the iodometric. Biological determination or colorimetric methods, *e. g.* according to Boxer and Everet [7] or Henstock[8], require standard substances and entail rather considerable errors. They are thus not suitable as comparative methods for the determination of purer preparations. Another conceivable method is one in which potassium ferricyanide is used instead of iodine. Such a method has been published by Hiscox [9]. This method has also been tested and found to be very unsatisfactory. Its greatest drawback probably lies in the fact that the penicillin must be treated with the reagent at a high temperature and an unsuitable pH. Thus, during the determination a non-reproduceable destruction of penicillin occurs which causes large errors. For fairly pure substances, like the crystalline preparations included in Table 3, there remains, however, the possibility of carrying out polarimetric determinations of content.

The specific rotation for pure substance must then be known. Available data for sodium benzyl penicillin vary between + 290° and + 305°. Leigh [10] gives the specific rotation, in sodium light, for *n*-amyl penicillin in 1 % neutral water solution at + 23° C as + 319°, and Salivar, Howard and Brown [11] measured a 1 % solution of benzyl penicillin procaine in 50 % aqueous acetone and found the rotation to be + 173° at 25° C. If these values are converted to benzyl penicillin sodium, one obtains the rotations + 301° in water and + 286° in 50 % aqueous acetone. The specific rotation for Pc VI, which does not contain any moisture, is + 302° ± 0.7. This value is the average of seven determinations, which gave $\varepsilon = \pm 0.7$ and $\sigma = \pm 1.8$ (*i. e.* ± 0.6 %), and were carried out in 1 % water-solutions with pH between 5.1 and 7.2 in sodium light at room-temperature. In this pH range it was not possible to observe any systematic change in rotation with pH. A single determination on a 1 % solution in a mixture of equal volumes of water and

acetone gave for Pc VI $[\alpha]_D^{20} = + 289°$. As may be seen from Table 3, however, the preparation contains 1.6 % penicillin F. The specific rotation for pure benzyl penicillin sodium would then be somewhat lower, *viz.* + 301° ± 1 in the water-solution and + 288° ± 2 in 50 % aqueous acetone.

The values + 301° for the specific rotation in sodium light for the water-free sodium salt of benzyl penicillin in 1 % water-solution with pH 5 to 7 and + 173° for procaine penicillin with one mole of water of crystallization in 50 % aqueous acetone has been used below for calculation of contents. From the first value, a specific rotation of + 240° has been calculated for the N-ethyl piperidine salt of the benzyl penicillin. For benzylpenicillin Diisopropyl Etherate, Nelson, Trenner, and Buhs [12] give the value + 241°, which corresponds to + 296° for the sodium salt. They consider that it is in satisfactory agreement with the value of + 290° for crystalline sodium benzyl penicillinate. The agreement is just as good, however, if 290 is replaced by 301.

In Table 3 the polarimetric contents are calculated from the quotients between rotations observed in the single cases and the above specific rotations. This is of course theoretically correct only if the optical rotation of the substances is derived exclusively from active penicillin. For the preparations in Table 3, this condition is probably satisfactorily complied with. In those cases in which the polarimetric contents are based upon only one determination, the same percentual error, ± 0.6 %, has been ascribed to them, as has been calculated for a single determination of specific rotation. Both the iodometric and the polarimetric contents have been calculated as if benzyl penicillin were the only penicillin component. If the actual composition is taken into aocount, the values may be changed by a few tenths of one per cent.

If, however, the polarimetric method of determination is used for a penicillin preparation that has been partly disintegrated, the penicillin-content can not be calculated as the quotient between the observed and the theoretical specific rotation.

When the inactivation takes place in strongly alkaline solution (about 0.3 *N* sodium hydroxide), the specific rotation at room-temperature is rapidly reduced, as may be seen from Table 5, to about half the original value. The continued inactivation, on the other hand, takes place very slowly. For Pc VI, for example, 39 % of the rotation remains after twenty-four hours, 20 % after 3 days, 14 % after 5 days and still 7 % after 12 days. Thus, if for a penicillin partially inactivated in a basic solution, the specific rotation is + $B°$, and for the corresponding 100 % preparation + $A°$, it is probably more correct to calculate the content of penicillin (x %) that remains in the preparation from the equation $X = \frac{100\ (B—0.5\ A)}{A—0.5\ A}$ than from $X = \frac{100\ B}{A}$. The

Table 5. Inactivation of crystalline penicillin preparations. 1 % penicillin preparation, 0.5 N sodium hydroxide, + 20° C.

Inactivation period in minutes	Remanent specific rotation in % of original				Remaining activity accord. to iodomet. determ.			
	PcI	PcIV	PcVI	PcIX	PcI %	PcIV %	PcVI %	PcIX %
Approx. 5	58 (= 18 % pc undestroyed)	62 (= 25 % pc undestroyed)	56 (= 12 % pc undestroyed)	66 (= 29 % pc undestroyed)	17	22	—	26
10	51	52	—	55	0	0	—	1
20	50	52	54	53	0	0	0	0
40	49	50	52	52	0	0	0	0

polarimetric contents given in parentheses in Table 5 have been calculated according to the first formula. On disintegrating in strongly acid solution (about 0.3 N sulphuric acid), the rapid reduction of the rotation, as may be seen from Table 6, seems to terminate when the value has diminished approx. 25 %. Within parentheses is given the remanent amount of penicillin calculated on analogy with the above from the equation $X = \frac{100\ (B—0.73\ A)}{A—0.73\ A}$.

Table 6. Inactivation of crystalline sodium penicillin. 0.3 % Pc VI, 0.2 normal sulphuric acid. + 20° C.

Inactivation period in minutes	Remanent specific rotation in % of original	Remaining penicillin in % of original		
		calculated from rotation	determined	
			iodomet.	biolog.
2 1/4	92	(70)	73.4	70
7	84	(41)	41,2	39
10	81	(30)	32.5	—
15	79	(22)	17.2	17
20	77	(15)	10.5	—
90	70	—	1.2	—
18 × 60	64	—	0	—

Table 7. Inactivation of crystalline sodium penicillin. 0.3 % Pc VI, 0.4 normal sodium carbonate pH 10.6, + 20° C.

Inactivation period in hours	Remanent specific rotation in % of original	Remaining penicillin in % of original		
		calculated from rotat.	determined	
			iodomet.	biolog.
1	85	(70)	71.6	79
3	71	(42)	46.0	50
4.5	61	(22)	33.2	—
6	52	—	21.4	22
8	43.4	—	10.7	—
20	31.4	—	5.5	—

Even disintegration at pH 10.6 (in 0.4 *N* carbonate) evidently takes place in at least two distinct steps at different rates. If the content of penicillin remaining is calculated according to the same formula as for the inactivation in strongly basic solution, the values given in parentheses in Table 7 are obtained.

The above observations concerning the reduction of the rotation permit a quantitative calculation of remanent penicillin only if the pH is constant and known during the disintegration. In practice, however, the majority of penicillin preparations are either not at all or only weakly buffered, and thus the pH will vary during the disintegration.

Polarimetric determination of the content of a penicillin solution should thus only be carried out if it is known that the penicillin in the solution exists in the active form, and that the solution does not contain other optically active compounds. A rotation value that is lower than the theoretical can scarcely be regarded as anything other than a qualitative test for disintegration. If the rotation amounts to only 50 % of the theoretical value, it is, in general, probably justifiable to assume that the preparation is biologically completely inactive. The polarimetric determination also suffers from the drawback that it requires relatively large amounts of substance.

AGREEMENT AND SPECIFICITY OF THE METHODS OF DETERMINATION

As has been mentioned, the polarimetric method is very unspecific, and may be used only for pure substances. Table 3, however, shows that the agreement between iodometric and polarimetric determinations on pure prepara-

Table 8. Comparison between iodometric and polarimetric determinations of commercial crystalline sodium and procaine benzyl penicillin and of N-ethylpiperidin penicillin.

Preparation type	Number of determinations	Iodometric content : polarimetric content				
		highest value	lowest value	average	ε	σ
Sodium penicillin	15	1.043	0.953	1.001	± 0.006	± 0.02
Procaine penicillin	15	1.016	0.983	0.998	± 0.003	± 0.01
N-ethyl piperidin penicillin	15	1.055	0.996	1.020	± 0.005	± 0.02

tions is very satisfactory. This has been further confirmed by a pair of experimental series carried out on Astras commercial sodium and procaine salts of benzyl penicillin. In Table 8 are given the number of parallelly determined substances, the highest and lowest values for the quotients between the contents, the mean values of the quotients, as well as ε and σ. The table also gives the results of some determinations on N-ethyl-piperidine preparations.

The quotient (iodometric content) : (polarimetric content) for ethyl-piperidine penicillin is 1.020, which indicates that the high iodometric value in Table 3 is not due to chance. As the iodometrically determined contents for ethyl-piperidine penicillin are sometimes considerably over 100 %, the error is probably to be sought in this determination. Ethyl piperidine itself, however, does not consume iodine under the experimental conditions used, and in four determinations of Pc VI, in the presence of an equivalent amount of ethyl piperidine, the same content was obtained (100.6 *W* 0.7 %) as earlier. All the ethyl-piperidine penicillins tested have, however, been produced in the same way, and approximately unchanged amounts of the same impurities may be conceived to occur in all the preparations. Experiments with highly purified ethyl-piperidine penicillin have not been made.

Iodometric determinations on partially disintegrated penicillin preparations, give good values. The Tables 5, 6, and 7 include a comparison between biological, iodometric and polarimetric determinations after disintegration in sodium hydroxide, sulphuric acid, and sodium-carbonate solution at pH 10.6.

The iodometric determinations so far discussed have been carried out on rather pure penicillin preparations. In many cases, however, the method is

Table 9. Comparison between biological and iodometric determination on two different substrate series.

Substrate	l_1	b_1	c_1	d_1	e_1	f_1	g_1	Mean of the quotient	ε
Biol. assay / Direct iodom.	0.80	0.85	1.11	0.83	1.01	1.13	1.17	0.97	± 0.05
Biol. assay / Iodom. after extraction	0.86	0.85	1.07	0.85	1.15	0.83	1.07	0.95	± 0.05
Direct iodom. / Iodom. after extraction	1.08	1.00	0.96	1.03	1.14	0.74	0.92	0.98	± 0.05
Substrate	a_2	b_2	c_2	d_2	e_2	f_2	g_2	—	—
Biol. assay / Direct iodom.	0.85	0.77	0.66	0.72	0.86	0.54	0.79	0.74	± 0.04
Biol. assay / Iodom. after extraction	0.91	0.91	0.93	0.88	1.04	0.94	0.88	0.93	± 0.02
Direct iodom. / Iodom. after extraction	0.94	0.84	0.70	0.82	0.82	0.65	0.90	0.81	± 0.04

usable also for very impure preparations, even for broth. The amount of iodine is then increased to 15 or 20 ml, while the sodium hydroxide and acid amounts are increased to 2 or 3 ml. Iodometric determinations were carried out in this way, in parallel with biological determinations, on a number of broths of a certain type. The agreements were satisfactory. For certain other broths the agreement was poor, and deviations of ± 25 % or more were not uncommon. After a simple shaking with an organic solvent (chloroform, pH about 2) and subsequent transfer to a buffer (pH 7—7.5), however, it was possible to determine the penicillin even in these broths with satisfactory reproducability. This does *not*, however, mean that the iodometric method is, in general, especially well suited for determinations of the penicillin content in broth. The quotients between the biological and iodometrical contents of the series may be seen in Table 9.

The comparison between the iodometric determinations and other determinations thus shows that the iodine method is surprisingly specific, and it also supports the assumption that under the above-described conditions one mole of penicillin consumes 8 or very near 8 equivalents of iodine.

SUMMARY

A modified iodometric method for the determination of penicillin is described.

The accuracy of the modified method is greater than that of the earlier methods. The standard deviation for a single determination is about $\pm$ 1.5 %.

The pH value in the iodine reaction mixture is of great importance and should be approx. 4.5.

The blank is treated with iodine for the same time, 20 minutes, as the alkaliinactivated sample.

The alkaliinactivated penicillin consumes, under the conditions described, 8 equivalents of iodine per mole.

Iodometric determinations on partially disintegrated penicillin agree well with biological determinations of potency.

The iodometric determination can, in certain cases, be used directly on filtered broth.

Polarimetric determinations are satisfactory if active penicillin of a known type is the only unknown rotating component.

Only under special conditions is it possible to calculate quantitatively the inactivation of penicillin from the reduction of rotation.

With neither the iodometric nor with the polarimetric methods is it possible to distinguish between the different types of penicillin, unless they are present as pure substances.

The biological determinations of type and content have been carried out at the Physiological Department of Astra's Central Laboratories. Cordial thanks are extended to the head of the department, fil. lic. Sten Wiedling.

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Received Dezember 20, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 536–542

Methylene Blue as an Inhibitor of Acetylcholine-Esterase

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Methylene blue is a strong inhibitor of the enzymic hydrolysis of acetylcholine. This was first demonstrated with serum cholinesterase [1–6], later on also found for the acetylcholine-esterase * of leech muscle extract [8] and of erythrocytes, brain, *Helix* blood, and other sources [9]. The action of methylene blue on the serum cholinesterase has been discussed in relation to the positive oxidation-reduction potential of the dye [10, 11].

It is known that compounds with a quaternary ammonium ion are strong inhibitors of both serum cholinesterase and acetylcholine-esterase of various sources. The action of methylene blue is due to the presence of such an ion, for the leuco form of the dye has no inhibiting effect. Regarding the action of the quaternary ammonium bases on acetylcholine-esterase, the present author has recently postulated [9], that such compounds interact with the enzyme (E) by competition with the substrate (S) in the formation of the ES complex. According to the Murray-Haldane hypothesis, an enzyme inhibition by excess of substrate may be explained by assuming the formation of an ES_2 complex which is incapable of the breakdown. It actually seems to be the case for the acetylcholine-esterase and its physiological substrate acetylcholine. This view satisfactorily explains the bell-shaped curve obtained by plotting the enzyme activity against the minus logarithm of the molar substrate concentration (pS). The characteristic substrate optimum (pS_{opt}) for the acetylcholine-esterase (2.4) has been found to shift to higher substrate concentration in the presence of choline [9] and prostigmine [12]. Both these compounds are quaternary ammonium bases. The shift of optimum becomes increasingly stronger with increasing inhibitor concentration. Prostigmine in 2×10^{-6} *M* solution, for instance, inhibits the enzyme 10 per cent at 3.3×10^{-2} *M* acetylcholine; when

* The term acetylcholine-esterase is equivalent to the previously used "specific", "true", and "e" cholinesterase [7].

the acetylcholine concentration is 10 times lower, the inhibition by the same concentration of prostigmine is as high as 88 per cent. It has now been postulated that such a shift of optimum acetylcholine concentration is characteristic of the action of quaternary ammonium bases on the acetylcholine-esterase.

It is the purpose of this paper to extend these observations with the inhibitory effect of methylene blue, also to show that the action of this compound is much the same as that of prostigmine on this specific enzyme.

METHODS

The enzyme activity was measured by the Warburg manometric method described previously [9]. Substrate, enzyme, and inhibitor were dissolved in bicarbonate-Ringer's solution containing 0.12 *M* NaCl, 0.034 *M* $NaHCO_3$, and 0.0012 *M* $MgCl_2 . 6H_2O$. The unit, b_{30}, an expression of esterase activity, is the amount of CO_2 in μl evolved during 30 minutes minus the corresponding value for non-enzymic hydrolysis. Measurements were made at 25° C. The substrate solution (1.60 ml) or a mixture of substrate and inhibitor (0.8 + 0.8 ml) was placed in the main compartment of the vessel, and 0.4 ml of the enzyme solution or a mixture of enzyme and methylene blue solutions (0.2 + 0.2 ml) in the side bulb.

The enzyme preparation was obtained by washing cow erythrocytes four times with 0.9 per cent NaCl solution and then haemolysing with distilled water to the same volume as the original blood volume. One part of the haemolysate was diluted with two parts of the bicarbonate solution.

Acetylcholine chloride was used as substrate in all experiments. Methylene blue (mol. wt. 373.89) was a commercial product (Merck & Co.).

INHIBITION AS FUNCTION OF SUBSTRATE CONCENTRATION

The inhibition of acetylcholine-esterase by methylene blue at various acetylcholine concentrations is shown in Fig. 1. In these experiments the enzyme came in contact with acetylcholine and methylene blue at the same time (*i. e.*, no incubation of enzyme with inhibitor). The final concentration of methylene blue in the reaction mixture in the two series of experiments shown was 1.07×10^{-5} *M* and 3.22×10^{-5} *M* respectively.

In the presence of methylene blue the optimum acetylcholine concentration is changed to a higher concentration. The higher the concentration of methylene blue the higher is the substrate optimum (pS_{opt} lower). In an 1.10×10^{-2} *M* acetylcholine solution when no inhibitor is present, this substrate concentration is higher relative to the substrate optimum. When methylene blue is added in increasing concentrations to such a solution, the new ("apparent") pS_{opt} approaches that corresponding to 1.10×10^{-2}. When methylene blue is present in still higher concentrations this substrate con-

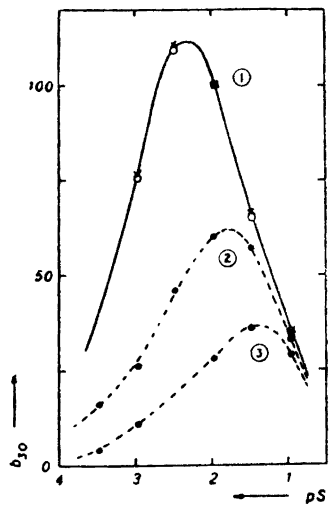

Fig. 1. Activity-pS curves for the enzymic hydrolysis of acetylcholine by acetylcholinesterase (cow erythrocytes) in the presence of added methylene blue (MB). No incubation, i. e., MB and acetylcholine in contact with the enzyme at the same time.

1. *Control; haemolysate* O———O, *intact cells* X———X; pS_{opt} *2.4*
2. *1.07 × 10⁻⁵ M MB* pS_{opt} *1.8*
3. *3.22 × 10⁻⁵ M MB* pS_{opt} *1.4*

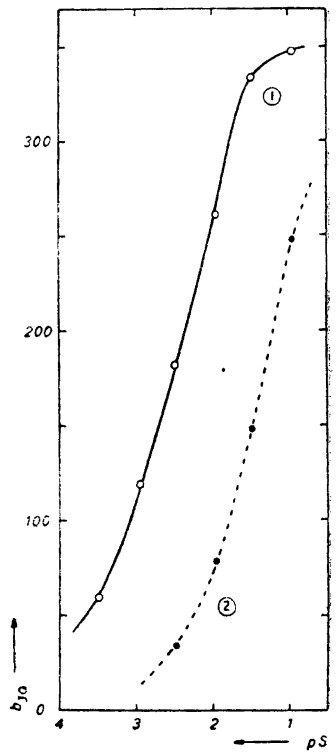

Fig. 2. Activity-pS curves for the enzymic hydrolysis of acetylcholine by human serum cholinesterase in the presence of added methylene blue (MB).

1. *Control*
2. *0.27 × 10⁻⁵ M MB*

centration is too low to give optimum enzyme activity (curves 2 and 3). Starting with a 1.10×10^{-2} *M* substrate solution, therefore, the enzyme activity increases with increasing substrate concentration when such a high concentration of the inhibitor is present, it decreases on the other hand in the absence of inhibitor or in the presence of low inhibitor concentration.

This change in activity-substrate concentration relationship in the presence of an inhibitor is important when the degree of inhibition of acetylcholinesterase is measured for an inhibitor as methylene blue. The inhibition for a given methylene blue concentration varies greatly with the substrate concentration. At 3.3×10^{-2} *M* acetylcholine, the enzyme is inhibited 12 per cent by 1.07×10^{-5} *M* methylene blue; at 10 times lower acetylcholine concentra-

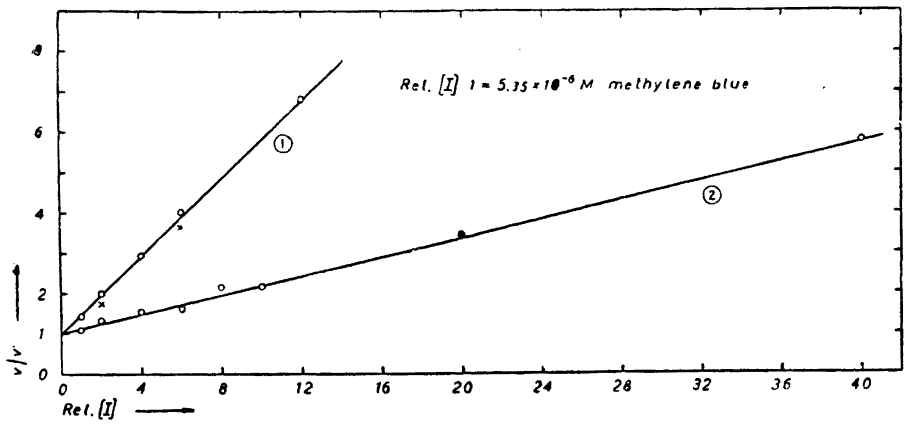

Fig. 3. Inhibition of acetylcholine-esterase (cow erythrocytes) by methylene blue (MB) as function of inhibitor concentration. v = velocity in the absence, v' = velocity in the presence of MB, expressed in µl CO_2 evolved in 30 minutes (b_{30}). Acetylcholine concentration 1.10 × $10^{-2}M$.

1. Without incubation; the two values marked with a cross were obtained in the experiments with varying substrate concentrations (Fig. 1)

2. Enzyme solution incubated 40 minutes with MB before mixed with the substrate

tion the inhibition by the same concentration of the dye is 58 per cent. The activity-pS curves are principally similar to those obtained for the electric tissue acetylcholine-esterase in the presence of prostigmine [12].

For comparison the action of methylene blue on the serum cholinesterase is illustrated in Fig. 2. The dye inhibits this esterase also by true competition. The relative degree of inhibition at various acetylcholine concentrations is markedly different from that found for the erythrocyte acetylcholine-esterase. Comparing the inhibitory effect of a compound on these two types of esterases, such differences must be considered. The corresponding value mentioned above for the acetylcholine-esterase and 1.07×10^{-5} M methylene blue are for the serum cholinesterase 89 and 98.5 per cent respectively (compared with 12 and 58 respectively).

INHIBITION AS FUNCTION OF INHIBITOR CONCENTRATION

In the case of competitive inhibition and constant concentrations of enzyme and substrate, a plot of the degree of inhibition (expressed as v/v') against the concentration of the inhibitor I gives a straight line:

Table 1. Comparison between the inhibitory action of some "anticholinesterases" on acetylcholine-esterase. Values calculated according to Equation 1. $K_I = 6.3 \times 10^{-4}$. I_{50} *= molar inhibitor concentration giving 50 per cent inhibition. In calculating the* K_I *values (in brackets) for the irreversible inactivators, the slope of the tangents of the curved lines (v/v'; I diagram) has been used* [12].

Inhibitor	Values obtained in incubation/no incubation experiments	Enzyme	[S] molarity	K_I	I_{50} molarity	Values calc. from
True competitive inhibitors						
Choline chloride	Inc. and no inc.	Erythrocytes (cow)	3.3×10^{-3}	2.5×10^{-3}	1.6×10^{-2}	Ref. 9
» »	» » » »	» »	1.1×10^{-3}	2.4×10^{-3}	6.3×10^{-3}	» 9
Prostigmine bromide	» » » »	Electric tissue	3.3×10^{-3}	1.6×10^{-7}	1.0×10^{-6}	» 12
Physostigmine sulphate	» » » »	» »	3.3×10^{-3}	6.1×10^{-8}	4.2×10^{-7}	» 12
Methylene blue	No incubation	Erythrocytes (cow)	1.1×10^{-2}	5.7×10^{-7}	1.1×10^{-5}	Fig. 2
» »	40 min. incub.	» »	1.1×10^{-2}	2.3×10^{-6}	4.3×10^{-5}	» 2
Irreversible inactivators						
Diisopropyl fluorophosphate (DFP)	Incubation	Electric tissue	3.3×10^{-3}	(2.1×10^{-7})	1.3×10^{-6}	Ref. 12
Tetraethyl pyrophosphate	»	» »	3.3×10^{-3}	(1.3×10^{-9})	7.3×10^{-9}	» 12
» » (TEPP)	»	Erythrocytes(man)	1.1×10^{-2}	(0.9×10^{-9})	7.0×10^{-9}	Unpubl.

$$\frac{v}{v'} = 1 + [I]\frac{K_S}{K_I([S] + K_S)} \qquad (1)$$

The reaction velocities v and v' represent the enzyme activities in the absence of the inhibitor I and in its presence respectively. K_S and K_I are the dissociation constants of the complexes enzyme-substrate and enzyme-inhibitor respectively. The intercept on the v/v' axis is 1 (one). From known values of [S] and K_S and the slope of the line (*i. e.*, K_S/K_I ([S] + K_S)), K_I can be calculated. The present author has applied this method in evaluating the action of choline [9] and other inhibitors [12] on various esterase systems.

Fig. 3 shows the results obtained with methylene blue as an inhibitor of the acetylcholine-esterase of cow erythrocytes. Table 1 compares these results with those obtained for other powerful inhibitors, analysed according to the graphical method described.

The fact that methylene blue gives a straight line according to Equation 1 shows that the dye acts as a competitive inhibitor. The K_I value, 5.7×10^{-7}, obtained in experiments when inhibitor and acetylcholine are mixed simultaneously with the enzyme (no incubation; curve 1, Fig. 3), is of the same order of magnitude as that of prostigmine (1.6×10^{-7}). As pointed out above the I_{50} value (defined in Table 1) greatly varies with the substrate concentration.

This is in contrast to the irreversible inactivators (DFP and TEPP) for which I_{50} is independent on the substrate concentration.

The I_{50} value is higher when the enzyme has been in contact with methylene blue before mixing with substrate (incubation experiments). A similar observation has been made with the irreversible inactivators. In the latter cases the affinity of the enzyme for acetylcholine is much greater than for the inactivators, *i. e.*, acetylcholine protects the enzyme against the action of these compounds when both substrate and inhibitor are coming in contact simultaneously with the enzyme. This is, however, not the case for methylene blue for which the inhibition is a true competitive one. The fact that the incubation of the enzyme solution with the dye leads to a weaker inhibitory action is due to the lowering of that action by the enzyme solution. It is, however, not the enzyme itself which has this effect. This has been proved by destroying the enzyme by heating, after which the enzyme solution still has the power to weaken the inhibitory action of methylene blue. Probably the oxidation-reduction potential is altered in an unfavorable way regarding the inhibitory action, *i. e.*, a part of the dye is converted to the inactive leuco base. This fact is important to consider when methylene blue is used therapeutically in order to depress the activity of acetylcholine-esterase *in vivo*.

SUMMARY

The kinetics of the inhibition of the acetylcholine-esterase by methylene blue has been studied using cow erythrocytes as enzyme source.

Methylene blue is a true competitive inhibitor and behaves in a similar way found previously for other quaternary ammonium bases. The optimum acetylcholine concentration, characteristic of this esterase, is changed to higher concentration in the presence of the dye. The higher inhibitor concentration the higher is the optimum acetylcholine concentration. Great differences in degree of inhibition are obtained when the action of the dye on the acetylcholine-esterase is compared with that on the serum cholinesterase.

Evaluating the inhibition as function of methylene blue concentration the dissociation constant (K_I) of the inhibitor-enzyme complex was found to be 5.7×10^{-7}. A higher value of K_I is obtained when the enzyme solution has been incubated with the inhibitor before mixed with the substrate.

This work has been supported by a grant from *Statens Naturvetenskapliga Forskningsråd*. I gratefully acknowledge the assistance of Mrs. M. Grahn.

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Received February 27, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 543 – 548

The Isolation of Nitrogen 1 and 3 as Methylamine and Ammonia from Pyrimidine Ribosides

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During investigations on pyrimidine metabolism with the aid of N^{15} it became desirable to study the turnover of the nitrogen atoms separately.[1,2]

As a first attempt in this direction a method has been worked out that permits the preparation of the nitrogen in position 1 and 3 in pyrimidine ribosides separately from each other.

In order to make the separation of the two nitrogen atoms possible one of them had to be labelled in some way. This was done by methylating the pyrimidine riboside in position 1 with diazomethane as described by Bredereck for purine ribosides [3].

The methylation was preceded by acetylation of the sugar with ketene.

The ribose was then split off with formic acid according to Vischer and Chargaff [4] and the methylated pyrimidine separated from uracil and from degradation products by chromatography on a starch column with butanol-water.

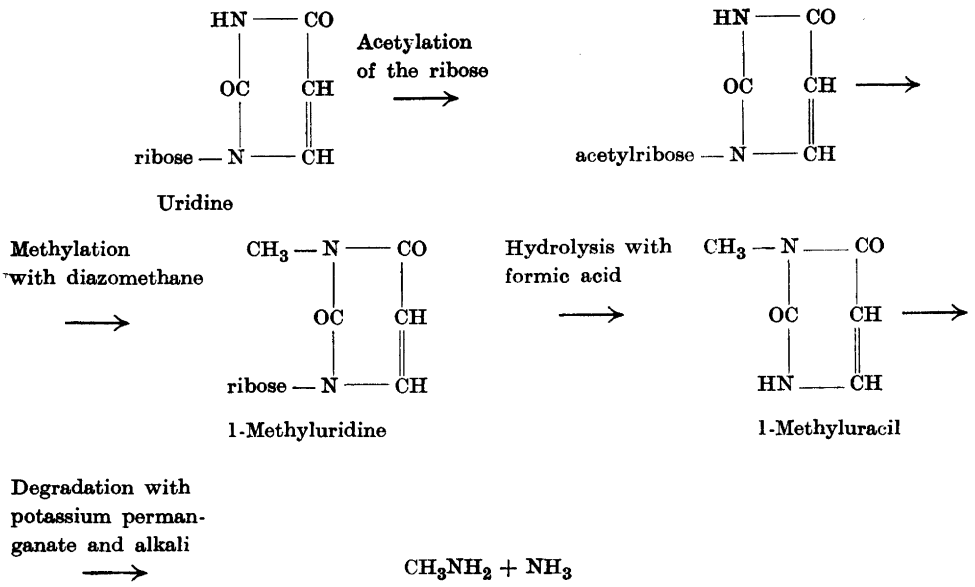
The pyrimidine ring was opened by means of potassium permanganate in neutral solution and then degraded by distillation with 50 % sodium hydroxide solution. The nitrogen in position 1 was split off as methylamine and that in position 3 as ammonia.

Methylamine and ammonia were separated by chromatography on a starch column with propanol — 0.5 *N* HCl, 2 : 1 as described by Moore and Stein for the amino acids [5,6].

The method has been worked out for uridine using an amount of 20 mg.

EXPERIMENTAL

Acetylation. The uridine (20 mg) was dissolved in 2 ml water + 20 ml acetone and acetylated for 2 hours with ketene.



Methylation. After acetylation the solution was evaporated to dryness *in vacuo*. The acetylated riboside was dissolved in 5 ml metanol + 10 ml acetone. A solution of diazomethane in ether prepared from 5 g nitrosomethylurea was poured into the riboside solution. After 3 hours the remaining diazomethane was destroyed with glacial acetic acid, the solution transferred to a bomb tube and evaporated to dryness on a steam bath.

Hydrolysis. The ribose was split off by hydrolysing for 6 hours with 5 ml conc. formic acid (98—100 %) at 175° C.

Chromatography of hydrolysate of methylated uridine. The hydrolysate was washed out with 5 ml + 3 ml + 3 ml water, centrifuged and the supernatant repeatedly evaporated to dryness *in vacuo* to remove the formic acid. Before the last evaporation the solution was neutralized with a few drops of 0.1 *N* NaOH. It was taken up in 10 ml + 2 ml + 2 ml of butanol-water (13.5 parts water + 86.5 parts butanol) and added to a starch column, length 200 mm diam. 40 mm. The chromatogram was developed with the same solvent. The size of each fraction was approximately 10 ml.

A chromatogram of the hydrolysate showed a high peak in the position expected for 1-methyluracil and a small one in the position expected for uracil (Fig. 1).

A chromatogram of synthetic 1-methyluracil (F. 174° — 175° C)[9] and uracil is shown in Fig. 2.

The *R*-value for synthetic 1-methyluracil was 1.63 and that for uracil 0.83 [2]. The substance that was formed during the hydrolysis had an *R*-value of 1.60 while the compound that was thought to be unmethylated uracil had $R = 0.79$. The light absorption curves for 1-methyluracil and the unknown compound had identical shapes with E_{max} at 2580 Å. For these reasons the substance formed during hydrolysis was thought to be identical with 1-methyluracil.

The overall yield calculated as uridine was 70 %.

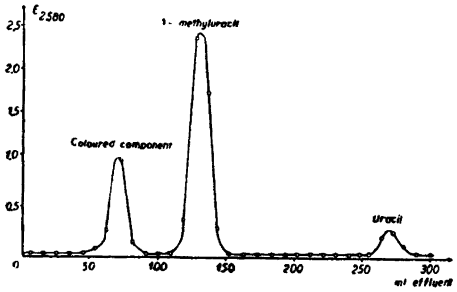


Fig. 1. Chromatography of hydrolysate of methyluridine. Length of column: 204 mm, diameter: 36 mm.

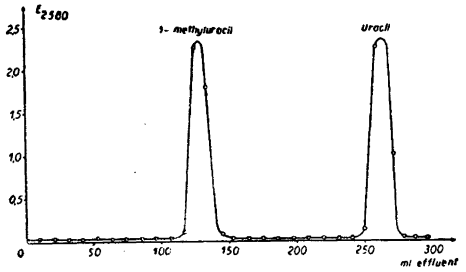


Fig. 2. Chromatographic separation of 1-methyluracil and uracil. Length of column: 205 mm, diameter: 36 mm.

Degradation. The fractions from the chromatogram that contained the methyluracil were evaporated to dryness *in vacuo*. The methyluracil was dissolved in 20 ml water and about 50 mg of Ba Cl_2 were added. A solution of $KMnO_4$ in 20 ml water was added to the solution of methyluracil. The amount of $KMnO_4$ used was such that the ratio $\frac{\text{mg } KMnO_4}{\text{mg methyluracil}} = 2$. The amount of methyluracil was calculated from the light absorption read in the Beckman spectrophotometer at 2580 Å. $E_{max_{2580\,Å}} = 1.000$ for 17.2 γ methyluracil/ml. After about 30 min. the permanganate-colour had disappeared. The MnO_2 was centrifuged off and washed twice with a few ml water. The centrifugate and the washing were transferred to the reaction flask and the solution was made to be 50 % with respect to NaOH.

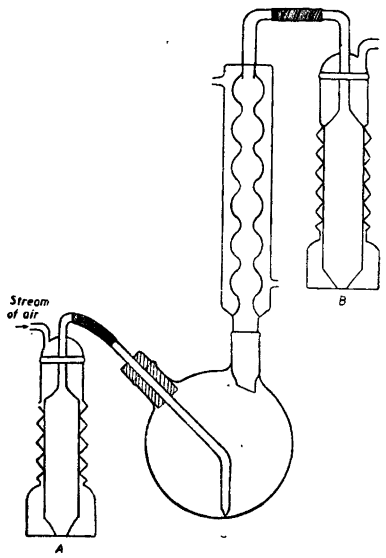


Fig. 3. Apparatus for the distillation with alkali.
A. Washbottle containing HCl for removal of ammonia from air stream.
B. Washbottle containing 0.1 N HCl for collection of methylamine and ammonia.
C. Degradation vessel.

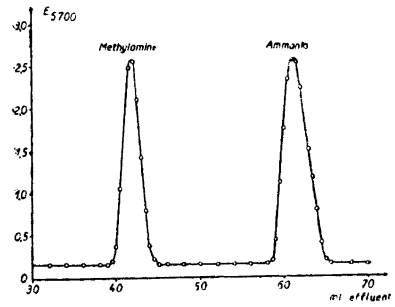


Fig. 4. Chromatographic separation of methylamine and ammonia. Length of column: 300 mm, diameter: 10 mm.

The solution was now distilled for 6 hours in the apparatus shown in Fig. 3. A stream of air which had been freed from ammonia by passing through a washbottle filled with HCl (A) was lead through the apparatus.

Methylamine and ammonia were collected in washbottle (B) which contained 0.1 *N* HCl.

Chromatography of methylamine and ammonia. Methylamine and ammonia were separated by chromatography on a starch column with propanol — 0.5 *N* HCl, 2 : 1. The technique was the same as that described by Moore and Stein [5,6] for the aminoacids. The solution of methylamine hydrochloride and ammonia chloride obtained after distillation was evaporated to dryness *in vacuo*. The residue was taken up in 5 ml + 2 ml + 2 ml of propanol — 0.5 *N* HCl, 2 : 1 and added to a starch column, length 200 mm diam. 40 mm. The chromatogram was developed with the same solvent. The positions of the peaks were determined by means of ninhydrin. A chromatogram of synthetic methylamine and ammonia is shown in Fig. 4.

R for methylamine was 0.52 and for ammonia 0.36. This value for ammonia agrees with that found by Moore and Stein [6].

A chromatogram of the degradation products revealed two peaks in the same position as those in the chromatogram of synthetic methylamine and ammonia. *R* for the first peak was 0.52 and for the second 0.35.

Characterization of the compounds as free from foreign nitrogen

Methylamine. Methylamine was determined quantitatively according to Alexander [7], the only difference being that different proportions of methylamine and chromotropic acid were used. After the methylamine had been oxidized to formaldehyd, distilled over and diluted to 10 ml, 0.5 ml of the solution was diluted to 5 ml with chromotropic acid in 21 *N* H_2SO_4 (2 mg/ml) and heated in a steam bath for 30 min. The colour was read in the Beckman spectrophotometer at 5700 Å. The ratio $\frac{\text{mg N from } CH_3NH_2}{\text{mg total N}}$ was determined and found to be 0.98—0.99.

Ammonia. Ammonia was determined by nesslerization [8]. The colour obtained was read in the Beckman spectrophotometer at 4000 Å and compared to the colour given by

a standard solution of NH_4Cl. The ratio $\frac{\text{mg N from } NH_3}{\text{mg total N}}$ was determined and the value 0.96 was obtained.

The overall yield of the method from uridine to methylamine and ammonia was 45—50 % of the theoretical.

DISCUSSION

Acetylation. In order that methylation with diazomethane in ether could be performed, the pyrimidine riboside had to be made soluble in a mixture of methanol and acetone. This was accomplished by acetylation of the ribose. Bredereck in his work on methylation of purineribosides [3] used acetic anhydride in pyridine as an acetylating agent, but the present author preferred to use ketene. Contamination with foreign nitrogen from the pyridine could thus be avoided.

Methylation. Diazomethane was thought to be the most selective methylating agent as Bredereck states that it only methylates the NH-group in the neighbourhood of a CO-group. Methylation of the sugar did not occur and the possibility that methyl groups might be transferred from the sugar to position 3 in the pyrimidine when the ribose was split off with formic acid could be neglected.

Hydrolysis. Chargaff states that 2 hours hydrolysis is sufficient [4] but later experiments by Reichard and Estborn at this laboratory (unpublished) have shown that the ribose is not completely split off after such short time.

Degradation. Before degrading with alkali the pyrimidine ring was opened with $KMnO_4$ in neutral solution. If this was not done more methylamine then ammonia was formed while theoretically they should appear in equal proportions. This suggested either that methylamine is formed to some extent from the nitrogen in position 3 as well as from that in position 1 or that under these conditions the nitrogen in position 3 is split off more slowly than that in position 1 and the reaction has not yet gone to completion.

As an experiment in which uracil was degraded in NaOH in the absence of permanganate showed that no methylamine is formed from the nitrogen in position 3 the second possibility seemed more likely. Thus the function of the $KMnO_4$ is believed to be the speeding up of the release of nitrogen from position 3. On the other hand if an excess of $KMnO_4$ was used more ammonia than methylamine was formed making it probable that ammonia was also formed from the nitrogen in position 1. If the treatment with $KMnO_4$ was carried out at room-temperature and if just so much $KMnO_4$ was used that no permanganate colour was present after 30 min. methylamine and ammonia after subsequent degradation with alkali appeared in the same amounts.

When just so much $KMnO_4$ was used that the ratio $\frac{\text{mg } KMnO_4}{\text{mg methyluracil}} = 2$ the change in the light absorption indicated that 90 % of the ring was opened. The oxidation was more readily achieved in the presence of Ba-ions.

SUMMARY

A method has been described that permits the isolation of the nitrogen in position 1 in pyrimidineribosides as methylamine and the nitrogen in position 3 as ammonia.

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Received February 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 549–558

Short Communication

The Factor in Vitamin K-Deficient Plasma which Accelerates the Coagulation of Dicumarol Plasma

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Several published studies [1–4] support the assumption that the coagulation anomaly produced by the administration of dicumarol is not a mere hypoprothrombinemia.

In experiments with chicken plasma Dam and Søndergaard [4] observed that small amounts of vitamin K-deficient plasma added to dicumarol plasma shortened the prothrombin time of the latter and vice versa. They explained this effect by assuming that the two plasmas are primarily lacking one and the same component (prothrombin) whereas in addition to this vitamin K-deficient plasma is lacking in another component and dicumarol plasma in a third.

By means of adsorption and elution procedures we have now isolated a small protein fraction from plasma of vitamin K-deficient chicks. This fraction contains the agent which shortens the prothrombin time of dicumarol plasma.

The protein thus isolated shows the following properties:

1. It is completely adsorbed from oxalated plasma of vitamin K-deficient chicks when the plasma is treated with one per cent of its weight of barium carbonate. It can be eluted from the adsorbent with citrate.

2. Addition of small amounts of this protein to dicumarol chicken plasma reduces the prothrombin time of the latter to a certain extent; the addition of larger amounts of the protein causes no further reduction of the prothrombin time.

3. Addition of the protein to plasma from vitamin K-deficient chicks does not alter the prothrombin time.

4. The protein has no effect on the prothrombin time of stored chicken plasma.

5. When stored chicken plasma is supplied with small amounts of the most labile factor of chicken plasma (corresponding to factor V in mammals), the addition of the new factor shortens the prothrombin time further.

In the dicumarol plasmas used in the present investigations the amount of the most labile factor is not reduced below its optimal concentration.

Fig. 1 shows how the curve (A–B) – representing the prothrombin time of mixtures of a dicumarol plasma with a vitamin K-deficient plasma – is straightened out (A–C) by the addition of a constant amount of the new factor so that the curvature otherwise occuring at the high concentrations of dicumarol plasma is eliminated.

Even when the preparation contains some prothrombin, the curve is straightened out in the same way (A′–C′), in this case shifted to somewhat lower prothrombin times, compared with the curve (A–C) resulting from a mere addition of the new factor.

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* Fellow 1949–50. Foundation for Danish-Norwegian Cooperation.

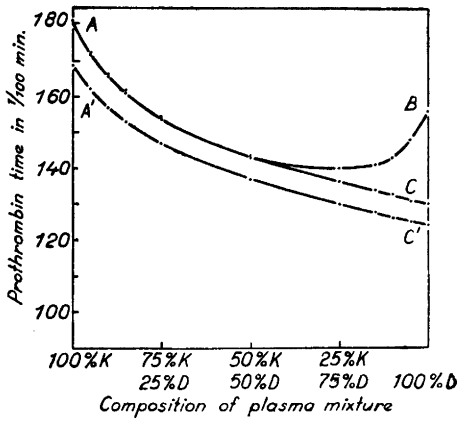

Fig. 1. Prothrombin time as a function of composition of plasma mixture.
K: plasma from vitamin K-deficient chick.
D: » » dicumarol-poisoned »

We have called this new factor the *x*-factor in absence of an appropriate name characterizing its function in prothrombin activation. Its adsorption characteristics are very similar to those of prothrombin, but it is distinctly different from this protein. It is also different from the most labile factor in chicken plasma (corresponding to factor V of mammalian plasma). It seems possible that the *x*-factor of chicken plasma could have the same function as the factor in mammalian plasma, which on coagulation gives rise to the SPCA (serum prothrombin conversion accelerator) of Alexander and co-workers [5]. The synthesis of the *x*-factor is obviously independent of vitamin K.

The properties and function of the *x*-factor are being studied further.

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Received March 24, 1950.

Dihydroxyphenylalanine and Hydroxytyramine in Mammalian Suprarenals

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In addition to adrenaline, extracts of mammalian suprarenals have recently been shown to contain *nor*adrenaline (Holtz and Schümann [1], Goldenberg, Faber, Alston and Chargaff [2], Bergström, Euler and Hamberg [3]). Hitherto, there has been no indication in the suprarenals of other catechols (Goldenberg *et al.*[2]). However, it is said that hydroxytyramine occurs in urine (Holtz) and has recently been found in extracts of heart (Goodall)[4].

When the suprarenal extracts of sheep were subjected to paper chromatography, an additional catechol spot was observed. This spot was identical with hydroxytyramine both as to color and to position. Further, this identity was substantiated by exposing the extract chromatogram to two different solvent systems, *i. e. N*-butanol/*N*HCl and phenol/H_2O.

In the suprarenal extracts prepared from thyroidectomized sheep, another spot has been observed. This spot, however, agreed in position and color with dihydroxyphenylalanine (DOPA). Again the identity was substantiated by preparing the paper chromatogram with different effluents, butanol and phenol.

The presence of hydroxytyramine in normal sheep suprarenals and of DOPA in the suprarenals of thyroidectomized sheep is suggestive of the role that hydroxytyramine and DOPA may play as precursors to *nor*adrenaline, and also the possible importance of thyroxin to such a change. Blaschko [5] first suggested these compounds as precursors to *nor*adrenaline.

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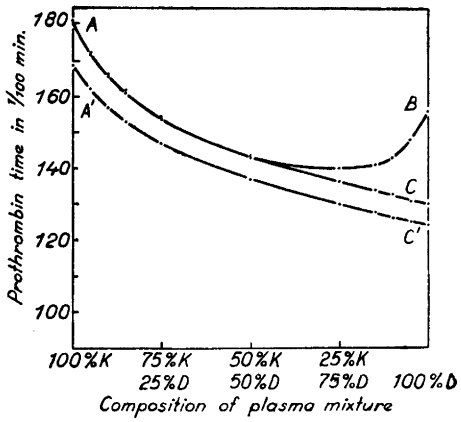

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On Tetramolybdates and Metatungstates

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If the polymolybdates and the polytungstates are divided into groups with different definite mole ratios $R_2O : MoO_3$ (WO_3), there appear in both cases groups with the ratio 1 : 4, tetramolybdates and tetratungstates.

The tetratungstates were already called metatungstates [1] in 1854 in analogy with the metatungstic acid $H_2O \cdot 4WO_3 \cdot xH_2O$ described by Laurent [2]. This name has later been used in order to draw attention to their close relations to the heteropolyacids, with which they are isomorphous [3], as is confirmed by X-ray powder photographs [4]. There is no doubt as to the analogy in structure between the metatungstates and such heteropolyacids as phosphotungstic acid $H_3PW_{12}O_{40}$, the structures of which have been determined [5–7]. The very complex ion $PW_{12}O_{40}^{3-}$ is described in modern text-books on inorganic chemistry [8–9] and will not be treated here; nor will we discuss the role of hydrogen in the metatungstate ion, but write it as $W_{12}O_{40}^{8-}$. It may be added, however, that a cesium compound $Cs_3H_3(H_2O_4)(WO_3)_{12} \cdot nH_2O$ has been found to be isomorphous with the heteropolyacids [10]. As this compound can also be written $Cs_2O \cdot 8WO_3 \cdot n\,H_2O$, the octotungstates obviously contain the metatungstate ion $W_{12}O_{40}^{8-}$ and must be considered as acid metatungstates.

The question then arises whether the tetramolybdates should be considered as heteropolymolybdates, containing a metamolybdate ion $Mo_{12}O_{40}^{8-}$, as has been stated by Mylius [11] and Rosenheim [12] (although with an incorrect opinion about the structure of such an ion). Unsuccessful efforts to prepare a metamolybdic acid have, however, been reported [4]. Another theory has been put forward by Jander [13], who describes the tetramolybdates as acid hexamolybdates, containing an ion $H_3Mo_6O_{21}^{3-}$. Finally the existence of an ion $Mo_4O_{13}^{2-}$ has been stated [14].

A complete structure determination of some tetramolybdates would of course give an answer to the question of the nature of the tetramolybdate ion. Two such structure determinations have been started by us. The choice of the substances was governed by a desire to get crystals stable in air. All tetramolybdates are described as efflorescent [14], but as the ammoniumtetramolybdates were reported to be the most stable ones, we prepared them by two different methods, following Wempe [15] and Barbieri [16]. We obtained crystals, stable in air of 30 % relative humidity, with the compositions $(NH_4)_2O \cdot 4MoO_3 \cdot 2H_2O$ and $(NH_4)_2O \cdot 4MoO_3 \cdot 2.5H_2O$. (The analytical methods have been described earlier [17]):

$(NH_4)_2O \cdot 4MoO_3 \cdot 2H_2O$:
Calc. 5.12 % NH_3, 86.8 % MoO_3, 8.08 % H_2O
Found 5.15 % NH_3, 86.5 % MoO_3, 8.35 % H_2O

$(NH_4)_2O \cdot 4MoO_3 \cdot 2.5H_2O$
Calc. 5.05 % NH_3, 85.7 % MoO_3, 9.25 % H_2O
Found 5.08 % NH_3, 85.6 % MoO_3, 9.32 % H_2O

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Received April 6, 1950.

Powder photographs taken with monochromatized CuK_α radiation immediately after the crystals had been taken from their mother-liquors, clearly indicated the existence of two different compounds. Rotation and Weissenberg photographs were taken with CuK radiation. Both compounds are triclinic, for which reason a structure determination probably would be very intricate and timeconsuming, if it is even possible.

From the photographs a, b^*, c^*, and a^* were determined, however, and thus the volume of the unit cell could be calculated. The density values were obtained by means of the buoyancy method. The knowledge of the MoO_3 content then permits a calculation of the number of Mo atoms in the unit cell, n.

$(NH_4)_2O \cdot 4MoO_3 \cdot 2.5H_2O$: $a = 9.78$ Å, $b^* = 0.134$ Å^{-1}, $c^* = 0.105$ Å^{-1}
$a^* = 80°$ V = 707 Å^3 $d = 3.1$ $n = 7.9$
$(NH_4)_2O \cdot 4MoO_3 \cdot 2H_2O$: $a = 10.0$ Å
$b^* = 0.143$ Å^{-1} $c^* = 0.108$ Å^{-1}
$a^* = 70°$ V = 686 Å^3 $d = 3.1$ $n = 7.8$

Although the accuracy of the determinations is not very great, it is undoubtedly established that there are eight Mo atoms in the unit cell, and we may consider the consequences of this result.

It can be explained in two essentially different ways. There may be discrete ions containing 8, 4 or 2 Mo atoms, or else infinite ions of some kind. In the first case it seems almost certain, that the ions in the mother-liquor must contain 8,4 or 2 Mo atoms. In the second case we have the further possibility, that there are ions containing multiples of 8 Mo atoms in the solution. If we assume that the diffusion experiments by Jander [13] (giving hexamolybdate ions) are at least approximately correct there remain only the alternatives with 8 or 4 atoms. It is not probable that the molecular weights obtained by Jander are too large (*cf.* the discussion of the method by Souchay [18] and of the paramolybdate ion $Mo_7O_{24}^{6-}$ by Lindqvist [19]). Therefore it is most probable that the tetramolybdates crystallize from solutions containing octomolybdate ions. In any case the meta- and hexamolybdate theories are undoubtedly ruled out by this investigation. We intend to study these problems further.

The author wishes to thank Miss Britta Svensson for her valuable aid during this work. *The Swedish Natural Science Research Council* has supported the investigation financially, which support is gratefully acknowledged.

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Received April 3, 1950.

Utilization of α-Ketoadipic Acid by Lysineless *Ophiostoma* Mutants

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Recently Borsook *et al.*[1] found that lysine was degraded to α-aminoadipic acid, α-ketoadipic acid and glutaric acid in mammalian tissue. Mitchell and Houlahan[2] then found that one lysineless *Neurospora* mutant could utilize α-aminoadipic acid in the place of lysine whereas three other genetic types were unable to do so. α-Ketoadipic acid or glutaric acid did not support growth.

As mentioned in an earlier note[3] we have also found that a number of the lysineless *Ophiostoma* mutants isolated by Fries[5] can utilize α-aminoadipic acid.

We now wish to report that the growth of a number of these mutants also can be supported by α-ketoadipic acid as shown by the following data.

Table 1. Growth response of Ophiostoma mutant 1320.

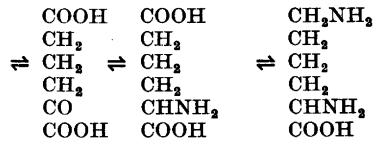
Addition to 25 ml basal medium (millimole)	Mg dry weight of mold after three weeks
0	0, 0
0.002 L-lysine	11, 12
0.020 L-lysine	99, 102
0.020 D, -α-aminoadipic acid	27, 29
0.020 α-ketoadipic acid	27, 9
0.050 » »	52, 54
0.100 » »	76, 74
0.020 glutaric acid	0

The technic used has been described by Bergström and Sjöbeck[4].

α-Ketoadipic acid was prepared according to Gault[6] except that the intermediate trimethyl-2-oxaloglutarate was decomposed by a milder method *i. e.* heated in 4 *N* hydrochloric acid 45 minutes on the water bath. After recrystallization from ether or acetone-ether, the α-ketoadipic acid melted at 123—125°.

$C_6H_8O_5$
Calc. C 44.98 H 5.04 Eq.wt. 80.0
Found » 44.95, 45.05 » 5.00, 5.16 » » 79.3

These results thus lend further support to the assumption that lysine can be formed or degraded in different types of organisms according to the general scheme.



In different organisms the synthesis can be blocked at different points due to the lack of a specific enzyme.

A full report will appear in *Physiologia Plantarum.*

This work is part of an investigation supported by *Magn. Bergvalls Stiftelse,* Stockholm.

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Received April 22, 1950.

The Use of Polarography for Automatic Recording in the Chromatography of Proteins

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During the last years several papers have appeared on the subject of protein chromatography [1–4], and more work is in progress. As a part of the work performed in this Institute a method has now been developed for the automatic recording of protein concentration in eluates. Since the adsorption systems mentioned below are not ideal for elution procedures, this paper should be considered mainly as a preliminary report on the method of recording.

The method is an application of the polarographic protein reaction [5,6] to small volumes of liquid emerging from the chromatographic column. All proteins which contain cysteine or cystine give this reaction and can be analysed in this way. * Instead of plotting current-voltage curves, as in the usual polarography, the voltage is fixed at a suitable value, and the current continuously recorded as a function of time. The use of polarography for flowing liquids has been reported by others [7,8], but not for small volumes at variable flow rate, and not together with chromatography.

Except for the adsorption column the apparatus consists of the following parts: a cuvette with a number of drilled holes, a mercury reservoir with rubber tube and capillary, a saturated calomel electrode, an electric circuit including a voltmeter and a microammeter, and a 20 μA strip chart recorder.

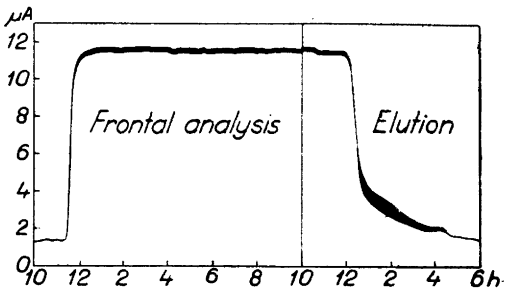

Fig. 1. Frontal analysis of 0.05 % serum albumin, followed by an elution. For data, see the text.

The cuvette is made of perspex (ICI, England) in such a way that all parts are readily visible from outside. Eluates and rinsing solution, if any, are introduced through two openings at one side, while the other side has an opening for the connection to the calomel electrode. The drop tip is introduced from above into the center cylindrical hole, which is vertical and open at the bottom. This hole contains 0.1–0.2 ml of liquid which is held by adhesion. Thus, eluates and mercury leave the cuvette through a common opening. The reason for this arrangement is that it was found impossible to siphon off the mercury through a separate tube because even traces of protein prevent the mercury from forming a coherent body. Instead, a great number of drops will form a plug in the mercury tube, which is then very soon completely shut up.

In order to find out the most suitable regions for the recording, a number of calibrations have been made by manual plotting of current-voltage curves. It was found possible at a dropping mercury potential of −1.7 to −2.0 volts to read protein concentrations as low as about 0.001 %. The most suitable range for recording seems to be 0.005–0.1 % which means that the method may be considered a micro one. Above 0.1 %, however, the

* Of course, if proper modifications are introduced, the method described here may be used also for other substances than proteins.

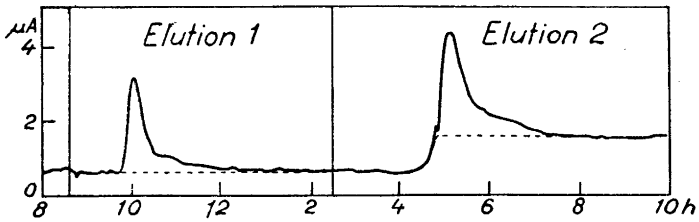

Fig. 2. Stepwise elution of 0.2 mg each of serum globulin and serum albumin. The base line, above which the protein concentration is recorded, has been drawn as a dashed line. For data, see the text.

current-concentration curves are very curved and the method is not well suited for accurate recording.

As the method is intended also for use in salting out adsorption [1, 2], some calibrations were made at high salt concentrations. Even in this case the sensivity is high; for example it is possible to detect a protein concentration of 0.003 % in 80 % saturated phosphate buffer. This means that the specificity is greater than in the interferometric method, where a minute variation of the salt concentration during a single run will completely mask the variation in protein concentration.

As examples of what the method can perform, some curves reproduced from actual strip chart records are given. Fig. 1 shows a frontal analysis of 0.05 % serum albumin in 0.35 *M* phosphate buffer (pH 6.7) on Hyflo Super Cel in a 1000 π cmm. (3.1 ml) perspex filter. The frontal analysis (total, 21 ml) is followed immediately by an elution (total, 8 ml) with the solvent. In this case the adsorption is very small, and probably this system could be used to separate proteins from other substances which are adsorbed at the conditions given. Fig. 2 shows the stepwise elution of serum albumin and serum globulin from a 2000 π cmm. (6.3 ml) column of a mixture (1+4) of tricalcium phosphate * and Super Cel. The globulin was eluted with 21 ml of 0.035 *M* phosphate buffer and the albumin with 20 ml of 0.105 *M* phosphate buffer, both at pH 6.7. The peak concentrations in the two elution zones were estimated to about 0.02 %.

This work is being continued in order that the method can be made more generally adaptable as a technique for analysis and separation of proteins. A report in greater detail will be published later.

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Received April 23, 1950.

* This adsorbent was generously supplied by Dr. S. Swingle, who, following Sumner and Dounce [9], is studying the application of a special preparation of it to protein chromatography [10].

Activities of the Components in Ion Exchangers

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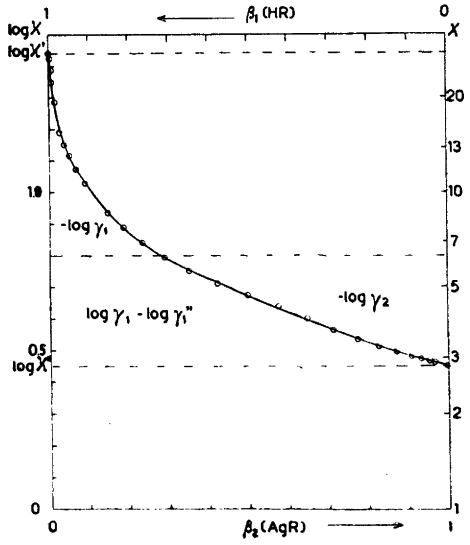
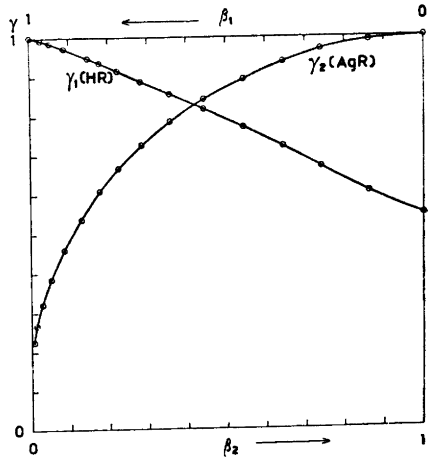
An ion exchanger (R^-) containing two different ions A_1^+ and A_2^+ certainly cannot be considered as an ideal solid solution of the components A_1R and A_2R. If it were, the law of mass action could be applied to the exchange reaction with an aqueous solution:

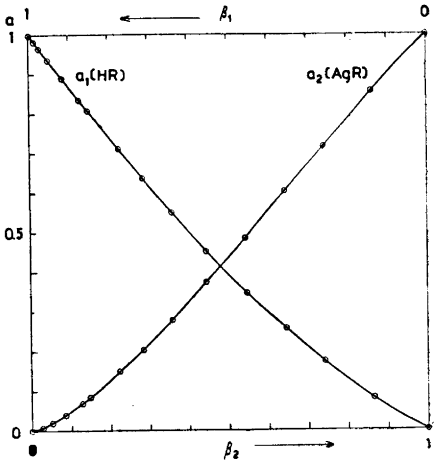
$$A_2^+ + A_1R \rightleftharpoons A_1^+ + A_2R \qquad (1)$$

inserting the molar fractions β_1 and β_2 of A_1R and A_2R for their activities. The quotient $\varkappa$ in equation (7) below is, however, found to vary considerably with β_1 and β_2.

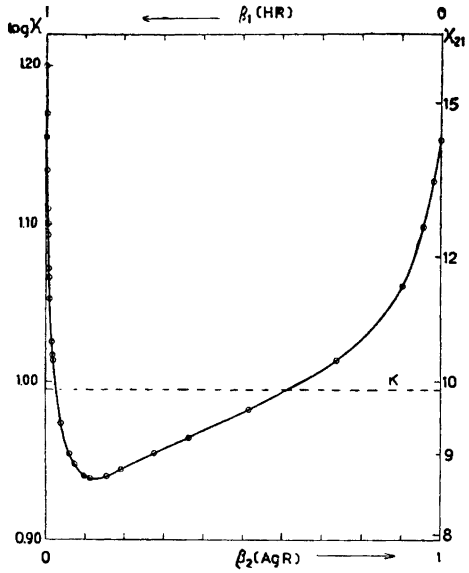
In describing the deviations from ideality, it seems desirable to eliminate the aqueous solution and use quantities that refer to the ion exchanger only. This can be done if the ion exchanger is formally regarded as a solid solution of A_1R and A_2R, and the activities a_1 and a_2, and activity factors γ_1 and γ_2 of the components are given for varying composition. This way of representation will be analogous to that used for the components of liquid mixtures.

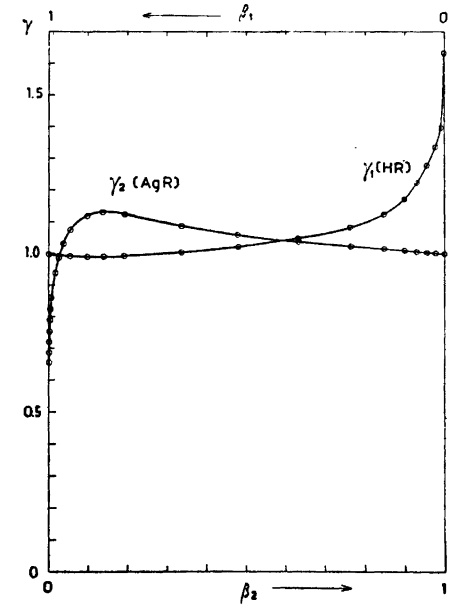




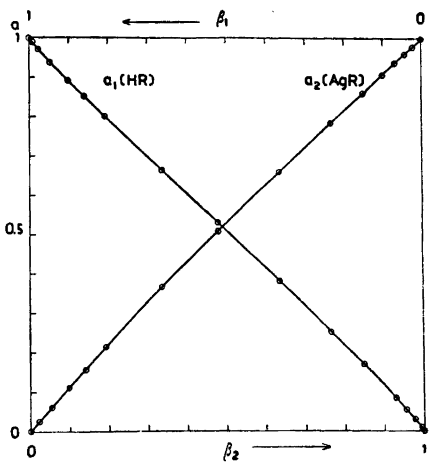


Figs. 1—3. Equilibria of H+ (1) and Ag+ (2) on Wofatit KS.
Fig. 1: Log $\varkappa$ as a function of β. For $\beta < 0.04$ the points were calculated from elution curves, for the smaller β from static measurements. Fig. 2: Activity factors γ_1 and γ_2. Fig. 3: Activities a_1 and a_2 of the components HR and AgR of the solid.





Figs. 4—6. Equilibria of H+ (1) and Ag+ (2) on Dowex 50.
Fig. 4: Log $\varkappa$ as a function of β. Fig. 5: Activity factors γ_1 and γ_2. Fig. 6: Activities a_1 and a_2 of the components HR and AgR of the solid. — Observe that log K is the average value of log $\varkappa$. (Fig. 4).



Let us assume that the two ions are univalent, that the total concentration is a_0 in the aqueous solution and s_0 in the sorbent, and that their molar fractions are a_1, a_2 in the solution and β_1, β_2 in the sorbent. Then in the aqueous solution the concentrations are:

$$[A_1^+] = a_1 a_0;\ [A_2^+] = a_2 a_0;\ a_1 + a_2 = 1 \quad (2)$$

and the activities, introducing the activity factors f_1 and f_2

$$\{A_1^+\} = f_1 a_1 a_0;\quad \{A_2^+\} = f_2 a_2 a_0 \quad (3)$$

In the sorbent, using pure A_1R and A_2R as standard states

$$[A_1R] = \beta_1 s_0;\ [A_2R] = \beta_2 s_0;\ \beta_1 + \beta_2 = 1 \quad (4)$$
$$\{A_1R\} = a_1 = \gamma_1 \beta_1;\ \{A_2R\} = a_2 = \gamma_2 \beta_2 \quad (5)$$

We define the thermodynamic equilibrium constant K_{21} of (1) and its inverse value K_{12} by

$$K_{21} = K_{12}^{-1} = \frac{\{A_1^+\}}{\{A_2^+\}} \cdot \frac{\{A_2R\}}{\{A_1R\}} = \frac{f_1 a_1 \cdot \gamma_2 \beta_2}{f_2 a_2 \cdot \gamma_1 \beta_1} \quad (6)$$

Equilibrium measurements will not give K_{21} directly but the equilibrium quotient $\varkappa_{21}$

$$\varkappa_{21} = \varkappa_{12}^{-1} = \frac{\{A_1^+\}}{\{A_2^+\}} \cdot \frac{[A_2R]}{[A_1R]} = \frac{f_1 a_1 \beta_2}{f_2 a_2 \beta_1} = \\ = K_{21} \cdot \frac{\gamma_1}{\gamma_2} \qquad (7)$$

Gibbs-Duhem's equation gives

$$\beta_1 \, d\ln (\gamma_1 \beta_1) + \beta_2 \, d\ln (\gamma_2 \beta_2) = 0 \qquad (8)$$

From (4), (7), and (8) we find

$$d\ln \gamma_1 = \beta_2 \, d\ln \varkappa_{21}; \; d\ln \gamma_2 = -\beta_1 \, d\ln \varkappa_{21} \quad (9ab)$$

For brevity we set $\varkappa_{21} = \varkappa$; $\beta_2 = \beta$, $\beta = 1-\beta$ and denote by $\varkappa'$, γ_1' (= 1) and γ_2' the values at $\beta = 0$ (pure A_1R), by $\varkappa''$ γ_2'' and γ_2'' (= 1) those at $\beta = 1$ (pure A_2R). Moreover we transfer (9a, b) to decadic logarithms and get:

$$\log \gamma_1 = \int_{\varkappa'}^{\varkappa} \beta \, d\log \varkappa \qquad (10\ a)$$

$$\log \gamma_2 = -\int_{\varkappa''}^{\varkappa} (1-\beta) \, d\log \varkappa \qquad (10\ b)$$

We have measured the equilibria of H^+ (= 1) and Ag^+ (= 2) on two ion exchangers, Wofatit KS and Dowex 50, for a wide range of β values. The curves $\varkappa(\beta)$ are given in Figs. 1 and 4. For Wofatit KS the calculations were made in the following way. First, $\log \varkappa$ was calculated for round β values by means of (10 a). The corresponding area is marked out in Fig. 1. Then the thermodynamic constant K was obtained from (7) which gives:

$$\log K = \log \varkappa'' - \log \gamma_1'' \qquad (11)$$

Now for each β, $\log \gamma_2$ was obtained from (7)

$$\log \gamma_2 = \log K + \log \gamma_1 - \log \varkappa \qquad (12)$$

For Dowex 50 the calculations of γ_1 and γ_2 were analogous though complicated by the minimum in $\varkappa$. (Fig. 4.)

The results are given in Figs. 2, 3, 5 and 6. When a number of curves a_i (β) are known, it will be tempting to try to explain them in analogy with liquid mixtures, by association, dissociation, compound formation (and perhaps by inhomogeneity of one of the components).

The generalization to multivalent ions is easily made.

Received April 31, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 559–577

Determination of the Equivalent Point in Potentiometric Titrations

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The equivalent point in potentiometric titrations is generally determined by plotting E or pH as a function of volume (V) of reagent added [1a]. The equivalent point is said to lie at the inflexion point of the curve, *i. e.*, at the point of maximum slope. This, however, is not strictly true for some ion-combination and oxidation-reduction titrations.

In the titration of very weak acids, for example, the slope of pH = f(V) at the equivalent point can be so small that it is difficult to determine the exact point of equivalence with any great degree of accuracy. In these cases it is many times possible to obtain greater accuracy by using various graphical or numerical methods [2]. The most common of these is the plotting of $\Delta E/\Delta V$ or $\Delta\mathrm{pH}/\Delta V$ as a function of V [1a]. In this method only a few points in the immediate vicinity of the equivalent point are of any significance. With ions of different charges the curve becomes asymmetrical, and the maximum is slightly displaced from the theoretical equivalent point [1b,3a] as shown in Fig. 7.

If $\Delta V/\Delta E$ or $\Delta V/\Delta\mathrm{pH}$, instead of $\Delta E/\Delta V$ or $\Delta\mathrm{pH}/\Delta V$, is plotted against V, certain advantages are obtained. Such curves are shown in Figs. 3 to 10. It will be noticed that, in the vicinity of the equivalent point, the curves consist of two straight lines which intersect each other and the V-axis at the equivalent point. The fact that the lines intersect on the V-axis can be of great importance in cases where ideal curves are not obtained, *e. g.* in precipitation reactions in systems with relatively high solubility products. The slopes of the lines are also of importance. In the titration of ions of similar charge, the curves are generally almost symmetrical about the equivalent point. In most reactions of ions of different charges, the slopes of the two lines are not the same.

If the curve $\Delta V/\Delta E = f(V)$ is plotted during the actual titration, the approximate position of the end point can generally be estimated long before it is reached and the increments (ΔV) accordingly reduced.

Under some conditions a deviation from the straight lines is found just at the end point. If the straight lines, however, are extrapolated, they will intersect on the V-axis. A comparison of the $\Delta E/\Delta V$ and $\Delta V/\Delta E$ curves for a precipitation titration is shown in Fig. 7. As can be seen, the $\Delta E/\Delta V$ curve deviates by about 1 %, while the $\Delta V/\Delta E$ lines intersect at the theoretical equivalent point.

THEORY

The theoretical background of the above discussion will be outlined in the following section.

Neutralization titrations

1. Strong acid — strong base. If V_o ml of a strong acid, with original concentration C_A, are titrated with a strong base, of concentration C_B, the concentration of hydrogen ions, after the addition of V ml of base, will be:

$$C_{H^+} = C_A \frac{V_o}{V_o + V} - C_B \frac{V}{V_o + V} \tag{1}$$

pH is defined by:

$$pH = -\log a_{H^+} = -\log f_+ \cdot C_{H^+} \tag{2}$$

where a_{H^+} is the hydrogen ion activity and f_+ is the activity coefficient. As the ion strength is scarcely altered during a titration, f_+ may be considered constant and (2) can be written:

$$pH = k - \log C_{H^+} \tag{3}$$

where $k = -\log f_+$. (1) and (3) together give:

$$pH = k - \log \left(C_A \frac{V_o}{V_o + V} - C_B \frac{V}{V_o + V}\right) \tag{4}$$

which is differentiated with respect to pH and V to give:

$$\frac{dV}{dpH} = \ln 10 \cdot \frac{V_o + V}{V_o} \cdot \frac{C_A V_o - C_B V}{C_A + C_B} \tag{5}$$

If the volume of base added at the equivalent point is V_e,

$$C_A V_o = C_B V_e \tag{6}$$

and (5) can be rewritten to give:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V}{V_o + V_e} (V_e - V) \tag{7}$$

In the vicinity of the equivalent point, $V_o + V$ is approximately equal to $V_o + V_e$ and (7) is approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot (V_e - V) \tag{8}$$

which is the equation of a straight line, intersecting the V-axis at $V = V_e$.

When the equivalent point has been exceeded in the titration,

$$C_{OH^-} = C_B \frac{V}{V_o + V} - C_A \frac{V_o}{V_o + V} \tag{9}$$

pH is here defined by:

$$pH = pK_w + \log a_{OH^-} = pK_w + \log f_- \cdot C_{OH^-} \tag{10}$$

As f_- will change only to a very small extent during a titration, $\log f_-$ may be considered constant and (10) can be written:

$$pH = k_1 + \log \left(C_B \frac{V}{V_o + V} - C_A \frac{V_o}{V_o + V}\right) \tag{11}$$

where $k_1 = pK_w + \log f_-$. Differentiation and simplification give:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V}{V_o + V_e} (V - V_e) \tag{12}$$

which, in the vicinity of the equivalent point, can be approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot (V - V_e) \tag{13}$$

The dV/dpH curves will not be straight lines, if the dilution can not be neglected. If, however, $\frac{dV/dpH}{V_o + V}$ is calculated instead of dV/dpH, the curves are transformed to straight lines, as can be seen from the following expressions:

$$\frac{dV/dpH}{V_o + V} = \frac{2.30}{V_o + V_e} (V_e - V) \tag{14}$$

and

$$\frac{dV/dpH}{V_o + V} = \frac{2.30}{V_o + V_e} (V - V_e) \tag{15}$$

where $V_o + V$ is equal to the total volume of the reaction solution.

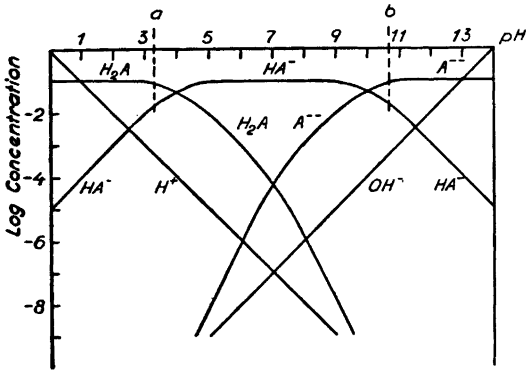

Fig. 1. Logarithmic diagram for a dibasic, weak acid.

2. Weak acid — strong base. Formulas will here be derived for weak, dibasic acids with such values of pk'_a and pk''_a that C_{H^+} and C_{OH^-} can be neglected in comparison to other terms in the range a to b in Fig. 1.

V_o ml of a weak acid (H_2A), with original concentration C_A, are titrated with a strong base of concentration C_B. For weak acids:

$$\mathrm{pH} = \mathrm{p}k_a + \log \frac{C_{salt}}{C_{acid}} \tag{16}$$

After the addition of V ml of base:

$$C_{H_2A} = C_A \frac{V_o}{V_o + V} - C_B \frac{V}{V_o + V} \tag{17}$$

and

$$C_{HA^-} = C_B \frac{V}{V_o + V} \tag{18}$$

Therefore, from (16), (17), and (18):

$$\mathrm{pH} = \mathrm{p}k'_a \mp \log \frac{C_A\ V_o - C_B\ V}{C_B\ V} \tag{19}$$

Differentiation and simplification give:

$$\frac{dV}{d\mathrm{pH}} = 2.30 \cdot \frac{V}{V_e}(V_e - V) \tag{20}$$

In the vicinity of the equivalent point, (20) can be approximated by:

$$\frac{dV}{d\mathrm{pH}} = 2.30 \cdot (V_e - V) \tag{21}$$

After the equivalent point has been exceeded ($V_e < V < 2V_e$):

$$C_{HA^-} = 2C_A \frac{V_o}{V_o + V} - C_B \frac{V}{V_o + V} \tag{22}$$

and

$$C_{A^{--}} = C_B \frac{V}{V_o + V} - C_A \frac{V_o}{V_o + V} \tag{23}$$

(22) and (23) together with (16) give:

$$pH = pk''_a + \log \frac{C_B V - C_A V_o}{2C_A V_o - C_B V} \tag{24}$$

which can be differentiated and simplified to:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{2V_e - V}{V_e} (V - V_e) \tag{25}$$

In the vicinity of the equivalent point, (25) can be approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot (V - V_e) \tag{26}$$

Equations (7), (12), (20), and (25) are parabolic functions of V. In the vicinity of the equivalent point, they approximate straight lines. Equations (7) and (12) were transformed to straight lines by dividing by $V_o + V$ (equations (14) and (15)). In a similar manner, (20) and (25) can be transformed to straight lines by dividing by V and $(2V_e - V)$, respectively:

$$\frac{dV/dpH}{V} = \frac{2.30}{V_e} \cdot (V_e - V) \tag{27}$$

$$\frac{dV/dpH}{2V_e - V} = \frac{2.30}{V_e} \cdot (V - V_e) \tag{28}$$

The value of $2V_e$, in the denominator in (28), can be obtained with sufficient accuracy from the intersection between (21) or (27) and the V-axis.

Near the second equivalent point, (25) can be written:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V - V_e}{V_e} \cdot (2V_e - V) \tag{29}$$

which, in the vicinity of the equivalent point, can be approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot (2V_e - V) \tag{30}$$

By dividing dV/dpH by $(V - V_e)$, (29) is transformed to a straight line:

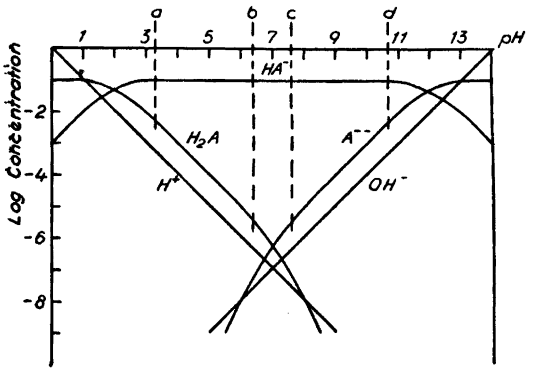

Fig. 2. Logarithmic diagram for a dibasic acid of intermediate strength.

$$\frac{dV/dpH}{V-V_e} = \frac{2.30}{V_e} \cdot (2V_e - V) \tag{31}$$

After exceeding the second equivalent point, equations (12), (13), and (15) should be used.

If the weak acids have such pk_a-values that their logarithmic diagrams are of the type shown in Fig. 2, the hydrogen and hydroxyl ion concentrations can not be neglected in comparison to the other terms, and it can not be conveniently demonstrated that dV/dpH is a simple function of V. However, a demonstration can be made from the expression for the buffer capacity [4], β. If the titration is made with a strong base, *e. g.* sodium hydroxide,

$$\beta = \frac{dC_{Na^+}}{dpH} = 2.30 \cdot (C_{H^+} + C_{OH^-} + \frac{C_a \cdot C_s}{C}) \tag{32}$$

where, in the range a to b in Fig. 2, C is the total concentration of the weak acid, C_a is the concentration of undissociated H_2A, and C_s is the concentration of HA^-.

In the range a to b, C_{HA^-} is approximately equal to C, and C_{OH^-} and $C_{A^{--}}$ can be neglected in comparison to C_{H^+} and C_{H_2A}. Therefore, (32) can be simplified to:

$$\frac{dC_{Na^+}}{dpH} = 2.30 \cdot (C_{H^+} + C_{H_2A}) \tag{33}$$

Under the same conditions, from:

$$C_{H_2A} = C - C_{HA^-} - C_{A^{--}} \tag{34}$$

and the expression for the electro-neutrality conditions in the solution:

$$C_{H^+} + C_{Na^+} = C_{HA^-} + 2C_{A^{--}} + C_{OH^-} \tag{35}$$

the following expression can be derived:

$$C_{H^+} + C_{H_2A} = C - C_{Na^+} \tag{36}$$

which, together with (33), gives:

$$\frac{dC_{Na^+}}{dpH} = 2.30 \cdot (C - C_{Na^+}) \tag{37}$$

Using the same terminology as above,

$$C = C_A \cdot \frac{V_o}{V_o + V}, \tag{38}$$

$$C_{Na^+} = C_B \frac{V}{V_o + V}, \tag{39}$$

and

$$dC_{Na^+} = C_B \frac{V_o}{(V_o + V)^2} \cdot dV \tag{40}$$

The last four equations give, after simplification:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V}{V_o} \cdot (V_e - V) \tag{41}$$

which, in the vicinity of the equivalent point, can be approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V_e}{V_o} (V_e - V) \tag{42}$$

which shows that dV/dpH approximates a straight line. $\frac{dV/dpH}{V_o + V}$ will be a straight-line function of V, as can be seen from the expression:

$$\frac{dV/dpH}{V_o + V} = \frac{2.30}{V_o} \cdot (V_e - V) \tag{43}$$

In the range c to d in Fig. 2, C_{HA^-} is approximately equal to C, and C_{H_2A} and C_{H^+} can be neglected in comparison to the other terms. Therefore, (32) can be simplified to:

$$\frac{dC_{Na^+}}{dpH} = 2.30 \cdot (C_{A^{--}} + C_{OH^-}) \tag{44}$$

Under these conditions, it can be deduced from (34) and (35) that:

$$C_{A^{--}} + C_{OH^-} = C_{Na^+} - C \tag{45}$$

which, together with (44), gives:

$$\frac{dC_{Na^+}}{dpH} = 2.30 \cdot (C_{Na^+} - C) \tag{46}$$

This equation, together with (38), (39), and (40), gives:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V}{V_o} \cdot (V - V_e) \tag{47}$$

which, in the vicinity of the equivalent point, can be approximated by:

$$\frac{dV}{dpH} = 2.30 \cdot \frac{V_o + V_e}{V_o} \cdot (V - V_e) \tag{48}$$

to show that dV/dpH very nearly approximates a straight line. As before, the dV/dpH curve is transformed to a straight line by dividing by $(V_o + V)$:

$$\frac{dV/dpH}{V_o + V} = \frac{2.30}{V_o} \cdot (V - V_e) \tag{49}$$

It should be noticed that formulas (41), (42), (43), (47), (48), and (49) are derived only for a range in the vicinity of the equivalent point. As shall be shown later experimentally, the curves will be straight lines within a rather large range.

Ion-combination titrations

1. Precipitation titrations. When V_o ml of a solution of a substance (A), with original concentration C_{A_o}, are titrated with a solution of a substance (B), of concentration C_{B_o}, a precipitate (A_xB_y) is formed according to the reaction:

$$xA + yB = A_xB_y \tag{50}$$

As long as A is present in excess:

$$C_A = C_{A_o} \cdot \frac{V_o}{V_o + V} - \frac{x}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V} \tag{51}$$

where V is the volume of solution B added. (51) and

$$C_A^x \; C_B^y = S \tag{52}$$

together give:

$$C_B = \frac{S^{\frac{1}{y}}}{(C_{A_o} \cdot \frac{V_o}{V_o + V} - \frac{x}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V})^{\frac{x}{y}}} \tag{53}$$

At the equivalent point, V is equal to V_e, and

$$yC_{A_o} \cdot V_o = xC_{B_o} \cdot V_e \tag{54}$$

(53), after taking logarithms and differentiating, gives:

$$\frac{dV}{d\ln C_B} = \frac{y}{x} \cdot \frac{V_o + V}{V_o + V_e} \cdot (V_e - V) \tag{55}$$

$d\ln C_B$ can be substituted according to:

$$d\ln C_B = \frac{n_B \cdot F}{RT} \cdot dE \tag{56}$$

where n_B is obtained from the following formula:

$$B_{ox} = B_{red} + n_B e^- \tag{57}$$

Equation (55) can be written:

$$\frac{dV}{dE} = \frac{y}{x} \cdot \frac{n_B \cdot F}{RT} \cdot \frac{V_o + V}{V_o + V_e} \cdot (V_e - V) \tag{58}$$

After the equivalent point has been exceeded, B is present in excess, and

$$C_B = C_{B_o} \cdot \frac{V}{V_o + V} - \frac{y}{x} \cdot C_{A_o} \cdot \frac{V_o}{V_o + V} \tag{59}$$

Taking logarithms, differentiating, and inserting (56) gives:

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot \frac{V_o + V}{V_o + V_e} \cdot (V - V_e) \tag{60}$$

Near the equivalent point, (58) and (60) can be approximated by:

$$\frac{dV}{dE} = \frac{y}{x} \cdot \frac{n_B \cdot F}{RT} \cdot (V_e - V) \tag{61}$$

and

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot (V - V_e) \tag{62}$$

respectively. By dividing (58) and (60) by $(V_o + V)$ straight-line functions of V are obtained:

$$\frac{dV/dE}{V_o + V} = \frac{y}{x} \cdot \frac{n_B \cdot F}{RT} \cdot \frac{V_e - V}{V_o + V_e} \tag{63}$$

$$\frac{dV/dE}{V_o + V} = \frac{n_B \cdot F}{RT} \cdot \frac{V - V_e}{V_o + V_e} \tag{64}$$

It can be seen that precipitation titrations are very similar to titrations of strong acids with strong bases.

2. Complex-formation titrations. When V_o ml of a solution of a substance (A), with original concentration C_{A_o}, are titrated with a solution of a substance (B), of concentration C_{B_o}, a complex is formed according to the reaction:

$$xA + yB = A_x\,B_y \tag{65}$$

This reaction is governed by the complex-constant K_c, defined by:

$$\frac{C_A^x \cdot C_B^y}{C_{A_xB_y}} = K_c \tag{66}$$

As long as A is present in excess,

$$C_A = C_{A_o} \cdot \frac{V_o}{V_o + V} - \frac{x}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V}, \tag{67}$$

$$C_{A_xB_y} = \frac{1}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V}, \tag{68}$$

and

$$C_B^y = K_c \cdot \frac{\frac{1}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V}}{\left(C_{A_o} \cdot \frac{V_o}{V_o + V} - \frac{x}{y} \cdot C_{B_o} \cdot \frac{V}{V_o + V}\right)^x} \tag{69}$$

Taking logarithms of (69) and differentiating the expression thus obtained gives, together with (56):

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot \frac{yV \cdot (V_o + V) \cdot (V_e - V)}{V_o \cdot (V_e - V) + xV \cdot (V_o + V_e)} \tag{70}$$

If $x = y = 1$, (70) can be simplified to:

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot \frac{V}{V_e} \cdot (V_e - V) \tag{71}$$

in full agreement with (20).

Compared to similar equations for the previous systems, (70) is rather complicated. However, in the vicinity of the equivalent point, $V_o \cdot (V_e - V)$ can be neglected in comparison to $xV \cdot (V_o + V_e)$. Therefore (70) can be approximated by:

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot \frac{y}{x} \cdot (V_e - V) \tag{72}$$

After the equivalent point has been exceeded, B is present in excess, and

$$C_B = C_{B_o} \cdot \frac{V}{V_o + V} - \frac{y}{x} \cdot C_{A_o} \cdot \frac{V_o}{V_o + V} \tag{73}$$

This, after taking logarithms and differentiating, gives, together with (56):

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot \frac{V_o + V}{V_o + V_e} \cdot (V - V_e) \tag{74}$$

This expression is of the strong acid — strong base type and can, in the vicinity of the equivalent point, be approximated by:

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot (V - V_e) \tag{75}$$

By dividing dV/dE by $(V_o + V)$ in the usual manner, a straight-line function of V is obtained:

$$\frac{dV/dE}{V_o + V} = \frac{n_B \cdot F}{RT} \cdot \frac{V - V_e}{V_o + V_e} \tag{76}$$

Oxidation-reduction titrations

When V_o ml of a solution of a substance (A) in its reduced state, with original concentration C_A, are titrated with a solution of a substance (B) in its oxidized state, of concentration C_B, the following reactions take place in the solution:

$$A_{red} = A_{ox} + n_A e^- \tag{77}$$

and

$$B_{ox} + n_B e^- = B_{red} \tag{78}$$

When V ml of solution B has been added

$$C_{A_{ox}} = \frac{n_B}{n_A} \cdot C_B \cdot \frac{V}{V_o + V} \tag{79}$$

and

$$C_{A_{red}} = C_A \cdot \frac{V_o}{V_o + V} - \frac{n_B}{n_A} \cdot C_B \cdot \frac{V}{V_o + V} \tag{80}$$

The potential in the solution is governed by:

$$E = e_A^\circ + \frac{RT}{n_A \cdot F} \cdot \ln \frac{C_{A_{ox}}}{C_{A_{red}}} \tag{81}$$

Inserting (79) and (80) in (81), taking logarithms, and differentiating the resulting expression gives:

$$\frac{dV}{dE} = \frac{n_A \cdot F}{RT} \cdot \frac{V}{V_e} \cdot (V_e - V) \tag{82}$$

which is of the weak acid — strong base type. In the vicinity of the equivalent point, it can be approximated by:

$$\frac{dV}{dE} = \frac{n_A \cdot F}{RT} \cdot (V_e - V) \tag{83}$$

By dividing dV/dE by V a straight-line function of V is obtained:

$$\frac{dV/dE}{V} = \frac{n_A \cdot F}{RT} \cdot \frac{V_e - V}{V_e} \tag{84}$$

When the equivalent point has been exceeded

$$C_{B_{red}} = \frac{n_A}{n_B} \cdot C_A \cdot \frac{V_o}{V_o + V} \tag{85}$$

and

$$C_{B_{ox}} = C_B \cdot \frac{V}{V_o + V} - \frac{n_A}{n_B} \cdot C_A \cdot \frac{V_o}{V_o + V} \tag{86}$$

The potential is determined by:

$$E = e^\circ_B + \frac{RT}{n_B \cdot F} \ln \frac{C_{B_{ox}}}{C_{B_{red}}} \tag{87}$$

(85) and (86) are inserted in (87). Taking logarithms and differentiating give:

$$\frac{dV}{dE} = \frac{n_B \cdot F}{RT} \cdot (V - V_e) \tag{88}$$

which will be a straight line under all conditions.

THE INCREMENT ΔV

The formulas in the preceeding section have been derived for infinitely small increments, ΔV. In practice it is, of course, impossible to make the increments infinitely small. It is therefore necessary to calculate in as simple a manner as possible the values of V against which $\Delta V/\Delta$pH or $\Delta V/\Delta E$ should be plotted. If ΔV is not too large, no great error ensues, if $\Delta V/\Delta$pH or $\Delta V/\Delta E$ is plotted against $V + \frac{1}{2}\Delta V$. For greater values of ΔV it may be necessary to make a correction. $\Delta V/\Delta$pH or $\Delta V/\Delta E$ is then plotted against $V + c\frac{1}{2}\Delta V$. The correction factor *(c)* is determined by the ratio between ΔV and the absolute value of $V_e - V$. In Table 1 the factor c is shown as a function of the ratio $\Delta V/|V_e - V|$.

Table 1. Correction factor c as a function of the ratio $\Delta V/|V_e - V|$.

$\frac{\Delta V}{\lvert V_e - V\rvert}$	c	
	$V_e > V$	$V > V_e$
0.01	1.0016	0.9984
0.02	1.0033	0.9967
0.05	1.0086	0.9914
0.10	1.0176	0.9824
0.15	1.0271	0.9729
0.20	1.0374	0.9626
0.25	1.0480	0.9520
0.30	1.0594	0.9406
0.40	1.0848	0.9152
0.50	1.1146	0.8854
0.60	1.1506	0.8494
0.70	1.1959	0.8041
0.80	1.2573	0.7427
0.90	1.3536	0.6464

In cases where the solution reaches the equilibrium potential very slowly, much time might be saved by making the increments rather large and applying the corrections to the values thus obtained. If ΔV is less than about 20 % of $|V_e - V|$, there is generally no need to make any corrections. The value of V_e, which is needed to calculate the factor c, can be obtained with sufficient accuracy from the titration curve or from the $\Delta V/\Delta$pH or $\Delta V/\Delta E$ curves, calculated without corrections.

The use of the correction factor is shown in the following example:

50 ml of a strong acid are titrated with a strong base. From the titration curve, V_e can be estimated to be about 20 ml. If a 3.00 ml increment of reagent was added between V = 15.00 and V = 18.00 ml, the pH-values obtained being 2.041 and 2.463 respectively, ΔV is about 60 % of $|V_e - V|$ and therefore the correction factor c is 1.15.

$$\Delta V/\Delta\text{pH} = 3.00/0.422 = 7.109$$

$$V + c\tfrac{1}{2}\Delta V = 15.00 + 1.15 \cdot 0.5 \cdot 3.00 = 16.73$$

$$V_o + V + c\tfrac{1}{2}\Delta V = 50 + 16.73 = 66.73$$

$$\frac{\Delta V/\Delta\text{pH}}{V_o + V + c\tfrac{1}{2}\Delta V} = \frac{7.109}{66.73} = 0.1065$$

In the $\Delta V/\Delta$pH vs. V diagram, 0.1065 is plotted against 16.73. Without corrections, 0.1069 would have been plotted against 16.50.

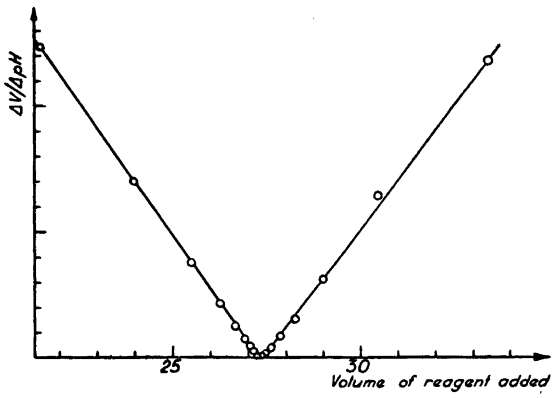

Fig. 3. $\Delta V/\Delta pH$ curve for the titration of HCl with NaOH.

If the titration had been made on a weak acid instead of on a strong one, the following value should have been calculated:

$$\frac{\Delta V/\Delta \mathrm{pH}}{V + c\tfrac{1}{2}\Delta V} = \frac{7.109}{16.73} = 0.4249$$

and 0.4249 should have been plotted against 16.73. Without corrections 0.4308 would have been plotted against 16.50.

It should be pointed out here that the same formula must be used for the calculation of the entire $\Delta V/\Delta$pH or $\Delta V/\Delta E$ curve. For example, it is not possible to calculate part of the curve using (8) and part using (14), since the values thus obtained differ by a factor of $(V_o + V_e)$. If, however, (14) is written:

$$\frac{\mathrm{d}V/\mathrm{dpH}}{\dfrac{V_o + V}{V_o + V_e}} = 2.30 \cdot (V_e - V), \qquad (14\,a)$$

(8) and (14 a) can be used for different parts of the curves, *e. g.*, (14 a) for the first part and (8) for the range in the vicinity of the equivalent point.

EXPERIMENTAL

In the following section some $\Delta V/\Delta$pH and $\Delta V/\Delta E$ curves will be shown. These curves have been obtained in actual titrations using a *Radiometer* potentiometer, type PHM 3f.

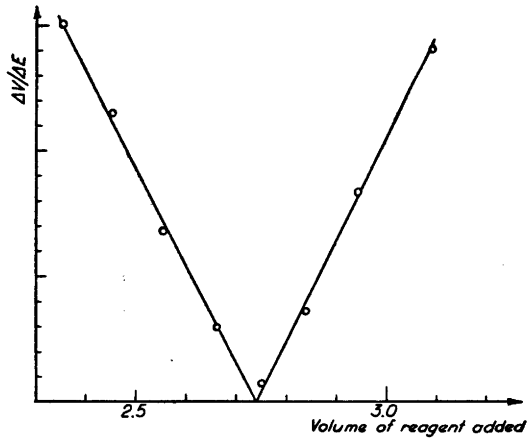

Fig. 4. $\Delta V/\Delta E$ curve for the titration of benzoic acid in ethylenediamine with an ethylenediamine solution of sodium aminoethoxide.

Neutralization titrations

1. *Strong acid — strong base.* 25.00 ml of 0.1087 *N* hydrochloric acid were diluted with distilled water to about 100 ml and titrated with 0.0995 *N* sodium hydroxide solution. The pH of the solution was measured with a glass electrode against a saturated calomel electrode. The data found in Fig. 3 were calculated from the readings using (8) and (13). The pH values above 9 have been corrected with the help of a nomograph supplied with the potentiometer.

2. *Titration of benzoic acid with sodium aminoethoxide in ethylenediamine solution.* 34.40 mg of benzoic acid were dissolved in 40 ml of dry ethylenediamine and titrated with a 0.1029 *N* ethylenediamine solution of sodium aminoethoxide [5], according to a slightly modified method of Moss, Elliott, and Hall [6]. The measurements were made with an ignited, bright platinum electrode against a calomel electrode with ethylenediamine, saturated with lithium chloride, as solvent [5]. The $\Delta V/\Delta E$ curve was calculated from (8) and (13) and corrected (see page 572). The curve is shown in Fig. 4.

3. *Weak acid — strong base.* 10.00 ml of 0.1118 *M* phosphoric acid were diluted with distilled water to 150 ml and titrated with 0.0995 *N* sodium hydroxide solution. The pH values were determined by a glass electrode against a saturated calomel electrode. In Fig. 5 $\Delta V/\Delta$pH curves, calculated from (42), (26), (21), and (48), are shown (pH values uncorrected). In Fig. 6 are shown $\Delta V/\Delta$pH curves for the same titration calculated from (43), (28), (31), and (49). As can be seen, the parabolic part of the curve in Fig. 5 has been transformed to straight lines.

Precipitation titrations

1. *Titration of silver ions with chromate.* 10.00 ml of 0.0999 *N* silver nitrate solution were diluted to 50 ml and titrated with 0.1020 *N* potassium chromate. A silver wire was used as indicator electrode and measurements were made against a calomel electrode with a saturated ammonium nitrate bridge. In Fig. 7 both $\Delta V/\Delta E$ and $\Delta E/\Delta V$ curves are shown. As can be seen, the $\Delta E/\Delta V$ curve reaches a maximum about 1 % before the

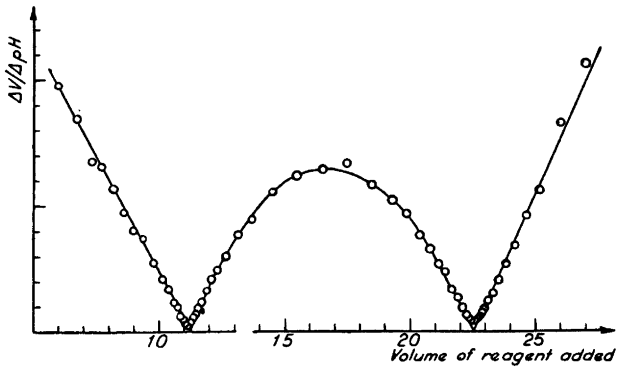

Fig. 5. $\Delta V/\Delta pH$ curve for the titration of H_3PO_4 with NaOH.

extrapolated $\Delta V/\Delta E$ curve reaches its minimum. The value obtained from the $\Delta V/\Delta E$ curve coinsides with the calculated equivalent point.

2. *Titration of iodide, bromide, and chloride ions with silver ions.* 10.00 ml of 0.0938 N potassium iodide, 10.00 ml of 0.0981 N sodium bromide, and 10.00 ml of 0.1087 N potassium chloride solutions were mixed and diluted to 100 ml, and 2 grams of ammonium nitrate were added. The solution was titrated with a 0.0999 N silver nitrate solution in the same manner as in the preceeding experiment. The $\Delta V/\Delta E$ curves obtained are shown in Fig. 8.

Complex-formation titration

To 20.00 ml of 0.1128 N potassium cyanide solution, 2 ml of 2 N ammonium hydroxide were added. The solution was diluted to 100 ml and titrated with 0.0999 N silver nitrate in the same way as before. The $\Delta V/\Delta E$ curve obtained is shown in Fig. 9. The first part of the curve is not in accordance with the theoretical requirements for the formation of $Ag(CN)_2^-$ ions, probably due to the fact that ammonium hydroxide was added. Nevertheless, the equivalent point is indicated very sharply.

Oxidation-reduction titration

A mixture of 15.00 ml of 0.0914 N ferrous sulfate and 5 ml of 5 N sulfuric acid were diluted to 100 ml with distilled water and titrated with 0.1009 N potassium permanganate solution. Measurements were made with an ignited, bright platinum electrode against a saturated calomel electrode. The $\Delta V/\Delta E$ curve for the titration is shown in Fig. 10. An enlargement of a section of the curve in the vicinity of the equivalent point is also shown in Fig. 10. It can be seen that the curve is parabolic before and straight after the equivalent point, in full agreement with (82) and (88). The slope of the straight line, however, should have been much greater — about 5 times the slope of the parabolic curve in the vicinity of the equivalent point. The discrepancy is probably due to the fact that the reaction

$$MnO_4^- + 8\ H^+ + 5\ e^- = Mn^{++} + 4\ H_2O$$

is not strictly reversible [3b,7].

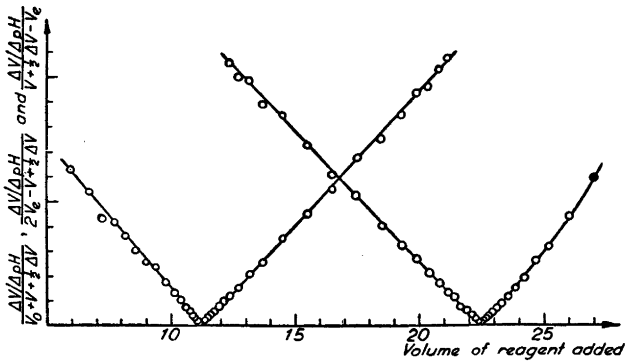

Fig. 6. $\Delta V/\Delta pH$ curve for the titration of H_3PO_4 with NaOH. The parabolic part of the curve in Fig. 5 has been transformed to straight lines. (See text.)

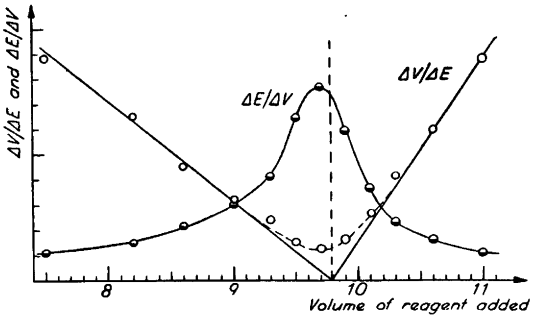

Fig. 7. $\Delta V/\Delta E$ and $\Delta E/\Delta V$ curves for the titration of Ag^+ with CrO_4^{--}.
—○———○— $\Delta V/\Delta E$; —●———●— $\Delta E/\Delta V$.

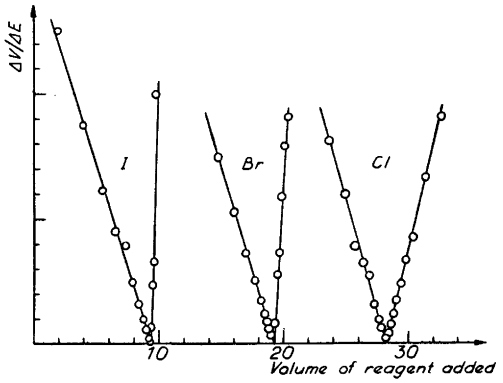

Fig. 8. $\Delta V/\Delta E$ curves for the titration of I^-, Br^-, and Cl^- with Ag^+.

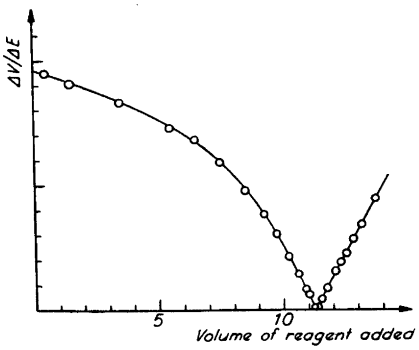

Fig. 9. $\Delta V/\Delta E$ curve for the titration of Ag^+ with CN^-.

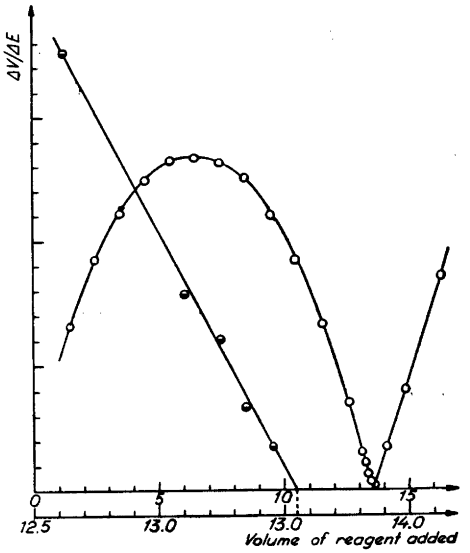

Fig. 10. $\Delta V/\Delta E$ curves for the titration of Fe^{++} with MnO_4^-.
—●———●—enlarged section of curve.

SUMMARY

It has been shown that by plotting $\Delta V/\Delta$pH or $\Delta V/\Delta E$ versus V, the equivalent point in potentiometric titrations can be determined very accurately. In the vicinity of the equivalent point, the $\Delta V/\Delta$pH and $\Delta V/\Delta E$ curves are very nearly straight lines. From the appearance of the curves some conclusions can be drawn concerning the reactions taking place during the titration. The application of a correction factor, for use in very accurate work, is discussed. The method has been verified by actual potentiometric titrations of various kinds.

The author wishes to thank Dr. Axel Johansson for his interest in this work and for many valuable discussions, and Mr. J. N. Ospenson for his revision of the English text.

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Received February 13, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 578–581

Aminoacyl Derivatives of 9-Aminofluorene

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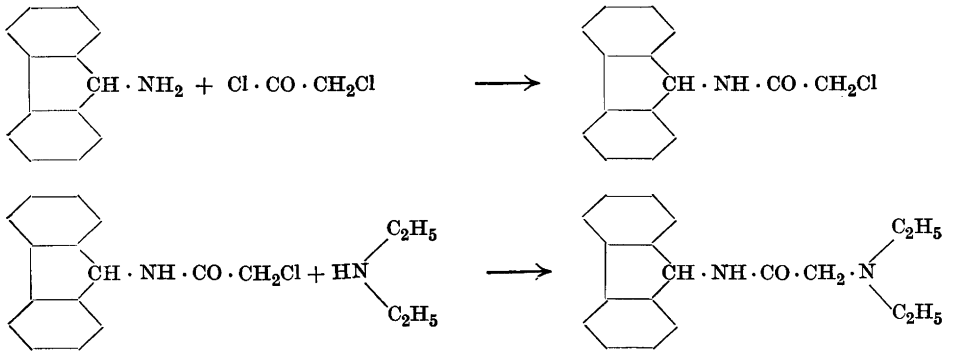
Some time ago we synthesised in this laboratory a series of aminoacyl-substituted phenothiazine derivatives [1]. As they possessed rather interesting pharmacological properties, we thought it worth while preparing similar compounds containing other condensed ring systems. Derivatives of fluorene have proved to possess interesting features, especially as antispasmodics [2], and we have now prepared several aminoacyl derivatives of 9-aminofluorene, analogous to the phenothiazine compounds.

Haloacylhalides react easily with 9-aminofluorene to form haloacylaminofluorenes. The reaction can be carried out using either sodium acetate [3] or pyridine as condensing agent, the latter method giving a somewhat higher yield. The haloacylamines are then treated with excess of the appropriate amine, which gives the desired aminoacyl compounds. The procedure may be examplified by the following reactions:



The compounds have been subjected to preliminary pharmacological tests (S. Wiedling). They all showed a high local anesthetic power, 1—12 times the activity of Xylocaine when tested on rabbit cornea. However, the time of

onset was much longer, and they all exhibited an irritant action. When tests were carried out on isolated guinea pig ileum, the compounds showed a very weak antihistaminic and antispasmodic effect.

EXPERIMENTAL

Halogenoacylaminofluorenes

9-(Chloroacetylamino)-fluorene. 9-Aminofluorene hydrochloride[4] (11.0 g) was suspended in water (125 ml) and made alkaline with sodium hydroxide. The precipitated base was extracted with ether (2 × 100 ml), the ether solution dried over solid NaOH and filtered. Pyridine (4.0 ml) was then added to the ether solution and chloroacetyl chloride (6.8 g) was added drop by drop with stirring and cooling with ice water. A white precipitate was formed, which was collected and washed with dilute acetic acid. After drying in air the crude product (11.0 g) was recrystallised twice from ethanol. M. p. 238–239°.

$C_{15}H_{12}ClNO$ (257.7)	Calc.	C	69.9	H	4.70	N 5.44
	Found	»	70.0	»	4.81	» 5.35

9-(β-Chloropropionylamino)-fluorene. From 9-aminofluorene hydrochloride (6.7 g) and β-chloropropionyl chloride (3.8 g) in the same way as described above. M. p. 202–203.5° (dec.) after recrystallisation of the crude product (7.2 g) from acetone.

$C_{16}H_{14}ClNO$ (271.7)	Calc.	C	70.7	H	5.19	Cl 13.1
	Found	»	70.8	»	5.36	» 13.1

9-(α,β-Dibromopropionylamino)-fluorene. From 9-aminofluorene hydrochloride (4.3 g) and α,β-dibromopropionyl chloride (4.8 g). M. p. 227–228° after recrystallisation of the crude product (5.5 g) from light petroleum-ethanol (1 : 2).

$C_{16}H_{13}Br_2NO$ (395.1)	Calc.	C	48.6	H	3.31	Br 40.4
	Found	»	49.0	»	3.21	» 40.0

9-(α-Bromopropionylamino)-fluorene. 9-Aminofluorene (18.1 g) was mixed with glacial acetic acid (85 ml) and α-bromopropionyl bromide (23.0 g) was added. An aqueous solution of sodium acetate (33 g in 140 ml) was then added in portions with stirring. The mixture was stirred for half an hour and the resulting precipitate was collected and dried in air. The crude product (21.0 g) was recrystallised twice from ethanol. M. p. 243–244°.

$C_{16}H_{14}BrNO$ (316.2)	Calc.	C	60.8	H	4.46	N 4.43
	Found	»	60.5	»	4.44	» 4.40

9-(α-Bromobuturylamino)-fluorene. This compound was prepared from 9-aminofluorene (15.8 g) and α-bromobuturyl bromide (22.0 g). The crude product (21.0 g) was recrystallised from ethanol. M. p. 226–227°.

$C_{17}H_{16}BrNO$ (330.2)	Calc.	C	61.8	H	4.89	Br	24.2	N 4.24
	Found	»	61.3	»	4.91	»	23.9	» 4.15

Aminoacylfluorenes

These compounds were all prepared in the same manner. The halogenoacyl derivative (1 mol) was heated under reflux in benzene or toluene with the appropriate amine (usually 2.6 mol). The hydrobromide or hydrochloride of the amine was separated and the filtrate extracted with *N* hydrochloric acid. The acid extract was made alkaline with sodium hydroxide and the precipitated base, which usually soon solidified, collected and recrystallised.

9-(Diethylaminoacetylamino)-fluorene. 9-(Chloroacetylamino)-fluorene (3.0 g) and diethylamine (2.2 g) were heated under reflux in toluene (50 ml) for five hours. The reaction mixture was treated according to the general procedure outlined above. The resulting product (3.3 g) was crystallised from light petroleum. M. p. 92.5—93.5°.

$C_{19}H_{22}N_2O$ (294.4)	Calc.	C	77.5	H	7.53	
	Found	»	77.6	»	7.48	

9-(Dimethylaminoacetylamino)-fluorene. 9-(Chloroacetylamino)-fluorene (2.0 g) was heated with dimethylamine (1.4 g) in benzene (30 ml) in a sealed vessel at 100° over night. After extracting the solution with hydrochloric acid the slightly soluble hydrochloride of the aminoacylfluorene was separated, suspended in water, and the suspension made alkaline. A further quantity of base was obtained in the same manner from the filtrate, and the combined products (together 2.0 g) recrystallised twice from 50 % ethanol. M. p. 135—136°.

$C_{17}H_{18}N_2O$ (266.3)	Calc.	C	76.7	H	6.81	N	10.5
	Found	»	76.9	»	6.92	»	10.7

9-(β-Hydroxyethyl-ethylaminoacetylamino)-fluorene. From 9-(chloroacetylamino)-fluorene (2.0 g) and ethylaminoethanol (2.7 g) by boiling in benzene (25 ml) for six hours. The crude base (2.3 g) was crystallised from light petroleum-acetone (10 : 1). M. p. 96.5—97.5°.

$C_{19}H_{22}N_2O_2$ (310.4)	Calc.	C	73.5	H	7.15
	Found	»	73.6	»	7.19

9-(Piperidinoacetylamino)-fluorene. From 9-(chloroacetylamino)-fluorene (1.5 g) and piperidine (1.33 g) by boiling in benzene (25 ml) for six hours. The base (1.75 g) was recrystallised from 50 % ethanol. M. p. 139.5—140.5°.

$C_{20}H_{22}N_2O$ (306.4)	Calc.	C	78.4	H	7.24	N	9.15
	Found	»	78.3	»	7.36	»	9.31

9-(Morpholinoacetylamino)-fluorene. 9-(Chloroacetylamino)-fluorene (1.5 g) and morpholine (1.3 g) were boiled in benzene (25 ml) for six hours. The crude base (1.7 g) was crystallised from light petroleum. M. p. 162.5—163.5°.

$C_{19}H_{20}N_2O_2$ (308.4)	Calc.	C	74.0	H	6.54	N	9.09
	Found	»	73.8	»	6.63	»	9.07

9-(α-Diethylaminopropionylamino)-fluorene. 9-(α-Bromopropionylamino)-fluorene (2.4 g) and diethylamine (1.5 g) were heated in toluene (40 ml) in a sealed vessel over night at 110°. The base obtained was an oil which was extracted with ether and a saturated ethereal solution of oxalic acid added until no more precipitate was formed. The oxalate (1.5 g) was purified by dissolving in acetone and precipitating with light petroleum when it melted at 174—176° (dec.). The analysis indicated that three moles of oxalic acid were bound to two moles of the base. The percentage of oxalic acid could also be determined directly. A sample of the salt was decomposed with alkali, the base removed by extraction and the water solution acidified and titrated with permanganate.

$2 \cdot C_{20}H_{24}N_2O \cdot 3(COOH)_2$ (887.0)	Calc.	C	62.3	H	6.14	N	6.32	$(COOH)_2$ 30.5
	Found	»	62.3	»	6.13	»	6.35	» 30.6

9-(α-Piperidinopropionylamino)-fluorene. 9-(α-Bromopropionylamino)-fluorene (1.1 g) and piperidine (0.8 g) were boiled in toluene for six hours. The base (1.1 g) was recrystallised from 50 % ethanol. M. p. 102.5—103.5°.

$C_{21}H_{24}N_2O$ (320.4)	Calc.	C	78.7	H	7.55	N	8.75
	Found	»	78.6	»	7.48	»	8.65

9-(α-Diethylaminobuturylamino)-fluorene. From 9-(α-bromobuturylamino)-fluorene (1.5 g) and diethylamine (0.9 g) by boiling in toluene (25 ml) for six hours. The crude base (0.6 g) was recrystallised from light petroleum. M. p. 115—116°.

$C_{21}H_{26}N_2O$ (322.4)	Calc.	C	78.2	H	8.13
	Found	»	78.4	»	8.15

SUMMARY

The synthesis of five halogenoacyl and eight aminoacyl derivatives of 9-aminofluorene is described.

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Received February 20, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 582–588

A Method for Titration of Small Amounts of Iodine in Acetic Acid Medium

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In iodimetric determinations of peroxides in fat the liberated iodine is titrated in a strong acetic acid medium. In Lee's method [1], for instance, the composition of the solution to be titrated is as follows: 20 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume), 30 ml distilled water, 1 g potassium iodide, and about 1 g of the sample to be tested. The two-layer system obtained is titrated with highly diluted sodium thiosulphate solution until the iodine color has disappeared. Some authors state that the titration can be performed using starch as an indicator. However, starch may be used in the acid medium described above only when larger amounts of iodine are present. With the small amounts of iodine liberated in the determination of peroxides in pure lard (amounting to 10^{-5}—10^{-6} equivalents of iodine per sample) starch is not a suitable indicator in our experience. However, neutralization of the acetic acid solution with sodium bicarbonate increases the sensitivity of starch as to iodine. The use of starch as an indicator in peroxide determinations, as so often mentioned in the literature, may have arisen from experiments with, for example, benzoylperoxide in such high concentrations as seldom correspond to the peroxide content of normal quality fats.

In such solutions, weakly yellow with fat, it is very difficult to titrate iodine without the aid of an indicator. For this reason we have investigated the possibilities of titrating iodine in acid medium with the use of a fluorescent indicator in ultraviolet light. Gotô [2] has earlier shown that iodine can be titrated with thiosulfate in hydrochloric acid solution using α-naphthoflavone or rhodamine B as a fluorescent indicator. For determination of iodine in neutral solution a fluorimetric method has been described by Harlay [3].

The attempts to titrate small amounts of iodine in acid medium with the aid of a fluorescent indicator were made for use in connection with peroxide

determinations. Therefore the titrations were performed in the two-layer system obtained by using Lee's mixture of acetic acid, chloroform, distilled water, and in some cases potassium iodide. As the indicator used by Gotô, α-naphthoflavone, was not obtainable, some 30 other dyestuffs available in our laboratory (including rhodamine BP) were examined with respect to their usefulness in the titration of iodine in ultraviolet light.

The source of light used was a mercury vapour lamp with nickel-glass bulb. The maximum intensity of the light was at 365 mμ.

Among the dyestuffs investigated rhodamine BP, thioflavin, pinacryptol yellow, and dicyanin A showed a distinct change in intensity of fluorescence in the vicinity of the equivalence point when titrating iodine in the described two-layer system. Further experiments showed that the change in fluorescence of thioflavin was more obvious than that of the other dyestuffs. Because of this the experiments were directed to a closer investigation of its usefulness in peroxide determinations and to an attempt to explain its action as an indicator *.

THIOFLAVIN AND ITS BLUE-FLUORESCING COMPONENT

The thioflavin used was obtained from a dye manufacturer in London. Its fluorescence in both water and chloroform solutions was bluish green. In the progress of the experiments the thioflavin proved to have one component with a very strong whitish-blue fluorescence in ordinary organic solvents, and one with a faintly yellowgreen fluorescence. In order to separate the blue-fluorescing component a mixture of 45 ml of 0.1 % aqueous thioflavin solution and 5 ml glacial acetic acid was extracted three times with 15 ml of benzene in a separatory funnel. The benzene extract was filtered and dried over calcium chloride. As the dyestuff was easily absorbed by the drying agent the benzene solution was dried for only one hour. From the filtrate the solvent was evaporated in vacuum on a water bath. The minute residue was dissolved in 1 000 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume). The solution of the blue-fluorescing thioflavin component thus obtained has been used throughout the experiments described below. The dyestuff solution has been used as solvent in the determination of peroxides in fats and oils according to Lee [1].

* Kocsis, *et al.*[4] earlier made use of thioflavin S as an adsorbtion indicator when performing precipitation titrations on halogen ions with silver nitrate in ultraviolet light.

EXPERIMENTS WITH BENZOYL PEROXIDE

Benzoyl peroxide was recrystallized three times according to Gatterman [5]. From the purified substance a standard solution was prepared containing about 100 mg of benzoyl peroxide dissolved in 100 ml of *n*-propanol. The solution was kept in a glass-stoppered bottle in a dark place.

In order to establish the accuracy in the determination of benzoyl peroxide, samples of the following mixture were titrated:

20 ml of a mixture of HAc + $CHCl_3$ (2 + 1) containing indicator
5.00 ml benzoyl peroxide solution
1 ml saturated KI solution
30 ml distilled water.

When the potassium iodide solution had been added the sample was allowed to stand in the dark for ten minutes. Distilled water was then added and the sample titrated with 0.01 *N* thiosulphate solution. In the two-layer system obtained in this way, the blue-fluorescing thioflavin component dissolved in the chloroform layer only. The end point is reached when the fluorescence in the chloroform suddenly increases to a maximum. This is best observed when the chloroform by gentle agitation is suspended in the water. At this point the chloroform phase, when illuminated from above, appears as a whitish-blue luminous fog separating from the water phase.

In the experiments described above the following relationships prevail:

$$2\ S_2O_3^{2-} \longleftrightarrow I_2 \longleftrightarrow 1/2\ O_2 \longleftrightarrow (C_6H_5CO)_2O_2$$

and the amount of active oxygen is obtained from:

$$\%\ \text{active oxygen} = \frac{8 \times \text{ml thiosulphate sol.} \times \text{its normality} \times 100}{\text{mg benzoyl peroxide per sample}}$$

The results obtained are tabulated below (Table 1).

Nos. 6—8 were titrated four days later than nos. 1—5, the same benzoyl peroxide standard solution being used. The results show that the standard solution is stable during this period of time and that the end-point in titration can be determined with sufficient accuracy.

EXPERIMENTS WITH FATS

In order to estimate the usefulness of this new method in the determination of peroxides in various fats, samples of lard and tallow were titrated in visible light, without indicator, and in ultraviolet light with the thioflavin component

Table 1. Titration of 5.00 ml benzoyl peroxide solution containing 5.44 mg benzoyl peroxide. Theoretically calculated active oxygen = 6.606 %.

Sample no.	Ml. 0.01 *N* thiosulphate sol. used (corr. for blank)	Active oxygen found, %	$\frac{\%\text{ found}}{\%\text{ calc.}} \times 100$
1	4.26	6.62	100.2
2	4.26	6.62	100.2
3	4.26	6.62	100.2
4	4.26	6.62	100.2
5	4.21	6.54	99.0
6	4.26	6.62	100.2
7	4.26	6.62	100.2
8	4.25	6.61	100.1
			Average value: 100.0

as an indicator. These preliminary experiments were performed without using an inert atmosphere.

The samples to be tested were prepared in the following way: The fat was melted on a water bath at 60° C and samples of 1 gram were weighed out in Erlenmeyer flasks. 1 g of potassium iodide and 20 ml of an acetic acid-chloroform-mixture (2 + 1), with and without indicator, were added. The samples were gently boiled over an open flame for 30 seconds. The fat dissolved and the reaction between iodide and peroxides took place. The solutions were immediately cooled to room temperature and 30 ml distilled water added. The samples were titrated with 0.01002 *N* thiosulphate solution.

From Table 2 it is obvious that the values obtained with titrations in visible and in ultraviolet light are not satisfactorily agreeable. The deviations are especially great in the experiment with tallow, probably due to the small amount of thiosulphate required. Considering the experience with benzoyl peroxide, the results obtained from titrations in ultraviolet light are probably more correct than those obtained in titrations in visible light where no indicator was used. In the latter titrations the error is probably due largely to the natural yellowish color of the fat in the chloroform solution which interferes with the end point.

As regards the titration of iodine in ultraviolet light attention has been payed to the possibility that photochemical reactions might interfere with the

Table 2. Determination of peroxides in 1.00 g fat. (1 ml 0.01 N thiosulphate sol. = 0.080 mg active oxygen).

Sample no.	Titration in visible light			Titration in U. V. light in the presence of the blue-fluoresc. compound		
	Ml thiosulphate sol. used (corr. for blank)		% active oxygen × 10^3	Ml thiosulphate sol. used (corr. for blank)		% active oxygen × 10^3
Lard 1	0.24	0.25	1.96	0.26	0.28	2.16
» 2	0.21	0.20	1.64	0.19	0.20	1.56
» 3	0.27	0.28	2.20	0.34	0.35	2.77
» 4	0.29	0.30	2.36	0.29	0.30	2.36
Tallow 1	0.10	0.11	0.84	0.05	0.06	0.44
» 2	0.04	0.07	0.44	0.03	0.00	0.12
» 3	0.04	0.04	0.32	0.06	0.07	0.52

determination. During the time of titration the authors observed no disturbances due to the ultraviolet light. Only after a longer time of exposure to the light an effect was revealed by an increase in the thiosulphate requirement.

THE PRINCIPLE OF THE FLUORESCENCE TITRATION OF IODINE IN THE TWO-LAYER SYSTEM

Experiments to explain the indicator action of the blue-fluorescing thioflavin component showed that neither a reduction-oxidation reaction within the dyestuff molecule, nor the formation of a non-fluorescent complex with the liberated iodine would explain the strong increase in fluorescence when the iodine was transformed into iodide. As the light has to pass through the water layer to reach the dyestuff dissolved in the chloroform layer, the absorption behaviour of the test solutions in ultraviolet light was investigated. In the presence of a large excess of potassium iodide, the water layer contains the iodine in the form of the complex I_n^--ions. It was supposed that the absorption of ultraviolet light by the I_n^- ions was so great that the fluorescent indicator in the chloroform layer was not exposed to the activating light. A simple experiment indicated this to be the case. A long narrow glass tube was filled with a sample of the same composition as used in the experiments with benzoyl peroxide. When the tube was held in slanted position and examined in ultraviolet light, the chloroform layer fluoresced with full brilliance whether the water layer contained large amounts of iodine or excess of thiosulphate.

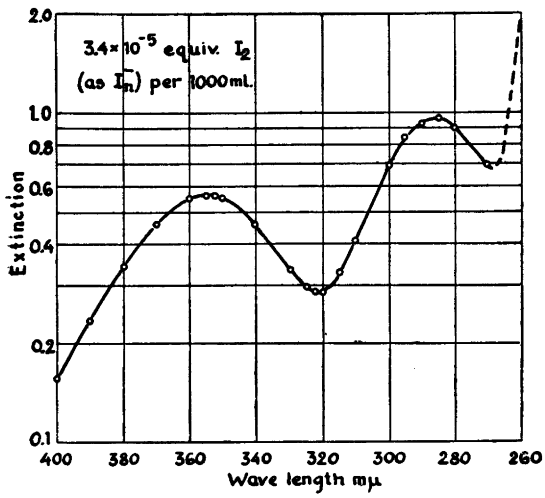

Fig. 1. Absorption curve in ultraviolet light for a solution containing 3.4 × 10^{-5} equivalents I_2 (as I_n^-) per 1000 ml.

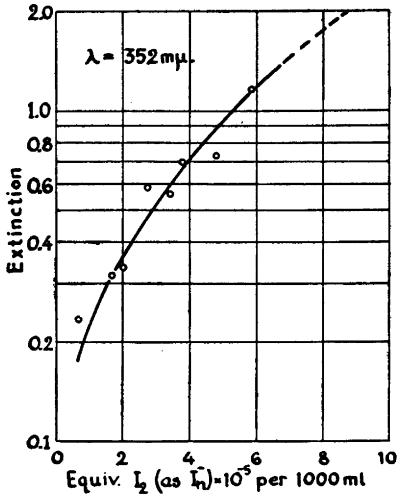

Fig. 2. Extinction at 352 mμ of solutions with varying iodine concentrations.

On this account the absorption curve in ultraviolet light for a solution containing 3.4 × 10^{-5} equivalents of iodine (as I_n^- ions) per 1 000 ml was determined (Fig. 1) and also the extinction at 352 mμ of solutions with varying iodine concentrations (Fig. 2). The apparatus used was the Beckmann spectrophotometer. The measurements were made on the water layer with the same composition as given in the experiments with benzoyl peroxide. The solutions were read against blanks which instead of benzoyl peroxide contained an equivalent amount of benzoic acid.

Fig. 1 shows that the I_n^- ions have an absorption maximum at 350—360 mμ which is very close to the intensity maximum of the mercury-vapour lamp used in the titrations. The results aggree with the findings of Custer and Natelson [6] and of Brode [7]. In Fig. 2 it is shown that the absorption increases very rapidly with increasing iodine concentration. At a concentration of only 10^{-4} equivalents per 1 000 ml the absorption is too high to be measured. The measurements thus confirm the assumption that the I_n^- ions, even in the small concentrations present, *e. g.*, in the determinations of peroxides in fats, absorb the ultraviolet light which activates the fluorescent indicator in the chloroform layer.

In iodine titrations in the presence of fat the impurities in the fat may interfere by absorbing the ultraviolet light. Of the fats examined, the peroxide content could be determined without disturbances in lard, tallow, butter, and

various edible oils of vegetable origin. On the other hand peroxide determinations in strongly colored feeding oils, for example, could not be made. Absorption measurements were made on both layers from solutions of the following composition: 20 ml of a mixture of 2 parts of acetic acid and 1 part of chloroform (by volume), 1 g fat and 30 ml distilled water. The measurements showed that the absorption at around 360 mμ in both the water and the chloroform layers was negligible in all cases except in the case of feeding oils where the absorption was very high in the chloroform layer.

The principle of the method suggested for the determination of small amounts of iodine in acetic acid medium may be of interest. The method has been only briefly described as the dyestuff used at the present time is difficult to obtain. The sensitivity of this method may be improved by using some other indicator. The shape of the titration vessel may also have some effect on the results.

SUMMARY

A new principle for titration of small amounts of iodine has been described. The titration is performed in ultraviolet light with thiosulphate solution in a two-layer system obtained by mixing acetic acid, distilled water, and chloroform. The end point is characterized by the appearance of fluorescence from a dyestuff in the chloroform layer. Because of the strong ultraviolet absorption of the iodine (in the form of the complex I_n^- ions) the chloroform layer does not show fluorescence with full brilliance until all the iodine has reacted with thiosulphate. The sensitivity of the method is about 25 micrograms or 2×10^{-7} equivalents of iodine per sample.

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Received February 24, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 589–596

Zur Konstitution des Fichtenlignins

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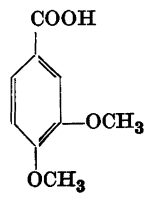
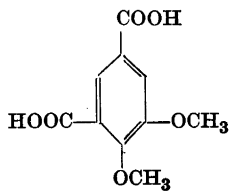
Bei der energischen Oxydation von isoliertem Lignin (Salzsäure-Phosphorsäure-Lignin), welches mit Diazomethan oder Dimethylsulfat methyliert war, erhielten Freudenberg und Mitarb. [1] bisher nur 1—2 % Veratrumsäure (I). Aus methyliertem Holz wurden in analoger Weise etwa 4 % des Ligninanteils als Veratrumsäure erhalten [2]. Das zweite charakteristische Abbauprodukt des Lignins, die Isohemipinsäure (II) trat erst auf, wenn das Lignin zur Aufsprengung seiner Ätherbindungen vor der Methylierung und Oxydation mit starker Kalilauge gekocht wurde. In diesem Fall wurden etwa 14 % I und 4 % II isoliert [3]. Der analoge Abbau von methyliertem Holz ergibt etwas bessere Ausbeuten an I und II [4]. Auch bei der Oxydation aller Ligninpräparate, welche durch Aufschluss von Holz oder isoliertem Lignin mit Bisulfit, Thioglykolsäure, Alkohol und Chlorwasserstoff, Eisessig und Magnesiumchlorid oder mit Kalium in flüssigem Ammoniak erhalten wurden, erhielt Freudenberg nur 2,5—6,5 % I und 1,5—4 % II [5].

Vor kurzem gelang es mir nun erstmalig, beim Abbau von methyliertem Alkohollignin ausser Veratrum- und Isohemipinsäure auch noch 1,3 % Metahemipinsäure (III) und etwas Bernsteinsäure zu isolieren [6]. Wie inzwischen weiter festgestellt wurde, liefert auch der aus dem Holz mit alkoholischer Salzsäure nicht auslösbare Ligninanteil (isoliert als Salzsäurelignin) nach der Methylierung beim Abbau mit Permanganat ähnliche Ausbeuten an III. In



I



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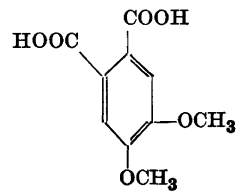


III

beiden Fällen sind noch nicht alle Oxydationsprodukte in reiner Form isoliert worden. In der Metahemipinsäure war zum ersten Mal ein Abbauprodukt gefunden worden, dessen Entstehung auf Grund der Freudenberg'schen Formelbilder [7] nicht zu erwarten war. Es erhob sich daher die entscheidende Frage, ob die die Metahemipinsäure liefernde Konfiguration bereits im nativen Lignin vorkommt oder ob sie erst bei der Auslösung des Lignins aus dem Holz durch Kondensationsreaktionen entstanden ist. Dass hierbei in gewissen Fällen durch Kondensationen neue Kohlenstoff-Kohlenstoff-Bindungen geschaffen werden können, hat kürzlich Wacek für das Phenollignin bewiesen [8]. Dass dagegen, wie Lautsch [9] behauptet, schon bei der Auslösung des Lignins mit alkoholischer Salzsäure eine Kondensation des Alkohols mit dem Lignin stattfindet, ist meines Wissens bisher nicht bewiesen. Eher lag natürlich eine Kondensation von Lignineinheiten untereinander im Bereich der Möglichkeit und Holmberg [10] und Freudenberg [11] nahmen deshalb an, dass das aus dem Holz mit alkoholischer Salzsäure nicht auslösbare Lignin durch die Säurewirkung kondensiert ist.

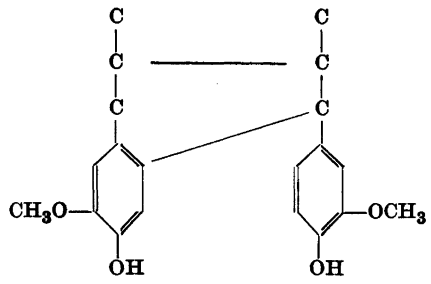
Zur Klärung der Herkunft der Metahemipinsäure wurden nun die früher von Freudenberg durchgeführten Oxydationsversuche von isoliertem Methyllignin unter dem Gesichtspunkt der Isolierung dieser Säure wiederholt. Da zur Zeit die Abbauversuche an methyliertem Holz und an anderen in Frage kommenden Ligninpräparaten noch nicht abgeschlossen sind, sollen vorläufig nur die wesentlichen Ergebnisse über den Abbau des Salzsäurelignins mitgeteilt werden. Das untersuchte Salzsäurelignin wurde dargestellt durch 36-stündige Behandlung von entharztem Fichtenholz bei 0° mit einer Salzsäure, welche ebenfalls bei 0° mit Chlorwasserstoff gesättigt war. Das so erhaltene Lignin enthält 15,4 % Methoxyl und ist rasch und praktisch vollständig sulfitierbar. Wie hier bereits vorweggenommen werden kann, ist es das erste Säurelignin, welches bei der Alkoholyse wie das Lignin des Holzes die von Hibbert und Mitarb. [12] aufgefundenen Guajacyl-propanderivate (hauptsächlich α-Äthoxypropiovanillon) liefert. Es dürfte daher dem nativen Lignin zumindest recht nahe stehen. Wird dieses Lignin mit Alkali und Dimethylsulfat methyliert und dann mit Kaliumpermanganat abgebaut, so erhält man neben 2,9 % Veratrumsäure auch 1,9 % Isohemipinsäure, 1,3 % Metahemipinsäure und etwas Bernsteinsäure.

Da sich diese Versuchsergebnisse von den früheren Freudenberg's wesentlich unterscheiden, fragt man sich, worauf dieser Unterschied beruhen kann. Verständlich ist, dass bei den hier beschriebenen Versuchen eine bessere Gesamtausbeute an Oxydationsprodukten erzielt wurde, da bei ihnen die Oxydation etwas früher abgebrochen wurde und weil ausserdem die Oxydationsprodukte durch erschöpfende Ätherextraktion vollständiger erfasst wurden,

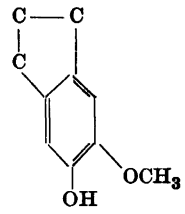
als es früher durch mehrmaliges Ausschütteln möglich war. Im letzteren Fall wird von der in Wasser recht gut, in Äther dagegen ziemlich schwer löslichen Metahemipinsäure nur ein Teil erfasst. Da diese ausserdem in Gemischen nicht besonders krystallisationsfreudig ist, könnte sie sehr wohl bei der Reinigung von I und II verloren gegangen sein. Für die Aufarbeitung der Oxydationsprodukte hat es sich als vorteilhaft erwiesen, die entstandenen Säuren zunächst mit Diazomethan zu verestern, die Ester zu fraktionieren und nach anschliessender Verseifung die Säuren zu isolieren. Iso- und Metahemipinsäure lassen sich auf Grund ihrer verschiedenen Wasserlöslichkeit oder über ihre Bariumsalze trennen. Das Bariumsalz von II ist in kaltem Wasser leicht, das von III ziemlich schwer löslich. Die Ursache für das Auftreten von Isohemipinsäure ohne vorhergehende Kalikochung des Lignins wurde zunächst darin gesucht, dass sich mein Lignin anders verhält als das von Freudenberg oxydierte Salzsäure-Phosphorsäure-Lignin. Da jedoch auch ein solches Präparat ohne Kalikochung Isohemipinsäure liefert, kann dieser Widerspruch vorerst nicht erklärt werden.

Welche Schlüsse ergeben sich aus diesen Versuchen für die Konstitution des Lignins? Solange nach einer früheren Mitteilung von Freudenberg und Walch [13] der Phenolgruppengehalt des Salzsäurelignins zu 1,8 % angenommen werden konnte, d. h. auf etwa 10 Einheiten eine phenolische Gruppe kam, war es einigermassen verständlich, dass ohne eine Aufsprengung der Ätherbrücken durch die Kalikochung bei der Oxydation nur so wenig Veratrumsäure erhalten wurde. Nachdem Freudenberg heute auf Grund verschiedener methodischer Verbesserungen bei der Bestimmung der Phenolgruppen annimmt, dass schon jede vierte Einheit eine freie Phenolgruppe besitzt [14], muss man selbst unter Berücksichtigung einer teilweisen Zerstörung der Oxydationsprodukte eine viel höhere Ausbeute an aromatischen Säuren erwarten. Diese Forderung ist auch jetzt noch nicht erfüllt, aber das Missverhältnis der isolierten Abbauprodukte zu den theoretisch erwarteten ist nicht mehr so krass.

Mit der Isolierung von Metahemipinsäure durch Abbau des methylierten Salzsäurelignins ist der Beweis erbracht, dass diese Säure nicht aus einer Konfiguration stammen kann, die erst sekundär durch Kondensationen des Lösungsmittels mit dem Lignin entstanden ist, wie man hätte einwenden können, wenn sie nur aus Alkohollignin entstanden wäre. Wie diese Metahemipinsäure liefernde Konfiguration im Einzelnen aussieht, kann vorerst noch nicht gesagt werden. Das nächstliegende ist natürlich eine Annahme von Lignaneinheiten (IV), denen schon früher Vanzetti eine entscheidende Rolle beim Aufbau des Lignins zugeschrieben hat [15]. Ebenso muss man in Betracht ziehen, dass die Metahemipinsäure einer Indankonfiguration (V)

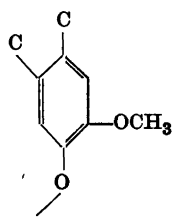


IV



V

entstammen könnte, welche durch Ringschluss aus einem Guajacylpropanderivat entstanden sein kann. Nachdem nun erstmalig das Vorkommen einer Metahemipinsäurekonfiguration im Lignin nachgewiesen ist, erhebt sich sofort die Frage, ob diese Gruppierung nur mit freier Phenolgruppe vorkommt oder ob sie gemäss der Formel VI auch veräthert genau wie die Gruppierungen VII und VIII am Gesamtaufbau des Ligninmoleküls teilnimmt. Versuche zur Klärung dieser Frage sind im Gang.



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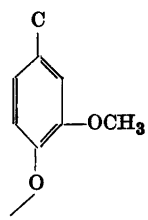

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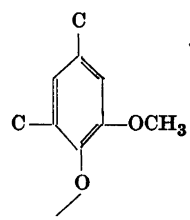
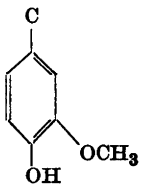


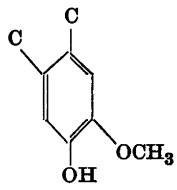
VIII

Die Isolierung von Metahemipinsäure verdient auch noch in andrer Hinsicht Interesse. Freudenberg hat in der letzten Zeit verschiedentlich darauf hingewiesen [14, 16], dass das aus Coniferylalkohol durch Dehydrierung in Gegenwart von Champignondehydrase entstehende Produkt grosse Ähnlichkeit mit Lignin besitzt. So entstehen daraus bei der Oxydation nach geeigneter Vorbehandlung Dehydro-diveratrumsäure, Veratrumsäure und Isohemipinsäure. Wenn nun aus diesem Dehydrierungsprodukt auch noch Metahemipinsäure gefunden würde, wäre seine Verwandtschaft, vielleicht sogar seine Identität mit dem Lignin noch offensichtlicher. Es sei in diesem Zusammenhang daran erinnert, dass ich bereits früher in einem auf enzymatischem Weg dargestellten Dehydrierungsprodukt des 5-Allyl-pyrogallol-1,3-dimethyläthers eine entsprechende Kondensation in der Nachbarschaft zur Seitenkette durch den Abbau zur entsprechenden Phthalsäure nachweisen konnte [17].

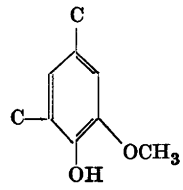
Von nicht geringerer Bedeutung als die Isolierung der Metahemipinsäure ist die der Isohemipinsäure. Bisher hat man immer annehmen müssen, dass die Phenolhydroxyle des Salzsäurelignins nur in der Gruppierung IX vorkommen. Nach der hier beschriebenen Isolierung von Iso- und Metahemipinsäure ist jedoch erwiesen, dass sich die Phenolhydroxyle auch noch auf die Gruppierungen X und XI verteilen. Da von den Gruppierungen IX—XI nur IX, welche wohl etwas häufiger vorkommen dürfte, wirklich den Charakter einer Endgruppe besitzt, möchte ich diese Gruppierungen nicht als Endgruppen sondern zweckmässiger als »phenolische Gruppen» bezeichnen. Durch den Nachweis von XI braucht man nicht mehr anzunehmen, dass die Verätherung von Guajacylpropan-einheiten die erste Stufe bei der Ligninbildung



IX



X



XI

ist, auf welche später die Kondensation in der 5-Stellung unter Bildung von Chroman- oder Cumaranringen folgt [18]. Unter der Voraussetzung, dass solche Ringe nicht bei der Isolierung des Salzsäurelignins aufgespalten worden sind, kann die erste Stufe der Ligninbildung ebensogut in einer Kondensation von Guajacylpropan-einheiten in der 5-Stellung bestehen. Das Phenolhydroxyl kann dann durch Bildung cyclischer Äther verschwinden, aber es muss nicht. Eine solche Reaktion stellte ich z. B. bei der enzymatischen Dehydrierung des Guajacyl-acetons fest [19].

Die Auffindung der Isohemipinsäure ohne vorhergehende Kalikochung erlaubt nun auch Rückschlüsse auf die Reaktionen des Lignins im allgemeinen. Durch zahlreiche Modellversuche [18, 20, 21] war die Möglichkeit erwiesen, dass Verbindungen wie Bisulfit, Thioglykolsäure oder alkoholische Salzsäure mit dem Lignin sowohl an den alkoholischen Hydroxylgruppen, besonders solchen in α-Stellung zum Phenylrest, wie auch mit deren offenen oder ringförmigen Phenoläthern reagieren können. Die zuletzt genannte Reaktion, die Aufspaltung cyclischer Phenoläthergruppierungen wurde von Freudenberg in Betracht gezogen, weil er die Bildung von Isohemipinsäure aus methylierter Ligninsulfosäure, methylierter Ligninthioglykolsäure und methyliertem Alkohol- oder Essigsäurelignin nur erklären konnte, wenn bei der Darstellung dieser Präparate durch eine Ätherspaltung Phenolgruppen der Anordnung

XI freigelegt werden. Da jedoch nach den vorliegenden Versuchen das Lignin — zumindest das Salzsäurelignin — diese Anordnung bereits enthält, ist es meines Erachtens nicht mehr erforderlich, unter den Ligninreaktionen eine Aufspaltung von cyclischen Äthern in nennenswertem Umfang zu diskutieren. Für den speziellen Fall einer bei 70° stattfindenden Sulfitierung des Lignins hatten Freudenberg, Lautsch und Piazolo [22] auch schon die Möglichkeit erörtert, dass diese Reaktion nicht in einer Aufspaltung von Benzofuranringen besteht, ohne damit allerdings von der allgemeineren Annahme abzugehen, in der Aufspaltung cyclischer Phenoläther eine der wesentlichen Ligninreaktionen zu sehen.

EXPERIMENTELLER TEIL

Zu einer am Wasserbad erwärmten Suspension von 32 g mit Alkali-Dimethylsulfat kalt methyliertem Salzsäurelignin in 900 ml Wasser gibt man unter lebhaftem Rühren fein gepulvertes Kaliumpermanganat in Portionen von 9 g. Jedesmal nach Verschwinden der Permanganatfarbe stellt man das pH der Lösung durch Zusatz von verd. Schwefelsäure auf 7 ein. Bei einer Oxydationstemperatur von etwa 90° werden 23 Portionen Permanganat gebraucht, bis die Farbe 5 Min. beständig ist. Drei solche Ansätze werden gemeinsam aufgearbeitet. Der Braunstein wird abgetrennt und mit heissem Wasser gewaschen. Filtrat und Waschwasser werden nach dem Einengen mit verd. Schwefelsäure angesäuert, wobei eine flockige, sich rasch zusammenfallende Fällung entsteht.

Diese Fällung wird abgetrennt und solange mit ätherischer Diazomethanlösung behandelt, bis keine Reaktion mehr zu erkennen ist. Von den hierbei entstandenen ätherlöslichen Estern lassen sich bei einer Badtemp. bis 265° insgesamt 3 g im Vakuum (0,01 Torr) abdestillieren.

Das Filtrat von dieser Fällung wird mit Äther erschöpfend extrahiert. Nach Einengen des Extrakts krystallisieren 0,98 g reine Isohemipinsäure aus, welche abgetrennt wird. Der nicht krystallisierende Anteil wird mit überschüssiger Diazomethanlösung behandelt. Die entstandenen Ester werden im Vakuum destilliert, wobei man neben einem Vorlauf von 0,93 g (bis 120°/12 Torr) noch 9,3 g abdestillieren kann (bis 270° Badtemp./0,01 Torr).

Der Vorlauf besteht im wesentlichen aus Bernsteinsäureester.

Die Ester aus Fällung und Extrakt werden gemeinsam destilliert und in folgende Fraktionen zerlegt:

1.	—128°/0,1 Torr	1,4 g	zum Teil krystallisiert
2.	128°—158°/0,1 »	7,13 g	» » »
3.	158°—190°/0,1 »	3,25 g	sehr zähes Öl

Fraktion 1 wird mit methylalkoholischer Kalilauge verseift. Nach Entfernung des Methanols fallen beim Ansäuern 0,75 g Veratrumsäure aus. Die in Lösung bleibende Substanz ist zum Teil in Benzol löslich, zum Teil darin unlöslich. Sie wird noch untersucht.

Fraktion 2 wird ebenfalls mit methylalkoholischer Kalilauge verseift. Nach Entfernung des Methanols fallen beim Ansäuern 2,83 g eines Gemisches aus, welches in 2,01 g benzollösliche Veratrumsäure und 0,82 g benzolunlösliche Isohemipinsäure getrennt werden. Das Filtrat dieser Fällung wird mit Äther extrahiert. Aus dem Extrakt krystalli-

sieren 0,47 g reine Metahemipinsäure aus, welche bei 190—193° u. Zers. schmilzt und deren Mischschmp. mit synthetisch dargestellter Säure [23] keine Depression zeigt. Der Dimethylester schmilzt bei 88°. Das Filtrat von der Metahemipinsäure wird nach Verjagen des Äthers mit warmem Benzol behandelt, wobei ein kristallisierender, aber noch nicht aufgeklärter Anteil ausgelöst wird. Werden die nun verbleibenden 2,75 g benzolunlöslicher Substanz mit 10 ccm absol. Äther behandelt, so erfolgt eine erneute Krystallisation von 0,75 g, welche neben etwas Isohemipinsäure 0,67 g Metahemipinsäure enthält. Aus dem Filtrat dieser Krystallisation lassen sich über das Bariumsalz noch weitere 0,1 g Metahemipinsäure abscheiden.

Aus der restlichen Substanz der Fraktion 2, sowie aus der verseiften Fraktion 3 wurden zwei krystallisierte Bariumsalze isoliert, welche noch untersucht werden.

ZUSAMMENFASSUNG

Beim Abbau von methyliertem Salzsäurelignin mit Kaliumpermanganat wurden im Gegensatz zu den Litteraturangaben ausser Veratrumsäure auch Isohemipinsäure und Metahemipinsäure aufgefunden. Dadurch ist bewiesen:

1. dass im isolierten Lignin als Baugruppen neben unkondensierten und in der 5-Stellung kondensierten Guajacylpropaneinheiten auch noch solche vorkommen, die in der 6-Stellung kondensiert sind,

2. dass sich die Phenolgruppen des Salzsäurelignins auf 3 verschiedene Gruppierungen verteilen,

3. dass bei der Reaktion des Lignins mit Bisulfit, Thioglykolsäure und alkoholischer Salzsäure die Aufspaltung cyclischer Äthergruppierungen keine wesentliche Rolle spielen kann.

Frl. Birgitta Abrahamson habe ich für die fleissige und geschickte Unterstützung bei der Durchführung der Versuche zu danken.

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Eingegangen am 13. Februar 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 597–600

X-Ray Data and Configuration of some Alcohols Derived from *Cyclo*hexane

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A great deal of work has been done in later years in order to elucidate the structure of *cyclo*hexane and its derivatives, especially by Hassel and co-workers [1]. Both electron diffraction and X-ray methods have been employed. In all the compounds hitherto examined it is found that the *cyclo*hexane ring has the staggered "chair" form, with a C—C distance of 1.54 A. and bond angles near the tetrahedral angle. In some cases deviation from tetrahedral angles have been observed [2, 3], presumeably due mainly to repulsive forces between atoms in the positions 1ε 3ε. However, no complete X-ray structure analysis leading to accurate values of the individual bond lengths and bond angles for this basic carbon skeleton has yet been carried out. Halogen derivatives are not quite suitable for this purpose, as the monosubstituted *cyclo*hexanes are liquids, whereas in compounds with two or more halogen atoms it would probably not be possible to obtain a very accurate determination of the carbon ring. Thus the alcohols of *cyclo*hexane would appear to provide the best point of attack. X-ray data on some of these are given by Patterson and White [4, 5], and Hassel and co-workers [6, 7], and in one case, α-phloroglucitol dihydrate, the arrangement and configuration of the molecules in the crystal is worked out [7]. The molecules are linked to one another by hydrogen bonds, a feature which we must expect to find in all these compounds.

The results of some of these earlier investigations are given in the table below. We have undertaken a preliminary X-ray investigation of some more alcohols of *cyclo*hexane, with the aim mainly to find a suitable compound for a detailed structure analysis. Space group determinations are, however, also of interest in connection with the possibility of resolving some of these substances into optical antipodes.

Oscillation and Weissenberg photographs were taken, using Fe Kα radiation ($\lambda = 1.934$ A). The cell dimensions obtained are accurate to about 1 %. The densities were determined by the flotation method in mixtures of carbon tetrachloride and benzene.

The following compounds were investigated:

Cis-Resorcitol, m. p. 87°. The crystals have the shape of monoclinic plates, with (010) dominating, and the a and c axes lying along the edges, joining at an angle of 111°. The extinction direction in polarised light lies about 16° from the a axis in the acute angle. The cell dimensions are: $a = 6.74$ A, $b = 19.6$ A, $c = 5.37$ A and $\beta = 111°$. The density is 1.14_4 g/cm^3, and there are four molecules (calc. 3.98) in the unit cell.

The only systematic absences occur in the $h0l$-reflexions for odd values of $h + l$, and in the $0k0$-reflexions for odd values of k. The space group is therefore $P2_1/n$.

101 is by far the strongest reflexion, and most atoms are therefore likely to be situated near this plane, a conclusion which is also justified by space considerations.

Trans-(dl)-Resorcitol, m. p. 118°. The crystals are orthorhombic, elongated along the b axis. (100) is a perfect cleavage plane. The following cell dimensions were derived: $a = 15.45$ A, $b = 8.41$ A and $c = 9.96$ A. The density was found to be 1.18_4 g/cm^3, and there are eight (calc. 7.97) molecules in the unit cell.

Absent spectra (from Weissenberg photographs) uniquely determine the space group to be *Pbca*.

We had also some *trans-d*-resorcitol at our disposal, but did not succeed in growing single crystals. Only some fibre-like crystalline formations were obtained, which had a repeat unit of 6.35 A along the fibre axis.

Cis-Chinitol, m. p. 106°. The crystals available were needle-shaped, apparently triclinic, but not true single crystals. Only the length of the axis along the needle was determined. It was found to be 6.44 A.

β-Phloroglucitol, m. p. 145°. The crystals are orthorhombic, elongated along the b axis, and bounded by faces (101) and ($\bar{1}01$). The cell dimensions are: $a = 9.00$ A, $b = 10.68$ A and $c = 6.97$ A. The density is 1.30 g/cm^3, and the unit cell contains four molecules (calc. 3.98).

Reflexions $h00$, $0k0$ and $00l$ are absent for odd values of h, k and l respectively, and as no other systematic absences occur, the space group is $P2_12_12_1$.

In Table 1 X-ray data are given on all *cyclo*hexane-diols (except for the optically active forms), as well as on the two 1,3,5-*cyclo*hexane-triols. The molecular structures are also included, using the nomenclature introduced by Hassel, where $\varkappa$ stands for a nearly horizontal, ε for a vertical, orientation of the C—O bond with respect to the rough plane of the carbon ring.

Table 1.

Compound	Structure	Space group	a	b	c	Number of molecules
α-*Cyclo*hexane-diol-(1,2) m. p. 99°	$1\varkappa 2\varepsilon$	*Pcab*	7.62	8.55	19.57	8
γ-*Cyclo*hexane-diol-(1,2) m. p. 104°	$1\varkappa 2\varkappa$	$I2/a$	19.13	9.92	7.23	8
Cis-Resorcitol	$1\varkappa 3\varkappa$	$P2_1/n$	6.74	19.6	5.37	4
Trans-Resorcitol	$1\varkappa 3\varepsilon$	*Pbca*	15.45	8.41	9.96	8
Cis-Chinitol	$1\varkappa 4\varepsilon$	—	—	—	6.44	—
Trans-Chinitol	$1\varkappa 4\varkappa$	$P2_1/n$	6.31	21.23	7.26	6
α-Phloroglucitol	$1\varkappa 3\varkappa 5\varkappa$	$R3c$	r = 8.71, α = 56°			2
β-Phloroglucitol	$1\varkappa 3\varepsilon 5\varkappa$	$P2_12_12_1$	9.00	10.68	6.97	4

There are only two possible isomers (optical antipodes not included) of the 1,2, 1,3 and 1,4-diols, and of the 1,3,5-triols. In each case it is therefore essential to know the configuration of one isomer only.

It will be seen that conclusions regarding the symmetry of the molecules can be drawn from the X-ray data above only for two of the compounds, namely *trans*-chinitol and α-phloroglucitol. In the others all molecules occupy general positions in the unit cell. In the case of *trans*-chinitol, two of the six molecules in the cell must possess a centre of symmetry, as pointed out by White [5], and this substance therefore has the configuration $\varkappa\varkappa$ or $\varepsilon\varepsilon$. For the molecules of α-phloroglucitol trigonal symmetry is required, showing that it is a $\varkappa\varkappa\varkappa \rightleftharpoons \varepsilon\varepsilon\varepsilon$ compound. A further analysis proved that the form present in the crystal is the $\varkappa\varkappa\varkappa$ form [7]. The configurations of the 1,2 and 1,3-diols can not be deduced from these preliminary X-ray data. However, optical resolution has been obtained for γ-*cyclo*hexane-diol-(1,2) and for *trans*-resorcitol[8], thus proving the first one to be $\varkappa\varkappa \rightleftharpoons \varepsilon\varepsilon$, whereas the latter is $\varkappa\varepsilon \rightleftharpoons \varepsilon\varkappa$. For the *cyclo*hexane-diols-(1,2) this conclusion is confirmed also by an electron diffraction investigation [9].

The space groups are all consistent with the structures given in the table. In the crystals of *trans*-resorcitol and γ-*cyclo*hexane-diol 1,2 there is an equal number of *d*- and *l*-molecules, in agreement with the possibility of resolving these compounds. The molecules of *cis*-resorcitol, the 1,4-diols and the 1,3,5-triols all have a plane of symmetry, and can therefore not be resolved. Any space group would be permitted for these compounds. The last substance, α-*cyclo*hexane-diol-(1,2), is interesting, as the crystals contain both *d*- and *l*-molecules, but no resolution has nevertheless been obtained. This is due to the transposition of the *cyclo*hexane ring, which transfers $1\ \varkappa\ 2\ \varepsilon$ into its optical antipode $1\ \varepsilon\ 2\ \varkappa$, thus making it impossible to resolve the compound.

As mentioned before, we were interested in finding a substance suited for a detailed analysis of the carbon skeleton of *cyclo*hexane. The best one would appear to be *cis*-resorcitol, its main advantages being centres of symmetry and two relatively short axes.

SUMMARY

The space groups of the resorcitols and of β-phloroglucitol have been determined, and all X-ray data given in the literature on diols and triols of *cyclo*hexane are collected. It is shown that the chemical and physical information available at this stage suffices to deduce the configurations of these compounds, and that the space groups found are all compatible with the assigned structures.

We are indebted to Dr. W. Rigby of Birkbeck College, London, for placing samples of the resorcitols at our disposal, and to mag. scient. K. Lunde of this laboratory for preparing the other substances. One of us (S. F.) acknowledges a grant from *Norges Teknisk Naturvitenskapelige Forskningsråd.*

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Received March 11, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 601–609

Isodynamic Pyrophosphatases in Rat Liver

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Since the investigations on the liver pyrophosphatases of pig and man by Bamann and Gall [1] in 1937 the existence of three isodynamic pyrophosphatases in human and animal tissues seems to have been generally accepted. In experiments on the pH-activity correlation in rat liver homogenates, however, I repeatedly found four peaks. A closer investigation was therefore carried out. The experiments which are presented in this report demonstrate the existence of a fourth pyrophosphatase in rat liver.

To begin with the assay was performed essentially according to the method of Gall [6] where the orthophosphate produced by the enzyme is determined directly on an aliquot of the deproteinized digestion mixture by means of the ceruleomolybdate reaction of Fiske and Subbarow (see *e. g.* Norberg [12]). Later on this technique was modified in many respects, and for the most acid enzymes the final method involves precipitation of the liberated orthophosphate.

As source of enzyme a homogenate (see *e. g.* Potter [13]) of rat liver in glass-redistilled water was used. It was always employed as soon as possible after the death of the animal.

EXPERIMENTAL

First method of assay

The substrate was prepared from 0.1 *M* sodium pyrophosphate, adjusted to the proper pH, by dilution 1 : 100 with 0.05 or 0.1 *M* buffer of the same pH. $MgSO_4$ at a final concentration of 0.002 *M* was also included. Five ml of the substrate were warmed to 37° C and 0.20 ml of the homogenate added. The digestion was allowed to proceed until 10 to 50 micrograms of phosphorus had been liberated, usually 15 to 30 minutes. Then the process was stopped by addition of 1.8 ml normal trichloracetic acid. 3.5 ml of the proteinfree filtrate were used for phosphate estimation. As pyrophosphate interferes with the Fiske-Subbarow reaction the micrograms of phosphorus were read from a calibration curve prepared with similar amounts of pyrophosphate as were present in the

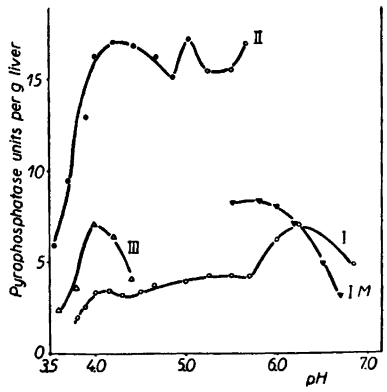

Figure 1. Activity-pH correlation for rat liver pyrophosphatases.
Curve I: with phthalate buffer.
» *I M: same homogenate with maleic acid buffer.*
» *II: with acetate buffer.*
» *III:* » » » *and second method of assay.*

samples. Duplicates or triplicates were run with each sample, and also duplicate blanks (homogenate added to substrate + trichloracetic acid at the moment of stopping the action of the enzyme). *The enzyme units* are the number of micromols of phosphorus liberated per minute under the experimental conditions used. In this paper all values are given in units per g of fresh liver.

The buffers used were: 0.05 *M* phthalate for pH 3.8—6.8, 0.1 *M* acetate for pH 3.5 to 6, 0.1 *M* maleic acid (Smits [19]) for pH 5.8—6.7, 0.1 *M* collidine (Gomori [7]) for pH 7—8, 0.1 *M* ammonium chloride-ammonia for pH 7.6—10, and 0.0028 *M* veronal-acetate (Michaelis [9]) for pH 4.4—9.2. All pH-values were controlled by the glass electrode.

The pH-activity curve. Figs. 1 and 2 show some of these experiments. In all curves there are indications of optima at pH 4—4.2, around pH 5, at pH 5.8—7, and on the alkaline side at pH 8—8.4. However due to the influence of the different ions in the buffers the peaks are more or less pronounced, and the pH optima may also be displaced. Thus phthalate clearly is more toxic to the pH 4 and 5 enzymes than is acetate. Also veronal seems to inhibit the pH 6 pyrophosphatase very strongly at the same time displacing its optimum towards pH 7. Maleic acid buffer on the other hand tends to displace the optimum of this enzyme to pH 5.8.

Table 1. Units of alkaline pyrophosphatase per g rat liver calculated from experiments with different digestion periods.

Digestion period in minutes	Amount of fresh liver used		
	22 mg	33 mg	66 mg
2	4.4	3.1	3.0
4	2.7	1.8	1.8
6	1.7	1.6	1.4
8	1.3	1.4	1.3

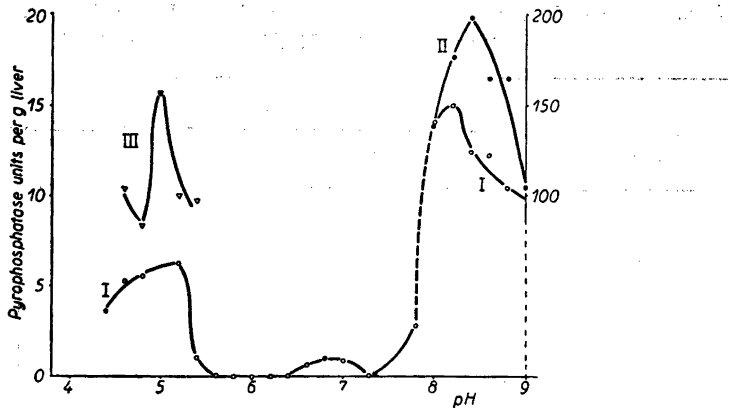

Figure 2. Activity-pH correlation for rat liver pyrophosphatases.
Curve I: with diethylbarbituric acid buffer. Five rats were used in order to get the same time from killing to assay in all points. Note that the alkaline pyrophosphatase is about ten times as active (right hand ordinate) as the pH 5 pyrophosphatase.
Curve II: with ammonium chloride buffer.
» III: » acetate buffer and second method of assay.

In order to study the other optimum conditions for these enzymes the three acid ones were investigated with acetate buffers at pH 4.0, 5.0, and 5.8, and the alkaline pyrophosphatase at pH 8.4 in ammonium chloride-ammonia buffer.

Time of digestion was next studied with the alkaline pyrophosphatase. It turned out that the enzymatic activity undergoes a rapid decay with time so that there is very poor proportionality between amount of enzyme and quantity of phosphorus liberated. This is evident from Table 1.

It was decided to try a method of assay with the shortest possible time of digestion. For practical reasons this time was fixed at 120 seconds.

Second method of assay

In order to enable an exact timing of the reaction the substrate was divided into two reagents, 1) the activating solution, and 2) the substrate proper. For the alkaline enzyme the *activating reagent* is a 0.1 *M* ammonium chloride buffer containing magnesium ions in the concentration stated as optimum, initially 0.002 *M*, later 0.2 *M* (see below). The *substrate* is a pyrophosphate solution adjusted to the same pH as that of the activating reagent and 1.7 times the intended final concentration of pyrophosphate.

For every sample one tube with 2.0 ml of the activating reagent and one with 3.0 ml of the substrate are placed in the water thermostat. To the activating reagent tubes 0.20 ml of the samples are added. The enzymatic process is started by pouring the substrate into the tube with the sample. When a number of reactions are started (5 seconds interval) the empty substrate tubes get 1.8 ml normal trichloracetic acid each. The digestion is stopped by pouring the trichloracetic acid in the digests and mixing back and forth between the tubes. With this technique 12 pairs of tubes can be handled at a time.

Table 2. Change in liver pyrophosphatase units with varying substrate concentration. Activation a) 4 mM Mg, b) 200 mM Mg.

$Na_4P_2O_7$ concentration m*M*	Assay at pH					
	4.0	5.0	5.8		8.4	
			a	b	a	b
0.25			10.5	73	16	131
1			15.5	75	5.6	148
2	7.2— 8.4					137
3		20.6		57		
4	11.4—13		21.7		1.1	
6		22.5		64		
8	7.5— 7.8		20.3			
9		25		52		
12		21.2				
16	2— 5	9	16.2			

Blanks are run in the same way pouring the substrate into the sample only after the addition of trichloracetic acid.

Due to the small volume adhering to the walls of the substrate tube the substrate concentration in the digestion mixture will be a little less than calculated. This does not seem to be of any significance, however.

Substrate concentration. This had to be investigated since fairly different concentrations have been used, from 1 m*M* by Gall [6] and others up to 20 m*M* by Desruisseaux [4], and because increasing pyrophosphate concentration may inhibit both pyrophosphatase and other enzymes, as shown inter alia by Schmidt and Thanhauser [18] and by Naganna and Menon [11].

Studies with the first method of assay had indicated different substrate optima at the four pH-optima. This supporting evidence for the existence of four pyrophosphatases was therefore followed up. The results differ somewhat in different experiments, but on the whole the enzymes of pH 8.4 and 5.8 have their optimum at about 1 m*M* pyrophosphate, whereas the pH 5 enzyme shows optimum activity at 10 m*M* pyrophosphate, and the pH 4 enzyme is most active at 1—4 m*M* P_2O_7. Table 2.

After these experiments the substrate reagents were prepared at such concentrations that the final pyrophosphate levels were 1 m*M* at pH 5.8 and 8.4, 10 m*M* at pH 5 and 4 m*M* at pH 4.

Activation and inhibition. The strong activation of alkaline pyrophosphatase by magnesium is well known (see *e. g.* Bamann and Gall [1]) whereas the pH 4 pyrophosphatase is not influenced by this ion (Bamann and Gall) or even inhibited (Roche, Thoai and Durand [15], Chevillard [2]).

With magnesium fairly weak concentrations are generally used, 1—10 m*M*, although Chevillard [2] used as much as 100 m*M* without stating, however, if this is an experimentally found optimum condition. With erythrocyte pyrophosphatase Naganna and Menon [11] find the optimum range to be 20—50 m*M* magnesium.

Table 3. Activation of pyrophosphatases by magnesium ion at 1 mM substrate concentration.

Concentration of $MgSO_4$, m*M*	Activity at pH 5.8	Activation in %	Activity at pH 8.4	Activation in %
0	29		3.7	
2	31	7	4.7	27
20	39	35	12.7	240
50	81	180	15.5	320
100	99.5	240	65.0	1 660
200	112	290	151	4 000
400	98	240	56.5	1 430
800			18	390

In our experiments, however, both the alkaline and the pH 5.8 pyropshophatase required 0.2 *M* $MgSO_4$ in the activating reagent for full activation. Some experiments are reported in Table 3.

The pyrophosphatase activity at pH 4 is not at all or only very slightly influenced by magnesium ions, the highest observed activation being 30 % at 20 m*M* $MgSO_4$. The pH 5 pyrophosphatase, on the other hand, has shown activation up to 345 %, so a real effect can hardly be doubted. In other experiments, however, magnesium had no effect. The behaviour of this enzyme is thus very irregular. Examples are given in Table 4.

Combination of 10^{-3} *M* Mg and 10^{-4} Zn, shown by Roche and Thoai [16] to activate intestinal pyrophosphatase, was tried at pH 5. The response to this combination was also very irregular, varying between no influence and activation by 150 to 250 %.

In this connection some other factors were investigated. Potassium cyanide which at higher concentrations inhibits certain phosphomonoesterases (Cloetens [3]) and erythrocyte pyrophosphatase activates the pH 5 liver pyrophosphatase slightly from 0.02 to 2 m*M* . 2 m*M* phenanthroline has the same effect. This substance according to Dumazert, Lévy and Marszak [5] also increases the activity of the alkaline pyrophosphatase from fntestine. Sodium fluoride is a potent inhibitor for the activity at pH 5. At 0.02 m*M* luoride, only about fifty per cent of the activity is left.

Table 4. Influence of magnesium on pyrophosphatase activity at pH 5.

Concentration of $MgSO_4$, m*M*	Assay with second method	Assay with third method		Activation in b) in %
		a	b	
0	3.19	4.05	1.59	
2	3.19	4.01	2.31	45
20	3.19		7.08	345

Table 5. Apparent stability of the rat liver pyrophosphatases.

Time from excision of liver to assay	At pH 4	At pH 5	At pH 5.8	At pH 8.4
5 minutes	7.3	25.2	75.8	134
10 »	3.55	10.8	67.5	—
15 »	5.8	7.2	68.1	129
20 »	4.0	4.1	66.2	122
25 »	3.12	7.4	61.7	111
30 »	4.3	6.4	61.1	121

As amino acids and some of their derivatives are activators for certain monophosphatases some such substances were tried with all four pyrophosphatases. In 0.5 m*M* concentration, however, alanine, betain hydrochloride, choline chloride, DL-alanylglycin, DL-leucylglycin, and DL-leucylglycylglycin showed no significant effect. The inhibitory effect of diethylbarbituric acid mentioned above was also tested with all four enzymes. It was found to be different for each enzyme. Thus at pH 4 a slight activation of about 20 % over acetate is observed whereas at pH 5 a moderate inhibition of the same order is found. At pH 5.8 the inhibition is very strong leaving but 10 % of the activity with acetate. The alkaline pyrophosphatase shows a moderate inhibition ranging from 15 to 45 % compared with ammonium chloride buffer. Inhibition of erythrocyte pyrophosphatase by veronal at pH 7.6 has been observed by Naganna [10].

Stability. The activity of the four pyrophosphatases was investigated at different times after the excision of the liver under the optimum conditions arrived at by means of the foregoing experiments. At pH 8.4 and 5.8 there is a small decrease in activity during the first 30 minutes which may reach 20 %. It is therefore advisable to perform the digestion within 15 minutes, when the activity seems to be between 90 and 100 % of the maximum measurable. The activities at pH 4 and 5 show much faster decay. Some of these results are presented in Table 5.

From the rapid change in activity at pH 4 and 5 it is clear that an adequate estimation of these enzymes is impossible. Attempts to stabilize the enzymes by the addition of $MgSO_4$ to the homogenate did not result in any improvement. Preparation of the homogenates in different buffers (pH 4.3, 5.0 and 6.1) gave even a somewhat lower assay than the homogenate in redistilled water. Obviously a new method of assay had to be found.

Third method of assay

During studies on another hydrolytic enzyme it was found convenient to precipitate the orthophosphate before applying the colour reaction. This method also proved useful for the pH 4—5 pyrophosphatases. With this modification no fall in activity either during the digestion period (up to 10 minutes) or with time after excision of the liver (within 30 minutes) could be detected. The rapid formation of an *inhibitor for the cerulemolybdate reaction* is therefore assumed.

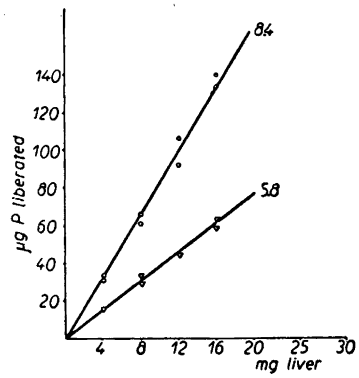

Figure 3. Proportionality for the alkaline and the pH 5.8 pyrophosphatases. Second method of assay.

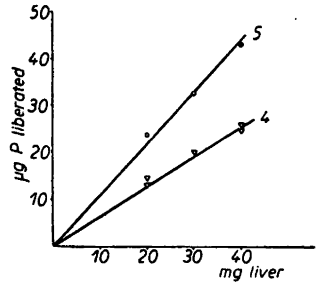

Figure 4. Proportionality for the acid pyrophosphatases assayed according to the third method.

The third method of assay, used at pH 5 and 4, consists of digesting the samples as in method 2 but for 10 minutes. The pyrophosphate concentration is kept at 1—4 m*M*. An aliquot of the trichloracetic acid filtrate is neutralised and the phosphate precipitated with 0.2 volumes of 10 % $CaCl_2$ containing sufficient $Ca(OH)_2$ to bring the solution to pH 9. This technique is adapted from the method for "true phosphate" given in the manual of Umbreit, Burris and Stauffer [22]. The precipitate is centrifuged down and washed twice with 2 % $CaCl_2$ containing $Ca(OH)_2$. Finally the precipitate is dissolved in a few ml of 0.1 N HCl and the Fiske-Subbarow reaction applied.

Proportionality. When analyzing a rat liver homogenate for pyrophosphatases at pH 8.4 and 5.8 with method 2 and at pH 4 and 5 with method 3 a reasonably good proportionality is obtained. This is evident from Figs. 3 and 4.

The standard deviations were calculated from duplicates. At pH 5.8 the standard deviation on the mean of duplicates is 3.5 %. At pH 8.4 the error is less. At pH 5 the calculated standard deviation for the mean value of duplicates is 3.9 %. The same applies for the more acid enzyme.

DISCUSSION

According to the results presented above there seems to be no doubt about the presence of four different isodynamic pyrophosphatases in rat liver. Two of them have their optima at distinctly acid pH values, about 4 and 5 respectively. One has an optimum pH between 5.8 and 7 depending on the buffer and may be referred to as the neutral pyrophosphatase. Finnaly there is the alkaline pyrophosphatase with optimum activity at pH of about 8. The acid pyrophosphatases differ in respect to optimum substrate concentration and the action of diethylbarbituric acid. They both are apparently unstable so that their activities may easily be overlooked. This is the case particularly

with the pH 5 enzyme. The pH 4 pyrophosphatase seems to be quite insensitive to magnesium ions whereas the pH 5 enzyme is sometimes considerably activated.

The neutral and alkaline pyrophosphatases are consistently and strongly activated by magnesium. They have similar substrate optima but the alkaline enzyme is only moderately inhibited by veronal whereas the neutral one is almost completely inhibited.

The pertinent data to the four pyrophosphatases are collected in the adjacent table:

Optimum pH	4	5	5.8—7	8—8.4
Substrate optimum	4	10	1	1 m*M*
Mg^{++} optimum	0	0—20	200	200 m*M*
Effect of diethylbarbituric acid	Weak stimulation	Slight inhibition	Strong inhibition	Moderate inhibition

In earlier investigations on the pyrophosphatases much longer digestion periods of 30 minutes to 24 hours have been used. The preliminary treatment of the enzyme may also seriously affect the results and even change the optimum pH as was found for the pyrophosphatases of basidiomycetes by Thoai [20]. Finally the natural occurrence or formation of inhibitors (Thoai [21]) makes comparison with the present results difficult.

Nevertheless the alkaline pyrophosphatase with the optimum pH at 7.4—9.4 is certainly the same enzyme or group of enzymes in all investigations. The acid pH 4 pyrophosphatase also has a fairly well characterized counterpart in most earlier investigations, where the optimum pH has been stated to be from 3.6 to 4.2. The interval from pH 5 to 7 is more difficult to judge. Bamann and Gall [1], Roche [14], Roche, Thoai and Marcelet [17], Desruisseaux [4], and others find an optimum around pH 6 (5.5—6.4). This enzyme is activated by magnesium. In leucocytes Chevillard [2] finds a plateau at pH 5 to 5.8, but on addition of magnesium ions the optimum is displaced to pH 6.3. Simultaneously an activation of several hundred percent takes place. It seems reasonable to assume that this enzyme belongs to one and the same group as the neutral pyrophosphatase of the present study.

In some investigations only two pyrophosphatases have been found, *e. g.* by Kroon, Neuman and Veerkamp [8]. In the past never more than three pyrophosphatases seem to have been observed, although Chevillard [2] in bone marrow found optima at pH 3.6, 4.4 and 8.7 plus a hint in the region where magnesium activates at an optimum of pH 5.2. This last enzyme should correspond to the »neutral» pyrophosphatase. Then the pH 4.4 enzyme of Chevillard would match the pH 5 pyrophosphatase of the present study.

The above considerations suggest that the pH 5 pyrophosphatase found in rat liver has previously been overlooked.

SUMMARY

The pyrophosphatase activity of rat liver homogenate has been studied with the necessary modifications in the methods of assay. Four distinct pH optima have been found, *viz.* at pH 4, 5, 5.8—7 ("neutral pyrophosphatase"), and about 8 (alkaline pyrophosphatase). The corresponding enzymes have been characterized by their differences reagarding substrate optima and activators or inhibitors. The pH 5 enzyme seems previously to have been overlooked.

This investigation has been supported by funds from the State Medical Research Council. I am indepted to E. Hiller and M. Wenner for technical assistance.

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Received April 14, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 610–612

Synthesis of N-(*p*-Chlorophenyl)-amidophosphoric Acid

SI-OH LI

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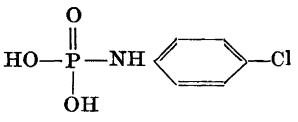
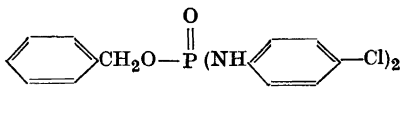
In 1933 Ichihara [1] suggested that N-(*p*-chlorophenyl)-amidophosphoric acid (I) might be used as a substrate for certain enzymatic investigations. The synthesis of this compound had previously been reported by Otto [2], and Ichihara and later Gomori [3] used his directions for the preparation of a compound which they believed to be I. However, Rorig [4] has recently shown that Otto's compound is in fact N-(*p*-chlorophenyl)-diamidophosphoric acid.

The present communication describes the synthesis of I by hydrogenolysis of dibenzyl-*p*-chloroanilinophosphonate, which was prepared by a procedure used by Atherton, Openshaw and Todd [5] for the synthesis of similar compounds. I could also be synthesized by Otto's method, using sodium hydroxide instead of ammonium hydroxide for the hydrolysis of the dichloride. These routes were found equally convenient for the preparation of I and have since been used for the synthesis of other similar compounds.

The hydrogenolysis of dibenzyl-*p*-chloroanilinophosphonate to I was carried out with Adams' palladium oxide catalyst. If Adams' platinum oxide catalyst was used, a different compound with the empirical formula $C_{19}H_{17}O_2PN_2Cl_2$(408), was obtained. The equivalent weight, found by acidimetric titration was 426. On the basis of this analytical evidence, the structure II is proposed for the compound.



(I)



(II)

EXPERIMENTAL

(Microanalyses by G. Cornali)

Preparation of dibenzyl *p*-chloroanilinophosphonate

Thirteen g of crude dibenzyl hydrogen phosphite (Atherton, Openshaw and Todd [6]) in 15 ml of dry carbon tetrachloride were added at − 10° (slowly, with stirring) to a solution of 6 g of *p*-chloroaniline in carbon tetrachloride. After the addition had been completed the reaction mixture was left at room temperature, when some heat was evolved and a precipitate separated out. Next day water was added and the carbon tetrachloride layer separated, washed with dilute hydrochloric acid and with a 10 % solution of sodium hydrogen carbonate and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue evaporated with alcohol After cooling in the ice box the residue crystallized in needles. The crude product was recrystallized from aqueous alcohol; yield 11.1 g = 63 %; m. p. 92—105°. A small amount of the sample was recrystallized for analysis. The pure crystals melted partially at 93° and totally at 110.5—111° (Hershberg apparatus).

$C_{20}H_{19}O_3PNCl$ (387.8)	Calc.	C	61.9	H	4.94	N	3.61	Cl	9.14	P	7.99
	Found	»	62.6	»	5.19	»	3.88	»	9.23	»	7.59

Hydrogenolysis of dibenzyl-*p*-chloroanilinophosphonate

Preparation of N-(p-chlorophenyl)-amidophosphoric acid. Dibenzyl-*p*-chloroanilinophosphonate (7.8 g) was hydrogenated in absolute alcohol (120 ml) with Adams' palladium oxide catalyst (0.180 g) under 1 atmosphere at 22°. A little more than the theoretical amount of hydrogen was taken up during 60 minutes. The reaction stopped spontaneously. The catalyst was then filtered off and washed with alcohol. After removal of the solvent in vacuum, the white residue was collected and washed repeatedly and successively with water and ether. Yield 2.5 g = 60 %. For purification the crude product was washed thoroughly with hot water and ether and dried at 80°/0.1 mm for 15 hours. The compound did not melt sharply but darkened at 260° and decomposed at 292°.

$C_6H_7O_3PNCl$ (207.6)	Calc.	C	34.7	H	3.40	N	6.75	Cl	17.1	P	14.9
	Found	»	34.8	»	4.23	»	6.71	»	16.1	»	14.8

Isolation of a compound m. p. 128°. Ten g of dibenzyl-*p*-chloroanilinophosphonate were hydrogenated in 150 ml of absolute alcohol with 0.20 g of Adams' platinum oxide catalyst under 1 atmosphere at 22°. 1395 ml of hydrogen were taken up during 4 hours. The catalyst was then filtered off and washed with alcohol. The solvent was removed in vacuum on a water bath of 37°, and the residue evaporated with 20 ml of alcohol. After cooling the residue crystallized. The crude product was recrystallized from aqueous alcohol in small white needles; m. p. 130—131°; yield 2.8 g = 27 %. After a second recrystallization the yield was 1.9 g = 18 %; m. p. 128°.

$C_{19}H_{17}O_2PN_2Cl_2$ (408)	Calc.	C	55.8	H	4.16	N	6.86	Cl	17.4	P	7.59
	Found	»	53.6	»	4.48	»	6.69	»	17.1	»	7.54

Preparation of N-(p-chlorophenyl)-amidophosphoric acid from *p*-chloroanilidophosphoryl-dichloride

One g of pure *p*-chloroanilidophosphoryl-dichloride, prepared according to Otto [2], was dissolved in 9 ml of 3 N sodium hydroxide, and N hydrochloric acid was added, whereby a white precipitate was formed. The addition was continued until nothing more separated. The precipitate was then filtered off and washed repeatedly and successively with water and ether. Yield 0.45 g = 53 %. The crude product was purified and dried as described above. When the pure compound was heated in a tube it behaved in the same way as the product obtained by hydrogenolysis. The identity of the two compounds was further ascertained by analysis.

$C_6H_7O_3PNCl$(207.6)	Calc.	C	34.7	H	3.40	N	6.75	Cl	17.1	P	14.9
	Found	»	35.1	»	3.56	»	6.53	»	16.2	»	14.5

SUMMARY

N-(*p*-Chlorophenyl)-amidophosphoric acid has been prepared by two independent methods. A compound melting at 128°, which is believed to be benzyl-di-*p*-chloroanilinophosphonate, was obtained by hydrogenolysis of dibenzyl-*p*-chloroanilinophosphonate with Adams' platinum oxide catalyst.

During the investigation the author has received funds from *The Danish Committee on Training of Foreign Scientists in Danish Laboratories*. The author is indebted to *Sadolin and Holmblad A/S, Copenhagen*, for permission to carry out this work in their research laboratory. I am sincerely grateful to Dr. H. Holter for his suggestion of the problem and to Dr. N. Clauson-Kaas for his kind interest and helpful advices.

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Received March 16, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 613 – 627

The Crystal Structures of Ag_2PbO_2 and $Ag_5Pb_2O_6$

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1. Ag_2PbO_2

Ag_2PbO_2 was first synthesized by Bullnheimer[1] in the following way: $AgNO_3$ is mixed with KOH and enough NH_3 is added to dissolve the precipitate. A small amount of this solution is mixed with about the same amount of a potassium plumbite solution of the same molarity as the silver solution. If a precipitate is formed, more NH_3 is added to the silver solution. When enough NH_3 is present to prevent precipitation, the two main solutions are mixed and warmed on a steambath. If the surplus of NH_3 is not too large, a crust of crystals of Ag_2PbO_2 is formed almost immediately. Ag_2PbO_2 forms brown prismatic crystals with metallic lustre. The density was determined by Bullnheimer to be 8.60.

X-ray investigation with the Laue method showed that the symmetry is monoclinic. Rotation photographs around [010], [100] and [11$\bar{1}$] and Weissenberg photographs of the reflexions $h0l$, $h1l$, $0kl$, $1kl$ and h, k, $h + k$ (Cu-K radiation) showed that the structure can be described in a body-centered cell with the dimensions: $a = 6.082 \pm 0.003$ Å, $b = 8.715 \pm 0.003$ Å, $c = 6.556 \pm 0.003$ Å, $\beta = 93.69°$. The dimensions are determined from powder photographs, taken with focussing cameras with Cr-K radiation (Table 4).

The following reflexions appeared:

hkl for $h + k + l = 2n$
$h0l$ for $h = 2n$ and $l = 2n$
$0kl$ for $k + l = 2n$
$hk0$ for $h + k = 2n$

If it is assumed that the structure is centro-symmetric, then the space group is C_{2h}^6—$I^2/_c$.

With a cell content of 4 Ag_2PbO_2 the density can be calculated to be 8.72, in satisfying agreement with Bullnheimer's experimental value of 8.60. Thus

there are 8 Ag, 4 Pb, and 8 O atoms in the unit cell. In C_{2h}^6 only the following arrangement of the metal atoms gave calculated intensities in agreement with those observed:

(000; 1/2 1/2 1/2) +
4 Ag_1 in 4 (*b*) : 0 1/2 0; 0 1/2 1/2.
4 Ag_2 in 4 (*d*) : 1/4 1/4 3/4; 3/4 1/4 3/4.
4 Pb in 4(*e*) : 0 y 1/4; 0 $\bar{y}$ 3/4. $y = 0.125$

The Weissenberg photographs showed strong absorption effects, especially those with [010] and [11$\bar{1}$] as rotation axis.

There is, however, one discrepancy among the *h*0*l* reflexions which seems difficult to explain, *viz.* that 604 is visible but not 60$\bar{4}$. If the small contribution from the oxygen atoms to the intensities is not taken into account, then it follows that $I(hkl) \sim I(hk\bar{l})$. As no deviation from this condition appeared among the 1*kl* reflexions, only those with *l* positive are listed in the table. However, the difference in intensity between 604 and 60$\bar{4}$ might be due to some deviation from the centrosymmetry. As the atomic positions in the *yz*-plane are ascertained beyond doubt by the Fourier synthesis, this difference might imply that there are some deviations from the suggested coordinates in the *a*-direction. In the space group C_s^4—*I*2, which would then apply, the *x*-parameter of the lead atoms can be arbitrarily fixed to 0. A variation of the *x*-parameter of the Ag_1 and Ag_2 atoms shows, however, that these atoms can be moved at the most $\sim \pm 0.03$ from the suggested positions, but that the agreement between observed and calculated intensities is not improved for any combination of the *x*-parameters within these limits. As there is no structural indication, that these small displacements from the positions in C_{2h}^6 really exist, it seems more probable that the difference in observed intensity between 604 and 60$\bar{4}$ has another origin than an error in the suggested atomic positions.

In all the photographs the inner reflexions are considerably weaker than was calculated. Therefore, it could hardly be expected that it would be possible to derive the oxygen atoms from the intensities. A Fourier synthesis using the 0*kl* reflexions (Fig. 1) showed, in addition to the metal atom maxima, several minor peaks, but the number of these peaks is larger than the number of oxygen atoms in the cell. However, a careful inspection of the intensities of the reflexions (Table 2) disclosed a couple of discrepancies which must be due to the fact that the influence of the oxygen atoms is not taken into account. These discrepancies are:

1) 020 is visible, although $F_{\text{Met}} = 0$
2) 011 is visible but not $\bar{1}10$, although for both reflexions $|F_{\text{Met}}| = 44$.

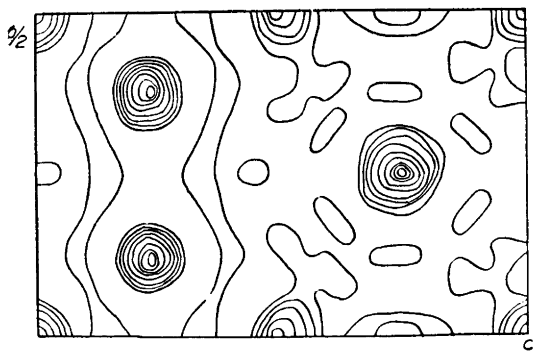

Fig. 1. Ag_2PbO_2. Projection of the electron density on the y z-plane. Compare Fig. 2.

Obviously, the oxygen atoms must be placed so that $F_{oxyg}(020)$ will be large and so that $|F_{Met}(011) + F_{oxyg}\ (011)| > |F_{Met}(\bar{1}10) + F_{oxyg}\ (\bar{1}10)|$. These conditions do not suffice to determine the positions of the oxygen atoms. However, from previous investigations of lead oxides it is known that the distance Pb^{2+}—O is 2.15 Å or larger. The distance Ag^{+}—O can hardly be shorter than 2.0 Å, as 2.05 Å is found for two-coordinated silver atoms in Ag_2O.

To account for the visibility of 020, the oxygen atoms must be situated approximately at $y = 0$ and 1/2 or at $y = \pm 1/4$. An inspection of the positions with $y \sim 0$ and 1/2 shows that no reasonable arrangement of the oxygen atoms can be obtained for these z-values. However, for $y \sim \pm 1/4$, an arrangement of the oxygen atoms can be worked out, which gives reasonable distances to both the Pb and the Ag atoms. The oxygen parameters are:

(000; 1/2 1/2 1/2) +
8 0 in 8 (f): $\pm (x, y, z)$; $\pm (x, y, 1/2-z)$ with $x = 0.311$, $y = 0.195$ and $z = 0.446$.

The following F-values are obtained:

$h\,k\,l$	F_{Met}	F_{oxyg}	ΣF	$\lvert F_{obs}\rvert$
020	0	− 38	− 38	48
011	− 44	− 17	− 61	50
$\bar{1}10$	44	− 7	37	< 50

As will be seen, this arrangement of the oxygen atoms accounts for both the visibility of 020 and the higher intensity of 011 compared with $\bar{1}10$.

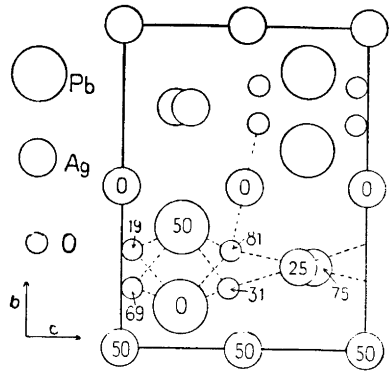

Fig. 2. Ag_2PbO_2. Projection of the structure on the yz-plane. Large circles denote lead atoms, medium circles silver atoms and small circles ogygen atoms. The figures denote the height of the atoms in percentages of a. The metal-oxygen bonds are shown with dotted lines. Superimposed silver atoms in a = 1/4 and 3/4 are symmetrically displaced.

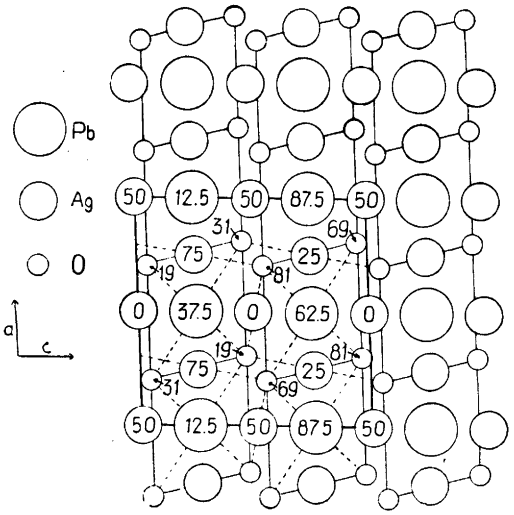

Fig. 3. Ag_2PbO_2. Projection on the xz-plane. Large circles denote lead atoms, medium circles silver atoms and small circles oxygen atoms. The figures denote the height of the atoms in percentages of b. Compare Fig. 2.

The interatomic distances are:

Pb — 2 O	= 2.28 Å	Ag_1 — 2 O	= 2.08 Å	O—O = 2.79 Å	
Pb — 2 O	= 2.37 Å	Ag_2 — 2 O	= 2.10 Å	(shortest distance)	
Pb — 2 Pb	= 3.74 Å	Ag_1 — 2 Ag_1	= 3.28 Å		
Pb — 2 Pb	= 3.94 Å	Ag_2 — 2 Ag_2	= 3.04 Å		
Pb — 2 Ag_1	= 3.65 Å	Ag_1 — 2 Ag_2	= 3.07 Å		
Pb — 2 Ag_2	= 3.61 Å	Ag_2 — 2 Ag_1	= 3.18 Å		

The structure is given in Figs. 2 and 3. The coordination figure of the lead atom is of the same type as in tetragonal PbO (Dickinson and Friauf [2], Moore and Pauling [3], Byström [4]) but here the oxygen atoms are displaced a little from the corners of a square, so that they form a very distorted tetrahedron with the lead atom outside the tetrahedron. The mean distance Pb—O, which is 2.32 Å is very close to the value in tetragonal PbO (2.30 Å). The low oxygen tetrahedra form chains in the *a*-direction with lead atoms alternately above and below the chains. Thus every chain forms a one-dimensional complex of the formula $(PbO_2^{2-})_\infty$ (Fig. 4), whereas in tetragonal PbO there are two-dimensional layers of the formula $(PbO)_\infty$ (Fig. 4). These $(PbO_2^{2-})_\infty$-

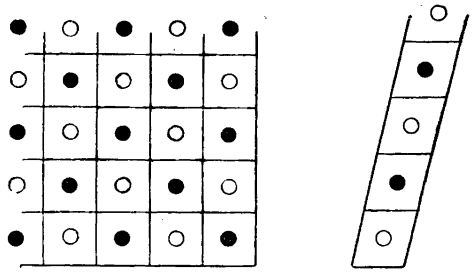

Fig. 4. A two-dimensional layer of the composition $(PbO)_\infty$ in tetragonal PbO and a one-dimensional chain of the composition $(PbO_2^{2-})_\infty$ in Ag_2PbO_2. Filled circles denote lead atoms below the layer or chain respectively and open circles lead atoms above the layer or chain.

chains are linked together by the Ag-atoms, each silver atom forming two linear bonds of the length 2.08 Å (Ag_1) or 2.10 Å (Ag_2) with two oxygen atoms belonging to different chains. Thus the Ag-atoms knit the chains together to a three-dimensional framework.

It is also of interest to note that the prism axis of the crystals and the direction of the $(PbO_2^{2-})_\infty$-chains coincide, *i. e.* the crystals grow fastest in the chain direction.

2. $Ag_5Pb_2O_6$

In the course of the attempts to produce single crystals of lead oxides hydrothermally (Byström [5]) a substance was obtained in the form of large hexagonal plates or hexagonal prisms. These crystals were first assumed to belong to a new lead oxide and gave beautiful X-ray photographs. The unit cell is hexagonal with $a = 5.939 \pm 0.003$ Å and $c = 6.428 \pm 0.003$ Å. The structure could not, however, be solved assuming only lead atoms (and oxygen atoms) in the unit cell. As the syntheses were carried out in silver crucibles, the only possible metal constituent besides lead in the substance is silver. As the yield of the phase by these first syntheses was only some ten mg, an analysis without a micro balance was not possible. However, by assuming five silver atoms and two lead atoms in the unit cell, a reasonable agreement between observed and calculated intensities was obtained.

As the substance was of considerable interest for the crystal chemistry of both silver and lead, an attempt has been made to solve the structure completely. Obviously the first step was to establish the formula. A series of hydrothermal syntheses were carried out in the temperature range 250°—300° C. Mixtures of Ag_2O and PbO_2 in various proportions were heated either with H_2O only or with a 5 % NaOH solution for about seven days. Mostly only a few crystals of the hexagonal phase were obtained, and as the crystals grew from the silver oxide (or silver) powder, a separation of the phases was

Table 1. Ag_2PbO_2. *Powder photographs. Cr-K radiation. β-reflexions are omitted.*

I	$\sin^2\Theta_{obs}$	$h\,k\,l$	$\sin^2\Theta_{calc}$	I	$\sin^2\Theta_{obs}$	$h\,k\,l$	$\sin^2\Theta_{calc}$
m	0.1226	002	0.1226	m	0.4675	150	0.4671
st	0.1312	$12\bar{1}$	0.1309	m	0.4760	330	0.4757
st	0.1392	121	0.1394	w	0.4855	312	0.4858
w	0.1425	200	0.1424	st	0.4905	004	0.4904
st	0.1677	$11\bar{2}$	0.1669	st	0.5245	$24\bar{2}$	0.5242
st	0.1820	$21\bar{1}$	0.1818	m	0.5583	$30\bar{3}$	0.5581
m	0.1843	112	0.1839			242	0.5582
st	0.1861	031	0.1859	w	0.5702	400	0.5696
v st	0.1911	130	0.1909	v w	0.5792	$15\bar{2}$	0.5812
		022	0.1916			$41\bar{1}$	0.6004
m	0.1989	211	0.1988			$20\bar{4}$	0.5988
m	0.2116	220	0.2114	v w	0.5997	143	0.6004
st	0.3682	$12\bar{3}$	0.3676			152	0.5982
m	0.3931	123	0.3932			$23\bar{3}$	0.5990
m	0.3990	042	0.3939	w	0.6279	$32\bar{3}$	0.6271
st	0.4108	$21\bar{3}$	0.4100	v w	0.6593	$40\bar{2}$	0.6582
m	0.4187	240	0.4186			204	0.6668
st	0.4315	321	0.4328	m	0.6663	$13\bar{4}$	0.6643
		033	0.4312			$22\bar{4}$	0.6678
st⁻	0.4355	$31\bar{2}$	0.4348	w	0.6846	$16\bar{1}$	0.6833
st	0.4620	213	0.4610	v w	0.6921	161	0.6917
		051	0.4622	w	0.6985	134	0.6983

impossible. However, a reasonable amount of the substance could be separated from a sample with a large surplus of lead (Pb:Ag 2 : 1) and analyses could be made on 50 mg samples. The following percentages were obtained:

Ag	(as AgCl)	: 51.0 ± 0.5 %		Ag:	51.39 %
Pb	(as $PbSO_4$)	: 39.0 ± 0.5	Calculated for $Ag_5Pb_2O_6$:	Pb:	39.44
O	(peroxide)	: 2.32 ± 0.02		O:	2.29

The peroxide oxygen was determined by dissolving the substance in a measured amount of oxalic acid and by titrating the excess of oxalic acid with $KMnO_4$.

Because of the limited amount of material a reliable determination of water could not be carried out. However, the only possible modification of the formula would be $Ag_5Pb_2O_6 \cdot H_2O$ (calculated percentages: Ag: 50.51, Pb: 38.81, O: 2.25), but as the structure determination will show, this formula is very improbable for space and symmetry reasons.

The Weissenberg photographs showed that the Laue symmetry was D_{6h}—$6/mmm$ (at least for the arrangement of the metal atoms). As no systematic extinctions appeared, the possible space groups are D^1_{6h}, D^1_6, C^1_{6v}, D^1_{3h}, D^3_{3h}.

Table 2. Ag_2PbO_2. Comparison between observed and calculated intensities. The influence of the oxygen atoms on the intensities is not taken into account. Cu-K radiation. Observed intensities from Weissenberg photographs.

$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}	$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}
	$0\,k\,l$ reflexions				
002	160	− 287	190	< 120	34
004	300	484	1110	190	− 195
006	210	− 193	121	140	− 283
008	300	332	141	< 80	0
020	48	0	161	190	219
040	< 60	26	181	< 120	0
060	< 80	0	1101	180	− 168
080	210	392	132	80	51
0100	< 70	0	152	< 100	41
011	30	44	172	240	− 243
013	< 60	40	192	180	− 214
015	< 80	− 39	123	270	257
017	< 80	33	143	< 100	0
022	240	300	163	240	− 202
024	< 80	0	183	< 110	0
026	210	196	1103	130	162
028	< 60	0	114	< 100	39
031	250	− 355	134	340	− 267
033	320	298	154	330	− 246
035	232	− 249	174	< 120	33
037	220	210		$h\,0\,l$ reflexions	
042	236	246	200	230	280
044	< 80	9	400	320	470
046	180	180	600	220	184
051	280	315	202	—	26
053	290	− 268	402	280	− 222
055	270	242	602	—	8
057	190	− 200	$20\bar{2}$	—	22
062	140	224	$40\bar{2}$	320	− 216
064	< 75	0	$60\bar{2}$	—	− 18
066	140	174	204	220	252
071	< 75	37	404	320	408
073	< 75	− 36	604	—	185
075	< 75	34	$20\bar{4}$	330	240
082	170	− 186	$40\bar{4}$	260	392
084	290	352	$60\bar{4}$	300	162
091	< 75	− 30	206	—	8
093	100	34	406	480	− 176
	$1\,k\,l$ reflexions		$20\bar{6}$	—	3
130	300	− 338	$40\bar{6}$	450	− 168
150	230	− 294	208	420	162
170	< 120	37			

$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}	$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}
	$h\,1\,l$ reflexions		217	470	213
110	—	44	417	—	32
310	—	40	$21\bar{7}$	450	208
510	—	40	$41\bar{7}$	—	32
710	—	33		$h, k, h+k$ reflexions	
011	80	− 44	$\bar{1}76$	280	− 196
211	170	− 344	$\bar{2}75$	320	206
411	—	− 42	$\bar{3}74$	80	35
611	140	− 221	$\bar{4}73$	—	− 35
$21\bar{1}$	220	337	$\bar{5}72$	180	− 206
$41\bar{1}$	—	40	$\bar{6}71$	160	196
$61\bar{1}$	200	221	066	270	176
112	160	− 347	$\bar{1}65$	330	180
312	220	− 298	$\bar{2}64$	350	196
512	140	− 245	$\bar{3}63$	250	− 188
712	100	− 205	$\bar{4}62$	125	192
$11\bar{2}$	130	− 347	$\bar{5}61$	110	178
$31\bar{2}$	160	− 293	$\bar{6}60$	150	176
$51\bar{2}$	100	− 239	257	75	− 32
$71\bar{2}$	90	− 200	156	—	36
013	—	41	055	500	232
213	130	303	$\bar{1}54$	380	− 250
413	—	38	$\bar{2}53$	—	− 39
613	100	214	$\bar{3}52$	—	39
$21\bar{3}$	130	296	$\bar{4}51$	190	− 178
$41\bar{3}$	—	38	$\bar{5}50$	160	− 223
$61\bar{3}$	80	210	$\bar{6}5\bar{1}$	—	− 36
114	—	42	246	530	356
314	—	38	145	—	0
514	—	35	044	—	10
$11\bar{4}$	—	37	$\bar{1}43$	—	0
$31\bar{4}$	—	35	$\bar{2}42$	450	478
$51\bar{4}$	—	33	$\bar{3}41$	—	0
015	55	− 38	$\bar{4}40$	—	10
215	450	− 250	$\bar{5}4\bar{1}$	—	0
415	—	− 34	$\bar{6}4\bar{2}$	410	350
615	280	− 199	336	100	34
$21\bar{5}$	500	− 244	235	—	− 36
$41\bar{5}$	—	− 32	134	350	− 263
116	550	− 236	033	470	307
316	420	− 221	$\bar{1}32$	—	42
516	320	− 200	$\bar{2}31$	—	− 42
$11\bar{6}$	550	229	$\bar{3}30$	300	− 292
$31\bar{6}$	400	− 214	$\bar{4}3\bar{1}$	180	263
$51\bar{6}$	300	− 197	$\bar{5}3\bar{2}$	—	34
017	—	34	$\bar{6}3\bar{3}$	—	− 29

$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}	$h\,k\,l$	$\lvert F_{obs}\rvert$	F_{Met}
426	310	172	516	270	− 195
325	270	− 186	415	—	− 37
224	210	224	314	—	38
123	220	246	213	280	296
022	360	314	112	180	− 338
$\bar{1}21$	260	− 284	011	50	− 44
$\bar{2}20$	300	296	$\bar{1}10$	—	− 44
$\bar{3}2\bar{1}$	170	262	$\bar{2}1\bar{1}$	200	338
$\bar{4}2\bar{2}$	210	216	$\bar{3}1\bar{2}$	250	− 292
$\bar{5}2\bar{3}$	190	− 184	$\bar{4}1\bar{3}$	—	− 38
$\bar{6}2\bar{4}$	180	168	$\bar{5}1\bar{4}$	—	37

Only one arrangement of the metal atoms in the xy-plane was found which agreed with the observed intensities. This can be described in any of the above-mentioned space groups with the following x y coordinates:

2 Ag in 0 0 and 0 0
3 Ag in 1/2 0; 0 1/2; 1/2 1/2
2 Pb in 1/3 2/3; 2/3 1/3

The lead atom position and the three-fold silver atom position are parameter-free in the c-direction in D^1_{6h}, D^1_6, D^3_{3h} and D^1_{3h}. However, agreement between observed and calculated intensities could not be obtained be varying the z-parameters of the silver atoms in 0 0 and thus the space group for the metal atoms must be C^1_{6v}—$C6mm$. In C^1_{6v} the metal atoms occupy the following positions:

1 Ag_1 in 1 (a): 0 0 z_1
1 Ag_2 in 1 (a): 0 0 z_2
3 Ag_3 in 3 (c): 1/2 0 z_3; 0 1/2 z_3; 1/2 1/2 z_3
2 Pb in 2 (b): 1/3 2/3 z_4; 2/3 1/3 z_4

Because of the symmetry, the z-parameters can be arbitrarily chosen so that $z_1 = -z_2$. The intensity of the reflexions $00l$ showed that z_3 and z_4 must be close to the parameter-free values of the positions 2 (c) and 3 (g) in the space group D^1_{6h} and that z_1 must be approximately 1/4. In the Weissenberg photograph the reflexions $00l$, because of the habit of the crystal (a hexagonal plate), showed up very strong, as also the $h\,0\,l$ reflexions with l high. This influence of the crystal habit on the intensity could be easily traced in the photographs from the variation in the general blackening. By the final adjuste-

Table 3. $Ag_5Pb_2O_6$. Powder photographs. Cr—K radiation. The influence of the oxygen atoms on the intensities is not taken into account. β-reflexions are omitted.

$h\,k\,l$	$\sin\Theta^2_{obs}$	$\sin\Theta^2_{calc}$	I_{obs}	I_{calc}	$h\,k\,l$	$\sin\Theta^2_{obs}$	$\sin\Theta^2_{calc}$	I_{obs}	I_{calc}
011	0.0808	0.0814	w^-	3.9	004	0.5082	0.5082	m^-	3.8
002	0.1268	0.1270	w^+	9.1	014	0.5573	0.5578	v w	0.8
110	0.1489	0.1488	st	21	032	—	0.5734	—	0.3
012	0.1764	0.1766	st	34	220	0.5957	0.5952	st	11
111	0.1804	0.1806	st^-	29	221	—	0.6270	—	0.1
020	0.1985	0.1984	w	6.7	213	—	0.6330	—	0.1
021	0.2302	0.2302	st	25	310	—	0.6448	—	0.2
112	0.2745	0.2758	v w	0.7	114	0.6568	0.6580	w	6.0
003	—	0.2858	—	0.2	311	—	0.6765	—	0.7
022	0.3253	0.3254	v w	0.8	024	0.7070	0.7066	v w	2.4
013	—	0.3354	—	0.1	222	0.7231	0.7222	w	8.1
210	0.3474	0.3472	v w	0.4	033	0.7314	0.7322	w	8.8
211	0.3799	0.3790	w	1.0	312	0.7720	0.7718	m	15
113	0.4349	0.4346	st	10	040	—	0.7936	—	1.6
030	0.4462	0.4464	m	4.2	005	—	0.7940	—	0.0
212	0.4737	0.4752	st	16	041	0.8245	0.8254	m^-	9.5
031	0.4782	0.4782	m	7.2	015	—	0.8436	—	0.9
023	0.4837	0.4842	m	6.6	214	—	0.8554	—	2.2
					223	—	0.8810	—	0.8

ment of the z-parameters only reflexions from those parts of photographs were compared, where no noticible changes in the general blackening occured, *i. e.* the reflexions with $l = 5$, 6, 7 and 8. The following values of the z-parameters were obtained:

$$2\pi z_1 = 95^\circ \qquad z_1 = 0.264$$
$$2\pi z_2 = -95^\circ \qquad z_2 = -0.264$$
$$2\pi z_3 = 188^\circ \qquad z_3 = 0.522$$
$$2\pi z_4 = 8^\circ \qquad z_4 = 0.022$$

As will be seen (Table 4), the agreement between observed and calculated F-values is very good for these reflexions with high l-indices.

The positions of the oxygen atoms cannot be deduced directly from the intensities, as they have only very little influence on the intensity of these high-angle reflexions, the F-values of which can be estimated with the best accuracy. However, a consideration of the positions in C^1_{6h} shows that the one-fold positions cannot possibly be occupied by oxygen atoms, because in that case Ag—O distances of 1.7 Å or less would result. For the same reason the formula cannot be $Ag_5Pb_2O_6 \cdot H_2O$. The six-fold positions lead to unacceptable O—O distances $\sim$ 2 Å. A combination of three two-fold positions

Table 4. $Ag_5Pb_2O_6$. Comparison between observed and calculated intensities. Observed intensities from Weissenberg photographs. The influence of the oxygen atoms on the intensities is not taken into account. Cu—K radiation.

h k l	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$	*h k l*	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$
100	30	34	055	30	38
200	120	118	016	120	100
300	160	152	026	< 24	30
400	100	85	036	50	48
500	< 30	22	017	28	37
600	180	193	027	90	94
110	80	180	037	100	120
210	20	28	018	55	55
310	50	27	011	14	41
410	150	124	021	64	180
510	40	21	031	57	140
610	40	20	041	50	140
220	170	262	051	< 24	18
320	< 30	24	061	< 24	16
420	70	71	012	43	180
520	150	103	022	17	31
330	150	114	032	< 24	30
430	20	20	042	< 24	27
001	14	15	052	40	110
002	90	180	062	38	100
003	< 14	40	013	< 17	14
004	190	260	023	52	140
005	31	16	033	38	140
006	150	140	043	38	110
007	40	48	053	24	12
008	140	160	063	38	29
015	45	45	014	38	49
025	120	140	024	43	110
035	90	105	034	52	120
045	110	130	044	24	67
			054	19	36

is also impossible for space reasons and also 6 O in $2 \cdot 2$ (*b*) + 3 (*c*). Then the only possibility which remains is that the six oxygen atoms are placed in two three-fold positions: 1/2 0 *z*; 0 1/2 *z*; 1/2 1/2 *z*. However, if the distance between superimposed oxygen atoms is assumed to be 2.6 Å, which is reasonable, then the two Ag_3—O distances will be only 1.91 Å (*cf.* 2.05 Å in Ag_2O), and if the Ag_3—O distances are increased to 2.05 Å then the O—O distances will be only 2.3 Å. Further, this alternative would imply six lead bonds, directed to the corners of a trigonal prism: a rather unlikely configuration.

A far more satisfying distribution of the oxygen atoms is obtained if the symmetry is lowered to C_{3v}^2—$C31m$. Then the two oxygen triangles can be moved around the three-fold axis, so that the lead atom is linked to six oxygen atoms, situated at the corners of a distorted octahedron. The parameters of the oxygen atoms are:

$C_{3v}^2 - C31m$
3 O in 3 (c): x_5 0 z_5; 0 x_5 z_5; $\bar{x}_5$ $\bar{x}_5$ z_5; $x_5 = 0.603$, $z_5 = 0.205$
3 O in 3 (c): x_6 0 z_6; 0 x_6 z_6; $\bar{x}_6$ $\bar{x}_6$ z_6; $x_6 = 0.397$, $z_6 = -0.171$

The influence of the oxygen atoms on the intensities is in most cases too small to be observed. However, a recalculation of the $00l$ reflexions shows that the incorporation of the scattering of the oxygen atoms in the F-values leads to a still better agreement between observed and calculated intensities. The observed F-values have been multiplied with a factor, to allow for the stronger influence of the absorption on the inner reflexions. This factor is taken as $A = \left|\frac{F_{calc}}{F_{obs}}\right|$ for the reflexions with h even. On these reflexions the oxygen atom has only a negligible influence. The values for l odd are then extrapolated (for 001) or interpolated. The values of $|F_{obs}| \cdot A$ are compared below with the calculated F values:

$h\,k\,l$	001	002	003	004	005	006	007	008
$\|F_{obs}\| \cdot A$	40	180	<25	260	37	150	40	140
$\|F_{calc}\|$ (only metal atoms)	15	180	40	260	16	140	48	160
$\|F_{calc}\|$ (metal + oxygen)	31	160	14	260	28	150	42	150

As will be seen, the agreement for the reflexions with l odd is much improved when allowing for the influence of the oxygen atoms, and thus the arrangement of the oxygen atoms in the z-direction is substantiated.

The following interatomic distances are obtained:

Pb — 6 O = 2.19 Å	Ag_1 — 3 O = 2.39 Å	O_1 — O_2 = 2.75 Å
Pb — 3 Pb = 3.43 Å	Ag_2 — 3 O = 2.44 Å	O_1 — O_1 = 3.15 Å
Pb — 3 Ag_1 = 3.76 Å	Ag_1 — Ag_2 = 3.04 Å	O_2 — O_2 = 3.15 Å
Pb — 3 Ag_2 = 3.89 Å	Ag_1 — 6 Ag_3 = 3.40 Å	Ag_3 — 2O = 2.07 Å
Pb — 6 Ag_3 = 3.64 Å	Ag_2 — 6 Ag_3 = 3.27 Å	

The distance Pb—6 O is very close to the corresponding value in PbO_2 (2.15 Å) and must imply that the Pb atoms are in a tetravalent state. The

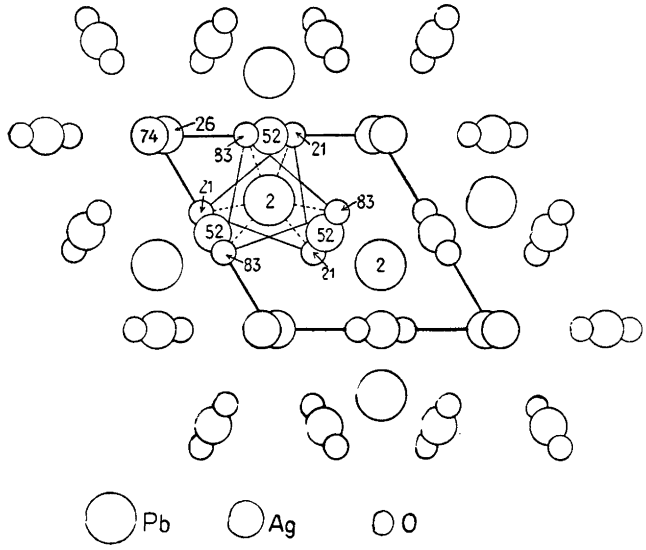

Fig. 5. $Ag_5Pb_2O_6$. Projection of the structure on the x y-plane. Large circles denote lead atoms, medium circles silver atoms and small circles oxygen atoms. The figures denote the height of the atoms in percentages of c. The dotted lines show the Pb—O bonds.

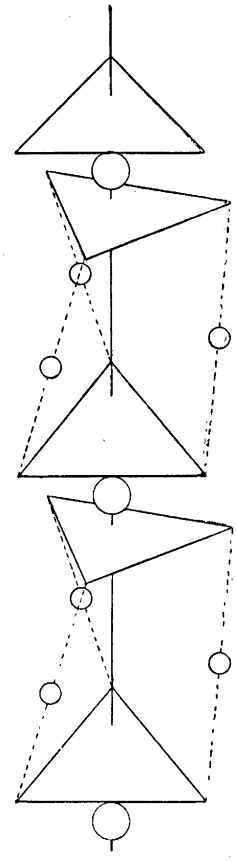

Fig. 6. The arrangement of the atoms in the vicinity of one of the three-fold axes. Large circles denote lead atoms and small circles silver atoms. The oxygen atoms are situated at the corners of the equilateral triangles.

two Ag_3 bonds are of practically the same length as the Ag—O bonds in Ag_2O (2.05 Å) and, as in Ag_2O, they are linear. Thus it is reasonable to assume that the Ag_3 atoms are in a monovalent state. Then it follows from the formula that the mean valency of the Ag_1 and Ag_2 atoms must be + 1/2. The coordination figure of these oxygen atoms is a triangle with the Ag atom a little displaced from the triangle plane and the three Ag—O bond angles are close to 120° (114° and 117°). As will be seen from the interatomic distances the Ag_1—Ag_2 distance is the shortest metal distance in the structure, being only 3.04 Å. It seems, that there might be covalent bonds also between the Ag_1 and Ag_2 atoms, and that thus there are four bonds from each Ag_1 and Ag_2 atom, three to oxygen atoms and one to a silver atom. Thus a binuclear komplex Ag_2O_6 is formed. A similar coordination has been found for silver atoms in Ag_2F (Ott and Seyfarth [6]) with a distance Ag—3 F = 2.4 Å, but in that compound

every silver atom is also in contact with three other silver atoms and the distance Ag—3 Ag is 2.9 Å.

The structure is given in Fig. 5. As will be seen from the figure, the octahedra around the lead atoms are linked together in layers, perpendicular to [001], by sharing edges. These layers form two-dimensional complexes of the composition $(Pb_2O_6^{4-})_\infty$. Octahedra from different layers are linked together by the Ag_3 atoms (Fig. 6). Around the origins of the unit cells, there are wide channels in the structure, running in the c-direction, and in these channels the Ag_1 and Ag_2 atoms are situated.

3. On the existence of other compounds in the system Ag—Pb—O

A number of compounds have been described in the system Ag—Pb—O, obtained by precipitating a solution containing both lead ions and silver ions with KOH or by precipitating a solution of $Pb(NO_3)_2$ in NaOH with $AgNO_3$ (Aston [7]). However, we have repeated these syntheses and the only crystalline Ag—Pb—O compound which is obtained is Ag_2PbO_2. It seems that the other compounds which have been described ($Ag_4Pb_7O_{11}$, $Ag_4Pb_3O_5$, Ag_4PbO_3) are in reality mixtures of Ag_2PbO_2 and PbO or Ag_2O.

SUMMARY

The crystal structures of Ag_2PbO_2 and $Ag_5Pb_2O_6$ have been determined.

Ag_2PbO_2 is monoclinic, with $a = 6.082 \pm 0.003$ Å, $b = 8.715 \pm 0.004$ Å, $c = 6.556 \pm 0.003$ Å and $\beta = 93.69°$. There are 4 Ag_2PbO_2 in the unit cell. The space group is C_{2h}^6—$I\,2/c$ and the atomic positions are:

(000; 1/2 1/2 1/2) +
4 Ag in 4 (b): 0 1/2 0; 0 1/2 1/2
4 Ag in 4 (d): 1/4 1/4 3/4; 3/4 1/4 3/4
4 Pb in 4 (e): 0 y 1/4; 0 $\bar{y}$ 3/4 with $y = 0.125$
8 O in 8 (f): $\pm$ (x, y, z); $\pm$ ($\bar{x}$, y, 1/2−z) » $x = 0.311$, $y = 0.129$ and $z = 0.446$.

The Pb atoms are bonded to four oxygen atoms, situated at the corners of a distorted square with a mean distance Pb—O of 2.32 Å. The Ag atoms form two linear bonds with a mean distance Ag—O of 2.09 Å.

$Ag_5Pb_2O_6$ is trigonal with $a = 5.939 \pm 0.003$ Å and $c = 6.428 \pm 0.003$ Å. There is one formula unit in the cell. The space group is C_{3v}^2—$C31m$. The atomic positions are:

1 Ag_1 in 1 (*a*): 0 0 z_1	with $z_1 = 0.264$
1 Ag_2 in 1 (*a*): 0 0 z_2	» $z_2 = -0.264$
3 Ag_3 in 3 (*c*): x_3 0 z_3; 0 x_3 z_3; $\bar{x}_3$ $\bar{x}_3$ z_3;	» $x_3 = 1/2$ and $z_3 = 0.522$
2 Pb in 2 (*b*): 1/3 2/3 z_4; 2/3 1/3 z_4;	» $z_4 = 0.022$
3 O in 3 (*c*): x_5 0 z_5	» $x_5 = 0.603$ and $z_5 = 0.215$
3 O in 3 (*c*): x_6 0 z_6	» $x_6 = 0.397$ and $z_6 = -0.171$

As will be seen, the metal atom positions have a higher symmetry than the oxygen positions and can be described in the space group C_{6v}^1—*C 6/mm*. The Pb atoms are surrounded by six oxygen atoms with Pb—O = 2.19 Å. The Ag_3 atoms form two linear bonds with Ag—O = 2.07 Å. The Ag_1 and Ag_2 atoms form three oxygen bonds with Ag—O = 2.39 and 2.44 Å respectively and probably one Ag—Ag bond with the distance Ag—Ag = 3.04 Å.

This work forms part of an investigation of the structure of metallic oxides. The work has been financially supported by *Statens Naturvetenskapliga Forskningsråd.*

We are indebted to mr. K. A. Wilhelmi and mr. S. Samson for valuable help with some of the experiments.

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Received March 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 628 – 640

Spectrophotometric Determination of Vitamin A by Means of Chromatography

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At the time being, three methods are available for the chemical determination of vitamin A: (1) the spectrophotometric method in which the absorption spectrum of vitamin A is measured and the concentration calculated from the height of the maximum in the 325—328 mμ range; and two colorimetric methods, (2) the Carr-Price reaction with antimony trichloride which gives a blue colour, and (3) the reaction with glycerol dichlorhydrin giving a red colour.

For the determination of vitamin A in substances containing very small amounts of light absorbing substances besides vitamin A, the spectrophotometric method will generally be preferred mainly for the following reason. When measuring the absorption curve in a wave length region around the absorption maximum the specificity of the measurement can be checked by comparison of the curve obtained with one for pure vitamin A. A control of this type cannot be made when using the colorimetric methods; an additional drawback of the Carr-Price reaction is the fact that the measurement cannot be performed with as high an accuracy as can the direct absorption measurement on vitamin A: The reaction with antimony trichloride is very unstable — in the course of 10 seconds the colour has reached its maximum and begins to fade. The reaction with dichlorhydrin does not show this defect but, on the other hand, it has not yet been tested sufficiently on different materials to allow the conclusion in which cases it can be regarded as satisfactorily specific.

Obviously, certain requirements must be put forth as to the course of the absorption curve in order that the spectrophotometric method can be used without correction. Unfortunately, however, none of the substances containing vitamin A usually available can completely satisfy our requirements in this respect. Nevertheless, for many years this method has been used and regarded

as the most satisfactory determination of vitamin A in fish liver oil. At the Second International Conference on Vitamin Standardization in June 1934, it was decided to acknowledge the application of this method, and to calculate the amount of vitamin A in international units per gram from $E^{1\%}_{1\,cm}$ at 328 mμ after multiplication by 1 600. As is well known, at that time the international unit of vitamin A was the biological effect of 0.6 μg β-carotene on rats; thus, the value of the conversion factor had to be based on comparisons between the biological effect on rats and the measured extinction for different vitamin-oils. In the course of time, the correctness of the factor 1600 has been under dispute, but it can now be regarded as well established that application of the factor 1600 is justified. In reality, the absorption spectrum of very few fish liver oils corresponds completely to the spectrum of pure vitamin A, a slight unspecific absorption is observed. Therefore, a correction of the measured extinction is always required, in accordance with the fact that the factor chosen is somewhat below the conversion factor for pure vitamin A found in recent years, namely ca. 1800.

The situation is somewhat changed since August 1949, when WHO has accepted the new international unit of vitamin A[1] as the biological effect of 0.344 μg vitamin A acetate, corresponding to 0.3 μg vitamin A alcohol. In fact, the conversion factor for spectrophotometric measurements can now be given disregarding the great uncertainty of biological determinations, because it is possible from the extinction coefficient of pure vitamin A to calculate the conversion factor to be 1894. It was decided to apply the factor 1900 which thus holds for absolutely pure vitamin A, contrary to the factor applied earlier which was used when working with fish liver oil and based on comparisons between biological and spectrographic investigations on this material. The new factor can therefore be applied only when the measured curve deviates but slightly from the accurately determined curve of pure vitamin A. The limits for permissible deviations at different wavelengths were established. When the deviations are greater than permissible a correction is required. If the deviations are not too great, *i. e.* if the measured curve reaches its maximum at the right wave length of 325—328 mμ, a correction is introduced according to the calculation suggested by Morton and Stubbs [2]. When the deviations from the vitamin A curve are considerable, Morton and Stubbs' method of correction will not lead to the correct result in most cases. This means that a spectrophotometric determination cannot be applied directly after saponification. A separation of vitamin A from interfering impurities has to be carried out. This separation can be performed by means of chromatography.

In recent years, chromatography has been applied rather frequently to a partial isolation of vitamin A, mainly in connection with determinations according to the Carr-Price method. Very recently, chromatography has been used in connection with the spectrophotometric determination of vitamin A. Gridgemann, Gibson and Savage [3] have published such a method for the investigation of whale liver oil; the same technique was applied by Barua and Morton [4].

However, the method of chromatographic-spectrophotometric determination of vitamin A described below, which for some time has been used in a great number of investigations, seems to have several advantages over the method outlined by Gridgemann and coworkers. Partly, it was found applicable to most substances containing vitamin A, partly much smaller quantities of vitamin A are required and, finally, it is more convenient as a routine method. In the investigations discussed here, calcium diphosphate has always served as an adsorber in the chromatography of fish liver oil. In the determination of substances containing less vitamin A it has been necessary sometimes to use the stronger adsorber aluminum oxide which was also applied by Gridgemann. In general the adsorbent applied should be as weak as possible, since this leads to the most effective separation.

EXPERIMENTAL

Prior to the chromatographic determination proper, the vitamin A must of course be brought into solution, *i. e.* it must be extracted from the present material; since, moreover, in the method here described it was considered preferable to avoid working with both vitamin A ester and vitamin A alcohol, saponification is necessary so that all of the vitamin A is present as alcohol.

When investigating fatty substances such as fish liver oil, vitamin A concentrates or alike as well as butter, saponification is performed directly with about twice the amount of alcoholic potassium hydroxide (ca. 2 *N* KOH in 80 % alcohol) subsequent addition of water and three times extraction with ether in the separatory funnel.

Solid material such as foddermixtures containing vitamin A and different vitaminized food products are also saponified with alcoholic potassium hydroxide; subsequently, the insoluble component is filtered off by means of a glass filter, boiled again twice with alcoholic posassium hydroxide until complete extraction of vitamin A is obtained.

The collected alcohol extracts are diluted with water and then extracted three times with ether in a separatory funnel.

To investigate the vitamin A content in milk equal parts of alcohol are added and the fat soluble constituents are extracted with ether in a separatory funnel. The ether is evaporated and, subsequently, saponification is performed with alcoholic potassium hydroxide and followed by renewed ether extraction in the separatory funnel.

In all materials investigated it was found of importance for the chromatography to remove as completely as possible fatty acids from the ether extract. Washing of the extract with water, as is usual in vitamin A determinations, was not sufficient. Instead, the following procedure is recommended: The extract is first washed once with water, then once or twice with $N/2$ KOH in 30 % alcohol and, finally, washed twice with water.

Subsequently, the washed ether solution is dried by filtration through a layer of dehydrated sodium sulphate and evaporated to dryness in a CO_2-atmosphere, the last traces of ether being removed at room temperature by means of a CO_2-stream.

The evaporation residue is dissolved in a suitable volume of petroleum ether and this solution is then used for chromatography.

Chromatography

Apparatus:

1. Glass tubing, for the adsorption column upper part 12 mm inner ø, 100 mm long, lower part ca. 8 mm outer ø, 50 mm long. Inserted with rubber stopper onto an ordinary suction flask.

2. U.-V. lamp, Philips HPW 120, emitting ca. 90 % of the light at 365—66 mμ and only ca. 1 % visible light.

Reagents:

1. Petroleum ether; boiling point below 70°.

2. Ethyl ether, peroxide free: peroxides are removed by 1—2 days storage with KOH and subsequent distillation.

3. Di-calcium phosphate. In chosing this reagent two properties have to be taken into consideration, *viz.* its adsorption capacity and grain size.

With regard to the adsorption capacity the following will be convenient. 300—400 μg pure vitamin A adsorbed in a column of the tube, dimensions given above, should move only slightly when 100 ml pure petroleum ether are poured through the column, while 100 ml of a petroleum ether — ethyl ether mixture (50 : 1) causes complete eluation.

With respect to the grain size it must be required that the adsorbent is not packed so tightly in the column that it is difficult to filter the solutions through in the course of a reasonable time. In a column of the above dimensions a sample of calcium phosphate which during suction with a suction

pump allows ca. 100 ml petroleum ether to pass in the course of 5—10 minutes can be used; it is possible, however, to obtain a preparation allowing a higher speed.

4. Aluminum oxide. When using commercial preparations meant for chromatography, an excellent filtration rate will always be obtained; therefore it is only necessary to specify requirements as to adsorption capacity. When pouring 25 ml of a petroleum ether-ethyl ether mixture (4 : 1) through the column of the above dimensions, 300—400 μg vitamin A should move only a few cm, while 25 ml petroleum ether-ethyl ether mixture (1 : 1) should eluate the vitamin A completely.

The adsorption capacity of both dicalcium phosphate and aluminum oxide can be increased by heating for some time to 100—110° C, it can be decreased by exposure to air at room temperature.

Procedure:

The column used for chromatography is prepared in the following way.

As a support of the adsorbent some scoured cotton wool is placed at the transition from the broader to the narrower part of the adsorption tube. Then the adsorbent is introduced into the tube in small portions suspended in petroleum ether. Each time the powder is allowed to settle and then pressed together by suction. However, the tube must never be sucked dry, some petroleum ether must always remain in the tube. The tube is filled with the adsorbent to 1—1 ½ cm from the top and then the column is ready for chromatography.

A convenient quantity of the extract in petroleum ether prepared as described above and corresponding to 150—600 μg vitamin A (in exceptional cases down to 50 μg) is sucked through the column and then washed with 25—50 ml pure petroleum ether. The column is inspected in U—V light where the vitamin A should be visible as a yellowish green fluorescent zone in the column. If such a zone is not visible, the reason may be that the solution contained too little or no vitamin A or that the adsorption capacity of the adsorbent used is too poor so that the vitamin A has run through the column. In the last mentioned case a more efficient adsorbent has to be applied.

If the vitamin A is adsorbed satisfactorily to the dicalcium phosphate, the chromatogram is developed by continued washing with a petroleum ether — ethyl ether mixture (50 : 1) until the yellowish green fluorescent vitamin A zone appears on the very bottom of the column, however, without any vitamin A having escaped. Then a clean suction flask is inserted and a mixture of petroleum ether-ethyl ether (50 : 1) is added until all vitamin A is eluated. This fraction is transferred to a volumetric flask of suitable size which is filled up with petroleum ether so that the solution contains 2—4 μg vitamin

A per ml. This solution can be used directly for the determination of the absorption curve in a Beckmann spectrophotometer. Evaporation of the chromatographed solution should be avoided, since it has been found that in a solution of vitamin A in petroleum ether unspecifically absorbing substances may be formed on evaporation which cause a deviation of the absorption curve from that of pure vitamin A.

If, however, vitamin A from the solution available for chromatography cannot be adsorbed on dicalcium phosphate, aluminum oxide must be used. Then, development of the chromatogram and eluation of vitamin A is performed, using other mixtures of petroleum ether-ethyl ether, starting, for example, with 25 ml 20 : 1, then 5 : 1, 3 : 1, 1 : 1 and changing the mixing ratio in the given sequence until the vitamin A moves at a suitable rate. When the vitamin A zone appears at the very bottom of the column, the suction flask is changed and eluation is continued with the last employed mixture until all vitamin A is eluated.

If dicalcium phosphate is found not to adsorb the vitamin A while aluminum oxide appears to be too strongly adsorbing, it is suggested to apply dicalcium phosphate which has been heat treated more efficiently than normal. In this case, a higher content of ethyl ether than the normally used 1/50 should be tried in the eluation mixture. In all cases, however, the solution must be brought to a volume giving — without evaporation — a concentration of vitamin A suitable for the measurements.

EXPERIMENTAL RESULTS

It is the aim of the experimental work presented here to determine the applicability of the method to the determination of vitamin A. The investigations were made partly on pure vitamin A, in order to detect the loss during chromatography, partly to demonstrate the applicability of chromatography to the purification of vitamin A from solutions containing unspecific, absorbing substances.

Experiments with pure vitamin A*

A prerequisite for the application of the method outlined above is the knowledge of the absorption curve of pure vitamin A in the employed mixture of petroleum ether and ethyl ether. Therefore, measurements were performed

* The vitamin A applied was produced synthetically and kindly put at the disposal of the State Vitamin Laboratory by Messrs. Hoffmann—la Roche, Basel, via Messrs. Wørum and Co., Copenhagen.

of solutions of known quantities of pure vitamin A in petroleum ether, ethyl ether, and absolute alcohol, respectively, the latter being the generally used solvent in vitamin A spectrophotometry. In Table 1 the results of such measurements are shown together with the measurements on pure vitamin A in absolute alcohol reported by Morton and Stubbs [5].

Table 1. Absorption curve for vitamin A in different solvents.

Wave length mμ	Petroleum ether		Ethyl ether		Abs. alcohol		Morton and Stubbs $\frac{E}{E_{325}}$ in abs. alcohol
	$E_1^{1\%}{}_{cm}$	$\frac{E}{E_{325}}$	$E_1^{1\%}{}_{cm}$	$\frac{E}{E_{325}}$	$E_1^{1\%}{}_{cm}$	$\frac{E}{E_{325}}$	
280	420	0.230	260	0.141	404	0.224	0.224
290	687	0.376	616	0.333	662	0.368	0.414
300	1 077	0.589	1 064	0.575	1 042	0.578	0.609
310	1 533	0.838	1 562	0.844	1 497	0.832	0.844
320	1 734	0.949	1 765	0.955	1 708	0.948	0.950
325	1 828	1.000	1 851	1.000	1 800	1.000	1.000
328	1 787	0.977	1 788	0.967	1 764	0.980	
330	1 732	0.947	1 728	0.934	1 704	0.964	0.957
340	1 325	0.725	1 319	0.713	1 335	0.742	0.772
350	819	0.448	782	0.423	824	0.457	0.509
360	441	0.241	409	0.221	458	0.255	0.278

The measurements indicate that the difference between the absorption curve of vitamin A in different solvents is only small in the range 310—350 mμ, which is the range in which the requirements of the new international rules for the determination of vitamin A must be fulfilled. Therefore it seems superfluous to take into consideration the deviation which appears when calculating the concentration of vitamin A on the basis of the absorption curve measured in the mixture of petroleum ether and ethyl ether. It should be mentioned that petroleum ether without any addition is used as a blank since it was found that a difference in the light absorption of ethyl ether and the petroleum ether used in the range above 280 mμ cannot be detected. At 270 mμ, however, the absorption of the petroleum ether was significantly higher than that of ethyl ether.

Furthermore, pure vitamin A was applied to the determination of losses during chromatography. A solution of vitamin A in petroleum ether was measured on the Beckmann spectrophotometer prior to and after chromato-

graphy. A column of dicalcium phosphate as described above was used, and a quantity of vitamin A of 150—200 μg which after chromatography was taken up in 50 ml. Four experiments of this type were performed. The loss was found to be ½—2 %. In Table 2 are given the measurements at different wave lengths for one of these experiments.

Experiments on solutions with other light absorbing substances

The applicability of chromatography to the purification of impure extracts was investigated on a series of vitamin A solutions from extracts of widely different materials. Some of these experiments are shown in Table 3 and the effect of chromatography is illustrated by the values of $\frac{E_{310}}{E_{325}}$ and $\frac{E_{350}}{E_{325}}$. It becomes clear from Table 3 that these ratios in the solution after chromatography are very much like those found for pure vitamin A dissolved in petroleum ether (Table 1).

Table 2. Loss during chromatography of 140 µg pure vitamin A.

Wave length mµ	$E_{1\text{ cm}}$	
	Directly, without chromatography	After chromatography
280	0.103	0.088
290	0.189	0.180
300	0.306	0.302
310	0.436	0.430
320	0.489	0.478
325	0.513	0.502
328	0.501	0.492
330	0.485	0.473
340	0.373	0.363
350	0.227	0.220
360	0.122	0.117

For the majority of the materials Table 3 shows a considerable decrease in $E_{1\text{ cm 325 m}\mu}^{1\%}$ due to chromatography. Of course, it cannot be decided on the basis of these experiments whether this decrease actually corresponds to unspecific absorption in the original solution. A comparison of these investigations with the experiments on pure vitamin A discussed above and showing

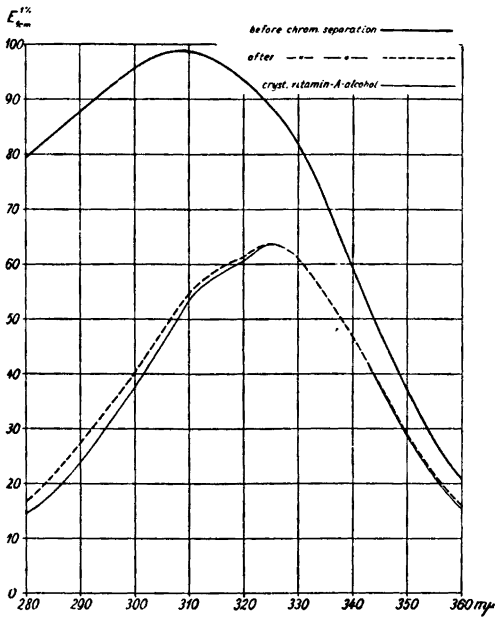

Fig. 1. Absorption-curves of extract from whale-liver-conc. before and after chromatography.

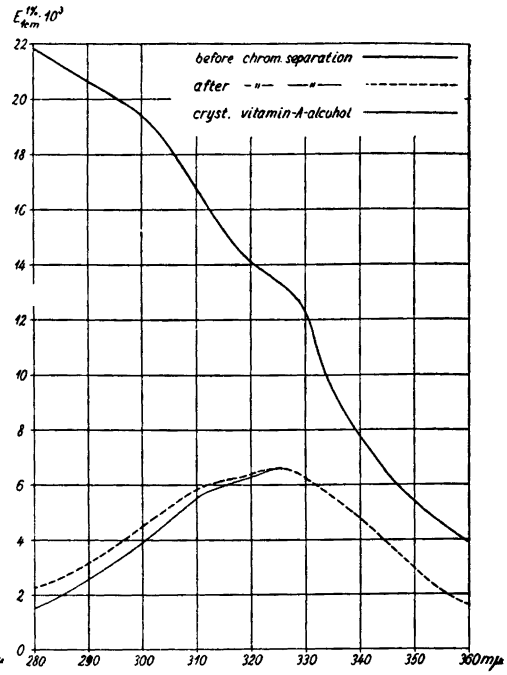

Fig. 2. Absorption-curves of extract from vitaminized oats before and after chromatography.

very small losses after chromatography leave no doubt that this is actually the case.

In order to demonstrate more clearly the applicability of chromatography to the purification of vitamin A solutions the absorption curve in the range 280—360 mμ before and after chromatography is given for three of the experiments shown in the table (Figs. 1—3).

Figs. 1—3 show that the absorption curves after chromatography fall very closely to the absorption curve of vitamin A, which seems to be an excellent criterion in favour of the specificity of the chromatographic-spectrophotometric method.

With regard to the applicability of chromatography to the purification of different extracts it should be mentioned that margarine extract is the only one among the materials investigated which could not be purified so effectively that a spectrophotometric determination could be performed, the reason being the sesame oil content of margarine which, according to Danish law, must be added to margarine as marking ingredient.

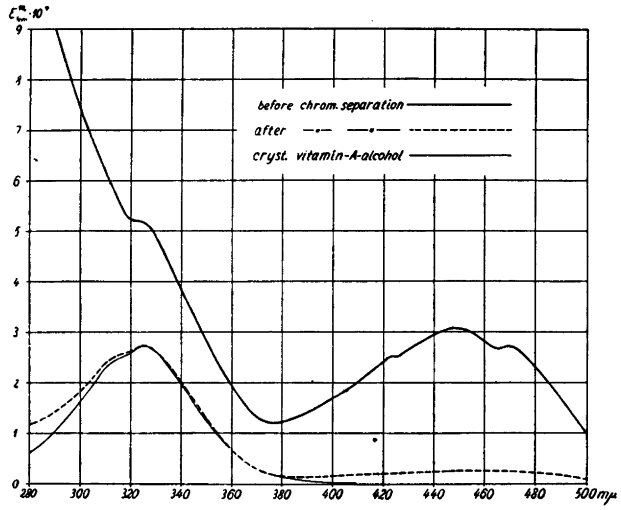

Fig. 3. Absorption-curves of extract from milk before and after chromatography.

DISCUSSION

Before the new international regulations for the determination of vitamin A had been accepted in 1949, it was possible to determine the content of vitamin A in fish liver oil without purification of the solution obtained after saponification and ether extraction prior to the measurement of the absorption spectrum. In this procedure the conversion factor 1600 compensated to some extent for the impurities present in the solution. For whale liver oil and its concentrates application of this conversion factor was more problematic because these products always show a considerably higher unspecific light absorption than does fish liver oil, which means that the maximum of the absorption curve may be found at a much lower wave length than that of pure vitamin A. Until recently, spectrophotometry has scarcely been applied to the determination of vitamin A in any other vitamin A containing materials.

In the new international regulations prescribing the conversion factor 1900 it was found necessary to formulate certain requirements with respect to the absorption curve forming the basis for the determination and the calculation by means of the given factor. It is required that the ratio between the extinction and the maximum extinction measured at any wave length in the range 310 to 350 mμ does not deviate by more than 0.02 from the same ratio for pure vitamin A. Here we are confronted with the question concerning the proper course of the absorption curve for pure vitamin A. In the communication published by the Medical Research Council it is stated that either

Table 3. Comparison between the absorption curves of different extracts before and after chromatography.

Materials	Before chromatography			Chromatography material	After chromatography			Calculated international units per gram
	$E_1^{1\%}{}_{\text{cm}}$ 328 mμ	$\frac{E_{310\ m\mu}}{E_{325\ m\mu}}$	$\frac{E_{350\ m\mu}}{E_{325\ m\mu}}$		$E_1^{1\%}{}_{\text{cm}}$ 325 mμ	$\frac{E_{310\ m\mu}}{E_{325\ m\mu}}$	$\frac{E_{350\ m\mu}}{E_{325\ m\mu}}$	
Whale liver oil	4.23	1.10	0.43	$CaHPO_4$	3.49	0.86	0.47	6 400
» » »	7.47	1.09	0.49	»	4.75	0.86	0.45	9 000
» » conc.	25.20	1.26	0.47	»	16.44	0.86	0.47	31 200
» » »	89.0	1.11	0.41	»	6.38	0.86	0.45	121 000
Cod liver oil	0.349	0.89	0.52	»	0.312	0.85	0.51	590
» » »	1.122	0.85	0.54	»	1.039	0.84	0.50	1 970
» » »	0.590	0.88	0.54	»	0.540	0.84	0.53	1 025
Butter	0.02075	1.02	0.50	»	0.01352	0.84	0.45	25.7
»	0.01715	1.00	0.51	»	0.01086	0.85	0.46	20.6
Liver paste	0.0703	0.89	0.57	»	0.0452	0.87	0.47	85.9
» »	0.0974	0.90	0.68	»	0.0468	0.87	0.45	88.9
Vitaminized oats	0.01480	1.23	0.50	»	0.00661	0.88	0.44	12.6
» »	0.01126	1.33	0.49	»	0.00446	0.91	0.46	8.5
Vitamine fodder	0.0492	1.45	0.55	Al_2O_3	0.0140	0.90	0.50	26.6
Milk	0.000517	1.19	0.54	$CaHPO_4$	0.000274	0.89	0.47	0.52
Liver oil paste	0.96	1.53	0.35	Al_2O_3	0.088	0.87	0.49	170

can the value given by Morton and Stubbs [5] be used or the curve can be redetermined by measuring a solution of the new biological standard against a solution of the oil from which the standard is made.

However, as is shown in Table 1 of the present paper, it becomes clear that there is no complete agreement between the author's measurements on pure synthetic vitamin A and the values given by Morton and Stubbs. Therefore, the question arises whether it is correct to use Morton and Stubbs' measurements; in which case the requirements as to the purity of the measuring solution are lower, the ratio $\frac{E_{350}}{E_{max}}$ (ca. 0.51) being higher than the corresponding ratio (0.46) measured on pure vitamin A as used in the present experiments.

If Morton and Stubbs' requirements are considered sufficient, many fish liver oils will in fact pass as being satisfactory, while none of the fish liver oils are satisfactory if the requirements correspond to what has been described in the present work (*cf.* the results on cod liver oil given in Table 3). The

requirement as to the course of the vitamin A curve given in the communications of the Medical Research Council, *viz.* measurement on the new standard solution, does not correspond to the curve for vitamin A alcohol, but for vitamin A ester, and is therefore applicable only when the determination is performed on the non-saponified oil solution. Without saponification, however, no material will lead to an absorption curve which is in satisfactory agreement with the curve for vitamin A acetate; consequently, as mentioned in the communication, it will always be necessary either to correct according to Morton and Stubbs [1] or to perform purification which, in practice, means chromatography.

Chromatography involves a separation of vitamin A ester from vitamin A alcohol and therefore the question arises whether the two components should be determined separately, which seems, however, very impractical. Saponification appears to be preferable and in this way the total vitamin A can be determined, as is the case in the present work.

Hence, if the curve for vitamin A acetate, measured as the international standard, is to be the basis for the evaluation of the specificity of the absorption curve, the determination must be performed applying Morton and Stubbs' correction to the directly measured curve. According to the communication of the Medical Research Council the correction is permissible only if the measured curve has a maximum at the proper wave length, *i. e.* at 325—328 mμ. If this does not hold, as in the case of most whale liver oils, a correct determination is possible only in connection with chromatography, but previous saponification is definitely to be preferred.

Since the above described technique of chromatography leads to absorption curves which are in good agreement with those measured on pure synthetic vitamin A, it appears reasonable to take this curve as the basis of the evaluation. If this curve is given preference, not one of the absorption curves for fish liver oil will show satisfactory agreement and, consequently, either the correction according to Morton and Stubbs or a purification through chromatography must be applied.

As chromatography is simple and not very time-consuming, and since there is little chance for the introduction of significant errors, it appears most convenient always to use chromatography, even in those cases where application of the correction method is permissible according to international rules. The very narrow wave length range applied in the correction method, which causes a multiplication of the spectrophotometric measuring uncertainty, no doubt results in an uncertainty of the calculated result that is much higher than the uncertainty of the chromatographic determination.

SUMMARY

A method for the chromatographic separation of vitamin A from petrol-ether-extracts of different materials is described. The method gives in connection with spectrophotometric measurement of the absorption curve a specific determination of vitamin A in a majority of materials containing this vitamin.

In experiments with pure vitamin A the losses during chromatography were found to be ½—2 %.

Values for the absorption at 310, 325 and 350 mμ, with and without chromatographic separation, of extracts from whale liver oils, cod liver oils, liver paste, butter, milk, vitaminized oats and fodder mixtures are given.

The determination of vitamin A in fish liver oils and whale liver oils is discussed in relation to the new international regulations concerning this problem and it is suggested that hereby the chromatographic separation should be preferred to the correction according to Morton and Stubbs.

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Received March 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 641–649

Attempts to Synthesize α-(3,4-Dimethoxyphenyl)-glycerol Derivatives

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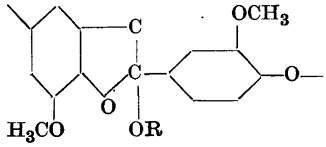
Holmberg showed that α-phenethyl alcohol reacts with sulphite cooking acid with formation of a sulphonic acid [1]. From this and other evidence [2] he concludes that the sulphonation of lignin is due to the substitution of sulphonic acid groups for hydroxyl groups of benzyl alcohol type.

In a recent paper, Erdtman, Lindgren, and Pettersson [3] showed that in the later phases of the sulphonation of lignin, the reaction involves the substitution of sulphonic acid groups for hydroxyl groups. Setting out from these results, the writer examined vanillyl alcohol, veratryl alcohol, and their homologues [4]. These benzyl alcohols were found to be suitable model substances for the sulphonation and condensation of lignin.

Poly-ethers containing benzyl alcohol groups were prepared by Erdtman and Leopold [5] and found to behave like lignin in their reactions with sulphite solutions. Erdtman [6], however, considers that there are in lignin two different groups which are responsible for the sulphonation. Only the most reactive of these groups is assumed to be of benzyl alcohol type, the other being a cyclic acetal group of the type

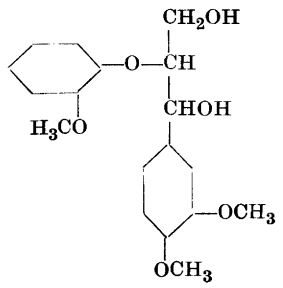


in which R represents a carbohydrate or lignin residue.

Enkvist and Moilanen [7] recently showed that vanillyl alcohol is also a good model substance for certain reactions of lignin during the sulphate digestion.

In the discussions of the structure of lignin, phenyl glycerol derivatives play an important *rôle* [2,8]. For this reason, the experiments (see Scheme 1)

which are described in this paper were initiated. The synthesis of α-(3,4-dimethoxyphenyl)-glycerol derivatives, particularly the guaiacyl ether I, was attempted. Although the synthesis of these compounds from 1-(3,4-dimethoxyphenyl)-propanone(-1) derivatives could not be accomplished, a series of interesting reactions were encountered.



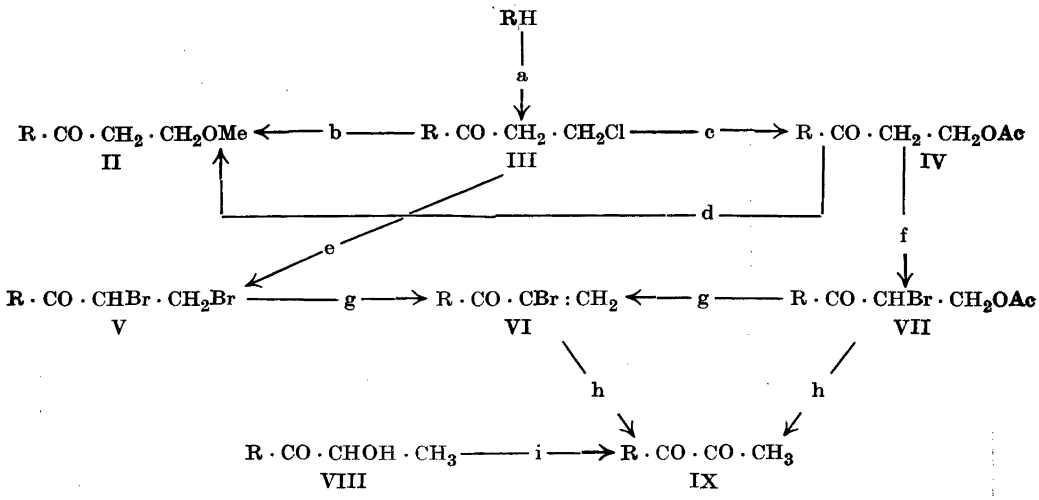
I

3-Acetoxy-1-(3,4-dimethoxyphenyl)-propanone(-1) (IV, see Scheme 1), synthesized according to the method of West, Hawkins, and Hibbert [9], was brominated to form 3-acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone-(-1) (VII). Several attempts were made to substitute acetoxyl or 2-methoxy-phenoxyl group for the bromine atom of the latter compound (VII). In all cases, however, the only substances obtained were the elimination products. When heated with potassium acetate in acetic acid, the bromo acetoxy compound VII yielded 1-(3,4-dimethoxyphenyl)-propandione(-1,2) (IX). On heating with guaiacol and a base, compound VII yielded 2-bromo-1-(3,4-dimethoxyphenyl)-propene(-2)-one(-1) (VI) and (or) the diketone IX.

The bromine atom in the 3 position of 2,3-dibromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (V) is also easily eliminated. With guaiacol and potassium carbonate, in acetone solution, the substance yields the unsaturated bromo compound VI.

The transformation of the bromo acetoxy compound VII into the diketone IX belongs to a type of reaction of which only a few examples have been described in the literature. The formation of a diketone has been observed by Bradley and Eaton [12] in the case of 3-chloro-1-acetoxy-1-phenyl-propanone(-2) (X Scheme 2), mild hydrolysis of the latter yielding 1-phenyl-propandione(-1,2) (XV). Gardner and Hibbert [13] showed that similar compounds, such as 3-chloro-1-acetoxy-1-(3-methoxy-4-acetoxy-phenyl)-propanone(-2) (XI) and 1,3-diacetoxy-1-(3-methoxy-4-acetoxy-phenyl)-propanone(-2) (XII), are converted by the action of mild hydrolytic agents into the diketone XVI. The formation of such diketones is of interest in lignin chemistry since Hibbert and co-

*Scheme 1. Schematic diagram of the synthesis *.*



* Except the synthesis of 2-hydroxy-2-ethoxy-1-(3,4-dimethoxyphenyl)-ethanone(-1).

R = 3,4-dimethoxyphenyl-

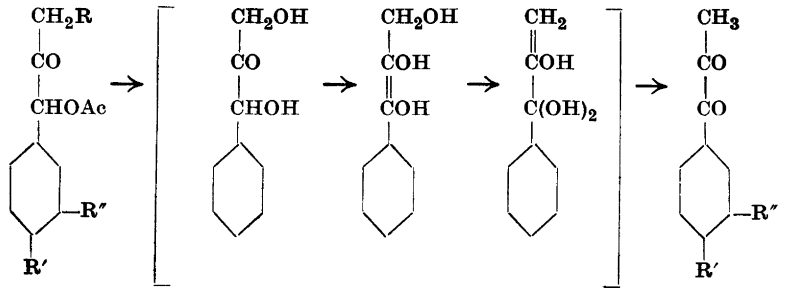
a) Friedel-Crafts synthesis, according to Freudenberg and Fikentscher [10]. b) Potassium hydroxide in methanol. c) Potassium acetate in acetic acid according to Hibbert and co-workers [9]. d) Potassium hydroxide in methanol according to Hibbert and co-workers [9]. e) Bromine in acetic acid. f) Bromine in chloroform. g) Potassium carbonate with acetone. h) Potassium acetate in acetic acid. i) Copper sulphate in pyridine according to Hibbert and co-workers [11].

workers have isolated the diketone XVI from the ethanolysis products of wood [14].

Gardner and Hibbert [13] concluded that the diketone XVI "is formed as the result of an allyl shift in a dismution ene-diol product XIV arising from the hydrolysis intermediate, 1,3-dihydroxy-1-(4-hydroxy-3-methoxy-phenyl)-propanone(-2)" (XIII). The transformation of the bromo acetoxy compound VII into the diketone IX by potassium acetate in acetic acid, however, does not necessarily involve a molecular rearrangement. The diketone IX may be formed via the unsaturated bromo compound VI, since this compound forms the diketone IX under the same conditions.

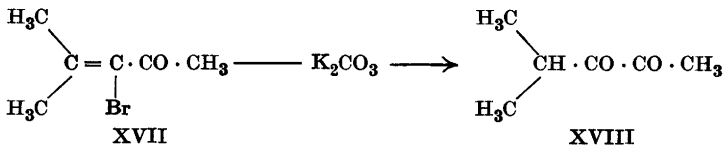
In general, there is a pronounced diminution in reactivity of a halogen atom adjacent to a double bond. The bromine atom of the unsaturated bromo compound VI, however, is very reactive. As mentioned above, it reacts with potassium acetate in acetic acid to yield the diketone IX. The high reactivity

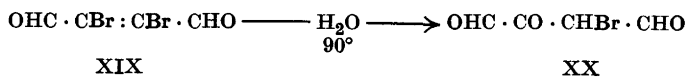
Scheme 2. The formation of diketones.



	R	R′	R″
X	Cl	H	H
XI	Cl	OAc	OMe
XII	OAc	OAc	OMe

	R′	R″
XV	H	H
XVI	OH	OMe





of a halogen atom in a similar position has been observed by Pauly and Lieck [15] in the case of bromo mesityl oxide (XVII Scheme 2) on heating with potassium carbonate this compound yielded the diketone XVIII. One of the bromine atoms of dibromo maleinic aldehyde (XIX) is also easily substituted. On heating with water at 90°, it yields the compound XX [16].

Attempts were made to synthesize the diketone IX by the oxidation of propioveratrone with selenium dioxide in dioxane or acetic acid solution. In no case was the diketone IX obtained. On the other hand, acetoveratrone is readily oxidized into 3,4-dimethoxyphenyl glyoxal by selenium dioxide in dioxane solution in the manner used by Fodor and Kovács [17] to synthesize several other, substituted phenyl glyoxals. 3,4-Dimethoxyphenyl glyoxal is transformed into a semiacetal, 2-hydroxy-2-ethoxy-1-(3,4-dimethoxyphenyl)-ethanone(-1), by heating with ethanol.

EXPERIMENTAL

3-Methoxy-1-(3,4-dimethoxyphenyl)-propanone(-1) (II)

3-Chloro-1-(3,4-dimethoxyphenyl)-propanone(-1) [10] (III) (8.6 g) was mixed with hot methanol (50 ml). Methanolic potassium hydroxide solution (77 ml, 0.488*N*) was added and the mixture was boiled for about one minute. After filtration and neutralization, the filtrate was evaporated to dryness in a vacuum. Recrystallization from ligroin yielded a substance, which had a m. p. of 69° (5.5 g, yield 65 %). The m. p. was increased to 71° by further recrystallizations. The substance gave no m. p. depression with the compound II (m. p. 71°), prepared according to West, Hawkins, and Hibbert [9].

3-Acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (VII)

3-Acetoxy-1-(3,4-dimethoxyphenyl)-propanone(-1)[9] (IV) (10 g) was dissolved in chloroform (50 ml), containing a drop of concentrated hydrochloric acid. Bromine (6.7 g) dissolved in chloroform (40 ml) was slowly added at room temperature. The reaction was started by means of ultra-violet irradiation. The red solution obtained was shaken with sodium bicarbonate solution and with water. After drying over anhydrous sodium sulphate the solution was filtered through a column of aluminium oxide. By this operation the colour of the solution was changed to pale brown. After evaporating the solution in a vacuum, the residue crystallized. Recrystallization from isopropyl ether yielded a substance which had a m. p. of 87—91° (7.6 g, yield 58 %). Further recrystallizations increased the m. p. to 94—95°.

$C_{11}H_9O_3Br(OCH_3)_2$	Calc.	OCH_3	18.7
	Found	»	18.8

2,3-Dibromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (V)

3-Chloro-1-(3,4-dimethoxyphenyl)-propanone(-1) [10] (III) (4 g) was dissolved in acetic acid (30 ml), containing a drop of concentrated hydrochloric acid. Bromine (2.8 g), dissolved in acetic acid (15 ml), was slowly added at room temperature. The reaction was started by means of ultra-violet irradiation. The solution was left over-night and then evaporated in a vacuum desiccator over sodium hydroxide. The residue crystallized and the crystals were washed with methanol (4.95 g, yield 80 %, calculated on the assumption that the product were the pure dibromo compound). Recrystallizations from methanol yielded a product which had the m. p. of 74°—78°.

$C_9H_6OBr_2(OCH_3)_2$	Calc.	Br	45.4
	Found	Halogen (determ. by titr. and calc. as Br)	46.8
	Calc.	OCH_3	17.6
	Found	»	18.4

The ratio halogen/OCH_3 is 1.01.
The product is evidently contaminated with the chloro derivative.

2-Bromo-1-(3,4-dimethoxyphenyl)-propene(-2)-one(-1) (VI)

1. 2,3-Dibromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (V) (2 g) was dissolved in acetone (20 ml). The solution was refluxed on a steam bath for one hour with anhydrous

potassium carbonate (2 g). The solution was then filtered and evaporated. The crystalline residue (1.2 g) was recrystallized from ethyl acetate. The substance obtained (0.6 g, yield 40 %) melted at 121—122°.

$C_9H_5OBr(OCH_3)_2$	Calc.	OCH_3	22.9	Br	29.5
	Found	»	22.6	»	29.9

2. From 3-acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (VII) the unsaturated bromo compound VI was similarly obtained. The yield was about 70 %.

1-(3,4-Dimethoxyphenyl)-propandione(-1,2) (IX)

1. 3-Acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (VII) (6.5 g) was heated with anhydrous potassium acetate (11.4 g) and acetic acid (30 ml) for 6 hours on a steam bath. Water was then added. After keeping for a few days in a refrigerator a yellow substance crystallized. The crystals were removed by filtration. By extraction of the mother liquor with chloroform, further amounts of the compound were obtained. Recrystallizations from ligroin yielded a product which had a m. p. of 69.5—70.5° (1.5 g, total yield 37 %). The compound contained 29.6 % methoxyl. The diketone IX requires 29.8 % methoxyl.

The compound gave no m. p. depression with the diketone IX (m. p. 71°), prepared by oxidation of 2-hydroxy-1-(3,4-dimethoxyphenyl)-propanone(-1) (VIII) with copper sulphate in pyridine [11], nor did the quinoxaline derivatives of these two samples give any depression in a mixed m. p. determination.

2. 2-Bromo-1-(3,4-dimethoxyphenyl)-propene(-2)-one(-1) (VI), treated in the same manner as the bromo acetoxy compound VII, gave the diketone IX in 80 % yield.

The quinoxaline derivative of 1-(3,4-dimethoxyphenyl)-propandione(-1,2) (IX)

The diketone IX and o-phenylendiamine were dissolved in alcohol-water (1 : 1) and heated to boiling for about one minute. On cooking the quinoxaline derivative crystallized. Recrystallization from methanol yielded a substance which had a m. p. of 117°.

$C_{15}H_{10}N_2(OCH_3)_2$	Calc.	OCH_3	22.1
	Found	»	21.8

Attempts to couple 3-acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (VII) with guaiacol

A mixture of the bromo acetoxy compound VII, a suitable base, guaiacol (x moles per a mole of VII, compare Table 1), and a solvent (only in the experiments 1 and 4) was heated on a steam bath for 2 hours with stirring.

The reaction mixture was diluted with water and extracted with chloroform or benzene (the latter solvent was used in those cases when the amount of bromide ion in the aqueous solution was to be determined). The extracted aqueous solution was acidified with nitric acid, and the bromide ion was precipitated with silver nitrate. The products recovered from the chloroform extract were examined in various ways.

Table 1 shows the yields of the substances obtained in these experiments.

In a substitution reaction bromide ion would of course be formed. Since the bromine atom of the unsaturated bromo compound is reactive, bromide ion could also be formed by hydrolysis of VI. For this reason the yield of bromide ion gives only a maximum value of the substitution.

In the experiments 2 and 3 no appreciable amounts of the substitution product, 3-acetoxy-2-(2-methoxy-phenoxy)-1-(3,4-dimethoxyphenyl)-propanone(-1), could be formed, as the yields of bromide ion were so low.

In experiment 4, on the other hand, the yield of bromide ion was high. By filtering a benzene solution of the reaction product through a column of aluminium oxide and evaporating the benzene, a yellow oil was obtained. The oil was dissolved in aqueous methanol and titrated against sodium hydroxide with phenolphthalein. The equivalent weight was 189 (that of the diketone IX is 208). By boiling a methanol solution of the oil and *o*-phenylendiamine for one minute, a precipitate of the quinoxaline derivative of the diketone IX was formed. Hence the oil was evidently impure diketone IX. Judging from the equivalent weight, the yield of the diketone IX was 45 %.

Table 1. The yields of the reaction products from coupling experiments with the bromo acetoxy compound VII and guaiacol.

Expt.	Moles of guaiacol per a mole of VII	Solvent	Base	The yields of %		
				Br^-	VI	IX
1	1	acetone	K_2CO_3		70	
2	53	no solv.	K_2CO_3	14		
3	53	» »	KOAc	17		
4	10	dioxane	MgO	76		45

Attempt to couple 2,3-dibromo-1-(3,4-dimethoxyphenyl)-propanone(-1) (V) with guaiacol

The bromo compound V was treated in the same manner as the bromo acetoxy compound VII in experiment 1 above. The only substance obtained was the unsaturated bromo compound VI. The yield was 40 %.

2-Hydroxy-2-ethoxy-1-(3,4-dimethoxyphenyl)-ethanone(-1)

Acetoveratrone (10 g) and selenium dioxide (11 g) were dissolved in dioxane (50 ml) containing water (2 ml). The reaction mixture was boiled for 4 hours on a steam bath. The separated selenium was filtered off, and the yellow solution evaporated in a vacuum at 40°. The residue was treated with water (75 ml) on a steam bath for 3 hours with constant stirring, and the resulting solution decolourized with charcoal, filtered, and concentrated to a small volume. The residue, a yellow oil, was dissolved in hot ethanol. On cooling, white crystals were obtained (7.4 g, yield 56 %, m. p. 60—64°). Recrystalization from ethanol yielded a product which had a m. p. of 69—71° (3.4 g, yield 25 %).

$C_{12}H_{16}O_5$	Calc. alkoxyl (as OCH_3) 38.7	C 59.9	H 6.66
	Found » » » 39.0	» 59.8	» 6.61

The quinoxaline derivative of 1-(3,4-dimethoxyphenyl)-ethandione(-1,2)

2-Hydroxy-2-ethoxy-1-(3,4-dimethoxyphenyl)-ethanone(-1) (0.5 g) was dissolved in water-ethanol (1 : 1, 10 ml). *o*-Phenylendiamine (0.5 g) was added and the solution boiled for a few minutes. On cooling, a crystalline product was obtained. Recrystallization from methanol yielded a substance, which had a m. p. of 120—121°.

$C_{16}H_{14}O_2N_2$	Calc.	N	10.5
	Found	»	10.4

SUMMARY

1. The following new substances have been synthesized (see Scheme 1): 2,3-dibromo-1-(3,4-dimethoxyphenyl)-propanone(-1), 3-acetoxy-2-bromo-1-(3,4-dimethoxyphenyl)-propanone(-1), 2-bromo-1-(3,4-dimethoxyphenyl)-propene-(-2)-one(-1), and 2-hydroxy-2-ethoxy-1-(3,4-dimethoxyphenyl)-ethanone(-1).

1-(3,4-Dimethoxyphenyl)-propandione(-1,2) and 1-(3,4-dimethoxyphenyl)-ethandione(-1,2) have been characterized as quinoxaline derivatives.

2. Attempts have been made to synthesize 2,3-dihydroxy-1-(3,4-dimethoxyphenyl)-propanone(-1) derivatives. They have, however, failed.

3. The bromine atom of 2-bromo-1-(3,4-dimethoxyphenyl)-propene(-2)-one(-1) is shown to react readily with mild hydrolytic agents with formation of 1-(3,4-dimethoxyphenyl)-propandione(-1,2).

The writer is indebted to *Statens Naturvetenskapliga Forskningsråd* and *Cellulosaindustriens Centrallaboratorium* for grants and to Miss Ulla Saedén and fil. kand. I. Åmark-Lindgren for their skilful assistence.

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Received March 19, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 650–657

The Crystal Structure of the Yellow Molybdic Acid, $MoO_3 \cdot 2H_2O$

On the Existence of an H_4O^{2+} Ion

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This study of the yellow molybdic acid $MoO_3 \cdot 2H_2O$ is a part of the series of investigations on polymolybdates, carried out at this institute. $MoO_3 \cdot 2H_2O$ is of special interest as a limiting compound in the series from normal molybdates to MoO_3 and its hydrates.

CRYSTALS

Crystals of $MoO_3 \cdot 2H_2O$ may be obtained mainly in three different ways. Thus it is formed after prolonged standing of HNO_3 solution of ammonium molybdate, which is used in the analysis of phosphorous. This product and that obtained by Rosenheim's [1] method are not pure, but contain small amounts of ammonium polymolybdates, as we have shown by powder photographs. A very pure acid is formed by the procedure described by Carpéni [2].

For this investigation a prismatic crystal was selected. Rotation and Weissenberg photographs were taken with Cu-K radiation around the a-axis ($h = 0$—4). The Weissenberg photographs indicated orthorhombic symmetry. The crystals have earlier been described as monoclinic [3] with $a : b : c = 1.0950 : 1 : 1.0664$ and $\beta = 90° 41'$ (*cf.* Groth [4]). The small difference of 41′ from 90° is very difficult to measure on single crystal photographs, but the symmetry of the intensities was obviously in agreement with an orthorhombic crystal. In order to get further information we also investigated powder photographs with Cr-K radiation in focusing cameras. The results are given in Table 1. Evidently the crystals are monoclinic, but it is not easy to detect any measurable differences in the intensities of hkl and $hk\bar{l}$. The cell dimen-

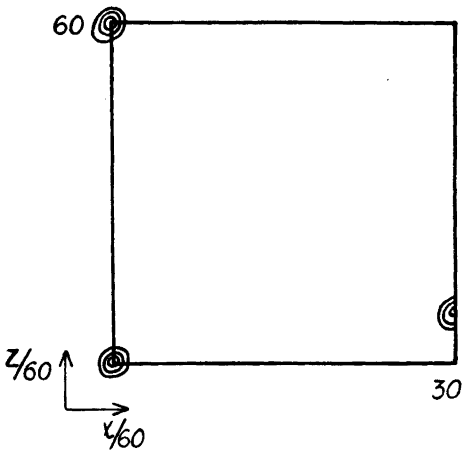

Fig. 1. P(x, 0, z).

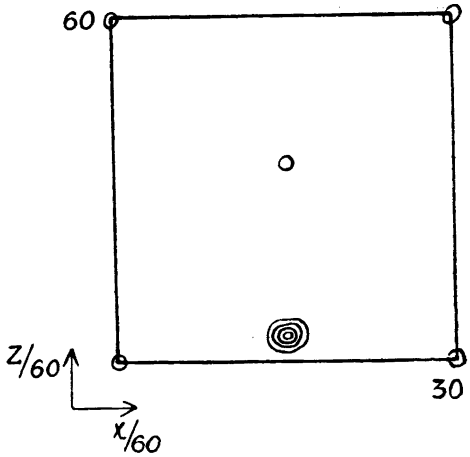

Fig. 2. ϱ (x, 0, z).

sions are $a = 3.77$ Å, $b = 6.91$ Å, $c = 7.34$ Å, and $\beta = 90° 40'$, (corresponding to $a : b : c = 1.09 : 1 : 1.06$ in the notation of Groth [4]). The density 3.124 [(3)] requires 2.02 formula units of $MoO_3 . 2H_2O$ in the cell. No $hk0$ reflections could be detected for h odd. Such a lack is, however, not typical for any monoclinic space-group, but must be due to the nearly orthorhombic structure. The only possible space-groups are C_2^1—$P\ 2$, C_s^1—$P\ m$ and C_{2h}^1—$P\ 2/m$. The crystallographic measurements [3,4] make the space-group C_{2h}^1—$P\ 2/m$ most probable.

The intensities were estimated visually and the values multiplied by $\cos^2 \mu \cdot \sin \Upsilon/(1 + \cos^2 2\Theta)$[(5)]. The absorption and temperature factors were neglected.

MOLYBDENUM POSITIONS

The positions of two Mo atoms in the unit cell are to be determined. They can be placed in two of the eight single-fold general positions 1(*a*)—1(*h*) or in one of the six two-fold positions 2(*i*)—2(*n*). 2(*i*)—2(*l*) should require a maximum in $P(0, y, 0)$ and 2(*m*)—2(*n*) in $P(x, 0, z)$. These cuts were therefore calculated. $P(0, y, 0)$ has only one maximum for $y = 0$, so the positions 2(*i*)—2(*l*) cannot be occupied by Mo atoms. In addition to the maximum in (0, 0, 0), $P(x, 0, z)$ (Fig. 1) has one other maximum in (30/60, 0, 8.5/60). As the coordinates for the general positions are 2(*m*) : $(\bar{x}, 0, \bar{z})$ $(x, 0, z)$ and 2(*n*) : $(x, \frac{1}{2}, z)$ $(\bar{x}, \frac{1}{2}, \bar{z})$ the values (30/60, 8.5/60) must correspond to $(2\,x_{Mo}, 2\,z_{Mo})$ giving $x_{Mo} = 15/60$ and $z_{Mo} = 4.25/60$. These parameters can easily be verified by a comparison with the intensities obtained. The lack of detect-

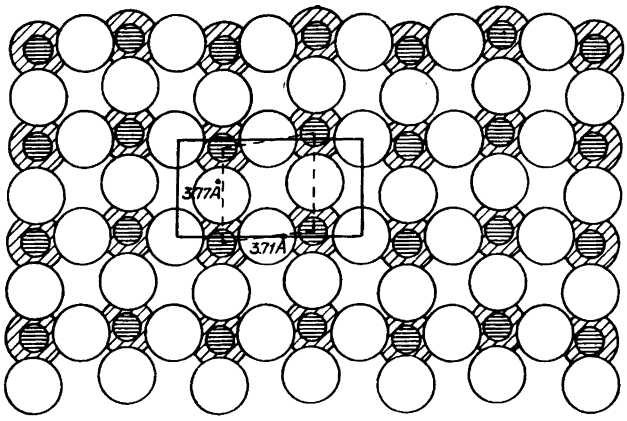

Fig. 3. MoO_4^{2-} *layer.*

Large circles = oxygen atoms
Small circles = molybdenum atoms
The oxygen atoms nearest to the spectator are omitted to show the underlying metal atoms.

able reflections $hk0$ for h odd is thus caused by $x_{Mo} = 15/60$. Of course the choice between $2(m)$ and $2(n)$ is quite arbitrary, and the two Mo atoms are placed in $2(m)$: $(x, 0, z)$ $(\bar{x}, 0, \bar{z})$ with $x = 15/60$ and $z = 4.25/60$. The signs of all F (hkl) could then easily be determined and ϱ $(x, 0, z)$ was calculated (Fig. 2). The Mo parameters definitely obtained are $x = 0.25$ and $z = 0.07$.

OXYGEN POSITIONS. MOLYBDENUM-OXYGEN COORDINATION

The Mo atoms in the planes $y = 0$ form distorted squares with the sides 3.71 Å and 3.77 Å. (Fig. 3). These distances agree very well with Mo—Mo and W—W distances obtained earlier for MoO_6 or WO_6 octahedra sharing corners [6-8]. As the zig-zag Mo—Mo strings are also found in WO_3[6], the structure may be built up of MoO_6 octahedra, which share corners. As a matter of fact oxygen maxima corresponding to such a structure occur in ϱ $(x, 0, z)$ (Fig. 2), although they are diffuse. It is not possible to fix the positions of these oxygen atoms more accurately by any method, so they have been placed halfway between neighbouring Mo atoms. The other two oxygen atoms may be situated at suitable distances on each side of the Mo atoms. It seems most reasonable to give these Mo—O distances values between 1.8 and 2.0 Å (the other Mo—O are about 1.86 Å and 1.89 Å). The parameters of the oxygen atoms will then be:

1 *O* in 1(*a*)	0, 0, 0
1 *O* in 1(*d*)	0.5, 0, 0
2 *O* in 2(*m*)	(x, 0, z) with $x = 0.25$ and $z = 0.57$
4 *O* in 4(*o*)	(x, y, z) with $x = 0.25$, $y = 0.27$, $z = 0.07$

This part of the structure is thus built up of parallel layers of MoO_6 octahedra sharing corners (Fig. 3). The formula of each layer is MoO_4^{2-}. The problem then remains of placing the additional oxygen atoms required by the formula $MoO_3 \cdot 2H_2O$. As the layers are identical, their unshared oxygen atoms are opposed to each other. If these atoms are given the distance 1.9 Å from Mo, their mutual distance is 3.1 Å, a little more than what corresponds to contact. The only space available for an ion with a radius larger than 1 Å is in the interstice between the eight oxygen atoms already placed in the cell, that is in 1(*f*): 0 ½ ½ and 1(*h*): ½ ½ ½. As a matter of fact maxima corresponding to these positions are found in ϱ (x, ½, z). The distances from such a central oxygen atom to its oxygen neighbours will then be $\sim$ 2.9 Å to four of them and $\sim$ 3.2 Å to the other four.

The structure finally obtained has a very great resemblance to the structure of $BaUO_4$[9]. The UO_4^{2-} ion forms layers very similar to those of MoO_4^{2-} and the Ba^{2+} ions are in contact with eight oxygen atoms, four of which are a little nearer to Ba^{2+}. In the determination of the structure of $BaUO_4$, space considerations were used to fix the positions of the oxygen atoms, so that the octahedral coordination is unequivocally correct.

EXISTENCE OF AN H_4O^{2+} ION

The great resemblance with $BaUO_4$ indicates immediately the possible existence of H_4O^{2+} ions in the crystal. However, such an ion has not been described previously, and other ways to distribute the four hydrogen atoms must be considered. Two of them may definitely be placed close to the central oxygen atom, forming H_2O, while the distribution of the other two must be discussed. At first it seems probable that they are attached to the MoO_4^{2-} layers, for example as hydroxyl bridges, giving $H_2MoO_4 \cdot H_2O$. Such a structure is a typical layer structure, having only neutral water molecules between the repelling layers. (Two oxygen atoms from different layers are pair by pair nearly in contact as described above.) In the habits of the crystals there are, however, no signs of cleavage, and the crystals have even been obtained as pseudo cubes (*cf.* Schulten [3]). This physical evidence seems to exclude definitely the $H_2MoO_4 \cdot H_2O$ structure theory. Another possibility would be to place the hydrogens between the repelling

Table 1. Powder photographs of $MoO_3 . 2H_2O$. Cr-K_α-radiation.

hkl	$10^4 \sin^2 \Theta$		*I* obs.
	calc.	obs.	
001	0919	0918	v st
200	0970	0973	st
020	1096	1095	v st
011	1193	1193	st
210	1244	1245	v st
111	1424	1435	v w
11$\bar{1}$	1447		diffuse
201	1866	1865	v st
20$\bar{1}$	1912	1911	v st
021	2015	2016	st
220	2066	2066	st
211	2140	2138	w
21$\bar{1}$	2186	2184	w
121	2250	2245	v w
030	2466	2470	v st

oxygens. Their distances (in any case larger than 3 Å) are, however, much too large to correspond to a hydrogen bond of this type, joining only two oxygen atoms. (Evans [10] gives the value 2.54 Å).

One explanation is that there are hydroxyl bridges in only one direction in the MoO_4^{2-} layers giving central H_3O^+ ions. Such an assumption cannot be definitely excluded, but there is no evidence in favour of this unsymmetrical. distribution of the hydrogen atoms, (*cf.* especially the analogy with $BaUO_4$).

All the hydrogen atoms may thus in some way be attached to the central oxygen atom. Then the question arises whether there are hydroxyl bonds between this central oxygen atom and its oxygen neighbours, or if there really exists an H_4O^{2+} ion. The distances are a little too large to be in agreement with earlier data on the hydroxyl bond length. (Evans [10] gives 2.7—2.8 Å.) The coordination of the oxygen atoms is, however, contradictory to the assumption of hydroxyl bonds, which requires a tetrahedral arrangement of oxygen around a central oxygen atom. Undoubtedly four of the oxygen atoms are nearer to the central atom than the others (2.9 and 3.2 Å), but the same distortion is found in $BaUO_4$[9] (2.7 and 3.0 Å), where no hydroxyl bond can exist. Consequently, we find the existence of an H_4O^{2+} ion to be the most reasonable explanation of all known facts. (The existence of H_3O^+ ion in

H_3OClO_4[11] has been established earlier by analogy with NH_4ClO_4). Of course a large amount of energy is required to attach an additional H^+ to H_3O^+. In this structure, however, there is a large gain of electrostatic energy, when an H_4O^{2+} ion is placed between the MoO_4^{2-} layers, which may be sufficient to explain its existence. Probably it can be considered as a rotating tetrahedral H_4O^{2+} ion (*cf.* NH_4^+). The acid will be written H_4OMoO_4. Possibly the yellow colour of the acid, unique among the molybdates, may be attributed to the H_4O^{2+} ion.

RELATIONS TO THE MOLYBDATES

The molybdates of well known structure with which a comparison can be made are the normal waterfree molybdates (*e. g.* $BaMoO_4$)[12] and the paramolybdates containing the ion $Mo_7O_{24}^{6-}$, described in two recent papers [13,14]. If we formulate the molybdic acid as H_4OMoO_4 it seems to be analogous to such compounds as $BaMoO_4$. The structure is, however, quite different, containing MoO_4^{2-} layers instead of discrete tetrahedral MoO_4^{2-} ions. This discrepancy is explained by the fact that H_4OMoO_4 is formed from highly acid solutions, where MoO_6 octahedra in some way sharing corners probably preexist (*v. infra*). The waterfree molybdates on the other hand often crystallise from solutions with pH > 7, which may contain tetrahedral MoO_4^{2-} ions [2]. (It must be mentioned in this connection that the structure of no hydrated normal molybdate is known.)

A comparison with the $Mo_7O_{24}^{6-}$ ion, which is rather compact and built up by MoO_6 octahedra preferentially sharing edges does not give any new relations. The equilibrium study by Byé [15,16] on the system Na_2O—MoO_3—H_2O reveals, however, some interesting facts, which may be combined with the knowledge of the two structures. Byé states, that in the highly acid region the polymolybdates are the stable compounds, and he indicates $MoO_3 \cdot H_2O$, $MoO_3 \cdot 2H_2O$ and MoO_3 as metastable. Of these three compounds the structure of MoO_3 is also known[6]. It contains zigzag rows of MoO_6 octahedra, which are coupled together by edges. These rows form layers by sharing corners. Transformations evidently take place rather easily in the solution which makes it possible for these two different compounds to be formed from very similar solutions. The transformation may have proceeded one step further to complexes with MoO_6 preferentially sharing edges, when the stable polymolybdates are formed, which probably in some way are derivatives of the paramolybdates. The most unstable compound is $MoO_3 \cdot H_2O$, which also may be built up by MoO_6 octahedra which share corners in some way. It is also interesting to study the relations to the tungstates. In WO_3[6] there is a three-dimensional net-work of WO_6 octahedra sharing corners. If this fact indicates that the

WO_6 octahedra have less tendency to share edges than the MoO_6 octahedra, it would also explain why there are only octatungstates but no deca- or higher tungstates described in the literature (*cf.* the great number of deca- and 16-molybdates).

These large ions of different kinds readily account for the high molecular weights obtained by diffusion [17] and dialysis [18] methods in very acid solutions, and for the polydispersity pointed out by Lamm [19]. A contradiction seems to arise in relation to the freezing point investigations [20,21], which indicate the existence of ions with 4 Mo atoms. It is, however, not possible to determine the molecular weights at high concentrations with freezing point methods, and probably the large ions are disaggregated to smaller complexes on dilution.

RELATIONS TO THE MOLYBDENYL ION

The reason why we also wish to direct attention to the solutions on the acid side of MoO_3 is in connection with a theory on oxygen bridgings put forward in a recent paper [22]. Electrochemical measurements combined with earlier crystal chemical data on several Bi compounds have led to the conclusion that in solutions on the acid side of $Bi(OH)_3$, there exist polynuclear complexes $Bi_nO_{n-1}^{(n+2)+}$, or $(BiO)_n^{n+}$. Such complexes are built up by Bi—O—Bi strings or as two-dimensional $(BiO)_n^{n+}$ sheets. It seems possible to apply similar discussions to acidified molybdic acid solutions, where there should be polynuclear complexes such as (MoO_{2n}^{2n+}) sheets. Of course such a theory must be verified by electrochemical measurements or X-ray investigations of molybdenum solutions, but it seems very probable.

SUMMARY

The crystal structure of $MoO_3 \cdot 2H_2O$ has been determined. It contains MoO_4^{2-} layers built up by MoO_6 octahedra, which share corners in two directions. In the interstices between the oxygen atoms of the parallel layers there are probably H_4O^{2+} ions, the existence of which has been discussed. The structure gives an answer to the main questions about the ionic conditions in very acid molybdate solutions, and it may also throw some light on the constitution of molybdenyl ions.

The author wishes to thank Miss Britta Svensson for valuable aid during the work. The Swedish Natural Science Research Council has supported the research financially, which support is gratefully acknowledged.

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Received March 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 658–665

Fine Structural Differences between Natural Cellulose Fibers as Revealed from Chain Length Distributions of Hydrolyzed Materials

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In previous publications [1,2], it was shown that during heterogeneous hydrolyses wood pulps behaved quite differently from cotton and cotton linters. Compared with the latter the degradation of pulps was considerably slower when measured as decrease in average degree of polymerisation (DP). The limit DP values, *i. e.* the constant DP's reached after prolonged time of hydrolysis, was higher for the pulps and greatly dependent upon the severity of the pulping conditions. For pulps swelled in alkaline solutions of increasing strength the rate of fall in DP increased and the limit DP decreased gradually, whereas the hydrolysis behavior of cotton remained unchanged until the alkali concentration was sufficient to cause intracrystalline swelling. A different arrangement in the less ordered regions and especially in the transition regions of the two fiber species was proposed as an explanation for these dissimilarities.

More information regarding these divergences might be acquired from knowledge of the chain length distribution diagrams of fibers degraded to their limit DP's. These diagrams will disclose the spread in length distribution of acid resistant fragments and also, with the reservations outlined below, of the ordered areas in the original fibers. As mentioned, the limit DP of wood fibers is sensitive to treatment with alkaline solutions which cause intercrystalline swelling only. It has recently been shown [3], that at the conditions employed in this investigation, intracrystalline swelling of pulp fibers starts by treatment with 6.5 per cent sodium hydroxide solution. To discover the changes in the intercrystalline domains of wood fibers causing the decrease in limit DP, distribution diagrams will have to be determined for samples swelled in alkaline solutions varying between zero and six per cent. Changes in the size

of ordered regions originating from intracrystalline swelling might be obtained from wood fibers swelled in eight or ten per cent and cotton linters swelled in ten per cent lye.

The present paper deals with such an investigation for a spruce sulfite pulp, an aspen holocellulose and cotton linters. The experimental findings will be seen to support the earlier proposed differences in the fine structural arrangements of purified wood and cotton fibers.

MATERIALS AND METHODS

The spruce pulp was an extracted sulfite pulp of relatively high DP (1650), carefully bleached with sodium chlorite to negligible lignin content. The aspen holocellulose was prepared from air dried chips, which were disintegrated in a Wiley-type mill and screened. The 20—40 mesh fraction was separated and air dried and holocellulose prepared according to the procedure described by Wise *et al.*[4]. Four chlorite treatments were found necessary to bring the lignin content to a low value of about one per cent. The cotton linters was an extracted acetate grade linters from Hercules Powder Co. All extractions were made with alcohol-benzene (1 : 2) for 24 h.

The swelling in sodium hydroxide solutions and the subsequent hydrolysis were as described earlier [2]. Nitration, fractionation of the nitrates in acetone solution and construction of the weight frequency distribution curves were carried out in the manner previously outlined in detail [1]. The DP's of the fractions were calculated from the intrinsic viscosities of the nitrates in acetone solutions using the Staudinger equation and $K_m = 10^{-3}$, c in g/l. The fractionation yielded 40—60 fractions of which 30—40 showed different intrinsic viscosities.

EXPERIMENTAL DATA AND DISCUSSION

Fig. 1 shows the weight frequency distribution curves for the hydrolyzed spruce pulp samples previously swelled in 0 (water) — 2—6 and 10 per cent sodium hydroxide solutions, respectively. Fig. 2 gives the similar curves for the aspen holocellulose samples swelled in 0—4—6 and 8 per cent sodium hydroxide and Fig. 3 represents the distribution curves for cotton linters swelled in 0 and 10 per cent lye and hydrolyzed. The loss in material during swelling and hydrolysis as well as the average DP's of the fractionated samples are found in Table 1.

On examination of the changes in the chain length distributions of the spruce pulp it is seen that the water swelled sample exhibits a rather heterogeneous distribution. A high, narrow peak is present at a DP of about 30, then comes another, but lower and broader maximum at about 130—150, followed by a low broad maximum at the 230 level. The most distinctive feature of the curve, is, however, the very long right hand tail extending to DP values of at least 7—800. The amount of material above D.P. 300 comprises almost 30 per cent of the total. The distribution curve for the two per cent

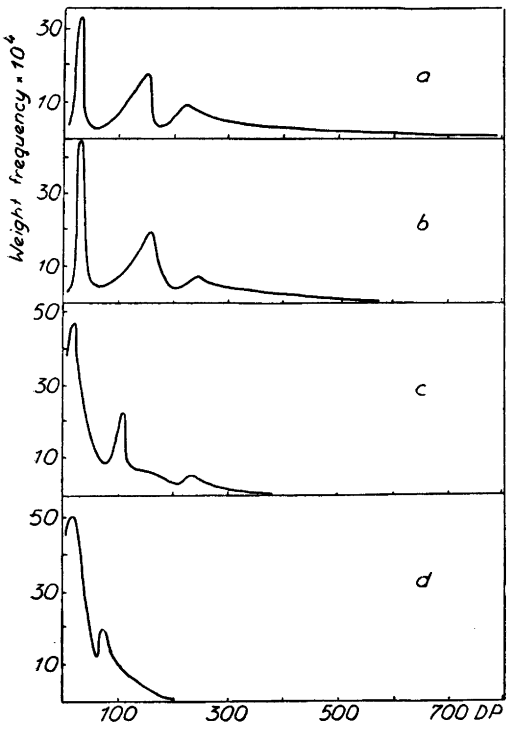

Fig. 1. Weight frequency distribution curves for hydrolyzed spruce pulp previously swelled in: a: Water, b: 2 per cent NaOH, c: 6 per cent NaOH, d: 10 per cent NaOH.

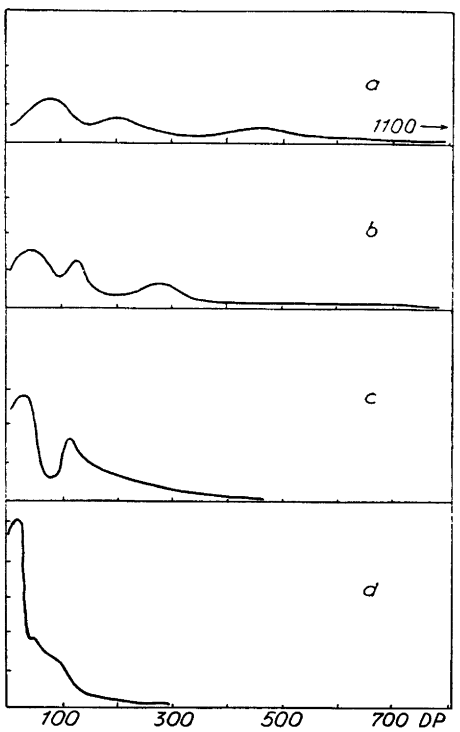

Fig. 2. Weight frequency distribution curves for hydrolyzed aspen holocellulose previously swelled in a: Water, b: 4 per cent NaOH, c: 6 per cent NaOH, d: 8 per cent NaOH.

sample has a somewhat larger peak at DP 30 and a broader peak at the 150 level as compared with the water swelled sample. The peak at DP 230 has diminished somewhat and the right hand tail comprises now less material and does not extend to the same high DP as it did in the first curve. Chains with DP of over 600 are, however, still present. A pronounced change has occured in the distribution of the six per cent sample. The maximum at DP 30 has increased considerably and comprises now 55—60 per cent of the material. The broad maxima found at DP 150 in the two previous distributions has moved to a lower DP of about 100—110 and is rather narrow. A small peak is still found at the 230 DP level. The tail to the right has now diminished considerably and comprises only 4—5 per cent of the material. No chains are found with DP above 350—400. As far as the amount of low DP material is concerned the ten per cent sample does not seem to be much different from the six per cent sample. The first maximum contains again about 60 per cent of the total but is somewhat narrower compared with the similar maximum for the six per cent material. The maximum previously at 110 has moved still

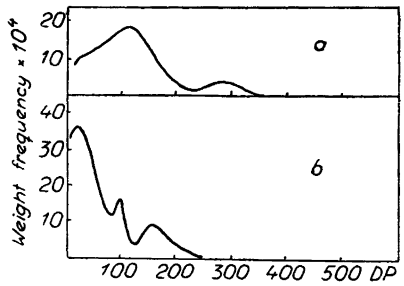

Fig. 3. Weight frequency distribution curves for hydrolyzed cotton linters previously swelled in: a: Water, b: 10 per cent NaOH.

further to the left and is found at a DP of 75. The extensive right hand tail has disappeared altogether and the longest chains have a DP of 175—200.

The water swelled aspen holocellulose exhibits a very large breadth of distribution (Fig. 2 a) with no such marked maximums as found for the similar spruce sample (Fig. 1 a). The extensive right hand tail is even more marked, reaching to DP 1100. A flat maximum is found at DP 450. Two broad modes are present in the low DP range, one containing some 20 per cent of the material at a DP of 100 and a smaller one at DP 200. The changes in the distribution caused by swelling in a four per cent lye are not very large (Fig. 2 b). The tail to the right is not so extensive and does not go beyond DP 800 and only ten per cent has a DP above 400. The small maximum previously at 450 is now at DP 275. The two peaks in the low DP range have moved to the left and increased somewhat in size. The first is seen at DP 60 and the second at DP 130. A striking alteration

Table 1. Material dissolved during swelling and hydrolysis and limit DP values for fractionated samples.

Material	Dissolved during swelling, %	Dissolved during hydrolysis, %	Total dissolved, %	Limit DP
Spruce pulp, swelled in:				
water	0	16.1	16.1	270
2 % NaOH	2.5	14.1	16.6	220
6 » NaOH	11.8	16.4	28.2	105
10 » NaOH	16.3	17.8	34.1	50
Aspen holocellulose, swelled in:				
water	0	—	—	315
4 % NaOH	30.4	20.0	50.4	240
6 » NaOH	32.6	19.2	51.8	130
8 » NaOH	28.6	28.1	56.7	60
Cotton linters, swelled in:				
water	0	7.3	7.3	135
10 % NaOH	2.2	13.1	15.3	75

has occured in the distribution curve of the six per cent sample. The long right hand tail has diminished considerably and reaches to DP 450 only. The main maximum is found at DP 30—35 and comprises 45 per cent of the total. Another maximum with a pronounced skewness to the right is seen at DP 100—110. The distribution is not too different from that of the six per cent spruce material. In the latter more material is concentrated in the first maximum (Fig. 1 c). This similarity is even more marked when the two fibers have undergone intracrystallin swelling, as for the eight per cent aspen and ten per cent spruce material (Fig. 1 d and Fig. 2 d). The peak at DP 25—30 comprises 55—60 per cent of the total. No other peak is found in the curve.

The water swelled cotton linters (Fig. 3 a) show a chain length distribution which in one respect differs markedly from those exhibited by the corresponding purified wood fibers. The long tail to the right so peculiar to the distribution of those fibers is not found in the curve for cotton linters. A rather small maximum is seen at DP 275 and the longest chains have a DP of about 375, compared with 800—1 100 for the wood fibers. A broad maximum with its peak at a DP of 110—125 comprises almost 85 per cent of the material. This part of the curve is somewhat similar to the distribution of the aspen holocellulose (Fig. 2 a). However, in the latter it includes 45 per cent only, of the total. In contrast to the spruce pulp (Fig. 1 a) the water swelled samples of cotton and aspen holocellulose (Fig. 2 a and Fig. 3 a) do not show any maximum in the very low DP range of 25—30. The distribution curve for the intracrystalline swelled cotton linters (Fig. 3 b) is rather similar to those of the corresponding wood fibers (Fig. 1 d and Fig. 2 d). A broad maximum comprising about 60 per cent of the chains is found at DP 25—30. Another minor peak is seen at DP 100 and still another low maximum at DP 130. The material does not seem to contain chains with DP above 250.

Cellulose chains may terminate within acid resistant aggregates and consequently the chain length distribution diagrams would not be identical with the length distribution of these fragments. However, the maximum length of the acid resistant fragments correspond at least to the length of the longest cellulose chains, and accordingly the amount of long chain material is a good indication of the amount of the longest aggregates. It is also reasonable to assume that pronounced peaks in the chain length diagrams correspond to similar peaks in the size distribution of the fragments. Regarding peaks in the lowest DP range it could be reasoned that these very short chains mostly end within aggregates. However, this does not necessarily always have to be the case. When comparing the size distribution of acid resistant fragments with that of the ordered domains in the fibers one must consider the changes which take place during hydrolysis. "The recrystallization" occuring when the cellulose chains are disrupted [5—7] might be expected to lead to a certain increase lengthwise in these domains. This increase might, however, be small and not serious if we realize that we are dealing with highly approximate values.

Interpreted on this basis it is evident that the purified, not alkali swelled wood fibers contain acid resistant aggregates of considerable length including cellulose chains with 800—1100 glucoseanhydride units or 4—5000 Å long,

which is several times the frequently considered average size of crystalline regions, 5—600 Å[8–10].

Upon swelling in alkaline solutions these long aggregates are made acid susceptible and a gradual decrease in their length takes place upon treatment in two, four and six per cent lyes.

The permanence of the x-ray diagrams has been established for wood pulp fibers swelled in alkaline solutions of this strength range [3]. No new maxima are observed in the distribution curves for samples swelled in lyes of these concentrations compared with the water swelled samples. This is especially pronounced for the spruce pulp. These findings indicate strongly that the very long aggregates found in the unswelled fibers consist of well arranged "crystalline" regions interconnected by throughgoing chains grouped in arrangements of somewhat lower order but still able to resist or greatly delay attack under the hydrolysis conditions applied. No such arrangements are present in the intercrystalline areas of cotton linters. The distribution diagrams for linters do not change upon swelling in lyes of these intermediate concentrations.

The resistance of the long aggregates is greatly dependent upon the conditions during hydrolysis. It has been shown [2] that the limit DP value change with the pulping conditions. We have also found that hydrolysis with stronger acids, especially if they exert greater swelling than the sulfuric acid applied in this investigation, will yield a lower limit DP. On the contrary weaker acids or milder conditions, *e. g.* lower temperature, lead to higher limit DP values for the wood fibers.

As mentioned wood pulp swelled in a six per cent lye still retains an unchanges x-ray diagram. Samples swelled in 6.5 per cent alkaline solutions will show sign of intracrystalline swelling. In six per cent samples all but the crystalline domains, as defined by x-ray diffraction, should therefore have undergone swelling, and in consequence the greater part of the intercrystalline acid resistant arrangements destroyed. The intercrystalline domains in the six per cent pulp fibers should hence be available for acid attack similar to that experienced by the linters. It was also found that the drop in DP for a six per cent wood pulp closely followed the course of cotton linters [2].

The length distributions of the acid resistant fragment in six per cent wood fibers and in linters show, however, a great variance. In the wood fibers an accumulation of very small fragments with a length of 100—200 Å is observed. No such accumulations is found in the linters. In spruce this part amounts to 60 per cent and in aspen to 45 per cent of the total. It seems reasonable to assume that regions of this dimension make out a considerable part of the highly ordered domains in wood fibers.

Several important differences in physical properties of wood fibers and cotton may be intimately connected with this large amount of very small crystalline regions in the former material. The easier mercerizing [3,11] of wood pulps compared with cotton might be due to an easier intracrystalline swelling of small crystalline regions when interconnected by long cellulose chains. In spite of similarity in amount of crystalline materials [12] the water regains of pulps is higher than for cotton, and this could very well be related, to the larger surface of many small compared with the smaller surface of fewer and larger crystallites. The higher constant rate of loss in material during hydrolysis of wood fibers may also be traced to their larger crystalline surface.

For all three materials, spruce, aspen and cotton fibers, the hydrolysis of the intracrystalline swelled samples leads to a considerable accumulation of small fragments 100—200 Å long only. In all three cases fragments of this size amount to about 60 per cent of the total. For the wood fibers this accumulation is a result of a continous change as it was found that the intercrystalline swelled samples contained already an agglomoration of these very small aggregates. For the linters the appearance of a large amount of small fragments is much more abrupt.

Considering the small differences in loss of material during hydrolysis of intracrystalline and intercrystalline swelled materials (Table 1) it might be assumed that the accumulation of these small fragments has been brought about through division of the larger ordered domains. This assumes that intracrystalline swelling has made the ordered regions acid susceptible at certain places, where the cellulose chains now may be disrupted. A final consequence of such a hypothesis would be that the areas exhibiting a true crystalline order are very small (100—200 Å), and that it is the arrangement in the regions between these small true crystallites which differs in various fibers, being of a highly gradual and diversified nature in wood fibers and exhibiting more abrupt changes in cotton fibers.

It is in this connection interesting to note that the average length of the hydrolysis products of regenerated cellulose fibers [13,14] compare well with the pronounced peak in the fragment size of the intracrystalline swelled materials.

The theory put forward above is in need of much more definite experimental evidence before it in any way should be regarded as established. The interpretation of the chain length distribution data do, however, strongly support and advance the earlier proposed fine structural difference between wood fibers and cotton.

SUMMARY

Spruce sulfite pulp, aspen holocellulose and cotton linters have been swelled in alkaline solutions varying from zero to ten per cent concentration and subsequently hydrolyzed. Chain length distribution determinations have been carried out on the hydrolyzed materials. Evaluation of the distribution curves show that the fine structure of purified wood fibers differs from that of otton linters by

1) the presence of well arranged chains interconnecting the highly ordered regions and resistant towards acid hydrolysis if not swelled in alkaline solutions

2) the presence of a large amount of very small highly ordered regions.

Upon hydrolysis all intracrystalline swelled fibers show an accumulation of very small fragments, 100—200 Å long. These are most probably formed through division of longer well ordered regions suggesting a subdivision of the latter into smaller perhaps more fundamental units.

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Received April 1, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 666–674

Studies on N-Substituted Barbituric Acid Derivatives. I

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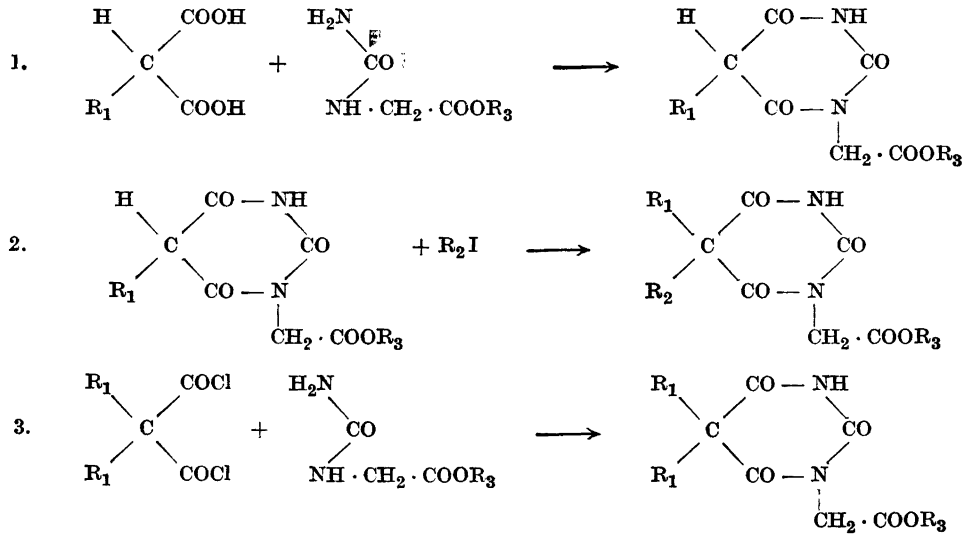
The pharmacological properties of a series of 1-carbethoxymethyl-5 : 5-dialkyl-substituted barbiturates have been described by Sandberg [1].

The syntheses and chemical properties of these derivatives and their parent compounds are described in the present paper.

The syntheses of the required substances were achieved according to the following scheme:



where R_1 = H, alkyl radicals with 1 to 5 C-atoms, or the phenyl group,
R_2 = allyl radical,
R_3 = alkyl radical with 1 to 3 C-atoms.

Part I of this paper deals with reaction 1 and some by-products, and Part II with reactions 2 and 3 and the final products.

EXPERIMENTAL

Syntheses

Malonic acid was condensed with hydantoic acid alkyl ester in the presence of $POCl_3$, on a water-bath (30 min), according to Rosén [2] to yield 1-carbomethox ymethyl-barbituric acid and its homologues (substances 1—3 in Table 1).

These compounds are soluble in hot water and alcohol, less soluble in benzene, and sparingly soluble in ether. Since they react as monobasic acids, their equivalent weights could be determined by titration with 0.1 *N* sodium hydroxide. Table 1 gives the equivalent weights and melting points.

1-Carbomethoxymethyl-barbituric acid (1) Found: N 14.01 per cent; $C_7H_8O_5N_2$ requires N 14.00 per cent.

1-Carbisopropoxymethyl-barbituric acid (3) Found: N 12.21 per cent; $C_9H_{12}O_5N_2$ requires N 12.28 per cent.

A few points concerning this synthesis are worthy of note. It was observed that if ten times or more of the usual quantities (0.05 moles of malonic acid, 0.10 moles of hydantoic acid alkyl ester and 0.055 moles of $POCl_3$) were used, the substances 1—3 were obtained only to a very small extent, and the main product was 1-carbomethoxymethyl-5-acetyl-barbituric acid and its homologues (derivatives 15—17 in Table 2).

If, on the other hand, equivalents of malonic acid and hydantoic acid alkyl ester were condensed, the compounds 15—17 were obtained as the main products, regardless of the quantities used.

In this case the yellow reaction mixture, after cooling, was a hard mass. This was crushed, washed with cold water and dried at room temperature. 5 g of this product was mixed with 50 ml of water. On heating to 70—80° C, an almost clear solution was obtained. After some minutes at this temperature, CO_2 was liberated, the solution became turbid, and a light-yellow precipitate appeared. This was filtered off and dissolved in alcohol, and the alcoholic solution was treated with charcoal. Repeated crystallizations from alcohol or benzene gave the pure substances 15—17.

These derivatives are insoluble in cold water, sparingly soluble in boiling water, and easily soluble in hot alcohol, benzene, and ether. As they are acids, the equivalent weights were determined by titration with 0.1 *N* sodium hydroxide (phenolphthalein as indicator). The equivalent weights and melting points are given in Table 2.

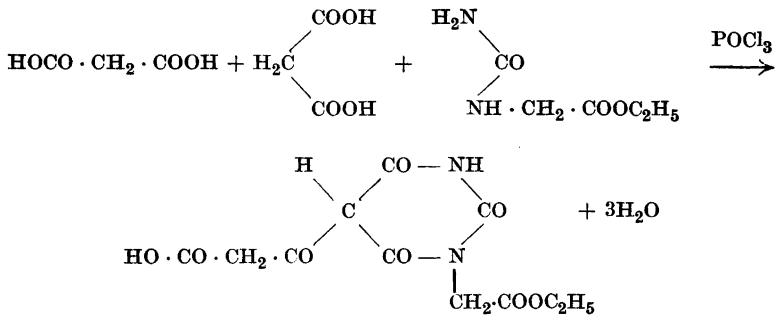
1-Carbomethoxymethyl-5-acetyl-barbituric acid (15). Found: N 11.54 per cent; $C_9H_{14}O_6N_2$ requires N 11.57 per cent.

1-Carbethoxymethyl-5-acetyl-barbituric acid (16). Found: C 46.70; H 4.74; N 10.95 per cent; $C_{10}H_{16}O_6N_2$ requires C 46.88; H 4.72; N 10.94 per cent. With 2:4-dinitrophenylhydrazine it gave a hydrazone melting at 247° C.

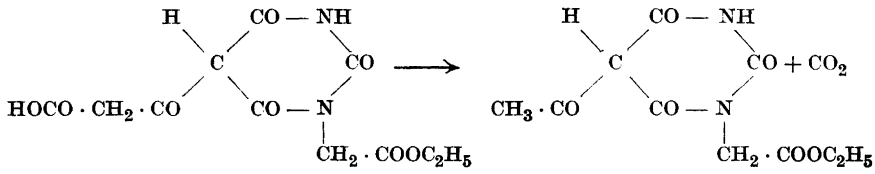
1-Carbisopropoxymethyl-5-acetyl-barbituric acid (17). Found: N 10.33 per cent; $C_{11}H_{18}O_6N_2$ requires N 10.37 per cent.

These compounds show an exceptional stability. They are not decomposed by boiling in water for one hour, and on acidification of their alkaline solutions (many hours old) they reprecipitate. These properties and the formation of a hydrazone indicate the 5-position of the acetyl group.

The first step in the formation of the 5-acetyl derivatives might be:



As mentioned earlier, this intermediate product is decomposed in water solution at about 80° C as follows:



The 5-acetyl derivatives were also obtained by the condensation of 1-carbomethoxymethyl-barbituric acid and its homologues with malonic acid in the presence of $POCl_3$.

These results are analogous with the findings of Conrad and Guthzeit [3], who obtained 5-acetyl-barbituric acid as a by-product in the synthesis of barbituric acid from malonic acid, urea, and $POCl_3$.

The structure of the substances 15—17 will be discussed again in connection with their ultra-violet absorption spectra.

The condensation of monoalkyl-substituted malonic acid with hydantoic acid alkyl ester was achieved by the two methods given by Rosén [2]:

1. Condensation in the presence of acetic anhydride at 60—70° C for 4 hours.
2. Condensation in the presence of $POCl_3$ on a water-bath for half an hour.

Table 1.

No.	Derivatives	M. p. uncorr.	Equiv. wt.		In 0.1 *N* NaOH	
			found	calc.	ε_{max}	Å
1	1-Carbomethoxymethyl-barbituric acid	168—169°	201.0	200.1	17 230	2 600
2	1-Carbethoxymethyl- » » *	137—138°	214.5	214.1	16 530	2 600
3	1-Carbisopropoxymethyl- » »	123—124°	229.3	228.1	16 100	2 600

* This substance is described by Rosén [2].

Table 2.

No.	Derivatives	M. p. uncorr.	Equiv. wt.		In 0.1 *N* NaOH	
			found	calc.	ε_{max}	Å
4	1-Carbethoxymethyl-5-methyl- barbituric acid *	137—138°	228.6	228.1	18 120	2 700
5	1-Carbomethoxymethyl-5-ethyl- » »	94— 95°	228.1	228.1	18 140	2 700
6	1-Carbethoxymethyl-5-ethyl- » » *	97— 98°	242.0	242.1	17 500	2 700
7	1-Carbisopropoxymethyl-5-ethyl- » »	95— 96°	257.0	256.1	16 300	2 700
8	1-Carbethoxymethyl-5-*n*-propyl- » »	138—139°	257.0	256.1	4 950	2 700
9	1- » -5-*iso*propyl- » »	80°	255.6	256.1	18 250	2 700
10	1- » -5-*n*-butyl- » »	124—125°	271.9	270.1	4 040	2 700
11	1- » -5-*sec*-butyl- » »	107—108°	272.4	270.1	—	—
12	1- » -5-*iso*-butyl- » »	100—101°	272.0	270.1	16 960	2 710
13	1- » -5-*iso*-amyl- » »	119—120°	285.8	284.1	4 140	2 700
14	1- » -5-phenyl- » »	142°	291.3	290.1	17 160	2 680
15	1-Carbomethoxymethyl-5-acetyl- » »	146—147°	241.1	242.1	13 700	2 810
16	1-Carbethoxymethyl-5-acetyl- » »	142—143°	256.7	256.1	12 900	2 810
17	1-Carbisopropoxymethyl-5-acetyl- » »	166—168°	271.8	270.1	11 700	2 810
18	1-Carbethoxymethyl-5-allyl- » »	oil	—	254.1	—	—
19	1-Carbethoxymethyl-3-acetyl-5-ethyl- » » *	184—187°	—	—	19 550	2 700
20	1-Carbethoxymethyl-3-acetyl-5-*n*-propyl- »	159—161°	—	—	18 400	2 700

* This substance is described by Rosén [2].

Derivatives 4—14 and 18 were obtained by both methods. Their melting points and equivalent weights are given in Table 2.

These compounds were crystallized from alcohol or benzene. They are also soluble in ether but only very slightly soluble in water. Difficulties were encountered in the crystallization of the higher homologues, but all but one were finally obtained in a crystalline form.

The following comments may be made on certain of the preparations involved. In synthesizing derivative 9, 1-carbethoxymethyl-5-isopropyl-barbituric acid, a by-product was obtained using the $POCl_3$-method. This proved to be: *2-methyl-buturyl-hydantoic acid ethyl ester,* $CH_3 \cdot \underset{\displaystyle CH_3}{\overset{\displaystyle |}{CH}} \cdot CH_2 \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOC_2H_5$

Found: C 52.3; H 7.81; N 12.05 per cent; $C_{10}H_{18}O_4N_2$ requires: C 52.2; H 7.89; N 12.17 per cent.

Crystallized from aqueous alcohol (30 per cent), the ester gave glistening plates, m. p. 125 to 127° C. It is also soluble in benzene and ether, and slightly soluble in boiling water.

It is obvious that in this case the *iso*propyl-malonic acid had been partly decomposed, giving an aliphatic compound on condensation with hydantoic acid ethyl ester.

Recrystallization of 1-carbethoxymethyl-5-phenyl-barbituric acid (14) from benzene sometimes gave a substance melting at about 100° C. This, however, was easily converted into the form melting at 142° C.

1-Carbethoxymethyl-5-allyl-barbituric acid (18) occurred as a viscous oil, which failed to crystallize. The product obtained has not been further investigated.

In the preparation of the derivatives 6 and 8 according to the acetic anhydride method, their corresponding 3-acetyl derivatives (19—20) were obtained as by-products.

As the substances 19 and 20 are insoluble in ether, they could easily be separated from their ether-soluble parent compounds. They were crystallized from alcohol or benzene. They are also soluble in alkali with decomposition.

1-Carbethoxymethyl-3-acetyl-5-n-propyl-barbituric acid (20)

Found: N 9.37 per cent; $C_{13}H_{18}O_6N_2$ requires N 9.38 per cent.

We have not succeeded in isolating the higher homologues of these 3-acetyl derivatives, although they were probably formed.

To obtain the sodium salts of compounds 2, 9, 12, and 13, the acids were dissolved in equivalent quantities of alcoholic sodium hydroxide. The sodium salts were precipitated on adding a considerable excess of ether. The sodium salts, however, deliquesced in the atmosphere.

Ultra-violet absorption spectra

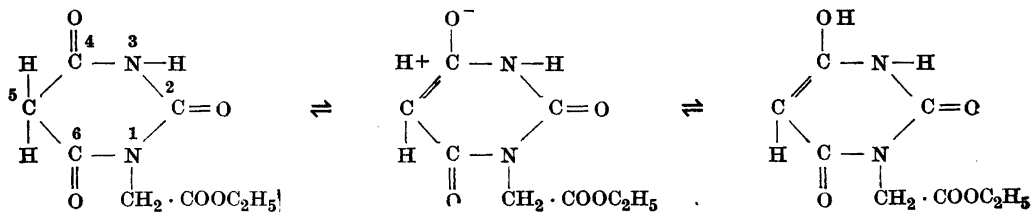
Measurements were made with a Beckman quartz Spectrophotometer. The molecular extinction coefficient ε is defined by the relation $\log I_0/I = \varepsilon \cdot c \cdot l$, where c is the molar concentration and l is the length of the tube in cm. In making the solutions, recently distilled, carbon dioxide-free water was used, and the solutions were examined immediately against blanks.

DISCUSSION

Fig. 1 shows the absorption spectra (in water) of 1-carbethoxymethyl-barbituric acid (2) under varying conditions. The concentration of all solutions is $4.324.10^{-5}$ molar.

The carbonyl groups in the molecule will act as chromophores and produce a small absorption. Tautomerism, however, producing an enolized structure with an olefinic linkage, would cause a much larger absorption than that due to the carbonyl groups.

From the absorption curves in Fig. 1 and the findings of Fredholm [4] and Stuckey [5] on the tautomerism of 1-methyl-barbituric acid, it can be concluded that the following mechanism is operative:



I ⇌ II ⇌ III

where only the hydrogen of the active methylene group in the 5-position is involved in the keto-enol tautomerism.

Two small peaks of low extinction value (approx. 1850) at 2750 Å and 2580 Å and a notable end-absorption are present in solutions of 0.1 *N* hydrochloric

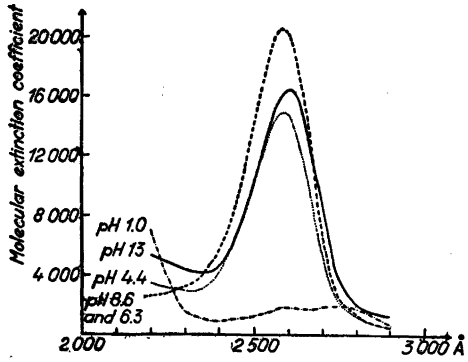


Fig. 1. Absorption spectra of 4.324 · 10^{-5} molar solutions of 1-carbethoxymethyl-barbituric acid at varying pH.
pH 1 : In 0.1 N hydrochloric acid.
pH 4.4: Pure aqueous solution.
pH 6.3: Two equivalents of sodium hydroxide added.
pH 8.6: Three equivalents of sodium hydroxide added.
pH 13 : In 0.1 N sodium hydroxide.

acid. The pure aqueous solution (pH 4.4) shows a marked increase in absorption, producing a peak at 2580 Å (ε_{max} = 15 000). The curve for the solution with one equivalent of NaOH added (pH 5.5), *i. e.* corresponding to a solution of the monosodium salt, shows a further augmentation of absorption, ε_{max} being 19 200 at 2580 Å (this curve is not drawn in Fig. 1). The addition of two and three equivalents of NaOH to the solution (pH 6.3 and 8.6) — which would correspond to the disodium and trisodium salts, if these exist — gave the same curve, the highest extinction coefficient figure being 20 600 at 2580 Å. In 0.1 *N* sodium hydroxide the absorption is decreased to ε_{max} = 16 500 at 2600 Å.

This shift of the band to a longer wave-length in strong alkaline solution might be due to the asymmetrical structure of the molecule, which is probably accompanied by a pronounced electro-asymmetry.

The interpretation of these curves may be the following: The strong band at 2580 Å is necessarily due to the ion II. At pH 1, the equilibrium has shifted and the keto-form I is mainly present (represented by the peak at 2750 Å), but the ion II is still present to a lesser extent (the peak at 2580 Å). In aqueous solution (pH 4.4), an intermediate equilibrium position is found where the enol-form II predominates. In slight alkaline solution (pH 8.6), the enolization rises to its maximum.

The decrease in absorption at pH 13 is due to the formation, to a certain extent, of the compound III as the sodium salt. This involves the assumption that the absorption of the undissociated sodium salt is less than that of the enolic ion II.

Reference to Table 1 shows that in 0.1 *N* sodium hydroxide the derivatives 1—3 all have the selective absorption at 2600 Å, the molecular extinction coefficient being decreased as the number of C-atoms in the side-chain in the 1-position increases.

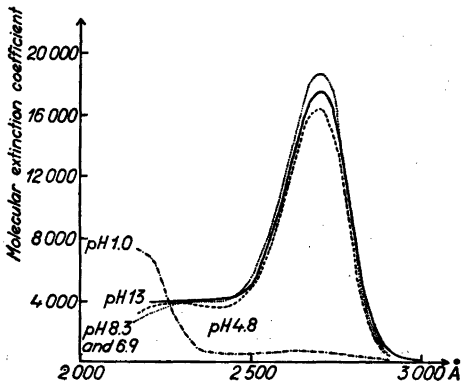
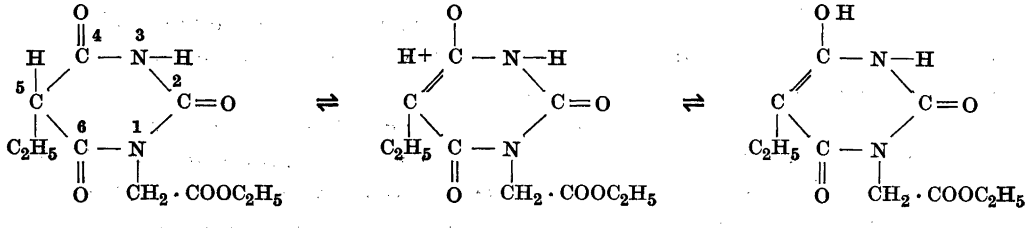


Fig. 2. Absorption spectra of 4.482 · 10^{-5} molar solutions of 1-carbethoxymethyl-5-ethyl-barbituric acid at varying pH.
pH 1 : In 0.1 N hydrochloric acid.
pH 4.8: Pure aqueous solution.
pH 6.9: One equivalent of sodium hydroxide added.
pH 8.3: Two equivalents of sodium hydroxide added.
pH 13: In 0.1 N sodium hydroxide.

5-Monosubstituted derivatives were next studied. These compounds still have a free hydrogen in the 5-position, and it is very probable that this will also undergo enolization.

Fig. 2 shows the absorption spectra of $4.482.10^{-5}$ molar solutions of 1-carbethoxymethyl-5-ethyl-barbituric acid (5) at varying pH. Examination of the curves shown in Fig. 2 reveals striking similarities with those in Fig. 1. From the close resemblance of the absorption spectra of the two derivatives at varying pH, it can be concluded that a similar tautomerism occurs, namely:



The enolic ion V gives a strong band at 2700 Å, ε_{max} being 18 600 at pH 8.3 (the curve at pH 6.9 not being measurably different from that of the absorption at pH 8.3). At pH 13, the compound VI is formed to a certain extent (ε_{max} = 17 500 at 2700 Å). A small peak at 2650 Å indicates the presence of the keto-form IV at pH 1, and the absorption in aqueous solution (pH 4.8) shows an intermediate equilibrium position (ε_{max} = 16 300 at 2700 Å) between IV and V.

The 5-monosubstituted derivatives in Table 2 (with the exception of 15—17: see the following) all have a peak at approximately 2700 Å. In the compounds 5—7, ε_{max} is diminished when the number of C-atoms in the sidechain

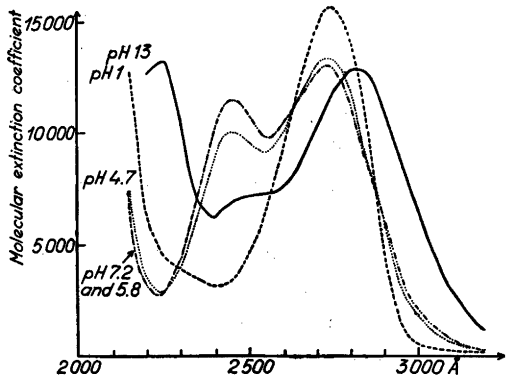


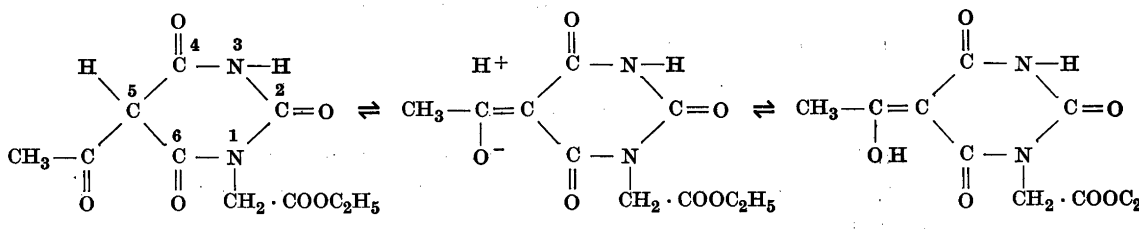
Fig. 3. Absorption spectra of 3.237 · 10⁻⁵ molar solutions of 1-carbethoxymethyl-5-acetyl-barbituric acid at varying pH.
pH 1: In 0.1 N hydrochloric acid.
pH 4.7: Pure aqueous solution.
pH 5.8: One equivalent of sodium hydroxide added.
pH 7.2: Two equivalents of sodium hydroxide added.
pH 13: In 0.1 N sodium hydroxide.

in the 1- position increases (*cf.* derivatives 1—3). It is further worth noticing that there is a remarkable decrease in the value of ε_{max} for some of the higher homologues.

As a representative of the three homologous compounds 15—17, the absorption spectra of 1-carbethoxymethyl-5-acetyl-barbituric acid (16) at varying pH are shown in Fig. 3. The concentration of all solutions is $3.237.10^{-5}$ molar.

The general pattern of the curves in Fig. 3 is in sharp contrast to those in Fig. 2. Thus, a similar tautomerism is excluded.

The following mechanisms are, then, possible:



At pH 1 there is a peak absorption (ε_{max} = 15 700) at 2730 Å, which is due to the compound VII. This band is diminished to a value of ε_{max} = 13 400 in the aqueous solution (pH 4.7), and a new strong band appears at 2450 Å (ε_{max} = 10 100).

The curves of the absorption at pH 5.8 and 7.2 (corresponding to the monosodium and disodium salts) are almost the same and show a further decrease in the band at 2730 Å (ε_{max} = 13 100) and an augmentation of the band at 2450 Å (ε_{max} = 11 500).

The two strong bands indicate the presence of two chromophores in the ion VIII, one of which is the double bond between the C-atom in the 5-position and the C-atom in the sidechain.

It is thus possible that tautomerism has occurred, affecting the hydrogen atom attached to the nitrogen in the 3-position, producing either a conjugated system with the double bond in the 3 : 4-position or a form with the double bond in the 2 : 3-position. This tautomerism (which is not marked in the above scheme) must then be most pronounced in VII and least in IX, a rather astonishing conclusion.

The absorption curve at pH 13 shows two peaks: one at 2810 Å (ε_{max} = 12 900) and the other at 2250 Å (ε_{max} = 13 300) and are referred to IX.

The above scheme is borne out to some extent by the following two facts:

In strongly acid solution, this derivative can be condensed with 2 : 4-dinitrophenolhydrazine forming a hydrazone, indicating the ketonic structure of the side-chain in the 5-position (VII).

In alkaline solution it was not possible (as will be reported in Part II of this paper) to substitute the free hydrogen atom in the 5-position with an allyl radical, thus showing that there is no free hydrogen owing to the formulae VIII and IX.

The pattern of the curves for the three 5-acetyl derivatives (15—17) is exactly the same in all details, and in 0.1 *N* sodium hydroxide solution ε_{max} decreases as the number of C-atoms in the side chain in the 1-position increases (*cf.* derivatives 1—3 and 5—7).

Further investigations on the tautomerism of the 5-acetyl derivatives will be carried out and published later.

SUMMARY.

1-Carbomethoxymethyl-barbituric acid, 1-carbethoxymethyl-5-methyl-barbituric acid, 1-carbomethoxymethyl-5-acetyl-barbituric acid, and some of their homologues have been prepared and their chemical properties and ultraviolet absorption spectra are reported.

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Received December 8, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 675–687

Studies on N-Substituted Barbituric Acid Derivatives. II

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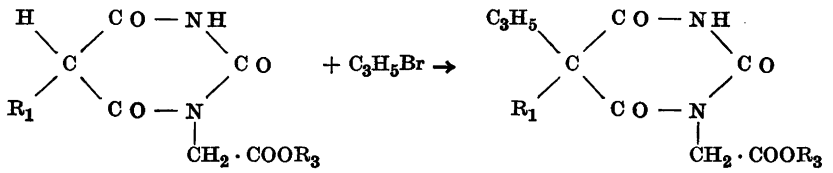
In Part I of this paper, the syntheses and chemical properties of series of 1-carbethoxymethyl-barbituric acid derivatives, which are mono-alkyl or nonsubstituted in the 5-position, are reported. Part II deals with those barbiturates of this type which are di-alkyl substituted in the 5,5-position.

Reaction 2



where: R_1 = H, or alkyl radicals with 1 to 5 C-atoms,
R_3 = alkyl radicals with 1 to 3 C-atoms,
mentioned in the introduction to Part I of this paper, was carried out in two modifications as follows:

For the synthesis of the substances 21—29 and 31 in Table 3 the method described in D. R. P. 526854 was applied.

As an example of this method, the synthesis of derivative 24, 1-carbethoxymethyl-5-*iso*propyl-5-allyl-barbituric acid, is described.

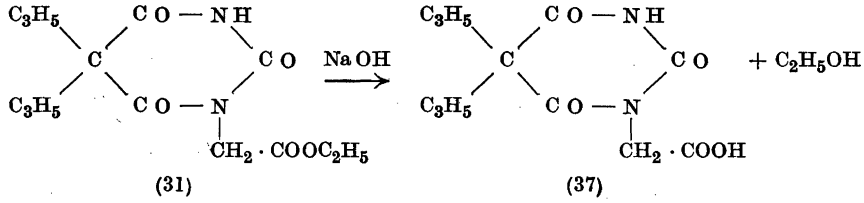
25.6 g of 1-carbethoxymethyl-5-*iso*propyl-barbituric acid (9) was dissolved in 101 ml of 1 *N* sodium hydroxide solution, and 0.75 g of copper sulphate and allyl bromide (12.7 g), in slight excess of the theoretical amount, were added. The reaction mixture was shaken for 2 hours at room temperature. An oil separated, which solidified on standing overnight. The crude product, blue-green in colour, was sucked off and dissolved in alcohol. On passing hydrogen sulphide through the solution, copper sulphide precipitated. This was filtered off. The filtrate was diluted with water and clarified with charcoal. Recrystal-

lization from aqueous alcohol gave the pure substance 24 (m. p. 75°) in a yield of 62 per cent.

A few noteworthy features of these syntheses may be mentioned.

The crystallization of the higher homologues presented considerable difficulties, but was accomplished except for the *n*-hexyl-allyl derivative.

In the synthesis of derivative 31, 1-carbethoxymethyl-5,5-diallyl-barbituric acid, especially when the shaking-time was prolonged to 3—3 ½ hours or an excess of sodium hydroxide solution was used for the dissolving of 1-carbethoxymethylbarbituric acid (2), only a good yield of *1-carboxymethyl-5,5-diallyl-barbituric acid* (37) was obtained at times. This substance is a dibasic acid which could, however, be titrated as a monobasic acid using methyl-red as an indicator. When using phenolphthalein, the end-point of the titration was not sharp. Found: equiv. wt. 267.1; C 54.6; H 5.38; N 10.3 per cent; $C_{12}H_{14}O_5N_2$ requires: equiv. wt. 266.2; C 54.1; H 5.30; N 10.5 per cent. M. p. 167—168°. It is therefore apparent that in this case the following hydrolysis had occurred:



(31) (37)

As mentioned in Part I of this paper, no allyl derivative could be formed from 1-carbethoxymethyl-5-acetyl-barbituric acid (16). This applied both when the reaction took place at room temperature and when the reaction mixture, with shaking, was heated in a bomb tube at 95° C for 2—3 hours.

The other modification of reaction 2, used for the synthesis of compounds 30—33 in Table 3, is an application of a method outlined in D. R. P. 268158. According to this method, substance 31, 1-carbethoxymethyl-5,5-diallyl-barbituric acid, was prepared as follows:

A solution of 53.5 g of 1-carbethoxymethyl-barbituric acid (2) in a mixture of 250 g of alcohol and 125 g of water was refluxed for two hours with a mixture of 61.5 g of allyl bromide and 68 g of sodium acetate in 125 g of water and 310 g of alcohol. The alcohol was distilled off under reduced pressure and a brown semisolid mass separated which, after filtering, solidified on washing with water. The crude product (93 per cent of the theoretical yield) was dissolved in aqueous alcohol, and the alcoholic solution was partially decolourized with charcoal. On diluting with water an oil again separated, which

was dissolved in 5 *N* sodium hydroxide solution; the alkaline solution being diluted with water. By careful addition of 5 *N* hydrochloric acid to about pH 7, the pure compound 31 crystallized on standing overnight. On further addition of hydrochloric acid, the oily mono-allyl derivative (18) formed.

The propyl- and butyl-homologues (32 and 33) occurred as viscous oils, which failed to crystallize even after repeated precipitation with hydrochloric acid from their alkaline aqueous solutions. However, the determination of the equivalent weights (Table 3) and nitrogen content (calcd. for der. 32: N 9.08, found: N 8.65 per cent and calcd. for der. 33: N 8.68, found 8.29 per cent) indicated that the products obtained were not pure.

Table 3.

No.	Derivatives	Melting point uncorr.	Equiv. weight		In 0.1 *N* NaOH	
			Found	Calc.	ε_{max}	Å
21	1-Carbethoxymethyl-5-methyl-5-allyl- barbituric acid	47— 48°	135.0	134.1	3720	2400
22	1- » -5-ethyl-5-allyl- » »	71— 72°	141.2	141.1	8200	2440
23	1- » -5-*n*-propyl-5-allyl- » »	85°	148.7	148.2	8580	2440
24	1- » -5-*iso*propyl-5-allyl- » »	75°	149.0	148.2	8410	2440
25	1- » -5-*n*-butyl-5-allyl- » »	60°	155.9	155.2	8290	2440
26	1- » -5-*sec*-butyl-5-allyl- » »	77— 78°	155.2	155.2	7960	2440
27	1- » -5-*iso*-butyl-5-allyl- » »	95— 96°	155.7	155.2	8530	2440
28	1- » -5-*n*-amyl-5-allyl- » »	100—102°	163.0	162.2	8390	2440
29	1- » -5-*iso*-amyl-5-allyl- » »	61— 62°	162.5	162.2	8350	2440
30	1-Carbomethoxymethyl-5,5-diallyl- » »	96— 97°	70.11	70.07	8180	2440
31	1-Carbethoxymethyl-5,5-diallyl- » »	67— 68°	73.66	73.58	8310	2440
32	1-Carbopropoxymethyl-5,5-diallyl- » »	oil	81.41	77.08	8400	2440
33	1-Carbobutoxymethyl-5,5-diallyl- » »	oil	82.24	80.59	8150	2440
34	1-Carbethoxymethyl-5,5-dimethyl- » » *	84— 85°	—	—	—	—
35	1- » -5,5-diethyl- » »	77— 78°	—	—	9020	2440
36	1- » -5-ethyl-5-*iso*-amyl- » »	oil	—	—	8310	2440
37	1-Carboxymethyl-5,5-diallyl- » »	167—168°	66.57	66.56	7590	2440
38	1,3-Di(carbethoxymethyl)-5,5-diallyl- » »	95— 97°	95.13	95.10	—	—
39	1,3-Di(carbomethoxymethyl)-5,5-diallyl- » »	113—115°	88.19	88.08	—	—

* This substance is described by Rosén [1].

On further acidification of the solutions of derivatives 30—33, after removal of the oils, the corresponding carbonic acid derivative, 1-carboxymethyl-5,5-diallyl-barbituric acid (37) precipitated.

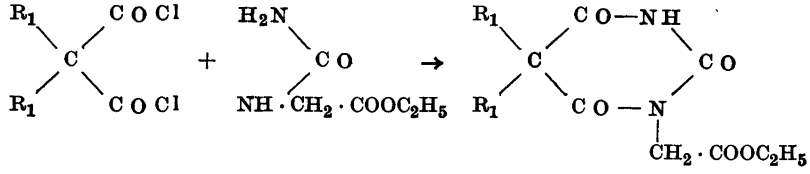
The identity of the compounds 21—33 was established by the method of synthesis, found to be reliable, and by the determination of the equivalent weights (see Table 3). The bromometric method of the Swedish Pharmacopeia Ed. XI for the assay of allyl group in Allypropymalum and Diallymalum was used for this purpose. Good results were obtained for derivatives 21—29,

containing one allyl group. On the other hand, the bromine addition was not complete in substances 30—33 and 37—39 with two allyl groups, and this method gave too high values even when using a reaction time of several hours. Neither did the method described by Schill [2] give correct analytical figures.

The following method, proposed by Schill, however, gave figures in close agreement with those required theoretically:

To a solution containing 0.1000—0.1500 g of the barbiturate in 15 ml of methanol (analytically pure, acetone-free) in a 300 ml glass-stoppered flask, 25 ml of 0.1 *N* potassium bromate and 5 g of potassium bromide were added. When the bromide was dissolved, 10 ml of 2 *N* sulphuric acid were added and the solution was allowed to stand for 5 minutes. After the addition of 1 g of potassium iodide, the liberated iodine was titrated with 0.1 *N* sodium thiosulphate using starch as an indicator. When approaching the end-point of the titration, 5 ml of chloroform were added to dissolve the precipitate of the bromination product. A blank determination was made simultaneously.

Derivatives 35 and 36 were prepared according to reaction 3:



wherein R_1 = alkyl radical with 1 to 5 C-atoms,
(mentioned in the introduction to Part I of this paper).

1-Carbethoxymethyl-5,5-diethyl-barbituric acid (35). 39 g of diethyl-malonyl chloride, obtained by the method of Fischer and Dilthey [3], was mixed with 64 g of hydantoic acid ethyl ester and heated on a water-bath for 20 hours. After cooling and addition of water, the aqueous solution was extracted with ether. On distilling the solvent, the ether extract left an oily residue, which was dissolved in 1 *N* sodium hydroxide solution. A precipitate appeared on the addition of hydrochloric acid to the filtrate. Recrystallization of this fraction by reprecipitation with hydrochloric acid from its alkaline aqueous solution gave the pure substance, m. p. 77—78° C. Found: C 53.4; H 6.57; N 10.29 per cent; $C_{12}H_{18}O_5N_2$ requires: C 53.3; H 6.72; N 10.37 per cent.

1-Carbethoxymethyl-5-ethyl-5-isoamyl-barbituric acid (36). 35 g of ethyl-*iso*amyl malonyl chloride (b. p. 225—235° C) was prepared from ethyl-*iso*-amyl malonic acid (52 g) and phosphorus pentachloride (148 g) according to the procedure of Fleischer and Siefert [4]. Ethyl-*iso*amyl malonyl chloride (35 g) and hydantoic ethyl ester (48 g) were mixed and heated on a water-bath for

20 hours. The mixture, after cooling, was then poured into water (70 ml), the aqueous solution being extracted with ether. On removal of the ether and cooling, an oil (40 g) remained. The oily product distilled at 187° to 189° C under 13 mm Hg pressure as a colourless oil which did not solidify on standing. — Found: N 8.69 per cent; $C_{15}H_{24}O_5N_2$ requires: N 8.97 per cent.

The following method of synthesis was found usable for obtaining a barbiturate with the carbethoxymethyl-group both in the 1-position and in the 3-position.

1,3-Di(carbethoxymethyl)-5,5-diallyl-barbituric acid (38). To a solution of 4.6 g of sodium (0.2 mole) in 200 ml of alcohol, 20.8 g of 5,5-diallyl-barbituric acid (0.1 mole) and 24.5 g of ethyl chloroacetate (0.2 mole) were added. The mixture was refluxed on a water-bath for three hours. Sodium chloride precipitated during the refluxing time. After heating it was sucked off. The filtrate was concentrated to about 100 ml by distilling off the alcohol. An oil, which hardened on standing, separated on acidification with 5 *N* hydrochloric acid. This product was partly soluble in 2 *N* sodium hydroxide solution. The pure substance 38 was obtained from the insoluble part (11.5 g) by recrystallization from aqueous alcohol. Table 3 gives the melting point and equivalent weight.

The compounds 1-carbethoxymethyl-5,5-diallyl-barbituric acid (31), 1-carboxymethyl-5,5-diallyl-barbituric acid (37), and unchanged 5,5-diallyl-barbituric acid were isolated from the alkaline solution.

Using molar equivalents of sodium, 5,5-diallyl-barbituric acid and ethyl chloroacetate, the same compounds were formed, but the yield of derivative 38 was decreased to 10.2 g (from 11.5 g). Found: N 7.42 per cent (Dumas): $C_{18}H_{24}O_7N_2$ requires: N 7.37 per cent.

1,3-Di(carbomethoxymethyl)-5,5-diallyl-barbituric acid (39) was obtained in a yield of 13.9 g from 4.6 g of sodium (0.2 mole), 200 ml of methanol, 41.6 g of 5,5-diallyl-barbituric acid (0.2 mole) and 21.7 g of methyl chloroacetate (0.2 mole) in an analogous way as described above for derivative 38.

In this case, 1-carbomethoxymethyl-5,5-diallyl-barbituric acid (30), 1-carboxymethyl-5,5-diallyl-barbituric acid (37), and unchanged 5,5-diallyl-barbituric acid were isolated from the alkaline solution.

Derivative 39 is remarkably less soluble in ethanol than substance 38 and its melting point and equivalent weight are given in Table 3. Found: N 7.98 per cent (Dumas); $C_{16}H_{20}O_7N_2$ requires: N 7.95 per cent.

A number of sodium salts (Table 4) were prepared as follows: The acids were dissolved in equivalent amounts of 0.8 *N* alcoholic sodium hydroxide solution. By the addition of a conciderable excess of ether the solutions became turbid. On standing overnight in a stoppered flask the sodium salts

crystallized. Yield 74—83 per cent. Their equivalent weights were determined by titration with 0.1 *N* hydrochloric acid using methyl red as an indicator. Potassium salts (Table 4) were prepared in an analogous way.

Table 4.

Derivatives	Melting point uncorr.	Equivalent weight	
		Found	Calc.
Na-salt of derivative 22	187—188°	305.5	305.3
Na- » » » 23	211—212°	318.8	319.3
Na- » » » 27	215—216°	331.2	333.3
Na- » » » 31	208—209°	316.2	317.3
K- » » » 22	191—192°	321.5	321.4
K- » » » 24	188—189°	337.5	335.4
K- » » » 31	186—187°	332.2	333.4

The stability of the aqueous solutions of these salts on storage is comparatively poor. Thus, the 10 per cent solution of the sodium salt of derivative 31, stored at room temperature for 8 days, showed some 50 per cent decomposition, ascertained by the ultra-violet absorption method (see the following). During the storage, the pH of the solution decreased from 9.45 to 9.21 and a precipitation of the acid (derivative 31) occurred.

Heating of the aqueous solutions caused rapid degradation, and it was not therefore possible to sterilize the solutions by heat.

Stable, concentrated (about 20 per cent) solutions of compounds 21—36 for pharmaceutical purposes were obtained by use of the following solvents:

diethylamine and alcohol (1 + 1)

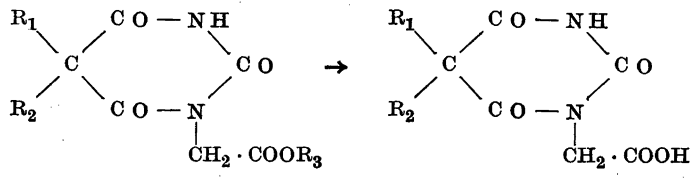
triethanolamine and alcohol (10 + 3)

methylacetamide and water (7 + 3).

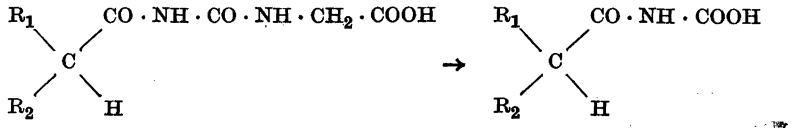
Xanthydrol is used for identifying barbituric acid derivatives [Fabre [5], Jespersen and Thoudahl-Larsen [6], Gad [7], McCutcheon and Plein [8]], but the N-substituted barbiturates included in this paper did not react with xanthydrol in glacial acetic acid solution. This is in agreement with the findings of McCutcheon and Plein [8], who failed to obtain any xanthyl derivative with the N-substituted barbiturates Evipal and Mebaral, which have a methyl group on the nitrogen.

The pharmacological properties of substances 21—27, 29, 31 and 35 are described by Sandberg [9], and those of the others will be reported later.

The alkaline hydrolysis was studied and the following scheme may be given:



A B



C D

A represents the compounds 1—36. 1-carboxymethyl-5,5-diallyl-barbituric acid (37), belonging to type B, was isolated from the syntheses of substances 30—33, as described above.

2-Methyl-valeryl-hydantoic acid, $CH_3CH_2CH(CH_3) \cdot CH_2CO \cdot NH \cdot CO \cdot NH \cdot CH_2COOH$, of type C, was isolated by hydrolysis of derivative 11, 1-carbethoxymethyl-5-*sec*-butyl-barbituric acid. The equivalent weight was determined by titration with 0.1 *N* sodium hydroxide solution using phenolphtalein as an indicator. Found: equiv. wt. 215.2 and N 12.75 per cent; $C_9H_{16}O_4N_2$ requires: equiv. wt. 216.2 and N 12.95 per cent. M. p. 215—216° C.

By hydrolysis of 1-carbethoxymethyl-5-*n*-butyl-barbituric acid (10), a substance of type D was isolated, namely: *caproylcarbamic acid,* $CH_3CH_2CH_2CH_2CO \cdot NH \cdot COOH$ with melting point 80—81° C. Its equivalent weight was determined by titration with 0.1 *N* sodium hydroxide solution with phenolphtalein as an indicator. Found: equiv. wt. 157.9 and N 8.74 per sent; $C_7H_{13}O_3N$ requires: equiv. wt. 159.2 and N 8.80 per cent.

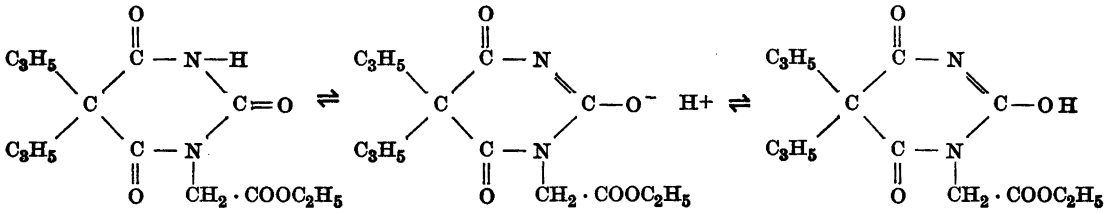
ULTRA-VIOLET ABSORPTION SPECTRA

Ultra-violet absorption spectra of the substances prepared were measured with a Beckman quartz spectrophotometer, using 1 cm silica cells. Molar extinction coefficients, ε, were calculated from the familiar equation $\log I/I = \varepsilon \cdot c \cdot l$, where I_0 is the intensity of the light passing through the sol-

vent; I is the intensity of the light passing through the solution; c is the molar concentration and l is the thickness of the cell in centimeters.

Fig. 4 shows the ultra-violet absorption curves for 1-carbethoxymethyl-5,5-diallyl-barbituric acid (31) at various hydrogen ion concentrations from pH 1.1 to pH 13. The concentration of all solutions is $4.970 \cdot 10^{-5}$ molar. The curve at pH 6.8 shows the superposition of two bands, indicated by the points of inflection. It is evident that the addition of base progressively increases the absorption of radiation at about 2430 Å, exhibiting a maximum value of $\varepsilon_{max} = 8\,600$ at pH 10.9. At pH 13 the absorption is decreased so that $\varepsilon_{max} = 8\,300$ at 2440 Å. On the other hand, in acid solution (pH 1.1) the 2430 Å band is quite weak, although there is an absorption peak in the shorter wave length region, ε_{max} being 7 000 at 2150 Å.

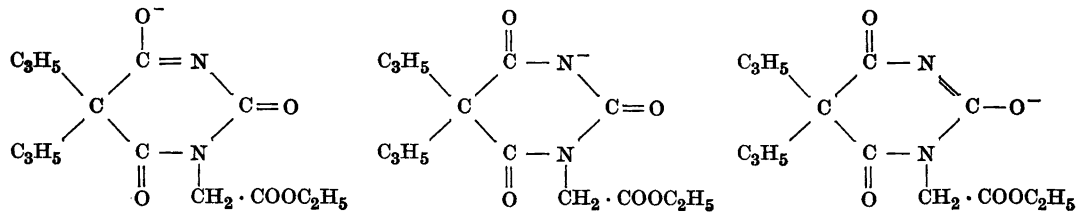
In agreement with the results obtained by Fredholm [10] on the tautomerism of 5,5-diethyl-barbituric acid, the following tautomeric equilibria may occur:



X XI XII

where the keto-form X predominates in strongly acid solution (the peak at 2150 Å) and the ion XI in slightly alkaline solution (the peak at 2430 Å). At pH 13, however, the equilibrium has shifted and the enol-form XII, as the sodium salt, is mainly present. According to this interpretation, the absorption of the undissociated sodium salt is less than that of the ion XI. This phenomenon will be discussed below.

On the other hand, the theory of resonance indicates that resonance may occur between the following ionic forms, in addition to that obtained in the resonance hybride of the carbonyl form X



XIII XIV XI

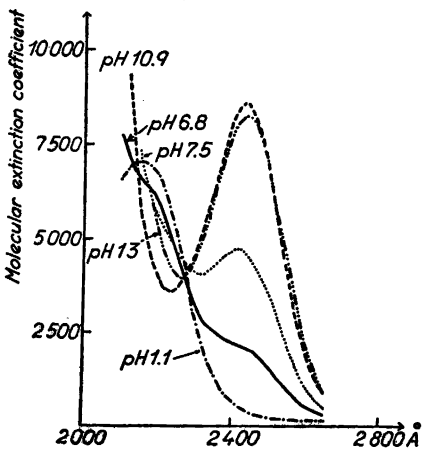


Fig. 4. Absorption spectra of 4.970 · 10^{-5} molar aqueous solutions of 1-carbethoxymethyl-5.5-diallyl-barbituric acid at various pH values.

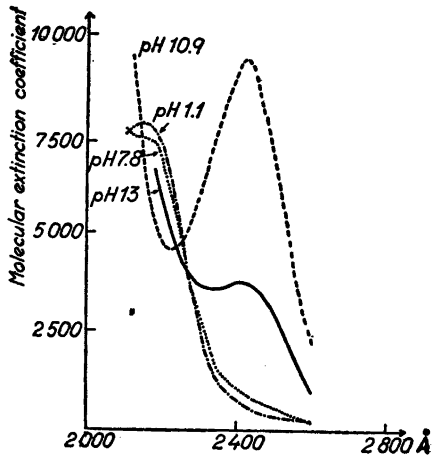


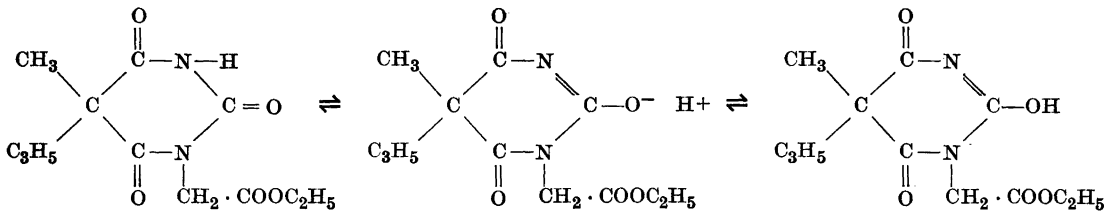
Fig. 5. Absorption spectra of 5.218 · 10^{-5} molar aqueous solutions of 1-carbethoxymethyl-5-methyl-5-allyl-barbituric acid at various pH.

From this point of view the absorption peak at 2430 Å may be attributed to the configurations XI, XIII, and XIV (not to any single one of them).

Table 3 gives the wave-lengths and ε_{max}-values in 0.1 *N* sodium hydroxide solution for the derivatives prepared. It will be seen that all the 1,5,5-trisubstituted barbiturates — with two exceptions (see the following) — have a maximum at 2440 Å, the molecular extinction coefficient being generally within the limits 7 500 and 9 000. Furthermore, there is no general relation between the substituent group and the magnitude of the molecular extinction coefficient. The relatively high value for the extinction coefficient was employed as the basis of a spectrophotometric method of assay of these barbiturates in different solutions. At pH 13 the barbiturates studied conform to the Lambert-Beer equation.

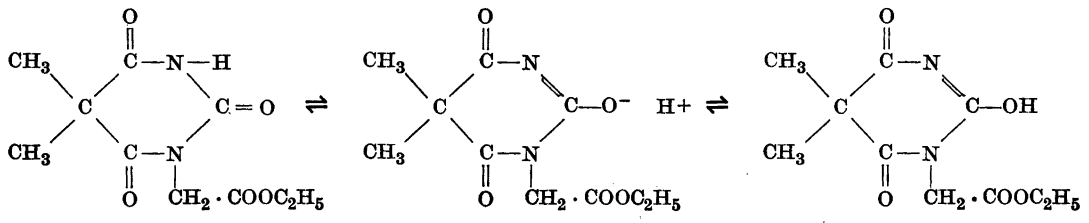
The two exceptions from the 2440 Å band at pH 13 will now be discussed.

The ultra-violet absorption spectra of 1-carbethoxymethyl-5-methyl-5-allyl-barbituric acid (21) as a function of pH in aqueous 5.218 · 10^{-5} molar solutions are illustrated in Fig. 5. The general shape of the curves in Fig. 5 is similar to that in Fig. 4. An important difference is that at pH 13, instead of an expected strong band at 2440 Å, there is a band of much reduced intensity at 2400 Å, ε_{max} being only 3 720. At pH 10.9, ε_{max} is 9 380 at 2420 Å and at pH 1.1 there is an absorption peak at 2150 Å (ε_{max} = 7 750). Under these conditions the following tautomerism would seem most likely:



XV XVI XVII

The absorption spectra of 1-carbethoxymethyl-5,5-dimethyl-barbituric acid (34) in aqueous $5.305 \cdot 10^{-5}$ molar solutions at various pH are summarized in Fig. 6. In analogy with the above findings the following tautomeric equilibria might be operative:



XVIII XIX XX

In acid as well as in neutral solution, the keto-form XVIII is present giving an absorption peak at 2150 Å with $\varepsilon_{max} = 6\,800$. The strong band at 2400 Å ($\varepsilon_{max} = 6\,850$) developing at pH 10.9 will necessarily be due to the ion XIX. At pH 13, however, this derivative shows no selective absorption other than the usual end absorption. The conclusion to be drawn from this finding is that the structure XX, as the sodium salt, does not cause the peak absorption common to all other higher homologues.

This interpretation seems to be substantiated by a comparative study of the three derivatives in Fig. 4—6, where the substituent groups in the 5,5-position change from di-allyl via methyl-allyl to di-methyl.

All the compounds have a strong band at pH 10.9, which may be attributed to the ions XI, XVI, and XIX (including structures analogous to the configurations XIII and XIV) with an enolised structure. At pH 13, however, there is a progressive change in the spectrum for the three substances. For the diallyl derivative, the absorption intensity at pH 13 is 96 per cent of that at pH 10.9. The corresponding figure for the methyl-allyl derivative is 40 per cent and finally for the di-methyl derivative only 19 per cent with no selective

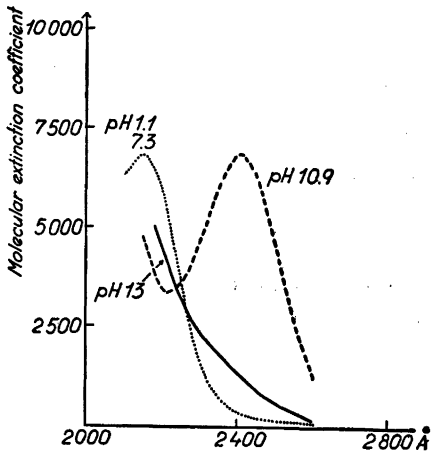


Fig. 6. Absorption spectra of 5.305 · 10⁻⁵ molar aqueous solutions of 1-carbethoxymethyl-5,5-dimethyl-barbituric acid at various pH values.

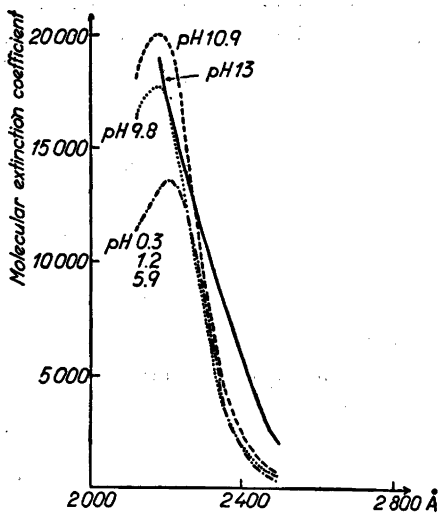


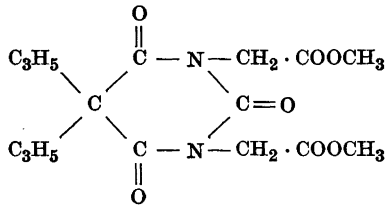
Fig. 7. Absorption spectra of 2.997 · 10⁻⁵ molar aqueous solutions of 1,3-di(carbomethoxymethyl)-5,5-diallyl-barbituric acid at various pH.

absorption. This gradual decrease in ultra-violet absorption of the configurations XII, XVII, and XX might be referred to an effect of the decreasing molecular weight of the substituent alkyl groups in the 5,5-position.

It is of interest to note that Stuckey [11] reported that 5,5-dimethyl-barbituric acid has an similiar absorption spectrum in that no peak is developed in 0.1 *N* sodium hydroxide solution. As an explanation of this phenomenon he states that the change from keto to enol ion does not take place. According to the forementioned results, however, this explanation cannot be correct.

The absorption spectra of $2.997 \cdot 10^{-5}$ molar aqueous solutions of 1,3-di (carbomethoxymethyl)-5,5-diallyl-barbituric acid (39) at varying pH values are shown in Fig. 7. In alkaline solution there is a strong band at 2180 Å (ε_{max} = 20 200 at pH 10.9 and ε_{max} = 17 800 at pH 9.8). In acid solution, the absorption is decreased so that ε_{max} = 13 600 at 2210 Å. It should be noted that this peak corresponds to that shown in acid solution by the derivatives in Figs. 4—6, the addition of a carbomethoxymethyl group in the 3-position having the expected effect of weighting the molecule and causing a slight shift of the curve to longer wave-lengths.

This substance is structurally incapable of undergoing tautomeric shifts. It must therefore exist predominantly in the following configuration:



It will only be added that the absorption curves for derivative 38, 1,3-di-(carbethoxymethyl)-5,5-diallyl-barbituric acid, are quite analogous to those shown in Fig. 7.

SUMMARY

A series of 1-carbethoxymethyl-5,5-dialkyl-barbituric acids and some of their Na and K salts have been prepared.

The alkaline hydrolysis and the stability of their solutions were studied. These barbiturates give no xanthyl derivative.

1,3-Di(carbomethoxymethyl)-5,5-diallyl-barbituric acid and its ethyl homologue have been synthesized.

The ultra-violet absorption spectra at varying pH for both types of barbiturates are reported.

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Received February 16, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 687–691

Some New Hydantoic Acid Derivatives

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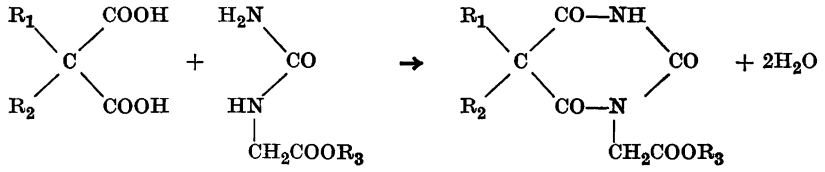
Ureides of certain brominated monocarboxylic acids have been used in medicine owing to their sedative effect. Most important among these compounds are α-bromo-*iso*valeryl-urea (bromyl, bromural) and bromodiethyl-acetyl-urea (carbromal, adalin), both of which are included in several modern pharmacopoeias. Barbituric acid, parent substance of the hitherto dominating group of synthetic hypnotics, is the ureide of the dicarboxylic malonic acid. Rosén and Sandberg [1,2] (1944, 1950) have shown that malonic acid, or a C-substituted malonic acid, and an ester of hydantoic acid can be converted under suitable conditions into barbituric acid derivatives:



If R_1 and R_2 both are alkyl radicals, such as ethyl, allyl or phenyl, the reaction product is a hypnotic one, though weaker and of shorter duration of action than the corresponding substance without any substituent on the N-atom. In this connection it would be of interest to synthesize analogous variants of the bromural-adalin-type and to test their sedative effect. For this reason α-bromo-*iso*valeryl bromide and bromodiethyl-acetyl bromide respectively were brought into reaction with an ester of hydantoic acid:

$$(CH_3)_2 \cdot CH \cdot CHBr \cdot COBr + NH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOR \rightarrow$$
$$\rightarrow (CH_3)_2 \cdot CH \cdot CHBr \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR + HBr,$$

$$(C_2H_5)_2 \cdot CBr \cdot COBr + NH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOR \rightarrow$$
$$\rightarrow (C_2H_5)_2 \cdot CBr \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR + HBr$$

ACTA CHEMICA SCANDINAVICA 4 (1950) 687–691

Some New Hydantoic Acid Derivatives

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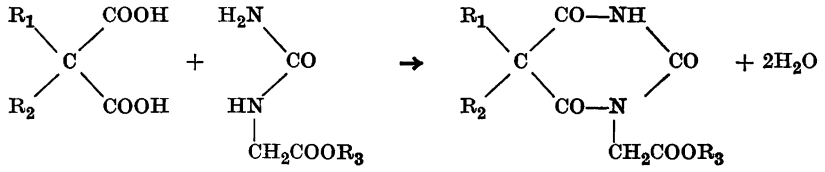
Ureides of certain brominated monocarboxylic acids have been used in medicine owing to their sedative effect. Most important among these compounds are α-bromo-*iso*valeryl-urea (bromyl, bromural) and bromodiethyl-acetyl-urea (carbromal, adalin), both of which are included in several modern pharmacopoeias. Barbituric acid, parent substance of the hitherto dominating group of synthetic hypnotics, is the ureide of the dicarboxylic malonic acid. Rosén and Sandberg [1,2] (1944, 1950) have shown that malonic acid, or a C-substituted malonic acid, and an ester of hydantoic acid can be converted under suitable conditions into barbituric acid derivatives:



If R_1 and R_2 both are alkyl radicals, such as ethyl, allyl or phenyl, the reaction product is a hypnotic one, though weaker and of shorter duration of action than the corresponding substance without any substituent on the N-atom. In this connection it would be of interest to synthesize analogous variants of the bromural-adalin-type and to test their sedative effect. For this reason α-bromo-*iso*valeryl bromide and bromodiethyl-acetyl bromide respectively were brought into reaction with an ester of hydantoic acid:

$$(CH_3)_2 \cdot CH \cdot CHBr \cdot COBr + NH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOR \rightarrow$$
$$\rightarrow (CH_3)_2 \cdot CH \cdot CHBr \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR + HBr,$$

$$(C_2H_5)_2 \cdot CBr \cdot COBr + NH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOR \rightarrow$$
$$\rightarrow (C_2H_5)_2 \cdot CBr \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR + HBr$$

In the first case four different esters of hydantoic acid were used, namely methyl-, ethyl-, *n*-propyl- and *iso*propyl-. Only the ethyl ester has been described before, as far as the author is aware, and some data on the other three hydantoic acid esters will now be reported. They have been prepared throughout by condensing glycocoll ester hydrochloride with potassium cyanate, as is described for the ethyl ester by Harries and Weiss [3]:

$$NH_2 \cdot CH_2 \cdot COOR,\ HCl + KOCN \rightarrow NH_2 \cdot CO \cdot NH \cdot CH_2 \cdot COOR + KCl$$

The ester hydrochlorides were obtained by passing dry hydrochloric acid into a boiling mixture of glycocoll and the alcohol in question (Harries and Weiss [3]; Abderhalden and Suzuki [4]).

EXPERIMENTAL

Hydantoic acid methyl ester

A cold solution of 126 g of glycocoll methylester hydrochloride (1 mol) in 120 g of water was poured under stirring into a cold solution of 90 g of potassium cyanate (1 mol + 10 per cent) in 145 g of water. The mixture soon got warm and after a while it was cooled. No precipitate appeared. The solution was first evaporated *in vacuo* and afterwards on a water-bath at atmospheric pressure until a solid residue was obtained, which proved to be the intended ester and potassium chloride. The ester was separated by extraction with boiling alcohol and crystallized as beautiful needles on cooling the filtrate. Yield: 76 g = 58 per cent. Easily soluble in alcohol and water, sparingly soluble in benzene, insoluble in ether. Melting point 97—98° C. The percentage content of carbon and hydrogen was determined in a Reihlen-Weinbrenner-apparatus, and that of nitrogen according to Kjeldahl.

Found	C	36.45	H	5.99	N	21.16
Calc.	»	36.36	»	6.10	»	21.21

Hydantoic acid *n*-propyl ester

A cold solution of 154 g of glycocoll *n*-propylester hydrochloride (1 mol) in 50 g of water was poured under stirring into a cold solution of 90 g of potassium cyanate in 145 g of water. The mixture got warm and turbid. On cooling a coarse precipitate appeared. This was filtered off, washed with water and dried. Weight 120 g. The product was then dissolved in the equal quantity of boiling water, the solution beeing treated with charcoal. From the filtrate pure ester was obtained as irregular, coarse prismatic crystals. Yield: 85 g = 53 per cent. The ester could also be recrystallized from alcohol, as it was slightly soluble in cold alcohol. Melting point 115—116° C. Found: N 17.37 per cent, calculated 17.49 per cent.

Hydantoic acid *iso*propyl ester

The synthesis was carried out in an analogous way to described above and it took place mainly in a similar manner. The *iso*propyl ester, however, crystallized much slower than the normal propyl ester and it sometimes formed supersaturated solutions. From 1 mol ester hydrochloride 77 g of pure hydantoic acid ester (= 48 per cent) was obtained. Feeble needles malting at 94—95° C. It is remarkable that the *iso*propyl ester has a lower melting point than the normal one. The *iso*-ester was easily soluble in cold alcohol, but it could be precipitated from the alcoholic solution by the addition of ether. Found: N 17.39 per cent, calculated 17.49 per cent.

α-Bromo*iso*valeryl-hydantoic acid ester

A mixture of 1 mol of bromo*iso*valeryl-bromide and 2 mols of hydantoic acid ester was kept at 75° C for 3 à 4 hours. The ester soon dissolved and the clear solution gradually darkened. Any evolution of hydrogen bromide was hardly discernible. The reaction product, after cooling, formed a viscid mass, which was mixed under stirring with an equal weight of water and neutralised with sodium bicarbonate. On the addition of water the desired substance, light yellow in colour, separated out. It was sucked off and washed. Yield almost quantitative. Easily soluble in alcohol, benzene and ether, sparingly soluble in water. Recrystallizations from aqueous alcohol (50 per cent) gave the pure esters. They occured as colourless needle-like crystalls, or as a white, crystalline powder. Table 1 gives the melting points and the percentage of bromine and nitrogen. In this series the melting point of the *iso*propyl ester is higher than that of the normal one.

Table 1. Analytical data of esters of α-bromo-iso-valeryl-hydantoic acid

Esters of α-bromo-*iso*-valeryl-hydantoic acid	Melting point uncorr.	Br per cent		N per cent	
		found	calc.	found	calc.
Methyl ester	113—114°	27.08	27.08	9.45	9.49
Ethyl ester	87—88°	25.72	25.85	9.02	9.06
n-Propyl ester	90—91°	24.61	24.73	8.63	8.67
iso-Propyl ester	101—111°	24.74	24.73	8.63	8.67

The hypnotic efficiency of the methyl-, ethyl- and *iso*propylesters of α-bromo-*iso*valeryl-hydantoic acid was tested on male albino rats weighing 150—190 g by observing the body righting reflexes.

The compounds were suspended in 5 per cent acacia solution, the suspension containing 100 mg/ml, and administered by stomach tube to the rats deprived of food 18—21 hours prior to administration.

All derivatives were ineffective in doses up to and including 1500 mg/kg bodyweight. — This biological test was carried out by F. Sandberg.

Bromodiethyl-acetyl-hydantoic acid ethyl ester

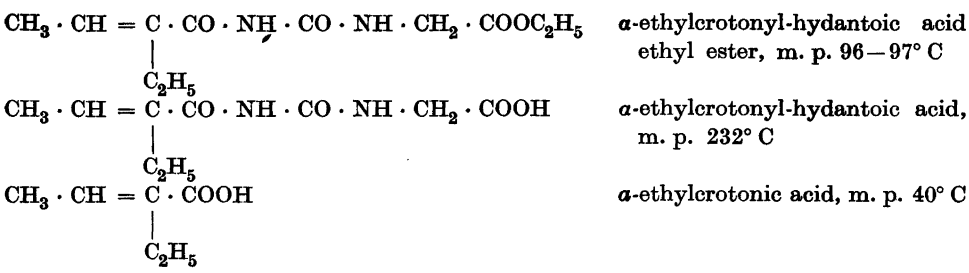
A mixture of 0.1 mols of bromodiethyl-acetyl bromide (25.8 g) and 0.2 mols of hydantoic acid ethyl ester (29.2 g) was heated on a water-bath for 4 hours. Firstly a clear, almost colourless solution was formed, which gradually darkened and got turbid by the formation of a precipitate. After cooling 50 ml water was added together with sufficient sodium bicarbonate to give a neutral reaction. The precipitate formed during the heating remained insoluble, and in addition to this a light brown, viscid oil separated. The solid substance mainly consisted of hydantoin. The oil was taken up in ether, and the ethereal solution was repeatedly extracted with water and dried with sodium sulphate. On distilling off the solvent 17 g of a light yellow, viscid oil remained, from which a crystalline precipitate separated very slowly and sparingly. However, the main part of the oil failed to crystallize. After 14 days only about 1 g of the solid substance was isolated. In contrast to the oil, these crystals (bromodiethyl-acetyl-hydantoic acid) were insoluble in benzene and could therefore be washed with this liquid. The remaining oil was probably bromodiethyl-acetyl-hydantoic acid ethyl ester, though not quite pure. If a solution of the oil in pyridine was boiled for some minutes, the following reaction took place:

$$(C_2H_5)_2 \cdot CBr \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR \rightarrow$$

$$\rightarrow CH_3 \cdot CH = \underset{\displaystyle C_2H_5}{\overset{\displaystyle |}{C}} \cdot CO \cdot NH \cdot CO \cdot NH \cdot CH_2 \cdot COOR + HBr$$

α-ethyl-crotonyl-hydantoic acid ester

The α-ethyl-crotonyl-hydantoic acid ester formed could be converted into the corresponding carboxylic acid and further into α-ethylcrotonic acid. These three decomposition products were isolated in pure form and analyzed. Only α-ethylcrotonic acid has been previously described. More data about these reactions will be reported in a later paper.



Bromodiethyl-acetyl-hydantoic acid ethyl ester was not tested biologically owing to its oily consistence.

Bromodiethyl-acetyl-hydantoic acid

The crystalline product isolated from the above mentioned oil was bromodiethyl-acetyl-hydantoic acid. After recrystallizations from alcohol or water the substance occurred as thin flat needles melting at 173—175° C. By titrating the alcoholic solution with 0.1 *N* sodium hydroxide solution, using phenolphthalein as an indicator, a satisfactory endpoint of the titration was obtained. Equivalent weight: found 293.4, calculated 295.1; Br: found 27.0 per cent, calculated 27.1 per cent.

SUMMARY

The methyl-, *n*-propyl- and *iso*propyl esters of hydantoic acid, bromodiethyl-acetyl-hydantoic acid and its ethyl ester have been prepared and their chemical properties are reported.

The methyl-, ethyl-, *n*-propyl- and *iso*propyl esters of α-bromo-*iso*valeryl-hydantoic acid have been prepared, their chemical properties determined and their hypnotic efficiency tested.

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Received March 7, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 692–702

Researches on Plant Growth Substances

I. On 1-Napthylacetaldehyde 1 *

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Recently Poul Larsen [1] presented evidence for the function of 3-indoleacetaldehyde as a growth hormone in higher plants. To procure more direct evidence that this compound is actually a growth hormone it would be of great interest to test the action of synthetically prepared 3-indoleacetaldehyde. This compound has, however, not hitherto been described in the literature, and experiments on synthesizing it were beset by such difficulties that we at last chose to try the different methods of preparing aldehydes on a model substance, which could be made from a more easily accessible starting material. For this purpose 1-naphthylacetaldehyde was selected. This substance would also be of interest from the point of view of growth hormone action, since 1-naphthylacetic acid is almost as active as 3-indoleacetic acid. Indeed our preparations of 1-naphthylacetaldehyde have been shown by Larsen [2] to possess activity as a growth hormone.

Of course not all methods leading to 1-naphthylacetaldehyde could be applied to the indole analogue, because the indole nucleus has a rather reactive hydrogen atom. But one could be fairly sure that there would be little use in trying methods of preparing 3-indoleacetaldehyde, which could not also be applied to the naphthalene analogue. As a matter of fact several of the usual methods of preparing aldehydes gave none or little 1-naphthylacetaldehyde, so this compound must be characterized as rather difficultly accessible.

1-Naphthylacetaldehyde has only been described once in the literature, by Tiffeneau and Daudel [3], who prepared it by the reaction between 1-vinylnaphthalene, mercuric oxide and iodine. The boiling point of the aldehyde was given as 163—166° at 13 mm and the melting point of the semicarbazone as

* This investigation has been supported by grants from *Danmarks tekniske Højskoles teknisk-kemiske Fond* and *Carlsbergfondet.*

208°. According to our investigations, however, this is nearer the melting point of 1-naphthaldehyde semicarbazone, and therefore the aldehyde of Tiffeneau and Daudel was not pure. On the other hand we were able to show that some naphthylacetaldehyde can be obtained by the method of Tiffeneau and Daudel.

1-Naphthylacetaldehyde may easily be oxidized to 1-naphthaldehyde, and in some preparations 1-naphthylacetaldehyde may be contaminated by 1-naphthaldehyde used as starting material or formed by side reactions. It was, therefore, of great importance for us to be able with certainty to distinguish between these two aldehydes. From 1-naphthaldehyde and from a pure sample of 1-naphthylacetaldehyde, prepared from 1-naphthyl-2,3-propanediol (see later), we prepared several derivatives, the melting points of which have been compared in the following table (the figures in parantheses are those found by Coles and Dodds [4] who previously prepared several derivatives of 1-naphthaldehyde). The melting points were determined on a Kofler stage.

Table 1.

	1-Naphthaldehyde-		1-Naphthylacetaldehyde
phenylhydrazone	82	(82)	—
as-methylphenylhydrazone	92		—
4-nitrophenylhydrazone	235	(237)	—
2,4-dinitrophenylhydrazone	260—62	(254)	184
oxime	98—99	(98)	123—24
semicarbazone	218—19	(219)	190—91
4-phenylsemicarbazone	205—06		166
thiosemicarbazone	222—23	(217)	179
3-nitrobenzhydrazone	217—18		150—51

As a rule the derivatives of 1-naphthylacetaldehyde are more soluble than the derivatives of 1-naphthaldehyde and with the exception of the oxime, their melting points are lower. The phenylhydrazone, *as*-methylphenylhydrazone and 4-nitrophenylhydrazone of 1-naphthylacetaldehyde are unsuited for identification; they were only obtained by addition of water to their alcoholic solutions as oily or semisolid precipitates.

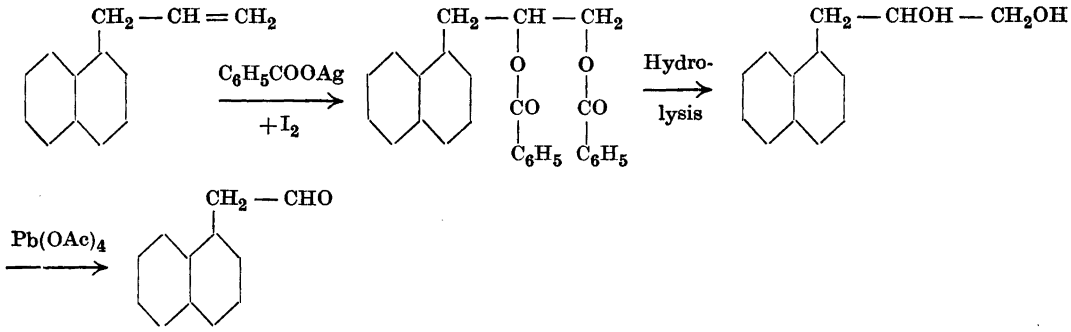
Especially characteristic for 1-naphthylacetaldehyde is the semicarbazone, which separates as white crystals by addition of semicarbazide hydrochloride (without addition of acetate) to aqueous solutions or suspensions of the aldehyde. The melting point is also suited for differentiation between the two aldehydes. The semicarbazone was prepared in most cases for isolating and

characterizing 1-naphthylacetaldehyde formed by different methods. From mixtures of the two aldehydes the semicarbazone of 1-naphthylacetaldehyde is easily obtained in the pure form. As the melting point of the semicarbazone of Tiffeneau and Daudel was given as 208°, their aldehyde must have contained an appreciable amount of 1-naphthaldehyde, and perhaps mainly consisted of this.

A point of great importance for the isolation of pure 1-naphthylacetaldehyde was the observation that the aldehyde is volatile with steam. The distillate has a very faint odour, somewhat resembling that of phenylacetaldehyde. By addition of semicarbazide hydrochloride to the aqueous distillate the semicarbazone separates in pure form.

The other derivatives mentioned under 1-naphthylacetaldehyde are also well suited for differentiation between the two aldehydes. For identification we have often used the 2,4-dinitrophenylhydrazone. It is, however, to be noted that the 2,4-dinitrophenylhydrazone of 1-naphthaldehyde is so slightly soluble, that this compound separates from mixtures of the aldehydes even when they only contain little 1-naphthaldehyde. The dinitrophenylhydrazone of 1-naphthaldehyde is darker red than that of 1-naphthylacetaldehyde.

As mentioned above the method of Tiffeneau and Daudel gives a little 1-naphthylacetaldehyde. This method is, however, not suited for preparation of the pure aldehyde and the same applies to most other methods we have investigated. The best method we have found for preparing pure 1-naphthylacetaldehyde is the aldehyde synthesis of Hershberg [5]. By application of this method to the problem in question, 1-allylnaphthalene was transformed into 1-(1-naphthyl)-2,3-propanediol, and this was oxidized with lead tetraacetate:



The preparation goes over two intermediary products, but the yields are good and the aldehyde is obtained in a high state of purity.

We have further investigated the following methods with a view to preparing 1-naphthylacetaldehyde (more details of some of the experiments are to be found in the experimental part).

a. Oxidation and dehydrogenation of β-1-naphthylethanol

The oxidation of β-1-naphthylethanol with chromic acid gave preparations consisting mainly of 1-naphthaldehyde. The aldehyde (isolated by extraction with ether, purifying *via* the hydrogensulphite compound and distilling) gave a semicarbazone, thiosemicarbazone, 4-phenylsemicarbazone, p-nitrophenylhydrazone, 2,4-dinitrophenylhydrazone and methylphenylhydrazone melting as crude products at 210°, 214°, 194°, 215°, 260° and 88—89° respectively. After recrystallization the melting points were the same as indicated in Table 1.

Shumeiko [6] has described a method of obtaining phenylacetaldehyde by oxidation of β-phenylethanol with chromic acid in the presence of an indifferent solvent (benzene, toluene *etc.*). By application of this method to β-1-naphthylethanol we obtained 1-naphthylacetaldehyde, but only in slight yield and containing an appreciable amount of 1-naphthaldehyde.

By oxidation of β-1-naphthylethanol with CrO_3 in acetic acid a little 1,4-naphthoquinone was formed; most of the naphthylethanol could be recovered unchanged.

By oxidation of β-1-naphthylethanol with lead dioxide and sulphuric acid, with hypobromite and with chloramine-T, 1-naphthaldehyde was obtained.

By the action of lead tetraacetate on β-1-naphthylethanol in benzene solution (*cf.* Criegee [7]) no reaction took place.

It was further attempted to prepare 1-naphthylacetaldehyde by catalytic dehydrogenation of β-1-naphthylethanol (the method of Bouveault [8]). An all-glass apparatus similar to that described by Weygand [9] was used. As catalyst we used either freshly reduced copper oxide (*cf.* Weygand [9]) or copper-silver on pumice (Davies and Hodgson [10]). The catalyst chamber was electrically heated to 240° and the apparatus thoroughly flushed with hydrogen. Then the alcohol was slowly distilled through the catalyst at a pressure of 12—15 mm. No aldehyde was formed, but from the distillate 1-ethylnaphthalene could be isolated. Evidently the alcohol is dehydrated to 1-vinylnaphthalene, which in its course is hydrogenated to 1-ethylnaphthalene. It was then attempted to use copper oxide as a catalyst; in this case, when removing the stopper at the top of the catalyst chamber, the characteristic odour of 1-naphthylacetaldehyde could be perceived, but no aldehyde could be detected in the distillate.

Finally we tried to repare 1-naphthylacetaldehyde by the Oppenauer method [11], *i. e.* oxidation of the alcohol with ketones (acetone, cyclohexanone) in the presence of aluminium *tert.*-butoxide, but no 1-naphthylacetaldehyde could be isolated.

b. From 1-vinylnaphthalene

By reaction of 1-vinylnaphthalene with iodine and mercuric oxide 1-naphthylacetaldehyde was obtained, but only in small yield.

1-Naphthylacetaldehyde could also be obtained by rearrangement of naphthylethyleneoxide, prepared from 1-vinylnaphthalene and perbenzoic acid.

c. 1-Naphthylmethylmagnesium chloride and ethyl orthoformate

This method failed, because the Grignard process resulted mainly in 1,2-dinaphthylethane.

d. 1-Naphthylmagnesium bromide and bromoacetal

No 1-naphthylacetaldehyde was obtained.

e. 1-Naphthylmagnesium bromide and ethoxyacetal

By the reaction of phenylmagnesium bromide with ethoxyacetal Späth [12] has prepared phenylacetaldehyde. No 1-naphthylacetaldehyde could be obtained by application of this method to 1-naphthylmagnesium bromide.

f. The glycidic ester synthesis of Claisen-Darzens [13]

Several reactions of ethyl chloro- or bromoacetate with 1-naphthaldehyde were performed using sodium, sodamide or sodium ethoxide as condensing agents. Only small yields of 1-naphthylacetaldehyde were obtained, the best ones when sodium ethoxide was used.

g. β-1-naphthylacrylic acid and hypochlorite or hypobromite

By reaction of cinnamic acid with hypochlorite or hypobromite good yields of phenylacetaldehyde are obtained (Erlenmeyer [14]); this is one of the best methods of preparing phenylacetaldehyde. By application to the naphthalene

analogue, however, no 1-naphthylacetaldehyde was obtained. Most of the β-1-naphthylacrylic acid remained unchanged (no addition of hypochlorous or hypobromous acid taking place), but some of it was oxidized to 1-naphthaldehyde.

h. Hydrolysis of 1-naphthylacetaldehyde oxime

By reduction of α-(1-naphthyl)-β-nitro-ethylene with aluminium amalgam, Mayer and Stieglitz [15] prepared the oxime of 1-naphthylacetaldehyde, but they did not hydrolyse this compound. By hydrolysis of the oxime with 1 *N* hydrochloric acid + ethanol (1 : 1) we prepared the aldehyde in a 70 % yield (isolated as the semicarbazone after steam distillation).

According to these experiments the most satisfactory method of preparing 1-naphthylacetaldehyde is the oxidation of 1-(1-naphthyl)-2,3-propanediol with lead tetraacetate. By distillation in a high vacuum the aldehyde was obtained as a colourless liquid (b. p. 112° at 0.7 mm) which crystallized by cooling in solid carbon dioxide and then had the melting point 33.5°. In the course of some weeks or months the crystalline substance is transformed into a viscous oil and later into a glassy mass. Without doubt this aldehyde undergoes polymerisations in the same manner as is known for phenylacetaldehyde (Stobbe and Lippold [16]), and accordingly the viscous oil and glassy substance are chain polymers.

EXPERIMENTAL

1-(1-Naphthyl)-2,3-propanediol-dibenzoate. A mixture of 15 g of 1-allylnaphthalene (prepared from 1-naphthylmagnesium bromide and allyl bromide. Yield 74 %. B. p. 135—136° at 15 mm), 40 g of dry silver benzoate, 21 g of iodine and 250 ml of dry benzene was refluxed for 14 hours. The silver iodide was filtered by suction and washed with benzene, and the benzene solution was washed with sodium hydrogen carbonate solution and with water and then dried over calcium chloride. The benzene was distilled off on a water bath, when the dibenzoate was left as a brownish oil. By dissolving the oil in petroleum ether and evaporating in vacuo the dibenzoate crystallized. It was purified by dissolving in hot methanol, cooling in ice and seeding with some of the crystals formed in petroleum ether solution. Yield 21.9 g = 60 %. M. p. 83°.

$C_{27}H_{22}O_4$ (410.4)	Calc.	C	79.01	H	5.40
	Found	»	79.62	»	5.37

1-(1-Naphthyl)-2,3-propanediol. The dibenzoate (20 g) was hydrolyzed by refluxing with a solution of 11 g of sodium hydroxide in 50 ml of water + 125 ml of methanol for 2 hours. The solution was then almost neutralized with sulphuric acid, the methanol removed in vacuo and water added. Most of the glycol separated, a little more could be

isolated by extraction of the solution with ether. The melting point of the crude product was 110°, after recrystallization from water the m. p. was 111°. Yield 8.9 g = 90 %.

$C_{13}H_{14}O_2$ (202.2)	Calc.	C	77.21	H	6.98
	Found	»	77.23	»	6.97

1-Naphthylacetaldehyde. The glycol (3.2 g) was treated with lead tetraacetate (8.9 g) in benzene (27 ml) for 1 ½ hour at room temperature. Then acetic acid (8 ml) was added and the mixture was heated at 50° for ½—1 hour. The solution was cooled, washed with water and sodium hydrogen carbonate solution. The aqueous layer was extracted with ether. The ether and benzene layers were combined, the solution dried with sodium sulphate, filtered and evaporated in vacuo. The residue was a faintly yellow oil. Yield 2.5 g = 92 %.

By distillation in a high vacuum of the crude aldehyde obtained by oxidation of 8.9 g of the glycol 3.7 g of 1-naphthylacetaldehyde = 60 % was obtained. The loss by distillation could presumably be much diminished. M. p. 33.5°. B. p. 112° at 0.7 mm.

$C_{12}H_{10}O$ (146.2)	Calc.	C	84.69	H	5.92
	Found	»	84.98	»	5.72

Derivatives of 1-naphthylacetaldehyde

2,4-Dinitrophenylhydrazone: Red-yellow precipitate by addition of the aldehyde to an alcoholic solution of 2,4-dinitrophenylhydrazine containing a little hydrochloric acid. Recrystallized from toluene it forms orange-red crystals with m. p. 184°.

$C_{18}H_{14}O_4N_4$ (350.3)	Calc.	N	15.99
	Found	»	16.02

Oxime: The aldehyde was added to a concentrated aqueous solution of hydroxylamine hydrochloride + sodium acetate, the solution heated to boiling and alcohol added to obtain a clear solution. Colourless crystals were obtained on cooling, m. p. 123—124°.

$C_{12}H_{11}ON$ (185.2)	Calc.	N	7.56
	Found	»	7.46

Semicarbazone: White crystals by addition of an aqueous solution of semicarbazide hydrochloride to an alcoholic solution of the aldehyde. Recrystallized from alcohol it had m. p. 190—191°.

$C_{13}H_{13}ON_3$ (227.2)	Calc.	N	18.49
	Found	»	18.38

4-Phenylsemicarbazone: White crystals from rather concentrated alcoholic solution (1 ml/0.15 g) of the components. Recrystallized from alcohol. M. p. 166°.

$C_{19}H_{17}ON_3$ (303.3)	Calc.	N	13.85
	Found	»	13.86

Thiosemicarbazone: An alcoholic solution of the aldehyde and an aqueous solution of thiosemicarbazide were mixed and heated to boiling. Water was added to opalescence and then alcohol to obtain a clear solution. By cooling colourless crystals, m. p. 179°.

$C_{13}H_{13}SN_3$ (243.3)	Calc.	N	17.27
	Found	»	17.54

m-Nitrobenzhydrazone: Yellow crystals from a solution of the components in aqueous alcohol. M. p. 150—151°.

$C_{19}H_{15}O_3N_3$ (333.4)	Calc.	N	12.63
	Found	»	12.33

Derivatives of 1-naphthaldehyde. For comparison the following derivatives of 1-naphthaldehyde were prepared:

Phenylhydrazone: Crystallized from a concentrated alcoholic solution of the components. M. p. 82°.

$C_{17}H_{14}N_2$ (246.3)	Calc.	N	11.38
	Found	»	11.29

α-Methyl-phenylhydrazone: Small yellow crystal aggregates from an alcoholic solution of the components. M. p. 92°.

$C_{18}H_{16}N_2$ (260.3)	Calc.	N	10.76
	Found	»	11.10

p-Nitrophenylhydrazone: From the components in dilute alcohol (1 : 1). Small yellow needles. M. p. 235°.

$C_{17}H_{13}O_2N_3$ (291.3)	Calc.	N	14.42
	Found	»	14.34

2,4-Dinitrophenylhydrazone: Recrystallized from toluene. Orange-red crystals. M. p. 260—262°.

$C_{17}H_{12}O_4N_4$ (336.3)	Calc.	N	16.66
	Found	»	16.69

Oxime: Colourless needles from dilute alcohol. M. p. 98—99°.

$C_{11}H_9ON$ (171.2)	Calc.	N	8.18
	Found	»	8.38

Semicarbazone: Colourless needles from alcohol. M. p. 218—219°.

$C_{12}H_{11}ON_3$ (213.2)	Calc.	N	19.71
	Found	»	19.78

4-Phenylsemicarbazone: White crystals from alcohol. M. p. 205—206°.

$C_{18}H_{15}ON_3$ (289.3)	Calc.	N	14.52
	Found	»	14.60

Thiosemicarbazone: Faint yellow, thin needles. Recrystallized from alcohol. M. p. 222—223°.

$C_{12}H_{11}SN_3$ (229.3)	Calc.	N	18.32
	Found	»	18.39

m-Nitrobenzhydrazone: Light yellow crystals from a hot alcoholic solution of the components. M. p. 217—218°.

$C_{18}H_{13}O_3N_3$ (319.3)	Calc.	N	13.17
	Found	»	13.31

Oxidation of β-1-naphthylethanol with chromic acid. To a solution of 6.5 g of sodium dichromate and 10 g of conc. sulphuric acid in 25 ml of water were added 50 ml of benzene and then, with vigourous shaking, a solution of 10 g of 1-napthylethanol [17] in 15 ml of benzene. The temperature rose to ca. 55°. The mixture was shaken until the temperature had fallen to room temperature; then 50 ml of water was added and the mixture was steam distilled. The distillation was continued until the distillate gave no further reaction with dinitrophenylhydrazine. The benzene layer was separated and the water layer extracted with ether. The combined extracts were dried with sodium sulphate and distilled *in vacuo*. Between 164 and 172° at 12 mm a small amount (1.9 g) of a faint yellow oil distilled. The aldehyde was analyzed by dissolving in ethanol, precipitating with an aquoeous solution of semicarbazide hydrochloride and weighing the precipitate; according to this analysis the aldehyde contained 81 % of naphthylacetaldehyde. After one crystallization the semicarbazone melted at 186—187° and contained 18.16 % N (calc. 18.49). The oxime prepared from the oil had the correct melting point (123°). The 2,4-dinitrophenylhydrazone, however, after recrystallization from toluene had the melting point 261—262°, *i. e.* the melting point of 1-naphthaldehyde 2,4-dinitrophenylhydrazone. The crude product of the dinitrophenylhydrazone melted partially at 175°. By chromatographic adsorption on aluminium oxide the two dinitrophenylhydrazones could be separated (that derived from 1-naphthaldehyde forming the lower zone) and isolated after elution they had the melting points 262° and 184°.

Transformation of 1-vinylnaphthalene into 1-naphthylacetaldehyde

a. With cooling in ice and vigourous shaking, 3 g of yellow mercuric oxide and 3.5 g of iodine were added to a solution of 1 g of 1-vinylnaphthalene [18] in 10 ml of ether. Yellow mercuric iodide was formed at first, but when the mixture reached room temperature it was transformed into the red modification. The ethereal layer was decolourised with sodium thiosulphate, the ether was removed *in vacuo* and the residue steam-distilled. During the distillation some iodine was formed which was reduced with sodium thiosulphate. By addition of semicarbazide hydrochloride the semicarbazone of 1-naphthylacetaldehyde (m. p. 190°) was precipitated. The yield was only 0.110 g $\sim$ 12 %. The residue from the distillation was obviously a polymeride.

b. 1-Naphthylethylene oxide was prepared from 1-vinylnaphthalene by reaction with perbenzoic acid analogous to the preparation of styrene oxide [19] as a colourless oil with boiling point 106° at 1.4 mm.

$C_{12}H_{10}O$ (170.2)	Calc.	C	84.69	H	5.92
	Found	»	84.28	»	5.70

One gram of 1-naphthylethylene oxide was added to a solution of anhydrous magnesium bromide in ether (prepared by addition of the calculated amount of bromine and a trace of iodine to 0.5 g of magnesium under 100 ml of anhydrous ether). The solution became turbid. The ether was removed by distillation and the residue steam distilled. The distillate gave 0.2 g of the semicarbazone of 1-naphthylacetaldehyde.

Condensation of 1-naphthaldehyde with ethyl bromoacetate

A mixture of 15.6 g of 1-naphthaldehyde [20] (0.1 mol) and 16.7 g (0.1 mol) of ethyl bromoacetate was added to a suspension of 0.1 mol of ethanolfree sodium ethoxide in ether (prepared from pulverized sodium and the calculated amount of ethanol in ether). After the addition the mixture was heated for one hour on a steam bath, then it was cooled, water was added and the ether layer dried with potassium carbonate and the ether removed by distillation. To the residue alcoholic sodium hydroxide was added. After standing for some days a white salt had separated which was filtered and washed with ether. Yield 10.2 g (+ 1.5 g from the filtrate). Analysis: 0.2241 g of the salt gave by steam distillation 0.085 g of a semicarbazone melting at 187—188°. The salt accordingly contained 39 % of the sodium salt of β-1-naphthylglycidic acid, and the yield of 1-naphthylacetaldehyde was 27 %, calculated on the 1-naphthaldehyde used.

After one crystallization the semicarbazone melted at 190°. The 2,4-dinitrophenylhydrazone, thiosemicarbazone and oxime prepared from the aldehyde formed by steam distillation melted at 181°, 180° and 118—120° respectively, showing the compounds to be derivatives of 1-naphthylacetaldehyde.

The oxime may also be prepared directly by boiling an aqueous solution of the salt with hydroxylamine hydrochloride, when the oxime separates.

By hydrolysis of the sodium salt of 1-naphthylglycidic acid with hydrochloric acid we once isolated a small amount of an acidic substance, which was insoluble in ether and melted at about 200° with evolution of carbon dioxide and formation of 1-naphthylacetaldehyde. According to these properties and analyses the compound is β-1-naphthylglyceric acid, $C_{10}H_7 \cdot CHOH \cdot CHOH \cdot COOH$.

$C_{13}H_{12}O_4$ (232.2)	Calc.	C	67.24	Eq.wt.	232
	Found	»	67.44	» »	233

SUMMARY

1-Naphthylacetaldehyde was for the first time prepared in a pure state by oxidation of 1-(1-naphthyl)-2,3-propanediol with lead tetraacetate and characterized through derivatives. Other possible methods of obtaining this

aldehyde were investigated, but most of them gave none or little of the required product.

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Received March 16, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 703–709

Researches on Plant Growth Substances

II. On 1-Napthylacetaldehyde 2 *

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In continuation of our work on l-naphthylacetaldehyde (preceding publication) we have investigated the applicability of the following reactions to the preparation of this aldehyde:

a. Decarboxylation of l-naphthylpyruvic acid.
b. Hydrolysis and decarboxylation of α-benzoylamino-β-l-naphthylacrylic acid.
c. Oxidation of β-l-naphthylalanine with ninhydrin.
d. Heating of barium 1-naphthylacetate with barium formate.
e. Rosenmund reduction of 1-naphthylacetyl chloride.
f. Reduction of ethyl 1-naphthylthiolacetate with Raney nickel.
g. Stephen reduction of 1-naphthylacetonitrile.
h. Hydrolysis of β-1-naphthyl-α,α-dicarbethoxyamino-ethane (β-1-naphthylidene diurethane).
i. Hydrolysis of ethyl 1-naphthylformyl acetate.
j. Oxidation of α-1-naphthylacrylamide with sodium hypochlorite.
k. Reaction of β-1-naphthylacrylic acid with hydrazoic acid.

We did not succeed in reducing the thiol ester (f) or in preparing the aldehyde by the Schmidt reaction (k), which applied to cinnamic acid gives a fair yield of phenylacetaldehyde (Oesterlin [1]). In all other cases 1-naphthylacetaldehyde could be isolated as the semicarbazone, but in most cases the yields were only small. Only by the reactions h and j could good yields of the aldehyde be obtained. The value of these methods, however, is severely dimi-

* This investigation has been supported by grants from *Danmarks tekniske Højskoles teknisk-kemiske Fond* and *Carlsbergfondet.*

nished by the circumstance that the starting materials, *i. e.* β-1-naphthylidene diurethane and β-1-naphthylacrylamide, are not too easily accessible. None of these methods are decidedly superior to the Hershberg reaction described in the preceding paper. The most serious drawback to this reaction is the troublesome transformation of 1-allylnaphthalene into 1-(1-naphthyl)-2,3-propanediol. Therefore we tried to prepare this glycol directly by the reaction of allylnaphthalene with hydrogen peroxide and osmium tetroxide. The desired reaction took place, but the glycol was rather impure and after purification a yield of only ca. 20 % was obtained. It was found that the crude product could be oxidized directly with lead tetraacetate, but without essentially increasing the yield. We also tried to prepare 1-naphthylacetaldehyde by ozonization of 1-allylnaphthalene, but, although 1-naphthylacetaldehyde was formed, the reaction product was very impure and difficult to purify.

As a conclusion we think that only the three methods mentioned can be considered as convenient for preparing 1-naphthylacetaldehyde; using naphthalene as starting material the three routes proceed via the following reactions:

1. Naphthalene $\xrightarrow{85\ \%}$ Bromonaphthalene $\xrightarrow{74\ \%}$ 1-Allylnaphthalene $\xrightarrow{60\ \%}$ 1-(1-Naphthyl)-2,3-propanediol-dibenzoate $\xrightarrow{90\ \%}$ 1-(1-Naphthyl)-2,3-propanediol $\xrightarrow{90\ \%}$ 1-Naphthylacetaldehyde. Over-all yield ca. 33 %.

2. Naphthalene $\xrightarrow{80\ \%}$ Chloromethylnaphthalene $\xrightarrow{72\ \%}$ 1-Naphthylmethylmalonic ester $\xrightarrow{95\ \%}$ 1-Naphthylmethylmalonic dihydrazide $\xrightarrow{90\ \%}$ β-1-Naphthyl-α,α-dicarbethoxyethane $\xrightarrow{88\ \%}$ 1-Naphthylacetaldehyde. Over-all yield ca. 43 %.

3. Naphthalene $\xrightarrow{80\ \%}$ 1-Chloromethylnaphthalene $\xrightarrow{67\ \%}$ 1-Naphthaldehyde $\xrightarrow{58\ \%}$ β-1-Naphthylacrylic acid $\xrightarrow{90\ \%}$ β-1-Naphthylacroyl chloride $\xrightarrow{90\ \%}$ β-1-Naphthylacrylamide $\xrightarrow{78\ \%}$ Ethyl β-1-naphthylvinylcarbamate $\xrightarrow{87\ \%}$ 1-Naphthylacetaldehyde. Over-all yield ca. 16 %.

The third method gives the smallest over-all yield, because of the many intermediate products, but the reagents used are rather cheap and readily accessible, and the operations involved are less delicate than in the first two methods. For preparation on a larger scale, therefore, the last method may well be the most convenient.

EXPERIMENTAL

1-Naphthylpyruvic acid. This compound was prepared either from the corresponding azlactone or from ethyl β-1-naphthyl-β-cyanopyruvate analogous to the preparation of phenylpyruvic acid (Bistrzycki [2]). The latter is the most satisfactory method.

a. A mixture of 7.5 g of 1-naphthaldehyde, 4.7 g of anhydrous sodium acetate, 7.0 g of hippuric acid and 19 ml of acetic anhydride was heated for one hour at 100°. On cooling yellow crystals of 2-phenyl-4-(naphthylmethylene)-oxazolone-(5) separated; these were filtered off and washed with water and ethanol. M. p. 170—171°.

$C_{20}H_{13}O_2N$ (299.3)	Calc.	N	4.68
	Found	»	4.53

Five g of the oxazolone was boiled for 5 hours with 50 ml of 10 % sodium hydroxide. The solution was acidified and the benzoic acid removed by steam distillation. The hot solution was filtered and cooled. The crystals, which separated, were recrystallized from hot water. Yield 1.5 g = 46 %. M. p. 157°.

$C_{13}H_{10}O_3$ (214.2)	Calc.	C	72.85	H	4.67	Eq.wt.	214
	Found	»	72.98	»	4.77	» »	215

b. A mixture of 400 ml of conc. sulphuric acid, 736 ml of water and 16 g of ethyl β-1-naphthyl-β-cyanopyruvate was heated to boiling. As soon as the ester had dissolved, the solution was filtered through glass wool and cooled. 1-Naphthylpyruvic acid separated as almost white crystals. Yield 70 %. Melting point 157° after recrystallization from water.

Decarboxylation of 1-naphthylpyruvic acid. A solution of 1-naphthylpyruvic acid (80 mg) in diethylaniline (3 ml) was heated for 20 min. On addition of hydrochloric acid and steam distillation a distillate was obtained, from which 1-naphthylacetaldehyde was isolated in form of the semicarbazone (m. p. 191°). Yield 50 %.

With quinoline instead of diethylaniline the aldehyde was also obtained (yield 38 %).

Ethyl β-1-naphthyl-β-cyano-pyruvate. This compound was prepared in the same way as the corresponding 2-naphthylderivative [3]. To a solution of 0.85 g of sodium in 10 ml of absolute ethanol 6.1 g of 1-naphthylacetonitrile and 5.4 g of ethyl oxalate was added. The mixture was stirred for about 12 hours at room temperature and was then poured into water. The aqueous solution was extracted twice with ether to remove unreacted nitrile and ethyl oxalate and the pyruvate was precipitated by addition of acid. Recrystallized from benzene the pyruvate was obtained as colourless crystals with melting point 115°. Yield 60 %.

$C_{16}H_{13}O_3N$ (267.3)	Calc.	N	5.24	
	Found	»	5.41	5.08

1-Naphthylacetaldehyde from β-1-naphthyl-α-benzoylamino-acrylic acid. This compound has previously been prepared by Kikkoji [4] by hydrolysis of the azlacetone of 1-naphthaldehyde with 5 % sodium hydroxide.

By heating with diethylaniline, addition of hydrochloric acid and steam distillation a 42 % yield of 1-naphthylacetaldehyde was obtained (isolated as the semicarbazone, m. p. 191°).

1-Naphthylacetaldehyde from β-1-naphthylalanine (*a*-naphthylalanine). This amino acid was first synthesized by Kikkoji by the azlactone method, reducing the acrylic acid with sodium amalgam, and has recently been synthesized from 1-chloromethylnaphthalene by the acetamidomalonic ester method [5]. We have prepared this compound in good yield by at the same time hydrolyzing and reducing the azlactone with hydriodic acid and red phosphorus in the same way as described for phenylalanine (Gillespie and Snyder [6]). Yield 61 %. M. p. 238°.

By addition of ninhydrin (45 mg) to a suspension of β-1-naphthylalanine (25 mg) in water (5 ml) and steam distillation 1-naphthylacetaldehyde was formed (yield 5.4 mg of the semicarbazone, m. p. 190°).

Rosenmund reduction of 1-naphthylacetyl chloride. The acid chloride (Boiling point 174° at 15 mm) was prepared from 1-naphthylacetic acid and thionyl chloride, and was reduced under the usual conditions (*cf.* Mosettig and Mozingo [7]), using a 4 % palladium-barium-sulphate catalyst (2 g to 4 g of the acid chloride in 16 ml of toluene). By steam distillation of the reaction mixture a 35 % yield of 1-naphthylacetaldehyde was obtained.

Ethyl 1-naphthylthiolacetate. To a solution of 3 g of 1-naphthylacetyl chloride in 10 ml of dry ether 2.5 g of lead ethylmercaptide was added. There was a feeble evolution of heat. After standing over night the solution was filtered, the ether evaporated and the residue distilled *in vacuo*. B. p. 137—139° at 0.5 mm. Yield 2.45 g = 73 %. Colourless oil.

$C_{14}H_{16}OS$ (230.3)	Calc.	C	73.01	H	6.13	S 13.92
	Found	»	72.77	»	5.74	» 13.72

We did not succeed in reducing this thiolester with Raney nickel according to the method of Wolfrom and Karabinos [8].

Stephen reduction of 1-naphthylacetonitrile. The reduction was carried out in accordance with the directions for the reduction of β-naphthonitrile [9], and the aldehyde formed isolated by steam distillation of the reaction product. Only 0.5 g of the semicarbazone (m. p. 190—191°) was obtained from 4 g of 1-naphthylacetonitrile. This is presumably due to the sensitivity of 1-naphthylacetaldehyde towards strong acid.

Ethyl 1-naphthylmethylmalonate. This compound was prepared by v. Braun and Nelles [10] by reaction of 1-chloromethylnaphthalene with sodium ethylmalonate. Following these directions we obtained the ester as a viscous oil which crystallized after standing for several weeks and then had m. p. 32°.

1-Naphthylmethylmalonic dihydrazide. This and the following compound were prepared in the same way as the corresponding phenyl derivatives (Curtius [11]): To 50 g of ethyl 1-naphthylmethylmalonate, 25 g of hydrazine hydrate and 5 ml of abs. alcohol were added and the mixture heated for one hour on a water bath. By the reaction the mixture was transformed into a solid mass, which was ground with water, filtered and washed with water, alcohol and ether. Yield 39 g = 91 %. M. p. 215—216°. The compound is very slightly soluble in most solvents. A small portion was recrystallized from ethanol without change of the melting point.

$C_{14}H_{16}O_2N_4$ (272.3)	Calc.	N	20.57
	Found	»	20.38

β-1-Naphthyl-a,a-dicarbethoxyamino-ethane. The dihydrazide (11 g) was dissolved in conc. acetic acid (20 ml), the solution was cooled in ice and sodium nitrite (10.3 g in 20 ml of water) added slowly with stirring. After the addition of the nitrite, 50 ml of water was added, which caused the azide to separate completely as a yellowish solid or semisolid mass. The azide is rather unstable, so we did not isolate it, but after standing for some minutes the acetic acid was removed by decantation and 100 ml of abs. alcohol was added to the azide and the solution boiled on a water bath for three hour. On cooling the diurethane crystallized and was recrystallized from ethyl acetate as white plates, m. p. 206—208°. Yield 11.3 g = 85 %.

$C_{18}H_{22}O_4N_2$ (330.4)	Calc.	N	8.48
	Found	»	8.45

Hydrolysis: A suspension of 2 g of the diurethane in 25 ml of 2 % sulphuric acid was steam distilled. By addition of semicarbazide hydrochloride to the distillate 1.15 g of 1-naphthylacetaldehyde semicarbazone (melting point 191—192°) was obtained, *i. e.* a yield of 87 %.

1-Naphthylacetaldehyde by hydrolysis of ethyl 1-naphthylformylacetate. Wislicenus and Elvert [12] prepared two forms (keto and enol form) of ethyl 1-naphthylformylacetate by condensation of ethyl 1-naphthylacetate with ethyl formate. We have prepared these two forms and transformed both into 1-naphthylacetaldehyde by boiling with 90 % acetic acid for 4 hours, removing of the acetic acid *in vacuo* and steam distilling. Yield 70 % of 1-naphthylacetaldehyde, isolated as the semicarbazone (melting point 191—192°). By hydrolysis with 2 % sulphuric acid instead of acetic acid the yields were much smaller (12 and 8 %).

β-1-Naphthylacrylic acid chloride. Naphthylacrylic acid (10 g) was suspended in benzene (25 ml), pure thionyl chloride (5 ml) was added, and the mixture heated cautiously untill the acid had dissolved (5 min.) Benzene and excess of thionyl chloride were removed in vacuo. The residue crystallized spontaneously and was recrystallized from petroleum ether. Yield 90 %. M. p. 52°.

$C_{15}H_9OCl$ (218.7)	Calc.	C	72.06	H	4.18	Cl	16.37
	Found	»	71.93	»	3.95	»	16.28

β-1-Naphthylacrylic amide. The acid chloride was melted and poured into 200 ml of aqueous ammonia. The suspension was heated for ½ hour at 100°, cooled and filtered. Yield 8 g. M. p. 172—173° after recrystallization from water. From the mother liquor 1 g of naphthylacrylic acid could be recovered, making the yield of amide 90 %.

$C_{13}H_{11}ON$ (197.2)	Calc.	C	79.16	H	5.62	N	7.10
	Found	»	79.20	»	5.43	»	7.16

Methyl β-1-naphthylvinylcarbamate. The amide was dissolved in methanol (80 ml) by warming, and the equivalent amount of 1 N sodium hypochlorite solution was added in one portion. After 5 minutes the solution began to deposit crystals. and was then cooled

in ice. The crystals were filtered and recrystallized from methanol. Yield 6.2 g = 78 %. M. p. 120–121°.

$C_{14}H_{13}O_2N$ (227.3)	Calc.	N	6.16
	Found	»	6.09

1-Naphthylacetaldehyde by hydrolysis of the urethane: 4.3 g were dissolved in 30 ml of warm ethanol, 7 ml of 6 N oxalic acid was added and the solution distilled with steam. The distillate was extracted with ether, the ether solution dried with sodium sulphate and evaporated in vacuo at room temperature. The residue crystallized in colourless needles. M. p. 34°. Yield 2.6 g = 81 %.

Oxidation of 1-allylnaphthalene with hydrogen peroxide. To a solution of 5 g 1-allylnaphthalene in 50 ml of methanol were added 3.4 ml of 30 % hydrogen peroxide and 0.40 ml of a solution of osmium tetroxide, containing 0.1 g OsO_4 in 9 ml of *tert.*-butanol. The solution was left for 2 days in ice box and then for 12 hours at room temperature. Most of the methanol was removed *in vacuo*, and water was added, which caused a green semisolid precipitate to separate. By repeated recrystallizations from water this gave 1.2 g of 1-(1-naphthyl)-2,3-propanediol with melting point 111° = 20 %.

$C_{13}H_{14}O_2$ (202.2)	Calc.	C	77.21	H	6.98
	Found	»	77.66	»	7.10

Other experiments with acetic acid or acetic acid + acetic anhydride as solvents gave no better yield.

SUMMARY

Several reactions were investigated with a view to preparing 1-naphthylacetaldehyde. From most of the reactions some 1-naphthylacetaldehyde was obtained, but only two of them are suited for preparative purposes, *viz.* the hydrolysis of β-1-naphthyl-α, α-dicarbethoxyamino-ethane and the Hofmann degradation of β-1-naphthylacrylic amide.

The following new compounds are described: 1-Naphthylpyruvic acid, ethyl β-1-naphthyl-β-cyano-pyruvate, ethyl 1-naphthylthiolacetate, 1-naphthylmethylmalonic dihydrazide, β-1-naphthyl-α,α-dicarbethoxyamino-ethane, β-1-naphthylacrylic acid chloride, β-1-naphthylacrylic amide, methyl β-1-naphthylvinylcarbamate.

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Received March 16, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 710–717

Short Communications

The Preparation of 3-Nitro-*p*-Toluic Acid from *p*-Cymene

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In the course of other experimental work, it became necessary to prepare 3-nitro-*p*-toluic acid from *p*-cymene. The literature advocates the use of fuming nitric acid, a nitric-sulfuric acid mixture, or oxidation to *p*-toluic acid (with dilute HNO_3) followed by nitration [1].

In view of the results of Senseman and Stubbs [2] in the catalytic oxygen oxidation of *p*-cymene, in the liquid phase, to *p*-toluic acid, a study of the effect of manganese dioxide on the nitric acid oxidation of cymene was undertaken.

The products obtained from conc. nitric acid (d. 1.40), cymene, and MnO_2 varied widely with the HNO_3-cymene ratio. With a ratio of 50 ml HNO_3 per ml cymene, quite pure crystalline 3-nitro-*p*-toluic acid (M. P. 186°, Eq. wt. 175 for the raw product) was obtained in a yield of 50–60 %. The pure acid could be easily obtained by a single recrystallization from boiling water. If the ratio were increased to 100 : 1, the yield was sharply reduced, probably due to the formation of dinitro products. Ratios of 10 : 1 and 25 : 1 yielded heterogeneous products from which alkali insoluble aldehydes and ketones, *p*-toluic acid, terephthalic acid, in addition to nitro-*p*-toluic acid, were isolated. The yields of pure nitro acid from these ratios were small.

The reaction was generally complete after one hour. Increasing the reaction time had little effect on either the yield or the composition of the products obtained.

A comparison test, using the optimium HNO_3-cymene ratio but no MnO_2, yielded a product (in 19 % yield) which showed a melting point of 160 to 180° (pure 3-nitro-*p*-toluic acid melts at 189°).

Thus it can be seen that the use of MnO_2 increases the yield of the nitro acid and decreases the formation of oxidation by-products.

However, in the oxidation of cymene to *p*-toluic acid with dilute HNO_3 (d. 1.19), the MnO_2 appears to act in an adverse manner, since the yields obtained (approx. 35 %) are inferior to those reported by Tuley and Marvel [3] (approx. 50 %).

An attempt was made to replace the MnO_2 with mercury salts, but the results were unsatisfactory. No precipitate at all was obtained after cooling the refluxed solution.

The following table summarizes the results:

Density of HNO_3 used	Time of reflux hr.	Ratio ml HNO_3 : Cymene	Product	Average Yield*	Remarks
1.40	1	50:1	3 nitro-*p*-toluic acid	53 %	
1.40	2	50:1	»	51 »	
1.40	1	100:1	»	15 »	
1.40	1	10:1	»	24 »	
1.40	1	25:1	»	26 »	
1.40	1	50:1	»	19 »	no MnO_2
1.19	1	50:1	*p*-toluic acid	35 » **	
1.19	1	250:1		40 » **	
1.19	1	250:1	—	—	$HgNO_3$ used

* Yield calculated from weight of raw product and quotient of found equivalent weight and true equivalent weight of product.

** Solubility of *p*-toluic acid in water (0.034 g/100 ml at 5° C) taken into account.

Inability of Guanosine to Act as a Precursor of Polynucleotides

EINAR HAMMARSTEN and
PETER REICHARD

Biochemical Department, Karolinska Institutet, Stockholm, Sweden

Recent work from this laboratory [1] has shown that the pyrimidineribosides, which contain ribose bound to cytosine or uracil are metabollically different from free pyrimidines. Isotope experiments with the latter [2, 3] proved that these compounds cannot be utilized for the synthesis of polynucleotides while isotopic cytidine and also to some extent uridine can be used by the rat for the synthesis of pyrimidines of both ribonucleic acid and desoxyrinucleic acid [1]. It was thought that perhaps in the same way guanine coupled to ribose might be a precursor for purines while free guanine is not [2]. For that reason guanosine containing N^{15} was prepared by allowing *B. coli* to grow in a $N^{15}H_4^+$ containing medium. After isolation of PNA from the bacteria [4] guanosine was prepared and purified by starch chromatography [5]. It had an excess of N^{15} of 4.20 atom per cent. After cristallisation from water the N^{15}-guanosine was injected subcutanously into rats at a level of 10 mg/100 g of body weight per day. The injections were carried out twice daily and over a period of 3 days. After that the rats were killed, the polynucleotides prepared and separated. Purines and pyrimidines were prepared from each fraction and analyzed for N^{15} (5). None of the fractions contained any significant amount of N^{15}. It is thus concluded that guanosine does not act as a precursor for the synthesis of polynucleotides.

1. Hammarsten, E., Reichard, P., and Saluste, E., *J. Biol. Chem.* **183** (1950) 109.
2. Plentl, A. A., and Schoenheimer, R. *J. Biol. Chem.* **153** (1944) 203.
3. Bendich, A., Getler, H., and Brown, G. B. *J. Biol. Chem.* **177** (1949) 565.
4. Hammarsten, E. *Acta Med. Scand.* Suppl. **196** (1947) 134.
5. Reichard, P. *J. Biol. Chem.* **179** (1949) 763.

Received June 2, 1950.

The general procedure used is as follows:

30 ml of pure *p*-cymene (B. P. 175.5—176.5°) were added to a mixture of 1500 ml conc. HNO_3 (d. 1.40) and 18 g MnO_2. This mixture was then refluxed for approximately one hour. Care must be taken to dispose of the oxides of nitrogen liberated during the reaction. The clear yellow solution was allowed to cool, whereupon pale yellow crystals separated. These were removed by filtration through a sintered glass filter and washed with cold water. If this wash water is added to the filtrate a further amount of slightly less pure 3-nitro-*p*-toluic acid separates.

The precipitate was then dissolved in dilute ammonia, filtered, and reprecipitated by neutralization with conc. HCl. This precipitate was removed, after cooling, and recrystallized from boiling water.

M. p. (lit.)	189°
M. p. (found)	188°—189°
N (calc.)	7.73 %
N (found)	7.70 »

The methyl ester was prepared by means of methanol and hydrochloric acid.

M. p. (lit.)	49°
M. p. (found)	48°—49°

1. Noad, H. *Ann.* **63** (1847) 297; Fittica, F. *Ann.* **172** (1874) 309; Kermack, W. O. *J. Chem. Soc.* **125** (1924) 2827; King, H. *J. Chem. Soc.* **127** (1925) 2639; Alfthan, J. *Ber.* **53** (1920) 83; Fittig, R., and Ramsay, W. *Ann.* **168** (1873) 251.
2. Senseman, C. E., and Stubbs, J. J. *Ind. Eng. Chem.* **24** (1932) 1184.
3. Tuley, W. F., and Marvel, C. S. *Org. Syn.*, Vol. 27 (1947) 86.

Received May 8, 1950.

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Received May 8, 1950.

On the Chlorine Content in Human Muscle and Skeletal Tissue

A rectification

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In *Acta Orthop. Scand.* 1949 [1] the present writer published an article with the above title. As Friberg [2] and Mutt [3] have already pointed out, the method employed for the chlorine determination embraces a source of error, which renders the results duly obtained, unreliable. The author wishes to express regret for the unfortunate occurrence, and at the same time give an account of the error and its effect on the final conclusions.

When ascertaining the quantitative determination of chlorine, it is usual for practically all methods to determine this element as silver chloride. This procedure, of course, is under the presumption that the investigated preparation contains no appreciable quantity of other halogens or cyanides as, obviously, cyanide as well as bromide and iodide, in association with silver, forms insoluble precipitations in nitric acid. In the material investigated by the writer none of these substances were present. The possibility of the formation of cyanide during the analysis itself, is a source of error which has not been reported in earlier literature and to which, hitherto, attention has not been paid. In spite of the same, it has been revealed in tests relating to biological material that cyanide is formed during the determination when performed with the Berg method. The precipitation of silver chloride and silver cyanide achieved by means of silver nitrate has since been looked upon as silver chloride, with the result that too high values have been obtained. When Berg's method is used for organic compounds containing chlorine but not nitrogen, the values are fully correct, whereas in determinations relative to preparations which, in addition to chlorine contain protein, cyanide is formed during the analysis and consequently the values attained, are too high. The same source of error is met with when determining the chlorine content, at least of casein, according to the Grote-Krekeler method. Analyses performed at different laboratories, have given values ranging from 0.11 to 1.1 % chlorine in casein. When the Grote-Krekeler method was applied as a control in order to verify values acquired by Berg's method, this source of error remained undetected. The possibility of cyanide formation and of too high values must moreover be counted with in respect to chlorine analysis according to Thompson and Oakdale, and also Robertson (see Chap. III of the foregoing work [1]).

In order to determine the magnitude of this error, it has been necessary to work out a chlorine determination method in which the error has been eliminated, and then compare the values with both the analytic methods. In doing so the writer used a modification of the chlorine determination method employed by Zacherl and Krainick [4]. With this procedure the combustion of the dried material is brought about with concentrated sulphuric acid and chromium trioxide in the presence of silver chromate, at which, as repeated tests have shown on the combustion of different protein substances (casein, cartilage, connective tissue and muscular protein), cyanide is not formed. As a further precautionary measure a receiver was used (potassium arsenite and acetic acid) which selectively retains chloride and eliminates cyanide. The chloride determination was afterwards made in the usual

manner according to Volhard. A more detailed account of this method for determining chlorine will be published later. On employing this new method (Zacherl and Krainick), in comparison with the foregoing (Berg) for the analysis of different preparations, the following values have been obtained:

Substance	% Cl according to Berg Cyanide formation	% Cl according to Zacherl and Krainick No cyanide formation
Liquefied subcutaneous fat	0.0	0.0
Toluenesulfonyle chloride (18.64 % Cl)	18.5	18.5
Dichlorosuccinic acid+glycose (1.05% Cl)	1.02	1.01
As above (0.10 % Cl)	0.12	0.10
Tyrosine "Merck"	4.03	0.08
Crude casein	1.09	0.04
Purified casein	0.96	0.04
Dialysed muscular protein	1.29	0.07
Connective tissue protein	2.50	0.97
Cartilage from intervertebral disc	1.0—2.5	0.4—0.6

Hence, it is apparent that although Berg's method when applied for determining the chlorine content in nitrogen-free organic chlorine compounds and in inorganic chlorine compounds gives correct values, the values given when the organic material contains nitrogen are too high, due to the formation of cyanide. The cyanide formation is then constant for the same preparation (casein. See the foregoing work, Chap. III [1]), but varies with different preparations. The method is therefore not applicable for determining the chlorine content of biologic material. The conclusions published in my foregoing work [1] can therefore not be adhered to, and the working hypothesis, based on study of pertinent literature relevant to the connection between so-called dry chloride retention and degeneration of the collagen system, is not supported by the analyses. Regarding the question as to whether any difference exists between the chlorine content of healthy and degenerated cartilage tissue, it can be said that the difference obtained with Berg's method (difference in chlorine content + the cyanide formation capacity) can either depend on an existing difference in chlorine content (in which event the cyanide formation should be identical in the compared series), or variableness in the cyanide formation (thus involving similarity of chlorine content in both series). Investigations on this point are continuing and will be published later. The "bound chlorine" detected by the author might probably be explained by the too high values which occurred on account of the cyanide formation. Hence, from the investigations made, there is no longer any reason to assume the presence of organic chlorides in the human body. The writer hopes to return to this question also in a later work.

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Received June 2, 1950.

Enzymatic Preparation of D.P.N. H_2 and Determination of D.P.N. *

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Methods have been described for both chemical and enzymatic determination of D. P. N.[1-3]. In their work on yeast alcohol dehydrogenase, Negelein and Wulff used the enzyme to prepare reduced D. P. N.[4]. They were, however, not able to reduce more than 85 % of the coenzyme.

Reduction with dithionite has been widely used but has the disadvantage that it is very difficult to free the solution completely from sulphite and sulphate, which will interfere with many enzyme reactions. The determination of D. P. N. with dithionite is not possible when alcohol, acetone and pyridine are present, since these substances will give a cloudy solution with dithionite. The method is also time consuming when many determinations have to be done; new buffers must be prepared each time.

The kinetics of the animal alcohol dehydrogenase shows [5] that an alkaline pH will be favourable for the complete reduction of D. P. N. This offers a means for the rapid determination of a certain amount of D. P. N.**

The following method for the preparation and determination of reduced D.P.N. is based upon the fact that in the presence of an excess of alcohol, at a pH of 10, all the D. P. N. will be reduced by alcohol dehydrogenase. T. P. N. will not interfere since the enzyme only reacts with D.P.N.[6]

Preparation of dihydro-D.P.N. The D.P.N.H_2 is prepared as follows: About 20 mg of D.P.N., usually about 55 % pure, is dissolved in water and the pH brought to 10—11 with dilute sodium hydroxide. Then 0.5 ml of absolute alcohol and about 5 mg of enzyme are added. During the reduction the pH is kept constant by the addition of a few drops of dilute sodium hydroxide.

After all the D.P.N. has been reduced the solution is heated to about 90° C to denature the enzyme. The solution is then dried under reduced pressure.

Determination of D.P.N. To a 1.0 cm cuvette containing 0.1 *M* NaOH-glycine buffer pH 10, a suitable amount of D.P.N. solution is added (about 10 to 80 gamma of pure D.P.N.). Then 0.1 ml of absolute alcohol and 1 to 2 mg of enzyme are added. If heavy metals or other enzyme inhibitors are suspected in the coenzyme solution, more enzyme is added. After about 10 to 15 minutes the reaction reaches completion. The difference in the extinction coefficient (log I_0/I) at 340 mμ before and after the addition of the enzyme multiplied by 118 gives percent D.P.N.[7].

We have compared this method with the chemical procedure described by Le Page [3] and complete agreement has been found.

The author wishes to thank *Stiftelsen Thérèse och Johan Anderssons Minne* for a grant.

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3. Le Page, G. A. *J. Biol. Chem.* **168** (1947) 623.
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Received June 7, 1950.

* A previous account of this work has been presented before the Physiological Society, Stockholm, on February 25th, 1950.

** The enzyme is commercially available (A. B. Astra, Södertälje, Sweden).

Crystalline Animal Alcohol Dehydrogenase 2

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Recently we reported purification and crystallization of alcohol dehydrogenase from horse liver [1]. In this paper we wish to describe the new method, which has given consistently good results, and some of the properties of the enzyme.

Preparation. One horse liver is ground and extracted with a double weight of distilled water for about 3 hours. We have generally used fresh livers, but frozen livers, 2—3 weeks old, give about the same yield.

The extract is heated to 52° C and kept at this temperature for 15 minutes [2], cooled to room temperature and centrifuged.

To the turbid red-brown solution is added ammonium sulphate to give a saturation of 0.5, and the solution centrifuged. The protein precipitate, which contains catalase, ferritin and the aldehyde oxidase activity, is discarded. The clear-red mother liquor contains the alcohol dehydrogenase activity.

The enzyme is precipitated when ammonium sulphate is added to give a saturation of 0.8. The precipitate is dissolved in 0.01 *M* phosphate buffer pH 7 and dialyzed against distilled water. The solution is then centrifuged, since some protein will denature on dialysis against water.

To the dialyzed solution is added phosphate buffer pH 7 untill the solution is 0.01 *M* with regard to phosphate. At this step the solution is clear red and contains about 150—200 mg of protein per ml.

The solution is now shaken vigorously with a mixture of alcohol and chloroform. To 1 liter solution we add 160 ml 99.6 % alcohol and 80 ml of chloroform. This procedure denatures nearly all of the hemoglobin. After centrifugation the alcohol and chloroform are evaporated under reduced pressure and the faint red solution is dialyzed against 0.01 *M* phosphate buffer pH 7.

The solution is again fractionated with ammonium sulphate and the precipitate between 0.50 and 0.80 % saturation is collected. The precipitate is dissolved in water and dialyzed against phosphate buffer as before.

The purity at this step is usually 0.06 to 0.1, the pure enzyme taken as 1.

The rest of the purification is carried out with alcohol at low temperature. We keep the temperature just above the freezing point of the mixture, though never below — 10° C. The protein concentration has varied from 10 to 50 mg per ml. As buffer solution we use 0.01 *M* phosphate at pH 7 throughout the whole procedure. The alcohol concentration referred to is volume percent 99.6 % alcohol.

From the first alcohol fractionation the precipitate between 20 and 60 percent alcohol is collected. The precipitate is dissolved in ice-cold phosphate. The purity at this step is 0.15—0.2.

The solution is then refractionated; this time the precipitates appearing at 30 and at 40 percent alcohol are collected separately and dissolved as before. Although the purity of these two fractions is about the same it is advantageous to separate them and refractionate them individually.

With the first fraction, the initial precipitate appearing at about 20 percent alcohol is discarded and the enzyme precipitated with 50 % alcohol. With the second fraction, the first precipitate is collected and the remaining protein is discarded. The two precipitates are dissolved as before and mixed. The purity is about 0.5—0.7.

On slow addition of alcohol to this solution the enzyme starts to crystallize at a alcohol concentration from 15 to 20 per-

Fig. 1. Crystalline alcohol dehydrogenase.

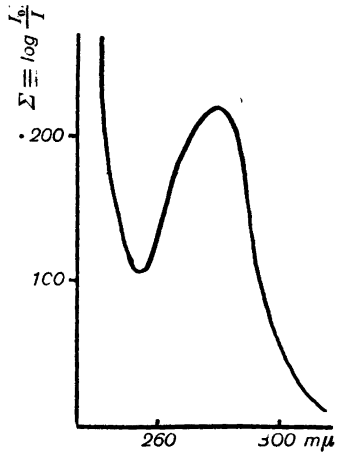

Fig. 2. Spectrum of alcohol dehydrogenase. 0.48 mg/ml; d = 1 cm.

cent. The purity of these crystals is about 0.8—0.9. We usually recrystallize three times before we get a pure enzyme. The enzyme solution is then dialyzed against water to remove all the alcohol from the solution.

The alcohol fractionations do not have to be carried out on the same day. We have kept different fractions for weeks in alcohol at low temperature without any loss in activity.

During crystallization all the activity leaves the solution and appears in the crystals. The protein is uniform on electrophoresis. The enzyme from different preparations has always shown the same properties. The crystals are shown in Fig. 1.

The total amount of enzyme present in the liver is difficult to determine on account of the large blank in the crude extract. However, as a result of determinations from many preparations we estimate the total amount of enzyme to be about 1 ‰ of the wet weight of the liver. A horse liver weighing 5.5 kg thus should contain 5.5 g of enzyme. From one horse liver we usually get 7—800 mg of crystalline enzyme. This yield can be increased considerably by refractionation of the discarded fractions.

Spectrum. The enzyme is a colorless protein. In the ultraviolet there is no other band than the usual protein band at 280 mμ (Fig. 2).

The band at 280 mμ is very low per mg of enzyme, about three times as low as that given for the yeast enzyme [3]. We use the extinction coefficient of 0.455 per mg of protein per cm at 280 mμ for determining the protein concentration in all our experiments.

Stability. The enzyme is stable from pH 5.4 to about 11. We store it in the form of crystals at low temperature or in solution in 30 % ammonium sulphate. Drying destroys the enzyme.

The author wishes to thank *Stiftelsen Thérèse och Johan Anderssons minne* for a grant.

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Received June 10, 1950.

Chondrosamine as a Component of Gangliosides and of Submaxillary Mucin

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According to Klenk [1] the gangliosides are composed of fatty acid, sphingosine, hexoses (in the main galactose) and a hydroxyamino acid called neuraminic acid (»Neuraminsäure») with the composition $C^{10}H^{19}NO^9$. In 1938 one of us [2] suggested the presence of hexosamine in the gangliosides and a recent analysis by paper chromatography [3] indicated that the hexosamine present might be chondrosamine.

We have now succeeded in isolating chondrosamine (as hydrochloride) from gangliosides prepared from normal brain tissue. After several recrystallisations it was obtained in a reasonably pure form and identified by qualitative reactions, elementary analysis, optical rotation and X-ray diffraction pattern.

A striking agreement in certain qualitative reactions between gangliosides and a carbohydrate occurring in submaxillary mucin [4] was noted in 1938 [2]. This carbohydrate was supposed to be a disaccharidic substance composed of a N-acetylated hexosamine and a polyhydroxy acid with six carbon atoms, the latter not being a hexuronic acid, however. The disaccharide in question (or polymers of it) is the dominating carbohydrate in the submaxillary mucin. From a submaxillary mucin hydrolysate we have now been able to isolate a hexosamine as the hydrochloride, and also in this case it proved to be chondrosamine.

Substances giving the same qualitative reactions as the gangliosides and the submaxillary mucin carbohydrate are also present to some extent in other epithelial mucins and in the glycoproteins of the blood serum. The carbohydrate group responsible for these reactions, which is believed to be composed of N-acetylated chondrosamine and a polyhydroxy acid with six carbon atoms, thus seem to have a rather wide distribution. The neuraminic acid, the isolation procedure of which includes prolonged heating with barium hydroxide, is probably to be regarded as a degradation product of the native disaccharidic compound.

A detailed report will be given elsewhere. The investigations are continued.

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Received June 4, 1950.

New Books

Advances in protein chemistry. Volume V. Edited by M. L. Anson and J. T. Edsall. Associate editor for the British Isles K. Bailey. Academic Press Inc., New York, 1949. ix + 481 pp. $ 7.50.

It is surely not necessary to repeat what has been said in praise of the preceding volumes of this series; everybody interested in the field of protein chemistry knows that in the "Advances in protein chemistry", he can find reliable information on a great variety of questions concerning proteins, their chemistry and their biochemistry. It may suffice here to give a short survey of the contents of the new volume: J. S. Fruton, Yale University, New Haven, gives an exhaustive review of methods used for peptide syntheses (305 references!). G. R. Tristram, Cambridge, England, discusses the amino acid composition of purified proteins, the methods used in the analyses and conclusions drawn from the results. J. B. Allison, Rutgers University, New Brunswick, has written on the biological evaluation of proteins. Milk proteins are treated by Th. L. McMeekin and B. D. Polis, Philadelphia, and plant proteins by J. W. H. Lugg, Melbourne, Australia. H. P. Lundgren, Albany, California, surveys the work on "synthetic fibers made from proteins", and K. H. Gustavson, Garveriindustriens forskningslaboratorium, Stockholm, communicates "some protein-chemical aspects of tanning processes". Finally we find in this volume an interesting paper by A. Claude, Rockefeller Institute, New York, on "proteins, lipids, and nucleic acids in cell structures and functions".

K. Myrbäck.

ACTA CHEMICA SCANDINAVICA 4 (1950) 719–724

Zur Kenntnis der Reaktion zwischen Chlorhydrinen und Ammoniak oder Aminen. II. Glycerinmonochlorhydrin und Amine

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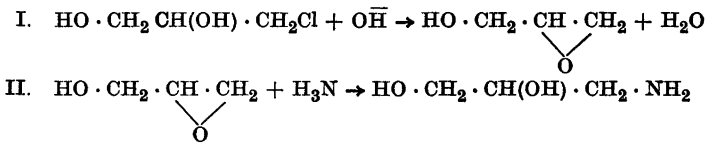
In einer früheren Mitteilung ist u. a. gezeigt worden, dass die Reaktion zwischen Ammoniak und Glycerinmonochlorhydrin höchst wahrscheinlich in folgenden zwei Stufen vor sich geht:



Wurde das Fortschreiten der Reaktion durch Bestimmung des gebildeten Chlorids verfolgt und die Geschwindigkeit für Stufe I nach dem bimolekularen Schema berechnet, so wurden nämlich Reaktionskoeffizienten ohne Gang erhalten, die ausserdem innerhalb der Versuchsgenauigkeit mit den Koeffizienten übereinstimmten, die Monochlorhydrin mit Alkali gibt. Das Ammoniak war in Überschuss vorhanden, weil dadurch der Einfluss des durch Stufe II gebildeten primären Amins auf die Hydroxylionenkoncentration weitgehend aufgehoben wird. Dieses Amin ist nämlich ohne Zweifel eine viel stärkere Base als Ammoniak.

In Mitteil. I hatten wir nur die Absicht, die annähernde Richtigkeit dieses Reaktionsmechanismus festzustellen. Die Zahl der Messungen war sehr klein (2 Tabellen mit Ammoniak und Glycerinmonochlorhydrin) und die mathematische Behandlung vereinfacht. Hier werden Versuche mit Ammoniak,

* Mitteil. I (von Lennart Smith unter Mitw. von Torsten Nilson) in *J. prakt. Chem. N. F.* **162** (1943) 63.

Methyl-, Dimethyl- und Trimethylamin veröffentlicht, die sowohl ohne als bei Gegenwart von entsprechenden Aminsalzen ausgeführt worden sind.

Zur mathematischen Behandlung wollen wir folgendes bemerken: der Reaktionsmekanismus ist bei Mono- und Dimethylamin genau derselbe wie bei Ammoniak, bei Trimethylamin aber etwas verschieden. Bei der Addition dieses Amins an das Glyceringlycid wird nämlich eine *quartäre* Base gebildet, was eine Vermehrung der Geschwindigkeit der Reaktionsstufe I zustande bringt. Näher hierüber unten (S. 00).

Im folgenden bedeuten: a, b, x und c Konzentrationen von Chlorhydrin, Amin (Ammoniak), gebildetem Chlorid und zugesetztem Ammoniumnitrat. K_d ist die thermodynamische und k_d die stöchiometrische Dissoziationskonstante des Amins (Ammoniaks).

Der Einfluss des Aktivitätsfaktors wurde nach folgender Formel berechnet (f = Aktivitätskoeff.; μ = Ionenstärke i z_i = Ionenvalenz):

$$-\log f_i = 0.5\, z_i^2 \cdot \frac{\sqrt{\mu}}{1+\sqrt{\mu}}; \qquad \log k_d = \log K_d + \frac{\sqrt{\mu}}{1+\sqrt{\mu}}$$

Für Stufe I gilt: $\frac{dx}{dt} = k\,(a—x)\cdot[OH]$;

$$\text{integriert} \int_0^{x_1} \frac{dx}{(a—x)\cdot[OH]} = k \cdot t_1$$

Der Verlauf der Reaktion wurde durch Bestimmung des gebildeten Chloridions x (Volhardtitrierung) bestimmt. Die Hydroxylionenkonzentration erhält man aus dem Ausdrucke:

$$[OH]\cdot\frac{c+x+[OH]}{b-x-[OH]} = k_d$$

Da die Abhängigkeit des Produktes $(a—x)\cdot[OH]$ im Integral von x somit bekannt ist, kann das Integral graphisch bestimmt werden.

Die kinetischen Gleichungen sind, wie ersichtlich, ohne Zuhilfenahme von Aktivitäten berechnet worden. Wir fanden dies überflüssig, in Betracht der Unsicherheit, die immerhin das in Stufe II gebildete Amin herbeiführt.

MESSUNGEN

Zu den Messungen ist zuerst zu bemerken: Methyl- und Trimethylamin waren Präparate von Merck »für wissenschaftliche Zwecke»; Dimethylamin wurde aus Dimethylanilin dargestellt. Die Reinheit dieses Präparates wurde

durch Bestimmung der Dissoziationskonstante kontrolliert. Das Chlorhydrin stellten wir aus Epichlorhydrin durch Wasseraddition dar. Es wurde durch Vakuumdestillation gereinigt.

Alle Messungen bei 20°; Zeit in Minuten.

I. Ammoniak

Tabelle 1.

$a = 0{,}0426$ $b = 0{,}1273$ $c = 0$ $K_d = 1{,}80 \cdot 10^{-5}$

Zeit	25,5	50	100	175,5	276	437	742	1067	1808
$\frac{x}{a} \cdot 100$	11	16	23	30	37	45	55	62	72
k	5,66	5,60	5,61	5,63	5,70	5,72	5,60	5,59	(5.31)

$k_{\text{Mitt.}} = 5{,}64 \pm 0.02$

Tabelle 2.

$a = 0{,}03699$; $b = 0{,}1109$; $c = 0{,}01733$; $K_d = 1{,}80 \cdot 10^{-5}$

Zeit	160	373	554	900	1084	1321	1631	1943	2417	3161
$\frac{x}{a} \cdot 100$	12	23	29	40	44	49	55	60	65	72
k	5,83	5,90	5,72	5,68	5,68	5,67	5,64	5,68	5,61	(5,54)

$k_{\text{Mitt.}} = 5{,}71 \pm 0{,}03$

Eine dritte Messung (Bedingungen etwa wie in Tabelle 2) ergab $k = 5{,}75$.

II. Methylamin

Tabelle 3.

$a = 0{,}02309$; $b = 0{,}06063$; $c = 0$; $K_d = 4{,}38 \cdot 10^{-4}$

Zeit	4,65	9,7	13,1	19,1	26,2	43,0	63,0
$\frac{x}{a} \cdot 100$	13	23	27	35	42	53	60
k	6,34	6,55	6,31	6,42	6,21	6,38	5,90

$k_{\text{Mitt.}} = 6{,}30 \pm 0{,}08$

Tabelle 4.

$a = 0{,}01689$; $b = 0{,}05057$; $c = 0{,}05480$; $K_d = 4{,}38 \cdot 10^{-4}$

Zeit	76	107	147	190	232	284	337	421
$\frac{x}{a} \cdot 100$	24	31	38	46	52	59	64	70
k	6,36	6,20	6,11	6,25	6,18	6,23	6,11	6,23

$k_{\text{Mitt.}} = 6{,}21 \pm 0{,}03$

Eine dritte Messungsserie ergab $k = 6{,}09 \pm 0{,}06$.

III. Dimethylamin

Tabelle 5.

$a = 0{,}02014$; $b = 0{,}06018$; $c = 0$; $K_d = 5{,}92 \cdot 10^{-4}$

Zeit	7	11	16	22,2	28,8	36,8	45,8	55	70
$\frac{x}{a} \cdot 100$	21	28	36	43	49	55	61	65	71
k	6,34	6,29	6,18	6,22	6,17	6,02	6,00	5,84	5,84

$k_{\text{Mitt.}} = 6{,}10 \pm 0{,}08$

Tabelle 6.

$a = 0{,}02602$; $b = 0{,}08501$; $c = 0{,}02644$; $K_d = 5{,}92 \cdot 10^{-4}$

Zeit	17	25,1	38,8	53,0	71,3	89,4	110,8	133,2
$\frac{x}{a} \cdot 100$	20	27	36	44	52	59	64	69
k	6,07	6,20	6,02	5,99	5,89	5,88	5,83	5,66

$k_{\text{Mitt.}} = 5{,}94 \pm 0{,}06$

Eine dritte Serie ($c = 0{,}055$) ergab $K_{\text{Mitt.}} = 5{,}84 \pm 0{,}06$.

IV. Trimethylamin

Wir bezeichnen kurz: Glycerinmonochlorhydrin Glm, Glyzid Gl, Trimethylamin R_3N, die gebildete quartäre Base $R_3NR'OH$. Diese Base ist als vollständig dissoziert anzusehen. Übrige Bezeichnungen wie oben, [ ] gibt Konzentrationen an.

$$\underset{a-x}{\text{I } Glm} + OH^- \rightarrow \underset{x-y}{Gl} + H_2O + \underset{x}{Cl^-}$$

$$\underset{x-y}{\text{II } Gl} + R_3N + H_2O \rightarrow \underset{y}{R_3NR'^+} + \bar{O}H$$

Weiter ist zur Zeit t: $b = [R_3N] + [R_3NH^+] + [R_3NR'^+]$ und
$[R_3NH^+] + [R_3NR'^+] = [\bar{O}H] + [Cl^-]$

Da $[Cl^-] = x$ und $[R_3NR'^+] = y$, bekommen wir folgende Gleichungen:

$$\frac{dx}{dt} = k\ (a-x)\ [\bar{O}H] \tag{1}$$

$$\frac{dy}{dt} = k_1\ (x-y)\ (b-x-\ [OH]) \tag{2}$$

$$[\bar{O}H]\ \frac{x-y+[\bar{O}H]}{b-x-[\bar{O}H]} = k_d \tag{3}$$

Der Geschwindigkeitskoeffizient k_1 ist bekannt und $= 0{,}28$ [1].

Mit Hilfe von (1), (2) und (3) erhält man schliesslich:

$$y = x + \frac{dx}{dt} \cdot \frac{1}{k\ (a-x)} + k_d - \frac{k_d(b-x)\ k\ (a-x)}{\frac{dx}{dt}} \tag{4}$$

Wir derivieren (4) in Bezug auf t. Aus der resultierenden Gleichung $\left(\frac{dy}{dt} = \frac{dx}{dt}\right.$ + u. s. w.) kann $\frac{dy}{dt}$ mit Hilfe von (2) eliminiert werden, wodurch schliesslich eine Gleichung dritten Grades in Bezug auf k, welche auch $\frac{dx}{dt}$ sowie $\frac{d^2x}{dt^2}$ enthält, entsteht. Wird, wie es hier der Fall ist, x empirisch für entsprechende t-Werte bestimmt, so ist es möglich, z. B. mittels der Methode der kleinsten Quadrate, eine empirische Funktion mit diesen Variablen zu berechen, die durch Derivation die nötigen Werte von $\frac{dx}{dt}$ und $\frac{d^2x}{dt^2}$ geben kann. Wir halten es für überflüssig, diese Funktionen hier zu wiedergeben (eine solche wurde berechnet für $t = 35-50$, eine andere für $t = 100-150$)*. (Für Berechnung von k_d muss man zuerst einen annähernden Wert von $[\bar{O}H]$ berechnen.)

Bei $t = 42$ wurde gefunden: $k = 5{,}8$
» $t = 125$ » » $k = 5{,}2$

[1] L. Smith, Lunds Universitets Årsskrift, N. F. Avd. 2, Bd. 42, Nr. 7.

* Diese mathematische Auseinandersetzung rührt von Grimsson her.

Eine einfachere Lösung ergibt sich aus der Tatsache, dass die hydroxylionenerzeugende Stufe II sich bald mit der dieselben Ionen verbrauchende Stufe I in Gleichgewicht setzt, welches bedeutet, dass $\frac{dx}{dt}$ etwa gleich $\frac{dy}{dt}$ wird. Nimmt man aus der x—t-Kurve den Wert von $\frac{dx}{dt}$ $(= \frac{dy}{dt})$ in einem bestimmten Augenblicke, erhält man mit Hilfe von (2) den Wert von $(x—y)$, welchen dann in (3) eingesetzt wird, wonach (wie oben) unter Vernachlässigung von [OH] neben $(b—x)$ im Zähler [OH] berechnet werden kann. Schliesslich gibt (1) den Wert von k. Für $t = 70$ wurde $k = 5{,}0$ erhalten, was leidlich mit den obigen Resultaten übereinstimmt.

ZUSAMMENFASSUNG

Wenn die hier dargelegte Auffassung vom Reaktionsmechanismus richtig ist, muss die Geschwindigkeitskonstante vom benutzten Amin unabhängig sein. Berücksichtigt man die Versuchsfehler, den schwer zu schätzenden Einfluss der Stufe II sowie die grosse Abhängigkeit der berechneten Geschwindigkeitskoeffizienten von den Dissoziationskonstanten der Amine, kann man behaupten, dass diese von der Theorie geforderte Unabhängigkeit vorhanden ist. Wir fanden folgende Geschwindigkeitskoeffizienten:

NH_3	$k = 5{,}7$
CH_3NH_2	$k = 6{,}2$
$(CH_3)_2NH$	$k = 5{,}9$
$(CH_3)_3N$	$k = 5{,}5$**

Die Reaktion zwischen Aminen (Ammoniak) und Glycerinmonochlorhydrin ist somit nach dem Seite 719 skizzierten Schema eine Stufenreaktion, in welcher zuerst eine Oxydoverbindung entsteht, die dann das Amin Ammoniak addiert. Höchst wahrscheinlich reagieren andere Chlorhydrine in ähnlicher Weise.

** Der durch das vereinfachte Verfahren gefundene Wert 5,0 ist hier nicht mitgenommen.

Eingegangen am 18. April 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 725–737

The Raman Spectrum and the Potential Function of Cyanogen

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From investigations on cyanogen, diacetylene and dimethyldiacetylene by electron diffraction Pauling, Springall and Palmer have concluded that the C—C single bond in these compounds has a considerable amount of double bond character [1]. It seems worth while to try these results confirmed from calculation of the potential functions, and in the case of C_2N_2 where Pauling *et al.* find 40 per cent double bond character such have already been carried out on basis of the valence force system [2–4]. However, it is to be expected that this can only give a rough approximation and that a more general type of potential function including "cross terms" should be applied. But even in the simple case of C_2N_2 not enough vibration frequencies can be observed in the infrared and Raman spectra to make a calculation of the complete harmonic potential function possible. This difficulty could be overcome by considering also frequencies from the isotopic molecule $C^{12}C^{13}N_2^{14}$ which constitutes about 2.2 per cent of natural cyanogen. In fact, by taking the Raman spectrum with a spectrograph of high resolving power and dispersion we have been able to detect two frequencies one of which can be ascribed to this molecule. In the following will be given the results of new electron diffraction experiments on cyanogen, and of a reinvestigation of the Raman spectrum including depolarization measurements of the two strongest lines. Finally, using data also from the infrared spectrum obtained by others [4] the force constants in the harmonic potential function are calculated and the nature of the valence force field is discussed.

EXPERIMENTAL

Preparation. The cyanogen used for these experiments was prepared according to Kistiakowsky [5] and A. Klemenc [6] by dripping a concentrated solution of potassium cyanide into a concentrated solution of cupric sulphate under reduced pressure. The gas evolved was purified by repeated distillations and finally dried over P_2O_5.

Electron diffraction *. Electron diffraction photographs were taken of C_2N_2 by the rotating sector method. From the microphotometer traces of four plates the radial distribution function $\frac{\sigma(r)}{r}$ was obtained by a procedure previously described in this journal [7]. This curve had very pronounced maxima at $r = 1.16$ Å, $r = 2.53$ Å and $r = 3.68$ Å corresponding to a linear model with distances: C—C: 1.38 Å, N≡C: 1.15 Å which is in complete agreement with the investigations of Pauling *et al.*[1] The probable error is about ± 0.02 Å.

Raman spectrum. The apparatus and methods for obtaining the Raman spectrum were those usually employed in this laboratory and which have been described elsewhere [8]. The observed frequencies are given in Table 1 together with their visually estimated intensity and comments on the general appearance of some of the Raman lines.

As far as we know the Raman lines 521.4 cm^{-1}, 561.4 cm^{-1}, 841 cm^{-1} and 2 298.4 cm^{-1} have not been observed before [9-11]. On the other hand we have not been able to detect the very weak lines 304 cm^{-1}, 696 cm^{-1}, 1 102 cm^{-1} and 1 386 cm^{-1} found by Reitz and Sabathy in their investigation of cyanogen [11].

A one prism spectrograph of high light-gathering power (camera lens f 1 : 2.5) was used for polarization measurements. The Raman tube was irradiated with approximately parallel light from two Philip's HO 2 000 Hg-lamps. By aid of a Rochon prism the scattered light was split into two beams with the polarization planes at right angles. In this way we got two separated pictures on the slit of the spectrograph and hence two spectra on the photographic plate. On the same plate and with the same set up were

Table 1.

Frequency in cm^{-1}	Intensity	Remarks
c. 240	weak	Broad diffuse
c. 475	very weak	Diffuse
507.2	strong	Somewhat diffuse
521.4	weak	
561.4	very weak	
750	very weak	
841	weak	
850.6	medium	Sharp
1 028.5	weak	Sharp
2 298.4	weak	
2 328.5	very strong	Sharp

* One of us (C. K. M.) is very indebted to Professor O. Hassel and his staff for a stay at the Physical-Chemical Laboratory of the University at Oslo during which this part of the work was done.

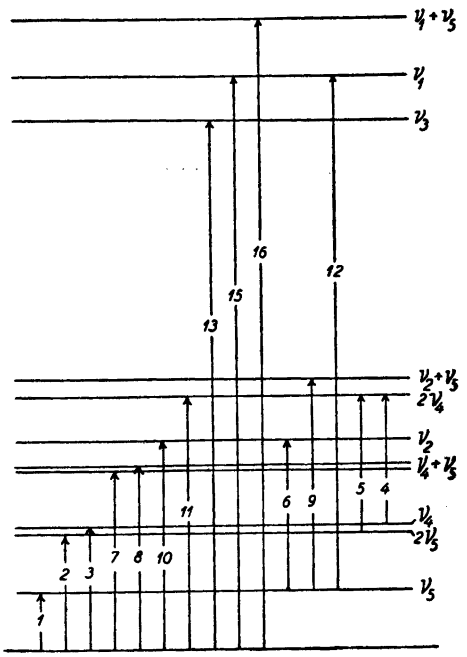

Fig. 1. Vibrational energy level diagram of the cyanogen molecule. The numbering of the different transitions refer to Table 2.

next photographed the two analogous spectra from a very pure CCl_4-sample and finally without the Rochon prism, the continuous spectrum of the hot filament of a small lamp with a Pt-step-filter before the slit. The plates were photometered by aid of a photoelectric microphotometer. The adjacent continuous step spectra photometered at the wave length of the Raman line considered gave the relation between blackening and energy. On basis of this and from the microphotometer traces of the Raman lines apparent depolarization degrees were calculated for C_2N_2 and CCl_4. Finally, using the known values for CCl_4 as standards [12] the true depolarizations were obtained. (It may be added here that the spectrograph slit was so wide that the heights of the maxima of the photometer curves could serve directly as a measure for the energy.) The mean values from four plates gave for:

$$\nu_4 = 507.2 \text{ cm}^{-1}, \quad \varrho = 0.83 \text{ and } \nu_1 = 2\,328.5 \text{ cm}^{-1}, \quad \varrho = 0.33$$

As the error is estimated to about ± 5 per cent it is concluded that ν_4 is depolarized. The line $\nu_2 = 850.6$ cm^{-1} was too weak for quantitative measurements to be made. However, it seemed to be polarized which one would also conclude from the sharpness of this line.

ASSIGNMENT OF FREQUENCIES

From classical stereochemistry as well as from considerations of the elctronic structure the molecule is expected to be linear and symmetric. As mentioned above, this is confirmed by the electron-diffraction measurements.

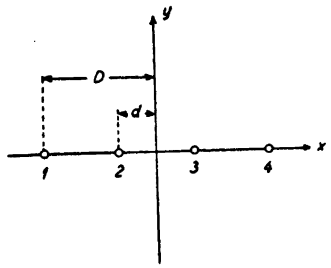

Fig. 2. Cartesian coordinates chosen for the symmetric C_2N_2-molecule. The four atoms placed along the X-axis are numbered from 1 to 4 and the dimensions of the molecule is given by the distances D and d.

Table 2. Raman and infrared frequencies of C_2N_2.

No.	Frequency in cm^{-1}	Activity observed	Remarks	Interpretation	Symmetry	Selection rule activity
1	c. 240	I–R (R)	σ-band	ν_5	E_{1u}	I–R (σ)
2	c. 475	R		$2\nu_5$	A_{1g}, A_{2g}, E_{2g}	R (pol)
3	507.2	R	depol.	ν_4	E_{1g}	R (dep)
4	521.4	R		$2\nu_4-\nu_4$	E_{1g}, E_{3g}	R (dep)
5	561.4	R		$2\nu_4-2\nu_5$	A_{1g}, A_{2g}, E_{2g}	R (pol)
6	618	I–R	σ-band	$\nu_2-\nu_5$	E_{1u}	I–R (σ)
7	732	I–R	π-band	$\nu_4+\nu_5$	A_{1u}, A_{2u}, E_{2u}	I–R (π)
8	750	R				
9	841	R		$(\nu_2+\nu_5)-\nu_5$	A_{1g}, A_{2g}, E_{2g}	R (pol)
10	850.6	R	polarized	ν_2	A_{1g} *	R (pol)
11	1 028.5	R		$2\nu_4$	A_{1g}, A_{2g}, E_{2g}	R (pol)
12	2 092	I–R	σ-band	$\nu_1-\nu_5$	E_{1u}	I–R (σ)
13	2 149	I–R	π-band	ν_3	A_{2u}	I–R (π)
14	2 298.4	R		ν_1 isotope	$C_{\infty h}, A_1$	R (pol)
15	2 328.5	R	(polarized)	ν_1	A_{1g}	R (pol)
16	2 562	I–R	σ-band	$\nu_1+\nu_5$	E_{1u}	I–R (σ)
17	2 662	I–R	σ-band	$\nu_3+\nu_4$	E_{1u}	I–R (σ)

Hence, C_2N_2 has the symmetry $D_{\infty h}$. From group-theoretical considerations [13] it follows that there are 3 non-degenerate fundamental vibrations, two of which are symmetric (A_{1g}) and one antisymmetric (A_{2u}) with respect to the inversion, further 2 degenerate vibrations being respectively symmetric (E_{1g}) and antisymmetric (E_{1u}). The five modes of vibration are shown schematically in Fig. 3. According to selection rules all the antisymmetric fundamentals

* Fermi-resonance between the two A_{1g} components causes an enhanced activity of the overtone, 2 ν_4, which appears as a Raman-line of medium strength.

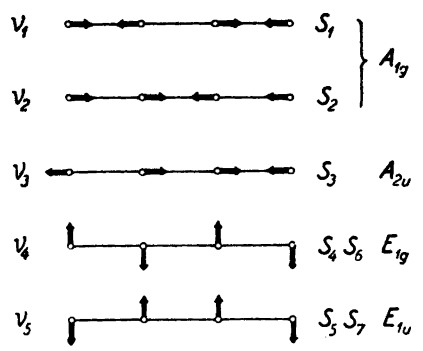

Fig. 3. Vibrational modes of the linear C_2N_2-molecule. The symmetry coordinates as well as the symmetry species are given for each normal vibration. Only one components of the degenerate vibrations is shown.

are infrared active while the symmetric ones are Raman active. Furthermore, A_{1g} vibrations give polarized Raman lines ($\varrho < 6/7$), all others depolarized ($\varrho = 6/7$).

The problem of assigning the observed frequencies to definite modes of vibration has caused some discussion in the literature [2], but must now be considered as settled. We shall therefore not go into any detail here. The assignment given in Table 2 (and Fig. 1) is in essential agreement with that given in Herzberg's book [2]. Two of the new Raman-lines 521.4 and 561.4 cm^{-1} can uncompelled be explained as the difference tones ν_4—$2\nu_4$ and $2\nu_5$—$2\nu_4$. Of the remaining new lines 2 298.4 cm^{-1} is undoubtedly due to ν_1 of the isotopic molecule $N{\equiv}C^{12}{-}C^{13}{\equiv}N$. As well the shift in frequency as the intensity ratio (I (2 328.5)/I(2 298.4)) is of the right order of magnitude. Furthermore, the line is sharp (strongly polarized) as should be expected. The remaining 841 cm^{-1} line is tentatively given the assignment ν_5—($\nu_2 + \nu_5$), because its intensity seems to be too high for interpreting it as an isotopic line. Below we are showing that neither its frequency is compatible with this assignment.

THE POTENTIAL FUNCTION OF THE C_2N_2 MOLECULE

The general harmonic potential function for vibrations belonging to the different symmetry classes may be formulated as:

$$
\begin{aligned}
&A_{1g}\ (\nu_1 \text{ and } \nu_2) && 2V_1 = a_{11}S_1^2 + a_{22}S_2^2 + 2a_{12}S_1S_2\\
&A_{2u}\ (\nu_3) && 2V_2 = a_{33}S_3^2\\
&E_{1g}\ (\nu_4) && 2V_3 = a_{44}S_4^2 + a_{66}S_6^2\\
&E_{1u}\ (\nu_5) && 2V_4 = a_{55}S_5^2 + a_{77}S_7^2
\end{aligned}
$$

where a_{11}, a_{22} . . . . . a_{77} are force constants and S_1, S_2 . . . . . S_7 the geometric symmetry coordinates. These are linear combinations of the atomic displacement coordinates with the property that they form basis for irreducible representations of the symmetry group and are independent of translations and rotations of the molecule. The symmetry coordinates for a simple molecule as C_2N_2 can be found by inspection (Fig. 3). The same coordinates may also be used for any isotopic species of this molecule, because the interatomic distances and the potential function are unchanged.

Linear vibrations (*Symmetry classes* A_{1g} *and* A_{2u}). Numbering the atoms as indicated in Fig. 2. we choose:

$$S_1 = x_1—x_4,\ S_2 = x_2—x_3 \text{ and } S_3 = x_1—x_2—x_3 + x_4$$

In order to exclude translation in the x-direction the following relation must be satisfied:

$$m_1x_1 + m_2x_2 + m_3x_3 + m_4x_4 = 0$$

Here m_i is the mass of the i-th atom. This enables us to express the displacement coordinates as linear combinations of the symmetry coordinates:

$$\begin{aligned}
x_1(2M) &= (m_2 + m_3 + 2m_4)S_1—(m_2—m_3)S_2 + (m_2 + m_3)S_3 \\
x_2(2M) &= —(m_1—m_4)S_1 + (m_1 + 2m_3 + m_4)S_2—(m_1 + m_4)S_3 \\
x_3(2M) &= —(m_1—m_4)S_1—(m_1 + 2m_2 + m_4)S_2—(m_1 + m_4)S_3 \\
x_4(2M) &= —(2m_1 + m_2 + m_3)S_1—(m_2—m_3)S_2 + (m_2 + m_3)S_3 \\
(M &= m_1 + m_2 + m_3 + m_4)
\end{aligned} \tag{1}$$

Lagrange's equations with the symmetry coordinates as generalized coordinates are:

$$\frac{d}{dt}\left(\frac{\partial \Delta T}{\partial \dot{S}}\right) + \frac{\partial \Delta V}{\partial S} = 0$$

where ΔT is the kinetic and ΔV the potential energy. Instead of inserting (1) into the kinetic energy function:

$$2\Delta T = m_1\dot{x}_1^2 + m_2\dot{x}_2^2 + m_3\dot{x}_3^2 + \mathrm{m}_4\dot{x}_4^2$$

we calculate the first member of Lagrange's equation from

$$\frac{\partial \Delta T}{\partial \dot{S}} = \sum_i m_i\dot{x}_i \frac{\partial x_i}{\partial S}$$

By using this procedure it is possible to set up the equations of motion for each symmetry class separately, considering only those symmetry coordinates which belong to that particular symmetry class.

In this case we want to determine the four "linear" force constants: a_{11}, a_{22}, a_{12} and a_{33}. This is possible because we in addition to the three fundamentals (ν_1, ν_2 and ν_3) in C_2N_2 now also know the frequency of one of the fundamentals (ν_1 (isot.)) in $C^{12}C^{13}N_2^{14}$. Because the symmetry of this molecule is lower ($C_{\infty}v$) all the linear fundamentals belong to the same symmetry class (A_1). Hence we cannot separate the variables S_1, S_2 and S_3. We get the following equations of motion:

$$\begin{aligned}
&\{(m_1+m_3)\ (m_1+m_4)+4m_1m_4\}\ddot{S}_1-(m_2-m_3)\ (m_1-m_4)\ddot{S}_2+[(m_1-m_4)\\
&(m_2+m_3)\ \ddot{S}_3+8a_{11}MS_1+8a_{12}S_2=0\\
&-(m_1-m_4)\ (m_2-m_3)\ddot{S}_1+\{(m_1+m_4)\ (m_2+m_3)+4\ m_2m_3\}\ddot{S}_2-[(m_2-m_3)\\
&(m_1+m_4)\ddot{S}_3+8a_{12}MS_1+8a_{22}MS_2=0\\
&(m_2+m_3)\ (m_1-m_4)\ddot{S}_1-(m_1+m_4)\ (m_2-m_3)\ddot{S}_2+(m_1+m_4)\ (m_2+m_3)\ddot{S}_3\\
&+8a_{33}MS_3=0
\end{aligned} \tag{2}$$

Trying solutions of the type $S_i=A_ie^{2\pi i\nu ct}$ and requiring non-trivial solutions we get the secular equation:

$$\begin{vmatrix}
2Ma_{11}-\{(m_1+m_4)(m_2+m_3)+4m_1m_4\}\varkappa & 2Ma_{12}+(m_2-m_3)\ (m_1-m_4)\varkappa & \\
& -(m_1-m_4)(m_2+m_3)\varkappa & \\
2Ma_{12}+(m_1-m_4)(m_2-m_3)\varkappa & 2Ma_{22}-\{(m_1+m_4)(m_2+m_3)+4m_2m_3\}\varkappa & \\
& (m_2-m_3)(m_1+m_4)\varkappa & \\
-(m_1-m_4)(m_2+m_3)\varkappa & (m_1+m_4)(m_2-m_3)\varkappa & \\
& 2Ma_{33}-(m_1+m_4)(m_2+m_3)\varkappa &
\end{vmatrix}=0 \tag{3}$$

where $\varkappa=\pi^2c^2\nu^2$ (c is the velocity of light).

The above equations will of course also be valid for the symmetric molecule $C_2^{12}N_2^{14}$ when the proper values are inserted for the atomic masses. The secular equation (3) then reduces to:

$$\begin{vmatrix}
a_{11}-m_1\varkappa & a_{12} & 0 \\
a_{12} & a_{22}-m_2\varkappa & 0 \\
0 & 0 & a_{33}-\dfrac{2m_1m_2}{M}\varkappa
\end{vmatrix}=0 \tag{4}$$

which splits up into the two equations:

$$(a_{11}a_{22}-a_{12}^2)-\varkappa(m_1a_{22}+m_2a_{11})+\varkappa^2m_1m_2=0 \tag{5}$$

(Two roots, $\varkappa_1$ and $\varkappa_2$, corresponding to ν_1 and ν_2 of symmetry class A_{1g}) and

$$a_{33} \cdot M - 2m_1 m_2 \varkappa_3 = 0 \tag{6}$$

($\varkappa_3$ corresponds to ν_3 of symmetry class A_{2u}).

From (4) we obtain the following relations:

$$a_{11}a_{22} - a_{12}^2 = m_1 m_2 \varkappa_1 \varkappa_2 \tag{7}$$

and

$$a_{11}m_2 + a_{22}m_1 = m_1 m_2 \, (\varkappa_1 + \varkappa_2) \tag{8}$$

Inserting the following numerical values:

$m_1 = m_4 = 14.0075$, $m_2 = 12.0038$, $m_3 = 13.0075$, $M = 53.0263$, $N = 6.0228 \cdot 10^{23}$, $c = 3.10^{10}$, $\nu_1 = 2\,328.5$, $\nu_2 = 850.6$, $\nu_3 = 2\,149$ and ν_1(isot.) $= 2\,298.4$ into (6), (7), (8) and the evaluated determinant (3) we calculate:

$$\begin{aligned} a_{11} &= \ 3.920 \cdot 10^5 \text{ dyne·cm}^{-1} \\ a_{22} &= \ 7.520 \cdot 10^5 \text{ dyne·cm}^{-1} \\ a_{12} &= \pm\ 3.890 \cdot 10^5 \text{ dyne·cm}^{-1} \\ a_{33} &= \ 4.402 \cdot 10^5 \text{ dyne·cm}^{-1} \end{aligned}$$

The ambiguity caused by the $\pm$ sign for a_{12} will be discussed below.

In these calculations we have considered the structure of cyanogen to be $N \equiv C - C \equiv N$. Now, it is a possibility that the carbon and nitrogen atoms should be interchanged (though the isotopic atom should still be a carbon atom): $\overset{-}{C} \equiv \overset{+}{N} - \overset{+}{N} \equiv \overset{-}{C}$. This means that we have to interchange the masses m_1 and m_2, m_3 and m_4 in our calculations. But if we do so it is easily seen from equations (3), (4), (6), (7) and (8) *that we get exactly the same calculations as before, only that a_{11} and a_{22} have been interchanged.*

We might next try whether the observed Raman line $\nu = 841$ cm^{-1} should be an isotope line corresponding to $\nu_2 = 850.6$ cm^{-1}. By introducing the numerical values for the force constants into (3) we calculate:

$$\nu_2(\text{isot.}) = 846 \text{ cm}^{-1}$$

for the isotopic molecule $C^{12}C^{13}N_2^{14}$. The difference between this value and the observed 841 frequency is so large that we believe it to be outside as well experimental error as uncertainty in the calculation. This supports our conclusion, mentioned above that the 841 cm^{-1} line must be assigned to the $C_2^{12}N_2^{14}$ molecule.

Non-linear vibrations. These can be treated in a way quite analogous to the linear vibrations. We shall here only state the results obtained for the symmetric molecule $C_2^{12}N_2^{14}$.

Symmetry class E_{1g}. $\nu_4 = 507.2$ cm^{-1}

$$a_{44} = a_{66} = 4\ \varkappa_4 \frac{m_1 m_2}{m_2 + m_1 \left(\frac{D}{d}\right)}$$

Inserting numerical values, $d = 0.69$ Å and $D = 1.84$ Å (cp. Fig. 2), we calculate:

$$a_{44} = a_{66} = 0.229 \cdot 10^5 \text{ dyne.cm}^{-1}$$

Symmetry class $E_{1u} \cdot \nu_5 = 240$ cm^{-1}

$$a_{55} = a_{77} = 2\ \varkappa_5 \cdot \frac{m_1 m_2}{M}$$

giving

$$a_{55} = a_{77} = 0.055 \cdot 10^5 \text{ dyne·cm}^{-1}$$

DISCUSSION

In order to get a better understanding of what is the physical significance of the potential function it may be helpful to consider the forces called into action when we give the molecule certain deformations. To this purpose we choose the following:

1) atom 1 is given a small displacement, Δx,
2) atoms 1 and 2 are both given the same displacement, Δx, and
3) atom 2 alone is given the displacement Δx,

while all other atoms are kept fixed in their original positions of equilibrium.

For this calculation we express the part of the potential function involving only linear vibrations in cartesian coordinates:

$$\Delta V = (a_{11}+a_{33})(x_1^2+x_4^2) + (a_{22}+a_{33})(x_2^2+x_3^2) + 2(a_{12}-a_{33})(x_1x_2+x_3x_4) - 2(a_{12}+a_{33})(x_1x_3+x_2x_4) + 2(a_{33}-a_{22})x_2x_3 + 2(a_{33}-a_{11})x_1x_4$$

A certain displacement, Δx, from the equilibrium position of one or more of the atoms will produce a force on the i-th atom:

$$K(i) = -\left(\frac{\partial \Delta V(x_j)}{\partial x_i}\right)\left(\frac{\partial \Delta V(x_j)}{\partial x_j}\right)_{x\ =\Delta x}$$

The "restoring coefficients", defined as $dK(i)/dx$, are given in the following Table 3, while the numerical values of the forces are given in Table 4 for the two different models of the molecule and with a_{12} as well negative as positive.

Table 3. Restoring coefficients of the single atoms for special atomic displacements.

Atom	K_1	K_2	K_3
1	$-2(a_{11}+a_{33})$	$-2(a_{11}+a_{12})$	$-2(a_{12}-a_{33})$
2	$-2(a_{12}-a_{33})$	$-2(a_{22}+a_{12})$	$-2(a_{22}+a_{33})$
3	$2(a_{12}+a_{33})$	$2(a_{22}+a_{12})$	$-2(a_{33}-a_{22})$
4	$2(a_{11}-a_{33})$	$2(a_{11}+a_{12})$	$2(a_{12}+a_{33})$

Table 4. Forces acting on single atoms in C_2N_2 for special atomic displacements Δx, unit: 10^5 dyne.

Model	Atom	K_1		K_2		K_3	
		a_{12} neg.	a_{12} pos.	a_{12} neg.	a_{12} pos.	a_{12} neg.	a_{12} pos.
N≡C—C≡N	1	$-16.64\,\Delta x$	$-16.64\,\Delta x$	$-0.06\,\Delta x$	$-15.62\,\Delta x$	$16.58\,\Delta x$	$1.02\,\Delta x$
	2	$16.58\,\Delta x$	$1.02\,\Delta x$	$-7.26\,\Delta x$	$-22.82\,\Delta x$	$-23.84\,\Delta x$	$-23.84\,\Delta x$
	3	$1.02\,\Delta x$	$16.58\,\Delta x$	$7.26\,\Delta x$	$22.82\,\Delta x$	$6.24\,\Delta x$	$6.24\,\Delta x$
	4	$-0.96\,\Delta x$	$-0.96\,\Delta x$	$0.06\,\Delta x$	$15.62\,\Delta x$	$1.02\,\Delta x$	$16.58\,\Delta x$
⁻C≡N⁺—N⁺≡C⁻	1	$-23.84\,\Delta x$	$-23.84\,\Delta x$	$-7.26\,\Delta x$	$22.82\,\Delta x$	$16.58\,\Delta x$	$1.02\,\Delta x$
	2	$16.58\,\Delta x$	$1.02\,\Delta x$	$-0.06\,\Delta x$	$-15.62\,\Delta x$	$-16.64\,\Delta x$	$-16.64\,\Delta x$
	3	$1.02\,\Delta x$	$16.58\,\Delta x$	$0.06\,\Delta x$	$15.62\,\Delta x$	$0.96\,\Delta x$	$0.96\,\Delta x$
	4	$6.24\,\Delta x$	$6.24\,\Delta x$	$7.26\,\Delta x$	$-22.82\,\Delta x$	$1.02\,\Delta x$	$16.58\,\Delta x$

By inspection of the figures in Table 4 it is easily seen that *the only physically reasonable solution is the $N \equiv C—C \equiv N$ model* with a_{12} negative, leading to the values given in column 1, 3 and 5, upper part. However, the other possibilities are also quite interesting, if we try to interpret what they mean physically. With a_{12} negative and the CNNC model we see that there is a strong interaction between the C and N atoms as well as between the C atoms, but none between the N atoms; this must mean that there is a bond between the C atoms from one end of the molecule to the other while the adjacent N atoms are not bonded to one another. Similarly, the change of sign of a_{12}

(from —to+), which is mathematically equivalent to interchanging the atoms 2 and 3, physically must be interpreted as giving a strong interaction corresponding to a triple bond between the atoms 1 and 3 and between 2 and 4. We see, in fact, that the calculations, being performed without paying any attention to the possible distribution of the carbon and nitrogen atoms between the places 1, 2, 3 and 4, *in every case lead to the same dynamical formula for the molecule*. This is because the physical interpretation of the force constants — or the restoring coefficients — is independent of the numbering of the atoms and hence always will show between which atoms there is a single resp. a triple bond or no bonding at all.

Table 4 shows furthermore that there is quite a strong interaction between atom 1 and the atoms 3 and 4 in our selected NCCN model, the restoring coefficients being + resp. — ca. $1 \cdot 10^5$ dyne.cm^{-1}. (Of course, for symmetry reasons, there is a corresponding interaction between 4 and the atoms 1 and 2.) This is, as we will show in a following paper, what is to be expected from the molecular orbital theory.

The restoring coefficients play the rôle of force constants and we may take as characteristic force constant for the C≡N bond: $K_{CN} = K_1\ (2) = 16.6 \cdot 10^5$ dyne.cm^{-1} and for the C—C bond: $K_2\ (2) = 7.26 \cdot 10^5$ dyne.cm^{-1}. However, in the latter value is included a contribution of $1.02 \cdot 10^5$ dyne.cm^{-1} due to interaction between 2 and 4. Hence, if we want to get the force constant for the "pure" C—C bond we must subtract this amount and get

$K_{CC} = K_2\ (2)_{corr.} = 6.24 \cdot 10^5$ dyne.cm^{-1}.

Let us next apply different empirical rules connecting the interatomic distances and the corresponding characteristic force constants:

Badger's rule[15]:	$K(r-a)^3 = 1.86 \cdot 10^5$
Clark's » [3]:	$K \cdot r^6 = 1.266^6$
Linnet-Thompson's rule [3]:	$K \cdot r^6 = 1.31^6$

The two first relations are valid for diatomic molecules while the last should apply to polyatomic molecules. Therefore, one would expect to get best results with the corrected K_{CC} values when using Badger's or Clark's rule while Linnet-Thompson's formula should be applicable to the "raw" uncorrected value. The following Table 5 shows that this is so.

Previous calculations based on the valence force system have given [2] $K_{C\equiv N} = 17.6 \cdot 10^5$ dyne·cm^{-1} and $K_{C-C} = 5.22 \cdot 10^5$ dyne·cm^{-1} although Linnet and Thompson [3] for the same constants find 17.5 and $6.69 \cdot 10^5$ dyne. cm^{-1}. This is more or less what one would expect from consideration of the

"valence structure" of C_2N_2. However, a simple and more exact relation seems to exist between the restoring coefficients as defined above and the bond orders as calculated by the *LCAO*-method. This will be discussed in a following paper.

Table 5. Interatomic distances in Å as calculated from the force constants in 10^5 dyne·cm^{-1} according to different empirical rules.

Interatomic distances	$d_{C\equiv N}$	d_{C-C}	d_{C-C}
Force constant in 10^5 dyne.cm^{-1}	16.6	7.26	6.24
Badger	1.16	1.32	1.35
Clark	1.15	1.33	1.37
Linnet-Thompson	1.17	1.38	1.42
Observed value	1.15	1.38	1.38

SUMMARY

Investigation of C_2N_2 by electron diffraction using the rotating sector method confirms the results obtained by Pauling *et al.* The Raman spectrum has been reinvestigated and four new lines detected, one of which is assigned to the isotopic molecule NCC^{13}N. On the basis of measured Raman frequencies and one frequency taken from the infrared absorption spectrum the complete harmonic potential function has been calculated. An interpretation of the physical significance of the force constants is given. It is thereby shown that the spectroscopic method unambigously leads to the configuration NCCN. Different empirical rules giving the relation between force constants and bond lengths are tested.

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Received March 24, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 738–743

Oxidation Path of the *d*-Glucose Dehydrogenase

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In the mammalian liver, Harrison [1,2] discovered a dehydrogenase protein, which in presence of coenzyme 1 or 2 (Andersson [3], Das [4]), oxidizes *d*-glucose. The enzyme cannot use oxygen directly, but methylene blue can function as a hydrogen acceptor, and so can the cytochrome system. Hawthorne and Harrison [5] showed the participation of cytochrome c, and suggested that diaphorase (Straub's [6] heart flavoprotein), is necessary for the reaction. The full system is supposed to comprise: O_2 + cytochrome oxidase + cytochrome c + diaphorase + (dehydrogenase protein + coenzyme) + *d*-glucose. Eichel and Wainio [7] re-examined the function of the system, but were not able to decide if the diaphorase participates or not.

In the present investigation the advantage of a highly purified dehydrogenase protein preparation (Brunelli and Wainio [8]) was taken for spectrophotometric experiments, in order to examine the rôle of the diaphorase for the function of the dehydrogenase *. — The necessity of cytochrome c for the full system has been re-examined in manometric experiments.

MATERIAL AND METHODS

d-Glucose dehydrogenase protein. In manometric experiments a crude preparation according to Harrison [9] was employed. For spectrophotometrical experiments the dehydrogenase was further purified according to Brunelli and Wainio's method [8]. *Coenzyme 1* was prepared from baker's yeast by the method of Williamson and Green [10]. *Diaphorase* was obtained from pig heart muscle according to Straub's procedure [6]. *Cytochrome c* was prepared from horse heart muscle according to Keilin and Hartree's procedure [11,12]. The convention that the pigment contains 0.34 per cent Fe is adopted. *Cytochrome oxidase* was obtained from pig heart muscle, by following, with minor alterations, the method of

* The term dehydrogenase is used to denote the holoenzyme: *d*-glucose dehydrogenase protein + coenzyme.

Keilin and Hartree[13]. The grinding with sand was substituted by a 2 min Waring Blendor mincing. Used amounts of oxidase are referred to dry weight.

Oxygen uptake. All manometric experiments (Warburg technique) were performed at 30° C. Total flask charge: 3.8 ml in the main compartment + 0.3 ml. KOH in the bottom centre tube. Phosphate buffer of pH 7.1 and a final concentration of 0.066 *M* was used throughout. Under extreme conditions the formed gluconic acid may change the pH towards the end of the experiment to 6.9. Gas phase: air. For further experimental details see Borei[14].

Spectrophotometric measurements: The reduction of ferricytochrome c was observed by the rise of extinction at 549 mμ (Beckman spectrophotometer).

RESULTS

A. Participation of cytochrome oxidase and cytochrome c

The cyanide inhibitability of the full system (Hawthorne and Harrison[5] and others) points to the participation of the cytochrome oxidase. Similar experiments in this investigation are in full accordance with these results. In addition the full system has been found strongly inhibitable by NaN_3. On the other hand, if the cytochrome c complemented oxidase preparation is replaced by methylene blue, no inhibition is achieved either by KCN or NaN_3 additions. Instead a slight augmentation is observed.

The used cytochrome oxidase preparation supports maximal glucose dehydrogenase activity even when no cytochrome c is added. This suggests that sufficient cytochrome c is present in the oxidase preparation.

Keilin and Hartree[15] have shown that repeated freezing of cytochrome oxidase preparations in liquid air and thawing at room temperature impairs the accessibility of endogenous cytochrome c. The activity of the oxidase is, however, completely restored by addition of external cytochrome c. Oxidase treated in this way gives a very low oxidation rate with the glucose dehydrogenase system if no external cytochrome c is present. The effect of addition of cytochrome c is seen from Fig. 1.

Lineweaver-Burk[16] analysis applied to experiments, in which the amount of added cytochrome c was varied, shows a straight line relationship between the inverse function of reaction velocity and cytochrome c concentration.

Cytochrome c complemented cytochrome oxidase prepared according to Haas[17] is unable to mediate between oxygen and glucose dehydrogenase. Addition of diaphorase (see below) is of no effect in this case.

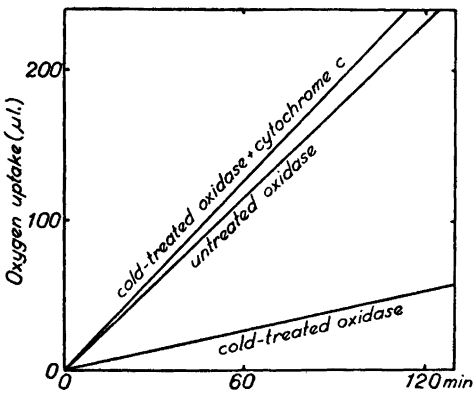

Fig. 1. Necessity of cytochrome c for the function of the d-glucose dehydrogenase system. Cytochrome oxidase preparation (untreated or 3 times frozen) (0.604 mg) + coenzyme 1 (0.8 mg) + d-glucose dehydrogenase protein (6.3 mg) + glucose (final concentration 0.16 M). Final concentration of cytochrome c, when added, 1.6 × 10^{-6} M. The addition of the same amount of cytochrome c to the untreated oxidase causes only a very slight augmentation.

B. Participation of diaphorase

If a complete system, containing the Keilin and Hartree cytochrome oxidase preparation, is tested manometrically, the omission of diaphorase proves to be of no significance for the activity, *cf.* Table 1. This is probably due to the content of diaphorase in the enzyme preparations.

Table 1. Significance of different components in manometric experiments with cytochrome oxidase preparations. The amounts or final concentrations of the various additions are denoted in the table.

Additions:	1	2	3	4	5	6	7
Cytochrome oxidase preparation (7.4 mg)	+	+	+	+	+	−	−
Cytochrome c (16 × 10^{-6} *M*)	+	+	+	+	+	−	−
Diaphorase (1 mg)	+	−	+	+	+	+	−
Coenzyme 1 (0.8 mg)	+	+	−	+	+	+	+
Glucose dehydrogenase protein (12.6 mg)	+	+	+	−	+	+	+
Glucose (0.16 *M*)	+	+	+	+	−	+	+
Oxygen uptake (µl)	450	447	12	− 2	15	72	4

If, on the other hand, the cytochrome oxidase and the cytochrome c are replaced by methylene blue, the oxidation rate is strongly dependent on the presence of added diaphorase. Moreover, the decolourization rate of methylene blue in Thunberg experiments is increased several hundred per cent if diaphorase is added.

It is obvious from Table 1 that the diaphorase content of the oxidase preparation excludes the possibility to decide from manometric experiments if the diaphorase is essential as a mediator between the cytochrome part of the full system and the dehydrogenase.

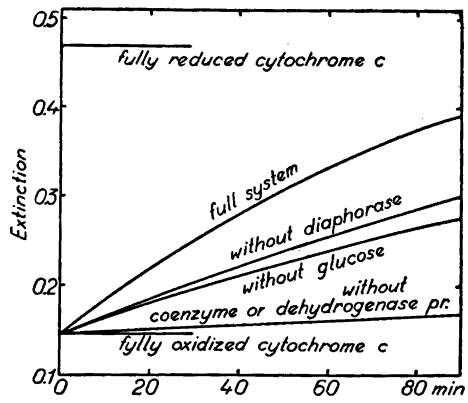

Fig. 2. Reduction of cytochrome c in the d-glucose dehydrogenase system. The rise in extinction at 549 mμ is followed spectrophotometrically. Full system: Cytochrome c (1 mg) + diaphorase (2.4 mg) + coenzyme 1 (0.8 mg) + d-glucose dehydrogenase protein (2.2 mg) + glucose (108 mg). Test volume 3.8 ml. Length of optical cell 1 cm. Room temperature.

It is, however, possible to follow spectrophotometrically the reduction of cytochrome c, and to study in such experiments, the effect of diaphorase addition. In those experiments, which are represented in Fig. 2, the diaphorase content of the test system itself was further depressed by using the purified glucose dehydrogenase preparation of Brunelli and Wainio [8]. Though this preparation is comparatively poor in both diaphorase and substrate, the controls with omitted external addition of these two reactants give quite appreciable reduction rates. Addition of diaphorase, however, doubles the activity of the system, thus establishing the significance of this carrier for the reaction.

DISCUSSION

The inhibition experiments with CN′ and N_3' support conclusively the view (Hawthorne and Harrison [5]) that the glucose dehydrogenase can function over the cytochrome system. The inhibitability by the named ions is bound to the presence of cytochrome oxidase and is not to be attributed to the dehydrogenase itself. This is shown by the noninhibitability of systems, where the cytochrome oxidase preparation is replaced by methylene blue.

The participation of cytochrome c as a mediator between the cytochrome oxidase and the dehydrogenase seems to be evident from Hawthorne and Harrison's [5] experiments. Eichel and Wainio [7] had, however, difficulties in confirming these results. Only with extremely diluted oxidase preparations was it possible for them to show any influence of added cytochrome c. In the present investigation it was found that the oxidase preparation (Keilin and Hartree's procedure) itself contains enough endogenous cytochrome c to give a maximal catalysis over the dehydrogenase system. External cytochrome c is

without influence. If, however, the accessibility of the endogenous cytochrome c is impaired through treatment in liquid air (procedure of Keilin and Hartree [15]) addition of external cytochrome c augments the oxidation strongly (*cf.* Fig. 1). The necessity of cytochrome c for the system used is thus clearly established. This result is furthermore supported by the fact that Lineweaver-Burk analysis shows that the cytochrome oxidase-cytochrome c complex, normally found in cytochrome oxidase-mediated systems (*cf.* Borei [14]), is functioning also in the glucose dehydrogenase system.

Yeast flavoprotein was found by Ogston and Green [18] and others, to mediate between oxygen and the dehydrogenase, and by Adler and Euler [19] to augment the decolourisation of methylene blue. The participation of diaphorase as a mediator between the cytochrome system and the dehydrogenase was suggested by Hawthorne and Harrison [5]. As the cytochrome oxidase preparations themselves apparently contain enough flavoprotein to maintain full activity in the system, it was not possible for Eichel and Wainio [7] to decide if the diaphorase is a necessary link or not.

In the present investigation, where Keilin and Hartree's cytochrome oxidase preparation was used, the same phenomenon was found (*cf.* Table 1, experiments 1—5). The addition of diaphorase is of no influence on the oxidation rate. This must mean either that diaphorase is not a link in the system, or that the enzyme preparations contain enough diaphorase themselves to support full activity. Furthermore it is confirmed that the diaphorase itself can mediate between oxygen and the dehydrogenase (Table 1, experiments 6 and 7). In this case, however, the oxidation rate is much lower than when cytochrome oxidase is present.

In methylene blue experiments, a positive effect was invariably found when diaphorase was added. This must imply a carrier function, of diaphorase between the dehydrogenase and the dye stuff. These findings agree with Adler and Euler's [19] results, but are in apparent contradiction to Eichel and Wainio's [7]. This discrepancy is probably due to high endogenous flavoprotein content of the dehydrogenase preparation used by the latter workers.

The spectrophotometric experiments on the reduction of oxidized cytochrome c through the dehydrogenase (Fig. 2) conclusively show, however, that diaphorase acts as a link between the two reactants.

SUMMARY

1. It is confirmed that the cytochrome system can mediate between the *d*-glucose dehydrogenase and oxygen (inhibition experiments with KCN and NaN_3).

2. Cytochrome c is necessary for this mediation (experiments with cytochrome oxidase preparations with impaired activity of endogenous cytochrome c).

3. Diaphorase is necessary as a link between cytochrome c or methylene blue and the dehydrogenase (spectrophotometric experiments on reduction of cytochrome c; Thunberg experiments).

4. The reaction path for *d*-glucose dehydrogenase activity thus comprises: $O_2 \rightarrow$ cytochrome oxidase $\rightarrow$ cytochrome c $\rightarrow$ diaphorase $\rightarrow$ (coenzyme 1 + *d*-glucose dehydrogenase protein) $\rightarrow$ *d*-glucose.

I wish to express my thanks to Dr. Hans Borei for discussions and advice, and for his critical examination of the manuscript.

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Received March 26, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 744–750

Antihistamine Agents

V. Aminoalkyl Derivatives of 2-Benzylthiazole

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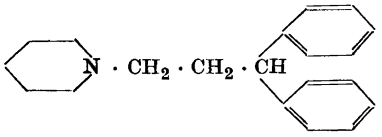
Several aminoalkyl derivatives of diphenylmethane have been reported to possess pronounced antispasmodic properties [1-3] and some of them, *e. g.* N-(γ,γ-diphenyl-propyl)-piperidine (I) have been used clinically. Replacement of a phenyl nucleus by a heterocyclic one has been carried out in the case of pyridine [4], and one of these compounds, 2-(γ-dimethylamino-α-phenyl-propyl)-pyridine (II), exerted a strong antihistaminic activity.



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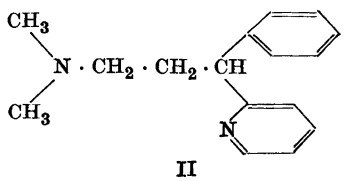
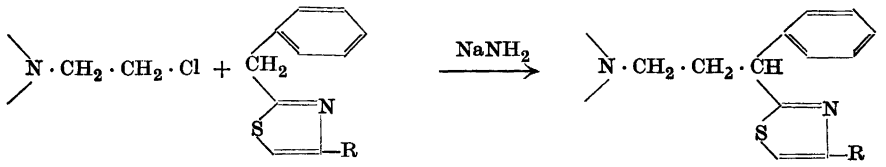


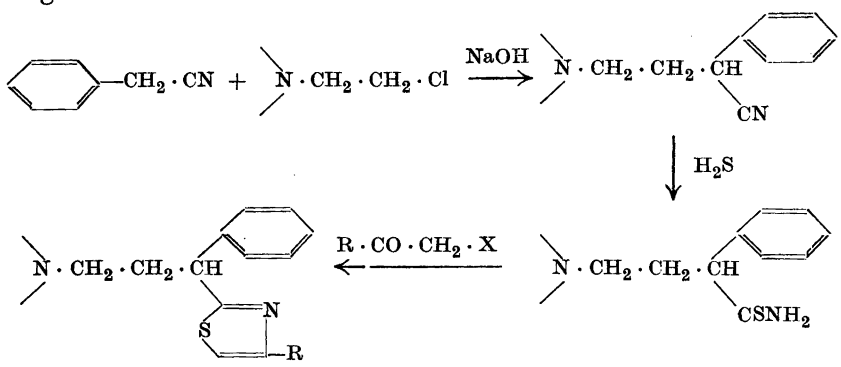
II

However, no other heterocyclic analogues appear to have been reported. As it is of great interest to study the spasmolytic and antihistaminic effect of such compounds, several derivatives of this type containing a thiazole nucleus have been prepared in this laboratory.

The thiazole compounds could be obtained by condensing the hydrochlorides of β-piperidino-, β-dimethylamino- and β-diethylaminoethyl chloride with 2-benzylthiazole and 2-benzyl-4-methylthiazole in the presence of sodium amide, two equivalents of the condensing agent being used to liberate the free aminoalkyl chloride from its hydrochloride.



They were, however, synthesised more conveniently and in better yields according to the scheme



The preparation of γ-diethylamino-α-phenylbutyronitrile by the condensation of β-diethylaminoethyl chloride and benzyl cyanide with sodium amide has been described by Eisleb [1]. The dimethyl derivative has been synthesised in the same way [5], and the piperidino compound has been prepared from γ-chloro-α-phenylbutyronitrile and piperidine [6].

Cusic showed recently [7], that sodium amide could be replaced by sodium hydroxide in a similar reaction. The basic nitriles, which were used as starting materials in the present work, were prepared in this way.

The nitriles reacted smoothly with hydrogen sulphide in ethanol at 70° to give thioamides. The reaction of the thioamides with chloroacetone, chloroacetaldehyde hydrate or ω-bromoacetophenone was best carried out in nitrobenzene as a solvent. In ethanol only ω-bromoacetophenone was reactive enough to give thiazole compounds.

After the experimental work had been completed, the synthesis of two of the thiazole derivatives described below was reported by Brown, Cook and Heilbron [8]. These investigators had prepared 4-methyl-2-(γ-diethylamino-α-phenylpropyl)-thiazole and 4-methyl-2-(γ-piperidino-α-phenylpropyl)-thiazole by condensing 2-benzyl-4-methylthiazole with the appropriate chloro tertiary amine in the presence of sodium amide. However, their attempts to convert γ-diethylamino-α-phenylthiobutyramide into a thiazole with chloroacetone or bromoacetal failed.

The new compounds have been tested for antihistaminic and antispasmodic activity. The tests were carried out on isolated guinea pig ileum, β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) being used as a standard *. It was found, that the highest antihistamine activity was exerted by

* Acknowledgement is made to Dr. S. Wiedling of Astra's Biological Department for performing these tests. Details will be reported elsewhere.

2-(γ-dimethylamino-α-phenylpropyl)-thiazole, which had 0.15 of the effect of Benadryl. Against barium chloride and acetyl choline, 4-methyl-2-(γ-piperidino-α-phenylpropyl)-thiazole was most potent, the activity being 1.25 and 1.8 resp. of the Benadryl activity.

EXPERIMENTAL

γ-Dimethylamino-α-phenylbutyronitrile

To a mixture of 36.0 g of powdered sodium hydroxide and 28.2 g of benzyl cyanide 43.2 g of β-dimethylaminoethyl chloride hydrochloride was added in portions. A vigorous reaction commenced and the temperature was not allowed to exceed 80°. The mixture was then heated on a water bath at 80—90° for eight hours. After cooling, the mixture was extracted with ether, the residue was dissolved in water and extracted with ether again. The combined extracts were extracted with 2 *N* hydrochloric acid, the aqueous layer was made alkaline and extracted with ether. The solvent was evaporated and the residue distilled. B. p. 90—100°/0.05 mm. Yield 17.4 g.

$C_{12}H_{16}N_2$ (188.3)	Calc.	C	76.5	H	8.56	N	14.9
	Found	»	76.6	»	8.63	»	15.0

γ-Dimethylamino-α-phenylthiobutyramide

0.25 g of sodium was dissolved in 60 ml of ethanol. 3.8 g of γ-dimethylamino-α-phenylbutyronitrile was added, the solution was cooled to — 10° and saturated with hydrogen sulphide. The mixture was then heated for seven hours at 70°. After cooling, the ethanol was evaporated at reduced pressure and the solid residue suspended in water and collected. Yield 3.6 g. After recrystallisation from ethanol the thioamide melted at 145—146° (dec.).

$C_{12}H_{18}N_2S$ (222.3)	Calc.	C	64.8	H	8.16	S	14.4
	Found	»	65.2	»	8.08	»	14.3

2-(γ-Dimethylamino-α-phenylpropyl)-thiazole

A mixture of 1.20 g of γ-dimethylamino-α-phenylthiobutyramide, 0.8 g of freshly distilled chloroacetaldehyde hydrate and 10 ml of nitrobenzene was refluxed for three hours (the refluxing temperature was 116—118°). After cooling, the reaction mixture was extracted with 100 ml of 2.5 *N* hydrochloric acid. The acid aqueous layer was then extracted with ether and made alkaline. The resulting oil was extracted with ether. An ethanolic solution of picric acid was added to the ether extract, until no more precipitate was obtained. The crude *dipicrate* (1.75 g) was recrystallised from water. M. p. 144—145°.

$C_{14}H_{18}N_2S \cdot 2\ C_6H_3N_3O_7$ (704.6)	Calc.	C	44.3	H	3.43	S	4.55
	Found	»	44.2	»	3.44	»	4.54

780 mg of the dipicrate, 30 ml of water, 5 ml of 5 *N* sodium hydroxide and 15 ml of chloroform was heated, and stirred on the water bath for one hour. The chloroform layer was separated and dried, and the solvent evaporated. The residue was distilled at 0.1 mm and 150° in the bath. Yield 205 mg of a viscous colourless oil.

$C_{14}H_{18}N_2S$ (246.4)	Calc.	C	68.3	H	7.36	
	Found	»	68.0	»	7.33	

4-Methyl-2-(γ-dimethylamino-α-phenylpropyl)-thiazole

A. A mixture of 1.20 g of γ-dimethylamino-α-phenylthiobutyramide, 2.0 ml of chloroacetone and 10 ml of nitrobenzene was refluxed for three hours. The reaction mixture was treated as in the preceding experiment. 1.28 g of a *dipicrate* was obtained, melting at 152—153.5° after recrystallisation from water.

$C_{15}H_{20}N_2S \cdot 2\ C_6H_3N_3O_7$ (718.6)	Calc.	C	45.1	H	3.65	S	4.46
	Found	»	45.0	»	3.64	»	4.34

From 610 mg of the dipicrate 125 mg of the base boiling at 0.1 mm at a bath temperature of 150° was obtained as a colourless oil.

$C_{15}H_{20}N_2S$ (260.4)	Calc.	C	69.2	H	7.74
	Found	»	69.0	»	7.60

B. To a suspension of sodium amide, prepared from 1.3 g of sodium and liquid ammonia, in 50 ml of toluene 4.8 g of 2-benzyl-4-methylthiazole [9] and 4.0 g of β-dimethylaminoethyl chloride hydrochloride were added. The mixture was refluxed for two hours, cooled and filtered. The toluene solution was then washed with water and extracted with 1 *N* hydrochloric acid. From the extract 3.5 g of a picrate was obtained by adding an aqueous solution of picric acid. It melted after crystallisation from water at 152—154° and showed no m. p. depression with the picrate from (A).

4-Phenyl-2-(γ-dimethylamino-α-phenylpropyl)-thiazole

A solution of 1.2 g of γ-dimethylamino-α-phenylthiobutyramide and 1.1 g of ω-bromoacetophenone in 10 ml of ethanol was refluxed for half an hour. The mixture was evaporated to dryness, treated with *N* sodium hydroxide and extracted with ether. By adding an ethereal solution of oxalic acid to the extract 1.3 g of *oxalate* was obtained. M. p. 154—157° after recrystallisation from acetone.

$C_{20}H_{22}N_2S \cdot (COOH)_2$ (412.5)	Calc.	C	64.0	H	5.86
	Found	»	64.1	»	6.04

From 330 mg of the oxalate 180 mg of the base was obtained. It distilled at 0.07 mm and 190° in the bath. The colourless oil partially solidified after some weeks.

$C_{20}H_{22}N_2S$ (322.5)	Calc.	C	74.5	H	6.88
	Found	»	74.6	»	7.02

γ-Diethylamino-α-phenylbutyronitrile

From 47 g of benzyl cyanide, 60 g of sodium hydroxide and 86 g of β-diethylaminoethyl chloride hydrochloride was obtained 27.9 g of this compound. B. p. 95—105°/0.1 mm.

$C_{14}H_{20}N_2$ (216.3)	Calc.	C	77.7	H	9.32	N 13.0
	Found	»	77.8	»	9.47	» 13.3

γ-Diethylamino-α-phenylthiobutyramide

This compound was prepared from 17.3 g of γ-diethylamino-α-phenylbutyronitrile in the same way as the preceding thioamide. As it was obtained as an oil, it was converted into the hydrochloride. The crude *hydrochloride* (14.0 g) was recrystallised twice from ethanol. M. p. 199—201°. Brown, Cook and Heilbron report m. p. 187°.

$C_{14}H_{22}N_2S$ HCl (286.9)	Calc.	N	9.77	S	11.2
	Found	»	9.84	»	11.1

2-(γ-Diethylamino-α-phenylpropyl)-thiazole

3.0 g of γ-diethylamino-α-phenylthiobutyramide hydrochloride was dissolved in water, made alkaline and extracted with ether. The ether solution was dried and evaporated. The oily residue was mixed with 1.6 g of chloroacetaldehyde and 20 ml of nitrobenzene and was then refluxed for three hours. The reaction mixture was treated in the usual way. An oily *dipicrate* was obtained, which was dissolved in boiling ethanol. On cooling 2.8 g of crystals melting at 144—145° were deposited.

$C_{16}H_{22}N_2S \cdot 2\ C_6H_3N_3O_7$ (732.6)	Calc.	C	45.9	H	3.85
	Found	»	46.2	»	4.11

From 2.7 g of the dipicrate 550 mg of the base was obtained. It distilled at 0.1 mm and 150° in the bath.

$C_{16}H_{22}N_2S$ (274.4)	Calc.	C	70.0	H	8.08
	Found	»	70.3	»	7.99

4-Methyl-2-(γ-diethylamino-α-phenylpropyl)-thiazole

This compound was prepared from 4.0 g of γ-diethylamino-α-phenylthiobutyramide hydrochloride and 4.3 ml of chloroacetone in the same way as the preceding thiazole. 6.6 g of *dipicrate* was obtained, melting at 141—142° after crystallisation from methanol. Brown, Cook and Heilbron [8] give the m. p. 142°.

$C_{17}H_{24}N_2S \cdot 2\ C_6H_3N_3O_7$ (746.6)	Calc.	C	46.7	H	4.05
	Found	»	46.6	»	4.00

5.1 g of the dipicrate was converted into free base following the usual procedure. It boiled at 0.1 mm and 150° in the bath. Yield 0.8 g.

$C_{17}H_{24}N_2S$ (288.4)	Calc.	C	70.8	H	8.39
	Found	»	71.1	»	8.31

γ-Piperidino-α-phenylbutyronitrile

This compound was prepared in the usual way from 80 g of β-piperidinoethyl chloride hydrochloride, 41 g of benzyl cyanide and 52 g of sodium hydroxide. Yield 23.3 g, b. p. 122—125°/0.05 mm.

$C_{14}H_{20}N_2$ (228.3)	Calc.	C	78.9	H	8.83
	Found	»	78.6	»	8.80

The picrate melted at 160—161°. Anker and Cook [6] report m. p. 161°.

γ-Piperidino-α-phenylthiobutyramide

From 12.0 g of γ-piperidino-α-phenylbutyronitrile 13.0 g of the thioamide was obtained. After recrystallisation from 50 % ethanol m. p. 144—145° (dec.).

$C_{15}H_{22}N_2S$ (262.4)	Calc.	C	68.7	H	8.45	N	10.7
	Found	»	68.8	»	8.64	»	10.8

2-(γ-Piperidino-α-phenylpropyl)-thiazole

4 g of γ-piperidino-α-phenylthiobutyramide, 2.3 g of chloroacetaldehyde hydrate and 20 ml of nitrobenzene was refluxed for three hours. After the usual treatment 5.45 g of a *dipicrate* was obtained. M. p. 159—161° after recrystallisation from 50 % ethanol.

$C_{17}H_{22}N_2S \cdot 2\ C_6H_3N_3O_7$ (744.7)	Calc.	C	46.8	H	3.79
	Found	»	47.0	»	3.81

From 2.0 g of the dipicrate 0.45 g of the thiazole, boiling at 0.1 mm and 180° in the bath, was prepared.

$C_{17}H_{22}N_2S$ (286.4)	Calc.	C	71.3	H	7.74	N	9.78
	Found	»	71.4	»	7.90	»	9.71

4-Methyl-2-(γ-piperidino-α-phenylpropyl)-thiazole

This compound was prepared from 4.0 g of γ-piperidino-α-phenylthiobutyramide and 6.3 g of chloroacetone in 20 ml of nitrobenzene. The *dipicrate* (5.2 g) was recrystallised from 30 % ethanol. M. p. 184—185°. (Brown, Cook and Heilbron [8] report 180°).

$C_{18}H_{24}N_2S \cdot 2\ C_6H_3N_3O_7$ (758.7)	Calc.	C	47.5	H	3.99
	Found	»	47.1	»	3.90

2.0 g of the dipicrate was converted into free base as usually. 0.40 g was obtained, distilling at 0.1 mm and 180° in the bath.

$C_{18}H_{24}N_2S$ (300.5)	Calc.	C	72.0	H	8.05	N	9.33
	Found	»	71.6	»	8.11	»	9.19

SUMMARY

Seven aminoalkyl derivatives of 2-benzylthiazole have been prepared and tested for antihistaminic and antispasmodic activity.

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Received April 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 751–761

An X-Ray Study of the Stereochemistry of the Nucleosides

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The nucleosides are compounds of great biological interest, and have therefore been subjected to comprehensive chemical investigations, as a result of which their chemical formulae are now well established. The *d*-ribose exists in the furanose condition [1], and the N-glycosidic linkage is almost certainly of the β-type [2], although direct confirmation of the latter point would appear very desirable. It still remains, however, to determine the actual shape of these molecules, especially the mutual orientation of the ribose ring and the pyrimidine or purine ring. The solution of this problem is not only of general stereochemical interest, but should also provide a clue to the structure of the nucleic acids. Only few physical investigations related to the structure of the nucleosides have been carried out. On basis of X-ray studies of fibres of Na-thymonucleate Astbury [3] has suggested that the base is parallel to the sugar, the bond joining the two rings making an angle with both ring planes. Hendricks [4] investigated the ions of adenosine and guanosine by measuring the interplanar cleavage spacings, d(001), of their salts with the clay mineral montmorillonite. He found that the atoms in these two compounds lie in or near two parallel planes 1.5 Å apart; one plane contains the purine radical, the other the ribose ring, and it is held that the hydroxyl groups as well as the primary alcohol group must be approximately in the plane of the purine. These investigations do not, however, give any detailed information about the structure of the molecules, and can hardly be regarded as conclusive even as far as their general shape is concerned. X-ray analysis of single crystals would appear to be essential. No such X-ray work on nucleosides is reported in the literature, but crystal structure determinations have been completed of some of their components, or related compounds. Valuable information relevant to the present problem is given by the work of Pitt [5] on hydroxypyrimidines, Clews and Cochran [6] on aminopyrimidines, Broomhead [7] on adenine

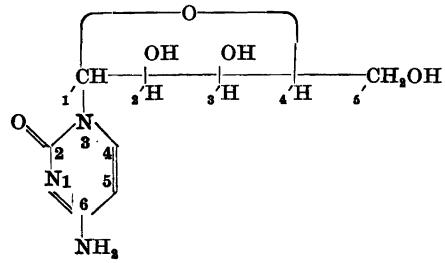
hydrochloride, and Beevers and Cochran [8] on sucrose sodium bromide dihydrate.

In the following crystal data will be given for each of the four nucleosides obtained from yeast nucleic acid, and their structures discussed.

CYTIDINE

Crystallographical. The crystals of cytidine are orthorhombic, elongated along the *c*-axis, bounded by faces (110) and (1$\bar{1}$0), with unit cell dimensions $a = 13.93$ Å, $b = 14.75$ Å and $c = 5.10$ Å. Density 1.53_2 g/cm^3. Space group $P\ 2_12_12_1$; four molecules (calc. 3.98) per unit cell.

Atomic co-ordinates were postulated by a careful consideration of available chemical, physical and crystallographic evidence, and the structure refined by two-dimensional Fourier syntheses. The structure determination is described in detail elsewhere [9], and here only points of stereochemical interest will be dealt with. The main results of the structure analysis of cytidine are evident from Fig. 1, which shows the molecule in the *c*-projection, with bond lengths



Cytidine

and bond angles. No great accuracy can be claimed, as only two-dimensional methods are employed and as rather bad molecular overlap occurs in the *a*- and *b*-projections. The agreement between $F_{obs.}$ and $F_{calc.}$ is, however, satisfactory, and the maximum possible error in the bond lengths is thought to be about 0.1 Å. The ribose ring is imperfectly resolved also in the important *c*-projection, and the greatest errors are therefore likely to occur in this part of the molecule.

Stereochemical. The investigation proves directly that the glycosidic linkage is of the β-type, as the bonds C_1'—N_3 and C_2'—O_2' are in *trans* position. The sugar is in the furanose condition, and cytidine can thus be described as cytosine-3-β-*d*-ribofuranoside, in accordance with the chemists findings.

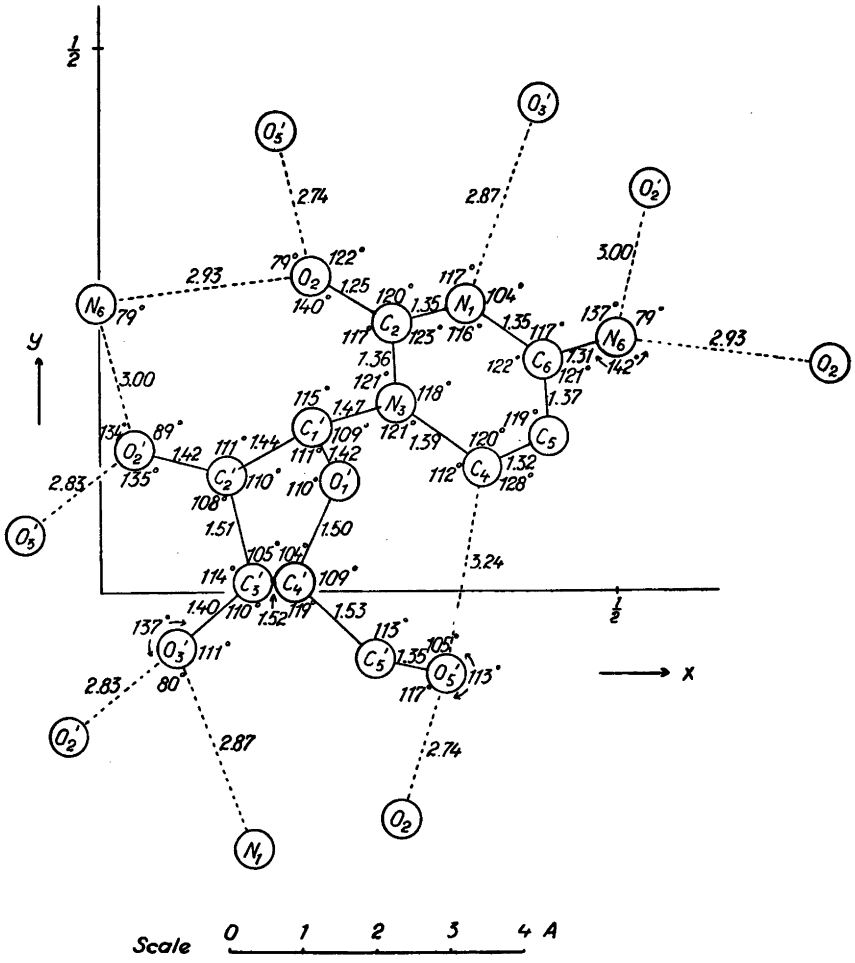


Fig. 1. The c-projection of a molecule of cytidine and its nearby contacts, with bond lengths (in Å units) and bond angles. Hydrogen bonds are dotted.

Consider first the pyrimidine ring and its substituents. The six ring atoms as well as O_2, N_6 and C_1' are all found to lie in the same plane within the experimental error. The bond lengths in the ring vary from 1.32 Å to 1.39 Å, and the bond angles are all near 120°. Although the rather wide limits of error make it impossible to discuss these results in detail, it would appear that we are confronted with a *resonating* system, in which the ring itself and the bonds to the keto (1.25 Å) and the amino group (1.31 Å) take part. The bond to the

carbohydrate does not seem to be involved, as it is found to have the length of a single bond (1.47 Å), but it lies in the plane of the pyrimidine ring in accordance with the double bond character of the ring bonds to N_3. Besides the "normal" structure with the groups $\diagdown C—NH_2$ and $\diagdown C = O$, structures with formal charges containing the groups $\diagdown C = \overset{+}{N}H_2$ and/or $\diagdown C—O^-$ must be supposed to take part, in order to account for the bond lengths. No estimation can be given from the present two-dimensional analysis as to which extent each canonical form contributes to the molecule, but the shortness of the bonds $C_2—O_2$ and $N_6—C_6$ indicate that important contributions may come from forms containing the groups $\diagdown C = O$ and $\diagdown C = \overset{+}{N}H_2$. It is in this connection interesting that both the external bonds from the latter group lie in the plane of the pyrimidine ring within the experimental error.

The shortness of the bond $C_6—N_6$ might also be explained by assuming that the molecule exists in its iminoform $—NH\diagdown C = NH$ in the crystal. This is, however, considered unlikely for chemical reasons, and also crystallographic evidence in favour of the aminoform is obtained from a study of the external bonding scheme in the crystal structure.

The *d*-ribose ring is found to be non-planar. Four of the atoms of the ring lie nearly in one plane, but the fifth member of the ring, C_3', is out of this plane by about 0.5 Å. It is very interesting that Beevers and Cochran [8] found the same feature in the case of fructo-furanoside, as it indicates that deviation from planarity may be a characteristic property of all saturated furanose rings. Some of their other results concerning the five-membered ring could, however, not be confirmed. They found all the bond distances in the ring to be short, 1.42 Å—1.45 Å, and a rather small bond angle of 104°. In addition the angles between the bonds to the hydroxyl groups and the adjacent ring bonds were definitely high at 113°—118°. The bond lengths found in the furanose ring in cytidine vary from 1.42 Å to 1.52 Å, and having in mind the rather wide limits of error these values do not differ significantly from normal single bonds. The mean bond angle in the ring is 108°, and the angles between the bonds to $(OH)_2'$ and $(OH)_3'$ and the ring bonds are not far from the tetrahedral angle, having the values 111°, 108°, 114° and 110°. *D*-ribose is thus found to be more "normal" than fructofuranoside, with bond angles nearer the tetrahedral angle, and bond lengths nearer to a single bond.

The central bond $N_3—C_1'$ lies in the plane of the pyrimidine ring and makes nearly tetrahedral angles with the adjacent ring bonds in the *d*-ribose. Con-

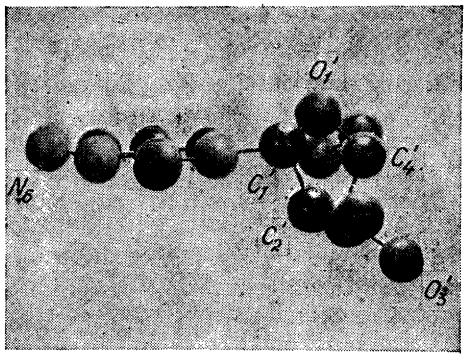


Fig. 2. A model of the cytidine molecule.

trary to Astbury's suggestion of the two rings being parallel, they are oriented in such a way that they are nearly *perpendicular* to each other as illustrated by the model in Fig. 2. Van der Waals forces would appear to play an important role in determining this mutual orientation. A study of a model shows that these forces will tend to impose restrictions on the freedom of rotation about the central bond, as the atoms in the *d*-ribose ring in many positions will come unfavourably near the keto group in the pyrimidine ring. With the *d*-ribose in *cis* position to the atom O_2 (referred to the bond N_3—C_1') the distances from O_2 to some of the atoms in this ring are only 2.4 Å—2.7 Å, and a *trans* arrangement, as actually found in the crystal structure, is therefore energetically more favourable.

The distance between the atom C_4 in the pyrimidine ring and O_5' in the primary alcohol group of the *d*-ribose is found to be as short as 3.24 Å, which would appear to be significantly less than the normal van der Waals approach of 3.4—3.5 Å. This may possibly be due to some kind of attraction between these two atoms, presumably of the hydrogen bond type. The relative position of C_4 and O_5' is also what we should expect in case of bond formation, as O_5' does not lie far away from the plane of the pyrimidine ring. The reason for the formation of the proposed bond may be sought in the possible polarisation of the group at C_4 in the pyrimidine ring to C^-—H^+, due to the inductive effect of the electronegative substituents in the pyrimidine ring (the keto and amino group).

Chemical evidence shows that in cytidine (and uridine) the carbohydrate is more firmly linked to the rest of the molecule than in other glycosides (inclusive of adenosine and guanosine). This abnormal behaviour may partly be related to the suggested intra-molecular bond, but the bond, if it exists, appears to be much too weak to offer a full explanation of the conduct of

cytidine. Another interesting fact in this connection is that 4,5-dihydrocytidine behaves chemically like a normal glycoside, quite unlike cytidine, and in this compound we should not expect such a bond to be formed, as the (CH_2)-groups can hardly be polarised to any extent.

The external bonding scheme in the crystal structure of cytidine consists of a very extensive system of hydrogen bonds, in which all its active groups are involved. Each molecule is linked to its neighbours by ten hydrogen bonds of lengths 2.74 Å—3.00 Å. The hydroxyl groups $(OH)_2'$ and $(OH)_3'$, as well as the amino group, are engaged in two external bonds, whereas the hydroxyl group $(OH)_5'$ makes only one external bond, possibly because of its participation in the intra-molecular hydrogen bond proposed above. For a fuller description of the external bonds, the reader is referred to a contemporary paper [9].

URIDINE

Crystallographical. The crystals of uridine are monoclinic, elongated along the *c*-axis, and bounded by faces $(1\bar{1}0)$ and (110). The cell dimensions are: $a = 13.88$ Å, b = 14.62 Å, $c = 5.00$ Å, $\beta = 95°$ (approx.). Cell volume 1010 Å^3; density 1.59 g/cm^3. Four molecules per unit cell (calc. 3.97).

The only systematic absences occur in the $0k0$-reflexions for odd values of k, and as uridine is optically active, the space group is $P\,2_1$. The asymmetric unit in the crystal consists of two molecules of uridine. Although uridine belongs to the space group $P\,2_1$, it has some of the features of cytidine's space group $P\,2_12_12_1$, as the reflexions 001, 100 and 300 are very weak. At the same time, there is a very great similarity in the cell dimensions of the two compounds, and the molecules of uridine are therefore placed roughly in the same positions in the unit cell as those of cytidine.

Stereochemical. Chemically, uridine differs from cytidine only in having a hydroxyl group in the 6-position instead of the amino group. The resemblance in the cell dimensions indicates that also the *shape* of the molecule is likely to be nearly the same in both cases, and our conclusions regarding the stereochemistry of cytidine can therefore probably be applied directly to uridine.

ADENOSINE

This purine nucleoside is of special importance on account of its participation in coenzyme molecules, and attempts to elucidate its structure would appear to be of great interest.

Crystallographical. The needle-shaped crystals are monoclinic, elongated along the c-axis, and bounded by faces (110) and $(1\bar{1}0)$. Even the best speci-

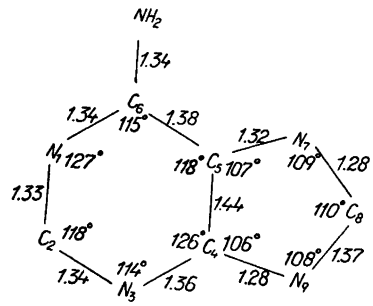


Fig. 3. Bond lengths and bond angles in adenine (J. M. Broomhead).

mens available were poorly developed and very small, with a cross-section not exceeding 0.04 × 0.04 mm. The unit cell dimensions are: $a = 11.94$ Å, $b = 10.22$ Å, $c = 4.82$ Å, $\beta = 103°$ (approx.). Cell volume 572 Å^3; molecular weight 267. The density was found to be about 1.57 g/cm^3, but an accurate determination was difficult because of the small, imperfect crystals. There are two (calc. 2.02) molecules in the unit cell.

The only systematic absences occur in the $0k0$-reflexions for odd values of k, and the space group is $P\ 2_1$. There is no centre of symmetry in the important c-projection, and no attempt was therefore made at this stage to work out the structure completely. In view of the very short c-axis, however, even only an estimate of the extension of the molecule in the c-projection may be of value. This was done by studying the low order reflexions, supported by a Patterson projection. The $h00$-reflexions show interesting intensity variations; 100 is absent, and 300 and 500 weak, whilst 400 is stronger than 200. This means that there must be atoms near $x = 0$, $x = 0.25$ and $x = 0.50$. A consideration of other reflexions indicates that the molecule must extend at least half a cell edge also in the y-direction, probably considerably more.

Stereochemical. The two components of adenosine have both been examined by X-rays before, adenine by Broomhead [7] and *d*-ribose by the author in his work on cytidine. The result of the former investigation is given in Figure 3, of the latter in Figure 1. It is proved that the glycosidic linkage is between N_9 in the purine and C_1' in the *d*-ribose [10], and that it is of the β-type [2]. One important remaining problem is the direction of the central bond N_9—C_1' connecting the two ring systems. Hendricks [4] and Astbury [3] have both suggested that it forms an angle with the plane of the purine (see above), so as to make the purine and the furanose ring parallel. In cytidine, however, this bond lies *in* or very near the plane of the pyrimidine ring, and the high degree of resonance in adenine, which is apparent from the bond lengths in Fig. 3, would seem to make this likely to be the case also in adenosine. Such a "cyti-

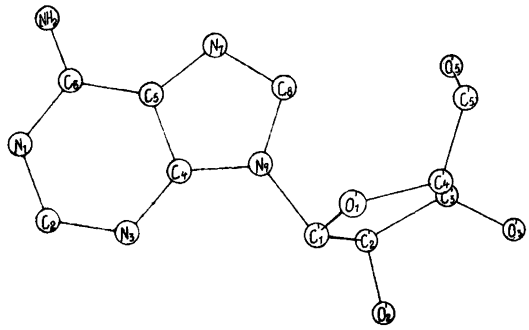


Fig. 4. The structure proposed for adenosine.

dine-like" structure is shown in Fig. 4. Here N_9—C_1' lies in the plane of the purine and forms tetrahedral angles with the adjacent ring bonds in the *d*-ribose; the two rings, far from being parallel, are nearly perpendicular to one another. Although other arrangements can not be definitely ruled out, this structure appears to give the most satisfactory explanation of the available data. Firstly, it is in agreement with the X-ray evidence, which indicates a rather flat ($c = 4.82$ Å) and widely spread-out molecule. The structure in Fig. 4 is about as flat as the molecule can be provided normal bond angles, and sufficiently extended. Secondly, it is correct from a chemical point of view, being adenine-9-β-*d*-ribofuranoside, whereas the sugar in the structure proposed by Hendricks actually is α-lyxose, not *d*-ribose, as pointed out by Gulland and coworkers [11]. Thirdly, Hendricks' results can be reinterpreted in terms of the proposed structure, in which also two parallel planes about 1.5 Å apart can be roughly recognised, the one containing the purine plus the atoms C_1', C_4' and C_5', the other passing through C_2', C_3' and O_2'. The remaining atoms do not lie in any of these planes, but none of them is far out. The structure appears thus to be consistent with the measurements of Hendricks.

In cytidine we found some evidence for a weak intramolecular bond, supposedly due to the polarisation of a (CH) group. There is no reason for assuming such a bond in adenosine, where the only substituent in the purine ring is far away from the group concerned, $(CH)_8$, actually in another ring.

GUANOSINE

Crystallographical. The crystals of guanosine are monoclinic, elongated along the *c*-axis, and bounded by {110}, {010} and {100}, the last form dominating. Their shape indicates monoclinic sphenoidal symmetry (class 2). The cell

dimensions are: $a = 17.45$ Å, $b = 11.44$ Å, $c = 6.65$ Å, $\beta = 98°$ (approx.). Cell volume: 1315 Å^3. Density: 1.60 g/cm^3. Guanosine crystallises with two molecules of water, and there are four (calc. 3.97) molecules $C_{10}H_{13}O_5N_5 \cdot 2H_2O$ in the unit cell.

The $0k0$-reflexions are absent for odd values of k, and the space group is $P\,2_1$. The asymmetric unit in the crystal consists of two molecules of guanosine and four molecules of water.

Stereochemical. No conclusion regarding the shape of the guanosine molecule can be drawn from these preliminary data. Also the guanosine ion was, however, investigated by Hendricks, who found it to be very similar to the adenosine ion. It appears therefore reasonable to assume that our discussion of the stereochemistry of adenosine is valid also for guanosine.

THE DEOXYRIBONUCLEOSIDES

The deoxyribonucleosides differ chemically from the ribonucleosides in that they contain 2-deoxy-*d*-ribose instead of *d*-ribose. No crystals of these compounds have been investigated, but the results from the study of cytidine and the other ribonucleosides can probably be applied to them. In cytidine the hydroxyl group $(OH)_2'$ is at a distance of as much as 3.75 Å from the keto group in the pyrimidine, and is unlikely to play any important role in determining the mutual orientation of the carbohydrate and the pyrimidine. The absence of $(OH)_2'$ should therefore not alter significantly the forces which act between the two ring systems, and the perpendicular relationship between them would appear likely to be maintained also in the case of the deoxyribonucleosides.

THE NUCLEIC ACIDS

Astbury [3] has on basis of X-ray studies of fibres and powders of nucleic acids suggested that these molecules are presumably stiff columns of nucleosides fitting closely on top of one another, with all the rings flat and parallel. In the present investigation it is established that the purine or pyrimidine is not parallel, but perpendicular to the ribose ring at least in one of the nucleosides, namely cytidine, and probably also in all the others. It appears likely that the nucleosides maintain their characteristic shape when they unite to form nucleic acids, and Astbury's theory is therefore in need of some modification. A full discussion of the problem is postponed pending the completion of the structure of a nucleotide, but one point will be made here. There is

evidence that the purines and the pyrimidines are nearly parallel to one another, and presumably *perpendicular* to the axis of the rod-like molecule, at least in thymonucleic acid. If this is the case, the ribose rings will consequently lie with their planes more or less *parallel* to the axis of the molecule, and it can be shown by models that a dominating spacing of about 3.4 Å, as found by Astbury, can be maintained in the molecule also under these circumstances.

EXPERIMENTAL

Considerable difficulties were encountered in getting single crystals big enough for X-ray work, and several techniques of growing crystals were tried. For cytidine the best results were obtained by slow evaporation of a solution in alcohol-water, and for adenosine by slow cooling of a concentrated solution in water. In the case of uridine and guanosine, crystals from the supplied samples could be used.

Density measurements were made by flotation of the crystals in a mixture of carbon tetrachloride and benzene.

The cell dimensions were derived from oscillation photographs, and are believed to be correct to at least within 1 %. Copper radiation ($\lambda = 1.54$ Å) is used throughout the investigation.

In the case of cytidine and adenosine also Weissenberg photographs were taken. The intensities were estimated visually and corrected for the Lorentz-polarisation factor in the usual way.

SUMMARY

Single crystals of cytidine, uridine, adenosine and guanosine have been studied by X-ray diffraction methods. The complete crystal structure of *cytidine* has been determined by two-dimensional Fourier syntheses, showing directly that cytidine is cytosine-3-β-*d*-ribofuranoside. The planar pyrimidine ring and the non-planar ribose ring are linked in such a way that they are nearly perpendicular to each other; the central bond N_3—C_1' lies in the plane of the pyrimidine ring. Preliminary investigations of the other three ribonucleosides, supported by other physical data, indicate that these molecules have a shape similar to that of cytidine, and it is considered likely that this holds also for the deoxyribonucleosides.

My best thanks are due to Prof. J. D. Bernal, F. R. S., and Dr. C. H. Carlisle for their continued interest and encouragement, to Prof. A. R. Todd, F. R. S., for an interview, and to Dr. D. O. Jordan and Dr. R. J. C. Harris for supplying the specimens. I also wish to thank The British Council for the award of a scholarship.

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Received April 1, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 762–771

An Electrophoretic Investigation of Vicilin and Legumin from Seeds of Peas

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Previous investigations at this institute have shown that the seeds of pea, *Pisum sativum*, contain two globulin components, vicilin and legumin [1] which are well-defined by ultracentrifugation. These components have been separated by different methods, for example precipitation at low temperature or fractionated precipitation with ammonium sulphate. The best method so far for the separation of the two components is iso-electric precipitation of legumin at pH 4.7. By dialysis of a solution containing both vicilin and legumin in a cellophane bag against a buffer at pH 4.7 legumin precipitates and vicilin stays in the supernatant solution. The preparation scheme for the isolation of vicilin and legumin from peas has been described in a previous paper which also deals with the differences between the ultra violet absorption of the two components [2]. By measurement of light absorption in the ultra violet region of the components in 0.1 N NaOH it is possible to calculate the tyrosine and tryptophan contents. Legumin and vicilin contain the same amount of tyrosine, but legumin contains five times as much tryptophan as does vicilin. Thus vicilin and legumin are different in their chemical composition. The molecular weights are also different; determinations have given the values $M = 186\,000$ for vicilin and $M = 331\,000$ for legumin [1]. In this paper the electrophoretic properties of the two components will be discussed. In a recent paper, published when most of the work described here was already completed, Wetter and McCalla have dealt with the same problem [3]. However, they have not investigated the isolated components. A comparison with their results will be made later on in this paper.

EXPERIMENTAL

Ground seeds from peas are extracted over night at + 4° C with 1 *M* NaCl buffered to pH 7. After centrifugation and filtration of the extract the proteins (vicilin, legumin and albumin) are precipitated with $(NH_4)_2SO_4$ to 70 % saturation. The resulting precipitate is dissolved in 0.2 *M* NaCl, pH 7, and the solution is then dialysed in a cellophane bag against water. (One night against running tap water, and then distilled water.) When the salt concentration is lowered by dialysis, the globulins precipitate. The precipitate is dissolved in 0.2 *M* NaCl, pH 7, and again dialysed against water. After this second dialysis the globulins, which are now free from albumins, are dissolved in unbuffered 0.2 *M* NaCl, and this solution is dialysed against a buffer solution of pH 4.7 (0.2 *M* NaCl). During this dialysis legumin is iso-electrically precipitated, and the vicilin remains in solution in the supernate. By repeated dialysis at pH 4.7, vicilin and legumin can be quantitatively separated, as indicated by the ultracentrifuge. The resulting vicilin and legumin preparations are finally precipitated in a salt free state by dialysis against distilled water, and freeze-dried in vacuum. They are obtained in the form of a white powder, readily soluble in 0.2 *M* NaCl at pH 7.

The same freeze-dried preparations of vicilin and legumin have been used in all the experiments described here. Comparisons have been made with other highly purified preparations but no differences have been observed. In other crude preparations, however, it was possible to show incomplete separation as will be mentioned later.

INVESTIGATIONS OF VICILIN

Solutions were prepared containing about 0.8 % dried vicilin in 0.2 *M* NaCl, buffered to different pH values ($\mu = 0.25$). The solution (18 ml), was dialysed against 2000 ml of the buffer for 48 hours at + 4° C, and then analyzed at + 5° C. in the Tiselius' electrophoresis apparatus [4] with the modifications indicated by Svensson [5]. Solutions of vicilin and especially legumin are very difficult to investigate at lower temperatures because they precipitate when the temperature is lowered. Legumin must be investigated at + 15° C. It seems, however, as if this sensitivity to low temperature is greater when the components are purified. Perhaps some stabilizing colloid is removed by purification.

A typical electrophoretic diagram of vicilin is shown in Fig. 1. One single peak is obtained. Thus vicilin, which is homogeneous by ultracentrifugation, is also homogeneous by electrophoresis. In investigations of other vicilin preparations which had not been sufficiently purified, a second peak appeared in the diagrams, probably consisting of legumin. Its concentration was about 5—10 % of that of the vicilin. Diagrams similar to Fig. 1 were obtained in the whole pH-interval investigated (pH 3.7—9.3). The results of the measurements are shown in Table 1 and Fig. 2. The isoelectric point for vicilin is determined from Fig. 2 to be pH 5.5.

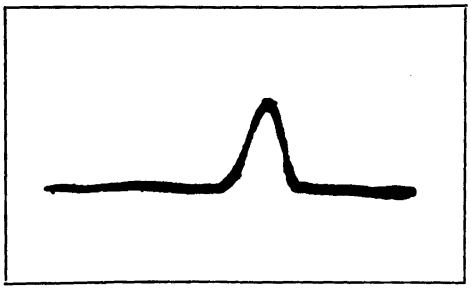

Fig. 1. Electrophoretic diagram of vicilin. pH 4.33. Concentration 0.7 %. Potential 3.01 volts/cm. Migration at + 5° C for 380 min. to the right. The descending peak has migrated 2.0 cm in the apparatus. $\Theta = 70°$. Θ = angle of inclination of the diagonal slit; this equals zero in the vertical position.

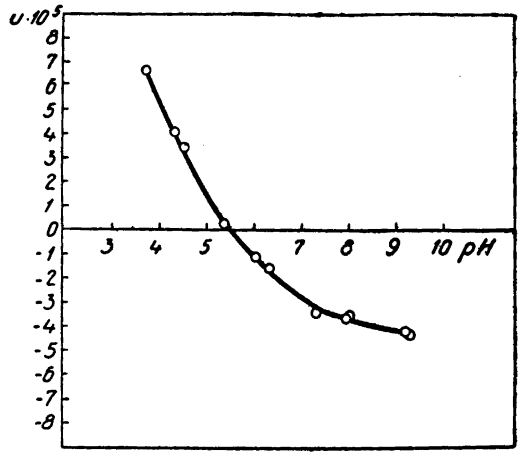

Fig. 2. Electrophoretic mobility of vicilin at different pH values.

INVESTIGATIONS OF LEGUMIN

In order to state whether vicilin and legumin have different electrophoretic properties, the following experiment was carried out. At pH 7.99 a mixture of vicilin and legumin in about the same concentrations (checked by ultracentrifugation) was analyzed in the electrophoresis apparatus at + 5° C. In this case the two components had never been separated from each other by iso-electric precipitation. Two peaks were obtained as can be seen in Fig. 3. The values for the mobilities were 6.95×10^{-5} and 3.79×10^{-5} cm²/volt. sec. The value 3.79×10^{-5} lies on the curve in Fig. 2, and this component must consequently be vicilin. The other component with the mobility 6.95×10^{-5} must be legumin. This experiment proves that vicilin and legumin behave differently in electrophoresis.

After this preliminary experiment some determinations were made on solutions containing purified legumin. As has been stated earlier in this paper, several properties of legumin make electrophoretic investigations rather diffi-

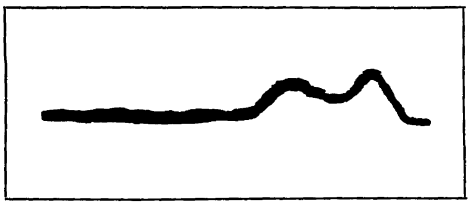

Fig. 3. Electrophoretic diagrams of vicilin and legumin. pH 7.99. Total concentration 0.6 %. Potential 3.65 volts/cm. Migration at + 5° C for 255 min. to the right. The descending peaks have migrated 2.1 cm (vicilin, on the left) and 3.9 cm (legumin, on the right) in the apparatus. $\Theta = 75°$.

Table 1. Electrophoretic mobility of vicilin at + 5° C at different pH Values. All buffer solutions contain 0.2 M NaCl.

Buffer (*M*)	pH	Mobility $\times 10^5$ cm^2/volt sec.
0.008 Na_2CO_3 0.008 $Na_2B_4O_7$	9.28	− 4.32
0.008 Na_2CO_3 0.008 $Na_2B_4O_7$	9.20	− 4.23
0.010 H_3BO_4 0.003 $Na_2B_4O_7$	8.04	− 3.56
0.010 H_3BO_4 0.003 $Na_2B_4O_7$	7.95	− 3.61
0.015 Na_2HPO_4 0.005 NaH_2PO_4	7.31	− 3.45
0.008 Na_2HPO_4 0.026 NaH_2PO_4	6.33	− 1.59
0.006 Na_2HPO_4 0.034 NaH_2PO_4	6.02	− 1.11
0.002 Na_2HPO_4 0.046 NaH_2PO_4	5.37	+ 0.24
0.063 HAc 0.050 NaAc	4.52	+ 3.47
0.100 HAc 0.050 NaAc	4.33	+ 4.04
0.400 HAc 0.050 NaAc	3.72	+ 6.65

cult. Firstly, legumin is iso-electrically precipitated in the pH range 4—6. The precipitation is most complete at pH 4.6—5.6. The solubility behaviour in this range will be described later in this paper. Secondly, legumin is precipitated at low temperature. In the experiments described here the temperature used was + 15° C, at which temperature no precipitation occurs. At this temperature very low current strength (about 8 mA) must be used (the potential

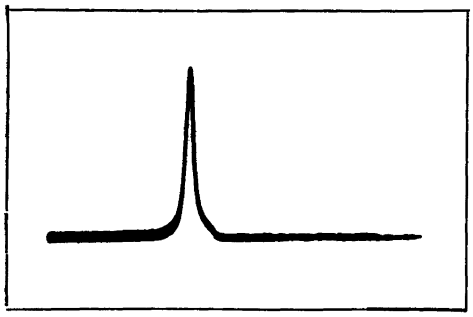

Fig. 4. Electrophoretic diagrams of legumin. pH 3.70. Concentration 1.7 %. Potential 0.99 volts/cm. Migration at + 15° C for 395 min. to the right. The descending peak has migrated 1.4 cm in the apparatus. Θ = 40°.

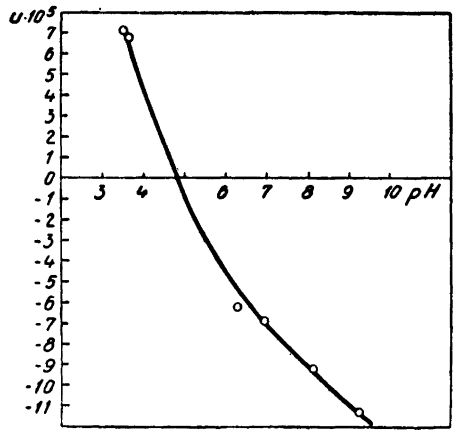

Fig. 5. Electrophoretic mobility of legumin at different pH values.

gradient $F = 0.6—1.0$ volts/cm instead of 2.6—3.6 volts/cm which could be used at + 5° C in the investigations of vicilin).

In electrophoresis of legumin solutions one main peak was obtained as is shown in Fig. 4. Thus legumin is homogeneous by electrophoresis. The results of the measurements are shown in Table 2 and Fig. 5, from which a determination of the isoelectric point of legumin gives the value pH 4.8. The error in this determination is of course rather large.

A COMPARISON BETWEEN THE ELECTROPHORETIC MOBILITIES OF VICILIN AND LEGUMIN AT + 5° C

In order to compare the mobilities for vicilin and legumin the following method was used. As described earlier, an electrophoretic analysis of a solution containing both vicilin and legumin was carried out at pH 7.99 and + 5°C. The mobilities obtained were 3.79×10^{-5} and 6.95×10^{-5} cm²/volt × sec. respectively. The value 3.79×10^{-5} lies on the curve for vicilin at + 5° C (Fig. 2). We can compare the mobilities for legumin obtained above at + 5° C $u = 6.95 \times 10^{-5}$, with the value from the experimental curve in Fig. 5 (which is run at + 15° C). For legumin, at pH 7.99 and + 15° C, was obtained from Fig. 5 $u = 9.05 \times 10^{-5}$. Thus, when the temperature is lowered from 15° C to 5° C, the electrophoretic mobility for legumin is lowered by 23 %. A later experiment showed the corresponding change for vicilin to be 28 %. This is probably largely a result of increased viscosity of the buffer. For water we

Table 2. Electrophoretic mobility of legumin at + 15° C at different pH values. All buffer solutions contain 0.2 M NaCl.

Buffer (*M*)	pH	Mobility × 10^5 cm^2/volt sec
0.008 Na_2CO_3 0.008 $Na_2B_4O_7$	9.27	— 11.3
0.013 H_3BO_3 0.002 $Na_2B_4O_7$	8.15	— 9.20
0.014 Na_2HPO_4 0.010 NaH_2PO_4	6.96	— 6.90
0.008 Na_2HPO_4 0.026 NaH_2PO_4	6.30	— 6.20
0.400 HAc 0.050 NaAc	3.65	+ 6.80
0.450 HAc 0.050 NaAc	3.60	+ 7.12

have the values: $\eta = 1.1404$ centipoises at 15° C and $\eta = 1.5188$ centipoises at 5° C [6]. According to Johnson and Shooter [7] the product $u \times \eta$ is a constant for a certain protein at any temperature. Thus $u_{15°} \times \eta_{15°} = u_{5°} \times \eta_{5°}$, or $\frac{u_{5°}}{u_{15°}} = \frac{\eta_{15°}}{\eta_{5°}}$ should hold in our case. We have $\frac{\eta_{15°}}{\eta_5^\circ} = 0.75$, and from the values above $\frac{u_{5°}}{u_{15°}} = \frac{6.95}{9.05} = 0.77$ for legumin. For vicilin, at pH 7.62, $u_{15°}$ was found to be 4.64×10^{-5} cm^2/volt × sec., and from Fig. 2 we obtain at pH 7.62 and + 5° C $u_{5°} = 3.40 \times 10^{-5}$ cm^2/volt × sec. These values give for vicilin $\frac{u_{5°}}{u_{15°}} = \frac{3.40}{4.64} = 0.73$. Thus $u \times \eta =$ constant holds for both vicilin and legumin and the electrophoretic mobilities of the two components can be compared at the same temperature. The mobility curve for legumin at + 5° can be calculated. After this correction has been made we can conclude that the mobility of legumin is about twice that of vicilin in the pH interval 6.3—9.3 at + 5° C. At pH 3.5, however, the mobility of legumin is lower than that of vicilin.

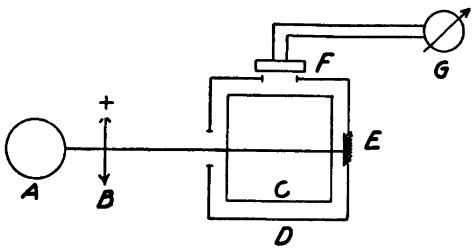

Fig. 6. Nephelometer.
A. Light source (Hg-lamp, "Philora").
B. Positive lens.
C. Glass cell, containing the solution (100 ml)
D. Metal box with windows, containing the cell.
E. Mirror.
F. Photoelectric cell (barrier-layer cell).
G. Reflecting galvanometer. Readings on a circular scale (in mm) with a radius of 90 cm.

DETERMINATION OF THE ISOELECTRIC POINT OF LEGUMIN BY MEANS OF ISO-ELECTRIC PRECIPITATION

From what has been said above, it is difficult to determine the isoelectric point of legumin electrophoretically, because legumin is iso-electrically precipitated. This precipitation was studied by means of a nephelometric method described below, (see Gibb [8] or any other textbook of optical methods). The Tyndall effect of the precipitate was measured at an angle of 90° in an apparatus shown in Fig. 6. There are many sources of error in quantitative nephelometry. Only the two most important will be mentioned: 1) the precipitate is inhomogeneous, *i. e.* the particles are of different sizes. This error can be largely eliminated by measurements on solutions of very low concentration in which case small particles are formed. The wave length of the incident light must consequently be short in order to have high intensity of the scattered light. In these experiments a Hg-lamp has been used. A concentration range where the measured turbidity is a linear function of the amount of substance precipitated must be used. 2) There is an age effect, *i. e.* the small particles form bigger ones. Thus the measurement must be done during the time interval when the age effect has little or no importance.

Determination of the dependence of the turbidity on time

25 ml of a 0.002 % legumin solution in 0.2 *M* NaCl were pipetted into 75 ml 0.2 *M* NaCl, buffered to pH 4.98 with acetate (total legumin concentration 0.0005 %). The solution was then immediately put into the nephelometer, and readings were made periodically as shown in Table 3. The same experiment was performed with a total legumin concentration of 0.002 %. Before and after all measurements in the nephelometer, 100 ml distilled water instead of

Table 3. The dependence of the turbidity on time.

Time after precipitation Minutes	Galvanometer readings $n-n_0$ mm.	
	Legumin conc. 0.0005 %	Legumin conc. 0.002 %
2	88	374
4	95	386
6	98	391
8	100	399
10	101	405
12	101	404
14	102	408
16	102	408
18	103	412
20	103	417
30	106	420
40	106	420
50	105	418
60	104	417

a protein solution were placed in the cell. The corresponding galvanometer reading is called n_0 (usually 0—10 mm) and the readings with protein solutions are called n. In the tables below the difference between the two readings, $n—n_0$ is shown.

It is evident from Table 3 that the measurement must not be made before 20—30 minutes.

Determination of the dependence of the turbidity on the amount of substance precipitated

Solutions containing legumin at different concentrations were iso-electrically precipitated at 3 different pH values. The results of the measurements are shown in Fig. 7. It can be seen that by iso-electric precipitation in the pH-range 4.5—5.6 and the total legumin concentration 0.0005—0.0025% the turbidity is a linear function of the protein concentration.

Determination of the maximum precipitation by iso-electric precipitation of legumin

In order to determine at which pH legumin is most completely precipitated, the following experiment was performed.

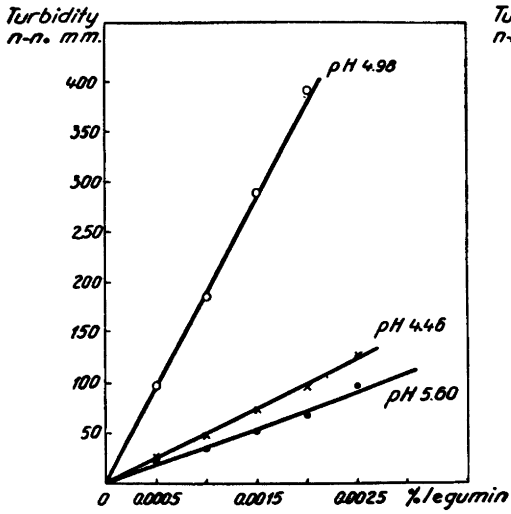

Fig. 7. The turbidity as a function of legumin concentration at different pH values.

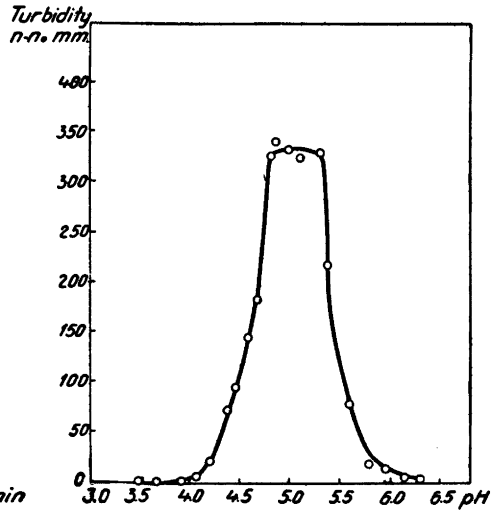

Fig. 8. Isoelectric precipitation of legumin at different pH values.

20 buffer solutions were made, containing acetate or phosphate ($\mu = 0.1$) and covering the pH range pH 3.3—6.3. These buffers also contained 0.2 *M* NaCl (total $\mu = 0.3$). The experiment was carried out in 10 Erlenmeyers flasks (200 ml) as described below. In each Erlenmeyer flask 75 ml of the buffer were pipetted. Every 2 minutes 22 ml 0.009 % legumin solution in 0.2 *M* NaCl were pipetted into one of the flasks, beginning in number one and finishing in number ten. After exactly 30 minutes the turbidity of each solution was measured in the nephelometer. The experiment was then continued in the same way with the 10 remaining buffer solutions. The results are given in Fig. 8.

As can be seen from Fig. 8 legumin is iso-electrically precipitated in a rather narrow pH interval. If we assume that a protein has minimum solubility at its isoelectric point [Rona and Michaelis [9]], we can conclude from this experiment that the isoelectric point of legumin is pH 5.0 $\pm$ 0.2. This result agrees rather well with the value pH 4.8 obtained by electrophoresis.

DISCUSSION

Vicilin and legumin are both practically homogeneous by electrophoresis but their electrophoretic properties are different. They are also different and homogeneous by ultracentrifugation. The author has never observed any dis-

sociation or association reaction by which vicilin is formed from legumin or legumin from vicilin. The differences in chemical composition, for instance the different tryptophan content, and especially the difference in sulfur content, 0.42 % S in legumin and 0.18 % S in vicilin from peas [Osborne and Campbell [10]] is a proof that we are dealing with two well-defined independent globulin components.

Wetter and McCalla [3] believe that it is very improbable that Osborne's methods can give well-defined, homogeneous protein species, and that it should be impossible to separate the different components with simple precipitation methods. The present author considers that with this and previous investigations he has proved this separation to be possible. It is rather simple to isolate vicilin and legumin with precipitation methods, and the resulting protein components are homogeneous by analysis in the ultracentrifuge and electrophoresis apparatus.

SUMMARY

1. Vicilin from peas has been analyzed in the Tiselius' electrophoresis apparatus. In the pH range 3.7—9.3 it is homogeneous.

2. The iso-electric point for vicilin has been determined to be pH 5.5.

3. Legumin from peas also is homogeneous by electrophoresis. Its electrophoretic mobility is higher than that of vicilin in the pH range 6.3—9.3. The iso-electric point for legumin has been determined electrophoretically to be pH 4.8. A nephelometric study of the iso-electric precipitation of legumin gave the value pH 5.0 of the isoelectric point.

The author wishes to thank Prof. A. Tiselius and Prof. T. Svedberg for the privilege of carrying out this work in their laboratories, and for the interest they have shown. He also wishes to thank Fil. mag. L. Graflund, who has constructed the nephelometer used in the work described in this paper. The investigation was supported by a grant from the Swedish Natural Science Research Council.

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Received April 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 772–781

Constituents of Pine Heartwood

XXI.* The Structure of Pinobanksin

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Pinobanksin, one of the most common phenolic constituents of pine heartwood, was first isolated from *Pinus Banksiana* by Erdtman [1]. This substance melted at 173—175° and was optically active, $[\alpha]_D^{20}$ + 15° **. Its composition agreed with the formula $C_{15}H_{12}O_5$, and oxidation with alkaline potassium permanganate yielded benzoic acid, thus showing the presence of an unsubstituted phenyl group. Pinocembrin, another common heartwood constituent, has the composition $C_{15}H_{12}O_4$ and is identical with (—)5,7-dihydroxyflavanone [2]. Erdtman assumed that pinobanksin and pinocembrin were structurally related and considered the most probable structure of pinobanksin to be 3,5,7-trihydroxy-flavanone [1], but no further experiments were carried out to prove this assumption.

A few 3-hydroxyflavanones have been found in nature during the last fifteen years, most of them by Japanese chemists. From the seeds of *Alpinia japonica* (Thunb.) Miq., Kimura and Hosi isolated a compound called alpinone, m. p. 178°, $[\alpha]_D^{20}$ + 79.1° (in pyridine), which they claimed to be 2-methyl-3,5-dihydroxy-7-methoxyflavanone [3, 4]. Fustin, a constituent of the wood from some *Rhus* species, was isolated as early as 1886 [5]. Its structure was elucidated by Oyamada in 1939.[6] It is a 3,7,3′,4′-tetrahydroxyflavanone, m. p. 216—218°. (The optical activity — if any — of fustin does not seem to have been determined.) Another 3-hydroxyflavanone, called ampelopsin, was isolated from the leaves of *Ampelopsis meliaefolia* Kudo. It has been shown to be a 3,5,7,3′,4′,5′-hexahydroxyflavanone, m. p. 245—246°, and is optically inactive [7]. It should be noted that both fustin and ampelopsin are accompanied by the corresponding flavonol, fisetin and myricetin, respectively. Two other

* XX. *Acta Chem. Scand.* **4** (1950) 448.

** The value + 1.5° given in Erdtman's paper is due to a misprint.

3-hydroxyflavanones were isolated by Pew [8], namely 3,5,7,3′,4′-pentahydroxyflavanone (taxifolin) from the heartwood and bark of *Pseudotsuga taxifolia* Britt. (Douglas-fir), m. p. 240—242°, $[\alpha]_D^{20}$ + 46° (in 50 % acetone)*, and 3,5,7,4′-tetrahydroxyflavanone, m. p. 237—241°, $[\alpha]_D^{20}$ + 45° (in 50 % acetone) from the heartwoods of *Notofagus dombeyi* Bl. (coigue) and of *Prunus serotina* Ehrh. The latter structure has also been ascribed to katsuranin, m. p. 224—225°, which has been isolated from the wood of *Cercidiphyllum japonicum* Sieb. et Zucc.[9]

In the course of the present author's investigations, pinobanksin has been isolated from the heartwood of several pine species [10]. By paper partition chromatography [11], it has been shown to be present in most pines.

The separation of pinobanksin from heartwood extracts is facilitated by its precipitation as an insoluble sodium salt with saturated sodium carbonate solution [1]. The pinobanksin is then liberated by acidification of the salt and purified by recrystallisation from toluene or from methanol-water. From toluene, it crystallises in large thick, colourless needles. After drying, the m.p.is 177—178°** and $[\alpha]_D^{20}$ +14.4° (in methanol). The loss in weight on drying at 110° corresponds to the composition 2 $C_{15}H_{12}O_5 \cdot C_7H_8$. Pinobanksin gives an orange-red colour when reduced with magnesium or zinc and hydrochloric acid. Pinocembrin gives an orange colour with magnesium but no colour with zinc, which confirms the statement by Pew[8] that the zinc colour reaction is specific to 3-hydroxyflavanones. (Flavanones containing hydroxyl groups on the side phenyl group give much deeper colours when reduced.)

Pinobanksin shows great resistance to racemisation. Treatment with 1 % sodium hydroxide for 43 hours at room temperature only depressed the specific rotation from + 14° to + 11°. (Pinocembrin is racemised completely under these conditions.) Boiling with alcoholic hydrochloric acid for one and a half hours did not change the rotation at all. Pew reports total racemisation of taxifolin after similar treatment [8]. When pinobanksin was treated with 15 % sodium hydroxide for seven days, a crystalline product, melting gradually at 180—200°, and having $[\alpha]_D^{20}$ + 5.5°, was obtained. Some decomposition of the molecule had evidently taken place in this experiment.

Pinobanksin gives a tribenzoate, m. p. 172—173°. Methylation with diazomethane yields a monomethyl ether, $C_{16}H_{14}O_5$ (II), m. p. 181—182°, $[\alpha]_D^{20}$—19° (in chloroform). A dimethyl ether, $C_{17}H_{16}O_5$ (III), can be prepared from the monomethyl ether by further methylation with one mole of dimethyl

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* In absolute ethanol, taxifolin has $[\alpha]_D^{20}$ + 13°, which lies close to the rotation of pinobanksin in methanol.

** All melting points uncorrected.

sulphate and anhydrous potassium carbonate in acetone solution. This method has been frequently employed by Seshadri and co-workers for the methylation of flavones and similar compounds [12]. The dimethyl ether can also be prepared by direct methylation of pinobanksin, but the overall yield is lower than for the two-step methylation. The dimethyl ether melts at 133—134° and has $[\alpha]_D^{20}$—31° (in chloroform). It is insoluble in dilute alkali and gives no colour with ferric chloride.

The structure of pinobanksin is shown by the catalytic dehydrogenation to the corresponding flavonol, galangin (IV). The dehydrogenation is carried out at 180° with a palladium-charcoal catalyst employing cinnamic acid as a hydrogen acceptor. The same method was used by Kotake and Kubota for the dehydrogenation of ampelopsin pentamethyl ether to the corresponding myricetin derivative [7].

Dehydrogenation by air, which has been successfully applied to taxifolin [8], failed with pinobanksin. The dimethyl ether can also be dehydrogenated by palladium-cinnamic acid to galangin-5,7-dimethyl ether (V). A spontaneous dehydrogenation takes place, when pinobanksin is methylated with an excess of dimethyl sulphate in acetone solution. Instead of pinobanksin trimethyl ether, galangin trimethyl ether (VI) is obtained in poor yield. A similar dehydrogenation during methylation has been observed with fustin [6].

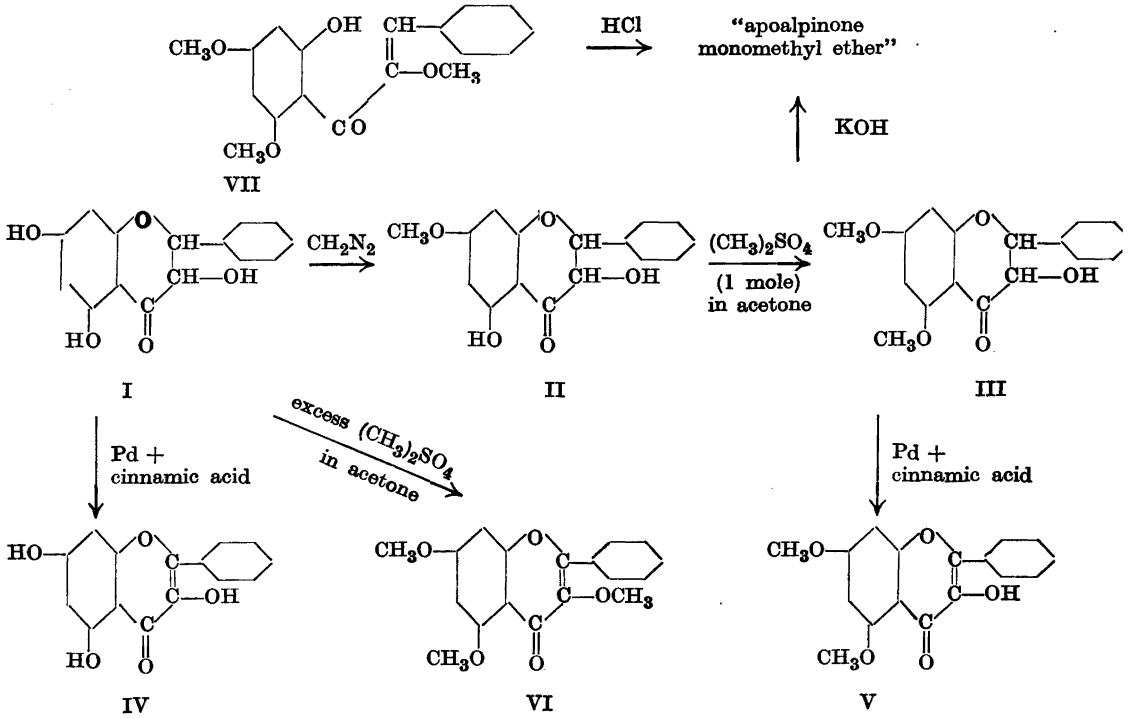
The methoxyl group in pinobanksin monomethyl ether certainly occupies the 7-position, since the hydroxyl group in the 5-position is much more difficult to methylate because of chelation with the carbonyl group [15]. It is to be noted that the isolation of a substance having the structure (II), apoalpinone, has been reported by Kimura and Hosi [3, 4]. It was obtained by treatment of alpinone with alkali and melted at 148°. Probably this compound differs from pinobanksin monomethyl ether only in the configuration at carbon atoms 2 and 3.

When pinobanksin dimethyl ether is boiled with alcoholic potassium hydroxide for a few minutes, it is rearranged to an optically inactive compound, m. p. 170—171°, which has the same composition as the optically active compound. This compound is identical with "apoalpinone monomethyl ether", which was first prepared by Kimura [13] by boiling 2'-hydroxy-4',6', α-trimethoxychalkone (VII) with alcoholic hydrochloric acid. Repetition of Kimura's synthesis (starting from 2-hydroxy-4,6, ω-trimethoxyacetophenone prepared according to Row and Seshadri [14]) yielded a colourless substance which melted at 169—170° (Kimura 169°). Its monoacetate (not described before) melted at 112—113°. The identity of the synthetic product with the substance obtained from pinobanksin dimethyl ether was established by the mixed melting points of the two substances themselves and their acetates. According to Kimura, "apoalpinone monomethyl ether" should be 3-hydroxy-5,

7-dimethoxyflavanone and thus should be a stereisomer of pinobanksin dimethyl ether. There are, however, some differences in the chemical behaviour of the two substances which cannot possibly be due only to different configuration at carbon atoms 2 and 3. For example, pinobanksin dimethyl ether gives an orange colour when reduced with magnesium-hydrochloric acid as does pinobanksin itself, while "apoalpinone monomethyl ether" gives no colour reaction at all. Furthermore, the latter substance dissolves readily in 2 *N* sodium hydroxide in the cold (and to a smaller extent even in more dilute alkali). Pinobanksin dimethyl ether is insoluble under the same conditions as would be expected from its structure. An attempt to dehydrogenate "apoalpinone monomethyl ether" to galangin 5,7-dimethyl ether failed completely. Thus, "apoalpinone monomethyl ether", apparently, is not a flavanone, and its formation by rearrangement of pinobanksin dimethyl ether will not be considered here as a proof of the structure of this substance.

Kimura's "apoalpinone monomethyl ether" has not been obtained by methylation of the apoalpinone mentioned above, but both substances yield the same product, "apoalpinone dimethyl ether", m. p. 108—109°, on exhaustive methylation [4, 13]. This substance is claimed to be 3,5,7-trimethoxyflavanone, but no rigorous proof of the structure has been given.

The following scheme shows the different reactions carried out with pinobanksin (I):



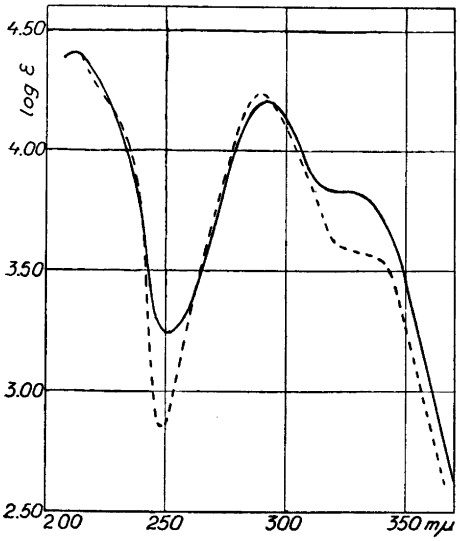


Fig. 1. Ultraviolet light absorption of pinobanksin (———) compared with pinocembrin according to Erdtman [2] (— — —).

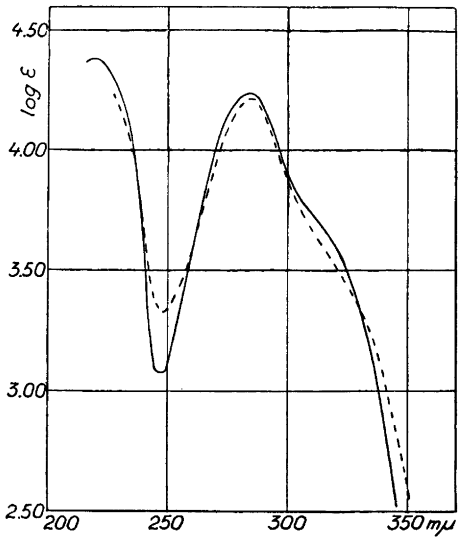


Fig. 2. Ultraviolet light absorption of pinobanksin dimethyl ether (———) compared with 5,7-dimethoxyflavanone according to Skarżyński [16] (— — —).

The ultraviolet absorption curve of pinobanksin (Fig. 1) shows a close resemblance to that of pinocembrin [2]. Pinobanksin dimethyl ether gives an absorption curve which resembles that of 5,7-dimethoxyflavanone [16] (Fig. 2). The absorption maximum lies at 292 mμ for pinobanksin and at 284 mμ for pinobanksin dimethyl ether. The wavelengths for the minima are 251 and 247 mμ respectively. The typical "shelves" for pinobanksin and pinocembrin at 320—330 mμ have been flattened out after methylation.

EXPERIMENTAL

The isolation of pinobanksin from pine heartwood extracts has already been described several times [1, 10]. After repeated recrystallisations from toluene, the pinobanksin forms thick colourless needles. When dried at 110°, they lost 15 % in weight and melted at 177—178° $[\alpha]_D^{20}$ + 14.4° ± 0.3° (methanol, c = 3.0). This rotation was determined in a 1 dm tube, whereas all previous determinations were made in a 0.5 dm micro tube. Colour reactions: Hot nitric acid, brownish-violet; ferric chloride, reddish-violet; diazotised benzidine, cherry red; magnesium-hydrochloric acid as well as zinc-hydrochloric acid, orange red.

$C_{15}H_{12}O_5$ (272.3)	Calc.	C	66.2	H	4.44
	Found	»	66.0	»	4.49

Benzoylation with benzoyl chloride and pyridine yielded the tribenzoate, colourless needles from ethanol, m. p. 172—173°.

$C_{15}H_9O_2$ $(OOCC_6H_5)_3$ (584.6)	Calc.	C_6H_5CO	53.9
	Found	»	52.4

Attempts to racemise pinobanksin

A. In dilute alkali: Pinobanksin (0.4 g, $[\alpha]_D^{20}$ + 14°) was dissolved in 1 % sodium hydroxide (50 ml), and the solution allowed to stand for 43 hours in a hydrogen atmosphere at room temperature. The solution was then acidified with acetic acid, and the pale yellow precipitate separated by filtration, washed with water and dried. M. p. 170—173°, yield 0.3 g. On recrystallisation from toluene, thick colourless needles were obtained. After drying at 110°, they melted at 174—176°. $[\alpha]_D^{20}$ + 11° ± 1° (methanol, c = 2.6).

B. In alcoholic hydrochloric acid: Pinobanksin (0.7 g, from *P. Banksiana*, $[\alpha]_D^{20}$ + 12°) was dissolved in hot ethanol (20 ml). Concentrated hydrochloric acid (10 ml) was added, and the solution boiled for one and a half hours under reflux. It was then diluted with water (100 ml), yielding a colourless crystalline precipitate which was filtered off, washed with water and dried. M. p. 171—174°, yield 0.6 g. After recrystallisation from toluene and drying at 120°, the m. p. was 173—175°. $[\alpha]_D^{20}$ + 12° ± 1° (methanol, c = 6.3).

C. In strong alkali: Pinobanksin (0.1 g, $[\alpha]_D^{20}$ + 12°) was dissolved in 15 % sodium hydroxide (10 ml) and allowed to stand for seven days in a hydrogen atmosphere at ordinary temperature. The yellow solution was then acidified, yielding a colourless precipitate, melting gradually between 150° and 170°. After recrystallisation from toluene and from 50 % acetic acid, the product melted between 180° and 200°, but no pure substance could be isolated. $[\alpha]_D^{20}$ + 5.5° (methanol, c = 2.5). Decomposition or rearrangement of the molecule must have taken place, since the reaction product melted higher than pinobanksin.

Pinobanksin monomethyl ether

Pinobanksin (3.0 g) was dissolved in ether (350 ml) and methylated by addition of a diazomethane solution (about 0.6 g diazomethane in 25 ml of ether). The solution, when left overnight at room temperature, deposited large colourless needles, which were collected. On evaporation of the ether, a second crop of crystals was obtained. Both crystalline fractions (2.3 g) were recrystallised twice from benzene, yielding pure pinobanksin monomethyl ether (1.8 g). Large colourless needles, m. p. 181—182°, $[\alpha]_D^{20}$ — 19° ± 1° (chloroform, c = 2.7). The substance dissolves in hot 1 *N* sodium hydroxide. The alcoholic solution gives a reddish-violet colour with ferric chloride (weaker than for pinobanksin itself) and an orange red colour with diazotised benzidine. Reduction with magnesium-hydrochloric acid gives an orange colour.

$C_{16}H_{14}O_5$ (286.3)	Calc.	C	67.1	H	4.93	OCH_3	10.8
	Found	»	67.0	»	4.93	»	10.9

Pinobanksin dimethyl ether

Pinobanksin monomethyl ether (1.0 g) was boiled with dry acetone (50 ml), dimethyl sulphate (0.37 ml) and freshly ignited potassium carbonate (5 g) on a steam bath, under reflux, for three and a half hours. The potassium carbonate was removed by filtration and washed with hot acetone. The acetone was then evaporated and the residue dissolved in ether. The ether solution (100 ml) was washed with 2 *N* sodium hydroxide (2 × 50 ml) to remove the monomethyl ether which had not reacted. It was then dried over anhydrous sodium sulphate and evaporated to dryness, leaving a yellow crystalline residue. This residue was recrystallised from chloroform-light petroleum and then from dilute methanol, yielding colourless needles (0.5 g), m. p. 133—134°. $[\alpha]_D^{20}$ — 31° (chloroform, c = 3.0). The crystals are insoluble in 2 *N* sodium hydroxide and give no colour with ferric chloride in alcoholic solution or with diazotised benzidine. Reduction with magnesium-hydrochloric acid gives an orange colour.

$C_{17}H_{16}O_5$ (300.3)	Calc.	C	68.0	H	5.37	OCH_3	20.7
	Found	»	68.3	»	5.41	»	20.4

Methylation of pinobanksin with an excess of dimethyl sulphate

Pinobanksin (0.85 g) was dissolved in dry acetone (40 ml). To the solution were added dimethyl sulphate (1.0 ml = 3.5 moles) and freshly ignited potassium carbonate (5 g). The solution was boiled under reflux for 20 hours. The acetone was then evaporated on the steam bath, and the residue acidified with 2 *N* sulphuric acid and then taken up in ether (150 ml). The yellow ether solution was washed with 1 *N* sodium hydroxide (2 × 50 ml) to remove any monomethyl ether. It was then dried over anhydrous sodium sulphate and the solvent evaporated, leaving a yellow syrup, which slowly deposited crystals. After stirring with ether, the crystals could be separated by filtration. More crystals were obtained from the filtrate on standing. The crystals were collected and recrystallised twice from methanol. Yield, 0.05 g of a pale yellow crystalline powder, m. p. 197—198°. No m. p. depression was observed on admixture with galangin trimethyl ether *.

$C_{18}H_{18}O_5$ (314.3)	Calc.	C	68.8	H	5.77	OCH_3	29.6
$C_{18}H_{16}O_5$ (312.3)	Calc.	»	69.2	»	5.16	»	29.8
	Found	»	69.3	»	5.17	»	30.3

The formula $C_{18}H_{18}O_5$ corresponds to pinobanksin trimethyl ether and $C_{18}H_{16}O_5$ to galangin trimethyl ether.

Dehydrogenation of pinobanksin

Pinobanksin (0.5 g), cinnamic acid (1.2 g), palladium-charcoal catalyst prepared as described by Ott and Eichler [17] (0.25 g) and water (25 ml) were heated in a stainless-steel bomb rotating in an oil bath at 180° for one hour. After cooling, the reaction mixture

* A sample of this substance was kindly supplied by Prof. T. R. Seshadri, Delhi, India.

was extracted with ether (50 ml) and the cinnamic acid removed by shaking with saturated sodium bicarbonate solution (4 × 50 ml). The brownish ether solution was dried over anhydrous sodium sulphate and filtered through a column of aluminium oxide. The brown impurities were adsorbed, and the pale yellow filtrate yielded a bright yellow residue on concentration. It melted at 200—210°. After two recrystallisations from dilute methanol, the m. p. was raised to 215—216°. Yield, 0.15 g.

$C_{15}H_{10}O_5$ (270.2)	Calc.	C	66.7	H	3.73
	Found	»	66.8	»	3.80

As no sample of galangin was available, this flavonol was prepared by demethylation of its 5,7-dimethyl ether with pyridine hydrochloride at 180°, a method which has recently been successfully applied to methylated phenols [18]. The galangin thus obtained was purified by vacuum sublimation and recrystallisation from dilute methanol. It melted at 213—215° and gave no m. p. depression with the sample obtained from pinobanksin.

Dehydrogenation of pinobanksin dimethyl ether

Pinobanksin dimethyl ether was dehydrogenated in the same way as described for pinobanksin. A pale yellow product was obtained, forming fibrous crystals from methanol. M. p. 172—173°. The m. p. was not depressed on admixture with a synthetic sample of galangin 5,7-dimethyl ether, prepared according to Kostanecki, Lampe, and Tambor [19]. These authors found a m. p. of 177—178°, but the sample prepared by the present author melted at 173—174°.

$C_{17}H_{14}O_5$ (298.3)	Calc.	C	68.5	H	4.73	OCH_3	20.8
	Found	»	68.3	»	4.79	»	20.6

Rearrangement of pinobanksin dimethyl ether to "apoalpinone monomethyl ether"

Pinobanksin dimethyl ether (0.1 g) was dissolved in 10 ml of methanol, containing 1 g of potassium hydroxide. The solution was boiled under reflux for 10 minutes. The yellow solution was then cooled and acidified with 2 *N* sulphuric acid. The methanol was evaporated, and the resulting aqueous suspension extracted with ether. The ether solution, after drying and concentration, yielded a yellow crystalline residue, which turned almost colourless on washing with methanol and ether (m. p. 169—171°). After recrystallisation from dilute methanol and from chloroform-light petroleum, colourless needles, m. p. 170—171°, $[\alpha]_D^{20}$ zero, were obtained. Yield, 0.05 g.

The substance gives a yellow solution in cold 2 *N* sodium hydroxide but gives no colour reaction when reduced with magnesium-hydrochloric acid.

$C_{17}H_{16}O_5$ (300.3)	Calc.	C	68.0	H	5.37	OCH_3	20.7
	Found	»	67.5	»	5.33	»	20.3

The acetate was prepared by acetylation with acetic anhydride-pyridine. Colourless crystals from dilute methanol, m. p. 112—114°.

$C_{17}H_{15}O_4$ ($OOCCH_3$) (342.3)	Calc.	CH_3CO	12.6
	Found	»	12.8

Synthesis of "apoalpinone monomethyl ether"

2'-Hydroxy-4',6',α-trimethoxychalkone (VII) was first prepared by condensing 2-hydroxy-4,6,ω-trimethoxy-acetophenone (prepared according to Row and Seshadri [14]) with benzaldehyde as described by Kimura [13]. 1.5 g of the ketone yielded 1.4 g of the chalkone, m. p. 111—112° (Kimura 112°).

The chalkone (0.85 g) was boiled with ethanol (100 ml) and 2 *N* sulphuric acid (20 ml) for 24 hours under reflux. The ethanol was then evaporated on a steam bath. On cooling, the remaining aqueous solution deposited a colourless, sticky solid, which was extracted with ether. The ether solution was shaken with 1 *N* sodium hydroxide solution (2 × 20 ml), and the alkaline extract acidified. A colourless precipitate appeared, which was taken up in chloroform. The chloroform solution was dried and concentrated, leaving an almost colourless crystalline residue. After two recrystallisations from chloroform-light petroleum and one from dilute methanol, the m. p. was constant at 169—170°. Yield, 0.3 g. Mixed m. p. with the product obtained by alkali treatment of pinobanksin dimethyl ether 170—172°.

$C_{17}H_{16}O_5$ (300.3)	Calc.	C	68.0	H	5.37	OCH_3	20.7
	Found	»	67.3	»	5.46	»	20.3

The acetate melted at 112—113° and gave no m. p. depression when mixed with the acetate of rearranged pinobanksin dimethyl ether.

An attempt to dehydrogenate "apoalpinone monomethyl ether" with palladium-cinnamic acid yielded a yellowish brown sticky product along with unchanged starting material.

Ultraviolet light absorption

The ultraviolet light absorption of pinobanksin and its dimethyl ether was measured in absolute ethanol with a Beckman Spectrophotometer, Model DU.

SUMMARY

Pinobanksin, a common pine heartwood constituent, is 3,5,7-trihydroxyflavanone (dihydrogalangin). The tribenzoate, the 7-monomethyl ether and the 5,7-dimethyl ether of pinobanksin have been prepared. The structure has been proved by catalytic dehydrogenation of pinobanksin and its dimethyl ether to the corresponding galangin derivatives. An attempt to prepare pinobanksin trimethyl ether led to galangin trimethyl ether, due to dehydrogenation.

Pinobanksin dimethyl ether has been rearranged by alkali to an isomeric compound earlier described as 3-hydroxy-5,7-dimethoxyflavanone ("apoalpinone monomethyl ether"). This substance, however, does not give the colour reactions characteristic to flavanones and cannot be dehydrogenated to the corresponding galangin dimethyl ether.

The ultraviolet light absorption of pinobanksin and its dimethyl ether has been studied.

This investigation has been financially supported by *Fonden för Skoglig Forskning*. The author is indebted to Mr. A. Misiorny for skilful experimental assistance and to Fil.lic. G. Aulin-Erdtman for determining the ultraviolet absorption of pinobanksin. The microanalytical work has been performed by Mr. W. Kirsten and Miss A. Renman, Institute of Medical Chemistry, University of Uppsala.

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Received May 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 782–788

Die Kinetik im Gitter des Silbersulfates

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Das Silbersulfat, Ag_2SO_4, ist als Teilnehmer einiger der vor etwa 25 Jahren bearbeiteten »Platzwechselreaktionen»[1] bekannt.

Es erschien uns lohnend, in diesem Zusammenhang Klarheit über die Beweglichkeitsverhältnisse im Gitter des Silbersulfates zu schaffen. Dazu erweist sich als am besten geeignet die Messung der Selbstdiffusion mit Hilfe radioaktiver Indikatoren sowie die Messung der Ionenleitfähigkeiten (spezifische Leitfähigkeit und Überführungszahl).

Im einzelnen wurde durch einen von uns (G. J.) die Selbstdiffusionskonstante mittels der Methoden der aktiven dünnen Schicht sowie der aktiven Teiltablette gemessen. Diese Messungen wurden ergänzt durch Bestimmung mittels der Kontaktmethode [2] sowie durch Versuche der Messung einer etwaigen Ionendiffusion des Sulfatrestes und Messung von Leitfähigkeit und Überführungszahl (R. L.).

DIE VERSUCHE

Bei der Bestimmung der Selbstdiffusionskonstante des Silbers mit Hilfe der Absorption radioaktiver Strahlung gelangte das Ag-Isotop 111 (7,5 Tage Halbwertzeit) zur Anwendung. Dieses entsteht durch einen (d, n)-Prozess * aus Palladium-110 und wird nach Auflösung des Palladiummetalls in Königswasser mit etwas Silber als Träger als Chlorid gefällt, zum Metall reduziert und schliesslich als Silbernitratlösung erhalten [3].

Daraus konnte, eventuell nach entsprechender Verdünnung mit inaktivem Silbernitrat, durch Fällen mit verdünnter Schwefelsäure ein Silbersulfat von geeigneter spezifischer Aktivität erhalten werden.

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* Die Bestrahlungen wurden mit dem Zyklotron des Nobelinstitutes für Physik in Stockholm-Frescati (Prof. M. Siegbahn) durchgeführt.

Sämtliche Versuche wurden an Tabletten von Ag_2SO_4 angestellt, die in der Handpresse hergestellt worden waren und deren Dichte (ca. 5,3) sich nur wenig von der theoretischen (5,45) unterschied.

Die Erwärmung auf die Diffusionstemperatur geschah in Vakuumöfen, bestehend aus einem Supremax-Schliffrohr mit aufgeschobenem Wärmekörper (Chromnickelband zwischen zwei Supremaxrohren) in definierter Gasatmosphäre, (entweder gereinigtem Stickstoff oder 10^{-2} mm Luftdruck).

Der Effekt der Heizwicklung wurde mit in Serie geschalteten Eisen-Wasserstofflampen stabilisiert, was eine ausreichende Temperaturkonstanz ($\pm$ 2° C) ergab.

Die Temperaturmessung geschah mit kalibrierten Platin/Platin-Rhodium-Thermoelementen, die bei den Methoden 1 und 2 unmittelbar oberhalb der Tabletten angebracht, bei der Methode 3 auf diesen aufgepresst waren.

Die radioaktiven Messungen wurden in gewohnter Weise mit einem Zählrohrverstärker nebst Untersetzer unter streng definierten Bedingungen durchgeführt. (Reproduzierbare Lage des Präparates, Apparaturkontrolle durch Standardmessungen.)

A. DIE BEWEGLICHKEIT DES SILBERS

1. Methode der aktiven Teiltablette

Hierbei wird auf eine lose gepresste inaktive Tablette ein bestimmter Bruchteil der Gesamtmenge, z. B. 1/4 oder 1/2, aktives Material fest aufgepresst. Die Diffusion wird verfolgt durch Messung der zunehmenden Aktivität auf der einen Seite und der abnehmenden Aktivität auf der anderen Seite, wonach die Diffusionskonstante graphisch ermittelt werden kann.

Diese Methode wurde zwischen 360° und 650° C angewendet, wobei Diffusionskonstanten zwischen $1{,}2 \cdot 10^{-9}$ und $1{,}5 \cdot 10^{-6}$ cm^2/sec erhalten wurden. Die einzelnen Werte sind in Fig. 1 eingetragen. (Einige derartige Versuche am Silbersulfat sind schon 1946 von Zimen und Hillert durchgeführt worden und zeigten ähnliche Werte.)

2. Methode der aktiven dünnen Schicht[2,4]

Hierbei wird eine sehr dünne Schicht des aktiven Materials, etwa durch Kondensation im Vakuum, gleichmässig auf der zu untersuchenden Tablette aufgebracht, und die im Laufe der Diffusion auf der aktiven Seite der Tablette auftretende Aktivitätsabnahme verfolgt.

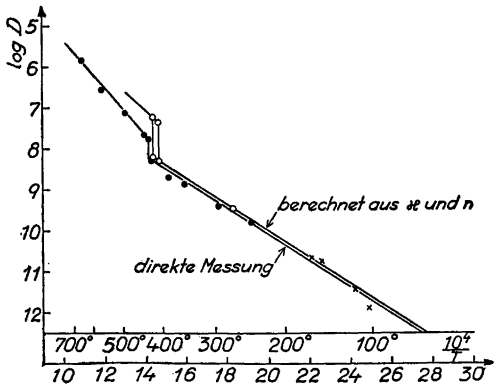

Fig. 1. Die Selbstdiffusionskonstante von Silber in Silbersulfat, logarithmisch dargestellt als Funktion der reziproken absoluten Temperatur.

Die Diffusionskonstante wird dann aus der graphischen Darstellung $A/A_0 = f(\mu\sqrt{Dt})$ entnommen [2]. Für den Absorptionskoeffizienten wurde der Wert 16 cm²/g für Ag-111 verwendet. Mit dieser Methode, die empfindlicher ist als die erstere, konnte zwischen 250° und 650° C gemessen werden, wobei sich Werte zwischen $2 \cdot 10^{-10}$ und $1{,}8 \cdot 10^{-6}$ cm²/sec ergaben. Die einzelnen Werte sind wiederum Fig. 1 zu entnehmen.

3. Methode des Überganges bei idealem Kontakt [2]

Diese Methode basiert auf dem Übergang radioaktiver Substanz von einer stark aktiven auf eine inaktive Tablette. Die beiden Tabletten sind in einem geeigneten Halter gegeneinander gepresst, wobei ihr Kontakt durch Messung der Leitfähigkeit kontrolliert werden kann [5].

In diesem Fall wurde Ag-110 als Indikator benutzt (250 Tage Halbwertzeit)*, dessen härtere Strahlung hier vorteilhaft ist, da im Gegensatz zu den beiden anderen Methoden hier möglichst keine Absorption vorliegen soll.

Die Streuung der Werte, in Fig. 1 durch Kreuze bezeichnet, ist allerdings in diesem Falle erheblich grösser als bei den anderen Methoden; dennoch scheint die Methode anwendbar zu sein, was uns wichtig war mit Rücksicht auf die weiter unten erwähnten Versuche zur Untersuchung einer etwaigen Beweglichkeit des Sulfatrestes.

Alle Diffusionswerte sind in Fig. 1 wiedergegeben in der üblichen Darstellung $\log D = f(1/T)$, die zwei Geraden verschiedener Neigung ergibt, getrennt durch eine Unstetigkeit bei etwa 430° C, welche auf die kristallographische

* Geliefert von der Isotope Division des Atomic Energy Research Establishment, Harwell (England).

Umwandlung des Silbersulfates zurückzuführen ist. Die Diffusionskonstante der Tieftemperaturmodifikation folgt der Gleichung:

$$D = 6{,}7 \cdot 10^{-5} \exp\ (-13400/RT)$$

während für die Hochtemperaturmodifikation gilt:

$$D = 2{,}5 \cdot \exp\ (-26500/RT)$$

B. DIE MESSUNG DER ELEKTRISCHEN LEITFÄHIGKEIT UND DER ÜBERFÜHRUNGSZAHL

Zur vollständigen Charakterisierung der Beweglichkeitsverhältnisse gehören auch Messungen der elektrischen Leitfähigkeit und der Überführungszahlen. Dies liefert einerseits einen weiteren Wert für die Diffusionskonstante [2], gibt andererseits aber auch Aufschluss über die elektrische Natur der wandernden Teilchen, also darüber, ob Diffusion von Ionen oder von Neutralteilchen oder aber direkter »Platztausch» benachbarter Gitterbausteine vorliegt.

Wir führten die Messung der Leitfähigkeit mit dem oben erwähnten Pastillhalter durch, wobei sowohl bei steigender als auch bei fallender Temperatur die Leitfähigkeit mit Hilfe einer Philips-Messbrücke (Philiskop) gemessen wurde. Auch bei der Leitfähigkeitsmessung macht sich ein Sprung der Werte am Umwandlungspunkte bemerkbar. Dabei ist zweckmässiger Weise mit steigender Temperatur zu arbeiten, da die Umwandlung leicht zu unterkühlen ist, bei rasch fallender Temperatur der Umwandlungspunkt also — bis zu 20 Grad — zu tief erhalten werden kann. (was die bisherige Literaturangabe 411° statt, wie hier gefunden, 430° erklären kann).

(Dieser Effekt ist mit der Methode der thermischen Differentialanalyse näher untersucht worden [6].)

Die Leitfähigkeit des Silbersulfat unterhalb des Umwandlungspunktes ist schon einmal von Jagitsch [7] gemessen worden, mit dessen Werten unsere Messungen befriedigend übereinstimmen.

Die Leitfähigkeitsmessungen wurden durch Bestimmung der Überführungszahl ergänzt, nachdem die annähernde Übereinstimmung [5] zwischen den direkt gemessenen und aus der spezifischen Leitfähigkeit berechneten [2] Diffusionskonstanten Grund zu der Annahme ergeben hatte, dass der Strom ausschliesslich durch Silberionen getragen wird.

Dabei war die Überführungszahl für Silber zu 1 angenommen worden, was nun hier gemessen werden sollte.

Auch zur Bestimmung der Überführungszahl wurde der mehrfach erwähnte Pastillhalter [5] benutzt und in Weiterentwicklung der »Kontaktmethode» die durch Einwirkung des elektrischen Feldes überführte Menge radioaktiv bestimmt *. Das Verhältnis dieser Menge zum Stromäquivalent war innerhalb der Fehlergrenzen (1—2 %) gleich 1. Es wurden Versuche bei 135° C und 145° C durchgeführt; die Anordnung der Tabletten war:

$$+ \text{Ag, AgJ, } Ag_2S, \text{ } Ag_2SO_4, \text{ } \overset{*}{Ag_2}SO_4, \text{ } Ag_2SO_4, \text{ } Ag_2S, \text{ AgJ, Pt} -$$

(Die Verwendung höherer Temperaturen verbietet sich wegen der zu festen Verschweissung der Tabletten, die sich dann nicht mehr voneinander trennen lassen.)

Aus den Leitfähigkeitswerten und der Überführungszahl sind die Selbstdiffusionskonstanten berechnet worden, die ebenfalls in Fig. 1 eingetragen sind.

C. UNTERSUCHUNG EINER ETWAIGEN BEWEGLICHKEIT DES SULFATIONS IM SILBERSULFAT

Bei Überführungsmessungen kann man mit einer Genauigkeit von ca 1 %, bei guten Ionenleitern auch von 0,1 % [8] rechnen; d. h. eine Überführungszahl kleiner als 10^{-3} entzieht sich den elektrischen Messungen. Eine kleinere relative Beweglichkeit einer Gitterkomponente lässt sich also nur durch Diffusionsmessungen feststellen.

Da nach den obigen Messungen keine oder nur eine sehr geringe Beweglichkeit des Sulfations zu erwarten war, wurde zunächst die empfindliche Kontaktmethode benutzt. Die Anordnung war völlig identisch mit der bei der Messung der Silberselbstdiffusion, nur dass diesmal die Aktivität der einen Tablette durch S-35 ** gegeben war.

Das aktive Silbersulfat wurde auf folgende Art hergestellt: [9]

Ca 2 g Schwefelpulver (aktiv) wurden in einem 200 ml-Erlenmeyerkolben mit 30 ml H_2O und ca. 15 ml Brom versetzt und das ganze umgeschüttelt. Nach etwa 15 min. Stehen (bis sich aller Schwefel im Brom gelöst hat) wurden 20 ml Äther hinzugefügt und umgeschüttelt, bis sich der Äther vollständig mit der Bromschicht vereinigt hatte. Eine leichte Erwärmung zeigt die beginnende Oxydation des Schwefels an, die in etwa 1/2 Stunde beendet ist.

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* Eine eingehende kritische Betrachtung des Verfahrens muss einer späteren Mitteilung vorbehalten werden.

** Geliefert von der Isotope Division des AERE, Harwell (England).

Äther und Brom wurden auf dem Wasserbade abdestilliert, und die Lösung darauf auf der Bunsenflamme auf halbes Volumen eingedampft. Die radioaktive Schwefelsäure wurde mit einem Überschuss von Silbernitrat gefällt, der (aus Silbersulfat und Silberbromid bestehende) Niederschlag durch Auskochen ausgelaugt und das herausgelöste Silbersulfat durch mehrfaches Umkristallisieren gereinigt.

Ein mit drei Tablettpaaren bei 180° C durchgeführter Versuch zeigte praktisch keinen Übergang radioaktiven Schwefels, woraus geschlossen werden kann, dass die Beweglichkeit des Sulfates im Silbersulfat (β-Modifikation) offenbar gleich Null, unter der Berücksichtigung der Fehlergrenzen aber jedenfalls kleiner als 10^{-4} von der des Silbers ist.

ZUSAMMENFASSUNG

1. Die Diffusionskonstante für Silber im Silbersulfat folgt in der Tieftemperaturmodifikation der Beziehung:

$$D = 6{,}7 \cdot 10^{-5} \cdot \exp\ (-13400/RT)$$

in der Hochtemperaturmodifikation der Beziehung:

$$D = 2{,}5\ \exp\ (-26500/RT)$$

2. Beim Übergang in die Hochtemperaturmodifikation springt die Diffusionskonstante auf etwa den 3,5 fachen Wert.

3. Messungen von Leitfähigkeit und Überführungszahl und deren Vergleich mit den Diffusionsmessungen ergeben, dass im Silbersulfat Silberionen die Leitung des Stromes übernehmen sowie für den Materietransport bei der Diffusion verantwortlich sind. Ein direkter Austausch zwischen benachbarten Gitterplätzen findet nicht in messbarem Masstabe statt.

4. Es liess sich keine messbare Beweglichkeit des Sulfations feststellen. Die kinetischen Vorgänge im Gitter des Silbersulfates werden demnach ausschliesslich durch Silberionen getragen.

(Aus experimentellen Gründen können die Aussagen unter 3 und 4 bisher nur für die Tieftemperaturmodifikation des Silbersulfates gemacht werden.)

Bei der Ausführung der Versuche war uns Ing. Percy Löwenhard behilflich. Wir danken Prof. J. A. Hedvall für sein Interesse an dieser Arbeit, Prof. M. Siegbahn und fil.lic. A. Hedgran für Bestrahlungen, und *Statens Tekniska Forskningsråd* für die Bereitstellung von Mitteln.

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Eingegangen am 8. Mai 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 789–792

The Thermosensitivity of Enterogastrone

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Very few facts are known about the enterogastrone (Eg) molecule. Öbrink [1] showed that the secretory depressant had no or a very slight electrophoretic mobility within wide pH-ranges. Katz, Dryer, Paul and Routh [2] obtained a similar result. This finding, together with the facts that the Eg activity is not destroyed by pepsin or trypsin [3] and easily dialyses through cellophane [4] makes it improbable that Eg is a protein or a protein derivative. Some of our active preparations lack any detectable amount of amino acids, whereas they all contain carbohydrate.

The present investigation of the thermosensitivity of Eg was undertaken in connection with work on the structure of the Eg molecule.

METHODS

Two enterogastrone preparations were used. They were both made from hog's intestine, but by two different methods which yielded preparations of different purity. The starting-material was in both cases the NaCl-precipitate or the "A-precipitate" [5]. One preparation (127 B) was in many respects similar to that of Gray, Bradley and Ivy [6]. The other one (111 B) was prepared according to a method which will be published in detail in this journal. Common to both the preparations was a dialysis in which the active material was recovered in the dialysate.

The activities of both the preparations were almost the same (one Eg unit in about 3 mg, cf. Öbrink [7]), but 127 B contained a large amount of amino-acids and peptides, whereas 111 B did not (ninhydrin and biuret reactions negative). The nitrogen content of 111 B was 0.24 % and of 127 B 14.8 %. NaCl formed more than 90 % of the 111 B.

The assay procedure was that worked out by Öbrink [7]. Heidenhain pouch dogs were used, and the secretion was induced by a continuous intravenous injection of histamine. When the secretion rate had become constant, the Eg was also injected intravenously at a slow constant rate during the rest of the experiment. After a latent period of about 60 minutes, the secretory rate decreased and a new steady level was reached. *The difference between the steady levels before and during the Eg administration was taken as a*

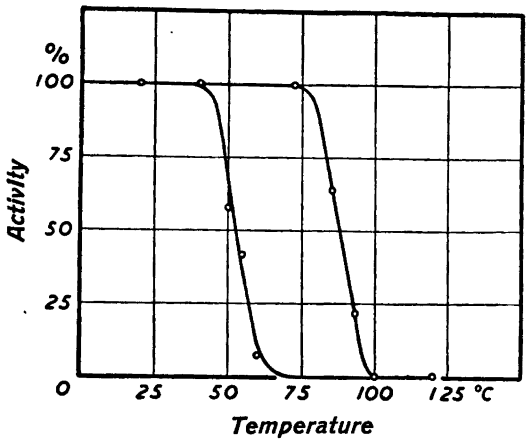

Fig. 1. The activity of two enterogastrone preparations after heating. Preparation 111 B to the left; 127 B to the right. The activity of unheated materials is defined as 100 %.

measure of the Eg activity. A curvilinear relationship was found to exist between the injection rate of Eg and its inhibitory effect. This inhibitory effect was expressed in per cent of the initial control secretion rate, which had to be kept within certain limits.

The Eg preparation was dissolved in 0.9 % NaCl to a concentration of 0.24 % (wt/v), and the injection rate was 6 mg Eg per hour in a 15 kg dog. When exposed to a high temperature, the solution was stored in a sealed vial of Pyrex glass. In most experiments the gas volume in the vial was air, but in some cases it was nitrogen.

RESULTS

It was found that enterogastrone cannot be kept for many days as an unbuffered and unheated water solution. Even after one day a decrease of activity was noticed, and after a month the preparation was completely inactive whether stored at room temperature or in an icebox (+ 4° C). Greengard, Atkinson, Grossman and Ivy [8] have kept a sterile solution potent for 10 days. In the present investigation no controls were made of sterility but all the solutions were freshly made. As a dry powder, however, the preparation has now been kept for some years, apparently without losing any activity.

The result of the investigations on the thermostability of the two preparations is seen in Fig. 1. The inhibitory effect of the Eg was determined with and without heating. The activity of the unheated preparations were defined as 100 %. The effect of the heated preparations was compared with the unheated ones and expressed in per cent of the original activity. *Cf.* Table 1.

It is obvious that the activity can be completely destroyed by heat and at some temperatures it was found that this happened whether oxygen was available or not (Table 1). It was also apparent that the two Eg preparations are characterized by different thermostabilities.

Table 1. Effect of heating of enterogastrone solutions.

Temp. °C	Time of heating, minutes	Inhibition of the secretion rate %	Activity of the heated solution %	Atmosphere in the vial
		Eg preparation 111 B.		
—	—	50.0 (average value)	100	air
41	60	50.0	100	»
50	60	29.0	58	»
55	60	21.0	42	»
60	60	4.0	8	»
100	10	0	0	»
100	10	0	0	N_2
		Eg preparation 127 B.		
—	—	60.8 (average value)	100	air
72	140	>60.8	100	»
84	120	39.0	64	»
93	120	13.2	22	»
100	120	0	0	»
100	60	0	0	»
120	120	0	0	»
120	120	0	0	N_2

DISCUSSION

In the present investigation it was found that two different enterogastrone preparations were unstable when stored as water solutions. When exposed to heat both the Eg preparations could be destroyed. This confirmed the earlier observations of Kosaka and Lim [9] that heating at 80—100° C for 10 minutes inactivated extracts derived from oil-treated intestinal mucosa.

The preparation 127 B showed a higher stability than 111 B, which was the purer with respect to organic constituents. The fact that the two preparations showed different stabilities to heat may be attributed to the higher content of organic impurities in the more stable 127 B. It may be thought that in this case the organic impurities have a protective effect on the thermolabile enterogastrone molecule.

It is clear that the Eg molecule is characterized by some very thermosensitive structure. The biological activity is completely lost if this labile structure of the molecule is destroyed by heat.

SUMMARY

Two enterogastrone preparations with different nitrogen contents (14.8 % and 0.24 %), but with equal biological activity, were shown to be very labile to heat. The preparation which had the lower content of organic material (N = 0.24 %) was the more thermosensitive one and did not withstand temperatures above 40° C.

The enterogastrone molecule is characterized by some not yet identified thermolabile structure.

Technical assistance was given by Miss M. Uhrström.

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Received April 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 793–797

On the Crystal Structure of Molybdenum Trioxide

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In 1931 two papers on the crystal structure of molybdenum trioxide appeared in the *Zeitschrift für Kristallographie* reporting the work on this subject carried out independently by Braekken [1] and N. Wooster [2]. The results obtained by these authors agree fairly well and there has been no reason to question the principal features of the structure as given by them. The structure has also been confirmed by the present investigation. Molybdenum trioxide thus crystallizes with four formula units of MoO_3 in the orthorhombic elementary cell and with the symmetry *Pbnm*. The molybdenum atoms coordinate six oxygen atoms to form MoO_6 octahedra, which are joined by sharing edges to form zigzag shaped rows. The rows are mutually connected by corners to layers, which are placed on top of each other without having atoms in common. Three of the six oxygen atoms surrounding every molybdenum atom are thus common to three MoO_6 octahedra, two of them belonging to two octahedra and the sixth one being unshared.

In connection with crystal structure investigations of a number of molybdenum oxides carried out at this Institute in recent years, it has been thought desirable to determine the interatomic distances for molybdenum trioxide with a higher degree of accuracy than was obtained in those early investigations in which the oxygen positions were settled from considerations of space only.

X-RAY DATA

Accurate unit cell dimensions of molybdenum trioxide have been calculated by Hägg and one of the present authors [3] from X-ray powder photographs obtained with focusing cameras and Cr-*K* radiation from a preparation (Kahlbaum *puriss.*) that had been heated for 15 hours at 200° C. The figures obtained in this way are in excellent agreement with those given by Braekken. *Cf.* Table 1.

Table 1. Unit cell dimensions *.

Authors	a Å	b Å	c Å	V Å^3
Braekken [1]	3.962	13.855	3.701	203.1
Wooster [2]	3.93	13.97	3.67	201
Hägg and Magnéli [3]	3.966	13.85	3.696	203.0

* The figures for the lengths of the axes given in the various papers are here multiplied by 1.002 in order to bring the dimensions on the true Å unit basis [4].

A needle-shaped crystal out of a sample obtained by sublimating the Kahlbaum preparation mentioned above was used for the single-crystal investigation. Mo-K radiation was used in order to get a large number of reflections and to minimize the influence of absorption. Due to the crystals being extremely thin it was only possible to obtain photographs with rotation around the needle axis. In this way Weissenberg photographs were registered for the layer lines 0—4. Multiple film technique was applied [5]. In order to bring about appropriate decrease of the blackening of the spots within subsequent photographs of the pack thin iron foils (about 20 μ) were inserted between the sheets of film.

Relative intensities of the reflections were estimated visually by comparison with an intensity scale obtained by exposing an interval of the zero layer line of the crystal with different exposure times on one pack of films. Correlation between the various layer lines was procured by means of a "Weissenberg oscillation photograph" as has been described elsewhere [6].

The structure amplitudes were computed by multiplying the intensity values by $\cos^2 \mu \cdot \sin \Upsilon/(1 + \cos^2 2\Theta)$ utilizing the curves given by Lu [7]. The temperature factor was neglected.

DETERMINATION OF THE ATOMIC PARAMETERS

According to the unanimous statements of Braekken and Wooster all the atoms of molybdenum trioxide are situated in the symmetry planes of the centrosymmetrical space-group *Pbnm* occupying fourfold point positions 4(c)*:

$$x\ y\ \tfrac{1}{4};\tfrac{1}{2}-x,\ \tfrac{1}{2}+y,\tfrac{1}{4}\ ;\ \tfrac{1}{2}+x,\ \tfrac{1}{2}-y,\ \tfrac{3}{4};\ \bar{x}\ \bar{y}\ \tfrac{3}{4}.$$

* Notations analogous to those of space-group D_{2h}^{16}-*Pnma* in *International tables for the determination of crystal structures*, Berlin (1935).

All the atomic parameters may thus be determined by investigating the section ϱ $(x\ y\ \frac{1}{4})$ of the three-dimensional electron density function.

On the basis of the parameter values given by Wooster it was possible to derive the signs of the structure factors of most reflections $h\ k\ l$. The section mentioned above could thus be preliminarily calculated *. Within the considered area ($0 \leq x \leq 60/60$, $0 \leq y \leq 60/120$) the resulting map showed one heavy maximum and three minor ones in approximate agreement with the positions assumed for the molybdenum and oxygen atoms. The atomic coordinates obtained in this way permitted a calculation of the signs of almost all of the registered structure factors. The section ϱ $(x\ y\ \frac{1}{4})$ derived on the basis of the entire number of observed reflections gave the parameter values listed in Table 2. The structure factor values corresponding to these atomic positions are in fair agreement with the observed ones.

Table 2. Atomic parameters.

	Parameter values according to					
	Braekken [1]		Wooster [2]		the present investigation *	
	x	y	x	y	x	y
4Mo in 4(c)	0.088	0.101	0.086	0.0985	0.0847	0.0998
$4O_1$ in 4(c)	0.588	0.43	0.586	0.4305	0.525	0.435
$4O_2$ in 4(c)	0.588	0.101	0.586	0.0985	0.56	0.100
$4O_3$ in 4(c)	0.088	0.250	0.086	0.250	0.015	0.230

* The molybdenum coordinates are very exact. The error in x is probably about $\pm$ 0.001 and the accuracy in y is even greater. The oxygen positions may be correct within about one per cent.

DISCUSSION

As is clear from Table 2 the molybdenum parameters obtained in this investigation are in close accordance with those derived by the previous authors. Concerning the oxygen atoms, however, there are certain considerable changes of the coordinates, *viz.* for the oxygen atoms denoted O_1 and O_3. Interatomic distances calculated on the basis of the new parameter values are listed in Table 3 (*cf.* Fig. 1). They are throughout of plausible lengths. The

* The Fourier summations were facilitated by using the Hägg-La urent calculating machine [8] and the Robertson strips [9].

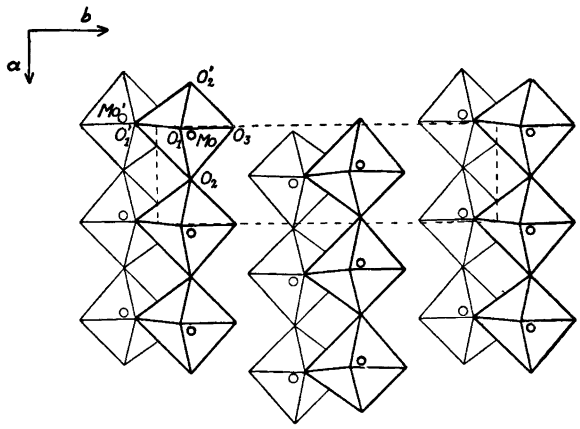

Fig. 1. Molybdenum trioxide viewed along [001]. Molybdenum atoms (small rings) at $z = \frac{3}{4}$ *and the surrounding octahedra are indicated by heavy lines, those at* $z = \frac{1}{4}$ *by thin lines. The extension of one unit cell is indicated by dashed lines. N. B. The oxygen atom* O_1 *is not identical with the one similarly denominated in Table 2, but related to the latter through a glide plane n normal to [010].*

Table 3. Interatomic distances (cf. Fig. 1).

Mo—Mo′	3.43 Å	$O_1 - O_1'$	2.6 Å
Mo—Mo (‖ [100])	3.97	$O_1 - O_2$	2.85
Mo—Mo (‖ [001])	3.70	$O_1 - O_3$	3.05
		$O_1 - O_2'$	2.65
$Mo - O_1$	2.05	$O_1' - O_2$	3.25
$Mo - O_1'$	2.35	$O_2 - O_3$	2.8
$Mo - O_2$	1.85	$O_3 - O_2'$	2.6
$Mo - O_2'$	2.1	$O_2' - O_1'$	2.85
$Mo - O_3$	1.8		

displacement of O_1 involves a decrease to 2.6 Å of the distance O_1—O_1' as compared with the value of about 2.75 Å calculated from the data of Braekken and Wooster. It seems quite reasonable that this edge, being shared by two MoO_6 octahedra, is relatively short. The shift in the position of O_3 makes the distance Mo—O_3 the shortest one within the octahedron. This also seems natural as the O_3 atom contrary to the other oxygen atoms is in contact with one metal atom only.

In a recent paper from this Institute[10], interatomic distances have been listed for molybdenum oxides and related compounds previously investigated by one of the present authors. For molybdenum trioxide the distance of 3.70 Å between the metal atoms of octahedra joined by sharing corners and belonging to the same row is in agreement with the normal value observed for a great number of molybdenum and tungsten compounds. The distance between molybdenum atoms of connected rows is considerably longer (3.97 Å) and approaches the figure observed for the distances between the puckered sheets of metal atoms in Mo_8O_{23} and Mo_9O_{26}. The distance for octahedra joined by edges (3.43 Å) exceeds the values observed for other molybdenum oxides. This is probably due to the repulsion between adjacent molybdenum atoms not being counter-balanced by further metal atoms in the trioxide.

The considerable variations of the molybdenum-oxygen and oxygen-oxygen distances of molybdenum trioxide evidently also depend on the layer character of the lattice.

SUMMARY

An investigation of the crystal structure of molybdenum trioxide by means of Fourier methods has confirmed the principal features of the structure reported by Braekken and N. Wooster, and given accurate atomic positions. The interatomic distances are compared with those previously obtained for other molybdenum oxides and related compounds.

The authors wish to thank Professor G. Hägg for his kind interest in this investigation and the Swedish Natural Science Research Council for financial support.

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Received May 17, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 798–805

Determination of Chloro-2-methylphenoxyacetic Acid by Infrared Spectrophotometry

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In 1941, Slade, Templeman and Sexton[1] found that 4-chloro-2-methylphenoxyacetic acid was very useful as a weedkiller. This product has found very extensive use especially in England, where it has been marketed by ICI under the trade names "Methoxone" and "Agroxone". It has also been widely used in many other countries including Sweden. The product is now made by many manufacturers and is generally called 4K2M.

The technical 4K2M is not a pure product and is usually sold dissolved in water as the sodium salt. It contains, in consequence of the preparation method [see *e. g.* Swedish patent no. 126 571[2]], in addition to 4-chloro-2-methylphenoxyacetic acid, varying amounts of the isomeric 6-chloro-2-methylphenoxyacetic acid, unchlorinated 2-methylphenoxyacetic acid and the higher chlorinated 4,6-dichloro-2-methylphenoxyacetic acid.

No method for the analysis of this mixture has been found in the literature. The task is not an easy one. It would be very difficult to find a chemical method for the analysis of the two isomers, 4-chloro-2-methylphenoxyacetic acid and 6-chloro-2-methylphenoxyacetic acid, because they can be anticipated to show very much the same chemical behavior. The physical properties, however, ought to be more suitable for the determination, but the presence of the two other components, the unchlorinated and the dichlorinated acid, makes the analysis difficult. It is not possible to employ isolated measurements of such physical properties as melting point or index of refraction for the analysis of the mixture. A method must be found by which data for each one of the components in the mixture can be determined.

Under these circumstances it seems reasonable to investigate the possibility of analyzing the mixture by spectrophotometric measurements in infrared

The conditions necessary for these measurements are that characteristic and not to closely located absorption bands can be found for the different substances. No investigations on the light absorption in infrared of chloro-2-methylphenoxyacetic acids seem to have been published.

The purpose of this work was to prepare the four compounds 2-methylphenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid, 6-chloro-2-methylphenoxyacetic acid and 4,6-dichloro-2-methylphenoxyacetic acid and to investigate their light absorption in infrared light. If possible a method for the determination of the four compounds would be worked out by spectrophotometrical means.

MATERIALS

2-Methylphenol: The crystalline *o*-cresol had a minimum melting point of 30.3° C.

4-Chloro-2-methylphenol: *o*-Cresol was chlorinated with sulphurylchloride as described by Sah and Anderson [4]. The reaction mixture was washed with sodium carbonate solution and water and dried with calcium chloride. During the following fractional distillation in vacuum, it was necessary to admit nitrogen through the capillary. Air caused a certain decomposition. Melting point 48.6—49.2° C.

6-Chloro-2-methylphenol: *o*-Cresol was chlorinated with chlorine at about 75° C and fractionated in vacuum. The first fraction containing 6-chloro-*o*-cresol and a small amount of unchanged *o*-cresol with almost the same boiling points was transformed into the corresponding acetates by Nord [5]. These acetates could be separated by distillation on account of a great difference in boiling point. The pure 6-chloro-2-methylphenyl acetate was hydrolysed to 6-chloro-2-methylphenol, which was further purified by recrystallization. Melting point 1.0—1.4° C.

4,6-Dichloro-2-methylphenol: This compound has almost the same boiling point as 4-chloro-2-methylphenol and therefore it cannot be prepared in pure form by chlorination of *o*-cresol. Pure 6-chloro-2-methylphenol was underchlorinated and 4,6-dichloro-2-methylphenol was obtained by distillation of the reaction mixture. It was purified by crystallization from petroleum ether. Melting point 52.5—52.7° C.

The corresponding phenoxyacetic acids were prepared by reaction of the phenols in alkaline solution with monochloroacetic acid in 30 % excess. The monochloroacetic acid was purified by recrystallization from glacial acetic acid, and was found free from dichloroacetic acid [6]. The reaction mixture was about 2*N* with respect to the phenols and the reaction temperature was kept at about 100° C. After complete reaction, the mixture was acidified to pH 7.0 and nonreacted phenol was extracted with ether. The solution was then acidified to complete precipitation of the acid which was extracted with ether and purified by recrystallization from benzene or chlorobenzene. Analysis data and melting points of the acids are shown in Table 1. The melting points were determined in accordance to Hershberg [7].

APPARATUS

A Beckman IR 2 Infrared Spectrophotometer with rock salt prism was used for the measurements in infrared. The recorder had been connected to an electrical compensation apparatus made in accordance to specifications by Foreman and Jackson [8]. The

Table 1. Analyses and melting points.

Compound	Elementary analyses						Melting point °C
	C %		H %		Cl %		
	calc.	found	calc.	found	calc.	found	
2-Methylphenoxy-acetic acid	65.05	64.98	6.07	6.13	—	—	154.8—154.9
4-Chloro-2-methyl-phenoxyacetic acid	53.88	53.66	4.52	4.53	17.68	17.69	120.0—120.2
6-Chloro-2-methyl-phenoxyacetic acid	53.88	53.78	4.52	4.46	17.68	17.69	109.5—109.8
4,6-Dichloro-2-methyl-phenoxy-acetic acid	45.98	45.87	3.43	3.36	30.17	30.30	187.9—188.2

determinations were made as follows. First the spectrum for solvent and cell was recorded. The paper was now reset to the starting point. The same cell was filled with the solution and the spectrum recorded. In doing so the spectrum of the solvent was followed by a horizontally movable pointer connected to the compensation apparatus. The recorded curve was the spectrum of the dissolved compound.

ABSORPTION CURVES

The selection of a solvent for infrared absorption measurements raised difficulties. The acids are almost insoluble in hexane and pentane. Carbon tetrachloride and carbon disulphide were not very suitable (Table 2).

Table 2. Solubility of 2-methylphenoxyacetic acid.

Compound	Solubility of 2-methylphenoxyacetic acid g/liter solvent
Carbon tetrachloride	0.50
Carbon disulphide	0.22
Chloroform	12.80
2-Nitropropane	10.04

From Table 2, it is evident that chloroform and nitropropane are better solvents. Such solvents as acetone, cyclohexanone and methylal are even better.

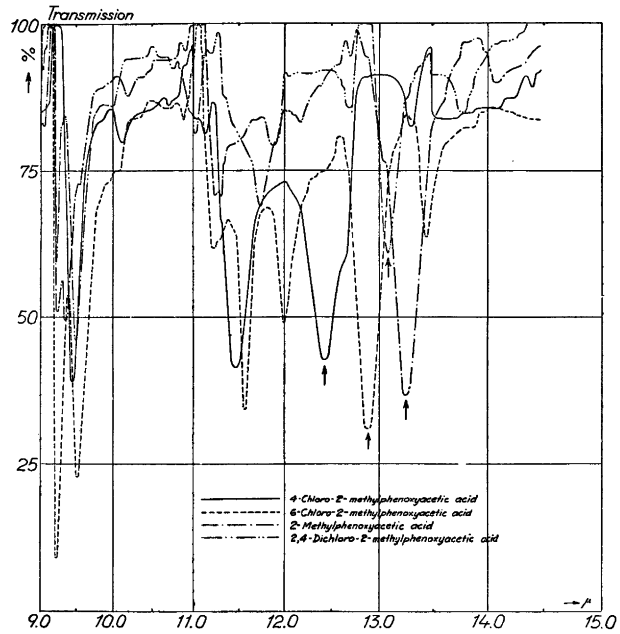

Fig. 1. Absorption curves of the acids.

Between 5—9 μ no measurements could be made because the solvents used had too high an absorption compared to the dissolved acids. Chloroform had the lowest absorption within this region, but its dissolving power for 2-methylphenoxyacetic acid and 4,6-dichlorophenoxyacetic acid was too low to make analysis possible.

Between 9—15 μ the absorption curves were recorded with acetone as a solvent. A 0.1 mm cell was used and the concentration for 4-chloro- and 6-chloro-2-methylphenoxyacetic acids was 90 g/liter. Owing to the lower solubility of 2-methylphenoxyacetic acid and 4,6-dichloro-2-methylphenoxyacetic acid, it was only possible to use a concentration of 45 g/liter. The obtained absorption curves are shown in Fig. 1.

Within this region there are no completely undisturbed absorption maxima. It is, however, possible to select maxima suitable for an analysis of the four mixed acids. These maxima are indicated by arrows in Fig. 1 and their wave lengths are as follows:

Table 3. Absorption maxima.

Compound	Wave lengths
4-Chloro-2-methylphenoxyacetic acid	12.38
6-Chloro-2-methylphenoxyacetic acid	12.86
4,6-Dichloro-2-methylphenoxyacetic acid	13.05
2-Methylphenoxyacetic acid	13.22

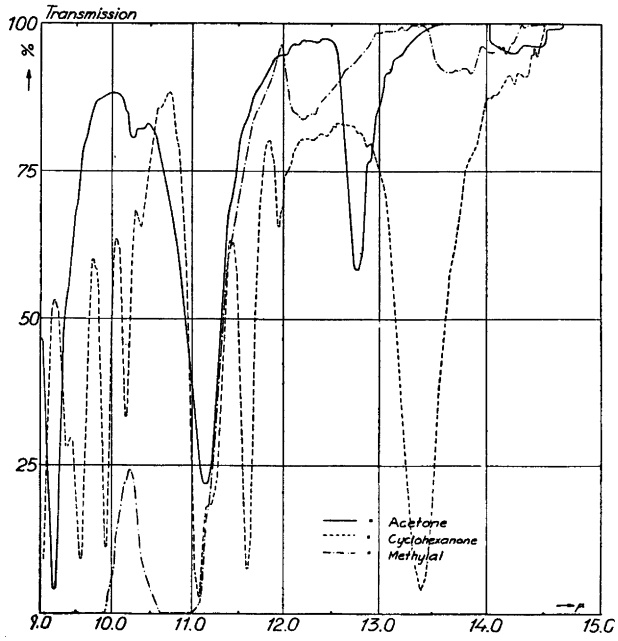

Fig. 2. Absorption curves of the solvents.

The absorption curves between 9—15 μ for acetone, cyclohexanone and methylal were recorded (Fig. 2).

Cyclohexanone had too high an absorption within the region 12.38—13.22 μ to be useful as a solvent. Methylal had a slight absorption but it was less suitable for quantitative measurements because of the low boiling point 42—43° C. Therefore we used acetone.

Acetone shows an absorption maximum at 12.75 μ which interferes with the determination of 6-chloro-2-methylphenoxyacetic acid but its extinction at the other wavelengths is comparatively low.

ANALYTICAL PROCEDURE

A number of solutions of each one of the four acids were prepared in different concentrations in acetone. The extinctions were determined at the four wavelengths recorded in Table 4. The same rocksalt cell of about 0.1 mm length was used for all determinations on solutions and solvents. In diagrams drawn on millimeter paper 450 × 600 mm, we could plot for each wavelength the extinctions of the four acids as a function of their concentrations (Fig. 3). In these diagrams the following abbreviations have been used: 2-methylphenoxyacetic acid 2M; 4-chloro-2-methylphenoxyacetic acid 4K2M; 6-chloro-2-methylphenoxyacetic acid 6K2M and 4,6-dichloro-2-methylphenoxyacetic acid 46K2M.

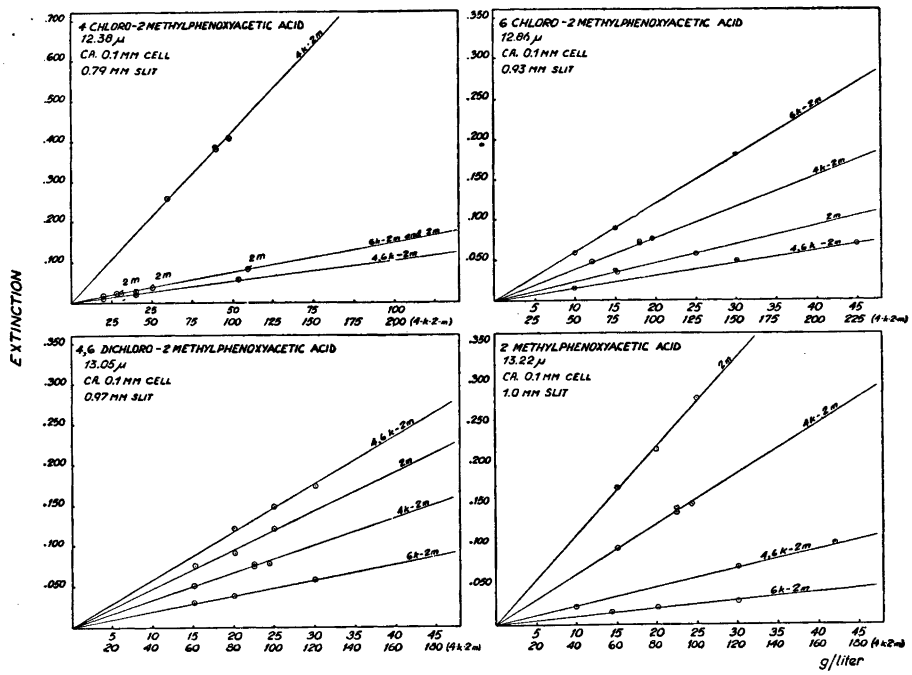

Fig. 3. Working curves.

The diagrams show a linear relationship indicating the validity of Beer's law:

$$E = \log I_0/I = K \cdot c \cdot l$$

where E = extinction, I_0 = light transmitted by the pure solvent, I = light transmitted by the solution, K = extinction coefficient of absorbing material, c = concentration in grams per liter and l = lengths, of solution path in centimeters.

The extinction coefficients of the four acids at each one of the four wavelengths can be calculated from values obtained in the concentration diagram. After determination of the extinction of a solution of the four acids in the same cell at the different wave lengths four equations of the following type are obtained.

$$E = K_1c_1l + K_2c_2l + K_3c_3l + K_4c_4l$$

With these equations the concentrations c_1, c_2, c_3 and c_4 of the four acids can be calculated. We have used a method of solving the equation system by successive approximations described by Daasch [9].

Table 4. Analyses of known mixtures.

Sample	2-Methylphen-oxyacetic acid %		4-Chloro-2-methylphen-oxyacetic acid %		6-Chloro-2-methylphen-oxyacetic acid %		4,6-Dichloro-2-methylphen-oxyacetic acid %		Total % found
	calc.	found	calc.	found	calc.	found	calc.	found	
1	0	0	90.1	91.0	5.0	4.4	4.9	4.9	100.3
2	5.0	5.3	77.6	77.0	11.2	10.0	6.2	6.6	98.9
3	4.0	3.5	59.4	60.4	29.7	29.7	6.9	7.8	101.4
4	35.0	35.4	45.0	45.0	20.0	20.0	0	0	100.4

EXPERIMENTAL RESULTS

A number of mixtures with varying known amounts of the four acids were dissolved in acetone (50—100 grams per liter, total) and analysed as described. The results are given in Table 4.

The accuracy obtainable depends on the reproducibility of the transmittance measurements. The wavelength of 6-chloro-2-methylphenoxyacetic acid must be set very carefully because it is on the side of the acetone (solvent) band.

With extinction measurements within the used range ($E = 0.3$—0.5), it is possible to reach a reproducibility of $\pm$ 0.02 with careful work. This means a maximum average error in the determination of $\pm$ 1.5 units in the calculated percentage. However, the error is generally considerably less which appears from the results obtained with mixtures of known composition.

We have analysed some of the commercial 4K2M preparations available in Sweden 1949. All of them were solutions and the acids were extracted as follows:

A 150 ml sample is acidified with hydrochloric acid to pH 7.0 and the liberated cresols extracted with 40 ml of ether. The pH in the extracted solution must not be higher than 8.0, if so more acid is added before separation. The solution is then extracted once more with 40 ml of ether. The solution which is now free from cresols is acidified with hydrochloric acid until precipitation is complete and the precipitate extracted twice with 40 ml of ether. The ether extract is washed twice with 25 ml of water, evaporated on a steam bath and dried at 110° C.

The found compositions of the different samples of 4K2M are shown in Table 5.

Table 5. Analyses of technical 4K2M.

Sample	2-Methyl-phenoxy-acetic acid %	4-Chloro-2-methyl-phenoxy-acetic acid %	6-Chloro-2-methyl-phenoxy-acetic acid %	4,6-Dichloro-2-methyl-phenoxy-acetic acid %	Total %	Chlorine content %	
						Calculated from infrared analyses	Determined according to Carius
1	4	61	26	8	99	18.4	18.29
2	10	74	12	3	99	16.3	16.01
3	4	62	23	9	98	18.1	18.29
4	52	32	6	2	92	7.4	7.73

The deficit of 8 per cent found in sample 4 indicates that unknown components are also present in this product.

SUMMARY

The four compounds 2-methylphenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid, 6-chloro-2-methylphenoxyacetic acid and 4,6-dichloro-2-methylphenoxyacetic acid have been prepared in very pure form, and their light absorption in infrared has been determined. The infrared region revealed suitable absorption maxima. A procedure for quantitative analysis has been worked out. This procedure has been successfully applied to the analysis of mixtures of the pure acids. A number of commercial products of chloro-2-methylphenoxyacetic acids have been analysed.

The author gratefully acknowledges the assistence of the following coworkers: Ing. Bengt Berglund, who made the infrared spectrophotometric measurements, Civ.Ing. Sven Nord and Ing. Sven Lundgren, who together with Ing. Berglund synthesized the compounds and Ing. Esse Zetterqvist and Ing. Åke Haster, who performed the chemical analyses.

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Received March 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 806–809

Le dosage de l'acide 4-chlore-2-méthylphénoxyacétique en composition avec des acides chlorés et nonchlorés

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En fabriquant les moyens pour détruire les mauvaises herbes, maintenant si actuels, de crésol chloré, on obtient un mélange d'acides, qui, sauf l'acide 4-chlore-2-méthylphénoxyacétique (4K2M) physiologiquement effectif, contient aussi l'acide 6-chlore 2-méthylphénoxyacétique (6K2M), des acides non-chlorés, dichloresubstitués et des crésols correspondants. Pour juger de l'efficacité du liquide il s'agit de déterminer la teneur de l'acide effectif.

Avec les méthodes habituelles on peut seulement séparer la quantité totale des acides et déterminer le dosage de chlore. On peut donc estimer la grandeur des teneurs des acides dichlorés et non-chlorés.

Pour séparer les acides différents les méthodes spectrophotométriques sont probablement les seules employables. Dans ce but le fil. dr. B. Sjöberg, Skoghall, a fait, selon ce qu'il m'a dit, une méthode pour cette détermination par l'absorption dans la région de l'infrarouge. Les investigations en l'infrarouge sont pratiquement difficiles et exigent des appareils extrèmement couteux, j'ai donc dû faire des investigations en l'ultraviolet pour obtenir une méthode pour le dosage de 4K2M.

Le laboratoire d'Essais de l'Etat en Suède voudrait comparer les deux appareils de Beckman et de Hilger afin de déterminer les avantages de l'un et de l'autre pour des buts spéciaux. Après avoir obtenu les courbes des acides en question, j'ai essayé de déterminer la teneur de l'acide 4K2M.

Les substances pures, obtenues de Skoghallsverken, sont, pour identification, investigées quant au point de fusion et l'acide 4K2M aussi au dosage de chlore.

Substance	Point de fusion, °C	Chlore, %
acide 4K2M	121	17,7
» 6K2M	110	
» nonchloré	155	
» dichloré	188	

Tableau 1. L'extinction des substances pures.

Substance	Acide 4-chlore 2-méthylphénoxyacétique				Acide 6-chlore 2-méthylphénoxyyacétique			Acide 4,6-dichlore 2-méthylphénoxyacétique		2-Méthylphénoxyacétique, nonchloré		
Concentr. mg/100 ml	12,9 H	10,0 Bl	12,9 Bl	10,0 B2	52,4 H	52,4 Bl	52,4 B2	10,0 Bl	10,0 B2	13,1 H	10,0 Bl	10,5 B2
Longueurs d'ondes en mμ	L'extinction log I_0/I											
310	0,016	0,004	0,064	0,002–0,002		0,026–0,004		0,005	0,004	0,073	0,001	0,002
300	0,028	0,011	0,091	0,010	0,00	0,038–0,005		0,007	0,006	0,083	0,001	0,002
290	0,649	0,446	0,623	0,508–0,01		0,050–0,002		0,070	0,075	0,087	0,005	0,005
288	0,916	0,655	0,91	0,712	—	0,058	0,005	0,117	0,131	0,094	0,007	0,010
287	—	0,706	—	0,737	—	—	0,012	0,149	0,161	—	0,012	0,016
286	—	0,719	—	0,727	—	—	0,022	0,184	0,200	—	0,019	0,026
285	0,915	0,714	1,03	0,717	0,008	0,084	0,035	0,221	0,238	0,128	0,033	0,040
282	1,02	0,776	1,10	0,796	0,099	0,180	0,130	0,251	0,245	0,335	0,154	0,197
280	1,06	0,819	1,16	8,83	0,216	0,299	0,261	0,234	0,234	0,692	0,379	0,485
279	—	0,818	—	0,805	—	—	0,345	0,238	0,243	—	0,539	0,665
278	1,02	0,801	1,15	0,799	0,389	0,473	0,440	0,246	0,250	1,065	0,692	0,83
275	0,87	0,691	1,03	0,676	0,635	0,73	0,700	0,262	0,265	0,997	0,746	0,76
270	0,670	0,524	0,82	0,508	0,733	0,87	0,82	0,199	0,196	1,18	0,89	0,95
265	0,440	0,340	0,60	0,326	0,69	0,87	0,78	0,148	0,147	0,94	0,684	0,71
260	0,276	0,207	0,441	0,197	0,515	0,71	0,612	0,098	0,099	0,665	0,455	0,478
255	0,173	0,122	0,339	0,116	—	0,54	0,436	0,063	0,067	—	0,285	0,287
250	0,114	0,076	0,291	0,076	0,190	0,372	0,276	0,044	0,054	0,284	0,148	0,157
240	0,74	0,593	0,93	0,695	0,085	0,320	0,182	0,237	0,295	0,205	0,057	0,067

H = appareil de Hilger, epaisseur des cuves = 100 mm, fente = 0,4 à 1,1 mm, correspondant une largeur de la bande spectrale de 5 Å

Bl = appareil de Beckman, epaisseur des cuves = 100 mm, fente = 0,5 à 1,1 mm, correspondant une largeur de la bande spectrale de ~ 22 Å

B2 = appareil de Beckman, epaisseur des cuves = 100 mm, fente = 0,1 à 0,3 mm, correspondant une largeur de la bande spectrale de 7 Å

Tableau 1. montre des valeurs d'extinction obtenues par des mesures d'absorption des acides différents en solution alcoolique. Les resultats obtenus sont corrigés concernant l'absorption des cuves.

L'investigation est faite avec deux appareils de Beckman, modèle DU, aux deux laboratoires differénts, et avec un appareil de Hilger, modèle »UV spek H 700». Les résultats obtenus sont pour comparaison calculés comme l'extinction ($\log \frac{I_0}{I}$) par 10 mg de la substance pro 100 ml de la solution, et alors les courbes sont designées.

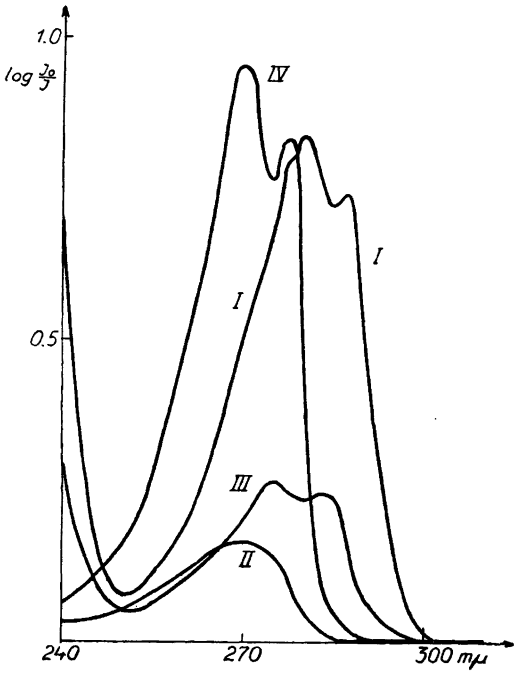

Fig. 1. L'extinction des mélanges connus (l'appareil B2).
I. 4K2M
II. 6K2M
III. 4.6-dichloré
IV. nonchloré

Celles-ci montrent que l'acide 4K2M surpasse un maximum à 287 mμ (courbe I), tandis que les autres y ont de l'absorption minimale. L'acide dichloresubstitué (courbe III) seulement a une absorption plus grande que les defauts de mesures, mais la teneur doit être peu importante. On peut introduire une correction, calculée du dosage de chlore.

Pour apprécier la précision des mesures quantitatives de l'acide 4K2M en mélange, j'ai fait des solutions avec des dosages connus et les résultats des mesures de l'extinction enregistrés à 280 st à 287 mμ sont inscrits au tableau 2.

Aux investigations avec les appareils de Beckman j'ai employé la même sensibilité, mais les fentes correspondantes étaient différentes, et par conséquent les détails des courbes apparaissent d'une manière différente. Les fentes sont inscrites au tableau 1.

Les resultats obtenus (tableau 2) montrent que les mesures d'absorption à 287 mμ de l'acide 4K2M sont égales, concernant la précision, en employant les 3 appareils. La méthode doit donner des resultats avec une précision de 3 unités en pourcentage.

Malheureusement je n'ai pas eu l'occasion de mesurer un plus grand nombre de mélanges connus pour justifier l'exactitude de la méthode.

Tableau 2. Investigation des mélanges connus.

Le mélange contient								
4K2M, mg par 100 ml	9,0	8,0	7,0	6,0	8,0	6,0	9,5	9,0
6K2M, » » 100 »	1,0	2,0	3,0	4,0	1,0	2,0	—	—
acide nonchloré, mg/100 ml	—	—	—	—	1,0	2,0	—	—
» dichloré, » /100 »	—	—	—	—	—	—	0,5	1,0
En appareil H:								
l'extinction à 280 mμ	0,762	0,676	0,590	0,516	0,638	0,488	—	—
dosage de 4K2M calculé, mg/100 ml	9,2	8,1	7,2	6,3	7,8	5,9	—	—
l'extinction à 287 mμ	0,679	0,590	0,509	0,432	—	0,435	0,709	0,675
dosage de 4K2M calculé, mg/100 ml	9,3	8,1	7,0	5,9	—	6,0	9,6	9,0
En appareil Bl:								
l'extinction à 280 mμ	—	—	0,585	0,511	—	—	—	—
dosage de 4K2M calculé, mg/100 ml	—	—	7,1	6,2	—	—	—	—
l'extinction à 287 mμ	—	—	0,496	0,424	—	—	—	—
dosage de 4K2M calculé, mg/100 ml	—	—	7,1	6,1	—	—	—	—
En appareil B2:								
l'extinction à 280 mμ	0,739	0,681	0,597	0,500	0,639	0,471	—	—
dosage de 4K2M calculé, mg/100 ml	9,0	8,1	7,2	6,0	7,6	5,7	—	—
l'extinction à 287 mμ	0,652	0,598	0,510	0,425	0,578	0,426	—	—
dosage de 4K2M calculé mg/100 ml	8,9	8,1	6,9	5,9	7,9	5,8	—	—

CONCLUSIONS

Il est possible de déterminer la teneur de l'acide 4-chlore-2 méthylphénoxyacétique, en mélange avec 6-chlore-2méthylphénoxyacétique et les acides dichloré et nonchloré, par évaluation de leur extinction à 287 mμ.

Je remereie monsieur fil.dr B. Sjöberg, Skoghall d'avoir mis à ma disposition les substances pures.

Je dois aussi remercier madame fil.lic. G. Aulin-Erdtman, messieurs fil.kand. B. Örtenblad et fil.lic. H. Arnfelt ainsique ses assistants pour leur amabilité de m'avoir permis d'utiliser leurs appareils.

Manuscrit reçu le 25 mars 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 810–815

Simultaneous Determination of Ionization Constant, Solubility Product and Solubility for Slightly Soluble Acids and Bases. Electrolytic Constants for Abietic Acid

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The following paper presents a simple and relatively reliable method for simultaneous estimation of ionization constant, solubility product and solubility of the undissociated molecules of slightly soluble weak acids or bases from a single potentiometric titration. As an example results for abietic acid are presented.

The idea of the method is to carry out a titration (performed with a strong titrator) starting from the range where the weak electrolyte is completely ionized and extend it over the flocculation point. From the first part of the curve the ionization constant is calculated, from the later part the solubility product. The solubility of the undissociated molecules is then obtained by dividing these two constants. A rough estimation of this intrinsic solubility can also be obtained from the equilibrium pH value where flocculation starts. The method also seems to be useful to determine the solubility of volatile weak acids and bases.

It is true that with decreasing solubility only a small part of the neutralization curve becomes useful for calculation of the ionization constant. Nevertheless the method seems to be as accurate for relatively insoluble weak acids and bases as the usual method, based on pH measurements of their salts at different concentrations. The accuracy of the method last mentioned is more influenced by omnipresent carbon dioxide especially at low concentrations, and in many cases formation of association ions at higher concentrations will limit the range of useful concentrations.

In the following the method is applied to monobasic acids, but can easily be extended to other cases.

Theory. The equations are derived for the acid HA dissolved in an excess of a strong base and titrated with a strong acid. Using common symbols, where C_a stands for the total concentration of weak acid HA + A^-, and C_s for the concentration of excess titrator added after the neutralization point, pK_s is calculated from

$$pK_s = pH + \log \frac{C_s + C_{OH^-} - C_{H_3O^+}}{C_a - C_s + C_{H_3O^+} - C_{OH^-}} + \frac{0.5\sqrt{\mu}}{1+\sqrt{\mu}} \quad (1)$$

After flocculation pK_L is computed from

$$pK_L = pK_s - \log a_{HA} = pH - \log (C_a + C_{H_3O^+} - C_s - C_{OH^-}) + \frac{0.5\sqrt{\mu}}{1+\sqrt{\mu}} \quad (2)$$

The solubility of the undissociated molecules L_{HA} is $= \frac{K_L}{K_s}$

At the point of beginning flocculation we obtain

$$L_{HA} = C_s + C_{OH^-} - C_{H_3O^+} \quad (3)$$

At higher concentrations the solubility of the undissociated acid L_{HA} can be reached already at the neutralization point. This salt concentration C'_a is determined by the equation

$$C'_a \approx C_{A^-} = \frac{K_s \cdot C_{HA} \cdot C_{OH^-}}{K_W} = \frac{K_s \cdot C_{HA}^2}{K_W} = \frac{K_s \cdot L_{HA}^2}{K_W} \quad (4)$$

Application to abietic acid

Abietic acid was isolated from American rosin (grade WW) principally according to Palkin and Harris[1]. 600 g rosin was dissolved in 500 ml ethanol and boiled with 5 ml concentrated hydrochloric acid and 1 g hydroquinone for half an hour. The abietic acid crystallized slowly on cooling. The acid was recrystallized as n-butylamine salt from benzene. After two recrystallizations 125 g of the salt was obtained as a faintly yellow product. The salt is relatively stable to oxidation and can be stored. The pure acid was prepared by dispersing the salt in ethyl ether, and crystallizing the free acid twice from acetone after decomposition of the salt with hydrochloric acid. Abietic acid was finally obtained in about 4 mm large colourless crystals, with a melting point of 168°—172°C, and an optical activity α_D = —103.8 to—105.1°/dm at 20°C in ethanol. The acid was vacuum dried and can be stored in vacuum and darkness for some days. Traces of oxidation products can be removed by recrystallization from acetone. All abietic acid used for the experiments described here was freshly prepared.

2.8—3.0 g abietic acid was dissolved at 90°—95° C in 0.2 N sodium hydroxide (about 10 % excess) under nitrogen atmosphere. Dissolving was complete in 10 minutes and after dilution to 500 ml a nearly colourless solution ($\sim$ 0.02 N) was obtained. Electrometric titrations were carried out on 150 ml portions of this solution in a nitrogen stream at 20° $\pm$ 1° C with 0.002 N hydrochloric acid during vigorous stirring. A Beckman low alkali error glass electrode (no. E 11903), a standard calomel reference electrode and an electronic potentiometer (Radiometer PHM3f) were used. The system was calibrated by a borate buffer according to Natl. Bur. Standards [2]. No correction for liquid junction potential has been applied. The liquid junction of the reference electrode was cleaned by letting out some KCl solution before each pH measurement. This was necessary because sometimes abietic acid was salted out at the liquid junction. The titration was extended over the flocculation point, which was observed. After measuring several points in the region of distinct precipitation the dispersion was retitrated with 0.02 N NaOH. The use of a dilute acid titrator was necessary to make sure of rapid equilibrium in the surroundings of the flocculation point. Activity coefficients were estimated according to equation (1) and (2). The pK_w value of Harned and Hamer [3] was used for the calculations.

Abietate solutions show the characteristic phenomena of long chain electrolytes [4]. A critical concentration of 0.015 N is obtained from conductivity measurements. Anomalies in the hydrolysis curve and formation of association ions are not observed until about this critical concentration [4,5]. The electrolytic constants are obtained from measurements below this concentration.

It is impossible to exclude small amounts of carbon dioxide. Further the acid is slowly oxidized. Both the carbonic acid and the oxidation products of abietic acid are stronger acids than abietic acid. Consequently they cause a decrease in the amount of titrator acid required to reach the first neutralization point from the alkaline side. Knowing the amount of all other electrolytes, it is possible to calculate the amount of interfering acids from the observed neutralization point. The first ionization constant of carbonic acid is about 15 times larger than that of abietic acid. Consequently only a slight amount of the bicarbonate ion is titrated in the first part of the neutralization. Thus this part will be most useful for accurate calculations. However, even then the contaminations show up as a slight upward trend in the pK_s value and downward trend in the pK_L value.

This is exemplified in Table 1 which shows the results of a complete titration (the last part carried out with a more concentrated titrator). Using the mean values of pK_s and pK_L obtained from this titration (7.55 and 11.41 respectively), a theoretical titration curve is calculated for comparison.

Table 1. Titration of sodium abietate with hydrochloric acid.

0.93445 g HAb in 15.00 ml 0.2046 *N* NaOH diluted to 150 ml.
Titration with 0.001994 *N* resp. 0.01994 *N* HCl.
Standard borax buffer; 0.01 *N* borax, 0.02 *N* NaCl (pH 9.20) gives — 77.8 mV.

ml HCl added	EMF observed mV	Calculated constants		EMF calculated mV *
0.00 *	— 197.9			— 189.5
25.00	— 185.2			— 182.7
40.0	— 176.7			— 174.2
50.0	— 168.8			— 167.7
60.0	— 158.8			— 158.5
70.0	— 148.3			— 150.7
80.0	— 134.4			— 133.3
90.0	— 118.9			— 119.6
92.5 †	— 113.7	pK_s	7.47	— 112.1
95.0	— 109.6	»	7.55	— 109.6
100.0	— 100.8	»	7.57	— 99.5
105.0	— 96.0	»	7.62	— 91.3
110.0 ††	— 93.1	L_{HA}	$1.54\ 10^{-4}$	— 85.8
120.0	— 90.5	pK_L	11.48	— 86.7
145.0	— 86.6	»	11.49	— 84.4
5.0 **	— 84.8	»	11.44	— 83.4
10.5	— 82.4	»	11.42	— 82.4
20.5	— 78.5	»	11.41	— 78.5
30.5	— 75.0	»	11.41	— 75.0
40.5	— 71.2	»	11.40	— 71.0
50.5	— 67.0	»	11.39	— 67.9
75.5	— 53.7		11.35	— 57.4
100.5	— 33.5		(11.26)	— 41.8
110.5	— 19.1			— 33.2
115.5	— 8.2		(10.82)	— 26.1
117.5	— 2.6			— 23.5
119.5	+ 4.9			— 20.2
121.5	+ 14.9			— 16.4
123.5	+ 28.0			— 11.8
125.5	+ 48.0		(10.48)	— 6.6
127.5	+ 73.8			— 0.0
129.5	+ 104.8			+ 9.1
131.5	+ 155.0			+ 23.1
133.5	+ 201.7			+ 55.8
135.5	+ 225.2			+ 208.0
137.5	+ 238.2			+ 236.3
139.5	+ 246.2			+ 244.1
149.5	+ 272.1			+ 270.8
174.5	+ 293.8			+ 293.8

* 0.001994 *N* HCl. ** 0.01994 *N* HCl. † Neutralization point. †† Opalescens. * From constants in preceeding column.

The agreement is good in the alkaline branch. In the acid branch the deviations are larger because the contaminating acids are titrated simultaneously with the abietic acid. In the test 2.782 milliequivalents abietate were added, but another 0.103 milliequivalents of acid were found from the position of the first equivalence point. At the second equivalence point another 0.056 milliequivalents had interfered.

Table 2 shows the average values of the electrolytic constants obtained in eight titration experiments.

Table 2. Electrolytic constants for abietic acid at 20° C.

Experiment	pK_s	pK_L	Intrinsic solubility L_{HA}	
			Calculated from $L_{HA} = K_L/K_s$	Observed at flocculation point
A I	7.69 ± 0.00	11.37 ± 0.03	$2.1\ 10^{-4}$	$L < 1.3\ 10^{-4}$
A II		11.37 ± 0.02		$L < 1.3\ 10^{-4}$
A III	7.88 ± 0.01	11.36 ± 0.02	$3.3\ 10^{-4}$	
B I	7.68 ± 0.04	11.40 ± 0.02	$1.9\ 10^{-4}$	$L < 1.1\ 10^{-4}$
B I retitr.	7.43 ± 0.06	11.41 ± 0.04	$1.0\ 10^{-4}$	$0.5 < L$
B II	7.66 ± 0.07	11.41 ± 0.02	$1.8\ 10^{-4}$	$L < 1.0\ 10^{-4}$
B II retitr.	7.79 ± 0.01	11.42 ± 0.03	$2.3\ 10^{-4}$	$0.6 < L < 1.3\ 10^{-4}$
C I	7.54 ± 0.07	11.45 ± 0.02	$1.2\ 10^{-4}$	
C I retitr.	7.55 ± 0.05	11.46 ± 0.05	$1.2\ 10^{-4}$	$0.4 < L < 0.8\ 10^{-4}$
C II	7.51 ± 0.09	11.43 ± 0.01	$1.2\ 10^{-4}$	$L < 1.0\ 10^{-4}$
C II retitr.	7.55 ± 0.03	11.46 ± 0.03	$1.2\ 10^{-4}$	$0.4 < L < 0.6\ 10^{-4}$
D (table 1)	7.55 ± 0.03	11.41 ± 0.02	$1.4\ 10^{-4}$	$L < 1.5\ 10^{-4}$
Mean value	7.62 ± 0.04	11.41 ± 0.01	$(1.6 \pm 0.3)\ 10^{-4}$	$0.5 < L < 1.0\ 10^{-4}$

The value for K_L and K_s are in good agreement. From what has been said earlier it could not be supposed that the value of L_{HA} calculated from K_L and K_s should coincide with that obtained from observation of commencing flocculation, in fact it is amazing that the results agree so well. The accuracy of the constants obtained is further influenced by liquid junction potentials, uncertainty in assumed activity factors, *etc.* The authors estimate the accuracy to be represented by

$$\begin{aligned} pK_s &= 7.6 \pm 0.2 \\ pK_L &= 11.4 \pm 0.1 \\ L_{HA} &= (1.6 \pm 1.0).\ 10^{-4} \text{ moles/l} \end{aligned}$$

Ekwall and Lindström [4] in a preliminary study, using crude acid, obtained as an upper limit $K_s L_{HA} = K_L = 1.19 \cdot 10^{-11}$ in reasonable agreement with our lower value on pure abietic acid $3.7 \cdot 10^{-12}$. In various works on paper sizing the ionization constant of rosin acids has been assumed to be between 10^{-4} and 10^{-11}.

SUMMARY

For slightly soluble acids and bases the ionization constant, solubility product and solubility can be determined by a single potentiometric titration. The method was applied to abietic acid and the values thus obtained are $pK_s = 7.6 \pm 0.2$, $pK_L = 11.4 \pm 0.1$ and the solubility of undissociated molecules $L_{HA} = (1.6 \pm 1) \cdot 10^{-4}$ moles/l.

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Received April 14, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 816–820

A Spectrophotometric Study on Complex Formation in Dilute Aqueous Solution of Cupric Bromide

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Cupric bromide in aqueous solution exhibits an absorption band at 281 mμ, at which wavelength cupric and bromide ions display practically no absorption. This can obviously be explained only be means of complex formation between cupric and bromide ions. Quantitative study proves difficult, however, because of the low degree of complex formation and of its complicated nature [1].

In sufficiently dilute solution, however, the complex $Cu(H_2O)_3Br^+$ must be practically the only one. The application of the law of mass action to such a solution gives

$$[Cu^{++}]\ [Br^-] / [CuBr^+] = K = K_0/F \tag{1}$$

where

$$f_{Cu^{++}} f_{Br^-} / f_{CuBr^+} = F \tag{2}$$

is the activity coefficient and K_0 is the thermodynamic dissociation constant. The extinction of a 1 cm layer of such a solution is, according to Beer's law

$$e = \varepsilon\ [CuBr^+] \tag{3}$$

where ε is the molar extinction coefficient. From (1) and (3) we obtain

$$\log \frac{e}{(c - e/\varepsilon)\ (2c - e/\varepsilon)} = \log\ \varepsilon/K_0 - pF \tag{4}$$

where c denotes the molarity of the cupric bromide solution. The activity coefficient may be represented by the Debye-Hückel equation

$$pF = \frac{2.04\ \sqrt{I}}{1 + a\sqrt{I}} - BI \tag{5}$$

In sufficiently dilute solution e/ε in equation (4) is negligibly small in comparison with c and $2\,c$. In such a case equations (4) and (5) can be used for the calculation of the quantities ε/K_0, α and B from the extinctions of a series of cupric bromide solutions, where the ionic strength is varied by addition of lithium or sodium perchlorate or perchloric acid [2]. In practice the magnitude of the molar extinction coefficient determines how dilute the cupric bromide solutions may be.

Table 1. Light absorption measurements in dilute cupric bromide solutions with perchloric acid additions at 25° C.

$\sqrt{I}$	$c_{Cu} \cdot 10^3$	e ($\lambda = 280$ mμ)	$\log \frac{e}{(c-e/\varepsilon)\,(2\,c-e/\varepsilon)}$
0.110	3.99	0.0274	2.938
0.151	7.62	0.0860	2.875
0.205	13.97	0.250	2.814
0.291	19.35	0.395	2.731
0.352	19.35	0.358	2.688
0.524	25.80	0.518	2.599
0.687	25.80	0.472	2.558
0.826	23.93	0.402	2.553
1.136	23.93	0.430	2.583
1.424	19.35	0.356	2.685
1.640	19.35	0.472	2.810
1.893	14.78	0.452	3.028

In Table 1 the measurements in perchloric acid solutions are recorded. Similar measurements were carried out in lithium and sodium perchlorate solutions. Wavelengths of 270, 280, 290, 300 and 310 mμ were used. The results were independent of wavelength. For the constants α and B the following values were obtained:

	α	B
$HClO_4$	1.476	0.252
$LiClO_4$	1.476	0.213
$NaClO_4$	1.476	0.190

In the calculation the value $\varepsilon = 1480$, obtained in a way described later in this paper, was used. These results did not, however, differ much from the results calculated using the value $\varepsilon = \infty$. For instance in perchloric acid solution the values $\alpha = 1.472$ and $B = 0.250$ were obtained when the value $\varepsilon = \infty$ was used. The values of α and B are of a reasonable magnitude. This is thus in agreement with the assumption made above that in the dilute cupric

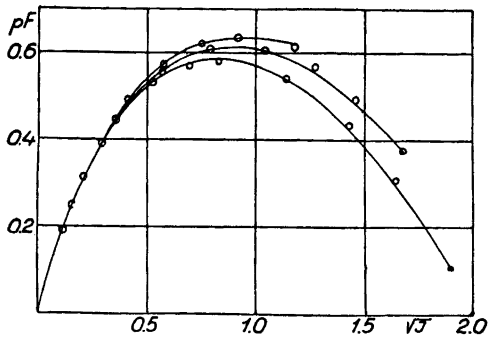

Fig. 1. Activity coefficient of the first cupric bromide complex in perchloric acid (lower curve), in lithium perchlorate (middle curve) and in sodium perchlorate (upper curve) solutions.

bromide solutions investigated, the first complex $Cu(H_2O)_3Br^+$ is practically the only one present. Supposing that the complex in question is a higher one we obtain from the experimental data an unreasonably steep curve representing the relation between pF and $\sqrt{I}$. In Fig. 1 the activity coefficient is plotted against the ionic strength.

For the quotient ε/K_0 the value 1340 was obtained by the above way. A more accurate value for this quantity can, however, be obtained by means of measurements in cupric bromide — sodium bromide solutions. In this case the equation

$$\log \frac{e}{(c_{Cu}-e/\varepsilon)\,(c_{Br}-e/\varepsilon)} = \log\, \varepsilon/K_0 - pF \qquad (6)$$

was used where c_{Cu} is the total copper and c_{Br} the total bromide concentration. This equation is analogous to equation (4) and the lower the bromide concentration the better is its validity. In Table 2 a series of experiments in cupric bromide — sodium bromide solutions is recorded. These experiments were also carried out at 280 mμ. It is significant that the value of ε/K_0 calculated by means of equation (6) proved to be nearly independent of the value of ε used. On the contrary α, and even more B, are highly dependent on the value of ε. For instance the following values were obtained:

ε	$\log\, \varepsilon/K_0$
∞	3.1183
1420	3.1202
1000	3.1206

By means of this extrapolation procedure the value of ε/K_0 may thus be determined irrespective of the unquestionable existence of higher complexes.

Finally the thermodynamic dissociation constant K_0 may be estimated from the data recorded in Table 2 by means of the equation

$$K_0 = \frac{e/c_{Cu}}{\varepsilon/K_0 - e/c_{Cu}\,[Br^-]\,F} \tag{7}$$

Table 2. Light absorption of mixed solutions of cupric bromide and sodium bromide. The estimation of the thermodynamic dissociation constant of the complex $Cu(H_2O_3)Br^+$.

$\sqrt{I}$	e	$c_{Cu} \cdot 10^3$	c_{Br}	pF	K_0 ($\varepsilon = \infty$)	K_0 ($\varepsilon = 1480$)
0.109	0.027	3.99	0.00798	0.190	6.76	0.95
0.205	0.247	13.97	0.0279	0.313	1.77	1.10
0.348	0.112	2.17	0.1143	0.446	0.80	0.73
0.410	0.825	13.97	0.1440	0.489	1.18	1.05
0.572	0.860	8.38	0.309	0.570	1.30	1.21
0.598	0.153	1.343	0.353	0.580	1.26	1.18
0.640	0.154	1.236	0.405	0.593	1.17	1.10
0.850	0.159	0.820	0.720	0.631	1.19	1.15
0.941	0.153	0.671	0.883	0.635	1.11	1.08
					mean value	1.06

which follows from equation (1). This equation is strictly valid only at a sufficiently low bromide concentration, but by means of an extrapolation procedure the value of K_0 can obviously be estimated. The calculation was carried out as follows. At first the activity coefficient in sodium perchlorate solutions was calculated by means of equation (4) using the value $\varepsilon = \infty$. Then the value of ε/K_0 was estimated by means of equation (6) using the value $\varepsilon = \infty$. Using these values of F and ε/K_0 the thermodynamic constant K_0 was now calculated by means of equation (7). These values are recorded in Table 2. As is seen these K_0 values are within the limits of experimental error independent of the bromide concentration except at low concentrations. With the aid of the mean value of these approximate K_0 values the molar extinction coefficient ε can now be calculated. With this ε value the calculation process was repeated and after a short series of approximations the values of the constants did not vary. The final values of K_0 are represented in the last column of Table 2. The mean valueof K_0 was $K_0 = 1.06$ and the corresponding value of the molar extinction coefficient was $\varepsilon = 1480$ ($\lambda = 280$ mμ).

It is interesting that the K_0 values obtained at 280 mμ are not dependent on the bromide concentration. This is not due to the absence of higher complexes because at other wavelengths, for instance at 310 mμ, the K_0 values obtained in the above way are highly dependent on the bromide concentration. The explanation is no doubt as follows. When the higher mononuclear complexes are considered, the equation

$$K_0 = \frac{e/c_{Cu}}{\varepsilon/K_0 - e/c_{Cu}\,[Br^-]\,F + \frac{1}{F\,[Br^-]}\sum \varepsilon_\nu\,[Br^-]^\nu/K_\nu - \frac{e}{c_{Cu}\,[Br^-]\,F}\sum [Br^-]^\nu/K_\nu} \qquad (8)$$

is obtained instead of equation (7). In this equation K_ν denotes the dissociation constant of the complex $CuBr_\nu^{(\nu-2)-}$ and is defined by

$$K_\nu = \frac{[Cu^{++}]\,[Br^{-\nu}]}{[CuBr_\nu^{(\nu-2)-}]} \qquad (9)$$

ε_ν being the corresponding molar extinction coefficient. In the sums only terms with $\nu \geqq 2$ are included. Equation (8) differs from equation (7) by two additional sum terms of which the first one is positive and contains molar extinction coefficients and is therefore dependent on the wavelength. The second one is negative. It is therefore possible that at a certain wavelength and in a limited range of bromide concentration the higher complexes have no effect on the results within the limits of the experimental error.

A Beckman Quartz Spectrophotometer Model DU with 1 cm and 5 cm cells was used in the light absorption measurements. The chemicals when not of the best quality obtainable, were recrystallized many times. Concerning further experimental details, the reader is referred to the previous papers by the present author [2,3].

SUMMARY

Complex formation in concentrated solutions of cupric bromide is very complicated but in sufficiently dilute solutions the complex $Cu(H_2O)_3Br^+$, obviously, is practically the only one. Complex formation in such dilute solutions is so slight that the concentration of the complex ion can be neglected as compared to the total copper concentration but the molar extinction coefficient is so large that it is possible to follow the change in the concentration of the complex. Therefore the determination of the activity coefficient of the complex in alkali perchlorate and perchoric acid solutions was possible spectrophotometrically and the accuracy obtained was considerable. Further a method for the estimation of the thermodynamic dissociation constant from extinction measurements is described and it is applied to dilute aqueous solutions of cupric bromide.

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Received May 16, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 821–827

The Oxidation of Phenyl Substituted Hydantoins

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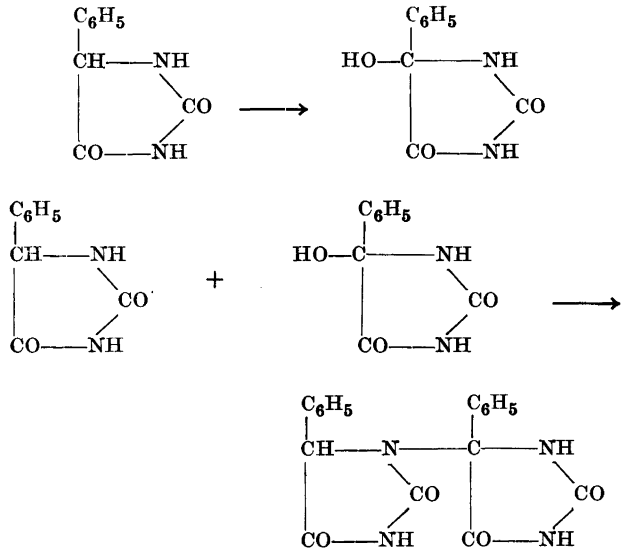
In two papers Aspelund [1] has shown that dialuric and hydurilic acids are formed when barbituric acids substituted with one alkyl, aryl, or aralkyl group in the 5-position are oxidized. The yields of these products vary with the oxidizing agent used. 5-Ethyl- and 5-benzylbarbituric acids give considerable amounts of the corresponding dialuric acids when aqueous hydrogen peroxide or potassium dichromate in acetic acid is used. Potassium permanganate in dilute sulfuric acid oxidizes these barbituric acids to both dialuric and hydurilic acids. In the oxidation of 5-phenylbarbituric acid aqueous hydrogen peroxide gives principally 5-phenyldialuric acid and a small amount of 5,5′-diphenylhydurilic acid. Potassium permanganate in dilute sulfuric acid causes the formation of 5,5′-diphenylhydurilic acid and a small amount of 5-phenyldialuric acid, whereas only the corresponding hydurilic acid is isolated when potassium dichromate in acetic acid is used.

As monosubstituted barbituric acids are very easily oxidized because the hydrogen attached to the central carbon atom between the two CO groups is mobile, it seemed to be of some interest to study the oxidation of the more stable hydantoins. Experiments with 5-methyl- and 5-phenylhydantoins have previously been carried out by Gabriel [2], who found that the hydantoins in question were oxidized by half an equivalent of bromine in acetic acid solution. The reaction products were pyruvinureide and "diphenylhydantil" (5-[5′-phenylhydantoin-1′-yl]-5-phenylhydantoin, *cf.* Formula I). Gabriel did not study the action of other oxidizing agents.

In order to avoid by-reactions only phenyl substituted hydantoins, *viz.* 5-phenylhydantoin, 1,5-diphenylhydantoin, and 3,5-diphenylhydantoin, were studied in the present work. Because the two last-mentioned substances are only sparingly soluble in water, they could not be oxidized in aqueous solution. In these cases only potassium dichromate in acetic acid was used, but 5-phenylhydantoin could also be oxidized with hydrogen peroxide both in the

presence of a small amount of sodium bicarbonate and in alkaline solution, and with potassium permanganate in dilute sulfuric acid.

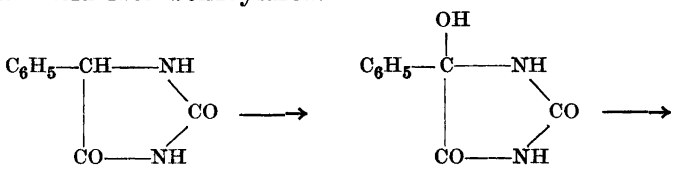
The last-mentioned oxidizing agent led to the formation of "diphenylhydantil" only. The same reaction product was also obtained when hydrogen peroxide together with a small amount of sodium bicarbonate was used. Oxidation with hydrogen peroxide in alkaline solution, however, leads to different results. With an excess of 0.15 equivalents of potassium hydroxide both "diphenylhydantil" and benzoylurea were formed. When the excess was increased to 1.12 equivalents of potassium hydroxide, benzoylurea and benzoic acid could be isolated, but no "diphenylhydantil". The formation of "diphenylhydantil" may be interpreted as an oxidation of the hydantoin to the corresponding hydroxy derivative and a reaction between this compound and unreacted 5-phenylhydantoin, *i. e.*

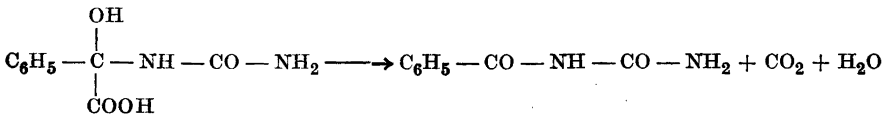


I

This sequence of reactions is supported by Gabriel's observation that "diphenylhydantil" is easily formed from a mixture of 5-hydroxy-5-phenylhydantoin and 5-phenylhydantoin. The formation of benzoylurea does not apparently derive its origin from "diphenylhydantil" because this substance cannot be converted into benzoylurea under the same experimental conditions. A plausible explanation is the following. 5-Phenylhydantoin is oxidized to 5-hydroxy-5-phenylhydantoin and the ring of the latter is opened by the alkali.

These reactions are then followed by another oxidation which converts the hydroxyureido acid into benzoylurea:





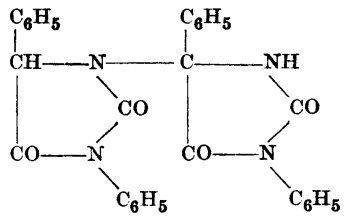
The carbon dioxide formed neutralizes the alkali and benzoylurea precipitates. This sequence of reactions is supported by the fact that 5-hydroxy-5-phenylhydantoin is converted into benzoylurea when it is oxidized in alkaline solution with hydrogen peroxide.

Potassium dichromate in acetic acid oxidizes the hydantoins mentioned in this paper to the corresponding hydroxyhydantoins. In all cases the reaction came to a standstill before all of the substance had reacted. The mixtures thus obtained were difficult to separate into their components, but by taking advantage of the fact that the hydroxyhydantoins are more readily soluble in water and alcohol than the corresponding hydantoins, fractional crystallization finally led to successful results. In the oxidation of 5-phenylhydantoin and 3,5-diphenylhydantoin the corresponding "hydantils" (*cf.* Formulae I and II) were formed as by-products. That the formation of these substances occurs in the way indicated in the first reaction sequence is evident from the fact that the "diphenylhydantil" is formed principally during the evaporation of the solvent after a half an hour's oxidation, *i. e.* when considerable amounts of 5-phenylhydantoin are still present.

Both 5-hydroxy-1,5-diphenylhydantoin and 5-hydroxy-3,5-diphenylhydantoin crystallize with one molecule of water. The hydrate of the latter is, however, much more stable than that of the former.

Although Gabriel prepared 5-hydroxy-5-phenylhydantoin by dissolving 5-bromo-5-phenylhydantoin in water, no melting point is given in his communication. In order to determine this constant for identifying purposes Gabriel's experiment was repeated.

3,5-Diphenylhydantoin was also oxidized with half an equivalent of bromine in acetic acid in order to obtain "tetraphenylhydantil" (5-[3′,5′-diphenylhydantoin-1′-yl]-3,5-diphenylhydantoin, *cf.* Formula II). The formation of this substance is, however, much slower than that of "diphenylhydantil".



II

This is also evident from the fact that "diphenylhydantil" is formed in considerable amounts and "tetraphenylhydantil" only in comparatively small amounts in the oxidations of the corresponding hydantoins with potassium dichromate in acetic acid.

EXPERIMENTAL

Preparation of 5-hydroxy-5-phenylhydantoin

Four grams of 5-phenylhydantoin were dissolved in 13.5 ml of acetic acid and to this solution 4.45 g of bromine in 8 ml of acetic acid were added. The mixture was warmed on the water bath for 20 minutes. The resulting colourless solution was allowed to stand until the next morning. The precipitated crystals of 5-bromo-5-phenyl-hydantoin (3.34 g or 58 per cent of the theoretieal amount, m. p. 210—212°) were collected and washed with a small amount of dry ether. The crystals were dissolved in 21 ml of water and the insoluble matter (0.22 g of "diphenylhydantil", m. p. > 300°) was filtered and washed with a small quantity of water. 5-Hydroxy-5-phenyl-hydantoin crystallized from the filtrate and was separated by suction. It melted at 180—181° (evolution of gas) and the yield was 1.71 g.

$C_9H_8O_3N_2$ Calc. N. 14.59 Found N 14.60

The filtrate was evaporated until almost dry. The substance that separated (0.49 g) could be identified as impure 5-hydroxy-5-phenylhydantoin. The total yield of the hydroxy derivative was accordingly 85 per cent calculated from the amount of the bromo derivative.

Oxidation of 5-phenylhydantoin by potassium dichromate

Two grams of 5-phenylhydantoin were dissolved in 25 ml of acetic acid, 2 g of finely powdered potassium dichromate were added and the mixture was heated on the steam bath. After five minutes' heating the colour of the mixture changed from orange to green. After two hours' heating the solution was left to cool. The solid substance that had separated was filtered together with unchanged potassium dichromate and washed with a small quantity of acetic acid. When the mixture had dried, it was treated with water to dissolve the dichromate. The insoluble "diphenylhydantil" (0.64 g, m.p. >300°) was filtered and the filtrate was three times extracted with ether, but the residue was very slight (0.01 g) and was not examined.

The acetic acid filtrate from the separation of "diphenylhydantil" and unchanged potassium dichromate was evaporated until dry. The residue was treated with dilute hydrochloric acid and the mixture was again evaporated in order to drive away most of the acetic acid. The solid substance remaining (0.15 g of "diphenylhydantil") was filtered and the filtrate was four times extracted with ether. The residue of the combined ether solutions (0.72 g) was treated with water, whereupon 0.40 g of rather impure 5-hydroxy-5-phenylhydantoin separated. After two recrystallizations from water the substance was found to be pure (m. p. 179—180°).

The aqueous filtrate was evaporated and the residue was treated with 1 ml of water. The insoluble substance was filtered and after recrystallization from water identified as 5-hydroxy-5-phenylhydantoin, m. p. 178—179° (0.13 g).

Another sample of 5-phenylhydantoin was heated for only a half an hour. "Diphenylhydantil" was isolated as the only product and this appeared principally during the evaporation of the solvent.

Oxidation of 5-phenylhydantoin with potassium permanganate in dilute sulfuric acid

Two grams of 5-phenylhydantoin were added to a solution of 0.70 g of potassium permanganate and 0.35 ml of conc. sulfuric acid in 20 ml of water and the mixture was boiled for half an hour *i. e.* until the colour of the permanganate had disappeared. The substance that had separated was filtered while the mixture was still hot and was identified as "diphenylhydantil" (0.88 g). From the filtrate 0.51 g of unchanged 5-phenylhydantoin separated. When the aqueous solution was evaporated 0.08 g of the same substance were obtained.

Oxidation of 5-phenylhydantoin with hydrogen peroxide

Experiment 1. Two grams of 5-phenylhydantoin were dissolved in 13 ml of water and a small quantity of sodium bicarbonate and 2.5 ml of hydrogen peroxide were added. The mixture was boiled. Almost at once crystals began to separate. The quantity of the solid substance gradually increased, so that when three-quarters of an hour had passed the mixture bumped considerably. After boiling for one hour, the mixture was filtered while hot. "Diphenylhydantil" (0.26 g) was obtained. Crystals began to separate in the filtrate and after the solution had cooled they were isolated and identified as 5-phenylhydantoin (1.70 g).

Experiment 2. Two grams of 5-phenylhydantoin were dissolved in 13 ml (1.15 equivalents) of *N* potassium hydroxide and 4 ml of 30 per cent hydrogen peroxide were added to the solution. The mixture was then allowed to stand at room temperature. As a consequence of the reaction the temperature rose a little. A solid substance gradually formed and after three hours and a half the substance was filtered and boiled with water. The insoluble part 0.25 g of "diphenylhydantil" was filtered off and from the filtrate 0.40 g of benzoylurea, m. p. 212—214°, crystallized.

When the alkaline filtrate was acidified 0.30 g of "diphenylhydantil" were isolated.

Experiment 3. Four grams of 5-phenylhydantoin were dissolved in 48.3 ml (2.12 equivalents) of *N* potassium hydroxide and 7.5 ml of 30 per cent hydrogen peroxide were

added. After four hours and a half impure benzoylurea that had formed was filtered. It weighed 0.85 g and melted at 206—209°, but on recrystallization from water the melting point rose to 211—212°.

When 7.5 ml of 30 per cent hydrogen peroxide were added to the filtrate, the reaction started again and the mixture became turbid. After twenty-three hours from the beginning of the experiment 0.91 g of benzoylurea, m. p. 214°, were filtered. The filtrate was slowly acidified. When 32 ml of *N* hydrochloric acid had been added, the solution was neutral to litmus and a solid substance had separated. It was identified as 5-phenylhydantoin (0.19 g). More hydrochloric acid was now added, and at first the solution remained clear, but when it was neutral to congo red a solid substance began to separate. When the solution was acid to congo red, the substance was filtered. It weighed 0.20 g and consisted of benzoic acid (m. p. 121—122°).

Oxidation of 1,5-diphenylhydantoin with potassium dichromate

Four grams of 1,5-diphenylhydantoin were dissolved in 50 ml of acetic acid, and 4 g of finely powdered potassium dichromate were added. The mixture was heated on the steam bath and turned green rather soon. After five hours' heating the solution was decanted from unchanged potassium dichromate. The solution was evaporated and the residue was treated with water containing a few drops of hydrochloric acid. At first an oily product separated that crystallized after some time. It was filtered and dissolved in alcohol containing a few drops of hydrochloric acid. The solution, which was slightly green-coloured, was placed in a refrigerator and after some time 1.27 g of unchanged 1,5-diphenylhydantoin, m. p. 202—204°, was separated by filtration.

Water was added to the filtrate and after a while a solid substance (1.80 g, m. p. 160—165°) was filtered. It was recrystallized several times from mixtures of alcohol and water, yielding colourless leaves, m.p. 170—171°.

$C_{15}H_{12}O_3N_2 \cdot H_2O$	Calc.	C	62.91	H	4.93	N	9.79	H_2O 6.30
	Found	»	63.13	»	4.98	»	9.79	» 6,44

The monohydrate of 5-hydroxy-1.5-diphenylhydantoin is readily soluble in alcohol, soluble in ether and insoluble in water and in inpolar solvents.

Oxidation of 3,5-diphenylhydantoin with potassium dichromate

Two grams of 3,5-diphenylhydantoin, 2 g of finely powdered potassium dichromate, and 25 ml of acetic acid were heated on the steam bath for three hours. The potassium dichromate that had not reacted was filtered off and water was added to the filtrate. The precipitate that formed was filtered at once and after it had dried in the air it was boiled with alcohol. The hot mixture was filtered. The substance that remained on the filter was "tetraphenylhydantil". The yield was 0.04 g.

$C_{30}H_{22}O_4N_4$ Calc. N 11.13 Found N 11.14

"Tetraphenylhydantil" does not melt under 300° and is insoluble in alcohol and water, but is slightly soluble in acetic acid.

The alcohol filtrate was diluted with water and the substance formed was filtered and identified as unchanged 3,5-diphenylhydantoin. It weighed 0.10 g.

More water was added to the acetic acid filtrate and next morning 1.05 g of solid substances had separated. After repeated fractional recrystallization 0.69 g of 5-hydroxy-3,5-diphenylhydantoin monohydrate, m. p. 168—169°, were isolated. The water is very firmly bound and its removal requires about one week over phosphorous pentoxide *in vacuo*.

$C_{15}H_{12}O_3N_2 \cdot H_2O$	Calc.	N	9.79	H_2O	6.30
	Found	»	9.67	»	6.11

The solubility of 5-hydroxy-3,5-diphenylhydantoin is approximately the same as the solubility of the corresponding 1,5-diphenyl derivative.

Oxidation of 3,5-diphenylhydantoin with bromine

Two grams of 3,5-diphenylhydantoin were dissolved in 25 ml of acetic acid. To this solution 0.635 g (0.5 equivalents) of bromine in 5 ml of acetic acid were added and the mixture was heated on the steam bath for one hour, *i. e.* half an hour after the disappearance of the bromine colour. The mixture was then diluted with water and the substance that separated was immediately filtered. When the substance was dry it was boiled with alcohol and filtered. In this way 0.43 g of "tetraphenylhydantil" were isolated. It had the same proporties as the substance isolated in the foregoing experiment.

From the filtrates 0.73 g of unchanged 3,5-diphenylhydantoin were isolated.

Action of hydrogen peroxide on 5-hydroxy-5-phenylhydantoin

Two grams of 5-hydroxy-5-phenylhydantoin were dissolved in 21 ml (2.02 equivalents) of potassium hydroxide, and 4 ml of 30 per cent hydrogen peroxide were added. After 24 hours 0.51 g of benzoylurea were isolated. When the corresponding experiment was carried out with "diphenylhydantil" no benzoylurea was isolated.

SUMMARY

The oxidation of 5-phenylhydantoin, 1,5-diphenylhydantoin, and 3,5-diphenylhydantoin has been studied.

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Received May 10, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 828–834

Short Communications

Activities of the Components in Ion Exchangers with Multivalent Ions

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A cation exchanger, R^-, containing two ionic species, A_1 and A_2, can be regarded as a solid solution of the components A_1R and A_2R. The activities of these components can be calculated from exchange equilibrium data, as we have recently shown for the case of univalent ions[1].

We shall now consider the general case with two ions: $A_1^{z_1+}$ and $A_2^{z_2+}$. We can choose the molecular weight of the components in two ways:

I. $(A_1)_{\frac{1}{z_1}}R$ and $(A_2)_{\frac{1}{z_2}}R$, using the *equivalent fractions* β_1 and β_2.

II. $A_1(R)_{z_1}$ and $A_2(R)_{z_2}$, using what we shall call the *molar fractions* x_1 and x_2. The activities are in both cases chosen so that they are unity in the pure components.

I. With I the reaction can be written:

$$z_1A_2^{z_2+} + z_1z_2(A_1)_{\frac{1}{z_1}}R \rightleftharpoons z_2A_1^{z_1+} + z_1z_2(A_2)_{\frac{1}{z_2}}R \quad (1)$$

Introducing the capacity s_0 in arbitrary units, and the activity factors γ_1 and γ_2:

$$[(A_1)_{\frac{1}{z_1}}R] = \beta_1 s_0;\ [(A_2)_{\frac{1}{z_2}}R] = \beta_2 s_0;\ \beta_1 + \beta_2 = 1 \quad (2)$$

$$\{(A_1)_{\frac{1}{z_1}}R\} = \gamma_1\beta_1;\ \{(A_2)_{\frac{1}{z_2}}R\} = \gamma_2\beta_2 \quad (3)$$

Now, assuming that we know the activities of the ions in the aqueous solution, equilibrium measurements give the equilibrium quotient $\varkappa_{21}$, which is related to the thermodynamic equilibrium constant K_{21} of (1) by:

$$\varkappa_{21} = \varkappa_{21}^{-1} = \frac{\{A_1^{z_1+}\}^{z_2}}{\{A_2^{z_2+}\}^{z_1}} \cdot \left(\frac{\beta_2}{\beta_1}\right)^{z_1z_2} = K_{21} \cdot \left(\frac{\gamma_1}{\gamma_2}\right)^{z_1z_2} \quad (4)$$

as is easily seen from (1), (2) and (3). Gibbs-Duhem's law gives:

$$\beta_1 \operatorname{dln} \gamma_1\beta_1 + \beta_2 \operatorname{dln} \gamma_2\beta_2 = 0 \quad (5)$$

From (5), (2) and (4) we find, remembering that K_{21} is a constant:

$$\operatorname{dln} \gamma_1 = \frac{\beta_2}{z_1z_2} \operatorname{dln} \varkappa_{21};\quad \operatorname{dln} \gamma_2 = -\frac{\beta_1}{z_1z_2} \operatorname{dln} \varkappa_{21} \quad (6)$$

Thus, as for univalent ions, γ_1 and γ_2 can be obtained by graphical integration of the curve $\log \varkappa_{21}$ (β).

II. If the "moles" chosen are $A_2R_{z_1}$ and $A_2R_{z_2}$, with molar fractions x_1 and x_2 and activity factors g_1 and g_2:

$$\beta_1 + \beta_2 = x_1 + x_2 = 1;\ \beta_1:\beta_2 = x_1z_1:x_2z_2,$$

thus

$$x_1 = \beta_1 z_2 (\beta_1 z_2 + \beta_2 z_1)^{-1};$$
$$\beta_1 = x_1 z_1 (x_1 z_1 + x_2 z_2)^{-1} \text{ etc.} \quad (7)$$

Since the standard states are the same as with I:

$$\{A_1R_{z_1}\} = \left\{\underset{z_1}{(A_1)_1R}\right\}^{z_1} \text{ or } g_1 x_1 = (\gamma_1\beta_1)^{z_1}$$
$$\{A_2R_{z_2}\} = \left\{\underset{z_2}{(A_2)_1R}\right\}^{z_2} \text{ or } g_2 x_2 = (\gamma_2\beta_2)^{z_2} \quad (8)$$

The reaction can be written:

$$z_1 A_2^{z_2+} + z_2 A_1R_{z_1} \rightleftharpoons z_2 A_1^{z_2+} + z_1 A_2R_{z_2} \quad (9)$$

The thermodynamic constant of (9) is the same as that of (1), K_{21}, because of (8). An equilibrium quotient λ_{21} for (9) may be defined by:

$$\lambda_{21} = \frac{\left\{A_1^{z_1+}\right\}^{z_1} x_2^{z_1}}{\left\{A_2^{z_2+}\right\}^{z_1} x_1^{z_2}} = K_{21} \cdot \frac{g_1^{z_2}}{g_2^{z_1}} \quad (10)$$

and from (10) and Gibbs-Duhem's law we can derive:

$$d\ln g_1 = \beta_2 z_2^{-1} d\ln \lambda_{21};$$
$$d\ln g_2 = -\beta_1 z_1^{-1} d\ln \lambda_{21} \quad (11)$$

To find γ_1, γ_2, g_1 and g_2 one need only make *one* graphical integration, either finding one of γ_1 and γ_2 with (6) and the other with (4), or one of g_1 and g_2 with (11) and the other with (10).

The other set of activity factors can be found from (8).

1. Ekedahl, E., Högfeldt, E., and Sillén, L. G. *Acta Chem. Scand.* **4** (1950) 556.

Received June 21, 1950.

Activities of the Barium and Hydrogen Forms of Dowex 50

ERIK HÖGFELDT, ERIK EKEDAHL, and LARS GUNNAR SILLÉN

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Högfeldt, Ekedahl and Sillén have recently developed equations for calculating the activities of the components in ion exchangers [1, 2].

For multivalent ions there is a choice whether to consider (I) equivalents $Ba_{\frac{1}{2}}R$, $Al_{\frac{1}{3}}R$ *etc.* or (II) molecules BaR_2, AlR_3 *etc.* Dependent on the choice, different mole fractions (I $\beta_1\beta_2$; II x_1x_2), equilibrium quotients (I $\varkappa$; II λ), and activity factors (I $\gamma_1\gamma_2$; II g_1g_2) should be used.

There are in literature very few measurements on ion exchange equilibria with multivalent ions. We have applied our formulae to two sets of measurements [3,4] on the exchange H^+ (= 1)$-Ba^{2+}$ (= 2) on Dowex 50. Marinsky [3] used Dowex 50 which had been treated with 6 C NaOH at 95° C for 48 hours, whereas Duncan and Lister [4] mention no pretreatment of their resin.

Fig. 1 gives log λ_{21} as a function of β, not of x, to facilitate the integration of equation (11). (For the numbers of equations see Högfelt at *al*[2]). The equilibrium quotient λ_{21} from Duncan's and Lister's measurements is seen to differ considerabl from Marinsky's, which is probably due to the pretreatment.

In Fig. 2 a and b, the g values have been calculated both from λ_{21} with (11) and from $\varkappa_{21}$ (Fig. 3) with (6) and (8). The deviations are small. They are of course due to the fact that the smoothed curves $\varkappa_{21}(\beta)$ and $\lambda_{21}(\beta)$ are not exactly equivalent.

It is interesting to note that Marinsky's values give a maximum in the activity factor g_2 of BaR_2, (and a minimum in g_1) not far from the composition of the

thus

$$x_1 = \beta_1 z_2 (\beta_1 z_2 + \beta_2 z_1)^{-1};$$
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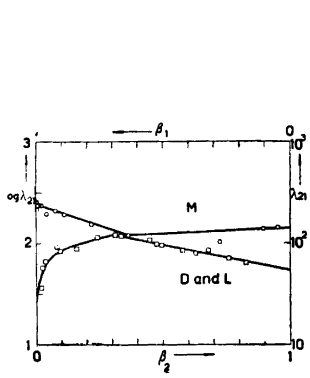


Fig. 1. Log λ_{21} as function of β for $Ba^{2+}-H+$ exchange on Dowex 50. ○: Marinsky [3]. □: Duncan and Lister [4].

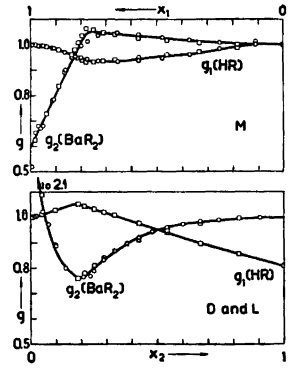


Fig. 2. Activity factors g_1 and g_2. ○: from (6) and (8), □: from (11). Fig. 2 a gives g from Marinsky's measurements and 2 b from Duncan's and Lister's.

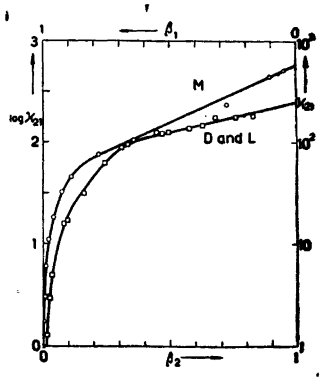
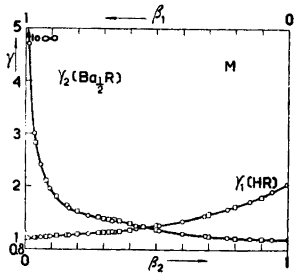
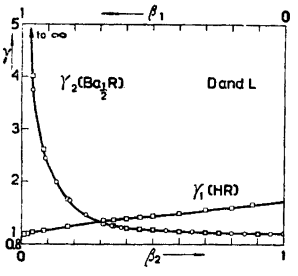


Fig. 3. Log $\varkappa_{21}$ as function of β. ○: Marinsky, □: Duncan and Lister.

compound $BaR_2(HR)_5$ proposed by Marinsky and Coryell [3]. At almost the same composition, Duncan's and Lister's values give a minimum in g_2 (maximum in g_1)!

Fig. 3 gives $\varkappa_{21}(\beta)$, and Figs. 4—5 give γ_1 and γ_2 calculated from (6) and from (11) and (8). This way of representation II, with molar sizes BaR_2 and HR, and the corresponding equilibrium quotient λ_{21} and activity factors g_1 and g_2, rather than I (with $Ba_{\frac{1}{2}}R$, $\varkappa_{21}$, γ_1 and γ_2). The curves obtained with II, by the way, resemble more those for univalent ions [1] (where I and II are of course identical).





Figs. 4—5. Activity factors γ_1 and γ_2. ○: from (11) and (8), □: from (6). Fig. 4 gives γ from Marinsky's measurements and Fig. 5 from Duncan's and Lister's.

does not bear out the difference between the two sets of measurements, since they give curves of essentially the same form.

Moreover, for $\beta_1 \to 1$, $\log \varkappa_{21}$ seems to tend towards $-\infty$, and $\gamma_2 \to +\infty$. This is to be expected since λ_{21} seems to have a finite value at $\beta_1 = 1$ (*cf.* (11) and (8)).

In this case it seems preferable to use

1. Ekedahl, E., Högfeldt, E., and Sillén, L. G. *Acta Chem. Scand.* **4** (1950) 556.
2. Högfeldt, E., Ekedahl, E., and Sillén, L. G. *Acta Chem. Scand.* **4** (1950) 828.
3. Marinsky, J. A. *Diss. Report to the Office of Naval Research NR-026—001* (1949).
4. Duncan, J. F., and Lister, B. A. J. *Faraday Discussion*, Reading, September (1949), preprint I : 11.

Received June 22, 1950.

The Factor in Dicumarol Plasma Which Accelerates the Coagulation of Plasma from Vitamin K-Deficient Chicks

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Department of Biology, Polytechnic Institute, Copenhagen, Denmark

Recently [1] we reported the concentration of a protein factor in plasma from vitamin K-deficient chicks accelerating the coagulation of plasma from dicumarolized chicks. This factor — tentatively called the $\varkappa$-factor — is essential for the understanding of the "anomalous" prothrombin times obtained for mixtures of plasmas from vitamin K-deficient and from dicumarol-poisoned chicks — as described by Dam and Søndergaard [2]. In dicumarol plasma the concentrations of prothrombin and $\varkappa$-factor are reduced; only after addition of optimal amounts of $\varkappa$-factor will a dicumarol plasma be a purely hypoprothrombinemic plasma. Prothrombin times of mixtures of plasma from dicumarolized and from vitamin K-deficient chicks also indicated that there is an agent in dicumarol plasma capable of accelerating the coagulation of vitamin K-deficient plasma.

It has now been possible to concentrate this agent — a protein factor — from plasma of severely dicumarolized chicks (prothrombin time 20 to 25 times the normal value) by means of adsorption and elution procedures. It shows the following properties:

1. It is completely adsorbed from plasma of dicumarolized chicks by treatment of the plasma with 1 % by weight of $BaCO_3$. It can be eluted from the adsorbent by a solution of sodium citrate.

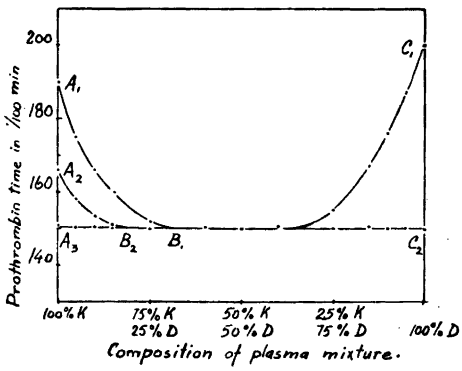


Fig. 1. Prothrombin time as a function of the composition of plasma mixtures.
K: plasma from a vitamin K-deficient chick.
D: plasma from a dicumarol poisoned chick.

2. Addition of small amounts of the protein to dicumarol chicken plasma has no influence on the prothrombin time.

3. It has no influence on the prothrombin time of fresh normal chicken plasma nor on the prothrombin time of stored chicken plasma.

4. Addition of small quantities of the protein to vitamin K-deficient chicken plasma reduces the prothrombin time to a certain extent. Addition of greater amounts has no further influence on the prothrombin time.

Highly active preparations of factor V (from chicken plasma) had no accelerating effect on the prothrombin time of the vitamin K-deficient plasma used in these investigations.

A typical form of the curve representing the prothrombin time for mixtures of a dicumarol and a vitamin K-deficient plasma as a function of the composition of the plasma mixture is shown in Fig. 1 (curve A_1-B_1-C_1). The result of the addition of a constant extremely small amount of the new coagulation protein to all mixtures is shown by the curve A_2-B_2-C_1.

The effect of optimal amounts of the protein added to all plasma mixtures is represented by the curve A_3-B_2-C_1.

* Fellow 1950—51: Norwegian Research Council for Technical and Natural Sciences.

We have called the new factor the δ-factor. This is meant to be a preliminary name until enough evidence concerning its exact function in prothrombin activation has accumulated to make a rational and systematic name possible.

The δ-factor is distinctly different from prothrombin, factor V and the ϰ-factor.

The effect of optimal amounts of the ϰ-factor on the prothrombin time of mixtures of a vitamin K-deficient and a dicumarolized plasma is represented by the curve A_1-B_1-C_2.

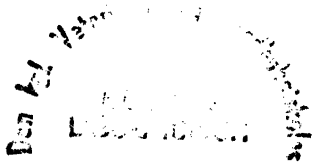
When optimal amounts of both the δ- and the ϰ-factors are added to all plasma mixtures, the curve is straightened out to give the line A_3-B_2-C_2. This is the type of curve that would be expected to represent the prothrombin time of mixtures of two purely hypoprothrombinemic plasmas having identical prothrombin content.

The deficit in δ-factor arises when chicks are fed on an artificial diet deprived of vitamin K. So far there are no indications that the δ-deficiency is caused by lack of another dietary factor than vitamin K. On the other hand, dicumarol — known as a potent inhibitor of the prothrombinogenic effect of vitamin K — does not seem to cause any significant reduction of the amount of the δ-factor in chicken plasma, at least not below the optimal level.

The properties and functions of the δ- and the ϰ-factors are being studied further.

1. Sørbye, Ø., Kruse, I., and Dam, H. *Acta Chem. Scand.* **4** (1950) 549.
2. Dam, H., and Søndergaard, E. *Biochem. et Biophys. Acta* **2** (1948) 409.

Received June 24, 1950.



ACTA CHEMICA SCANDINAVICA 4 (1950) 833–834

New Books

Gmelins Handbuch der anorganischen Chemie, 8. Auflage, System-Nummer 10. Selen, Teil B: Die Verbindungen des Selens. Gmelin-Verlag, Clausthal-Zellerfeld, 1949. DM 40.

For many decades, Gmelin's Handbuch has been *the* reference book of inorganic chemistry, unparallelled in breadth of scope and completeness of references. After the German collapse, the Gmelin Institute quite naturally met with considerable difficulties. All chemists will be pleased to know that it has been able to continue its work, as shown by the appearance, in 1949, of four new issues of Gmelin's Handbuch (Se B, Sb B2, Sb B3, and Pt A5).

Issue Se B treats the compounds of selenium. Since selenium has a low number (10) in Gmelin's system, only compounds of elements with very low numbers (H, O, N, the halogens, and S) are included. Following the traditions of Gmelin, not only the chemical properties in a narrow sense (preparation, reactions, equilibria, *etc.*) but also physico-chemical and physical properties are very thoroughly reported.

The text in all respects seems to be up to pre-war standards. A number of pages is devoted to the increasing use of selenium dioxide as an oxidant in organic chemistry.

On page IV we find the notice "Die Literatur ist berücksichtigt bis 1. Januar 1948". Had they but desired, Dr Pietsch and his collaborators could certainly have written a thrilling preface telling about all the difficulties they have had in accomplishing this result.

Lars Gunnar Sillén

E. Waldschmidt-Leitz. *Chemie der Eiweisskörper*. Sammlung chemischer und chemisch-technischer Vorträge, Neue Folge Heft 49. Ferdinand Enke Verlag, Stuttgart, 1950. vi + 187 pp. DM 16.–

The author states in the preface that this book is designed primarily for German readers who have had difficulty in finding the modern literature on protein chemistry. However, the book is of great intrest far outside German territory. Prof. Waldschmidt-Leitz has written a relatively short but most valuable monograph on the chemistry of proteins by discussing particularly the recent advances in this subject. The physico-chemical properties and the physiological metabolism of the proteins are only described in broad outline. The literature has been considered till the end of 1948 and the volume contains a comparatively large number of references (about 1500).

The first half of the book describes in five chapters the general chemical properties of proteins. Chapter I (23 pp.) describes the amino acids as constituents of proteins. The modern methods for the separation of amino acids are shortly mentioned and the most important ones for their quantitative determination are summarised with a comprehensive list of references. Chapter II (7 pp.) discusses shortly the synthesis, constitution, properties, occurrence, and isolation of peptides. In Chapter III (15 pp.) the general properties of the proteins as colloids are described.

The degradation of proteins is described in Chapter IV. In its first part (6 pp.) the non-hydrolytic reactions are briefly

mentioned. The second and main part of this chapter (24 pp.) discusses the hydrolysis of proteins. As expected, enzymic hydrolysis is exhaustively described and the result is a most valuable survey of the chemistry of the proteinases and peptidases.

The various hypotheses about the structure of proteins are discussed in Chapter V (29 pp.) As with most of the problems described, the author reviews this complex question by considering the most recent literature.

The second half of the book (Chapter VI; 70 pp.) contains a description of the different groups of proteins. The physical constants and the amino acid constituents are summarised clearly in many tables. The book ends with a survey of "physiologically active proteins", such as hormones, enzymes, viruses, toxins, and bacteriophages.

The generally accepted nomenclature for amino acids (D and L, instead of *d* and *l*) now in use in all modern textbooks and periodicals of biochemistry, has not been incorporated. There are only a few missprints (*e.g.*, p. 5, structure of iodogorgoic acid; p. 105, cytochrome c).

The book is well written and printed. It is recommended to all who want to get a short introductory survey of protein chemistry.

Klas-Bertil Augustinsson

ACTA CHEMICA SCANDINAVICA 4 (1950) 835–845

Studies on the Antibacterial Factors of Human Saliva

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Although the effect of saliva on different kinds of microorganisms had earlier been studied, it was not until 1934—1936 that Dold and collaborators [1-3] definitely showed that human saliva inhibits the growth of a great number of bacteria, *e. g. Corynebacterium diphtheriae*, staphylococci, streptococci, *Escherichia coli*, *Eberthella typhosa*, *Salmonella paratyphi B*, *Vibrio cholerae*, *Bacillus pyocyaneum*, and *Bacillus anthracis*. The antibacterial factor proved to be thermolabile, and sensitive to drying and ultraviolet radiation. It was also clear that it could not be identical with Fleming's lysozyme, present in small amounts in saliva and acting principally against a few airborn microorganisms, *viz. Micrococcus lysodeicticus*, and *Sarcina lutea*. According to Dold the antibacterial effect of saliva is due to a substance, possibly of enzymatic character, which is secreted with the saliva, and which he called inhibin. Moreover, Dold was of the opinion that microbial antagonism had no importance with respect to the antibiotic effect, although certain bacteria from saliva occasionally exhibit antagonistic action against other bacteria.

Other workers have ascribed greater importance to possible factors produced by the bacteria in the saliva. In 1937 Prica [5] asserted that the salivary bacteria had an antagonistic effect against at least the capsular bacteria *(Bacterium rhinoscleromatis*, *Bacterium Friedländer*, and *Bacterium ozaenae)*. He showed that culturemedia of staphylococci from saliva, which had been passed through Seitz filters, inhibited their growth, and assumed that the antibacterial effect was due to a secretory or metabolic product from the staphylococci. This product he found to be thermostabile. Mühlenbach [6] considered the antibacterial activity of saliva principally to be due to Dold's inhibin, but he pointed out that under certain conditions streptococci from saliva could antagonize staphylococci and *Corynebacterium diphtheriae*. Hölzl [7] made further studies of the antibiotic effect of salivary streptococci against *Corynebacterium diphtheriae*. By ether extraction of bouillon cultures

of salivary streptococci, he obtained an extract active against *Corynebacterium diphtheriae* both in vitro and in vivo. However, he was of the opinion that under natural conditions microbial antagonism was of slight importance. Thompson and Shibuya [8] found in 1946 that of 49 strains of streptococci cultivated from human saliva, 27 had an antagonistic effect against *Corynebacterium diphtheriae*, 24 of these being of the mitis type, 1 of the salivarius type, and 2 of the enterococcus type.

Other investigations of the antibacterial effect of saliva, sterilized by filtration have produced conflicting results. Dold, Lächele, and Hsing [1], and Weigmann and Noeske [9] found no antibacterial activity in saliva which had been passed through Seitz filters. On the other hand, Weigmann and Koehn [10] and Casassa [11] reported that the antibacterial factor passed through both Seitz and Berkefeld filters. Knorr [12] found antibacterial effect in saliva filtered through Jena glass filters "G 5 on 3". Kesteren, Bibby, and Berry [13] reported that saliva filtered through Berkefeld N filters had antibacterial activity, though it was low in comparison with the activity before filtration.

Dold, Lächele, and Hsing [1], and Weigmann and Noeske [9] were of the opinion that the antibacterial effect of saliva was not due to microbial antagonism, because there was no correlation between the number of salivary bacteria and antibacterial activity. And furthermore, Dold [14] reported that saliva collected directly from ductus submaxillaris in dogs had antibacterial action.

Therefore, it seemed probable from the literature that saliva contained a substance with antibacterial effect. In 1942 Kesteren, Bibby, and Berry [13] made resultless attempts to concentrate and purify it.

Most of the earlier investigators, who had been interested in these problems, found both interindividual and intraindividual variations in the antibacterial power of saliva. The present investigation was begun in order to ascertain the nature of the antibacterial factor, and possible causes of the variation, which has been reported. Were these problems solved, it might be possible to find means of increasing the natural antibacterial activity of the saliva.

EXPERIMENTAL METHODS

Agar plate method according to Dold [1]: Saliva, used directly, or after dilution with distilled water, was mixed with agar at a temperature of 45° C in 1 : 1 proportions (1 ml saliva: 1 ml agar). The mixtures were poured into small Petri dishes, 3 cm in diameter, the final concentration of saliva in the plates varying between 1 per cent and 50 per cent. The agar medium had the following composition: 2 g of agar, 0.4 g Bactopeptone (Parke Davis & Co.), 0.1 g KH_2PO_4, 0.1 g K_2HPO_4, and 0.5 g NaCl per 100 ml of medium at pH 6.8. When streptococci and pneumococci were used as test bacteria, the medium was fortified with 10 ml of human serum, and the pH adjusted to 7.2. The plates were inoculated with 0.05 ml of a suspension of the test bacterium. By cautious rotations of the

plates, the suspension was equally dispersed over the surface, and the plates were then incubated at 37° C for 16 hours. The growth of the test bacterium was classified as +, + +, or + + +. A + indicated slight, but macroscopically visible growth; a + + indicated intermediate growth, and a + + + indicated the same growth as in the controls. Two types of controls were used; plates with the agar medium, and plates with the saliva-agar mixture. In the latter, the postulated antibacterial factor of the saliva had been destroyed by heating at 100° C for 2 min.

Agar diffusion method [15]: A 0.5 ml layer of the same agar medium used in the agar plate method was poured into Petri dishes of 10 cm diameter. Inoculation with the test bacterium was accomplished by mixing 1 ml of a 24 hour old culture with 50 ml agar at 45° C, and pouring 2 ml of this mixture on the surface of each plate. Plates prepared in this way could be kept for use at a temperature of 4° C for about 2 weeks. 0.1 ml of the saliva to be tested was placed upon the agar plate in small, sterile glass cylinders (height 1 cm, inner diameter 0.5 cm) from which the saliva diffused into the medium; 6—8 cylinders could be placed on each plate. The plates were then incubated at 37° C for 16 hours. In this method antibacterial activity is indicated by zones of growth inhibition around the cylinders.

The following test bacteria were used: in the agar plate method *Staphylococcus H* *, *Staphylococcus* 209, *Pseudomonas aeruginosa*, one strain of an alpha and one of a beta streptococcus, *B. coli commune*, *B. coli beta polare*, and a pneumococcus strain, type 19; in the agar diffusion method only *Staphylococcus H*. Inocula for assays were prepared by centrifuging the cells from 1 ml of the bouillon media, washing once with water or 0.9 per cent NaCl, and diluting to a volume of 200 ml with 0.9 per cent NaCl. The two strains of streptococci, and the pneumococcus strain were cultivated for 16 hours in ascites bouillon, and the other test bacteria in peptone bouillon for 24 hours.

EXPERIMENTAL RESULTS

The effect of saliva on its own bacteria

According to Dold [1], the salivary bacteria did not show any growth in the plates for the first two days, owing to the presence of inhibin, which was also supposed to inhibit the common bacteria of the saliva. This hypothesis could not be confirmed in this investigation. After incubation at 37° C for 16 hours, the uninoculated agar-saliva plates assumed a turbid appearance which was shown, at a magnification of ten, to be caused by the formation of a great number of small colonies of bacteria from the saliva. Microscopical control of slides from these plates taken both immediately after pouring, and after incubation at 37° C for 16 hours showed that a great increase in the number of salivary bacteria had definitely occured. Another manifestation of the activity of the salivary bacteria was the change in pH value of the uninoculated plates during the incubation. From the initial value of 6.8 the pH changed

* *B. coli commune* and *B. coli beta polare* were received from professor Ragnar Nilsson, Ultuna, the other test bacteria from the Institute of Hygiene and Bacteriology at the University of Uppsala.

Table 1. Inhibitory effect on Staphylococcus H of saliva from 25 healthy individual 19—50 years of age. Normal diet. Dold's agar plate method.

Saliva no.	Growth of the test bacterium (*Staphylococcus H*)							Number of viable salivary bacteria per mm³
	Controls		Concentration of saliva in the plates per cent					
	agar medium	agar+ heated saliva	50	25	10	5	1	
1	+++	+++	—	—	—	+	++	
17	+++	+++	—	—	—	+	+	
18	+++	+++	—	—	—	—	+	
19	+++	+++	—	—	—	+	++	
20	+++	+++	—	—	—	+	++	
21	+++	+++	—	—	+	++	+++	
22	+++	+++	—	—	—	+	+	
23	+++	+++	—	—	+	++	++	
24	+++	+++	—	—	—	+	++	
25	+++	+++	—	+	+	++	+++	
26	+++	+++	—	—	+	++	+++	
33	+++	+++	—	—	—	+	+++	4 000
34	+++	+++	—	—	+	+	++	6 000
35	+++	+++	—	—	+	+	++	20 000
36	+++	+++	—	—	+	+	++	7 000
37	+++	+++	—	—	+	+	++	12 500
38	+++	+++	—	—	+	+	++	20 000
39	+++	+++	—	+	++	+++	+++	7 000
40	+++	+++	—	—	+	++	+++	10 000
42	+++	+++	—	+	++	+++	+++	500
44	+++	+++	—	—	—	++	+++	15 000
45	+++	+++	—	—	—	+	+++	20 000
46	+++	+++	—	—	—	—	++	25 000
47	+++	+++	—	—	—	++	+++	1 250
48	+++	+++	—	—	—	++	+++	7 500

+ indicates slight growth, ++ intermediate growth, +++ the same growth as in the controls, — indicates, no macroscopic growth of the test bacterium.

to values varying between 6 and 8, depending on the proportions of acid and alkali producing bacteria. If 0.5—1 per cent glucose was added to the agar medium, the lactic acid production changed the pH to about 4.5, in spite of the addition of buffer substances to the agar medium. If glucose was not added, however, the alterations in pH were not great enough to cause a non specific inhibition of the test bacteria.

Table 2. Inhibitory effect on Staphylococcus H of saliva from 10 healthy vegetarians 19—35 years of age. Dold's agar plate method.

Saliva no.	Growth of the test bacterium (*Staphylococcus H*)							Number of viable salivary bacteria per mm³
	Controls		Concentration of saliva in the plates per cent					
	agar medium	agar+ heated saliva	50	25	10	5	1	
27	+++	+++	−	+	++	+++	+++	1 000
28	+++	+++	+	++	+++	+++	+++	375
29	+++	+++	++	++	+++	+++	+++	100
30	+++	+++	++	+++	+++	+++	+++	100
31	+++	+++	−	−	−	+	++	2 500
32	+++	+++	−	−	−	+	++	6 750
41	+++	+++	−	−	−	−	+	100 000
43	+++	+++	−	+	++	+++	+++	500
49	+++	+++	−	+	++	+++	+++	5 000
50	+++	+++	−	−	−	+	++	12 500

+ indicates slight growth, ++ intermediate growth, +++ the same growth as in the controls, − indicates, no macroscopic growth of the test bacterium.

The rapid growth of the bacteria in fresh saliva could also be shown in the following way: Saliva, freed from heavy suspended particles such as epithelial cells and leucocytes by centrifuging at a low rate (1500 turns per minute), was, except for a slight opalescence, clear. After incubation for 24 hours at 37° C, however, it grew turbid due to the growth of salivary bacteria.

The effect of saliva on different test bacteria

Unstimulated saliva, collected 1 to 3 hours after meals, was used in experiments employing Dold's agar plate method. Two groups were investigated, one consisting of 25 healthy persons, 19 to 50 years of age, with a normal diet; the other, of 10 healthy vegetarians, 19 to 35 years of age. The results of these experiments are shown in Tables 1 and 2.

They agree with Dold's experiments to the extent that, with a few exceptions, all the tested salivas showed a strong antibacterial activity against *Staphylococcus H*. Furthermore, if the salivas were heated to 100° C for 2 minutes, or to 56° C for 30 minutes, the activity was completely destroyed. Of the other tested bacteria, active saliva inhibited *Staphylococcus 209*, a strain of an alpha, and a strain of a beta type streptococcus, a pneumococcus strain

of type 19, and two coli strains; *Coli beta polare*, and *Coli commune*. Against *Pseudomonas aeruginosa* the effect was slight, and sometimes completely absent.

The significance of the salivary bacteria for the inhibitory effect

In order to explain the relation of the salivary bacteria to the inhibitory effect of the saliva, a number of experiments were made with salivas, freed from bacteria by filtration through Seitz filters, Jena glass filters "G 5 on 3", and Pyrex filters U. F. In all cases, the salivas lost all their antibacterial activity, when tested with Dold's agar plate method. It seemed possible that the active factor might not have passed through the filters. Larger amounts of saliva were freed from bacteria by centrifugation at a rate of 10 000 turns per minute for two successive periods of 20 minutes each. Furthermore, the sterility of the centrifuged saliva was tested by putting a drop of saliva on an agar plate. As a rule, either no colonies, or at most a few, had developed after 24 hours' incubation at 37° C. Centrifuged saliva from 50 healthy individuals, 19 to 50 years of age, was tested by Dold's agar plate method, and the agar diffusion method. An antibacterial effect could not be shown against *Staphylococcus H*, *Staphylococcus 209*, the alpha and beta streptococci, *B. coli commune*, *B. coli beta polare*, *Pseudomonas aeruginosa*, or the type 19 pneumococcus strain . (In the agar diffusion method, only *Staphylococcus H* was used as test bacterium, as was previously stated). When the centrifuged sediments containing the salivary bacteria were added to the clear salivas, the whole inhibitory effect returned.

In another experiment, 5 uncentrifuged salivas, showing a low antibacterial activity, were tested with Dold's agar plate method before, and after incubation at 37° C for 16 hours. The results are seen in Table 3.

As might be expected, the inhibitory effect was stronger after incubation, owing to the increase in the number of salivary bacteria.

In 24 of the tested salivas the number of viable bacteria was determined in the following way: the salivas were diluted with 0.9 per cent NaCl in the proportions 1 : 100, 1 : 1000, 1 : 10 000, and 1 : 100 000. 0.05 ml of each of these solutions was dropped on the surface of agar plates. After incubation at 37° C for 24 hours, the number of colonies was counted, and the number of bacteria per mm^3 saliva calculated. The number of bacteria in the different salivas is indicated in Tables 1 and 2. There seemed to be a definite correlation between the number of bacteria and the antibacterial effect, for the salivas richest in bacteria had the strongest antibacterial effect, while the salivas with

Table 3. Inhibitory effect of different salivas before and after incubation at 37° C for 24 hours. Dold's agar plate method. Test bacterium Staphylococcus H.

Saliva no.	Growth of the test bacterium (*Staphylococcus H*)											
	Fresh saliva						The same salivas after incubation at 37° C for 24 hours					
	Controls	Concentration of saliva in the plates per cent					Controls	Concentration of saliva in the plates per cent				
		50	25	10	5	1		50	25	10	5	1
51	+++	+	++	+++	+++	+++	+++	−	−	−	+	++
52	+++	+	++	+++	+++	+++	+++	−	−	+	++	+++
53	+++	++	++	+++	+++	+++	+++	−	−	−	++	+++
54	+++	++	+++	+++	+++	+++	+++	−	−	−	+	+++
55	+++	−	+	++	+++	+++	+++	−	−	−	++	+++

\+ indicates slight growth, ++ intermediate growth, +++ the same growth as in the controls;
− indicates, no macroscopic growth of the test bacterium.

the smallest amount of bacteria had the weakest activity. There were, however, many salivas which had a weak inhibitory action, even though they contained a rather large number of bacteria. This fact might be due to a slight rate of growth of the bacteria in these salivas.

Since the great importance of the salivary bacteria for the inhibitory effect of saliva had been shown, it remained to find out how this effect is produced. As mentioned earlier, Prica [5], and Hölzl [7] have, among others, shown that certain bacteria from saliva, cultivated under special conditions, are able to produce substances with an antibiotic effect against other bacteria. To determine whether salivary bacteria under natural conditions can produce such substances, salivas from 50 healthy individuals, 19 to 50 years of age, were examined after incubation at 37° C for 24 hours. The bacteria were removed by centrifuging, as above, and the antibacterial effect was tested by the agar plate method, and the agar diffusion method. Here, as before, all antibacterial effect was removed from the salivas by the centrifugation of the bacteria.

To find out if the antagonistic effect of the salivary bacteria toward the testbacteria was due to competition for growth factors, present in insufficient quantities in saliva, the following experiments were performed: Saliva was sterilized by heating at 100° C for 5 minutes, or by filtration through Jena glass filters "G 5 on 3". 5 ml of the sterile saliva was placed into each of several

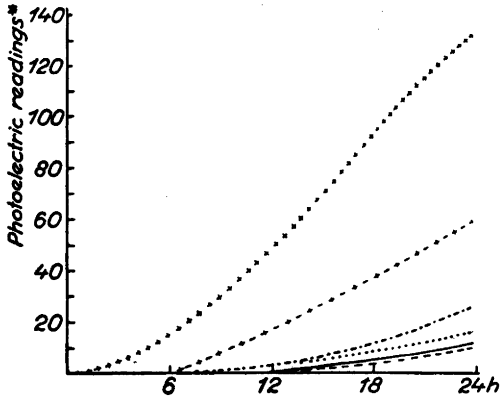

Fig. 1. Growth of different bacteria in sterile saliva at pH 7.

\+ + + + *Miscellaneous culture of salivary bacteria*
\+ — + — *Pseudomonas aeruginosa*
—·—·— *Staphylococcus H*
····· *B. coli commune*
——— *beta type streptococcus*
— — — — *Pneumococcus type 19*

* *Given as scale readings on the Klett-Summerson photoelectric colorimeter. Mean values from two tubes corrected for blank readings.*

sterile glass tubes, and the tubes were then inoculated with 0.05 ml of equally turbid suspensions of different bacteria (the inocula contained approximative three millions of bacteria). The growth of the bacteria was measured by turbidimetric analysis in a Klett-Summerson photoelectric colorimeter, after 6, 12, 18, and 24 hours. The growth rate varied somewhat in the different salivas. In all cases, however, the tubes inoculated with suspensions of miscellaneous salivary bacteria showed a considerably stronger growth than the tubes, which had been inoculated with the different test bacteria. A difference could not be observed between the growth rates in saliva sterilized by heating and in that sterilized by filtration; the growth factors, therefore, did not seem to be destroyed by short heating (100° C for 5 minutes). Fig. 1 shows typical curves of the growth of different bacteria in sterile saliva at pH 7. Streptococci, pneumococci, staphylococci, and *Escherichia coli* grew very slowly, apparently because of insufficient amount of essential growth factors in the salivas. By adding 10 per cent of blood serum as a source of growth factor, the growth rates of streptococci and pneumococci were considerably increased. The adding of 0.2 per cent peptone to the filtered saliva was sufficient to promote a strong growth of staphylococci and *Escherichia coli.*

By adding peptone to unfiltered saliva a similar result could be obtained. Saliva used directly or after dilution with distilled water was mixed in 1 : 1 proportions with agar solution containing 0.4 or 4 per cent of peptone as source of growth factor. The final concentration of saliva in the plates varied between 1 and 50 per cent. For each dilution of saliva two samples were used, one containing 0.2 per cent and the other 2 per cent of peptone. As usual the plates were inoculated with 0.05 ml of a suspension of the test bacterium (*Staphylococcus H*). The result is shown in Table 4.

Table 4. Inhibitory effect of saliva on Staphylococcus H in saliva agar plates with 2 and 0.2 per cent peptone.

Saliva agar plates with different amounts of peptone	Growth of the test bacterium (*Staphylococcus* H)					
	Controls	Concentration of saliva in the plates per cent				
		50	25	10	5	1
2 per cent peptone	+++	−	+	++	+++	+++
0.2 » » »	+++	−	−	−	+	++

+ indicates slight growth, ++ intermediate growth, +++ the same growth as in the controls. − indicates, no macroscopic growth of the test bacterium.

The addition of peptone to the saliva agar plates facilitated the growth of the test bacteria in spite of the presence of salivary bacteria.

In view of the above results, it is surprizing that some authors have found antibacterial effect in saliva sterilized by filtration. However, many of these workers have used as controls agar plates containing only a mixture of agar and unfiltered saliva, in which the antibacterial activity has been destroyed by heating. Since the filtration causes a marked decrease in the amount of organic substances available as nutriment for the test bacteria, the growth of test bacteria in agar plates containing filtered saliva will be less than in the controls with heated, unfiltered saliva. This fact may have been erroneously interpreted as due to the presence in the filtered saliva of substances with antibacterial effect.

The loss of organic substances in filtered saliva can be illustrated by a saliva, in which nitrogen determination was made before and after filtration through a Seitz filter. The quantity of nitrogen diminished from 0.46 to 0.18 mg per ml. By filtration through Jena glass filters "G 5 on 3" the loss of nitrogen was about 20 per cent.

DISCUSSION

The lack of antibacterial activity of saliva, from which the bacteria have been removed by centrifuging, indicates that the antibacterial effect of fresh saliva is due to bacterial antagonism of the salivary bacteria. Although certain bacteria isolated from the saliva are capable, under favorable conditions, of producing substances with an antibiotic effect toward other bacteria, the antagonistic effect of the salivary bacteria does not seem to be caused in this

way under natural conditions. The microorganisms normally found in human saliva seem to grow rather rapidly there. Most pathogenic bacteria, however, grow only very slowly in sterile saliva because of a deficiency of factors essential for their growth. When bacteria with relatively low growth rates as compared with the salivary bacteria enter the saliva, their growth will be inhibited by the lack of growth factors. When these factors are added, however, the inhibitory effect of the salivary bacteria is considerably weakened. Of the bacteria tested, Pseudomonas aeruginosa is least inhibited by the salivary bacteria. This agrees with the observation, that *Pseudomonas aeruginosa*, next to the salivary bacteria themselves, shows the most rapid growth in sterile saliva.

Furthermore, under physiological conditions, the pH of the saliva may also be of importance in the determination of the inhibitory effect toward pathogenic bacteria. The pH of human saliva normally varies between 6 and 7. If it is below 6.5 the growth conditions for many of the pathogenic bacteria is further reduced, since the optimal growth for most of them occurs at pH 7.0—7.4, and they grow very slowly if at all at a pH of less than 6.5. Many of the salivary bacteria still show a rather rapid growth at this pH.

Since the common salivary bacteria inhibit the growth of pathogenic bacteria, the therapeutical use of antibiotics may, by a simultaneous action on sensible microorganisms in the normal bacterial flora of the mouth, increase the possibility of an oral infection by those pathogens, which are to a lesser extent affected by the drug in question. Lipman, Coss and Boots [16] and Long [17] have recently studied the influence of penicillin therapy on the bacteria of the mouth. Before the treatment there were principally grampositive bacteria present. During the treatment, however, the number of these bacteria rapidly diminished and was followed by a flora of gram-negative, penicillin-resistant microorganisms. The changes were the same whether the penicillin was given orally, or by intramuscular injections, provided that in the latter case the drug was given in so high a dose, that it was secreted in the saliva ($>$ 500 000 units a day).

Louis Weinstein [18] has recently described five cases of new bacterial infections during the course of treatment with streptomycin or penicillin. In one patient, who probably had atypical pneumonia, administration of penicillin was followed by some apparent improvement, but on the fourth day of treatment *Hemophilus influenzae* was isolated from the throat and blood for the first time, and the condition became worse. In a second patient, who had faucial diphtheria, pneumonia developed. *Klebsiella pneumoniae*, not found at the beginning of treatment, was seen in throat and sputum after the fourth day of penicillin therapy, and was the only organism in the pulmonary tissues

revealed be postmortem culture. The other three patients were infected with *Staphylococcus aureus* during treatment with streptomycin for infections caused by *Hemophilus influenzae.*

SUMMARY

1. The antibacterial effect of human saliva toward staphylococci, streptococci, pneumococci, *Escherichia coli* and *Pseudomonas aeruginosa* has been investigated. All of these bacteria are inhibited by the saliva, *Pseudomonas aeruginosa* only slightly and inconstantly.

2. The antibacterial effect toward the above bacteria is due to the antagonistic action of the salivary bacteria.

3. This antagonistic effect seems to be connected with a competition between the salivary bacteria and pathogens for essential growth factors in the saliva. The normal bacterial flora require less of such factors, and will accordingly constitute an important defense mechanism against oral infections.

The author is greatly indebted to professor Gunnar Ågren for the impulse to this investigation, and for help and advice during its course.

The investigation was supported by a grant for medical research from the University of Uppsala. The technical assistance of Mrs. I. Kristenson is gratefully acknowledged.

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Received May 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 846–849

Studies Related to Pristane

IV. Infra-Red Spectra

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In the course of their work on the constitution of the saturated aliphatic hydrocarbon pristane from the liver oil of the basking shark, J. S. Sörensen and N. A. Sörensen [1,2] determined the physical constants of a series of aliphatic hydrocarbons C_{19}—C_{20}. On the basis of their careful measurements these authors concluded that pristane is probably identical with *nor*phytane, *i. e.* 2,6,10,14-tetramethylpentadecane.

In order to obtain conclusive confirmation of this identity, the infra-red spectra of pristane, *nor*phytane, phytane and crocetane were measured. The results of these measurements are discussed in the present communication.

EXPERIMENTAL

The isolation of *pristane* from the unsaponifiable matter of the liver oil of the basking shark and its purification has been described in the first communication of this series [3].

Norphytane and *phytane* were obtained from phytol as described in the third communication of this series [2].

Crocetane "A" was prepared according to Fischer and Löwenberg [4] as described in Part I [3].

Crocetane "B" was obtained from carefully purified 2,6,11,15-tetramethylhexadecatetraene-2,6,10,14 (digeranyl) [5].

The physical constants of the samples used for measurement are given in Table 1 (*cf.* also ref. 1–3).

Table 1. Physical constants of the samples investigated.

	d_4^{20}	n_D^{20}
Pristane	0.7827	1.4385
*Nor*phytane	0.7833	1.4386
Phytane	0.7907	1.44225
Crocetane "A"	0.7858	1.44055
Crocetane "B"	0.7888	1.4410

The infra-red spectra were measured on a Perkin-Elmer infra-red spectrometer, model 12B, with a 60° rock-salt prism. The substances were examined in the liquid state at room temperature.

RESULTS AND DISCUSSION

As may be seen from Fig. 1, the infra-red spectra of pristane (curve I) and *nor*phytane (curve II) are practically identical. Although this close coincidence of the spectra might have been considered proof of the identity of these hydrocarbons, it was thought worth while to examine the spectra of phytane (2,6,10,14-tetramethylhexadecane) and crocetane (2,6,11,15-tetramethylhexadecane) as well, to make sure that the differences between the infra-red spectra of such closely related hydrocarbons are sufficient to permit their safe identification.

As would be expected, the differences found were most marked in the case of hydrocarbons which differ in their terminal groups, *e. g. nor*phytane and phytane, or crocetane and phytane, since the terminal groups give rise to characteristic absorption bands; the strong band at 1170 cm^{-1} with a shoulder at about 1150 cm^{-1} is due to skeletal vibrations of the *iso*propyl groups, $(CH_3)_2CH$- [6]). In the spectrum of phytane a moderately strong band is found at 770 cm^{-1} which is characteristic of the terminal ethyl group [7].

On the other hand, the hydrocarbons *nor*phytane and crocetane differ only in the number of the CH_2-groups in the middle of the chain. Their spectra therefore show many common features. Nevertheless, even in this case the homologues may safely be distinguished from one another by means of their infra-red spectra. In the spectra of the crocetanes (curves IV and V), the strong bands at 735 cm^{-1}, which may probably be assigned to the CH_2-rocking vibrations in the chain R_1-$CH_2CH_2CH_2$-R_2 [8,9], show distinct and readily reproducible shoulders at about 727 cm^{-1}, whereas these shoulders are definitely absent in the spectra of pristane and *nor*phytane (as well as of phytane). Because of the natural width of these hydrocarbon bands it has not been possible to resolve the 735 cm^{-1} and the overlapped 727 cm^{-1} bands although our instrument is capable of resolving bands separated by about 2 cm^{-1} in this region. Resolution of these bands might be achieved at very low temperatures. The overlapped band at 727 cm^{-1} may be assigned to the CH_2-rocking vibrations in the group R_1-$CH_2CH_2CH_2CH_2$-R_2 [7,9]. Some further dissimilarities in the spectra may also serve to distinguish between *nor*phytane and crocetane, *e. g.* the distinct broad band at 970—80 cm^{-1} in the spectra of the crocetanes (*cf.* curves IV and V).

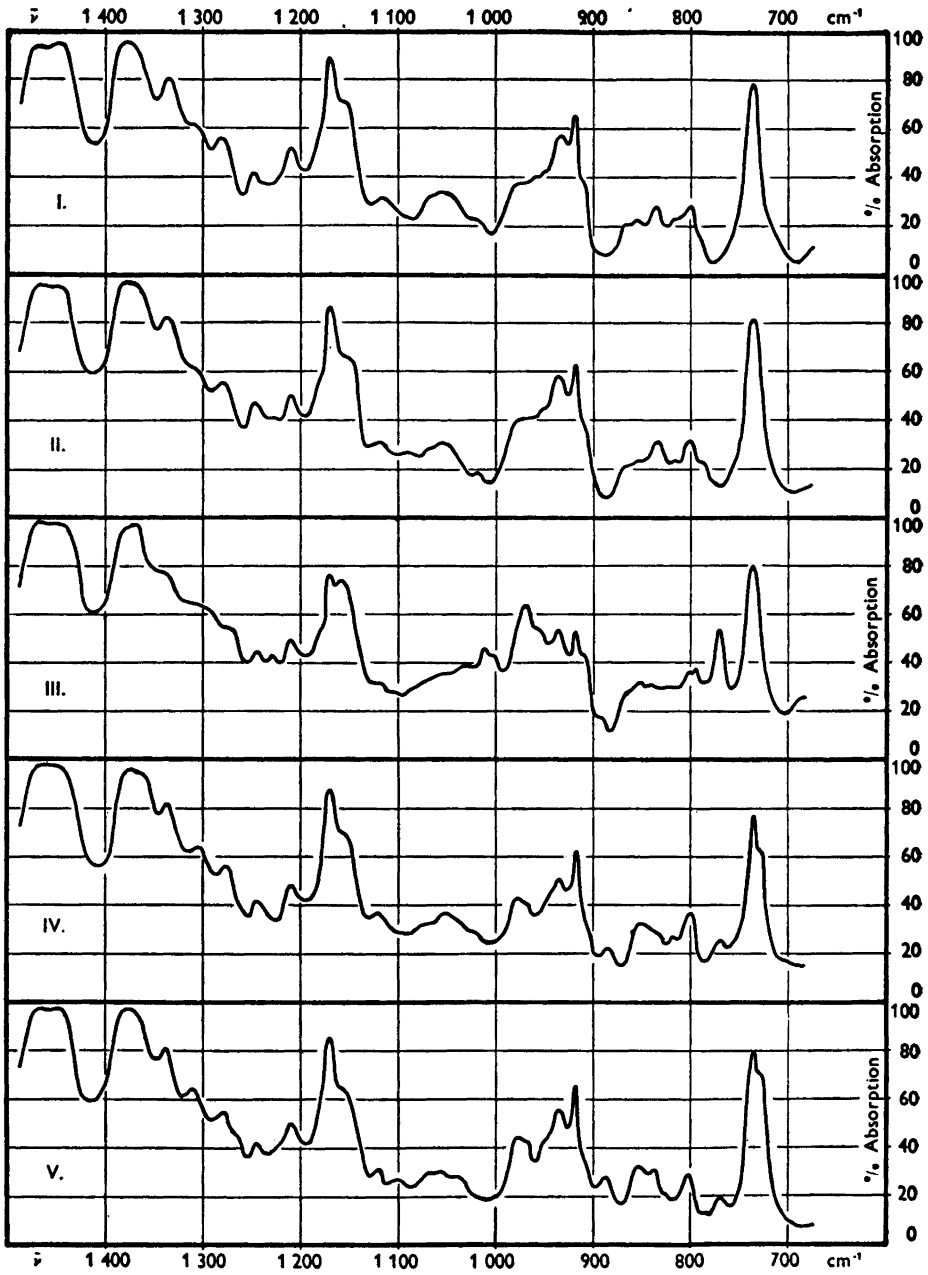

Fig. 1. I. Pristane. II. N o r phytane. III. Phytane. IV. Crocetane "A". V. Crocetane "B". Cell thickness 0.15 mm.

It may be concluded that the infra-red spectrum is sufficiently sensitive to small changes in constitution, even in the case of aliphatic hydrocarbons of chain length as great as C_{19} or C_{20}, to serve for distinguishing between such very closely related hydrocarbons, if properly applied.

The coincidence of the infra-red spectra is therefore considered conclusive evidence for the identity of pristane and *nor*phytane.

It may be added that the infra-red spectrum of phytane (2,6,10,14-tetramethylhexadecane) closely resembles the spectrum of farnesane (2,6,10-trimethyldodecane) and even that of hexahydromyrcene (2,6-dimethyloctane) which have been reported recently.[8] All these hydrocarbons possess carbon skeletons consisting of regularly arranged isoprene residues and differ only in the number of these units.

SUMMARY

The infra-red spectra of the saturated hydrocarbons pristane, *nor*phytane, phytane and of two samples of crocetane have been examined. These spectra furnish conclusive evidence for the identity of pristane and *nor*phytane (2,6,10,14-tetramethylpentadecane) which had previously been suggested on the basis of measurements of physical constants.

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Received May 5, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 850–855

The Synthesis of *trans*-Lachnophyllum Ester

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In 1935 the Russian chemists Wiljams, Smirnow and Goljmov [1] isolated a highly unsaturated aliphatic methyl ester m. p. 32.6—32.8° with the empirical formula $C_{11}H_{12}O_2$ from the plant *Lachnophyllum gossypinum* Bge, which belongs to the Compositae. On the basis of thorough degradation experiments they gave this compound the constitutional formula (I):

(IV) $CH_3-CH_2-CH_2-C\equiv CH$ $\quad$ $H-C\equiv C-CH=CH-CH_2OH$ (V)

(III) $CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-CH_2OH$

(I) $CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-COOCH_3$

In 1941 Sörensen and Stene [2] described a still more unsaturated methyl ester, m. p. 37°, containing 2 H less than the empirical formula of (I) ($C_{11}H_{10}O_2$) and having the same carbon skeleton, from scentless mayweed (*Matricaria inodora* L). On the basis of degradation experiments it was given the constitutional formula (II);

II) $CH_3-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3$

Since Wiljams *et al.* [1] could isolate maleic acid from the alkali decomposition mixture, (I) ought to occur in nature in the *cis* configuration and should be named *cis* lachnophyllum ester. The matricaria ester (II) may exist in four different stereoisomers. The exact configuration of the compound from scentless mayweed is as yet not known. The discoverers of this extremely unsaturated ester realized its transformation into another stereoisomer through the action of U. V. light. This isomeric matricaria ester showed higher molecular refraction and dispersion than the original compound and was named *trans* matricaria ester, a designation of only a preliminary nature.

No further occurrences, of these diacetylenic acids have been noted in literature, so they seem to be rather scarce in nature, at least in large amounts. The first compound of such structure encountered in nature, however, dates back to 1907, when there appeared a small note in the annals of the Ministry of Agriculture of the Dutch East Indies about etherial oils from plants collected in the Tenger Mountains of Java through Dr. Carthaus [3]. The oil from a plant which Dr. Carthaus named *Artemisia lavandulaefolia* yielded crystals of m. p. 32.5—33° on cooling. The chemical investigation of this crystalline compound, reported in the same annals for 1910 [4], in conjunction with our recent knowledge of the esters (I) and (II), substantiates that the crystalls of m. p. 32.5—33° and analysing for $C_{12}H_{14}O_2$ (misprint $C_{12}H_{14}O_3$ in Gildemeister, Hoffman) undoubtedly represent the first diacetylenic compound ever found in nature. Unfortunately the same interesting Dutch investigation from 1910, contains the remark that Dr. Carthaus must have been wrong in naming the starting material, since *Artemisia lavandulaefolia* was stated not to occur in the Tenger district.

In a forthcoming communication from this laboratory, however, we are going to demonstrate that at least among the compositae oils the matricaria ester (II) and very interesting relatives may be found in some cases, although mostly in small amounts.

With the intention of definitely establishing the constitutional formula given to the matricaria ester (II) one of us (C. M. H.) in 1942 began attempts to synthesize the all-*trans* compound with the constitution (II). When British periodicals became available after the war we became aware of the great progress in diacetylene syntheses in the laboratory of Sir Ian Heilbron at Imperial College, London. When Professor Heilbron learned about our experiments on the syntheses of (I) and (II) he kindly left the problem to us. Since the independent synthetic work at the Imperial College had developed very close to such compounds, we should like to express our sincere gratitude to him for this gesture.

Oxidative coupling of different 1-acetylenes to symmetrical diacetylenes has been carried out many times since C. Glaser introduced this synthesis in 1870 [5]. Zal'kind and Aizikovich [6] found that *tert.*acetylenic alcohols could be coupled to diacetylene glycols with good yields using molecular oxygen. *sec.* Acetylenic carbinols, however, gave bad yields [7]. In 1947 Bowden, Heilbron, Jones and Sargent [8] gave experimental conditions for the coupling of *primary* acetylenic carbinols with good yields.

The oxidative coupling of two different 1-acetylenes, first carried out by v. Baeyer [9], must give two symmetrical diacetylenes besides one asymmetrical compound. Since many synthetical experiments, *e. g.* the coupling of one Grignardized acetylene with the iodide of the other, failed completely, we tried the asymmetrical oxidative coupling of pentyne-1 (IV) and penten-3-yn-1-ol-5 (V) to decene-2-diyn-4,6-ol-1 (III), *i. e.* the alcohol corresponding to *trans* lachnophyllum acid. The two parts needed for the coupling synthesis have been made readily available through the investigations of Vaughn *et. al.* [10] and by Haynes *et. al.*[11].

The attempts at coupling the two above mentioned pentynes were begun using the conditions given by Heilbron *et. al.*[12] for the dimerization of penten-3-yn-1-ol-5 (V), but as work progressed, certain changes had to be made. The conditions given in the experimental part of this paper are those which have so far been found to be the best ones, but we do not claim them to be optimal.

Even in the best experiments most (79 %) of the penten-3-yn-1-ol-5 (V) was coupled to decadiene-2,8-diyne-4,6-diol-1,10. Of the pentyne-1 (IV) 23 % was converted to decadiyne-4,6. In the expected boiling range we got quite small amounts (12.7 % of theory) of a beautifully crystallizing carbinol m. p. 39—40° of the expected composition $C_{10}H_{12}O$.

The constitution of this carbinol (III) was confirmed through hydrogenation to *n*-decanol-1, characterized as the 3,5-dinitrobenzoate. Further, the U. V. spectrum of its hexane solution (Fig. 1, curve A) was in accordance with experience from the spectra of conjugated polyethylenes and related conjugated enynes, *viz.* the long wave absorption being weaker, but falling in the same region, the fine structure spacing, however, corresponding only to the dominant vibration frequency of bisubstituted acetylenes (67.10^{12} sec. $^{-1}$). See [13,14].

	λ_{max}	ε_{max}
$CH_3CHOH \cdot CH = CH-CH = C(CH_3) -CH = CH_2$ see [15]	2 590 ÅU	32 500
	2 670 »	40 500
	2 770 »	32 500
$CH_3-CHOH-CH = CH-CH = C(CH_3) -C \equiv CH$ see [15]	2 600 ÅU	35 500
	2 640 »	32 000
	2 690 »	27 500

	λ_{max}	ε_{max}	
$CH_3-CHOH-CH=CH-CH=CH-C\equiv CH$ see [16]	2 470 *	24 500	
	2 600	34 000	
	2 730 *	24 500	
$CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-CH_2OH$	2 395	7 300	$\Delta\nu \cdot 10^{-12}$
	2 520	14 600	62.1 cm^{-1}
	2 665	21 850	64.8 »
	2 825	19 440	63.9 »

* = inflections

The oxidation of the carbinol to acid was carried out according to the procedure of Bowden *et. al.* [17, compare 12]. Since the resulting acid, *n*-decene-2-diyn-4,6-oic acid, is very unstable, it was converted to the methyl ester with diazomethane. The ester distilled at 50—55° air bath/0.001 mm as a colourless liquid which

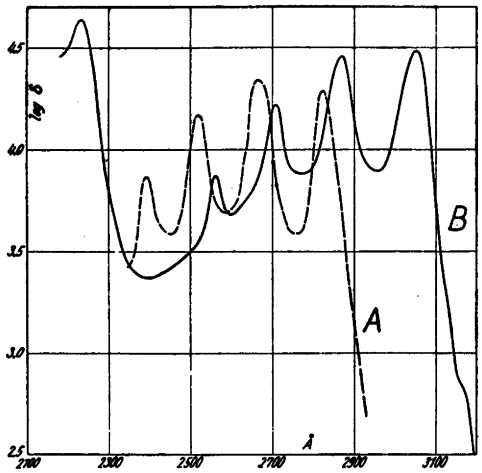

Fig. 1. Ultraviolet absorption of decene-2-diyn-4,6-ol-1 (III), curve A, and trans-lachnophyllum ester (I), curve B in hexan.

$(\varepsilon = \frac{lg\, I_0/I}{c \cdot d}$, *c in mol/l, d = cm)*

crystallized at low temperature. It was recrystallized from pentane at —10°, and then formed colourless needles, m. p. 18.5—19°.

We were rather surprised to find a m. p. definitely lower than that of the naturally occurring *cis* lachnophyllum ester which is reported to be 32.6—32.8°. As mentioned above the thorough degradation experiments of Wiljams *et. al.*[1] leave no doubt about the constitution of the naturally occurring ester.

However, the hydrogenation of the synthetic *trans* lachnophyllum ester gave methyl caprate with the consumption of exactly 5 moles of hydrogen. The methyl caprate was characterized, after saponification, as the amide, by m. p. and mixed m. p. with capric amide (94—95° and 94—96°, respectively).

Furthermore the *trans* lachnophyllum ester has a U. V. spectrum in hexane very similar to those of known conjugated diene-diynes. As set forth by Kuhn and Grundmann [18] the carbalkoxy group in conjugation with aliphatic unsaturated systems corresponds to one ethylene bond. The mean values of the long wave maxima for the known 5 conjugated diene diynes are:

$\lambda_{max,\ mean\ value}$ diene diynes	3 102	2 882	2 722	2 581 ÅU
trans lachnophyllum ester	3 053	2 872	2 710	2 568 »

Accordingly, the displacement of the ene diyne spectrum through a conjugated carbomethoxy group seems to be the same as in the polyene series.

EXPERIMENTAL

Pentyne-1 was prepared according to [11] and pentene-3-yn-1-ol-5 according to [12].

Decene-2-diyn-4,6-ol-1

Pentyne-1 (3.5 g) and penten-2-yn-1-ol-5 (4.1 g) were shaken with a solution of ammonium chloride (112.4 g), copper(I) chloride and conc. ammonia (2 ml) in water

(286 ml). To the solution was added iron(III) chloride (5.9 g) and air was bubled through for 6 ½ hours. The reaction product was isolated by repeated extraction with ether. Distillation of the reaction product at 70—80° (air bath)/0.001 mm yielded decadiyne-4,6 (0.8 g) in the trap (—80°), decene-2-diyn-4,6-ol-1 (III) (0.957 g) and decadiene-2,8-diyne-4,6-diol-1,10 (3.2 g) as a residue. The monocarbinol (III) which crystallized immediately, melted at 37.5—38.5°, raised to 39—40° by crystallization from petroleum ether. It formed long, thin needles

	$C_{10}H_{12}O$ (148.2)	Calc.	C 81.04	H 8.16			
		Found	» 80.80	» 8.1			
λ_{max}	2 395 ÅU		2 520 ÅU		2 665 ÅU		2 825 ÅU
ε_{max}	7 300 »		14 500 »		22 000 »		19 500 »

The 3,5-dinitrobenzoate of the acetylenic carbinol(III) melted at 105.5—106°, and crystallised in yellow needles from petroleum (b. r. 60—80°).

	$C_{17}H_{14}N_2O_6$ (342.4)	Calc.	C 59.65	H 4.12		
		Found	» 59.8	» 4.0		
λ_{max}	2 538 ÅU		2 691 ÅU			2 860 ÅU
ε_{max}	22 500 »		21 000 »			11 500 »

n-Decanol from decene-2-diyn-4,6-ol-1

The hydrogenation of decene-2-diyn-4,6-ol-1 (148 mg) was carried out in ethyl acetate with platinum as a catalyst.

$C_{10}H_{12}O$ (5)	Calc.	112 ml H_2	(0°, 760 mm)
	Found	115 » »	(0°, 760 »)

The 3,5-dinitrobenzoate of the hydrogenation product melted at 54—55° when recrystallized from petroleum ether; the mixture with the 3,5-dinitrobenzoate of n-decanol-1 (m. p. 56—57°) melted at 55—56.5°.

Methyl-*n*-decene-2-diyn-4,6-oate = *trans* lachnophyllum ester

Decene-2-diyn-4,6-ol-1(III) (2.5 g) in acetone (50 ml) was oxidized with a mixture of chromium trioxide (4.46 g) and sulphuric acid (3.86 ml conc.) in water (17.8 ml). The acetone solution was cooled in ice, and the oxidation mixture was added with stirring at such a rate as to keep the temperature at about 20°. The stirring was continued for 20 min, after all the oxidation mixture was added. At the end of this time, the reaction mixture was poured into much water and the reaction product was extracted with several portions of ether. The combined ether extracts were washed with water and extracted twice with sodium carbonate solution. From the sodium carbonate solutions the extracted acids were liberated with very dilute sulphuric acid and extracted with ether. To the ether solution was added a solution of diazomethane in ether. The ether solution was washed with water and dried with sodium sulphate for a few hours. The residue distilled at 50—55° (air bath)/0.001 mm (410 mg) and crystallized readily below 0°. Recrystallisation from pentane at —10 °C yielded colourless short needles, m. p. 18.5—19°.

$C_{11}H_{12}O_2$ (176.2)	Calc.	C 74.97	H 6.86	
	Found	» 75.1	» 7.0	

λ_{max}	2 235 ÅU	2 568 ÅU	2 710 ÅU	2 872 ÅU	3 053 ÅU
ε_{max}	44 000 »	6 850 »	16 500 »	29 000 »	30 100 »

Methyl caprate from *trans* lachnophyllum ester

trans Lachnophyllumester (32 mg) was hydrogenated with a 2 % palladium on barium sulphate catalyst.

Calc.	20.4 ml H_2	(0°, 760 mm)
Found	20.4 » »	(0°, 760 »)

The hydrogenation product was saponified with potassium hydroxide and the acid was converted through its acid chloride to the amide, which melted at 94—95° after crystallisation from petroleum (b. r. 60—80°). The mixture of this amide and the amide of capric acid (m. p. 97—98) melted at 94—96°.

SUMMARY

Decene-2-diyn-4,6-ol-1 was synthesized through an asymmetric oxidative coupling of pentyne-1 and penten-3-yn-1-ol-5. Decene-2-diyn-4,6-ol-1 was oxidized with chromium trioxide to the corresponding acid, *trans* lachnophyllum acid. The methyl ester of *trans* lachnophyllum acid has m. p. + 18.5—19°, compared with + 32.6—32.8° given for the methyl ester of *cis* lachnophyllum acid occurring in *Lachnophyllum gossypinum* Bge.

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Received April 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 856–860

The Iodine-Azide Reaction

IV. Chain Length of the Carbon Disulphide Catalyzed Reaction

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The kinetics of the iodine-azide reaction, which is a reaction between azide ions and iodine, has been studied by Dodd and Griffith [1], Hofman-Bang [2, 3], and Hofman-Bang and Szybalski [4], with potassium tetra- and pentathionate and carbon disulphide as the catalysts. The general picture is that the rate of reaction is proportional to the concentration of azide ions and catalyst, but independent of the concentration of iodine. The rate determining reaction step must therefore be a reaction between an azide ion and a catalyst ion or molecule. In the case of the carbon disulphide catalyzed iodine-azide reaction [4], the rate determining reaction step is without doubt the *first* reaction step in a sequence of reactions. The present author has mentioned [2] that the tetrathionate catalyzed iodine-azide reaction seems to be analogous, so that the *first* and rate determining reaction step is a reaction between an azide ion and a tetrathionate ion.

That a reaction takes place between azide ions and tetrathionate ions even if iodine is not present, can be shown by a rather simple experiment: Stock solutions of 0.1 *M* sodium azide, 0.05 *M* potassium tetrathionate, and 0.02 *N* iodine in 0.04 *M* potassium iodide, were kept at 25° C in a water thermostat. 10 ml potassium tetrathionate solution, 10 ml sodium azide, and 0.2 ml starch indicator were mixed in an Erlenmeyer flask and left for t minutes in the thermostat. 10 ml iodine solution was now added, and after an additional t' minutes the iodine-starch colour disappeared. From Table 1 it can be seen that when the time t was longer than approx. 20 minutes the reaction with iodine was instantaneous.

Quite analogous experiments could be carried out with carbon disulphide instead of potassium tetrathionate. In this case the product formed by interaction between azide ions and carbon disulphide molecules is known to be

Table 1. Reaction between sodium azide and potassium tetrathionate at 25° C. 10 ml 0.05 M potassium tetrathionate were mixed with 10 ml 0.1 M sodium azide solution and 0.2 ml starch indicator, and left for t minutes. 10 ml 0.02 N iodine solution was now added, and after an additional t′ minutes the iodine-starch colour disappeared.

Expt. no.	t Time in min	t' Time in min
1	0	12.18
2	5	10.02
3	10	7.18
4	15	3.87
5	20	0.78
6	25	0.00
7	30	0.00

azido-dithiocarbonate ions [4–6]. Such experiments with carbon disulphide showed that the ratio: number of iodine atoms consumed instantaneously per carbon disulphide molecule present, gradually increased with time to about 16. This shows that a chain reaction must take place.

CHAIN LENGTH OF THE CARBON DISULPHIDE CATALYZED IODINE-AZIDE REACTION

If a very diluted solution of sodium azide, a known amount of iodine, and starch indicator is titrated by dropwise addition of a diluted solution of sodium azido-dithiocarbonate until the iodine-starch colour disappears, it is to be expected that the ratio: atoms of iodine consumed per molecule of azido-dithiocarbonate should be about 16. It is, of course, a condition that the solutions are so diluted that any recombination of carbon disulphide molecules and azide ions to azido-dithiocarbonate ions can be neglected.

EXPERIMENTAL

Solutions used in the experiments: 1) A sodium azide solution, which was 0.1000 M; 2) an iodine solution, which was 0.00991 N with respect to iodine and 0.02 M to potassium iodide; 3) a sodium azido-dithiocarbonate solution, which was 0.0996 M. Sodium

Table 2. Determination of the chain length of the carbon disulphide catalyzed iodine-azide reaction. In each experiment were mixed 10 ml 0.1 M sodium azide, 10 or 20 ml 0.00991 N iodine, 1 ml starch indicator, and a considerable volume of water. This solution was titrated by dropwise addition of a 0.00198 M sodium azido-dithiocarbonate solution until the iodine-starch colour disappeared.

Expt. no.	Total volume ml	Sodium azide ml	Iodine ml	Sodium azido-dithio-carbonate used ml	Temp. of the solution °C	Chain length (atoms of iodine consumed per molecule of sodium azido-dithio-carbonate added)
1	500	10	10	3.18	23.2	15.7
2	250	10	10	2.93	23.1	17.1
3	500	10	10	3.10	14.1	16.1
4	500	10	20	5.99	23.7	16.7
5	500	10	10	3.10	34.2	16.1
6	500	10	10	3.06	24.1	16.4
7	1000	10	10	3.18	24.1	15.7

azido-dithiocarbonate was prepared according to Sommer [5] and analyzed according to Browne and Smith [7], who have worked out a modified Volhard titration for the determination of azido-dithiocarbonate. Before use 10 ml of the stock solution of sodium azido-dithiocarbonate were diluted with water to 500 ml. In each experiment 10 ml 0.1 *M* sodium azide, 10 or 20 ml 0.00991 *N* iodine, and 1 ml 0.5 % starch indicator were pipetted off into a 2 liter Erlenmeyer flask. After dilution with a considerable volume of water this solution was titrated with 0.00198 *M* sodium azido-dithiocarbonate solution. The flask was shaken vigorously after the addition of each drop of the azido-dithiocarbonate solution.

From Table 2 it is seen that the number of atoms of iodine consumed per molecule of sodium azido-dithiocarbonate added is fairly constant in all 7 experiments. It is independent of the azide ion concentration, the iodine concentration, and the temperature. Of course, there might be a slight dependency on temperature — which is not seen due to the experimental error. When the solution of sodium azide and iodine was titrated with a solution of sodium azido-dithiocarbonate 5 times stronger than the one used in the experiments, the results were about the same.

These results show that the carbon disulphide catalyzed iodine-azide reaction is a chain reaction with a chain length of 16—17 at room temperature.

DISCUSSION

Kinetic investigations [4] have shown that the rate determining reaction step of the carbon disulphide catalyzed iodine-azide reaction is formation of azido-dithiocarbonate ions:

$$N_3^- + CS_2 \rightarrow SCSN_3^-$$

Since Browne and Hoel [6] have shown that iodine reacts immediately with azido-dithiocarbonate ions with formation of azido-carbon disulphide, which again reacts with sodium azide solution with the evolution of nitrogen, it is reasonable to assume that the following reaction steps take place:

$$2\ SCSN_3^- + I_2 \rightarrow (SCSN_3)_2 + 2\ I^-$$
$$(SCSN_3)_2 + 2\ N_3^- \rightarrow 2\ CS_2 + 2\ N_3^- + 3\ N_2$$

Due to the above mentioned results, which show that the reaction is a chain reaction with a chain length of 16—17, the reaction mechanism must be assumed to be somewhat different. The chain reaction can be accounted for by the following sequence of reactions:

$$N_3^- + CS_2 \rightarrow SCSN_3^- \quad (1)$$

$$2\ SCSN_3^- + I_2 \rightarrow (SCSN_3)_2 + 2\ I^- \quad (2)$$

$$(SCSN_3)_2 + 2\ N_3^- \rightarrow 2\ \overset{*}{S}CSN_3^- + 3\ N_2 \quad (3)$$

$$\overset{*}{S}CSN_3^- \rightarrow CS_2 + N_3^- \quad (4)$$

$$\overset{*}{S}CSN_3^- \rightarrow SCSN_3^- \quad (4a)$$

In this sequence, reaction (1) is the rate determining, and all the other reactions are assumed to be very fast. The ion $\overset{*}{S}CSN_3^-$ in reaction (3) is assumed to be an activated ion, which can either decompose to a carbon disulphide molecule and an azide ion (reaction (4)) or give off energy to become an ordinary azido-dithiocarbonate ion (reaction (4a)). Two of these ions now can again react with iodine to give azido-carbon disulphide. Further it is necessary to assume that the activated ion $\overset{*}{S}CSN_3^-$ has a given probability of decomposing to carbon disulphide and azide, and consequently also a given probability of becoming an ordinary azido-dithiocarbonate ion. The probability must be independent of all concentrations and can according to the experiments only be very slightly dependent on the temperature. Let us

assume that the probability of decomposing into a carbon disulphide molecule and an azide ion is $\frac{1}{17}$. The chain length, S, would then be:

$$S = 1 + (1 - \frac{1}{17}) + (1 - \frac{1}{17})^2 + (1 - \frac{1}{17})^3 + \ldots\ldots = 17$$

From the proposed chain mechanism can be inferred that the energy of activation of the reaction between azide ions and carbon disulphide must be about the same as that of the carbon disulphide catalyzed iodine-azide reaction, because the chain length is rather independent of temperature. This has already proved to be true (*cf.* a following publication).

To decide if tetra- and pentathionate ions combine with azide ions to form ions analogous to azido-dithiocarbonate ions, is difficult because a chemical reaction takes place with the evolution of nitrogen [2, 3]. When a solution of potassium tetra- or pentathionate and sodium azide was left for some time and then allowed to run down into a solution of iodine and sodium azide, several molecules of iodine per molecule of polythionate were consumed immediately. Therefore it seems reasonable also in these two cases to assume a chain reaction taking place with intermediates analogous to azido-dithiocarbonate ions. A kinetic study of the nitrogen evolution from a solution containing tetrathionate and azide ions, could possibly give some valuable information.

SUMMARY

The catalytic effect of azido-dithiocarbonate ions on diluted solutions of iodine and sodium azide was studied. The reaction turned out to be a chain reaction with a chain length of about 17 at room temperature. A reaction mechanism has been proposed, in which the chain carrier is azido-dithiocarbonate ions. The chain length is independent of or only slightly dependent on the temperature. It was shown that tetra- and pentathionate ions react with azide ions with formation of a product which will give an instantaneous iodine-azide reaction.

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Received April 20, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 861–865

Purine and Pyrimidine Turnover of Ribo Nucleic Acid from Fractionated Rat Liver Cytoplasm

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In a recent publication Jeener [1] investigated the turnover of ribonucleic acid (PNA) in different parts of animal cells with the aid of isotopic phosphorus. For this purpose he applied the principles of the techniques of differential centrifugation as described by Hogeboom, Schneider and Pallade [2] for the separation of mitochondria, microsomes and "cell sap" from different organs. His results indicated that in all the organs investigated, PNA-phosphorus from various locations in one and the same cell contained different amounts of isotope, the results from the different fractions sometimes varying by as much as a factor of 40.

These results seemed to indicate the presence of at least metabolically different PNA's in one and the same cell. One might even suspect that the PNA in various parts of the cell might differ chemically. Such differences might be expected to show up on analysis for purine and pyrimidine bases.

The present investigation was carried out in order to investigate further these problems. Two different approaches seemed possible for this purpose. The first and more direct would have been to isolate mitochondria, microsomes and "cell sap" from some organ according to Hogeboom and co-workers' technique, isolate the PNA's from each fraction and analyze for purines and pyrimidines with the aid of existing methods [3]. This approach was not adopted, as it seemed that it would be difficult to avoid degradation of PNA during the preparation of the cell fractions. The second possibility was to investigate the turnover of the purines and pyrimidines with the aid of the stable isotope N^{15}. This approach was chosen and the isotope was administered in the form of glycine-N^{15} in order to permit correlation with previous experiments [4,5] which have been carried out in this laboratory with this substances. The most suitable organ for the present investigation seemed to be rat liver

because of its high content of PNA and the relative ease with which the different cytoplasmic fractions might be prepared. In order to get a higher and more significant labelling of the purines and pyrimidines without using too much of the isotope the synthesis of PNA was speeded up by partial hepatectomizing the rats before the administration of the isotope. After the rats had been killed the livers were rapidly removed and the cytoplasmatic cell fractions isolated according to the principles outlined by Hogeboom *et al*[2]. After preparation of PNA from the different fractions purines and pyrimidine nucleosides were isolated from each sample of PNA and analyzed for N^{15} in the mass spectrometer. The results are summarized in Table 1.

Table 1. Purines and pyrimidine nucleosides from PNA from rat liver cell fractions. The value $\frac{E_{max}}{\gamma N/ml}$ *is a test for purity with respect to foreign nitrogen. For standard values see Reichard*[7, 8].

	Excess N^{15}	$\frac{E_{max}}{\gamma N/ml}$
Adenine		
Mitochondria	0.224	0.177
Microsomes	0.224	0.183
"Cell sap"	0.234	0.177
Guanine		
Mitochondria	0.486	0.162
Microsomes	0.504	0.172
"Cell sap"	0.490	0.165
Cytidine		
Mitochondria	0.153	0.277
Microsomes	0.161	0.282
"Cell sap"	0.161	0.281
Uridine		
Mitochondria	lost	
Microsomes	0.176	0.332
"Cell sap"	0.185	0.312

As can be seen from the table the isotope contents of one and the same base from mitochondria, microsomes and cell sap are not significantly different from each other and thus under the present conditions do not indicate that a fractionation of cytoplasmatic PNA has occured.

EXPERIMENTAL

Administration of isotope. The isotope was administered as glycine-N^{15} (atom per cent excess = 32). 10 rats weighing about 200 g each were subjected to partial hepatectomy as previously described [4]. 16 hours after the operation each rat received a dose of 50 mg of glycine / 100 g of body weight by subcutanous injection. The same amount of isotope was given to each rat 2, 4, 6 and 8 hours after the first injection. Two hours after the last injection the rats were killed, the livers rapidly removed and after cutting into small pieces immersed in 200 ml ice cold 0.88 *M* solution of sucrose.

Isolation of cell fractions. The procedure was the same as that of Hogeboom *et al.*[2] only somewhat simplified. All operations described below were carried out in the cold. The pieces of liver in sucrose were homogenized in a Swedish mixer of the Waring blendor type ("hushållsassistent") at half speed until no macroscopic pieces could be distinguished. This usually took about 5—7 minutes. The resulting suspension was filtered through a double layer of muslin. Unbroken cells and cell nuclei were spun off by three more centrifugations at 600 g for 10 minutes in an »Ecco» centrifuge. The supernatant from the last centrifugation was poured off and centrifuged in a Korda angle centrifuge at 16.000 g for 1 hour. The sediment was considered to represent the mitochondria. The microsomes were obtained from the supernatant, which microscopically did not contain any mitochondria, by centrifugation at 60.000 g for 1 hour in an ultra centrifuge. The PNA in the supernatant from this centrifugation was precipitated by making the solution 70 % with respect to alcohol. None of the fractions were washed as the amount of material was rather limited and slight contamination of one fraction with another was not thought to be likely to effect the results seriously. Each fraction was dried with alcohol and ether.

Preparation of purines and pyrimidine ribosides. PNA's samples were prepared from each fraction according to the method of Hammarsten [9]. Purines and pyrimidine ribosides from each sample were then worked up according to methods worked out at this laboratory [6–8].

DISCUSSION

The present results sharply contrast with those obtained by Jeener using P^{32}. Before discussing this difference one must keep in mind that the experimental conditions in the present investigation were not the same as those used by Jeener. In the present work regenerating liver from the rat was the system investigated, whereas Jeener worked on mouse embryo, chicken embryo and pigeon crop gland. On the other hand the differences of turnover rate with P^{32} were obtained from such different organs that one might well expect them to be valid for all those organs from which the different cytoplasmatic fractions (mitochondria, microsomes and cell sap) can be isolated.

Another difference in the experimental conditions is the time chosen for the administration of the isotope. Because of the much smaller dilution which can be allowed for the stable nitrogen isotope as compared to radioactive phosphorus, the isotope administration was carried out over a period of 10 hours the total amount of isotope being divided into five doses. Jeener only

gave a single injection of isotope and killed the animals 2 hours later. These differences seem, however, to be far from enough to explain such fundamentally different results.

Neither are the slight differences in the methods of preparation of the cytoplasmatic fractions of any more considerable importance. As only one centrifugation has been applied it is not to be expected that complete separation of the fractions has been achieved. It seems especially likely that the isotope value of the mitochondrial purines and pyrimidines may have been considerably effected by the inclusion of microsomes in this fraction. This applies both to the present investigation and Jeeners original work. As the results, however, are more of qualitative than of quantitative significance this objection does not affect the main conclusion.

The main difference between Jeeners experiments and the present investigation, was the difference of the isotopic precursor used. The results obtained in this way as far as can be seen at present, seem to indicate that though phosphorus has a different turnover in different PNA fractions from cell cytoplasm, this is not true for the nitrogen of the purines and pyrimidines. This explanation can not of course be fully accepted until an investigation of fractionated PNA turnover from regenerating liver has been carried out with the aid of P^{32}.

The present results naturally do not indicate that there can not exist several PNA's within one and the same cell, each containing nitrogen with various different turnover rates. A significant difference between the turnover rates of the purines from cytoplasmic PNA and nuclear PNA has also been shown for the first time in an investigation from this laboratory on regenerating liver [4, 10]. The present author has also previously found some indirect evidence, that cytoplasmatic purines and pyrimidines may exist in different metabolic states [5]. The correct conclusions from the present experiment may therefore not necessarily be that there only exists one type of PNA in rat liver cytoplasm with respect to purine and pyrimidine turnover, but that the method of differential centrifugation may be too coarse for it to be possible to obtain a fractionation of metabolically different PNA's.

SUMMARY

After administration of glycine- N^{15}, PNA samples from mitochondria, microsomes and cell sap from regenerating rat liver were isolated. The isotope content from the corresponding purines and pyrimidines did not show any significant differences. The results were thus not in agreement with those obtained by Jeener [1] with P^{32}.

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Received April 27, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 866–869

Thiosulphate Catalysis on Mixtures of Tetrathionate and Monoselenotrithionate

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In neutral, weakly alkaline and weakly acid solutions tetrathionate undergoes a slow rearrangement into trithionate and pentathionate:

$$2\ S\begin{matrix}\diagup SO_3^- \\ \diagdown S_2O_3^-\end{matrix} = S(SO_3)_2^{--} + S(S_2O_3)_2^{--} \qquad (1)$$

This change is strongly catalyzed by thiosulphate [1–4]. The catalysis is due to the following equilibria [1–3]:

$$S\begin{matrix}\diagup SO_3^- \\ \diagdown S_2^*O_3^-\end{matrix} + S_2^*O_3^{--} \rightleftharpoons S(S_2^*O_3)_2^{--} + SO_3^{--} \qquad (2)$$

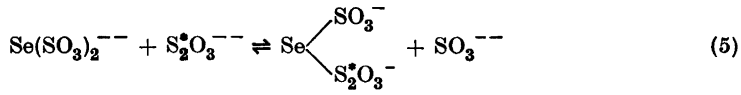
$$S\begin{matrix}\diagup SO_3^- \\ \diagdown S_2^*O_3^-\end{matrix} + SO_3^{--} \rightleftharpoons S(SO_3)_2^{--} + S_2^*O_3^{--} \qquad (3)$$

In presence of a slight excess of sulphite, the equilibria are displaced completely to the side of thiosulphate. If sulphite is removed as hydrogen sulphite [2, 5], or by use of formaldehyde in buffered solutions [6], the equilibria are displaced slowly to the side of sulphite. The reactions with sulphite are nucleophilic displacements of thiosulphate by sulphite [7, 8], as indicated through stars in Eqs. (2) and (3) above. By analogy, the reverse reactions can hardly be anything else than displacements also, *viz.*, of sulphite by thiosulphate.

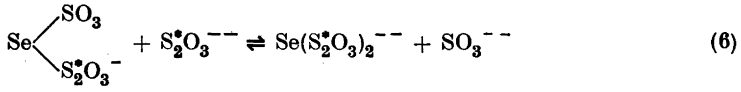
Monoselenotrithionate reacts more readily with thiosulphate than do trithionate and tetrathionate [9]:

$$Se(SO_3)_2^{--} + 2\ S_2^*O_3^{--} \rightleftharpoons Se(S_2^*O_3)_2^{--} + 2\ SO_3^{--} \qquad (4)$$

and this equilibrium is displaced farther to the side of sulphite than are the equilibria (2) and (3). It is, presumably, composed of two separate equilibria, in analogy with (2) and (3):



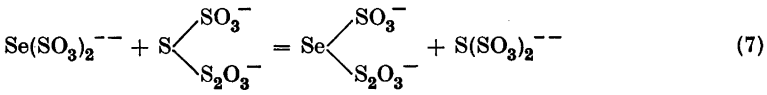
(5)



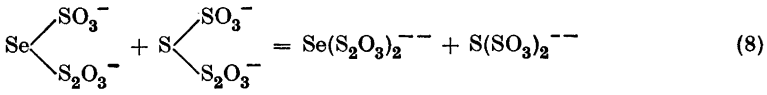
(6)

The intermediate, monoselenotetrathionate, has not been isolated in a pure state, although Heuer[10] stated to have obtained the potassium salt in mixture with potassium tetrathionate.

In a mixture of tetrathionate or pentathionate with monoselenotrithionate or monoselenotetrathionate, in presence of thiosulphate, there should, as a result of the equilibria (5) and (6) which are displaced to the side of sulphite, and the equilibria (2) and (3) which are displaced to the side of thiosulphate, take place a rearrangement, with a decrease of the sulphur content of the polythionate and an increase of the sulphur content of the monoselenopolythionate. *E. g.*, thiosulphate should catalyze the changes:



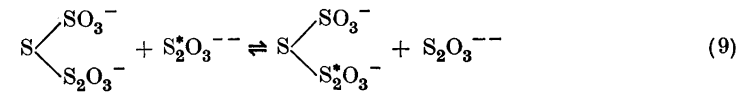
(7)



(8)

In fact, in a mixture of tetrathionate and monoselenotrithionate, in presence of thiosulphate, the content of monoselenotrithionate gradually decreases. This decrease is, presumably, due to the processes (7) and (8) or, depending on the relative amounts of tetrathionate and monoselenotrithionate present, to (7) alone. Of these processes, (8) bears a close resemblance with the rearrangement (1) of tetrathionate into trithionate and pentathionate.

In an earlier paper[7] it was suggested that a different equilibrium:



(9)

might be responsible for the thiosulphate catalysis on the rearrangement (1) of tetrathionate, in analogy with the catalysis of thiosulphate on the decompositions of pentathionate and hexathionate[7, 11]. Such an equilibrium probably exists; however, it now seems more likely that it proceeds simultaneously with and independently of the catalyzed rearrangement. If so, there are two modes of interaction of thiosulphate with tetrathionate, corresponding to Eqs. (2) and (9). Both reactions involve a nucleophilic attack by thiosulphate on one of the divalent sulphur atoms of tetrathionate, but in different directions. Each of the divalent sulphur atoms of tetrathionate forms a bridge between a sulphite and a thiosulphate group. In (2) the sulphite group is displaced by thiosulphate, whereas in (9) the thiosulphate group is displaced, also by thiosulphate. The nucleophilic reactivity of sulphite is higher than that of thiosulphate, in displacements on sulphur, as evidenced by the fact that the equilibria (2) and (3) are displaced far to the side of thiosulphate. If other effects do not enter, thiosulphate should thus displace the thiosulphate group (Eq. 9) markedly faster than it displaces the sulphite group (Eq. 2).

EXPERIMENTAL

5 millimole each of potassium tetrathionate and potassium monoselenotrithionate were used in each experiment, and 25 ml of a buffer, being 0.5 *M* with respect to dihydrogenphosphate and 0.1 *M* with respect to monohydrogenphosphate. Thiosulphate was added as a catalyst, and the volume adjusted to 100 ml. For analysis, 20 ml samples were pipetted out, into a mixture of 20 ml of the phosphate buffer described above, 20—25 ml of 0.1 *N* sodium thiosulphate, 1 ml of 40 % formaldehyde, and 100 ml of water. After standing for 5 minutes, 20 ml of 10 % acetic acid were added, and the excess of thiosulphate was back-titrated with 0.1 *N* iodine. This procedure[9] gives the content of monoselenotrithionate according to Eq. (4) and, presumably, the content of monoselenotetrathionate according to Eq. (6). Tetrathionate has no effect on this analysis, because the formaldehyde removes the sulphite before it can react with tetrathionate[9].

Table 1. Per cent decrease in amount of thiosulphate consumed (originally 20 ml of 0.1 N thiosulphate).

Thiosulphate added as a catalyst	Time of standing 30 min	1 hour	2 hours
None	1.25	2.15	2.50
1 ml 0.1 *N*	5.10	8.00	11.95
5 » 0.1 »	14.25	20.75	29.35
10 » 0.1 »	21.50	29.50	41.10

The catalysis manifested itself through the gradual appearance of a greenish colour in the solutions. During the analyses, in the cases where the decrease in titer value was more than about 20 %, a red selenium colour developed one or two minutes after the thiosulphate had been added; however, the end points in the titrations were sharp.

SUMMARY

Thiosulphate exerts a catalytic effect on mixtures of tetrathionate and monoselenotrithionate. The mechanism of the catalysis is analogous to that of the thiosulphate catalysis on tetrathionate alone.

The author is indebted to *Styret for Nansenfondet og de dermed forbundne fond* for a grant.

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Received May 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 870–877

Light Absorption Measurements on Turbid Solutions

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In a previously published paper [1], one of the authors described a simple and rapid method of measuring the turbidity of a suspension. The method is insensitive to large variations in the color of the continuous phase. One of the commercially available photoelectric colorimeters can be used without any alterations.

It is often of interest to measure the absorption of a more or less turbid or opalescent liquid photoelectrically. Usually the problem is solved by removing the turbidity by filtration or centrifugation. In special cases, however, these methods are not applicable, or at least difficult to perform.

A rational solution of the problem of how to make colorimetric measurements on turbid solutions is the use of an integrating sphere. Dognon [2, 3] describes an instrument consisting of a light source surrounded by the solution to be studied contained in a sample holder shaped as nearly as possible like a spherical shell, the whole being placed in the center of an integrating sphere. The principle is evidently sound, but due to the difficulties in realizing the theoretical requirements of the construction, Dognon was only able to use his instrument for relative measurements.

We propose to show how the problem can be solved with the aid of a photoelectric colorimeter provided with ample space for the sample holders.

THEORY

We assume that a beam of parallel light enters a suspension in a plane parallel sample holder at right angles. The continuous phase is light-absorbing. The light is received by a photocell on the other side.

The notations used are:

I_0 = intensity of light entering the suspension
I = intensity of transmitted (undeviated) light
I_s = total intensity of scattered light
αI_s = fraction of scattered light, striking the photocell
I_a = intensity of absorbed light
x = distance between suspension and photocell
d = length of light path through suspension
T_x = intensity of light received by the photocell when the distance from the suspension is x.

We can consider the sample holder as being divided into two compartments, each having the same thickness as the original one. The first is filled with the coloring matter, the second with the light scattering particles. This means that we tacitly assume the turbidity to be so low, that the average distance covered by a light ray in the colored and turbid medium is not appreciably increased.

After having passed through the first compartment, the light intensity is reduced to:

$$I_0 - I_a = I_0 \; e^{-k'c'd} \tag{1}$$

(k' = extinction coefficient and c' = concentration of the coloring matter). The problem is to determine either k' or c' when the other is known.

Part of the light intensity ($I_0 - I_a$) is scattered in the second compartment. If Lambert's law is valid, we have

$$I_t = (I_0 - I_a) \; e^{-\varkappa d} = I_0 \; e^{-(\varkappa + k'c')d} \tag{2}$$

($\varkappa$ = extinction coefficient for the turbid medium).

The intensity of light received by the photocell, when the sample holder is in position x_1 (close to the photocell) is

$$T_{x_1} = I_t + \alpha_1(I_0 - I_a - I_t) \tag{3}$$

or rearranged

$$I_a = I_0 - \frac{1}{\alpha_1}\left[T_{x_1} - I_t \; (1 - \alpha_1)\right] \tag{4}$$

By making measurements with the sample holder filled first with distilled water and then with the colored and turbid medium, relative values of the light intensities I_0 and T_{x_1} are obtained directly. By measuring T_x for increasing distances between sample holder and photocell and extrapolating to

infinity, I_t may also be calculated (see previous paper). Only if α_1 is known in advance, can I_a be calculated from (4) and introduced into (1). It is seen that the result is quite independent of the validity of (2). In other words it does not matter if the scattering follows Lambert's law.

The possibilities of determining α_1 will be discussed in detail. For convenience this quantity is, in the following, sometimes called "scattering coefficient". The value of α_1, *i. e.* the relative amount of the scattered light which strikes the photocell when the sample holder is in position x_1 close to the photocell, depends firstly on the experimental arrangements, especially on the solid angle through which the photocell is seen from the turbid solution. Secondly, the character of the suspension, especially the size and shape of the scattering particles, determines the intensity of the scattered light in different directions (see *e. g.* ref. 4—7). Thirdly, it will be seen, that α_1 also depends on the concentration of scattering substance, probably because the secondary scattering increases with the concentration.

It is not possible to calculate the value of the scattering coefficient theoretically, not even at infinite dilution. If the scattering followed Rayleigh's classical law, this might be possible, but Rayleigh had to presuppose that the scattering particles were small compared with the wavelength of the light. This condition is seldom fulfilled and although much work has been devoted, especially by Debye and by Mark and co-workers (for reviews see ref. 5—7), towards extending the theory to larger scattering particles, the theoretical approaches are far too complicated to be of any use in the cases considered here.

Values of α_1 are determined from an evaluation curve calculated by means of eq. (9) in the previous publication. It is necessary to have available suspensions of different turbidity (including a clear solution), either colorless or with a constant light absorption. In the actual absorption measurements the amount of scattering substance in grams per liter will seldom be known and is, moreover, without interest. It is therefore recommended that the scattering coefficient be plotted against I_s/I_0 instead. As the value of I_s cannot be calculated until I_a is known, the correct value of α_1 to be put into (4) must be determined by successive approximations.

In cases where an evaluation curve of the type described cannot be made, it may be possible to measure the scattering coefficient separately on the same suspension, the absorption of which should be determined, simply by changing the light filter to a wavelength with no appreciable light absorption. Although the amount of scattered light of course varies strongly with the wavelength as predicted by Rayleigh's law, measurements described below indicate that the variation in α_1 is much smaller.

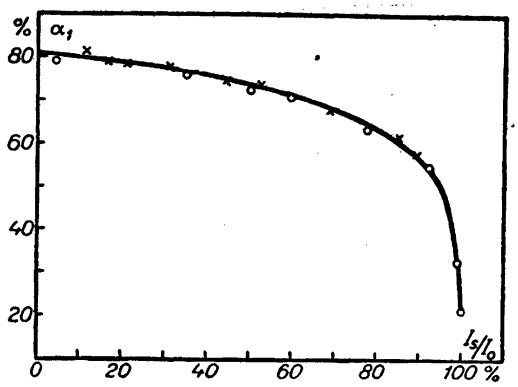

Fig. 1. The scattering coefficient α_1 as a function of the relative intensity of scattered light I_s/I_0 for dilute rubber latex and turbidity standard suspensions. Calculated from Figs. 3 and 6 in previously published paper.
× *rubber latex* O *turbidity standard*

Should none of the possibilities described above be practicable it is still feasible to determine acceptable values of the scattering coefficient on suspensions similar to the ones used in the absorption measurements.

The model experiments to be described show that formula (4) gives correct values as long as the turbidity is not too large. When the turbidity exceeds a certain limit, the values of I_a found from (4) tend to increase, *i. e.* the correction for turbidity is too small. An explanation is easily found. In deriving the formulas above we have assumed the turbidity to be so low, that the distance covered by a light ray in the sample holder is not increased by the turbidity. Due to the scattering, this distance must increase with the turbidity, which means that more light is absorbed.

It is outside the scope of this investigation to attempt a theoretical calculation of the effect. A semi-empirical correction can, however, be applied by assuming that in the formal division of the sample holder into two parts, the one filled with the coloring matter has a light path $(d + \Delta d)$ instead of d.

Formula (4) still gives the true amount of light absorption for the colored and turbid medium. For the light absorption corresponding to the light path d we get

$$I_a = I_0 - \frac{e^{k' c' \Delta d}}{\alpha_1}\left[T_{x_1} - I_t\,(1 - \alpha_1)\right] \qquad (5)$$

which is identical with (4) when $\Delta d = 0$.

EXPERIMENTS

In Fig. 1, values of the scattering coefficient for rubber latex and turbidity standard suspensions taken from the previous publication have been plotted against I_s/I_0. Although these types of suspensions are distinctly different with

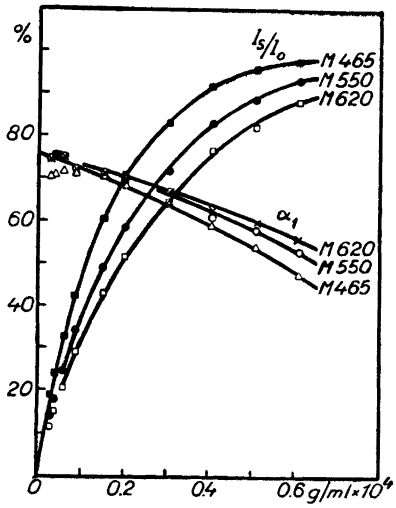

Fig. 2. Intensity of scattered light and scattering coefficient as a function of latex concentration for different light filters.

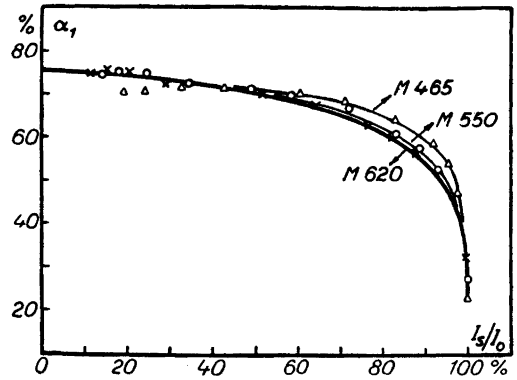

Fig. 3. Scattering coefficient plotted against relative intensity of scattered light. See Fig. 2.

regard to scattering properties, the values of α_1 practically coincide. The explanation probably lies in the very pronounced forward scattering, giving values of α_1 as high as 80 %. In the position x_1, close to the photocell, most of the scattered light is received by the photocell in any case. Differences in the intensity distribution begin to become appreciable at larger distances between the photocell and sample holder.

Figs. 2 and 3 show on dilute latex suspensions how I_s/I_0 and α_1 vary with the wavelength. When plotted against concentration, the variation of the scattering coefficient is much smaller than the variation of the percentage of scattered light. When α_1 is plotted against I_s/I_0 the differences almost disappear. It should be noted that the experimental errors in the determination of α_1 are largest at small turbidities.

In order to test eq. (4) model experiments were made on solutions of copper sulphate in ammonia with and without addition of rubber latex suspension. The copperammine complex has a distinct absorption maximum around 620 mμ [8]. Three different copper concentrations were chosen: 40, 160 and 400 ppm. Values of α_1 at different latex concentrations (Fig. 4) were found to be almost independent of the copper concentration between 0 and 160 ppm but slightly lower at 400 ppm. It seems therefore advisable to investigate the possible color dependence of α_1 in cases where large variations in absorption are expected.

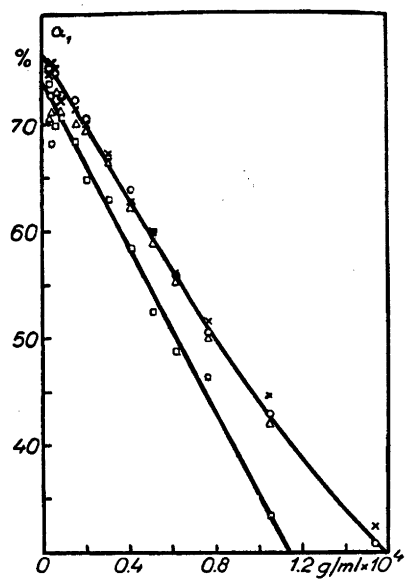

Fig. 4. Comparison between values of scattering coefficient for different copper concentrations. × = 0 ppm Cu^{++}. ○ = 40 ppm Cu^{++}. △=160 ppm Cu^{++}. □=400 ppm Cu^{++}.

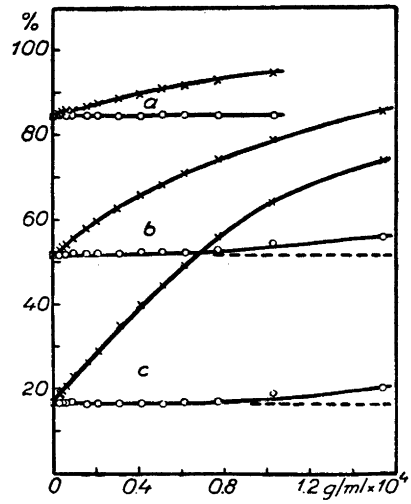

Fig. 5. Relative light absorption as function of latex turbidity. a: 400 ppm Cu^{++}; b: 160 ppm Cu^{++}; c: 40 ppm Cu^{++}. Filter M 620. × apparent value of absorption, the turbidity being neglected. ○ absorption I_a in percent calculated from (4).

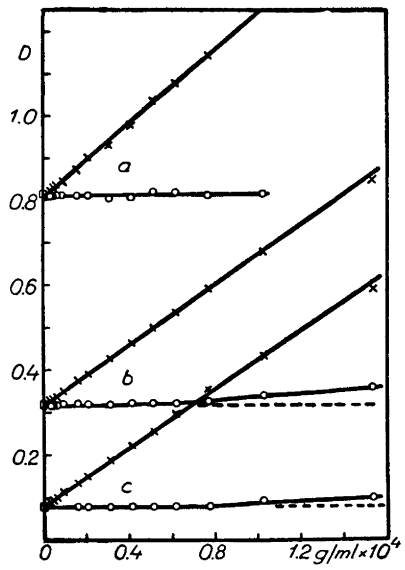

Fig. 6. Extinction as a function of latex turbidity (see Fig. 5).

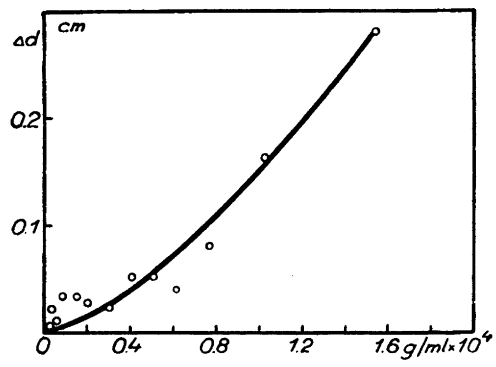

Fig. 7. Δd calculated from eq. (5) as a function of latex concentration. Copper concentration 160 ppm.

In Fig. 5, the values of $(I_0—T_{z_1})$, that is, the uncorrected values of the light absorption, are compared with I_a calculated according to (4). Fig. 6 gives the corresponding extinction values D. Eq. (4) is valid up to latex concentrations of $0.6—0.8 \cdot 10^{-4}$ g/ml, which corresponds to values of $I_s/(I_0—I_a)$ of around 90 %, a considerable turbidity.

For higher values of the turbidity the use of eq. (4) still gives fairly good values for the light absorption. By means of eq. (5) values of Δd have been calculated for one of the three copper concentrations used (Fig. 7).

DETAILS OF THE MEASUREMENTS

The instrument used was a Lumetron Photoelectric Colorimeter, Model 402 E (from Photovolt Corp., New York, USA).

Length of light path through the sample holder 2 cm. in all experiments.

The spectral transmittance of the light filters used was measured with a Beckman spectrophotometer.

Filter	Transmission range
M 465	435—480 mμ
M 550	530—570 mμ
M 620	610—640 mμ

The rubber latex (from Trelleborgs Gummifabriks AB) had a solids content of 61.5 % (drying 3 hours at 105° C) and was diluted with ammonia (approx. 5 %). Solutions of copper sulphate were also made with 5 % ammonia.

The extrapolation of the transmission readings to infinite distance between sample and photocell was made as previously described.

SUMMARY

When light absorption measurements are made with photoelectric colorimeters on turbid solutions, the values obtained will be too high due to the scattering. A way to overcome this difficulty is described. It is necessary to know a quantity, for convenience called "scattering coefficient", which gives the percentage of scattered light reaching the photocell when the sample holder is placed close to it. The possibilities of measuring the scattering coefficient are discussed in detail. Model experiments have been made on solutions of copper sulphate in ammonia with varying amounts of rubber latex added. Correct values of the light absorption are obtained up to turbidities corresponding to 90 % scattering of the non-absorbed light. At higher turbidities a moderate deviation is found. A possible explanation for this is given and a semi-empirical correction formula suggested.

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Received April 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 878–884

The Alkaline Hydrolysis of Phenyl β-Glucosides

A Kinetic Investigation

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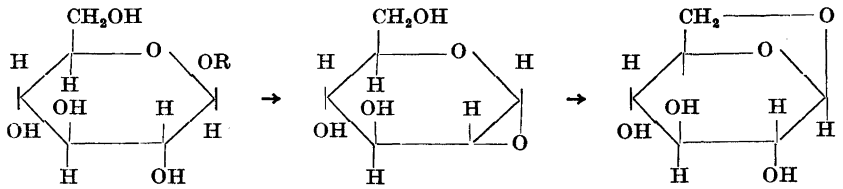
Glycosides generally are stable toward alkali, but several exceptions are known. The best known type of alkali-labile glycosides is phenyl β-glucosides. In 1894 [1] Tanret proved that picein and some other glucosides yielded 1,6-anhydro-β-glucopyranose or levoglucosan, by the action of alkali. Montgomery, Richtmeyer and Hudson [2] have proved that this is a general reaction for phenyl β-glucosides, and have used the reaction for the preparation of levoglucosan and similar 1,6-anhydrides in excellent yields [3].

The mechanism of this reaction has been studied by McCloskey and Coleman [4]. The starting material as well as the final product have β-configurations, so one must assume that the reaction involves an even number of Walden inversions. The direct formation of levoglucosan without any inversion is improbable, so the most simple assumption is that the reaction proceeds over an intermediate and involves two inversions. This intermediate, according to McCloskey and Coleman, is probably 1,2-anhydro-α-glucopyranose, because the reaction is blocked by the presence of a methoxyl group at carbon 2. Another consequence of this theory is that the phenoxyl group must be in *trans* position to the hydroxyl group at carbon 2 and in *cis* position to the terminal CH_2OH group if a phenyl glycoside is to be sensitive to alkali and yield a 1,6-anhydride. These restrictions are in excellent agreement with the experimental results.



Phenyl β-glucoside — 1, 2-Anhydro-α-glucose — Levoglucosan

* Billeruds AB Research Fellow 1949–1950.

Fisher, Hawkins and Hibbert [5] have studied the kinetics of the alkaline hydrolysis of some phenyl glucosides. All aglucones in their investigation, except phenol itself, contained methoxyl groups in *ortho* position to the hydroxyl groups and therefore, on account of steric effects, their material is not suitable for a quantitative treatment. From their experiments, however, as well as from the semiquantitative measurements of Montgomery, Richtmeyer and Hudson [2], it is clear that the velocity of the reaction increases with increasing acidity of the aglucone.

In the present investigation, the alkaline hydrolysis of a number of phenyl β-glucosides, substituted in *meta* or *para* position, has been studied kinetically. The reaction was found to be of second order, depending upon the concentrations of the hydroxyl ion and of the glucoside.

$$v = k\ [OH^-]\ [\text{phenyl } \beta\text{-glucoside}]$$

It was convenient to work with a large excess of alkali, so that the kinetics could be calculated as a first order reaction. The effect of the alkali concentration was therefore determined in a separate series of runs (Table 1). The excess of alkali was about 10-fold.

Table 1. Hydrolysis of p-chlorophenyl β-glucoside at 100° C.

c_{OH^-}	$k \cdot 10^3$
1.87	3.73
0.93	3.43
0.465	3.51

The glucosides of seven different phenols were investigated. All the runs were made att 100° C. The reactions were studied polarimetrically, except for *p*-nitrophenyl β-glucoside. This glucoside was so sensitive to alkali that very dilute solutions had to be used and the rotations of the solutions were consequently very small. In this case the concentration of nitrophenoxyl ion, formed by the hydrolysis, was determined photoelectrically. The results are summarized in Table 2.

For reactions which occur in the side chain of *meta* or *para* substituted benzene derivatives, Hammet's equation [6] should be valid.

$$\log k - \log k_0 = \varrho \cdot \sigma$$

Table 2. Velocity constants for the hydrolysis of some substituted phenyl β-glucosides at 100° C.

Substituent in the phenyl group	c_{OH^-}	σ *	k
$p-OCH_3$	1.87	− 0.268	$3.6 \cdot 10^{-4}$
$p-CH_3$	1.87	− 0.170	$3.0 \cdot 10^{-4}$
$m-CH_3$	1.87	− 0.069	$6.6 \cdot 10^{-4}$
None	1.87	0.000	$9.7 \cdot 10^{-4}$
$p-Cl$	1.87	+ 0.227	$3.7 \cdot 10^{-3}$
p-CH_3CO	0.208	+ 0.874	$7.5 \cdot 10^{-2}$
$p-NO_2$	0.0185	+ 1.27	2.0

* The values of σ are taken from Hammet [6].

Here k and k_0 are velocity (or equilibrium) constants for the substituted and unsubstituted benzene derivatives. ϱ is the reaction constant and σ is the substituent constant. This linear relationship was found to be valid for the alkaline hydrolysis of phenyl β-glucosides. In Fig. 1 the values of log k are plotted against the corresponding values of σ. The ϱ value was + 2.5.

When the values of log k are plotted against the pK_s values for the phenols (48.9 % ethanol, $t = 20°$ C), determined by Schwarzenbach and Rudin [7], a still better agreement is obtained (Fig. 2). This is quite natural since the systematic errors are smaller the more related the compared substances are. It is evident that the σ-value for p-OCH_3(—0.268) is too low in this case. Both the pK_s of the phenol and the log k for the glucoside indicate a higher value (about — 0.13). These irregularities are clearly shown if one compares the p-OCH_3 derivatives with the corresponding p-CH_3 derivatives. Of the acids, the p-CH_3 is the stronger, but if one compares the phenols, the p-OCH_3 is somewhat more acidic.

In none of the reactions studied was the final rotation in agreement with the assumption that all the phenyl β-glucoside had been converted to levoglucosan. If one assumes that the byproduct is glucose, which under these conditions is transformed into substances without any optical rotation, the yield of levoglucosan can be calculated to 80—90 % in good agreement with the values found by Montgomery, Richtmeyer and Hudson [2]. This side reaction does not seem to influence the kinetics of the reaction appreciably.

If, according to McCloskey and Coleman, 1,2-anhydro-α-glucose is an intermediate in this reaction, the formation of this substance must be the slow, rate determining step of the reaction, and its transformation into levo-

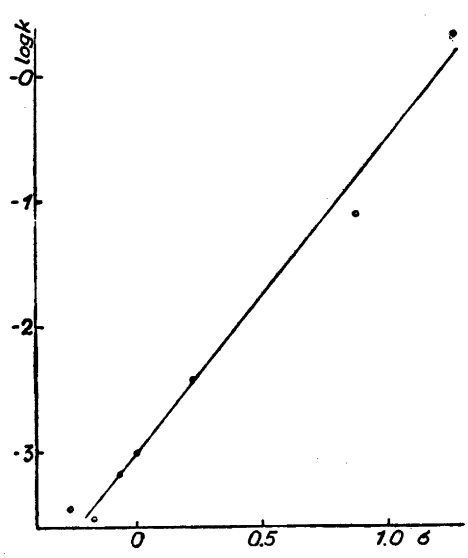


Fig. 1. Linear relationship between log k and Hammet's σ-values.

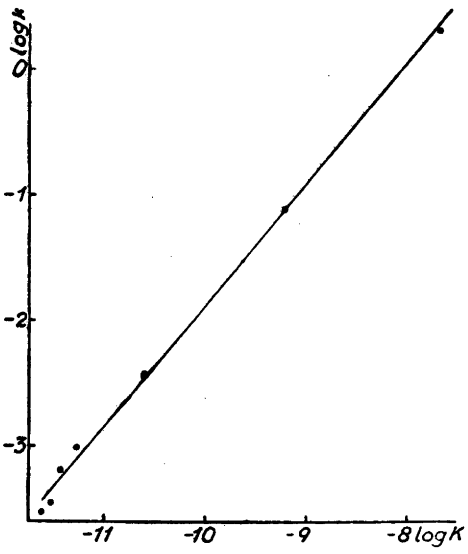


Fig. 2. Linear relationship between log k and log K for the corresponding phenols.

glucosan must be rapid. Only in this case could one expect the kinetics actually found. This hypothetical intermediate is not known * but its triacetate has been prepared by Brigl [8]. When this substance is treated with water, the ethylene oxide ring is split up and α-glucose triacetate is formed. If the substance were treated with strong alkali, however, one might expect that the ester groups (or at least a great deal of them) would be hydrolized before the ethylene oxide ring reacted. Then the solution would contain 1,2-anhydro-α-glucose, which, if the above theory were correct, would yield levoglucosan. When the 1,2-anhydride triacetate was dissolved in 1.25 *N* KOH in water-dioxane (2 : 1) at room temperature, the rotation of the solution decreased rapidly to negative values, indicating the formation of levoglucosan. This substance also was isolated from the reaction mixture. The theory of McCloskey and Coleman has thus been strongly supported.**

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* Pictet and Castan claim to have synthesized this substance (*Helv. Chim. Acta* **3** (1920) 645). Their results, however, are disputed by Brigl (*Ber.* **62** (1929) 1716). It seems rather certain that their substance, which could not be obtained in a crystalline state by other investigators, can not be 1,2-anhydro-α-glucose. The present investigation also supports Brigl's opinion.

** (Added in proof)

In the 25 May issue of *Chemtcal Abstracts*, **44** (1950) 4425, a paper by Bardolph and Coleman [*J. Org. Chem.* **15** (1950) 169] is abstracted. They have, previous to us, demonstrated that levoglucosan is formed by the action of alkali on triacetyl-1,2-anhydro-α-glucose.

EXPERIMENTAL

The glucosides used in this investigation are, with one exception, described in the literature. They were synthesized as tetraacetates by conventional methods and deacetylated catalytically with potassium ethoxide in ethanol [9]. For *m*-cresyl β-glucoside and its tetraacetate, we were not able to obtain the melting points recorded by Helferich and Philipp [10]. Our highest values for the glucoside and its acetate were 174—175° and 109—111° while Helferich and Philipp report 183—184° and 137—139°, respectively. Our values for the optical rotation, however, were in excellent agreement with those of H. and P.

p-Chlorophenyl β-glucoside tetraacetate

p-Chlorophenyl β-glucoside tetraacetate was prepared from β-glucose pentaacetate and *p*-chlorophenol, with *p*-toluenesulfonic acid as catalyst, using the fusion procedure of Helferich and Schmitz-Hillebrecht [11]. The substance was recrystallized from ethanol $[\alpha]_D^{20} = -20.0°$ (Chloroform $c = 2$). Yield 65 %. M. p. 123—124°, (Uncorr).

$C_{20}H_{23}O_{10}Cl$ (458.8)	Calc.	Acetyl CH_3CO	37.5
	Found	» »	37.3

p-Chlorophenyl β-glucoside

p-Chlorophenyl β-glucoside tetraacetate was deacetylated catalytically with potassium ethoxide in ethanol according to Bonner and Koehler [9]. The yield of deacetylated product was almost quantitative. After two recrystallizations from ethanol, the substance melted at 173—174° and further recrystallizations did not change this value. $[\alpha]_D^{20} = -69.5°$ (Water, $c = 1$).

$C_{12}H_{15}O_6Cl$ (290.7)	Calc.	Cl	12.2
	Found	»	12.1

Procedure for the kinetic determination

The alkaline hydrolysis of six phenyl β-glucosides were studied polarimetrically. The results are summarized in Table 2. Here only a typical series of runs is described.

Samples of phenyl β-glucoside (0.6 g) were dissolved in 1.87 *N* potassium hydroxide (10 ml), preheated to 100° in a thermostatically controlled glycerol bath, and were kept in the bath. At regular intervals a sample was taken out of the thermostat and was rapidly chilled and neutralized with 3.5 *N* sulfuric acid. For the faster reactions, the reaction was interrupted by rapid neutralization with 3.5 *N* sulfuric acid. For the 8 hours and longer runs, the neutralization had to be omitted on account of the voluminous precipitate of silicic acid formed. It was found, however, that the rotation was independent of the pH of the solution. The solution was diluted to 25.0 ml and the optical

rotation of the solution (if necessary filtered) was determined in a 40 cm tube. The velocity constant $k \cdot c_{OH^-}$ was calculated for a first order reaction, time in minutes, and expressed in Brigg's logarithms. The different runs are summarized in Table 3.

Table 3. Alkaline hydrolysis of phenyl-β-glucoside.

Time in minutes	a	$k \cdot c_{OH^-} \cdot 10^3$
0	— 6.12°	—
60	— 5.43	1.83
120	— 4.90	1.82
240	— 4.13	1.86
480	— 3.48	1.72
840	— 3.15	(1.68)
1440	— 3.03	—

Mean value 1.81

If all the glucoside had been converted to levoglucosan, the final rotation would be — 3.55°. If one assumes that optically inactive substances are formed as byproducts, the yield of levoglucosan can be calculated to be 85 %.

This method could not be used for the *p*-nitrophenyl glucoside. Instead a photo-electrical method was used. The instrument was a Spekker absorptiometer. (Filter, Ilford 601.) A calibration curve for *p*-nitrophenol in alkaline solution, in concentrations between 10 and 75 mg per liter was constructed. *p*-Nitrophenyl β-glucoside (30 mg) was then treated with 0.0185 *N* KOH (100 ml) at 100°, the reaction interrupted by neutralization and the free nitrophenol determined. The results are summarized in Table 4.

Table 4. Alkaline hydrolysis of p-nitrophenyl-β-glucoside.

Time in minutes	% *p*-nitrophenol	$k \cdot c_{OH^-}$
4	27.6	0.0353
6	39.8	0.0368
10	58.2	0.0379
20	84.5	0.0405

Mean value 0.038

Experiments with 1,2-anhydro-α-glucose triacetate

1,2-Anhydro-α-glucose triacetate (1.5 g) was dissolved in dioxane (12.5 ml) and 1.87 *N* potassium hydroxide (25 ml) was added. The initial positive rotation of the solution changed within a few minutes to a constant negative value. The solution was allowed to stand for half an hour and was then neutralized with 2 *N* sulfuric acid, concentrated to dryness under reduced pressure, and the residue acetylated with acetic anhydride (8 ml) and anhydrous sodium acetate (0.5 g) by heating on a steam bath for two hours. The mixture was poured into ice water and extracted with chloroform. The chloroform solution was washed with sodium bicarbonate solution and with water, dried over calcium chloride, filtered through aluminium oxide, which removed much of the dark brown color, and concentrated. The remaining sirup was dissolved in boiling water. After cooling, crystals separated, which after further recrystallizations melted at 107.5—108.5°, alone or in mixture with authentic levoglucosan triacetate. The yield was about 20 %.

SUMMARY

The alkaline hydrolysis of seven phenyl β-glucosides has been investigated kinetically. The reaction was found to be of the second order, depending on the concentrations of the glucoside and the hydroxyl ion. A linear relationship between the values of log *k* and Hammet's σ-values for the substituents has been demonstrated. The reaction constant, ρ, was 2.5.

1,2-Anhydro-α-glucose triacetate was found to yield levoglucosan by alkaline hydrolysis. This strongly supports the theory of McCloskey and Coleman that 1,2-anhydro-α-glucose is an intermediate in the alkaline hydrolysis of phenyl β-glucosides into levoglucosan.

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Received May 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 885–891

The Isolation of Deoxyribonucleic Acids from Rye and Wheat

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As part of a wide investigation of the chemical and physical properties of a range of deoxyribonucleic acids extracted under mild conditions from various sources, our attention has been directed to the problem of the isolation of deoxyribonucleic acids from vegetable tissues and grain. The presence of extractable amounts of deoxyribonucleic acid in such material was shown by Kiesel and Belozersky [1] who isolated from pea-germ a nucleoprotein which gave a positive Feulgen [2] reaction, and which contained thymine. However, owing to the drastic methods found necessary to disintegrate the pea-germ, marked degradation undoubtedly occurred during the isolation of the nucleoprotein. In attempts to overcome this difficulty, Feulgen, Behrens and Mahdihassan [3] separated nuclei from embryos of rye by grinding the latter under benzene in a specially constructed mill, followed by centrifuging the suspension in a mixture (S. G. 1.39) of carbon tetrachloride and benzene. From the nuclei thus obtained, a deoxyribonucleotide was isolated by a procedure which involved a preliminary extraction with boiling water, further extraction with sodium carbonate solution, and digestion with pancreatin. This method of Feulgen *et al.*[3] is obviously of limited application, since, apart from the disadvantages of the alkaline extraction, and the probable presence of deoxyribonuclease in the pancreatin preparation the use of organic solvents is liable to damage the nuclei (Glick [4]). Accordingly, alternative methods of extraction have been investigated. Following the work of Mirsky and Ris [5] in which cell nuclei were successfully disrupted by vigorous agitation in a high speed mixer, and of Overend and Webb [6] who isolated a deoxyribonucleic acid from *Haemophilus pertussis* cells disintegrated in a Mickle shaker, it appeared that a combination of these methods would permit the isolation of highly polymerised deoxyribonucleic acids from various grains. In this connection it should be

noted that Mirsky and Pollister [7] state that a deoxyribonucleohistone may be isolated by extracting with 1 *M* sodium chloride solution, wheat germ which had previously been extracted with petroleum-ether. The experimental details described leave much to be desired.

The present communication records the results obtained by the application of such methods to rye and wheat. From the latter source it was possible to obtain comparatively pure preparations of deoxyribonucleic acid. The isolation from rye was complicated by the apparent solubility of the initial deoxyribonucleoprotein in 0.14 *M* sodium chloride solution. Consequently it was difficult to separate the deoxyribonucleic acid from ribonucleic acid.

EXPERIMENTAL

1. Isolation of deoxyribonucleic acid from rye

The dry grain was crushed in a mill and the resulting powder (1.5 kg) was suspended in 100 g portions in 1 *M* sodium chloride solution (300 ml/100 g powder) and subjected to vigorous agitation in a high speed mixer. After 10 minutes the suspension was filtered through cheese-cloth to remove the hulls. The residue was re-extracted with 1 *M* sodium chloride solution (total volume 1200 ml) as above, and the combined filtrates were centrifuged to remove starch. The insoluble starch was washed at the centrifuge with 1 *M* sodium chloride solution (500 ml) and discarded. Ethanol (3 vols.) was added to the combined supernatent liquid and washings. (This solution was opalescent.) The precipitate formed was collected at the centrifuge after 18 hours at 0°. It was suspended in 1 *M* saline (300 ml) and vigorously agitated in the mixer for 5 minutes. The resulting viscous suspension was diluted with 1 *M* sodium chloride (200 ml) and then centrifuged. The clear supernatent (S_1) (See Scheme) was decanted and the residue re-suspended in 1 *M* saline (100 ml), homogenised in the mixer and again centrifuged. The supernatent liquid (S_2) was decanted and the solid residue mixed with glass (Ballotini) beads and disintegrated in the Mickle shaker. After dilution with 1 *M* saline (100 ml), the suspension was centrifuged. The supernatent liquor (S_3) was decanted off and the solid (R) was discarded. Dilution of each of the above solutions (S_1, S_2, and S_3) with water (6 to 10 vols.) (*cf.* Mirsky and Pollister [7]) failed to precipitate the deoxyribonucleoprotein. Consequently much ribonucleic acid accompanied the deoxyribonucleic acid throughout the subsequent stages of the preparation (*cf.* Table 1). Addition of ethanol (1.5 vols.) to S_1 afforded a fibrous precipitate (D_1-collected by winding around a glass rod) and a precipitate (D_2) composed of very short fibres and some non-fibrous material. This precipitate (D_2) separated rapidly and was isolated at the centrifuge after decanting the main bulk of the supernatent liquid. The latter on standing overnight, deposited more precipitate (D_3). Samples of D_1 and D_2 gave an intense blue colour with the Dische[8] diphenylamine reagent, which exhibited the characteristic absorption band (Max. at 5800 Å) (*cf.* Deriaz, Stacey, Teece and Wiggins [9]) confirming the presence of deoxyribonucleic acid. Subsequent operations were as shown in the accompanying scheme and require little further comment. The qualitative separation of fractions D_2A, D_2B, D_2C, D_3A and D_3B was carried out according to the method described above for the separations

Table 1. Deoxyribonucleic acid and ribonucleic acid content of rye fractions, as determined by the method of Schmidt and Thannhauser [10].

Fraction	*Deoxyribonucleic acid*	*Ribonucleic acid*
	mg P/100 mg material	
Residue H	0.0105	—
Residue T	0.007	—
Residue R	0.003	—
Nucleoprotein D_1	1.3	2.31
Nucleic Acid D_1A	1.88	2.62
» » D_2A	1.58	2.12
» » D_2B	1.36	2.98
» » D_2C	0.57	2.49
» » D_3A	1.07	1.98
» » D_3B	0.14	2.31
Fraction S_2	0.402	2.06
Fraction S_3	0.128	2.35

The figures in the table multiplied by 10 give as an approximation the percentage of deoxyribo- and ribo-nucleic acids in the fractions analysed

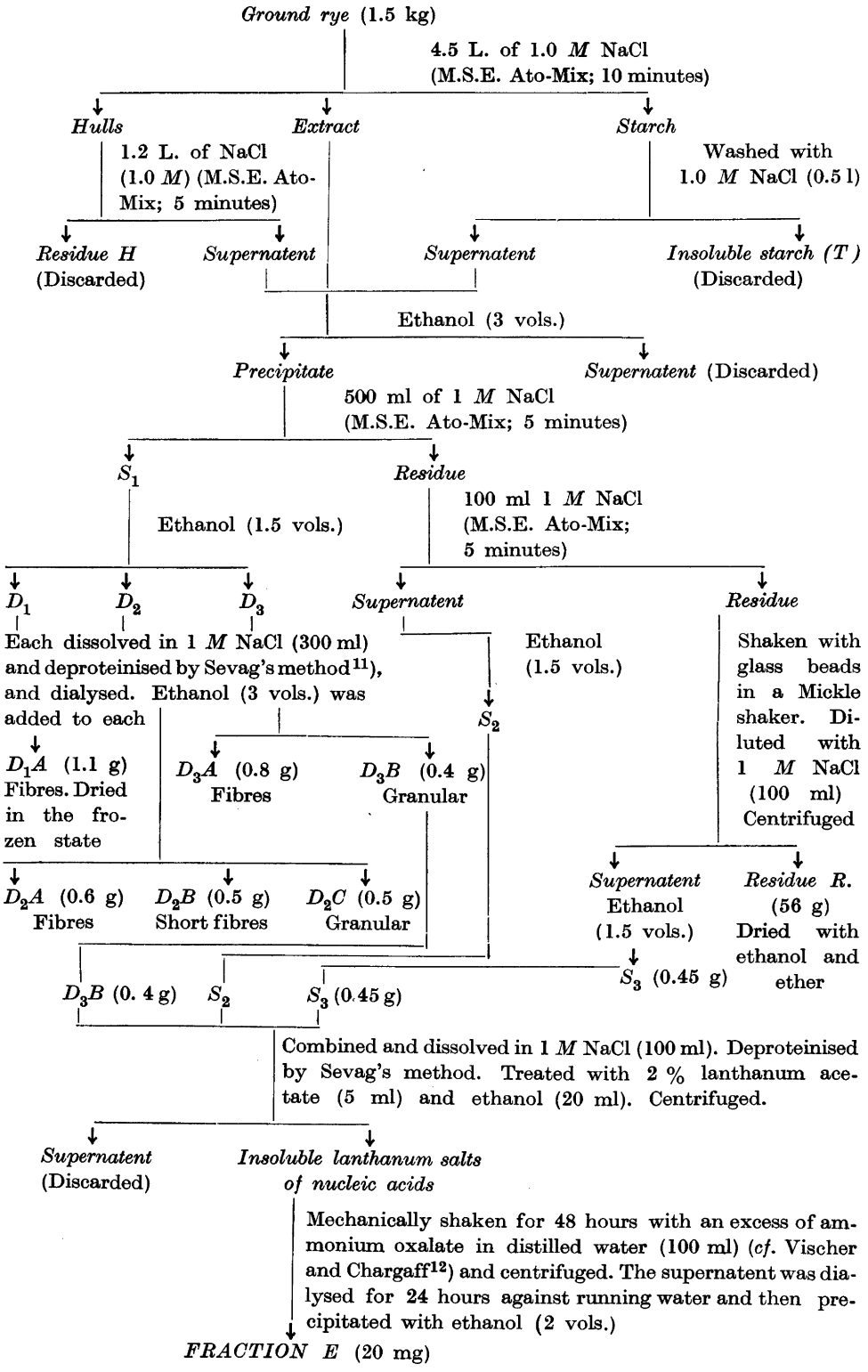
of fractions D_1, D_2, and D_3. The minimum volume of ethanol was used for precipitation at all stages, in order to minimise co-precipitation of polysaccharide material.

The analysis of the various fractions by the method of Schmidt and Thannhauser [10] gave the results recorded in Table 1. These indicate that the major part of the deoxyribonucleic acid of the rye was removed from the grain during the initial extraction and was concentrated in the fibrous precipitates. Attempts to concentrate the nucleic acids present in fractions S_2, S_3, and D_2B by first removing the protein and then precipitating the nucleic acids as lanthanum salts, gave insufficient material (Fraction E) to warrant further purification.

Purification of the crude deoxyribonucleic acid

Fractions D_1A, D_2A, D_2B and D_3A were combined and dissolved in distilled water (100 ml). Saturated calcium chloride solution (15 ml) was added and the precipitate of calcium phosphate removed at the centrifuge after 15 minutes. Addition of ethanol (2 vols.) to the clear supernatent solution resulted in the precipitation of fibres which were collected and dissolved in distilled water (80 ml) and 0.1 *M* veronal buffer (pH 7.8, 30 ml). To this solution 0.1 % (W/V) ribonuclease solution (previously heated to 100° at pH 4.0 to remove possible traces of deoxyribonuclease) and 1 : 1000 sodium ethylmercurithiosalicylate solution (14 ml) were added. The mixture was left at room temperature for 24 hours and the enzyme protein then removed by the Sevag [11] method. Five treatments with chloroform-octanol (9 : 1) served to remove the protein. The

Scheme of isolation.



aqueous solution was dialysed for 18 hours against running tap water, centrifuged and then added to ethanol (3 vols.). The resulting fibrous precipitate was removed rapidly from the small amount of granular material which accompanied it, washed with ethanol and dissolved in distilled water (10 ml) to give an extremely viscous solution. Absolute ethanol (14 ml) was added and the long fibres which immediately separated were removed on a glass rod. The fibres were dissolved in distilled water (30 ml) and re-precipitated with ethanol (75 ml). This procedure was repeated four times and the final product dried *in vacuo* in the frozen state. The product (0.5 g) dissolved extremely rapidly in water to give a viscous solution. It was free from protein (Sakaguchi and ninhydrin tests were negative) but contained some polysaccharide material as shown by the low analytical figures for nitrogen and phosphorus content, and the high reducing power value (as glucose) of the material after acid hydrolysis (see Table 2). The high content of polyribonucleotides remaining in the product (see Table 2) after its digestion with ribonuclease and subsequent dialysis, may be attributed to the fact that among the products from the action of ribonuclease on ribonucleic acid, is a relatively large non-diffusible polynucleotide fragment which is resistant to further action of the enzyme (Loring, Carpenter and Roll [13] *cf.* Overend and Webb [14]). It was not found possible to remove this polyribonucleotide from the deoxyribonucleic acid preparation, either by fractional precipitation with ethanol from aqueous solution, or by the precipitation of the nucleic acid as its insoluble lanthanum salt.

2. Isolation of deoxyribonucleic acid from wheat germ

From this material it was possible to isolate relatively pure preparations of deoxyribonucleic acid by the procedure outlined below. Except where stated to the contrary all operations were carried out in the cold room. In accordance with Mirsky and Pollister [7] it was found that a preliminary extraction of the wheat germ with petroleum-ether was essential in order to obtain workable amounts of the nucleoprotein. A suspension of the wheat germ (600 g) (supplied by Glaxo Laboratories Ltd) in petroleum-ether (b. p. 60—80°, 4 l) was kept at room temperature for 24 hours and then filtered. The dry solid was suspended in 0.14 *M* sodium chloride solution (5 l) and subjected to vigorous agitation in the high speed mixer. The suspension was kept for 24 hours and then separated by centrifuging. The supernatent liquid was decanted and the deposit homogenised with 1.0 *M* sodium chloride (2.5 l) in the mixer. After standing for 24 hours, this suspension was separated as before. The supernatent was diluted with seven volumes of water. The resulting precipitate which separated slowly was collected at the centrifuge after 1.5 hours, and transferred to 1.0 *M* saline (500 ml) when the main bulk of the material readily dissolved. The undissolved solid was removed (by centrifuging) and the clear solution obtained, was freed from protein by shaking seven times with chloroform-amyl alcohol (3.5 : 1) in the usual way. Addition of cold absolute ethanol (1.5 vols.) to the protein-free solution gave a fibrous precipitate which was collected at the centrifuge after 1.5 hours. A solution of the solid in distilled water (120 ml) was dialysed for 12 hours at 3° against distilled water (1600 ml) and then evaporated in the frozen state. The white powder (0.6 g) obtained, dissolved rapidly in water to give a viscous solution which was free from chloride ions and gave negative Sakaguchi and ninhydrin reactions. It contained 4.77 % of ribonucleic acid (see Table 2).

Table 2. Properties of deoxyribonucleic acid preparations from rye and wheat. Analyses performed on material dried in vacuo over phosphorus pentoxide at 80°.

Property	Source	
	Rye	Wheat
Ribonucleic acid content (method of Euler and Hahn [15])	16 %	4.77 %
Reducing value after acid hydrolysis	27 %	—
Phosphorus content (Allen's [16] method)	6 %	8.87 %
Nitrogen content (micro-Kjeldahl method)	10.3 %	13.66 %
N/P	1.70	1.55
Purine N/Pyrimidine N (method of Gulland, Jordan and Threlfall [17]	1.57	1.57
Ultraviolet absorption	λ max. 260 m/μ λ min. 230 m/μ	λ max. 260 m/μ λ min. 230 m/μ

SUMMARY

Deoxyribonucleic acids have been isolated from rye and wheat germ. Some characteristics of the products are reported.

Thanks are due to Professor M. Stacey F.R.S. for his interest in these investigations, to Glaxo Laboratories Ltd. for the gift of the wheat germ, and to Miss P. Hudson for technical assistance in the work with the rye.

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Received May 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 892–900

Azolone Studies

I. On the Reaction between Phenylglyoxal and Urea or Substituted Ureas in Alkaline Solution

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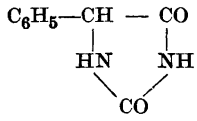
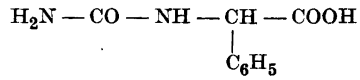
Some years ago Fisher, Ekeley and Ronzio [1] published a paper in which they reported on the reaction between phenylglyoxal and urea or substituted ureas in alkaline solution. Their experiments have been repeated in this laboratory and the structures of the reaction products re-examined and changed so as to be more in accord with the experimental findings.

The condensation between phenylglyoxal and urea "in cold basic solution"* was claimed [1] to give an 85 % yield of 5-phenylhydantoin (I), a compound which was first described in the literature by Pinner [2].



I.

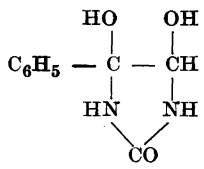


II.

In our hands, however, the condensation, which was performed under identical conditions, gave consistently lower yields (30-40 %) of (I). In addition there was isolated an 8-10 % yield of α-phenylhydantoic acid (II), identified by comparison with an authentic specimen prepared according to Pinner (*l. c.*). The reaction in question merely constitutes an extension of the classical Biltz synthesis [3, 12] of 5,5-diphenylhydantoin from benzil and urea.

* Quoted from ref. 1. From the experimental section, however, it appears that the reaction was performed by heating the solution for three minutes at a temperature just below boiling.

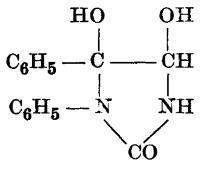
When the reaction was carried out by refluxing the reactants in alkaline solution, Fisher *et al.* (*l. c.*) obtained a low yield of a compound, $C_9H_{10}N_2O_3$, to which they ascribed the structure (III).



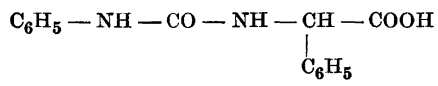
III.

This formula has now proved to be incorrect, the condensation product being in fact α-phenylhydantoic acid (II), presumably formed in a secondary reaction by ring-opening of (I). In keeping with this view is the readiness with which it recyclized to (I).

For the reaction product of phenylglyoxal and phenylurea in alkali the American authors [1] put forward the expression (IV).

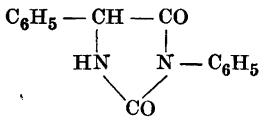


IV.

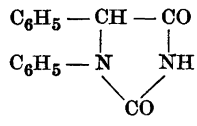


V.

This structure also has proved untenable. The reaction product gave analytical figures corresponding with the formula $C_{15}H_{14}N_2O_3 + \frac{1}{2}H_2O$. The water of crystallization was given off only after heating at 100° *in vacuo* over phosphorus pentoxide for 2 hours. The product was freely soluble in aqueous sodium bicarbonate and could be titrated as a mono-carboxylic acid. It was remarkably stable towards alkali whereas acidic reagents readily transformed it into a new compound (VI), $C_{15}H_{12}N_2O_2$, differing from (V) by the elements of water, and having all the characteristics of a hydantoin.



VI.

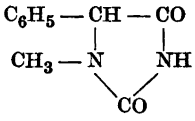


VII.

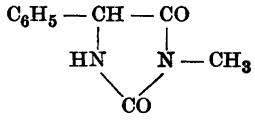
The ring-closed compound was formulated as 1,5-diphenylhydantoin (VII) by Fisher *et al.*[1] though this is obviously inconsistent with its insolubility in aqueous alkali. In the literature the authentic 1,5-diphenylhydantoin is described by Aspelund [4], the two compounds being distinctly different with regard to melting point and chemical properties *. A *levo*-rotatory stereoisomeride of 3,5-diphenylhydantoin (VI) has been reported previously by Ehrlich [5], whereas the corresponding racemic substance was a hitherto unknown compound.

The structure (V) was confirmed in this laboratory by an independent synthesis from DL-C-phenylglycine and phenyl isocyanate, yielding the hydantoic acid with m.p. 168°, thereby demonstrating the statement of Kossel [6], that the compound melts at 154°, to be erroneous. On treating the compound with ethanolic hydrogen chloride, water was readily split off and racemic 3,5-diphenylhydantoin (VI) resulted which was proved identical with the $C_{15}H_{12}N_2O_2$ compound mentioned above.

Analogously, Fisher *et al.*[1] pictured the condensation product (m. p. 174°) of phenylglyoxal and methylurea as 1-methyl-5-phenylhydantoin (VIII).



VIII.

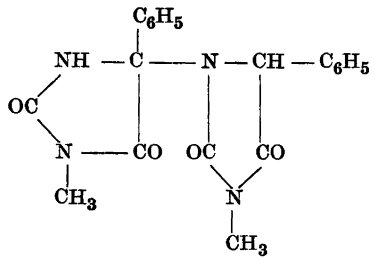


IX.

On repeating the reaction in this laboratory a 28 % yield of a substance, $C_{10}H_{10}O_2N_2$, melting at 163°, was obtained. Gabriel [7] prepared the authentic 1-methyl-5-phenylhydantoin many years ago by a procedure which left no doubt as to its correct structure, and reported the melting point to be 177°, corroborated recently by Long, Miller and Troutman [8]. The isomeric 3-methyl-5-phenylhydantoin (IX) (m. p. 161°-162°) has been described by Pinner [2]. The identity of the C_{10}-compound with (IX) was established by comparison with a sample prepared by methylation of 5-phenylhydantoin with methyl sulphate. (IX) was readily soluble in dilute alkali, a feature generally excluding substitution in the 3-position of the hydantoin ring. On standing, however, the alkaline solution soon deposited a high-melting crystalline compound (X), formed by oxidation of (IX), and this proved identical with dimethyldiphenylhydantil previously prepared by Gabriel [7], using a different procedure. The true nature of this behaviour in alkali may have been overlooked

* A sample of 1,5-diphenylhydantoin for comparison was kindly supplied by Professor Aspelund.

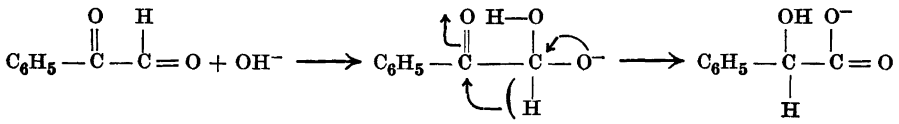
by Fisher *et al.*[1] * and may have influenced these authors in their choice of structure (VIII), whereas no satisfactory explanation can be given for the discrepancy in melting points between their and our product.



X.

DISCUSSION

Some comments may be made regarding the mechanism of the reactions in question. It is well known that phenylglyoxal rearranges in alkali to mandelic acid; this reaction has been studied *inter alia* by Alexander[9] who proved it to be of the second order. Doering *et al.*[10] presented evidence that the reaction proceeds without interruption of the linkage between the phenyl- and the keto-group, and suggested the mechanism



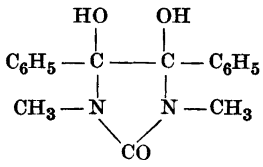
which involves the transfer of a hydride ion.

In the course of the present study it was shown that the condensation of phenylglyoxal with ureas precedes the rearrangement, because mandelic acid and phenylurea do not interact under the conditions used in the condensation.

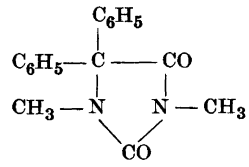
It will be remembered that phenylglyoxal with urea and methylurea yielded the hydantoins, whereas phenylurea reacted to form the hydantoic acid under similar conditions. The question therefore arises as to whether the rearrangements take place in an open or in a cyclic structure. It was proved that none of the hydantoic acids in question suffered ring-closure under the conditions used in working up the reaction mixtures. Submitted to an alkaline

* *e. g.* The statement from ref. 1 as to the reaction product forming "a monopotassium salt which hydrolyzes readily with water".

treatment, identical with the one used in performing the reactions, 3,5-diphenylhydantoin was ring-opened to the corresponding hydantoic acid, whereas 5-phenyl- and 3-methyl-5-phenyl-hydantoin were recovered in high yields. This fact makes it appear probable, that α,δ-diphenylhydantoic acid (V) is a secondary reaction product and that the rearrangement consequently is preceded by cyclization. Supporting evidence for this view may be found in the observation by Biltz [3], that 1,3-dimethyl-4,5-diphenyl-4,5-dihydroxy-2-imidazolidone (XI) could easily be transformed into 5,5-diphenyl-1,3-dimethyl-hydantoin (XII) in alkaline solution.

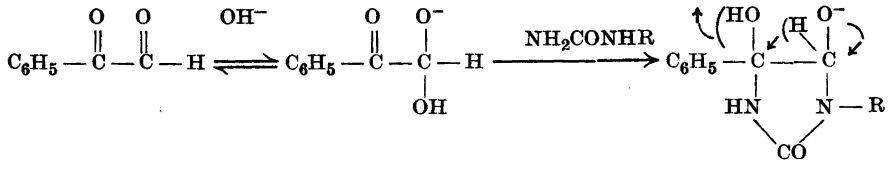


XI.

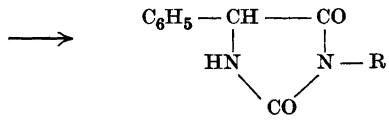


XII.

Thus, the reactions in question may be pictured as follows:



XIII. XIV.

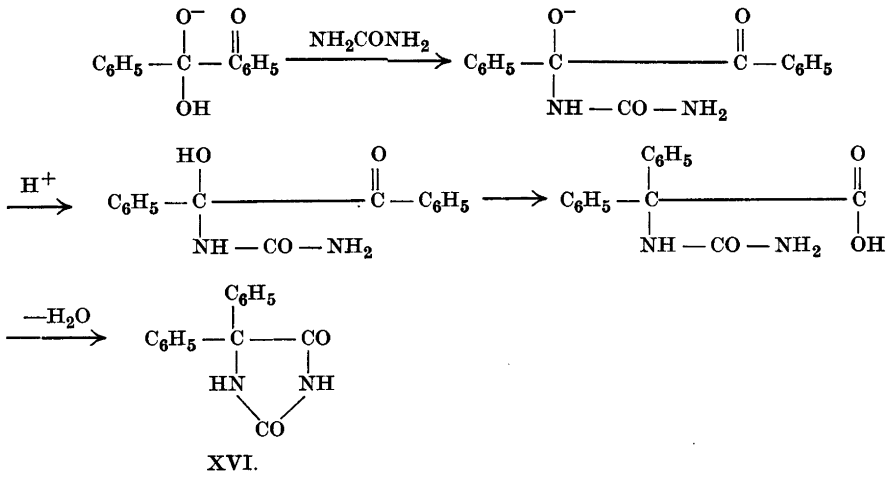


XV.

The reversible addition of a hydroxyl-ion to (XIII) is followed by addition and dehydration giving (XIV), which in turn rearranges by displacement of a hydroxyl-ion by the migrating hydride-ion. This hydride-shift may be greatly facilitated by the negative charge on the adjacent oxygen-atom.

Although the 1,2-hydride shift is a common and generally accepted feature in intramolecular rearrangements, the displacement reaction suggested here does not appear to be generally recognized. Alexander [9], in studying the mechanism of the homogeneous Cannizzaro reaction, has suggested a somewhat similar displacement, taking place, however, over a 1,3-system, although a more recent paper [11] by the same author does not seem to support this view.

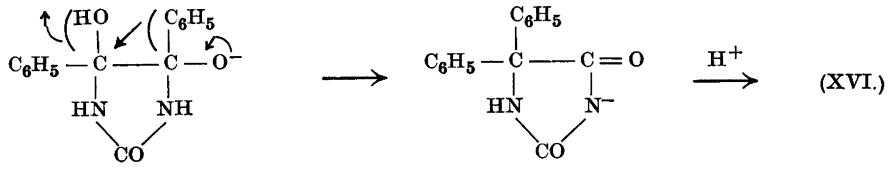
It may be worthy of note in this connection that Sikdar and Ghosh [12] recently suggested the following mechanism for the reaction between benzil and urea



XVI.

This series of steps, however, does not explicitly account for the necessity of maintaining alkaline conditions throughout the reaction, nor does the final spontaneous ring-closure appear to be a likely course of reaction.

By analogy with the suggestions presented above, this rearrangement may be pictured



(XVI.)

involving a hydroxyl-ion displacement by the phenyl group with its bonding pair of electrons.

EXPERIMENTAL *

The reaction between phenylglyoxal and urea

Following the procedure of Fisher *et al.* (*l. c.*), a mixture of phenylglyoxal [13] (12.16 g) and urea (4.80 g) in 100 ml of water was heated to boiling for three minutes after addition of 50 % potassium hydroxide (24 ml). On cooling and acidification 4.48 g (32 %) of

* All melting points are uncorrected. Analyses were carried out in this laboratory by Mr. A. Grossmann.

crude material separated. After recrystallization from aqueous ethanol and drying in the air, 4.40 g of 5-phenylhydantoin (I) was obtained as its monohydrate. M. p. 180°.

After two days the mother liquor had deposited 1.17 g (8 %) of α-phenylhydantoic acid (II), which after several recrystallizations from aqueous ethanol, separated in needles melting with strong effervescence at temperatures varying between 174° and 182°, depending on the rate of heating. Mixed m. p. with an authentic sample [2] was undepressed, whereas the m. p. on admixture with 5-phenylhydantoin was 160—64° (dec.).

The reaction between phenylglyoxal and phenylurea

The reaction between these compounds was conducted in accordance with the directions given [1], and a 72 % yield of crude α,δ-diphenylhydantoic acid (V) was obtained. An analytical sample was prepared by repeated crystallizations from ethanol. Colourless, silky needles, m. p. 168° (dec.).

$C_{15}H_{14}N_2O_3 + \frac{1}{2} H_2O$ (279.3)	Calc.	C	64.51	H	5.41	N 10.03
	Found	»	64.42	»	5.51	» 9.78

Neutr. equiv. 278.5

The water of crystallization was removed *in vacuo* over phosphorus pentoxide at 100° for two hours.

Calc.	H_2O	3.22 %
Found	»	3.22 »

The dried product (m. p. 168°) was analyzed for nitrogen.

Calc.	N	10.36
Found	»	10.52

α,δ-Diphenylhydantoic acid (500 mg) was dissolved in ethanol (7 ml), conc. hydrochloric acid (1 ml) added, and the solution refluxed for one hour. On cooling small needles, consisting of 3,5-diphenylhydantoin, separated in quantitative yield. Recrystallized twice from ethanol for analysis. M. p. 189°.

$C_{15}H_{12}N_2O_2$ (252.3)	Calc.	C	71.44	H	4.80	N 11.11
	Found	»	71.58	»	4.71	» 11.36

Synthesis of α,δ-diphenylhydantoic acid (V) and 3,5-diphenylhydantoin (VI)

To a well cooled and vigorously stirred solution of DL-α-aminophenylacetic acid (7.6 g) in 1 *N* sodium hydroxide (50 ml), phenyl isocyanate (5.5 ml) was dropwise added. A trace of *sym*-diphenylurea was filtered off and the filtrate carefully acidified, when a creamy separation resulted. Yield 95 %. Crystallized from aqueous ethanol as a mass of fine colourless needles containing half a molecule of water. M. p. 167—68° (dec.).

No depression of the m. p. was observed when mixed with the reaction product described above.

Calc.	*vide supra*					
Found	C	64.76	H	5.34	N	9.98

On treating the hydantoic acid in ethanol with hydrochloric acid in the manner described above, it was transformed into 3,5-diphenylhydantoin of m. p. 189°. The identity of the two products was secured by mixed m. p.

The reaction between phenylglyoxal and methylurea

An alkaline solution of equimolar amounts of these two substances was heated to boiling for one minute, then cooled and acidified, when a brown syrup separated, which gradually became crystalline on standing. The mother liquor slowly deposited an additional crop of the same purity. Total yield 28 %. A sample was recrystallized twice from aqueous ethanol for analysis. M. p. 162—63°.

$C_{10}H_{10}O_2N_2$ (190.2)	Calc.	C	63.14	H	5.30	N 14.73
	Found	»	62.94	»	5.22	» 14.72

Synthesis of 3-methyl-5-phenyl-hydantoin (IX)

Methylation of 5-phenylhydantoin was previously carried out by Pinner [2], who used methyl iodide. Methyl sulphate has now been found to be a more convenient methylation agent. To a stirred solution of 5-phenylhydantoin (2.5 g) in 2.11 *N* sodium hydroxide (6.75 ml), methyl sulphate (1.4 ml) was added in small portions. Crystallization started within a few minutes. Yield 84 %. Separated from aqueous ethanol as a mass of fine needles. M. p. 162—63° alone or in mixture with the reaction product described above.

Diphenyldimethylhydantil (X)

3-Methyl-5-phenyl-hydantoin readily went into solution in diluted aqueous or methanolic potassium hydroxide. Within a few minutes a crystalline powder separated, slightly soluble in organic solvents. M. p. 325—30° (dec.). A sample was triturated several times with hot methanol and analyzed.

$C_{20}H_{18}O_4N_4$ (378.4)	Calc.	C	63.46	H	4.79	N 14.81
	Found	»	63.18	»	4.50	» 14.78

Phenylurea and mandelic acid

A mixture of mandelic acid (3.04 g) and phenylurea (2.72 g) in water (40 ml) containing 50 % potassium hydroxide (2 ml) was heated at the boiling temperature for 5 minutes. On cooling a 89 % yield (2.42 g) of unchanged phenylurea separated. The filtrate was acidified and extracted with ether, when a 84 % yield (2.55 g) of mandelic acid was recovered.

Stability of the hydantoins

Treating a) 3,5-diphenylhydantoin b) 3-methyl-5-phenylhydantoin and c) 5-phenylhydantoin in aqueous solutions with 50 % potassium hydroxide, under exactly the conditions used in the condensation experiments described above, [a] gave a 98 % yield of α,δ-diphenylhydantoic acid while [b] and [c] were recovered in 82 % and 89 % yields respectively.

SUMMARY

The reactions between phenylglyoxal and urea, phenylurea and methylurea have been studied, and the structures of the products previously reported in the literature revised.

Suggestions are presented as to the course and mechanism of the reactions involved.

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Received June 1, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 901–906

Über die Umsetzung von Formaldehyd mit Allylacetat *

III. Eine neue Synthese des Erythrols, Crotonaldehyds und Allylcarbinols

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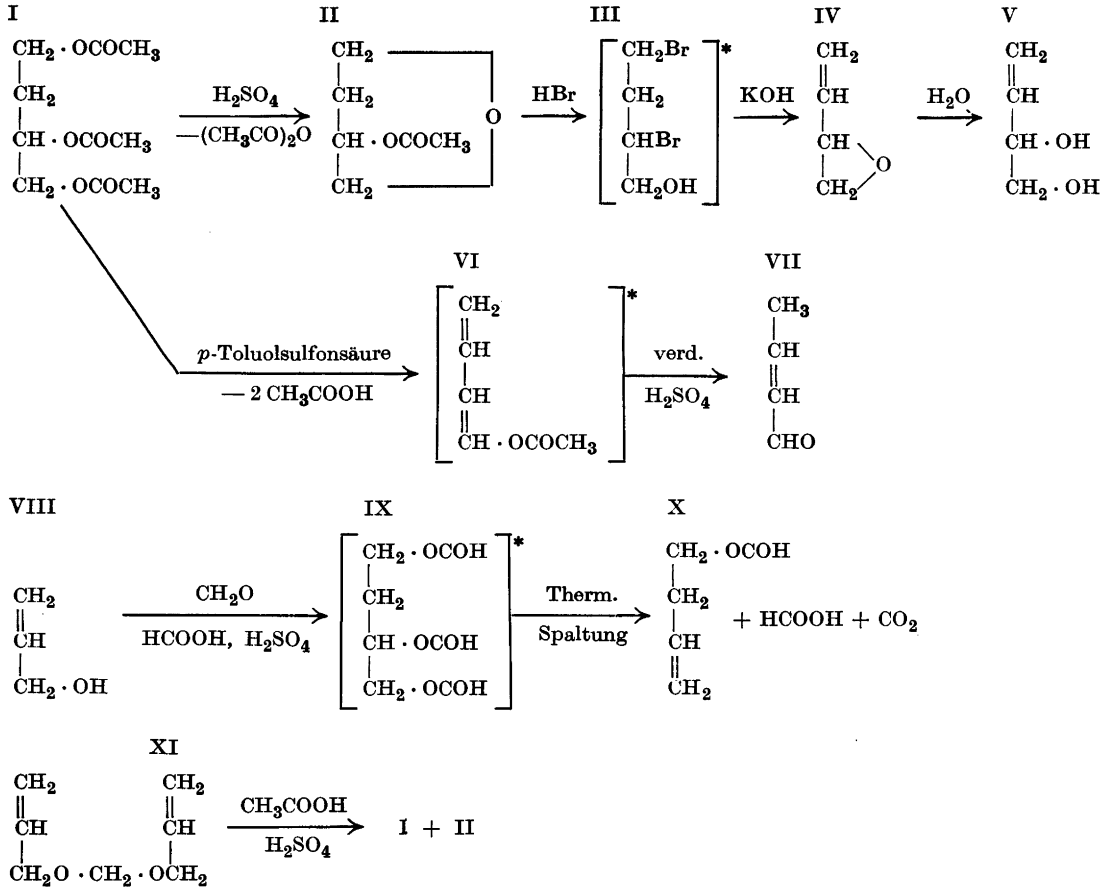
Um in der aliphatischen Reihe den beim Äthylen [1] begonnenen und bisher bis zum Butantriol-(1,2,4)-triacetat [2] durchgeführten schrittweisen »Formaldehyd-Olefin-Aufbau» weiter verfolgen zu können, haben wir untersucht, welche Olefinderivate der C_4-Reihe vom Butantriol-(1,2,4)-triacetat aus durch katalytische Essigsäureabspaltung zugänglich sind. Bei der Durchführung von Formaldehyd-Olefin-Reaktionen haben wir in der Regel beobachten können, dass neben dem normalen, um ein Kohlenstoffatom reicheren glykolischen Aufbauprodukt gleichzeitig ein mit diesem genetisch verwandtes Olefinderivat mit der gleichen Anzahl Kohlenstoffatome entsteht, das seinerseits durch erneute Reaktion mit Formaldehyd den Aufbau der Kette um ein *weiteres* Kohlenstoffatom ermöglicht. Dieser olefinische Trabant des Butantriol-(1,2,4)-triacetates könnte, wie bereits angedeutet [3], das Erythrol-diacetat [Buten-(1)-diol-(3,4)-diacetat] sein, um dessen Darstellung wir uns daher in der vorliegenden Untersuchung besonders bemüht haben. Durch Abspaltung von Essigsäure aus Butantriol-(1,2,4)-triacetat (I) könnten theoretisch mehrere Isomere entstehen, je nachdem die Abspaltung zwischen den Kohlenstoffatomen 1—2, 2—3 oder 3—4 erfolgt. Nach unseren früher mitgeteilten Erfahrungen mit anderen Verbindungen sollte bei der Vakuumdestillation mit konzentrierter Schwefelsäure die Bildung der isomeren Butendiol-diacetate begünstigt sein, allein weicht die Reaktion in diesem Falle in Richtung der Bildung von 3-Acetoxytetrahydrofuran (II)[4] aus. Wir haben daher zur Darstellung des Erythrols (V) den Umweg über das 3-Acetoxytetrahydrofuran

* 7. Mitt. über Formaldehyd-Olefin-Reaktionen.



* Die in eckigen Klammern stehenden Formeln stellen nicht isolierte hypothetische Zwischenstufen dar.

gewählt, das, nach Behandlung mit Bromwasserstoff bei der Destillation über Kaliumhydroxyd das 3,4-Epoxy-buten-(1) (IV) gab. Das Epoxybuten wurde mit verdünnter Salzsäure in das Erythrol [5] und dieses durch Kochen mit Essigsäureanhydrid in das Erythrol-diacetat übergeführt.

Während die Vakuumdestillation des Butantriol-(1,2,4)-triacetates mit konzentrierter Schwefelsäure vorwiegend Ringschluss veranlasste, lieferte dessen Destillation mit *p*-Toluolsulfonsäuremonohydrat bei gewöhnlichem Druck ein zwischen 106—121° übergehendes Destillat (J. Z. 46.7), das bei erneuter Destillation mit verdünnter Schwefelsäure Crotonaldehyd (VII) ergab. Dessen Bildung vollzieht sich möglicherweise über das Enolacetat des Vinylacetal-

dehyds (VI). Legt man zur Ausbeuteberechnung die gefundene Jodzahl zugrunde und berücksichtigt, dass es sich bei dem verwendeten Ausgangsmaterial um ein nicht ganz reines Präparat handelte, errechnet sich eine Ausbeute von ca. 30 % der Theorie.

Im Zusammenhang mit anderen geplanten Synthesen erschien uns das Allylcarbinol von Bedeutung, dessen Aufbau bisher nur Wagner [6] und Pariselle [5,7] auf metallorganischem Wege gelang. Nach einem Patent der I. G. Farbenindustrie [8] entsteht Allylcarbinol, neben etwas Crotylalkohol, beim Zutropfen von Butandiol-(1,3) zu Alaun bei 180—190°. Wir haben zunächst Butandiol-(1,3)-diacetat mit *p*-Toluolsulfonsäure bei gewöhnlichem Druck destilliert und dabei in glatter Reaktion [Allylcarbin]-acetat in einer Ausbeute von etwa 75 % der Theorie erhalten. Näher lag uns jedoch die Entscheidung der Frage, ob das Allylcarbinol nicht auch vom Butantriol-(1,2,4) aus erreichbar sei. Anfänglich dachten wir daran, dass auf dem früher beschriebenen Wege (*l. c.*) hergestellte Butantriol-(1,2,4) nach Veresterung mit Ameisensäure thermisch zum Allylcarbinol bzw. dessen Formiat zu spalten, vervollständigten jedoch diesen Leitgedanken zu einer direkten aufbauenden Synthese, indem wir, gemäss Formelreihe VIII—X, Allylalkohol mit Paraformaldehyd in Ameisensäure unter dem katalytischen Einfluss von konzentrierter Schwefelsäure umsetzten und das Reaktionsgemisch nach Neutralisation der Schwefelsäure ohne weiteres der thermischen Spaltung zum [Allylcarbin]-formiat unterwarfen. Dieses Verfahren ist bezüglich der Ausbeute an Allylcarbinol offenbar dem metallorganischen von Pariselle unterlegen, dagegen zeichnet es sich vor diesem durch grössere Einfachheit aus.

In einer früheren Publikation [9] wurde gezeigt, dass acetalartig gebundener Formaldehyd in Gegenwart von Eisessig-Schwefelsäure und eines Olefins zur Aufbaureaktion voll ausgenutzt wird. In Analogie zu der beschriebenen Darstellung von Hexahydrosaligenindiacetat aus Formaldehyd-dicyclohexylacetal haben wir nun das Formaldehyd-diallylacetal (XI) *ohne* Zusatz von Formaldehyd nur mit Eisessig-Schwefelsäure gekocht und dabei 3-Acetoxytetrahydrofuran und Butantriol-(1,2,4)-triacetat erhalten. Also auch in diesem Falle wurde acetalartig gebundener Formaldehyd säurekatalytisch für den «Glykolaufbau» ausgenutzt.

EXPERIMENTELLER TEIL

(Unter Mitarbeit von Birgit Sanne, Gunnar Aksnes und Eirik Hidle.)

I. Allylcarbinol aus Allylalkohol

Eine Mischung aus 1410 g Ameisensäure (85 %), 340 g Paraformaldehyd und 30 ml konz. Schwefelsäure wurden bis zur völligen Auflösung des Paraformaldehyds erwärmt und nach dem Abkühlen auf 70° mit 500 g Allylalkohol versetzt. Das Ganze erwärmte

man erneut auf 93°, wo die Reaktion unter Selbsterwärmung und Verfärbung einsetzte. Nach Abklingen der Selbstreaktion wurde eine Zeitlang unter Rückflusskühlung gekocht und nach Zusatz von 58 g wasserfreier Soda (zur Neutralisation der Schwefelsäure!) an der Raschigkolonne bei gewöhnlichem Druck destilliert. Nachdem zwischen 74—107° 1200 g Destillat (*A*) (J. Z. 56.2) übergegangen waren, erwies sich das Destillat gegen Brom-Eisessig als praktisch gesättigt (Ameisensäure), um dann kurz danach bei langsam fallenden Destillationstemperatur (infolge der nun einsetzenden thermischen Spaltung zu [Allylcarbin]-formiat!) wieder ungesättigt zu werden. Sobald das Destillat gegen Brom-Eisessig wieder ungesättigt wurde, fing man dieses für sich auf. Die Destillationstemperatur sank während der Spaltung bis auf 60°. Man erhielt so 196,5 g Destillat (*B*), das [Allylcarbin]-formiat neben Ameisensäure enthielt. Das Destillat A wurde zwecks Ausnutzung nicht umgesetzen Allylformiates gemäss der gefundenen Jodzahl erneut mit 106 g Paraformaldehyd und 25 ml konz. Schwefelsäure umgesetzt und wie oben beschrieben behandelt. Man erhielt so weitere 107.3 g [Allylcarbin]-formiat — Ameisensäure — Mischung, die mit Destillat B vereinigt und mit einer Lösung von 250 g Kaliumhydroxyd in 100 ml Wasser durch 1-stündiges Kochen verseift wurde. Das Verseifungsgemisch extrahierte man mehrfach mit Äther und destillierte die vereinigten Ätherauszüge über 200 g gebranntem Kalk. Nach Abdestillieren des Äthers erhielt man 37, 7g Destillat, das im wesentlichen zwischen 102—110° bei gewöhnlichem Druck überging und rohes Allylcarbinol darstellt. Durch weitere Reinigung gewann man ein Präparat vom Sdpkt.$_{748}$ 111—113°, $D_4^{21} = 0{,}8565$, $n_D^{21} = 1{,}4213$, Mol.-Refr. 21,38 (ber. 21,65). Pariselle gibt an: Sdpkt. 113—114°, $D^{17} = 0{,}848$, $n_D^{17} = 1{,}421$.

II. [Allylcarbin]-acetat aus Butandiol-(1,3)-diacetat

100 g Butandiol-(1,3)-diacetat (Hoechst a. Main) wurden mit 5 g *p*-Toluolsulfonsäure-monohydrat an der Widmerspirale bei gewöhnlichem Druck destilliert. Man erhielt im Siedeintervall zwischen 89—115° 96,5 g Destillat, das nach Zusatz von Äther zur Entfernung der Essigsäure mit gesättigter Sodalösung und Natriumsulfatlösung gewaschen und über wasserfreiem Natriumsulfat getrocknet wurde. Nach Entfernen des Äthers gingen bei der Destillation 60 g rohes [Allylcarbin]-acetat vom Sdpkt. 120—126° über. Die nochmalige Destillation ergab ca. 50 g einer völlig farblosen Flüssigkeit vom Sdpkt. 124—126°, $D_4^{21.8} = 0{,}9129$, $n_D^{22.5} = 1{,}4115$. Palomaa u. Juvala geben an: Sdpkt.$_{735}$ 124—126°. Pariselle gibt an: $D_0^{15} = 0{,}918$; $n_D^{15} = 1{,}411$.

Verwendet man an Stelle der *p*-Toluolsulfonsäure konzentrierte Schwefelsäure, so erhält man neben [Allylcarbin]-acetat bedeutende Mengen Butadien, das in Brom-Eisessig absorbiert und als Tetrabromid vom Schmp. 118—119° nachgewiesen wurde.

III. Crotonaldehyd aus Butantriol-(1,2,4)-triacetat

179 g Butantriol-(1,2,4)-triacetat (nicht ganz rein) wurden mit 10 g *p*-Toluolsulfonsäure-monohydrat an der Widmerspirale bei gewöhnlichem Druck destilliert. Man erhielt 112,4 g Destillat zwischen 106—121°, das zunächst farblos war, aber sich schon während der Destillation dunkelbraun färbte (J. Z. 46.7). Destillationsrückstand 71,5 g schwarze Krusten. Das Destillat wurde mit einer Lösung von 8 ml konz. Schwefelsäure in 70 ml Wasser versetzt und die Mischung erneut an der Widmerspirale destilliert, wobei mit den Wasserdämpfen Crotonaldehyd zwischen 65—100° überging. Das Destillat wurde mit Äther und gesättigter Sodalösung geschüttelt, die Ätherschicht abgetrennt und die wässrige Schicht 2-mal mit Äther ausgeäthert. Nach dem Trocknen über Calciumchlorid

wurden die vereinigten Ätherauszüge destilliert. Man erhielt 10 g Crotonaldehyd vom Sdpkt. 103—105°. Dieser wurde zur Identifikation in das *Semicarbazon* vom Schmp. 202° übergeführt. Mischschmelzpunkt mit einem authenischen Präparat unverändert.

$C_5H_9ON_3$ (127,2)	Ber.:	C	47,23	H	7,14	N	33,05
	Gef.:	»	47,28	»	7,13	»	32,70

IV. Erythrol aus 3-Acetoxytetrahydrofuran

a) *Behandlung mit Bromwasserstoff*

In 126 g 3-Acetoxytetrahydrofuran leitete man einen lebhaften Strom Bromwasserstoff ein, bis die Gewichtszunahme 183,8 g betrug. Das Reaktionsprodukt blieb über Nacht sich selbst überlassen und wurde dann an der Widmerspirale destilliert, wobei beim Sdpkt.$_{14}$ 120—125° 226,2 g Destillat übergingen (Destillat A).

b) *3,4-Epoxybuten-(1)*

In einem Claisenkolben mit Tropftrichter und angeschlossenem Liebigkühler wurde vorstehend beschriebenes Destillat A portionsweise auf einen reichlichen Überschuss feinpulverisiertes Kaliumhydroxyd getropft, wobei unter kräftiger Reaktion sofort eine farblose Flüssigkeit ab 60° zu destillieren begann. Die Destillationstemperatur hielt sich ziemlich konstant und stieg gegen Schluss auf etwa 100°. Man erhielt 51,8 g trübes Destillat. Die Trübung verschwand nach Zusatz von wasserfreiem Natriumsulfat. Bei der Destillation an der Widmerspirale erhielt man 40 g Destillat; das im wesentlichen beim Sdpkt.$_{753}$ 66° überging: $D_4^{20} = 0{,}8770$, $n_D^{20} = 1{,}4178$, Mol.-Refr. 20,0 (ber. 19,65). Pariselle gibt an: Sdpkt.$_{760}$ 70°, $D^{20} = 0{,}87$, $n_D^{20} = 1{,}416$.

c) *Erythrol*

40 g 3,4-Epoxybuten-(1) (vorstehend beschriebenes Präparat) wurden vorsichtig zu einer Mischung von 100 ml Wasser und 1 ml konz. Salzsäure getropft, wobei kräftige Wärmeentwicklung erfolgte. Das Reaktionsgemisch kochte man dann eine Stde. lang unter Rückflusskühlung und destillierte direkt an der Widmerspirale. Man erhielt 30,2 g Destillat vom Sdpkt.$_{13}$ 91°, $n_D^{19} = 1{,}4615$, $D_4^{19} = 1{,}0506$, Mol-Refr. 23,06 (ber. 23,27). Pariselle gibt an: Sdpkt.$_{13}$ 91—93°, $n_D^{14} = 1{,}469$; $D^{14} = 1{,}05$.

Bisphenylurethan Schmp. 125—126° (Pariselle gibt Schmp. 125—126° an).

$C_{18}H_{18}O_4N_2$ (326,3)	Ber.:	C	66,26	H	5,56	N	8,59
	Gef.:	»	66,58	»	5,74	»	8,39

Erythrol-diacetat Sdpkt.$_{13}$ 88—89°, $D_4^{23.5} = 1{,}065$.

V. Überführung von Formaldehyd-diallylacetal in 3-Acetoxytetrahydrofuran und Butantriol-(1,2,4)-triacetat

140 g Formaldehyd-diallylacetal (Sdpkt. 138—142°; Probe auf freien Formaldehyd mit Fuchsinschwefliger Säure negativ!) wurde mit 300 ml Eisessig und 5 ml konz. Schwefelsäure erhitzt. Bei 102° (in der Flüssigkeit gemessen!) trat Sieden ein. Infolge der freiwerdenden Reaktionswärme hielt sich die Flüssigkeit etwa 15 Minuten von selbst im Sieden unter zunehmender Dunkelfärbung. Das Gemisch wurde dann 2 Stunden lang unter Rückflusskühlung gekocht. Das schwarzbraune Reaktionsgemisch wurde zwecks

Neutralisation der freien Schwefelsäure mit 10 g wasserfreier Soda versetzt und zunächst bei gewöhnlichem Druck destilliert: 1) Sdpkt. 90—120°, 307 g, J. Z. 113,5, Reaktion mit Fuchsinschwefliger Säure auf Formaldehyd positiv!). Bei der Destillation des Rückstandes bei 9 mm Druck erhielt man u. a. ca. 10 g einer zwischen 60—70° übergehenden Fraktion, die vorwiegend aus 3-Acetoxytetrahydrofuran bestand (Perhydrolreaktion auf Furanderivate [2] positiv!) und ca. 10 g einer zwischen 150—157° übergehenden Fraktion (D_4^{19} = 1,1441, V. Z. 469,1), die fast reines Butantriol-(1,2,4)-triacetat darstellt. Durch Verarbeitung sämtlicher Fraktionen auf 3-Acetoxytetrahydrofuran nach der früher angegebenen Vorschrift [4] erhielt man dieses in reinem Zustande. Zwecks Identifizierung wurde es durch Umesterung in das 3-Oxytetrahydrofuran (Sdpkt.$_8$ 74—75°) und dieses in das

Phenylurethan vom Schmp. 118—120° überführt.

$C_{11}H_{13}O_3N$ (207,2)	Ber.:	C	63,75	H	6,32	N	6,76
	Gef.:	»	63,77	»	5,90	»	6,86

ZUSAMMENFASSUNG

Das durch Umsetzung von Formaldehyd mit Allylacetat in Gegenwart von Eisessig und Schwefelsäure erhältliche Butantriol-(1,2,4)-triacetat lässt sich durch Destillation mit *p*-Toluolsulfonsäure in *Crotonaldehyd* und auf dem Umwege über 3-Acetoxytetrahydrofuran in *Erythrol* überführen. — Butandiol-(1,3)-diacetat liefert bei der Destillation mit *p*-Toluolsulfonsäure [*Allylcarbin*]-*acetat*. Durch Umsetzung von Allylalkohol mit Formaldehyd in Ameisensäure und thermische Spaltung des Reaktionsproduktes entsteht *Allylcarbinol* bzw. dessen Formiat. Beim Kochen von Formaldehyddiallylacetal mit Eisessig-Schwefelsäure bildeten sich 3-Acetoxytetrahydrofuran und Butantriol-(1,2,4)-triacetat.

Dem *Fridtjof Nansen-Fond* und *Norges Almenvitenskapelige Forskningsråd* danke ich für finanzielle Unterstützung.

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Eingegangen am 3. Juli 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 907–913

Electrophoresis of Mucopolysaccharides in a Slab of Hyflo Super-Cel

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Most methods for the fractionation of mucopolysaccharides are based on differential precipitation with organic solvents from their aqueous solutions, in the presence of salts. Acid mucopolysaccharides are separated by means of the solubility in water of their alkaloid salts. Still other methods have recently been devised in which fractionation is achieved by means of partition between two immiscible solvents [1].

For the isolation of polysaccharides from various organs we have used the method described by Jorpes and Gardell [2] for the fractionation of the easily soluble barium salt of the crude heparin preparations.

It was however impossible with this method to obtain a good separation of the chondroitin sulphuric acid from the hyaluronic acid. The method of Meyer and Chaffee [3] likewise gave inadequate separation. Previous experiments on the other hand have shown that the electrophoretic mobilities of these two substances are widely different. Thus Blix working with synovial fluid found at pH 8 a mobility of -12 cm^2v^{-1} sec^{-1} $\times$ 10^5 for hyaluronic acid and working with cartilage extract a mobility of -17 $cm^2v^{-1}sec^{-1}$ $\times$ 10^5 for chondroitin sulphuric acid. The higher mobility of the chondroitin sulphuric acid is no doubt due to the fact that it carries both a sulfate and a carboxyl group per disaccharide unit whereas hyaluronic acid has only a carboxyl group.

An attempt to separate the two polysaccharides by electrophoresis seemed therefore to be called for.

An extremely simple apparatus for ionophoresis and electrophoresis has been described by Consden, Gordon and Martin [5] who were able to separate mixtures of amino acids and lower peptides in a slab of silica jelly.

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As however it seemed unlikely that molecules as large as those of chondroitin sulphuric acid and hyaluronic acid would be able to migrate through silica jelly it was decided to attempt a separation in a slab consisting of powdered quartz.

Preliminary experiments in which this stabilising agent was used, enabled the separation of the mixture of the two polysaccharides into two bands. The bands were however broad and their separation not quite satisfactory. The width of the bands and the degree of their separation were found to vary considerably with the amount of fluid held by the quartz powder slab during the experiment, a wetter plate resulting in broader bands. More finely ground samples of quartz also seemed to improve the results. Unfortunately the initial preparation of slabs of quartz powder of exactly the same degree of wetness was extremely difficult and in addition the degree of wetness usually tended to become accentuated during the electrophoresis. During the course of this work the design of an apparatus for continuous electrophoresis in media stabilised with glass powder was published by Svensson and Brattsten [6]. Difficulties such as those due to differing degrees of wetness are eliminated by the use of a slab of powder maintained in a vertical position between perspex plates.

During the experiments reported here however these difficulties were largely overcome by substituting Hyflo Super-Cel, a commercial kieselguhr for the quartz powder. The use of this material was suggested to one of us by Dr. A. J. P. Martin. In contrast to quartz powder this substance was easily packed homogenously into the apparatus and could contain an appreciable amount of fluid without the appearence of free liquid. Consden *et al.* investigated the results of the ionophoresis of amino acid mixtures by taking an imprint of the damp slab on filter paper and identifying the amino acids on the paper. An analogous procedure could not be used in the present work since no reaction sufficiently sensitive for the identification of the two polysaccharides was available. Instead the electrophoresis was discontinued after a suitable time and the slab cut up into strips, one to two cms. wide, parallel to the electrodes. The strips were eluated with water and the amount of carbohydrate in the eluates were determined according to Gurin and Hood's modification of Dische's carbazole reaction [7]. The course of the electrophoresis could be followed and the time for discontinuing determined, by initially placing a drop of acid fuchsin at the same distance from the electrodes as that of the polysaccharide mixture. During the experiment the fuchsin separated into four components, one of which moved only slightly slower than the fastest moving polysaccharide fraction.

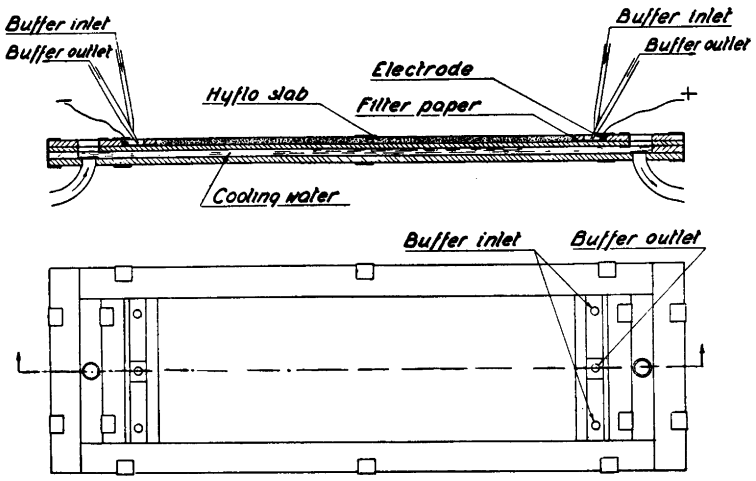

Fig. 1. Plan and cross section of the apparatus.

EXPERIMENTAL

A suitable amount of Hyflo Super-Cel is purified by boiling with concentrated sulphuric acid for one hour. Perhydrol is repeatedly added during the boiling. After cooling and diluting with several volumes of water the Super-Cel is filtered with suction and washed with water until the washings react neutral to litmus. It is then suspended in the buffer to be used in the experiment and again filtered. Should the pH of the filtrate deviate from the original value for the buffer washing is continued with buffer until the deviation disappears. The Super-Cel is then placed in the apparatus and packed by tapping with a large wooden spoon. The buffer, which is squeezed out during the packing, is blotted off with filter paper. By means of alternate packing and absorption of excess fluid a hard and even slab, moist but not wet, is prepared. When the apparatus has been filled with Super-Cel in this way a strip, three cm wide is cut off with a sharp knife from each end of the slab and replaced by a single layer of filter paper three cm wide. The ends of the slab are also supported by one cm wide barriers consisting of a further four layers of filter paper surmounted by strips of glass. The electrodes were a platinum anode and a brass cathode placed at a distance of 1.5 to 2 cm from each end of the Super-Cel slab and parallel to it. The buffer is made to flow in and out between the electrodes and the slab. For details see Fig. 1. The rate of flow of the buffer is regulated in such a way that though the fluid no more than covers the

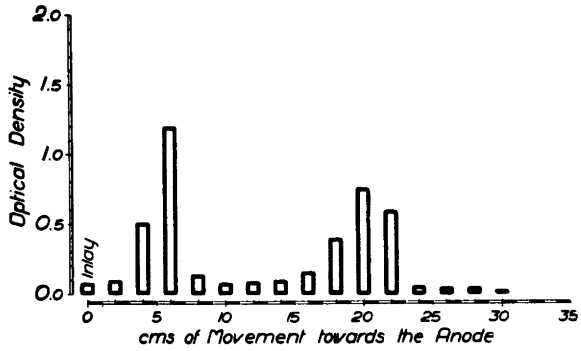

Fig. 2. Electrophoresis at pH 4.7 of a mixture of 20 mgs of chondroitin sulphuric acid and 20 mgs of hyaluronic acid.

electrodes which themselves lie on the filter paper covering the floor of the apparatus, no change in pH can be observed in the buffer in the proximity of the Super-Cel slab. The polysaccharide mixture to be subjected to electrophoresis, usually some 30—40 mg, is dissolved in buffer and made into a thick paste with Super-Cel, this is then packed carefully into a groove, 1 cm wide, which has been cut out from the slab, parallel to the electrodes. The groove does not extent across the whole slab but ends 2—4 cm from each side. A circular hole of diameter 2—4 mm is cut out at the same distance from the electrodes as is the groove and filled with acid fuchsin, mixed with buffer and Super-Cel. The position of the groove in the slab is chosen so that the substances under separation may have the maximum possible migration distance. The electrodes are connected to a source of D. C. supply, giving a potential gradient of from 6 to 7 volts per cm. When a 0.1 *M* acetate buffer was used with this potential, gradient a current of 100 to 150 mA was obtained. The voltage between the electrodes should not be so great that the slab becomes warm. The time for which the electrophoresis is conducted is estimated from the distance of movement of the acid fuchsin. At a suitable moment the slab is cut up into transverse strips 1—2 cm wide. Each strip is suspended in 20—40 ml of water and centrifuged or allowed to sediment for a few hours. The supernatant is decanted and the precipitate washed once with water. The combined mother liquor and washings are evaporated to dryness in vacuo, the temperature of the water bath being kept at + 70° C. The residue is dissolved in 4—10 ml of water. One ml of the solution is analyzed according to Gurin and Hood's modification of Dische's carbazole reaction. The colour density at 540 mμ is read off by means of Coleman junior spectrophotometer.

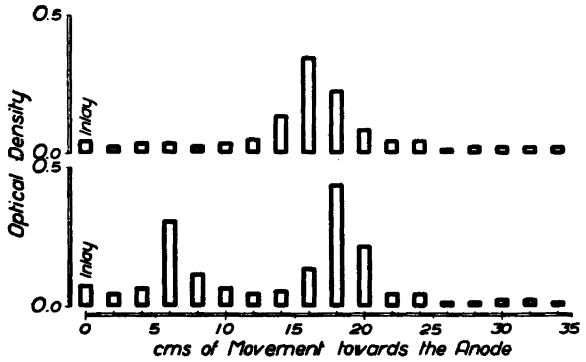

Fig. 3. Electrophoresis at the same time of 15 mgs of chondroitin sulphuric acid and a mixture of 15 mgs of chondroitin sulphuric acid and hyaluronic acid.

RESULTS

During the electrophoresis at three different pH values *i. e.* 3.7 (0.1 *M* citrate buffer), 4.7 (0.1 *M* acetate buffer) and 6.7 (0.1 *M* phosphate buffer) a mixture of chondroitin sulphuric and hyaluronic acid separated into two bands. In these experiments a mixture of 20 mg each of chondroitin sulphuric and hyaluronic acid was used. The result of a typical experiment is seen in Fig. 2. The chondroitin sulphuric acid was prepared from bovine tracheal cartilage according to the method of Jorpes [8] and the hyaluronic acid from umbilical cord by digestion with pancreatic extract and removal of the remaining protein with Lloyd's reagent. To ascertain, whether the bands were really due to a separation of the two components in the mixture, experiments were carried out with 40 mg of only one of the polysaccharides. These experiments showed that the hyaluronic acid and the chondroitin sulphuric acid yielded bands similar to the slower and the faster ones formed respectively from the mixture. To find out whether the two polysaccharides would influence each other's mobilities when together, a modification of the apparatus was made. After the slab had been packed it was divided into two halves by a vertical glass strip at right angles to the electrodes. A mixture of chondroitin sulphuric and hyaluronic acids was placed in one of the compartments thus formed and chondroitin sulphuric acid only in the other. The result is evident from Fig. 3. The faster moving fraction of the mixture moved at the same speed as the chondroitin sulphuric acid in the other compartment. In order to ascertain the recoveries from these experiments the amount of polysaccharide in each strip was estimated by means of a standard absorption curve made from each substance. The recovery usually was 80—90 per cent.

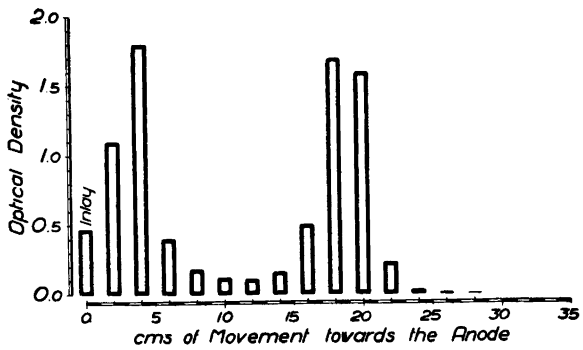

Fig. 4. Electrophoresis at pH 4.7 of hog skin polysaccharide.

Different preparations of both chondroitin sulphuric and hyaluronic acid behave identically in the apparatus. Chondroitin sulphuric acid, prepared according to a new method by Strandberg (9) and hyaluronic acid, prepared from the vitreous body, had the same mobilities as had the earlier preparations.

ESTIMATION OF CHONDROITIN SULPHURIC AND HYALURONIC ACID IN THE TISELIUS APPARATUS

Both polysaccharides were also examined in the Tiselius apparatus in acetate buffer at pH 4.7 and of ionic strength 0.1.

Although the presence of small peaks indicated that neither of these materials was quite pure the mobilities of the main components if the pH of the analysis is taken into account were not inconsistent with those of Blix mentioned above.

APPLICATION OF THE METHOD TO THE FRACTIONATION OF BIOLOGICAL MATERIAL

According to Meyer and Chaffee [3] chondroitin sulphuric and hyaluronic acids are found in hog skin. This could be verified with electrophoresis.

A polysaccharide was prepared from defatted hog skin by a method to be described later. It consists essentially of digesting the crude material with pancreatic extract and adsorbing the last traces of protein with Lloyd's reagent. The polysaccharides are then precipitated with alcohol. An almost protein-free preparation is obtained.

200 mg of this preparation was subjected to electrophoresis at pH 4.7 according to the method described above. Two bands with the mobility of

chondroitin sulphuric and hyaluronic acid respectively were obtained (Fig. 4). No analysis of the fractions was made.

DISCUSSION

The present method seems to provide a rather simple means for the electrophoretic separation of substances whose molecules are either to large to be able to penetrate through jellies or for which no appropriate jelly is at present available. If a larger apparatus or the apparatus of Svensson and Brattsten mentioned above is used it will be possible with this method to separate polysaccharides in amounts sufficient for chemical and structural analysis.

SUMMARY

A simple method for the electrophoretic separation of mucoplysaccharides is described. It is possible with this method to separate a mixture of chondroitin sulphuric acid and hyaluronic acid.

The presence of hyaluronic and chondroitin sulphuric acid in hog skin polysaccharide is demonstrated.

Modification in the technique for the application of the method for preparative purpose is suggested.

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Received June 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 914–921

On Hydrogen Peroxide Decomposition

II. The Ferric Salt Catalysis

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In a previous paper [1] it has been shown that the decomposition of hydrogen peroxide catalysed by ferric salts can be explained by the following reaction mechanism:

$$\begin{aligned} Fe^{+++} + HO_2^- &\rightleftharpoons FeOH^{++} + O \\ O \quad + HO_2^- &\rightarrow OH^- \quad + O_2 \end{aligned} \qquad \text{I}$$

or by:

$$\begin{aligned} Fe^{+++} + HO_2^- &\rightleftharpoons FeOOH^{++} && 12 \\ FeOOH^{++} &\rightleftharpoons FeOH^{++} + O && 25 \\ O \quad + HO_2^- &\rightarrow O_2 \quad + OH^- && 50 \end{aligned} \qquad \text{II}$$

The apparently illogical numbering of the processes has been introduced in a paper to be published later on. The number 12 in the first equation has to be read one, two, reaction 21 (two, one) will then be the opposite reaction etc.

From the expression which has been found for the reciprocal velocity of reaction:

$$-\frac{dt}{dx} = \frac{0.4343}{B} \cdot \frac{1}{x} + \frac{A}{B} \cdot \frac{1}{x^2} \qquad (6a)^{**}$$

it cannot be said right away which of the above reaction mechanisms affords the best explanation of the reaction, but in 1949 Evans, George and Uri [2] showed that even a rather acid solution of ferric salt is coloured brown by the addition of hydrogen peroxide; they think that the brown colour is due to the production of $FeOOH^{++}$ ions. On the basis of colorimetric measurements a complex constant for the ion has been calculated, *i. e.* an equilibrium

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** This equation and equation (16) were first introduced in the above mentioned paper.[1]

constant for reaction 12 in scheme II; furthermore they have investigated the dependence of the equilibrium constant on the temperature. In consequence of this finding reaction mechanism number II must be taken as the best representation of the reaction.

In the following an account will be given on the calculation of the velocity constants for some of the reactions in reaction scheme II and on some investigations concerning the dependence of these constants on the temperature.

1. CALCULATION OF SOME VELOCITY CONSTANTS AT 25° C

If it is assumed, that soon after the addition of hydrogen peroxide to the ferric salt solution a stationary state is attained the expression for the reciprocal velocity can be written (Christiansen [3]):

$$\frac{1}{s} = \frac{dt}{dO_2} = -\frac{2dt}{dH_2O_2} = \frac{1}{k_{12} \cdot c_{Fe^{+++}} \cdot c_{HO_2^-}} \cdot \left(1 + \frac{k_{21}}{k_{25}}\right) + \frac{1}{k_{12} \cdot c_{Fe^{+++}} \cdot c_{HO_2^-}} \cdot \frac{k_{21} k_{52} c_{FeOH^{++}}}{k_{25} k_{50} c_{HO_2^-}} \tag{16}$$

or when K_{Fe} is the equilibrium constant for: "$Fe^{+++} + H_2O \rightleftharpoons FeOH^{++} + H^+$" and $K_{H_2O_2}$ is the acid dissociation constant for hydrogen peroxide, and c_{Ferric} is the concentration of ferric salt:

$$-\frac{dt}{dH_2O_2} = \frac{1}{2} \cdot \frac{K_{Fe} + c_{H^+}}{c_{Ferric} \cdot K_{H_2O_2}} \cdot \frac{1}{k_{12}} \cdot \left(1 + \frac{k_{21}}{k_{25}}\right) \cdot \frac{1}{c_{H_2O_2}} + \frac{1}{2} \cdot \frac{K_{Fe} \cdot c_{H^+}}{K^2_{H_2O_2}} \cdot \frac{k_{21}}{k_{12}} \cdot \frac{k_{52}}{k_{25}} \cdot \frac{1}{k_{50}} \cdot \frac{1}{c^2_{H_2O_2}} \tag{18}$$

As shown in reaction mechanism II the $FeOOH^{++}$ion may be split up in two ways, : according to reaction 21 or 25. Assuming reaction 21 to be much more rapid than 25 we have: $k_{21} >> k_{25}$, *i. e.* (18) may be written:

$$-\frac{dt}{dH_2O_2} = \frac{1}{2} \cdot \frac{K_{Fe} + c_{H^+}}{c_{Ferric} \cdot K_{H_2O_2}} \cdot \frac{k_{21}}{k_{12}} \cdot \frac{1}{k_{25}} \cdot \frac{1}{c_{H_2O_2}} + \frac{1}{2} \cdot \frac{K_{Fe} \cdot c_{H^+}}{K^2_{H_2O_2}} \cdot \frac{k_{21}}{k_{12}} \cdot \frac{k_{52}}{k_{25}} \cdot \frac{1}{k_{50}} \cdot \frac{1}{c^2_{H_2O_2}} \tag{19}$$

By comparing (6a) and (19) we obtain:

$$\frac{0.4343}{B} = \frac{1}{2} \cdot \frac{K_{Fe} + c_{H^+}}{c_{Ferric} \cdot K_{H_2O_2}} \cdot \frac{k_{21}}{k_{12}} \cdot \frac{1}{k_{25}} \tag{20}$$

and:

$$\frac{A}{B} = \frac{1}{2} \cdot \frac{K_{Fe} \cdot c_{H^+}}{K^2_{H_2O_2}} \cdot \frac{k_{21}}{k_{12}} \cdot \frac{k_{52}}{k_{25}} \cdot \frac{1}{k_{50}} \tag{21}$$

When K_{12} is the equilibrium constant for reaction 12 we have:

$$\frac{k_{12}}{k_{21}} = K_{12} \tag{22}$$

and (20) may be written:

$$k_{25} = \frac{B}{0.8686 \cdot c_{Ferric}} \cdot \frac{K_{Fe} + c_{H^+}}{K_{H_2O_2}} \cdot \frac{1}{K_{12}} \tag{23}$$

In the above cited paper [1] Table 3 B/c_{Ferric} at $c_{H^+} = 0.01$ was found to be 4.43. $K_{Fe} = 2 \cdot 10^{-3}$ (*l. c.* page 10). $K_{H_2O_2} = 2.4 \cdot 10^{-12}$ (Joyner [4]). $K_{12} = 2 \cdot 10^9$ (Evans and coworcers [2]). From these numbers k_{25} is calculated by means of (23):

$$k_{25} = 13 \text{ mol/min. at } 25° \text{ C.}$$

(21) may be written:

$$\frac{k_{50}}{k_{52}} = \frac{B}{A} \cdot \frac{1}{2} \cdot \frac{K_{Fe} \cdot c_{H^+}}{K^2_{H_2O_2}} \cdot \frac{1}{K_{12} \cdot k_{25}} \tag{24}$$

From the experimental results in Table 3 in the above cited paper [1] B/A is found to be 10.6, when the hydrogen ion concentration is 0.01. Substituting in (24) this value for B/A together with the above mentioned values for the equilibrium constants and the above calculated value for k_{25}, the ratio between k_{50} and k_{52} may be calculated:

$$\frac{k_{50}}{k_{52}} = 7 \cdot 10^8 \text{ at } 25°\text{C}$$

By means of this value for the ratio $\frac{k_{50}}{k_{52}}$ the ratio $\frac{w_{50}}{w_{52}}$ can be calculated. w_{50} means the probability for the oxygen atoms to react according to 50 and w_{52} the probability to react according to 52.

$$\frac{w_{50}}{w_{52}} = \frac{k_{50} \cdot c_{HO_2^-}}{k_{52} \cdot c_{FeOH^{++}}} \tag{25}$$

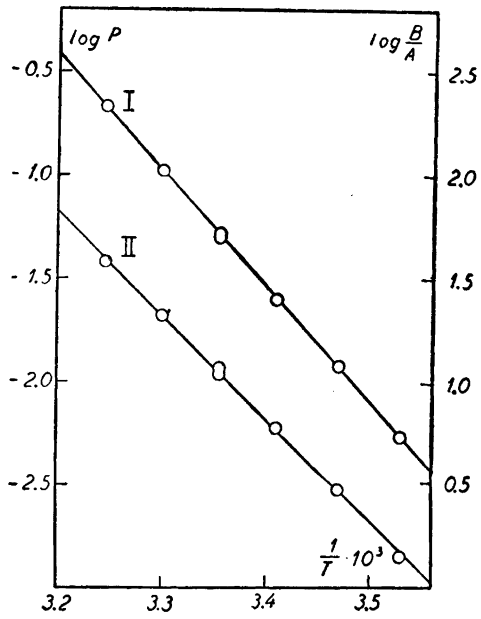

Fig. 1. Curve I: The dependence of $\log P = \log \frac{B}{c_{Ferric}} (K_{Fe} + C_{H^+})$ *on the temperature. Curve II: The dependence of* $\log \frac{A}{B}$ *on the temperature.*

In a mixture with $c_{H_2O_2} = 0.05$, $c_{H^+} = 0.01$ and $c_{Ferric} = 0.001$ the ratio will be about 50 and after 90 % of the hydrogen peroxide has been decomposed the ratio will be about 5.

2. DETERMINATION OF SOME ACTIVATION ENERGIES

Experiments have been carried out at 6 different temperatures between 10° C and 35° C. The experiments were all carried out as described in the above cited paper [1] page 2. The composition and the temperatures of the reacting mixtures are shown in Table 1, together with the values for the constants A and B.

According to (23) is:

$$\log k_{25} = \log \frac{B}{c_{Ferric}} + \log (K_{Fe} + c_{H^+}) - \log K_{H_2O_2} - \log K_{12} - \log 0.8686 \quad (26)$$

The values for $\log \frac{B}{c_{Ferric}}$ are given in Table 1 column 6 and the values for $\log (K_{Fe} + c_{H^+})$ are given in column 7. The values used for K_{Fe} at the different temperatures are calculated from the equation:

Table 1. The dependence of the constants A and B on the temperature. In all the experiments the hydrogen ion concentration was 0.01, and the initial concentration of hydrogen peroxide 0.05.

1	2	3	4	5	6	7	8
T	$\frac{1}{T} \cdot 10^3$	c_{Ferric}	$A \cdot 10^3$	$B \cdot 10^3$	$\log \frac{B}{c_{\text{Ferric}}}$	$\log(K_{\text{Fe}} + c_{\text{H}^+})$	$\log \frac{B}{A}$
283.15	3.532	0.004	1.50	2.12	− 0.284	− 1.972	0.150
288.15	3.470	0.004	1.50	4.40	0.041	− 1.960	0.467
293.15	3.411	0.004	1.50	9.00	0.352	− 1.945	0.778
298.15	3.354	0.002	0.80	8.70	0.639	− 1.921	1.036
298.15	3.354	0.004	1.50	17.60	0.644	− 1.921	1.069
303.15	3.299	0.002	0.80	16.60	0.912	− 1.892	1.316
308.15	3.245	0.002	0.80	30.80	1.188	− 1.854	1.586

$$\log K_{\text{Fe}} = -\frac{12300}{4.57\,T} + 6.33 \tag{27}$$

The heat of reaction: 12 300 cal/mol is calculated by Rabinowitch and Stockmayer [5]. Furthermore we use $K_{\text{Fe}} = 2 \cdot 10^{-3}$ at 25° C.

The values for $\log P = \log \frac{B}{c_{\text{Ferric}}} \cdot (K_{\text{Fe}} + c_{\text{H}^+})$ are plotted against $1/T$ in Fig. 1. From the figure is calculated

$$\log \frac{B}{c_{\text{Ferric}}} (K_{\text{Fe}} + c_{\text{H}^+}) = -5580 \cdot \frac{1}{T} + 17.24 \tag{28}$$

For $H_2O_2 \rightleftharpoons H^+ + HO_2^-$ Joyner [4] has measured $\Delta H = 8600$ cal/mol; when $K_{H_2O_2} = 2.4 \cdot 10^{-12}$ at 25° C we have:

$$\log K_{H_2O_2} = -\frac{8600}{4.57\,T} - 5.30 \tag{29}$$

From the data of Evans and coworkers [2] can be calculated:

$$\log K_{12} = -\frac{1800}{4.57\,T} + 10.65 \tag{30}$$

Substituting equations (28), (29) and (30) in (26) we get:

$$\log k_{25} = -\frac{15100}{4.57\,T} + 11.95 \tag{31}$$

or

$$k_{25} = 10^{11.95} \cdot e^{-\frac{15100}{RT}}\ \text{min}^{-1}$$

or

$$k_{25} = 10^{10.17} \cdot e^{-\frac{15100}{RT}}\ \text{sec}^{-1} \tag{31a}$$

i. e. the activation energy for the proces 25 is

$$E_k = \text{about } 15000 \text{ cal/mol}$$

According to (24) is:

$$\log \frac{k_{50}}{k_{52}} = \log \frac{B}{A} + \log 0.5 + \log K_{Fe} + \log c_{H^+} - 2 \log K_{H_2O_2} - \log K_{12} - \log k_{25} \tag{32}$$

The values for $\log \frac{B}{A}$ are given in Table 1 column 8; these values are plotted against $\frac{1}{T}$ in Fig. 1. From the figure is calculated

$$\log \frac{B}{A} = -5082 \cdot \frac{1}{T} + 18.08 \tag{33}$$

Substituting (27), (29), (30), (31) and (33) in (32) we have when $e_{H^+} = 0{,}01$:

$$\log \frac{k_{50}}{k_{52}} = -\frac{1425}{4.57\,T} + 10.11 \tag{34}$$

or

$$\frac{k_{50}}{k_{52}} = 10^{10.11} \cdot e^{-\frac{1425}{RT}} \tag{34a}$$

that is: the difference between E_k for process 50 and E_k for the process 52 is equal to about 1400 cal/mol. This figure is however so small that the activation energies for the two processes mentioned may be considered practically equal as the values of the figures used for the calculations are rather uncertain.

DISCUSSION

It is shown in the above section that the velocity constant for the process 25 can be written

$$k_{25} = 10^{10.17} \cdot e^{-\frac{15100}{RT}} \text{ sec.}^{-1} \tag{31a}$$

The frequency factor $10^{10.17} \cdot \text{sec.}^{-1}$ is less than the value which one normally finds for monomolecular reactions *i. e:* about 10^{12} — but it is not much less when one takes into concideration the errors in the figures used.

It is also shown that the ratio between the velocity constants for the bimolecular reactions 50 and 52 can be written:

$$\frac{k_{50}}{k_{52}} = 10^{10.11} \cdot e^{-\frac{1425}{RT}} \tag{34a}$$

The factor $10^{10.11}$ has an unexpectedly high value. Following from the simple collision theory one would have expected a value of about one [6]. This means that the reactions 50 and 52 deviate from the normal in such a way that the collision factor in k_{50} is greater and the collision factor in k_{52} is less than is expected from the collision theory. Such reactions are known and are not uncommon.

Moelwyn-Hughes includes in his book [7] the following as explanation of such deviations from the normal:

"Chief among the causes responsible for reducing below its normal value the rate of a second-order reaction in a homogeneous system are: (1) endothermic formation of a complex prior to the reaction proper; (2) the necessity for the ionization of either or both of the reactants; (3) deactivation by solvent molecules; (4) stringent conditions of orientation or of internal phase of the reacting molecules at the moment of impact, and (5) the existence of strong forces of repulsion. Factors which allow a faster reaction velocity than that permitted by the simple collision theory are: (a) exothermic formation of a complex prior to the reaction proper; (b) the distribution of the energy of activation among a number of internal degrees of freedom of the reacting molecules; (c) the propagation of reaction chains; (d) activation by some means other than by collision, *e. g.* by radiation, and (e) the existence of strong forces of attraction. Evidence from individual reactions could be quoted in support of each of these."

It cannot be said, which of the above mentioned factors can be applied in the present case. But that the collision factor in k_{52} is less than expected is

perhaps due to a deactivation of the oxygen atoms by collision with the water molecules which are combined with the $FeOH^{++}$ ions. And the fact that the collision factor in k_{50} is greater than expected is perhaps due to activation in a different way than by collision. It is possible that the magnetic properties of the oxygen atoms could have an effect upon the velocity of the process 50.

SUMMARY

1. It has been shown that the ferric salt catalyzed decomposition of hydrogen peroxide must be explained by the reaction mechanism:

$$\begin{aligned} Fe^{+++} + HO_2^- &\rightleftharpoons FeOOH^{++} && 12 \\ FeOOH^{++} &\rightleftharpoons FeOH^{++} + O && 25 \quad \text{II} \\ O + HO_2^- &\rightarrow O_2 + OH^- && 50 \end{aligned}$$

and some of the velocity constants have been calculated.

2. The activation energies for some of the processes in the scheme II have been calculated on the bases of experiments.

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Received May 26, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 922–925

Vitamin K in Germinating Peas

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A survey of the literature reveals only two instances of vitamin K formation in germinating seeds. Dam and Glavind [1] studied the effect of light on the formation of vitamin K in germinating peas and used the biological method for determining the vitamin. The plant material was dried and fed to test animals (chickens) either as such or as a petroleum ether extract. The vitamin amount was reported in Dam units and obtained by measuring the clotting time of blood. The findings showed that the vitamin K content of peas germinating in the presence of light for sixteen days was approximately treble the amount of vitamin K of seedlings kept a corresponding time in darkness. Dam *et al.*[2] later carried out a similar experiment with the germinating seeds of *Picea canadense* whose sprouts exposed to light were analyzed. Also the vitamin K-content of plants germinating in light was approximately double that of sprouts kept in darkness. In the latter case some vitamin K had formed in the darkness since the vitamin content after 14 days of germination was higher than in seeds.

The present paper relates to an experiment in which the vitamin K was determined colarimetrically by means of the redox method, the objective being to follow daily the vitamin K formation in peas and simultaneously compare the values obtained with those reported by Dam. The germinations were carried out both in the presence and absence of light.

EXPERIMENTAL

Vitamin K was determined according to the method of Scudi and Buhs [3] which is a colorimetric adaptation of the principles employed by Trenner and Bacher [4]. This method involves a catalytic hydrogenation of the vitamin K-quinone to the hydroquinone stage with Raney nickel catalyst in butanol solution, and use of phenosafranine as an indicator. Hydroquinone is reoxidized with 2,6-dichlorophenol indephenol in the

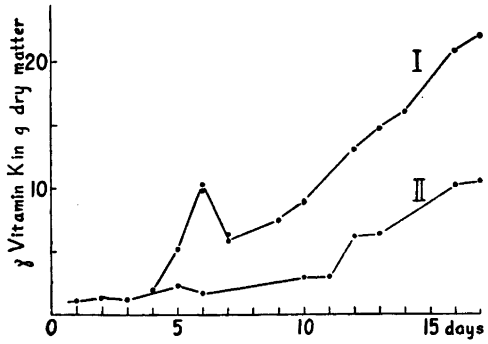

Fig. 1. Vitamin K content of peas germinating in (I) presence of light, (II) absence of light.

absence of air, and the decrease in colour is measured. Interfering colors present are removed by extracting the hydroquinone, after reduction with Claisen's alkali, in a special apparatus. The method is not specific since all quinones present give the same reaction.

The seedlings were allowed to germinate in damp quartz sand kept in a photothermostat. Two 200-watt lamps gave light during nine hours of the day. Of the 50 seedlings the best developed 40—45 were chosen for each determination. The seedlings were rinsed, dried between blotting paper and weighed. They were then cut into small pieces which were dried in a thermostat at 60° C for 12 to 18 hours and subsequently in a vacuum desiccator having nitrogen pressure of about 12 mm. The dried preparation was weighed and extracted in the dark with petroleum ether. Reduction in the modified apparatus of Scudi and Buhs lasted for 15 to 30 min, in some cases still longer, despite the relative rapidity of the hydrogen current.

The vitamin K content of peas does not change notably during the first four days, but from this time on the phylloquinone amount begins to increase in seedlings grown in light. Immediately after the epicotyl is exposed to light, the vitamin K amount in the seedlings increases five-fold during the fifth and sixth days, then decreases slightly during the seventh day but after that the vitamin amount of the seedlings exposed to light increases steadily until the seventeenth day.

In etiolated seedlings an increase in vitamin K content begins approximately at the end of the ninth germination day. In peas sixteen days old the vitamin content of those exposed to light was 20.8 and of those grown in the dark 10.1 γ per gram dry-material (Fig. 1).

DISCUSSION

Thayer *et al.*[5] define the activity of phylloquinone in a mg as 1000 Thayer-Doisy units, which corresponds to 10 Dam units. Hence, 1 Dam unit equals 0.1 γ of vitamin K. The 16 days old peas in the experiment of Dam and Glavind[1] consequently contained 33.5 γ and 9.5 γ vitamin K corresponding to our values of 20.8 γ and 10.1 γ. Our values for etiolated peas conform with

those of Dam and Glavind, but the ones for peas germinating in the presence of light are considerably lower. For removal of carotinoids the reduction prior to determination proper had to be repeated twice in our experiments which process probably caused some losses in green plants. Nor is the method quite specific as fairly large amounts of tocoferylquinone affect the result (Scudi and Buhs [6]).

Figure 1 is elucidative in showing the effect of light on the vitamin K formation. Vitamin K forms also in the absence of light and consequently its biosynthesis is not bound to chlorophyll formation as assumed by Dam *et al* [2]. Dam regards his findings of vitamin K in peas germinating in the dark so low that they hardly exceed the amount found in the seed itself. He explains that the vitamin K increase in the seedlings of *Picea canadense* germinating in the absence of light is due to chlorophyll-synthesis which, as is well known, is noted in evergreens despite the absence of light. The parallelism between vitamin K synthesis and chlorophyll synthesis is according to Dam, due to phytol occuring in both molecules. True, Dam himself remarks [2] that in the absence of light phytyl forms in connection with protochlorophyll which in turn can develope in the etiolated parts of plants. On the other hand, there is no evidence as to whether the material defined colorimetrically or by animal tests is, in reality, phylloquinone. In the animal tests also other naphtoquinone derivatives are active, and in the method employed all the quinones give the same reaction.

The vitamin K maximum occuring on the sixth day in peas germinating in light is interesting and deserves attention. As vitamin K accumulates in the chloroplasts [2] the great increase in vitamin K content can, during the time mentioned, be connected with the likewise intensive chlorophyll synthesis. Here mention is made of the fact that when peas germinate under conditions identical with those used in our experiments catalase, according to Virtanen *et al.*[7], also attains an activity maximum on the sixth day of germination. In a like manner this enzyme has greatly increased in the chloroplasts and its activity generally is proportional to the intensity of photosynthesis [8,9].

SUMMARY

The formation of vitamin K in peas germinating in the presence and absence of light has been studied. Vitamin K is also formed in the absence of light although in quantities considerably smaller than in the presence of light.

Between the fourth and sixth day the seedlings which developed in the presence of light were observed to increase greatly in vitamin content after

the exposure of the epicotyl. The maximum attained on the sixth day was followed by a slight drop and then by a gradual increase in the vitamin content beginning from the seventh day. Etiolated seedlings showed an increase in vitamin K only after the eleventh day.

The vitamin K content of ungerminated but swollen seeds was 1.2 γ per gram of dry matter. In 17 days the vitamin K content increased to 22.0 γ in the presence of light and to 10.3 γ in the absence of light.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 4 (1950) 926–936

The Molecular Structure of Biphenyl and some of its Derivatives. II

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NON-*ORTHO*-SUBSTITUTED BIPHENYLS

Electron diffraction studies of biphenyl and some of its non-*ortho*-substituted derivatives have been carried out by the sector method, showing that the molecules have non-planar configurations in the gaseous state [1,2]. It was, however, not possible in any of the cases treated to give the value of the angle φ between the two ring planes with very great accuracy. The best result was obtained by the investigation of 3,3'-dibromobiphenyl. It led to six practically independent values for the angle, the mean value being 54° and the fluctuation being 5° to either side. An accurate determination of the angle is very desirable; it would give us valuable information about the van der Waals distances between two hydrogen atoms. The 3,3',5,5'-tetrabromobiphenyl was expected to be well fitted for the purpose; experience, however, unfortunately showed that this was not true. The study of the 3,3',5,5'-tetrabromobiphenyl did not lead to a more accurate determination of the angle than the study of the dibromo-compound already mentioned. By comparing the two compounds 3,3'-dibromobiphenyl and 3,3',5,5'-tetrabromobiphenyl, it can easily be seen that the latter one contains the largest number of distances varying with the angle φ, but it also contains the largest number of distances which do *not* vary. The latter distances seem, unfortunately, to effect the electron diffraction pattern to a greater extent than do the former ones. In Fig. 1 the $\frac{\sigma(r)}{r}$-curve for 3,3',5,5'-tetrabromobiphenyl is given. At about 8 Å the peaks in the curve seem to disappear. For $r > 8$ Å the fluctuations from the abscissa axis are very small and the reproducibility found by studying curves originating from different diagrams is unsatisfactory. Though this part of the curve also certainly gives evidence for the non-planarity of the

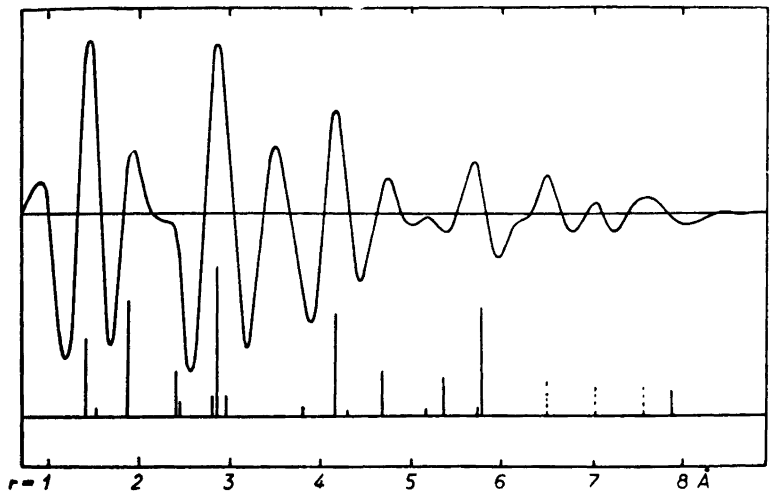

Fig. 1. $\frac{\sigma(r)}{r}$*-curve of 3,3',5,5'-tetrabromobiphenyl.*

molecule, it has been omitted in the discussion because of its uncertainty. Studying the $\frac{\sigma(r)}{r}$-curve for $r < 8$ Å we see that the inner part of the curve is easily explained by inter-atomic distances which do not vary with φ. (Solid lines in the line diagram.) The three outer peaks can be ascribed mainly to distances which do vary with φ. (Dashed lines in the line diagram.) In Table 1 the positions of the maxima and the corresponding distances and

Table 1. Values characterizing the magnitude of the deviation from coplanarity of 3,3',5,5'-tetrabromobiphenyl.

Observed r-values	Distances	φ	
6.49 Å	Br_3-C_2'	48°	
7.01 »	Br_3-C_5'	40°	mean value 49°
7.61 »	Br_3-C_3'	58°	

φ-values are given. For the last peak corrections for the fixed distance indicated have been carried through. The mean value of φ from these three peaks is found to be 49°.

Table 2. φ-values for different non-ortho-substituted biphenyls in the gaseous state.

Compound	
Biphenyl	45°
3,3'-Dibromobiphenyl	54°
3,3'-Dichlorobenzidine	52°
3,3',5,5'-Tetrabromobiphenyl	49°

In Table 2 we have listed the non-*ortho*-substituted biphenyls which have been studied by the electron diffraction method. The corresponding φ-values found are listed. If we now assume the actual angle to be the same for all the biphenyl derivatives of this type, φ can be given the value $50° \pm 5°$. If we further give the C—H-bond distance the value 1.05 ± 0.03 Å, the central C—C'-bond distance the value 1.50 ± 0.03 Å, and take into consideration the errors from all these three molecular parameters, but consider the C—C-bond distance in the ring to be correct, equal to 1.40 Å, we find the value for the 2,2'-H—H-distance to be 2.5 ± 0.2 Å. The value is perhaps somewhat larger than should be expected assuming the non-coplanarity to be caused by equilibrium between van der Waals forces and resonance phenomena. But considering the large limit of error it is not at all unreasonable.

I would like to draw attention to the fact that the calculation of the φ-value and consequently the 2,2'-H—H-distance is based upon the assumption that all the C—C-bond distances in the phenyl rings are the same. It has in several cases been claimed that this is far from correct. Fowweather and Hargreaves [3] report, in the case of *m*-tolidine dihydrochloride, variations in the C—C-bond length ranging from 1.31 Å to 1.40 Å, and Smare [4] finds in the case of 2,2'-dichlorobenzidine that the C—C-bonds are alternately long and short, with mean values 1.45 and 1.30 Å respectively. Both the investigations mentioned are based upon X-ray crystallographic work. We have, as will be mentioned later, never been able to observe such a great deviation by the electron diffraction method, though minor deviations can not be excluded. It is, on the other hand, easily seen that deviations of the type mentioned would not effect our calculation very much, and would not effect the main feature of the result at all.

ORTHO-SUBSTITUTED BIPHENYLS

It might also be worth while to study some *ortho*-substituted biphenyls to compare the molecular structure in the gaseous state with the result found in the crystals by the X-ray investigators. X-ray analysis of the crystal structure of m-tolidine dihydrochloride [4] and 2,2'-dichlorobenzidine [3,5] has shown that in each of these molecules the phenyl rings are rotated from the *cis*-planar configuration through approximately 36° in opposite directions around the central C—C'-bond, so that the angle between the ring planes is approximately 72°.

The structure of 2,2'-dichloro- and 2,2'-dibromobiphenyl has been studied by the sector method. The $\frac{\sigma(r)}{r}$-curves of these compounds are to a great

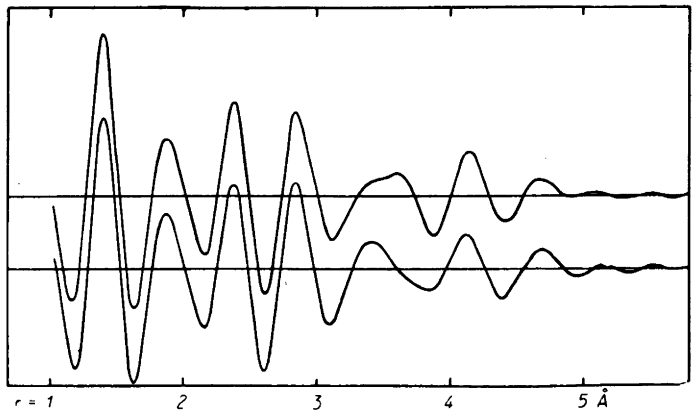

Fig. 2. The upper curve is the $\frac{\sigma(r)}{r}$*-curve of 2,2′-dibromobiphenyl, the lower one is the* $\frac{\sigma(r)}{r}$*-curve of monobromobenzene.*

extent composed of contributions from distances occurring within the phenyl rings. We should therefore assume that the $\frac{\sigma(r)}{r}$-curve of 2,2′-dichlorobiphenyl ought to be very similar to the $\frac{\sigma(r)}{r}$-curve of monochlorobenzene, and that the $\frac{\sigma(r)}{r}$-curve of 2,2′-dibromobiphenyl ought to be similar to that of monobromobenzene. — In Fig. 2 the upper curve is the $\frac{\sigma(r)}{r}$-curve of 2,2′-dibromobiphenyl, the lower one is that of monobromobenzene. The last curve has been multiplied by a factor of about 2 to get the proper normalization. The two curves are strikingly similar in the main features. The assumption made earlier that the phenyl rings in a biphenyl derivative are usually very nearly the same as the benzene ring itself is therefore shown to be reasonable. If, for instance, in the two curves we compare the peaks at 1.40 Å corresponding to the C—C-bond distance, we see no evidence of the existence of different C—C-bond lengths in the phenyl ring of the biphenyl derivative. In fact, a close inspection of the curves really shows that the peak mentioned is about 5 % wider for the 2,2′-dibromobiphenyl curve than for the bromobenzene curve. This is, however, easily explained by considering the contribution of the central C—C′-bond distance in the case of the 2,2′-dibromobiphenyl, the C—C′-bond distance being about 1.50 Å. The upper curve in

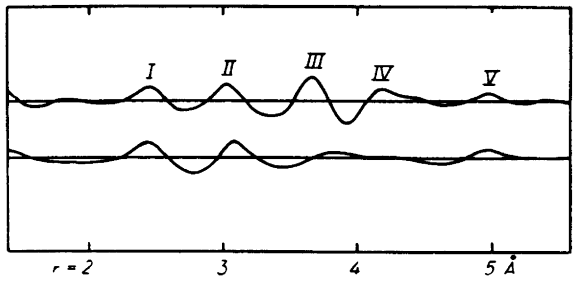

Fig. 3. The upper curve is the difference of the two curves of Fig. 2; the lower one is the theoretical $\frac{\sigma(r)}{r}$*-curve for distances between the two rings of the 2,2'-dibromo-biphenyl molecule, containing only distances which do not vary with* φ.

Fig. 3 is the difference between the two curves in Fig. 2. This difference $\frac{\sigma(r)}{r}$-curve contains contributions almost exclusively from the distances between atoms in different rings. These distances can be divided into two types: those which do not vary by rotation about the central C—C'-bond, and those which do vary. The contribution of the former kind to the difference $\frac{\sigma(r)}{r}$-curve can therefore, for the purpose of determining the angle φ, be subtracted to advantage from the curve. The lower curve in Fig. 3 is a theoretically calculated difference curve containing only distances which are independent of φ. The molecular structure parameters chosen to be used in these calculations are as follows: C—C-bond distance in the ring = 1.40 Å, C—Br-bond distance = 1.88 Å, valency angles = 120°, C—H-bond distance

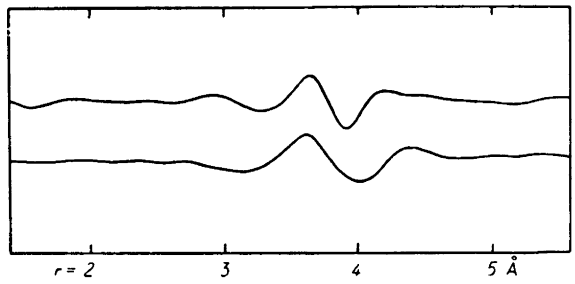

Fig. 4. The upper curve is the difference between the two curves of Fig. 3; the lower one is the $\frac{\sigma(r)}{r}$*-curve for distances in the 2,2'-dibromobiphenyl molecule which d o vary with* φ.

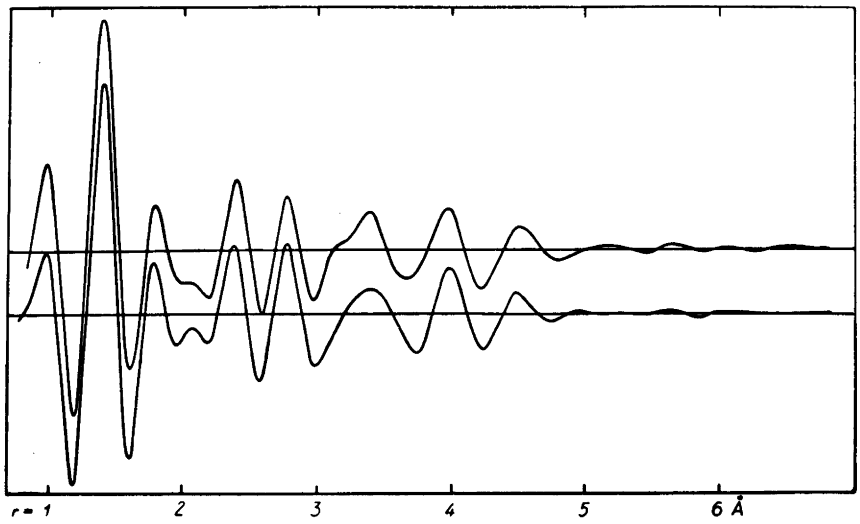

Fig. 5. The upper curve is the $\frac{\sigma(r)}{r}$*-curve of 2,2'-dichlorobiphenyl, the lower one is that of monochlorobenzene.*

= 1.05 Å, and the central C—C'-bond distance = 1.50 Å. By comparing the two curves in Fig. 3 we see that peak I, II, and V can be attributed mainly to distances independent of φ, while the peaks III and IV must be due to distances which vary with φ. In Fig. 4 the upper curve is the difference between the two curves in Fig. 3. A series of theoretical difference curves containing only distances varying by rotating about the central C—C'-bond has been calculated for different values of φ. ($\varphi = 0°$ for the *cis*-form). A value of $\varphi = 75°$ seems to lead to the best agreement with experimental data. The corresponding theoretical curve is given as the lower curve of Fig. 4. It has been multiplied by 0.5 to get a proper scale for the comparison with the upper curve. The real difference in the height of the main peak of the "experimental" and the theoretical curves of Fig. 4 can be explained by assuming that the molecule is not quite rigid. If the molecule is oscillating somewhat about the equilibrium position, the height of the main peak, which is due to the Br—Br-distance, must of course decrease. The minor disagreement in the shape of the two curves in Fig. 4 can also be partly explained by the assumption of an oscillation as mentioned, but other effects might contribute to a certain extent. For instance, the fact that the experimental $\frac{\sigma(r)}{r}$-curves are calculated using the temperature factor $e^{-0.007s^2}$ while the normal curves (Viervoll [6]) used for

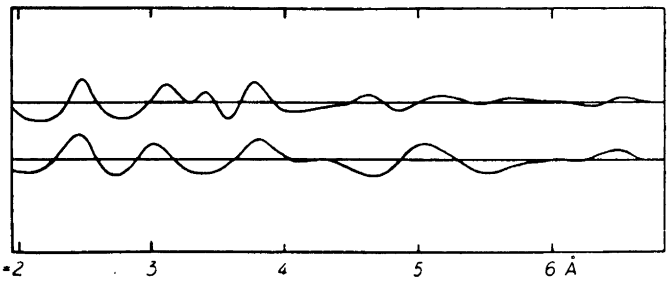

Fig. 6. The upper curve is the difference between the two curves of Fig. 5; the lower one is the theoretical $\frac{\sigma(r)}{r}$*-curve for distances between the two rings of the 2,2′-dichlorobiphenyl molecule, containing only distances which do not vary with* φ.

the theoretical curves are calculated with the temperature factor $e^{-0.01s^2}$ will be of some importance.

The same procedure carried through for 2,2′-dibromobiphenyl and bromobenzene has been applied to the corresponding chloro-compounds. In Fig. 5 the upper curve is the experimental $\frac{\sigma(r)}{r}$-curve of 2,2′-dichlorobiphenyl and the lower one that of monochlorobenzene. Here we also find the C—C-bond peak of the biphenyl derivative curve to be about 5 % wider than the corresponding peak of the chlorobenzene curve. The difference between the two curves of Fig. 5 is given as the upper curve in Fig. 6. The lower curve in this figure is the theoretical difference curve containing only distances which do not vary with the angle φ. The theoretical curve is calculated using a C—Cl-bond distance of 1.72 Å. The other parameters found to be in best agreement with experiments are the same as for the bromo-compound. — The curves of Fig. 7 are, as we see, a little more complicated than those of Fig. 4 to which they correspond. For 2,2′-dibromobiphenyl the halogen-halogen distance predominates, the carbon-halogen and the hydrogen-halogen distances being of less importance. In the case of 2,2′-dichlorobiphenyl, however, the carbon-halogen and the hydrogen-halogen distances must contribute to a relatively greater extent. This is easily seen in both the curves of Fig. 7. The φ-value which leads to the best agreement is, in this case, 74°. The Cl—Cl distance corresponds to peak I. On first inspection we might believe peak III to correspond to the Cl—Cl distance. This assumption leads to an angle of about 110°; however, resulting in a minimum at the r-value of peak I, it is easily excluded. In this case also the lower curve (Fig. 7) has been multiplied by 0.5 to make the height of the peaks comparable to those of the upper one.

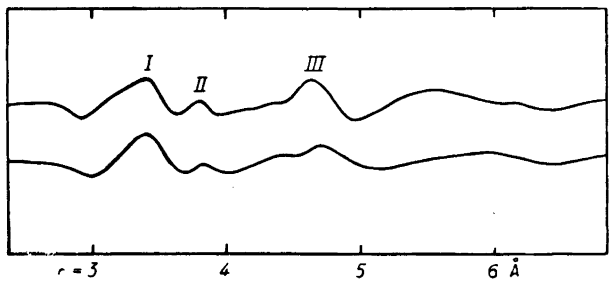

Fig. 7. The upper curve is the difference between the two curves of Fig. 6; the lower one is the $\frac{\sigma(r)}{r}$*-curve for distances in the 2,2'-dichlorobiphenyl molecule which do vary with* φ.

Here, therefore, as for the bromo-compound, there also seems to be an oscillation about the equilibrium position. The curves in this case give, however, still further indications for the existence of an oscillation: If we compare the relative height of the peaks of the curves of Fig. 7, remembering the factor 0.5, we see that peak I in the "experimental" curve (upper curve) is reduced considerably more than peak III, the last one being in fact only negligibly reduced. Now is peak I essentially due to the Cl—Cl distance while peak III is due to two Cl—C distances. By a rotation of, say, 10° from the equilibrium position, the Cl—Cl distance is increased or decreased about 0.18 Å, while *one* of the Cl—C distances contributing to peak III is *increased* about 0.06 Å and the other one is *decreased* by the same amount. It is therefore obvious that an oscillation about the equilibrium position must influence the height of peak I more than the height of peak III. This effect might be used to get a rough idea of the magnitude of the oscillation.

The 2,2'-diiodobiphenyl has also been studied. The electron diffraction diagrams taken were, however, not very good, and we got few diagrams, due to the small amount of substance available. The experiments ought therefore to be repeated. It might, however, be mentioned that the $\frac{\sigma(r)}{r}$-curve calculated shows the peaks which are expected due to the fixed distances, and in addition to that a pronounced peak appears at 3.82 Å. It can reasonably be interpreted as an I—I distance corresponding to a φ-value of about 79°.

If we include the 2,2'-diiodobiphenyl we can summarize our results in Table 3. In the fourth column the effective halogen-halogen radii found by our investigations are given. They are compared with the Pauling van der Waals radii [7] given in the fifth column. The latter are somewhat greater than the

Table 3. Values characterizing the amount of deviation from the coplanarity of some ortho-substituted biphenyls.

Compounds	φ	Halogen-halogen distances	Halogen-halogen radii	Pauling v.d. Waals radii	Difference
2,2′-Dichlorobiphenyl	74°	3.46 Å	1.73 Å	1.80 Å	0.07 Å
2,2′-Dibromobiphenyl	75°	3.62 »	1.81 »	1.95 »	0.14 »
2,2′-Diiodobiphenyl	79°	3.82 »	1.91 »	2.15 »	0.24 »

radii found by us. The difference increases in going from the chloro- to the iodo-compound.

The determination of the φ-value of the *ortho*-substituted biphenyls is probably more accurate than for the non-*ortho*-substituted biphenyls. The probable error of the halogen-halogen distance is presumably less than 0.05 Å. Taking into consideration the possible errors in the determination of the other parameters necessary for the calculation of the φ-value, we can conclude that the probable error of φ is about 5° or perhaps somewhat less than that. In this case also, however, an accurate estimation of the errors is rather difficult.

The φ-values found above are in good agreement with those found for the 2,2′-dichlorobenzidine by the X-ray investigation already mentioned. The φ-values for gaseous 2,2′-dichlorobiphenyl and solid 2,2′-dichlorobenzidine are, in fact, the same, within the limits of errors.

It might be objected that a comparison of a biphenyl derivative with the corresponding benzidine derivative is not permissible because of the additional resonance possibility caused by the amino groups in the latter type of compound. It is my opinion, however, that the φ-value of the *ortho*-substituted molecules under discussion is mainly determined by the interaction of the *ortho*-atoms, and is consequently not very much influenced by resonance phenomena.

The fact that the *ortho*-substituted biphenyls treated above, show a molecular structure more similar to the *cis*-form than to the *trans*-form seems perhaps a little surprising at first. According to Pauling [7] the electronegativities of hydrogen, carbon, and chlorine are 2.1, 2.5, and 3.0 respectively. This means that a repulsive force ought to act between two chlorine atoms and an attractive force ought to act between a hydrogen atom and a chlorine atom, both effects favouring a *trans*-form and decreasing the dipole moment of the molecule. Hampson and Weissberger [8], who have studied the dipole moment of 2-, 3-, and 4-mono-chlorobiphenyl and 2,2′-, and 3,3′-dichlorobiphenyl, were led to the conclusion that *London forces* play an important rôle in deter-

mining the inner configuration of the molecules. The three types of forces determining the structure of 2,2'-, and 3,3'-dichlorobiphenyl are, according to Hampson and Weissberger: a) Electrostatic repulsions and attractions, b) quantum mechanical attractions (London forces), and c) quantum mechanical repulsions (impenetrability of colliding atoms). To these we might add a fourth effect caused by resonance phenomena. This effect might, however, as earlier emphasized, be of less importance for the 2,2'-derivatives. In order to study the theoretical dipole moments of 2,2'-, and 3,3'-dichlorobiphenyl Hampson and Weissberger calculated the London forces between the two chlorine atoms. This procedure is essentially correct for the 2,2'-dichlorobiphenyl, but for the 3,3'-dichlorobiphenyl the 2,2'-hydrogen interaction, which was not at all taken into consideration by Hampson and Weissberger, must in fact be of much greater importance than the 3,3'-chloro interaction. — In the case of the 3,3'-compound Hampson and Weissberger believe the two phenyl rings to rotate almost unhindered about the central C—C'-bond. A completely free rotation leads to a dipole moment of 1.90 D, and if the weak chlorine-chlorine interaction is taken into consideration the calculated value is 1.89 D. The structure is, however, not at all unambiguously determined by the dipole moment measurement. The assumption made earlier [2] that the non-*ortho*-substituted biphenyls are non-planar with a φ-value of about 50° and with a fifty-fifty contribution of the *cis*- and the *trans*-like forms, also leads to a dipole moment of 1.89 D. For comparison the experimental value reported is 1.80 D. The calculated value is based upon Hampson and Weissberger's measurement of the dipole moment of 3-chlorobiphenyl (1.64 D). If the value for chlorobenzene is used (1.55 D), a still better agreement is obtained.

For 2,2'-dichlorobiphenyl a dipole moment calculation based upon Hampson and Weissberger's value for 2-chlorobiphenyl (1.45 D) and the φ-value given in this work (74°) leads to a value of 2.00 D. The measured value is 1.91 D. The agreement is very good, considering not only the rough approximation made when assuming a rigid model but also both the errors of the dipole moment determinations of 2-chlorobiphenyl and 2,2'-dichlorobiphenyl and the errors in our determination of the φ-value.

SUMMARY

Electron diffraction investigations of 3,3',5,5'-tetrabromobiphenyl, 2,2'-dichloro-, 2,2'-dibromo-, and 2,2'-diiodobiphenyl have been carried through. The first of these compounds shows a deviation from the coplanarity of about the same magnitude as was found earlier for other non-*ortho*-substituted bi-

phenyls [2]. — For the three *ortho*-substituted compounds under investigation the angle between the two ring planes is found to be 74°, 75°, and 79° respectively, the deviation from the *cis*-form being less than from the *trans*-form. The agreement with X-ray investigations and dipole moment measurements is very good, and well within the limits of error.

I should like to express my gratitude to Professor Dr. O. Hassel for his interest in my work and to Dr. William S. Emerson at the Monsanto Chemical Co, Dayton, Ohio, who has kindly supplied me with the 2,2′-dichlorobiphenyl. The other compounds studied in this investigation were purchased from L. Light & Co Ltd. Old Bowry Laboratories, Wraysbury, England. — I must also acknowledge my indebtedness to *Norges Teknisk-Naturvitenskapelige Forskningsråd* and the Fulbright Foundation for financial aid for a journey to U. S. A. during which the present investigation was finished. — A great deal of the elaborate calculation work has been carried through on I. B. M. machines which have kindly been placed at the disposal of the Chemical Department of the University in Oslo.

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Received May 23, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 937–941

Note on the Nature of Valence Forces

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Although several empirical rules connecting bond lengths and force constants exist [1] only few attempts have been made to derive such relations on a theoretical basis. However, a simple approximate formula can be obtained from classical-mechanical considerations as follows: Let us picture the valence force between two atoms "i" and "j" as a bundle of parallel arranged springs each of which has the same strength and modulus. A "single bond" may then be represented by a certain number of "unit" springs, a "double bond" by twice this number and so on. Assuming Hooke's law to be valid we must apply a force ΔK given by

$$\Delta K = C \cdot \frac{\Delta X}{d_{ij}}$$

in order to obtain the relative change in length, $\Delta X/\mathrm{d_{ij}}$, where d_{ij} is the distance between the atoms and ΔX the displacement of say atom "i". If we have P_{ij} such springs side by side between the two atoms the force required will be

$$\Delta K = C \cdot \frac{\Delta X}{d_{ij}} \cdot P_{ij}$$

Dividing by ΔX we get the force constant, K_{ij}, and we may write:

$$\frac{K_{ij} \cdot d_{ij}}{P_{ij}} = C \qquad (1)$$

Interpreting P_{ij} as the total bond order this relation may be expected to hold as long as we consider bonds between the same kind of atoms. Table 1 shows that — within the uncertainty inherent in the force constants as well as in the

Table 1.

Atom pair	Compound	Bond length	Bond order	Force constant	$\frac{K_{ij} \cdot d_{ij}}{P_{ij}}$	Mean value
C≡C	C_2H_2	1.20 Å	3.0	$15.7.10^5$	$6.3.10^{-3}$	
C=C	C_2H_4	1.33 »	2.11	9.6 »	6.0 »	
C:::C	C_6H_6	1.39 »	1.67	7.60 »	6.3 »	
C:::C	C_2N_2	1.38 »	1.46	6.24 »	5.9 »	
C−C	C_2H_6	1.54 »	1.11	4.5 »	6.2 »	$6.14 \cdot 10^{-3}$
C≡N	C_2N_2	1.15 »	2.5	16.6 »	7.6 »	
(C=N		1.30 »	2.0	12.1 »	7.85 »)[1]	
C−N	CH_3NH_2	1.47 »	1.0	5.0 »	7.35 »	
C−N	CH_3NC	1.47 »	1.0	5.5 »	8.1 »	
C···N	C_2N_2	2.53 »	0.34	1.02 »	7.5 »	7.65 »
C≡O	CO	1.13 »	3.0	18.9 »	7.1 »	
C:::O	CO_2	1.16 »	2.2	14.12 »	7.4 »	
C=O	CH_2O	1.21 »	2.0	12.3 »	7.4 »	
C−O	CH_3OH	1.42 »	1.0	5.0 »	7.1 »	7.25 »
N≡N	N_2	1.09 »	3.0	22.4 »	8.1 »	
N=N	N_3^-	1.24 »	2.0	11.7 »	7.3 »	
N···N	C_2N_2	3.68 »	− 0.46	− 0.96 »	7.7 »	7.7 »

bond orders — this is a rather good approximation. It is an interesting point that (1) also seems to be valid for bonds between non adjacent atoms. Such forces are expected from the molecular orbital theory and may be computed for some simple molecules. In several cases of linear molecules it has been possible to obtain both bond order, interatomic distances, and force constants between "separated" atoms within the same molecule so that the validity of (1) could be tested. Some examples have been included in Table 1.

For carbon-carbon links Coulson [2] has derived a formula connecting the π-bond order, p, and the length, r_a, of an actual bond with those of the single and double carbon bonds and involving also the force constants K_s and K_d for the latter ones. Calling the lengths of a single and double bond s and d respectively his formula may be written

$$\frac{d \cdot K_d - r_a \cdot K_d}{r_a \cdot K_s - s \cdot K_s} = \frac{1-p}{p} \qquad (2)$$

Table 2.

Atom pair	Compound	Bond length	Force constant	$K_{ij} \cdot d_{ij}$	Ionic refraction [3]
HF	HF	0.92 Å	$9.7 \cdot 10^5$	$8.9 \cdot 10^{-3}$	2.20 cc
HO	H_2O	1.02 »	7.6 »	7.7 »	4.06 »
HCl	HCl	1.28 »	5.15 »	6.6 »	8.45 »
HBr	HBr	1.41 »	4.11 »	5.8 »	11.84 »
HS	H_2S	1.33 »	4.25 »	5.65 »	15.0 »
HSe	H_2Se	1.53 »	3.37 »	5.16 »	17.3 »
HJ	HJ	1.60 »	3.14 »	5.0 »	18.47 »
HN	(NH_3)	1.01 »	6.86 »	6.9 »	(7) »
HC	(CH_4)	1.09 »	5.45 »	6.0 »	(12) »

Let us apply our relation (1) to an actual (resonating) bond with π-bond order p and force constant K and let us first calculate the constant C from a single bond and next from a double bond. Then we get

$$\frac{K \cdot r_a}{1+p} = K_s \cdot s \quad \text{and} \quad \frac{K \cdot r_a}{1+p} = \frac{K_d \cdot d}{2}$$

From these two equations we obtain:

$$\frac{dK_d - r_a K}{r_a K - sK_s} = \frac{1-p}{p} \tag{3}$$

The similarity with Coulson's formula is striking and in fact (3) seems to give at least as good results as (2).

One might ask whether the constant C in (1) is the same for all atoms. That this is not the case is seen from the hydrogen halides in Table 2.

It is tempting to relate C to the polarizability of the halide ions in these cases. Imagine a proton approaching a big halide ion, then it induces an electric moment αE where E is the electrical field strength due to the proton at the centre of the halide ion and α is the polarizability. The increase in electrostatic energy can be shown to be $\frac{1}{2} \alpha E^2$. Hence a force

$$K = -\frac{\partial}{\partial x} (\tfrac{1}{2} \alpha E^2)$$

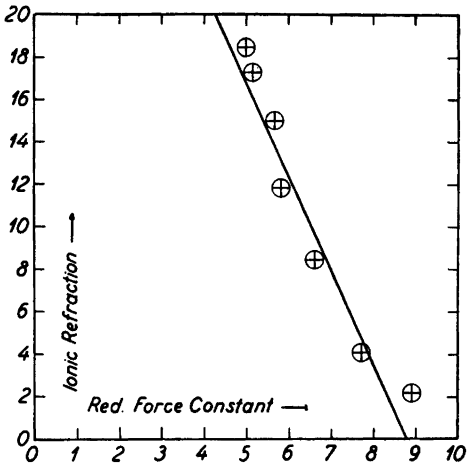

Fig.1. Relation between "reduced force constant" (see text) and ionic refraction of the X-atom for some H—X-bonds (cp. Table 2).

will act on the proton and for a small displacement ΔX there will be an additional force

$$\Delta K = -\frac{\partial^2}{\partial x^2}(\tfrac{1}{2}\alpha E^2)\Delta X$$

so that we get a contribution to the force constant K_{ij} of

$$\Delta K_{ij} = -\tfrac{1}{2}\alpha \frac{\partial^2}{\partial x^2} E^2$$

If we assume $\frac{\partial^2 E^2}{\partial x^2}$ to be approximately the same for the different atoms or ions we should expect the $C's$ to be roughly proportional to the ionic refractions $A = \frac{4\pi N}{3}\alpha$. The graph (Fig. 1) shows that this really is the case for a series of bonds involving hydrogen, and in fact we can put

$$\frac{K_{ij} \cdot d_{ij}}{P_{ij}} = 8.8 - 0.23\, A_j \qquad (4)$$

for compounds of this type. Unfortunately the ionic refractions are not known for carbon or nitrogen but if we postulate the validity of (4) also for CH and NH we obtain $A_C \approx 12$ and $A_N \approx 7$.

Relation (1) has already proved to be useful in problems where one wants the bond order for a special bond and the pure theoretical calculation cannot be carried through.

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Received June 1, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 942–947

A Method for the Determination of Tracer Phosphate in Biological Material

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The determining of the labelling in connection with studies of phosphate metabolism made with the aid of labelled phosphate, is generally performed in the following way:

In a sample containing orthophosphate the phosphate is determined *e. g.* according to Fiske and Subbarow [1] or to some modification of their method. The radioactivity is determined in a Geiger-Müller-counter after precipitation of the orthophosphate in the sample, together with phosphate carrier as $MgNH_4$-salt. The specific activity = number of impulses per minute emitted per microg. of P. is then calculated according to ex. Lindberg [2].

Three essential faults are connected with this method. The radioactivity and the phosphate are determined on different solutions, one of them acid and the other alkaline. In samples containing acid-labile phosphate correct values are obtained for the number of impulses, but the phosphate values are too high. If the sample contains alkali-labile phosphate, correct phosphate values but too high a number of impulses are obtained. If there are small amounts of orthophosphate in the sample, but great amounts of highly labelled esters, esterified phosphate is adsorbed on the phosphate precipitate and the number of impulses obtained is too high. The latter applies to an even greater extent if inorganic pyrophosphate is present in the solution.

We have found the method discussed below generally applicable.

Orthophosphate is determined according to Martin and Doty[3], whose method is a modification of that introduced by Beerneblom and Chain [4]. On determination according to Martin and Doty the derivatives of phosphomolybdic acid which have been formed, are shaken up into a mixture of isobutanol and benzene (1 : 1). The formed cerulomolybdate is determined

colorometrically, after the addition of H_2SO_4 dissolved in ethanol, and of $SnCl_2$ to the drawn-off isobutanol-benzene phase.

The method of Martin and Doty has been modified to make possible the direct determination of the specific activity of the orthophosphate fraction in a sample. In order to determine radioactivity, a sample is taken directly from the isobutanol-benzene phase, pipetted onto a small aluminium plate, and dried and placed under a Geiger-Müller tube.

METHOD

Reagents: According to Martin and Doty.

1. Isobutanol-benzene, 1 : 1.
2. 10 *N* H_2SO_4.
3. 10 % ammonium molybdate.
4. 32 ml conc. H_2SO_4 dissolved in 968 ml abs. (99.5 %) ethanol.
5. 10 g $SnCl_2.2\ H_2O$ dissolved in 25 ml conc. HCl, kept in a refrigerator.
6. Reagent 5 diluted 200 times with *N* H_2SO_4, is to be freshly prepared immediately before each determination.

If the sample is deproteinized, *e. g.* a TCA extract, the reagents mentioned above are sufficient. If, on the other hand, the sample is not deproteinized, or is a homogenate, the deproteinization can be performed by using silico-tungstic acid (according to Martin and Doty) in the tube in which the phosphomolybdic acid is formed.

RESULT

3 ml of a nearly sulphate-free sample (the content of sulphate in a biological extract does not interfere with the determination) containing 2—16 microgram P, is mixed with 5 ml of reagent 1, 0.5 ml reagent 2 and 0.5 ml reagent 3. The tube is stoppered, with a ground glass stopper for preference, and shaken vigorously for 15 seconds. After separation, which generally takes place rapidly without centrifugation, approximately 2.5 ml of the upper phase is sucked off with the aid of a tight, locked Krogh-syringe, which contains the desired volume when full. The syringe is washed quantitatively with reagent 4. The combined sample and wash solutions are mixed with 0.5 ml of reagent 6. The color-intensity may be determined at 640 mμ in a Klett-Summerson photometer. A Beckman-spectrophotometer shows two absorption-maxima in formed molybdenum-blue, one at 625 and one at 730 mμ. The color-intensity therefore should be measured in a Beckman-spectrophotometer at one of these wavelengths.

When determining the radioactivity, a smaller syringe which takes about 0.2 ml is used thus permitting a very accurate testing. The sample is drawn directly from the isobutanol-benzene phase and transferred onto an alumi-

nium plate containing a small filterpaper. When the sample is dry the radioactivity may be determined by placing the plate in an electron-counter. If the specific activity is low another 0.2 ml can be pipetted off and added to the dried sample. It is possible to carry out a determination at a very low specific activity in the following way: In a separatory funnel a 3 ml sample of orthophosphate in the form of phosphomolybdic acid is shaken with 5 ml of isobutanol-benzene. When the phases are well separated 1 ml 5 % Na_2CO_3 is added to the isobutanol-benzene phase and the mixture is shaken for 15 seconds, whereupon the phosphate migrates quantitatively into the water phase. From this 0.2 ml may be pipetted off for activity-analysis. If the whole amount is needed for determination, the whole solution may be precipitated with Fiskes reagent after the addition of orthophosphate as a carrier.

The latter procedure has the advantage that the magnesium ammonium phosphate precipitation does not adsorb highly labelled esterified phosphate, which would cause the specific activity of the orthophosphate to become too high.

In the beginning a considerably more concentrated sample may be taken. For a phosphate determination a suitable amount of the benzene-isobutanol phase is sucked off, after which it is diluted to 5 or 10 ml with reagent 4 and 0.5 ml of reagent is then added. The method is illustrated by the following experiment.

Fifteen hours before the experiment a white rat had been given 0.5 mC P^{32} intraperitoneally. The animal was anaesthetized with amytol. Muscles were then taken from both back-legs and immediately frozen in carbon dioxide chilled acetone. The muscles were extracted with cold 10 % TCA.

The amount of orthophosphate per unit volume present in the extract and the specific activity of the orthophosphate were determined immediately, before the hydrolysis of the creatinephosphate had time to take place.

If a cold sample containing ortho- and creatine phosphate is pipetted off into a reagent-solution and then cooled in ice-water, less than 1% hydrolysis of the creatinephosphate occurs, whereas the orthophosphate is transformed quantitatively into phosphomolybdic acid and as such migrates into the benzene-isobutanol phase.

Fig. 1 shows hydrolysis of creatine-phosphate in the presence of acid molybdate; after 30 minutes the creatine-phosphate is almost completely hydrolyzed. Under these circumstances ATP or ADP is not hydrolyzed [5]. This phase may be sucked off completely since the orthophosphate present in the sample as phosphomolybdic acid has been transferred into the isobutanol phase. The water-phase is then allowed to stand 30 minutes at room-temperature, while new phosphomolybdic acid is formed from the hydrolyzed creatinephosphate. The newly formed phosphomolybdic acid may

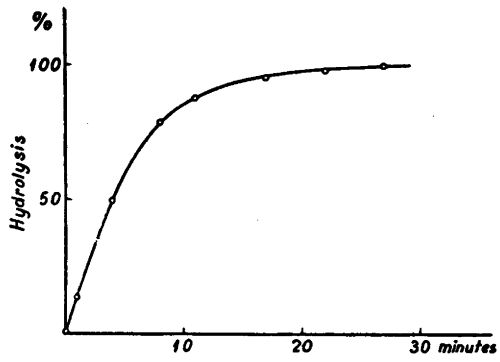

Fig. 1. Hydrolysis of creatine-phosphate at 20° C in presence of phosphate-determining reagent.

be shaken up into the upper phase, after the addition of a further 5 ml of isobutanol-benzene, thus obtaining a direct value for creatine phosphate.

In order to determine the labile ATP-phosphate, the sum of ortho- and creatine-phosphate should be deducted from the value obtained when the 3 ml sample is hydrolyzed for 20 minutes at 100° with 0.5 ml 10 *N* H_2SO_4. The total phosphate is determined after combustion with 0.05 ml in excess of the amount of H_2SO_4 necessary for phosphate analysis and one drop of H_2O_2.

Table 1 shows the result of the analyses carried out on the TCA extract of muscles.

Upon precipitation of radio-active orthophosphate with Fiskes reagent using added phosphate "carrier" and in the presence of large amounts of active esterified phosphate or active pyrophosphate, the specific activity of the orthophosphate is too high because of the adsorption on the magnesium-ammonium-phosphate crystals. Inorganic pyrophosphate seems to precipitate largely with Fiskes reagent in the presence of large amounts of orthophosphate.

Table 1. Phosphate fractions separated by acid hydrolysis, from a TCA-extract of muscles.

Number of impulses/minute and extinction for 1 ml of the original sample. 2.56 ml was taken from the isobutanolbenzene phase for the phosphate determination. According to the determination with standard orthophosphate under these conditions an estimation of 25 corresponds to one microgram P. 0.18 ml was used for radioactivity measuring.

	Microg P	Impulses/min.	Spec.act.
Ortho-P	2.8	3 160	1 130
Creatine-P	6.5	8 470	1 300
Labile ATP-P	7.8	10 400	1 330
Residual-P	8.4	2 540	320

Table 2 shows the analytical results of a sample containing small amounts of orthophosphate and comparatively large amounts of ATP. The value for the specific activity of the orthophosphate is in agreement with the values obtained directly from the TCA-extract. The specific activity of the orthophosphate will be approximately twice as high as that of the labile ATP-phosphate, when measuring radio-activity with the aid of precipitation, according to Fiske.

Table 3 shows a comparison between the values obtained with the two methods for specific activity on different hydrolysis fractions in the presence of radioactive inorganic pyrophosphate. The table shows that only the method described here is applicable under these circumstances. The inorganic pyrophosphate precipitation is almost complete when using Fiskes reagent in the presence of orthophosphate.

Table 2. Different hydrolysis fractions in an impure ATP-preparation.

The same TCA-extract as before was precipitated after spontaneous hydrolysis of creatine phosphate with mercury acetate at pH 4.0. The precipitate in TCA-solution was decomposed with H_2S, the mercuric sulfide was filtered off and H_2S removed by aeration. The rest of the procedure is the same as in the preceding experiment. Ext. 27.2 = 1 microg P.

	Microg P	Impulses/min.	Spec.act.
Ortho-P	1.0	1 110	1 110
Labile ATP-P	8.6	10 200	1 190
Residual-P	4.3	1 200	280

Table 3. Comparison of specific activities determined according to this method and as for example determined by Lindberg [2], in the presence of radioactive pyrophosphate.

The impure ATP-preparation, prepared according to Table 2, was hydrolized for 30 minutes at 100° with $Ba(OH)_2$, pH 9.0, thus the ATP is hydrolized into adenylic acid and inorganic pyrophosphate [6]. Barium is precipitated as sulphate, the sulphate excess is kept so low that the sulphate concentration cannot influence the phosphate determination.

Determination according to precipitating method (2).

	Impulses/min.	Microg P	Spec.act.
Ortho-P	6 880	3.5	1 940
Easily hydrolyzable P	3 200	7.0	460
Residual-P	1 170	4.0	300
Total P	11 200	14.5	770

Determination according to this method.

	Impulses/min.	Microg P	Spec.act.
Ortho-P	3 430	3.5	980
Easily hydrolizable P	7 780	6.9	1 130
Residual-P	967	4.2	230
Total-P	12 000	14.6	820

SUMMARY

1. Description of a method allowing a quick and exact determination of the specific activity of orthophosphate present in a sample. This can be done even in the presence of creatine phosphate and radioactive pyrophosphate.

2. After determination of the percentage of orthophosphate and of the specific activity of the orthophosphate, the amount of creatinephosphate and its specific activity may be determined in the same sample.

3. In a homogenate the amount of orthophosphate and its specific activity can be determined in the same tube and at the same time as deproteinization is being carried out.

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Received June 2, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 948–956

The Hydrolysis of Non-Choline Esters by Acetylcholine-Esterase from Human Erythrocytes

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It is now well established that the esterase which has been regarded as specific for choline esters also splits non-choline esters. This new approach to the specificity problem of the acetylcholine-splitting enzymes was initiated by the observation of Bodansky [1], who found that triacetin is hydrolysed by the acetylcholine-esterases of brain and erythrocytes. The esterase of certain snake venoms, the properties of which are similar to those of the acetylcholine-esterase of mammalian nervous tissue and erythrocytes, was shown by Bovet-Nitti [2] to hydrolyse a number of aliphatic esters, in particular acetyl esters. Augustinsson [3] has demonstrated that the acetyl-ester linkage in acetylsalicylcholine and acetylsalicylic acid itself are split by the brain and erythrocyte esterases. The splitting of acetylsalicylic acid has recently been confirmed by Zeller *et al.*[4] with erythrocytes and snake venom. A most careful study of the substrate specificity of these enzymes has been made by Adams and Whittaker [5,6]. The carbon analogue of acetylcholine, 3,3-dimethyl butyl acetate, is the most rapidly split next to acetylcholine itself. It was stated by these authors that in general the more closely the alcohol group simulates the choline configuration, the more rapidly is the ester hydrolysed. However, Zeller *et al.*[4] have shown that the acetylcholine-esterases of human erythrocytes and certain snake venoms catalyse the hydrolysis of the acetates of very different hydroxyl derivatives. Therefore, Zeller has considered his "e-Type" cholinesterase (equivalent to acetylcholine-esterase used in this paper) to be an "acetylesterase".

A study of the enzymic hydrolysis of triacetin by Blaschko and Holton [7] and Augustinsson [8] confirmed the assumption that the acetylcholine-esterases present in brain (caudate nucleus), electric organ, erythrocytes, and snake venom are responsible for the hydrolysis of this ester. The observation of

Bodansky [1] that human brain splits triacetin even more rapidly than acetylcholine was explained by Augustinsson [8]. He showed that there is a great difference between the activity-substrate concentration relationships for these two substrates.

The main purpose of the present investigation is to give further information about the hydrolysis by the acetylcholine-esterase of non-choline esters, exemplified by acetylsalicylcholine, acetylsalicylic acid, and triacetin.

METHODS

The Warburg manometric method was used for the determination of the esterase activity [3]. The enzyme activity is expressed in terms of the amount of CO_2 in μl ($= b_{30}$), evolved during 30 minutes minus the corresponding value for non-enzymic hydrolysis. Measurements were made at 25° C.

The enzyme preparation was obtained by washing human erythrocytes (the blood was taken up in heparin) four times with 0.9 per cent sodium chloride solution and then haemolysing them with distilled water to form a solution of the original blood volume. This haemolysate was subsequently diluted with distilled water to give a suitable activity for each substrate used.

The following substrates were employed:

Substrate	Abbreviation	Mol. wt.	
Acetylcholine chloride	ACh	181.66	Hoffmann-La Roche & Co.
Acetylsalicylcholine chloride	ASaCh	301.77	LKB, Stockholm (prepared according to Euler *et al.* [9])
Acetylsalicylic acid	ASa	180.15	Bofors AB
Triacetin	TA	218.20	Fisher Scientific Co.

The acetylsalicylic acid was neutralised with 0.1 *M* NaOH and then used immediately after neutralisation.

HYDROLYSIS OF ACETYLSALICYLCHOLINE (ASaCh)

Total hydrolysis at various substrate concentrations

The author [3] has recently found that ASaCh gives a familiar dissociation curve when the enzyme activity is plotted against the logarithm of molar substrate concentration. This is in sharp contrast to the bell-shaped curve obtained with acetylcholine. It has been suggested that at high ASaCh concentrations only the acetyl-salicylic linkage is split, the breakdown of the choline-ester linkage occurring no more than it does in the case of high con-

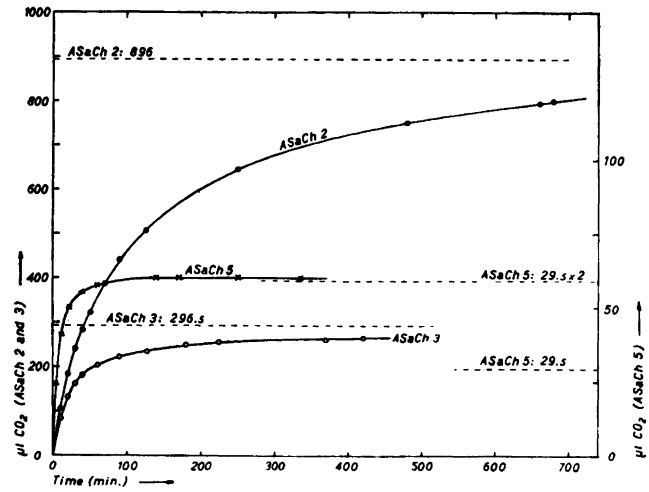

Fig. 1. Total (enzymic + spontaneous) hydrolysis of acetylsalicylcholine of various concentrations. Total volume of the reaction mixture 2.00 ml. Key to the various substrate concentrations in Table 1, which also gives the values found experimentally for spontaneous hydrolysis (30 minutes) are found.

centrations of acetylcholine. At low substrate concentrations, on the other hand, both ester linkages of ASaCh are supposed to split at the same time. Support of this hypothesis has now been obtained by the following experiments.

The hydrolysis of ASaCh was allowed to proceed for a time period long enough to give complete destruction of the substrate. Fig. 1 shows the results obtained with various substrate concentrations. It is seen that at high substrate concentrations (1.99×10^{-2} *M* and 6.62×10^{-3} *M*; ASaCh 2 and 3 respectively) only one of the ester linkages is split enzymically. At low substrate concentration (6.62×10^{-4} *M*; ASaCh 5), on the other hand, both ester linkages are broken, because the hydrolysis proceeds till the amount of CO_2 liberated corresponds to the calculated value for the breakdown of two ester linkages. Both linkages also are split when the ASaCh concentration is 1.99×10^{-3} *M* (ASaCh 4; not shown in the figure). After 1345 minutes, 155 μl CO_2 was evolved; the calculated value for complete hydrolysis of one of the linkages is 89.5 μl.

From these experiments we conclude that when the ASaCh concentration is below 1.99×10^{-3} *M* both ester linkages of this substrate are split. When the concentration is above that value only one of the linkages is broken. This substrate concentration is approximately the same as has been shown to be the optimum for the enzymic hydrolysis of acetylcholine by this enzyme

(2—3 $\times$ 10^{-3} M). We know that high concentrations of acetylcholine inhibit the enzymic hydrolysis. It is now suggested that a similar situation is valid for the hydrolysis of ASaCh, *i. e.*, at high ASaCh concentration the hydrolysis is retarded when only the hydrolysis of the choline-ester linkage is concerned. Therefore, at very high ASaCh concentrations only the acetyl-salicylic linkage is broken. Further support for this hypothesis has been obtained in experiments when choline was used as an enzyme inhibitor. Thus, it has been demonstrated [3] that at high substrate concentrations the inhibition by choline is much less than expected in a competitive inhibition. At low and medium ASaCh concentrations the inhibition is considerable or of the same order of magnitude as has been observed for the hydrolysis of acetylcholine.

The hypothesis put forward by Murray and Haldane [10] to explain the bell-shaped curve obtained by plotting the enzyme activity against the logarithm of molar acetylcholine concentration is based on the assumption of *two* active centres (in the following termed centre I and II) of the enzyme molecule. This proposal is well supported by the results described above and by those which will be discussed in the following.

Competition Experiments

A preliminary report on competition experiments carried out with mixtures of substrates and with esterases from various sources has recently been published by the present author [3]. A theory applicable to such experiments has been worked out by Whittaker and Adams [11]. The experimental results of the present investigations are summarised in Table 1.

Let us first consider the hydrolysis of mixtures of acetylcholine and ASaCh. In the presence of high concentrations of both substrates the rate of hydrolysis is between the rate of hydrolysis of each substrate measured separately, *i. e.*, lower than the ASaCh hydrolysis and higher than the acetylcholine hydrolysis. Hence, there is a competition between these two substrates. When the initial substrate concentrations are lower, the rate of hydrolysis of the substrate mixture becomes higher than each of the separate rates. At these concentrations also there is a competition between the two substrates, *i. e.*, acetylcholine and ASaCh compete for the same active groups of the enzyme molecule. The mechanisms of the two reactions, however, are not the same at high and low substrate concentrations. This is further evidence in support of the hypothesis put forward above for the enzymic hydrolysis of the two ester linkages of ASaCh.

Table I also shows that acetylsalicylic acid (ASa) is split by erythrocyte haemolysate, as demonstrated previously [3,4]. In the experiments with the

Table 1. Total (enzymic + spontaneous) hydrolysis of acetylsalicylcholine and acetyl- in molarity according

Time (min.)	μl CO_2											
	1				2				3			
	ACh	ASaCh	ASa	ACh + ASaCh	ACh	ASaCh	ACh + ASaCh	ASa + ASaCh	ACh	ASaCh	ASa	ACh ASa
6	6.5	24.5	5.5	23	12.5	24.5	19	10.5	19	26	2	2
12	16	45.5	11	41	26.5	44	33	28	39	38.5	4.5	4
18	23	63.5	17	55	39	60.5	42.5	42	60	54	7	6
24	31.5	79	21.5	67	52	75	53.5	53	80	66.5	8.5	8
30	38	91	26	77.5	65	88	62.5	63.5	98.5	77.5	10	9
36	45.5	105	30.5	88	77	101	72.5	74	114	88	12	11
42	—	117	—	100	—	111.5	84	85	—	99	—	12
88		200		166		185.5	154	152		148		21
100		—		181		199.5	173	169		158		24
130		—		—		234.5	213	203		181		29
163		310		268		269	257	237		199		34
181		330		278		286	282	253		210		37
237		—		—		332	353	—		—		44
Autohydrolysis μl CO_2 per 30 min.	9.5	13.5	16	23	6	8	14	20.5	4.5	4.5	9	
Calculated values for complete hydrolysis	4 928	2 966		7 894	1 479	896	2 375		493	296.5		79

Soln. no.	1	2	3	4	5
ACh chloride	1.10×10^{-1}	3.30×10^{-2}	1.10×10^{-2}	3.30×10^{-3}	1.10×10^{-3}
ASaCh chloride	6.62×10^{-2}	1.99×10^{-2}	6.62×10^{-3}	1.99×10^{-3}	6.62×10^{-4}
ASa (as Na salt)	1.11×10^{-1}	3.33×10^{-2}	1.11×10^{-2}	3.33×10^{-3}	1.11×10^{-3}

These values refer also to those of the substrate mixtures. Enzyme concentration: 0.013 ml haemolysate per ml reaction mixture (total volume 2.00 ml). The calculated values (for ASaCh) at the bottom of the table refer to the hydrolysis of one ester linkage.

mixtures of acetylcholine and ASa it is seen that even here competition occurs. This also happens when ASa and ASaCh are present at the same time.

The affinity constants for each substrate, using the formula introduced by Whittaker and Adams, have not been determined, mainly because it has been difficult to evaluate

alicylic acid and mixtures of these compounds with acetylcholine. Substrate concentrations the following:

μl CO_2

		4						5					
+ a	ASa + ASaCh	ACh	ASaCh	ASa	ACh + ASaCh	ACh+ ASa	ASa + ASaCh	ACh	ASaCh	ASa	ACh + ASaCh	ACh+ ASa	ASa + ASaCh
)	10	23.5	23	2	25	15	11	23	15	1	30.5	23.5	10
)	26	47.5	37.5	2	46	38.5	24	43	24	1	53	46.5	21
'	37	71	48.5	4	66.5	59.5	32	52	32	3	68.5	58	27
'	47	90	56.5	4.5	84	79	38	54.5	36	4	77.5	63.5	32
.5	58.5	108	63	5	101.5	95	43	56	38	5	85	65.5	36
:	64	122.5	68	6.5	115	110	49	56	42.5	6	89	67.5	38
	72	—	72.5	—	131	—	53	—	45	—	93	—	40
	123		93		183		71		57		108		49
	132		96		189		74		60		109		50
	150		102		201		80		64		111		—
	170		103		211		82		65		113		
	184		104		215		—		65		114		
	—		105		223				—		—		
.5	13.5	4	3.5	4	7.5	8	7.5	3	2.5	4	5.5	7	6.5
		148	89.5		237.5			49.5	29.5		79		

the initial rate of hydrolysis for acetylsalicylcholine and acetylsalicylic acid (the velocity falls off rapidly with time).

HYDROLYSIS OF TRIACETIN (TA)

Acetylcholine-esterases from various sources have been shown recently to split triacetin. This was demonstrated in competition experiments by Blaschko and Holton [7] using dog brain and cobra venom. With the purified electric tissue esterase (*Electrophorus*) the present author has given a similar evidence [8].

In order to get further information about the mechanism of action of the acetylcholine-esterase of human erythrocytes, competition experiments have

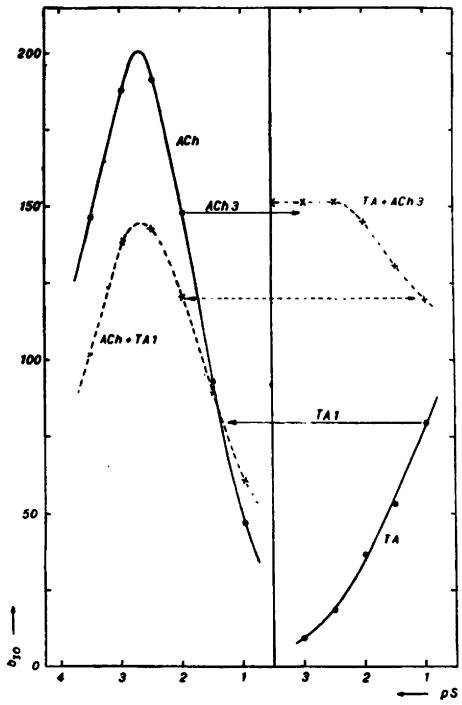

Fig. 2. Activity-pS curves for the enzymic hydrolysis of acetylcholine (ACh) and triacetin (TA) and mixtures of these esters by the human erythrocyte acetylcholine-esterase.

been carried out at various substrate concentrations. The results are shown in Fig. 2. Triacetin competes with acetylcholine both at low and high acetylcholine concentrations. The experimental results also demonstrate that triacetin of increasing concentrations decreases the enzymic hydrolysis of 1.10×10^{-2} *M* acetylcholine.

As pointed out above and according to the tentative model for acetylcholine esterase introduced by Zeller and Bissegger [10] there are two active centres in the enzyme molecule. One of them (centre I) is a negatively charged group which combines with the positive nitrogen atom of the choline ester, probably by an electrostatic attraction. This active enzyme centre is characteristic of the acetylcholine-esterase; cf. the discussion of Adams and Whittaker [11]. The second active centre (centre II) is supposed to attract the acyl group of the ester. Characteristic of centre II is its ability to split acetates at a higher rate than other esters. This is the reason why triacetin is split.

Further studies by the present author [12] have demonstrated that choline inhibits the enzymic hydrolysis of triacetin by this enzyme. This inhibition is not competitive. The inhibition is independent of the triacetin concentration. With increasing choline concentration the degree of inhibition increases up to

0.25 *M* choline; a still higher choline concentration does not further change the hydrolysis rate of triacetin.

As long as we consider only the centre II it is quite justifiable to speak about an acetylase or acetylesterase rather than a choline-esterase, as proposed by Bovet-Nitti [2] and Zeller *et al.* respectively [4]. This centre controls the specificity of the enzyme for the acyl portion of the substrate. We are indebted to the work of Adams and Whittaker for the observation that only a relatively small variation is possible on that part of the ester. The observation, especially that made by Zeller *et al.*, that the alcoholic part can be varied considerably without any appreciable loss of enzyme activity is not too surprising.

We must remember, however, that it is the centre I that is really characteristic of this esterase. No esters other than those of choline and probably those of the phosphorus and arsenic analogues of choline have yet been discovered which give the characteristic activity-substrate concentration relationship of acetylcholine-esterase. Centre I — in combination with centre II — is responsible for this. The distance between these two centres is probably about the same as that between the nitrogen atom (of choline) and the carbonyl group (of a choline ester), *i. e.*, 5.8 Å. As far as we know there exists in the living body only one compound which consists of a positively charged atom, a chain of two carbon atoms, and an alcoholic group esterified with acetic acid. That compound is acetylcholine. Most probably, it is the physiological substrate of the enzyme which therefore is called acetylcholine-esterase [13].

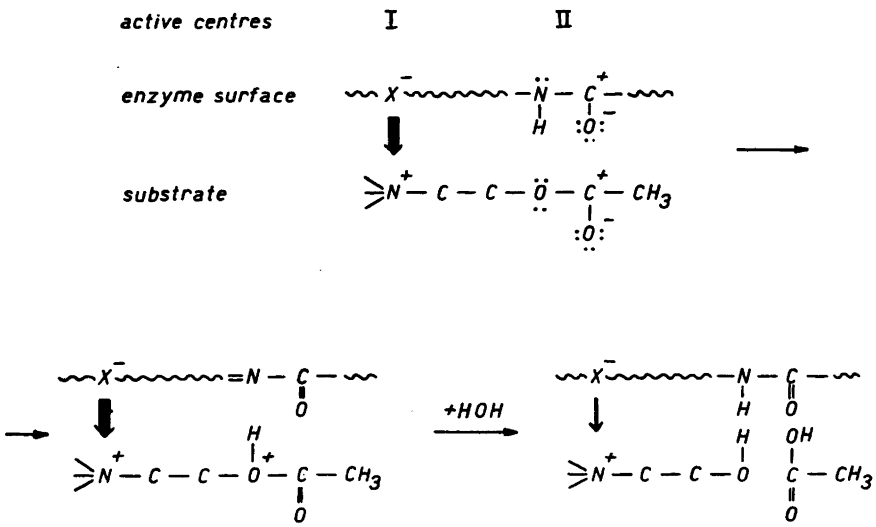

Fig. 3. Tentative model for the mechanism of enzyme action.

SUMMARY

The enzymic hydrolysis of acetylsalicylcholine, acetylsalicylic acid, and triacetin by acetylcholine-esterase has been studied using human erythrocytes as an enzyme source.

Acetylsalicylcholine at high concentration does not inhibit the enzyme. Both ester linkages of this substrate are split at low concentrations, while at high concentration the choline ester linkage only is split. In competition experiments it has been demonstrated that both acetylsalicylcholine and acetylsalicylic acid compete with acetylcholine for the active groups of the enzyme molecule. This occurs both at high and low substrate concentration.

Triacetin and acetylcholine compete for one of the two active groups (centre II) of the enzyme.

Proof has been given for the existence of two active centres of the acetylcholine-esterase. Centre I carries the active group which combines with the positively charged nitrogen atom of choline, and centre II the group which combines with the acyl (acetyl) group of the ester.

This work has been supported by a grant from *Statens Naturvetenskapliga Forskningsråd*. I am greatly indebted to Mrs. M. Grahn for technical assistance.

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Received June 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 957–962

Zone Electrophoresis in a Glass Powder Column *

Preliminary Report

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Stabilization by gels and powders has been much used in earlier electrophoresis studies with the moving boundary method. Electro osmosis, however, was found to make accurate mobility measurements impossible. Later on the method was applied again for preparative purposes. Kendall and Crittenden [1] used electrophoresis in a capillary system with the aim to separate isotopes. More recently Brewer, Madorsky and Westhaver [2] enriched one of the potassium isotopes electrophoretically in a packing of glass wool, cotton, glass beads *etc.* The method has also been applied to biochemical problems. Cooledge [3] separated albumin and globulin in a glass wool column, Consden, Gordon and Martin [4] made separations of amino acids and peptides in silica jelly, and later Gordon, Keil and Sebesta [5] reported similar effects with proteins in agar jelly. Butler and Stephen [6] studied the electrophoresis of amino acids in a horizontal asbestos column. Electrophoresis on filter paper has lately been described by several authors, see for example Cremer and Tiselius [7]. Recently Svensson and Brattsten [8] described an apparatus for complete and continous electrophoretic separation, with glass powder as the stabilizing principle. The apparatus described here has also glass powder as stabilizing agent, but is intended to be an analytical instrument.

The principle of the apparatus is shown in Fig. 1. The center glass tube is loaded with the capillary system, glass powder, and connected with the electrode tubes. The system is filled with the buffer in question and a "zone" of the solution to be investigated is made up in the glass powder column.

* It seems practical to distinguish between "boundary electrophoresis" as performed in the common moving boundary apparatus, and "zone electrophoresis" where the migration of more or less completely separated zones is studied, usually by application of some immobilizing medium to prevent convection.

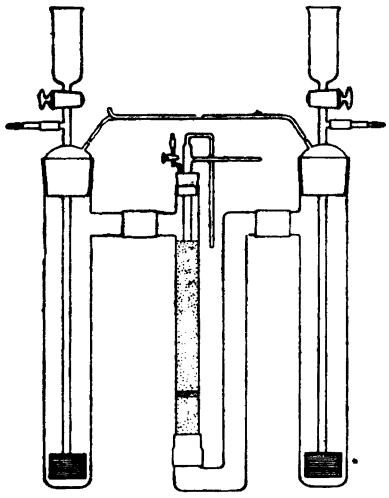

Fig. 1. The principle of the apparatus. The two horizontal tubes which makes it possible for the electro-osmotic flow to develop freely.

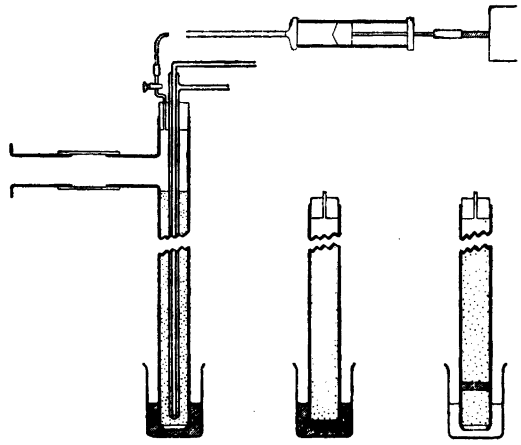

Fig. 2. Filling the apparatus. The central cooling tube is depicted in the left drawing.

When a potential is applied between the electrodes, charged molecules and particles migrate in the electrical field in the tube. If more than one electrophoretic component is present in the original zone, this will split up during the migration into a number of zones corresponding to the number of components.

An essential part of the apparatus is of course the capillary system. The ideal system should have, a low zeta-potential, low adsorptive power and a uniform structure. Those properties of a capillary system suitable in electrophoresis are discussed by Svensson and Brattsten [8]. The present apparatus uses almost the same capillary system as that described by Svensson and Brattsten. However, since the present system requires very uniformly sized particles, the glass powder has been fractionated extensively. Some preliminary attempts to get a glass powder with spherical particles have been carried out.

The electro-osmotic flow is usually a disturbing factor if a closed system is used. However, if the system is open and the glass powder has sufficient porosity to allow the electro-osmosis to develop without convection, the phenomenon has no influence on the electrophoretic separation and practically none on the sharpness of the zone boundaries. This "open" system can be established by the introduction of two horizontal tubes (in the same level)

each connected with the upper tube on the electrode joint (Fig. 1). The buffer in the vessels ends with a meniscus in the horizontal tubes respectively (the diameters must be small enough). Now an electro-osmotic flow may occur without altering the hydrostatic properties, since the two menisci move perpendicular to the gravitational field. This movement is easily measured and then also the direction and extent of the electro-osmotic flow.

For a sharp separation the temperature gradient in the tube with the capillary system during the experiment is of great importance. Temperature differences arising from the current between the peripherial and the central parts of the tube, make the zones parabolic in shape because of the great mobility temperature coefficient. In order to decrease these differences a cooling tube is placed in the center of the tube (Fig. 2). The thermostat water is forced through the cooling tube and back into the thermostat. It is not necessary to work at the density maximum of water to secure stability of the zones in this apparatus.

The analytical power of the apparatus is adversely influenced by the unsharpening effects at the zone boundaries (diffusion *etc.*) and by the height of the zone at start. In the latter case this is dependent upon the amount of material necessary for chemical analysis after the separation. Most of the experiments have been carried out with the solution under investigation in osmotic equilibrium with the buffer (dialysis). In some cases a special technique has been used to sharpen the zone boundaries. By continuous dilution of a buffer brought into the tube with the glass powder by the sucker-system (Fig. 2), a conductivity gradient is formed in the electrophoresis tube. When a zone migrates in this gradient, the lower parts of the zone move in a stronger field than the upper. This effect tends to press the zone together and make the boundaries sharper.

DESCRIPTION OF THE APPARATUS

The tube with the capillary system has an inner diameter of 20 mm, length of 200 mm, and has a glass filter plate in one end. The cooling tube has an outer diameter of 8 mm. The electrophoresis tube has rubber connections on one side and on the other rubber-glass with the electrode tubes as shown in Fig. 1. The electrodes and electrode tubes are the same as used in the standard Tiselius moving-boundary apparatus.

The glass powder has to be fractionated very carefully. After boiling with nitric acid, the powder is added in portions to a vertical glass cylinder with water flowing rapidly from the bottom (through a glass filter). The width of the cylinder was 80 mm and the velocity of water flow about 50 ml/sec. The

smaller glass-particles follow the streaming water and the heavier remain in the tube. With the aid of a siphon the powder flowing above the middle in the cylinder is sucked off after ten minutes. The remaining heavy particles are removed. This operation is repeated three times for the sucked off powder. The remaining "middle-fraction" is again put in the cylinder. The flo wof water in the cylinder is now made more rapid, and the water is directed to fall down in another vertical cylinder with weak counterflow of water. Only the heaviest particles of the middle fraction can reach the bottom of this last cylinder.

This last heavy fraction is used and the fractionated glass powder is put into the tube. In order to get a better close packing the tube is vibrated during filling with a commercial "massage vibrator". The cooling tube is centered manually during the addition of the glass powder. This method of centering is somewhat inaccurate and another arrangement is desirable. During the filling of the powder the liquid must always cover the glass powder, if not, air-bubbles may arise. In order to avoid gas-bubbles in the capillary system the buffer should be boiled before use.

The filling procedure with the solution under investigation is shown in Fig. 2. The syringe driven by a syncronous motor siphons buffer from the upper end of the tube with a speed of 0.5 ml, and the solution surrounding the lower part of the tube (in a beaker) flows gently through the glass filter plate up into the glass powder. When the solution-zone has reached a height of about 3 mm, the filter is washed with buffer. Then the latter surrounds the lower part of the tube (in the beaker) and the syncronous motor is started again. When the zone has migrated to a suitable level (about 3 or 4 cm) in the tube the lower rubber connection is put on. This operation must not force buffer into the tube. To avoid this the rubber has to be either somewhat greater in diameter than the tube or thin enough to be rolled over it. In the first case a tight connection must be assured.

For the localization of the zones no suitable optical method is now available. However, a sampling technique similar to that used in chromatography (see for example Stein and Moore [9]) has proved to be of great value for this apparatus, especially when the experimental arrangements can be made simpler. The solution is pressed downwards out of the column and is collected in successive portions in a rack of sample tubes, the movement of which is synchronized with that of the solution passing through the column. In this way the separated zones are collected, and the distribution of material in the column after the separation may be determined by analysis (according to some suitable method). During the sampling one of the electrode vessels is detached. (Fig. 3).

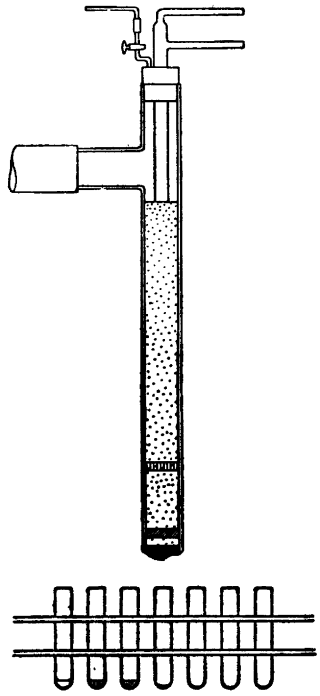

Fig. 3. Sampling after the electro-phoretic separation.

Several preliminary experiments have been carried out with the apparatus, both with organic indicators and protein pigments. A mixture of phycoerythrin and phycocyan in a phosphate buffer of ionic strength 0.025 and pH 7.6 is easily separated.

The described principle has proved applicable to the separation of some colored ions and some proteins. However, much more work is necessary to make the apparatus a good analytical instrument. The capillary system must be improved. A refined conductivity gradient technique would perhaps give still better results. A method for direct observation of the zones should be of great value.

SUMMARY

An apparatus for electrophoretic analysis is described, in which glass powder is used as a capillary system. A thin "zone" of the solution to be investigated is made up in the glass powder. This original zone will split up during the experiment in a number of zones corresponding to the number of electrophoretic components. The fractions are collected with a sampling technique similar to the one used in chromatography.

This investigation has been financially supported by a grant from the Swedish Natural Science Research Council.

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Received June 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 963–970

Short Communications

A Simplified Method for the Colorimetric Determination of Molybdenum in Steel

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The starting point for this investigation was an article about colorimetric determination of molybdenum in steel by Kapron and Hehman [1]. In this it was shown that an addition of butyl cellosolve (2-butoxyethanol) had a stabilizing effect on the red-brown molybdenum-thiocyanate complex; the proposed analysis procedure gives very reliable results and represents a decided step forward, but is somewhat circumstantial in regard to the final colouring of the steel solution.

By observations made with butyl cellosolve the present author got the idea that it might be possible to make a stable solution containing butyl cellosolve, potassium thiocyanate and stannous chloride at the same time; the colouring of the final solution might then be effected by mixing fixed amounts of this single reagent and the dissolved sample, thus simplifying the colouring process considerably.

Investigation did not bear out this idea completely, but sufficiently so for practical purposes, as the mixed reagent may be used for at least two months; the butyl cellosolve has a strongly retarding effect on the reaction between potassium thiocyanate and stannous chloride, but does not stop it.

The following procedure was set up:

Special reagents:

Perchloric acid-phosphoric acid: 1 volume of perchloric acid, 70 %, + 2 volumes of phosphoric acid, 85 %, + 3 volumes of water.

Perchloric acid-sulphuric acid: 1 volume of perchloric acid, 70 % + 1 volume of conc. sulphuric acid.

Mixed reagent for the blank solution *(blank reagent):*

a) 100 g stannous chloride is dissolved at less than 50° C in conc. hydrochloric acid; after cooling, water is added, while stirring, until the total volume is 1 litre. 24 hours later the solution is filtered, and 1–2 g granulated tin are added; it is kept under CO_2-atmosphere.

b) 800 ml sulphuric acid (50 %) is mixed with 600 ml butyl cellosolve and cooled.

c) Add 1400 ml b) to 800 ml a), while stirring, and cool. Add 1–2 g granulated tin and keep under CO_2. This solution is very stable.

d) *The blank reagent* is made by adding 100 ml water to 1100 ml c). Add 1–2 g tin and keep under CO_2. Very stable.

Mixed reagent for the colour reaction *(colouring reagent):*

e) 4 g potassium thiocyanate is dissolved in 100 ml water. Very stable.

f) *The colouring reagent* is made by adding 100 ml e) to 1100 ml c). Add 1–2 g granulated tin and keep under CO_2. It may be used for about 2 months.

Analytical procedure:

1. *Low-alloy steels (and carbon steels).* *Gently* dissolve 0.5 g of the sample in a

mixture of 5 ml hydrochloric acid (19 %) and 15 ml perchloric acid (70 %), heat to dense fumes and boil for 5 minutes. Cool until a precipitate begins to form, add about 50 ml water, boil a short time, cool to room temperature, transfer to a 250 ml volumetric flask, dilute to the mark and mix thoroughly. (During the above procedure the silicic acid has been quantitatively precipitated: it may be removed by filtering and directly used for a determination of the silicon content; in this case, however, it is better to dissolve 1 g of the sample.)

If the steel contains more than 0.5 % C or 0.05 % S, dissolve in 5 ml hydrochloric acid (19 %), oxydate completely by dropwise adding conc. nitric acid, boil, cool slightly, add 15 ml perchloric acid (70 %) *etc.*

Pipette 50 ml of the steel solution into a beaker, and, while stirring, 50 ml colouring reagent; five minutes later the solution is ready for measurement; the colour does not change for more than a week, and the solution keeps clear.

The blank solution is prepared by mixing 50 ml steel solution and 50 ml blank reagent. It is not necessary to use pipettes if the steel solution is only faintly coloured in itself.

It is possible to analyse 4 samples in 35 minutes.

2. *High-chromium and stainless steels.* The chromium content is reduced by adding 2 ml conc. hydrochloric acid to the fuming perchloric acid solution (let it cool somewhat before the addition) and boiling until the red-brown chromyl chloride vapours are no longer visible. This process is repeated until a new addition gives only a slight colouring of the fumes. A content of 1—2 % Cr is permissible.

If the sample dissolves with difficulty in the usual manner, proceed in the way described for steels with more than 0.5 % C, reduce the chromium content, etc.

3. *Tungsten steels.* 0.5 g is *gently* dissolved in 20 ml perchloric acid-phosphoric acid. When the solution becomes clear cool slightly, add 10 ml perchloric acid and reduce the chromium content. It is sometimes necessary during this process to add small portions of perchloric acid to compensate for evaporation losses. Add 5 ml perchloric acid-sulphuric acid, fume for 5 minutes, cool slightly, add 50 ml water, boil about 3 minutes, cool to room temperature, transfer to 250 ml volumetric flask, dilute to the mark etc.

It is possible to dissolve in the well-known sulphuric acid-phosphoric acid mixtures, oxydate with nitric acid *etc.*, if care is taken with the driving out of the nitrous gases. As a general procedure the perchloric acid method is preferable, because of the very low blank values for instance.

The above procedure was verified in the following way:

A. The Kapron and Hehman (K & H) method and the present modification (K—W) were compared by measuring the extinction of the same solutions by each method.

Three types of steel were used:

I.	0.40 C— 2.46 Cr— 2.42 Ni—	0.37 Mo
II.	0.46 C— 1.33 Cr— 0.24 V—	0.65 Mo
III.	1.3 C— 4.5 Cr— 2.67 V— 11.88 W—	1.00 Mo

I was dissolved using the high-chromium, II the low-alloy and III the tungsten method.

The measurements were taken on a Lange colorimeter with stabilized light source and green filter (photocell apparatus).

Table 1.

Method	Colour extinction less blank extinction		
	I	II	III
K & H	.199	.254	.323
K—W	.199	.255	.323

The "Naphtodioxanes"

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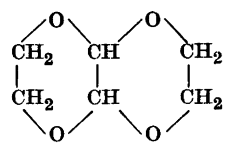
Of the two "naphtodioxanes" prepared from 2,3-dichlorodioxane and glycol, the lower melting isomer (m. p. 111°) is reported to have a considerable dipole moment (1.9 D.), whereas the moment of the isomer of m. p. 136° is found to be rather small (0.7 D.) [1]. The substances were thought to have the structure formula



and thus to be stereoisomeric and corresponding to the two decalins, and movable forms of the cyclohexane ring had therefore to be considered in order to explain the observed dipole moments [1]. The knowledge more recently obtained about the shape of the decalin molecules [2] has, however, made us doubt the correctness of this explanation, and we have therefore carried out some investigations of the two naphtodioxanes in our laboratory.

The coloured solution of steel II was kept for some time for observation purposes. After a lapse of two weeks the extinction was measured to 0.256. After filtration the same value was measured. The day afterwards a white precipitate began to form.

B. The stability of the colouring reagent was examined by measurements taken over a period of 9 weeks on the same steel solutions. The variations were insignificant and non-systematic:

Table 2.

Obs. time Weeks	Colour extinction less blank extinction I	II	III
9	.199 ± .001	.255 ± .001	.323 ± .001 ÷ .002

When the colouring reagent had aged for about 2 weeks it became possible to detect hydrogen disulphide by placing a strip of filter paper wetted with lead acetate solution in the neck of the reagent bottle; the evolution was slow, however, and did not upset the measurements.

C. Because of the price of butyl cellosolve a "blank reagent 2" was made in which the butyl cellosolve was entirely omitted and replaced with water. The two types gave exactly the same values.

In the course of a few days the "blank reagent 2" began to evolve hydrogen disulphide.

In Table 3 shows the connection between "% molybdenum in the interval 0—1 %" and "colour extinction less blank extinction".

Table 3.

% Mo	0	.09(3)	.18(5)	.24	.28	.37	.65	1.00
Extinction	.002	.075	.126	.162	.176	0.200	.255	.323

Seemingly the curve begins by being nearly straight, then bends through a transition zone into another straight line. This unusual aspect may be caused by different dominating colour complexes at lower and higher molybdenum contents.

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Received May 23, 1950.

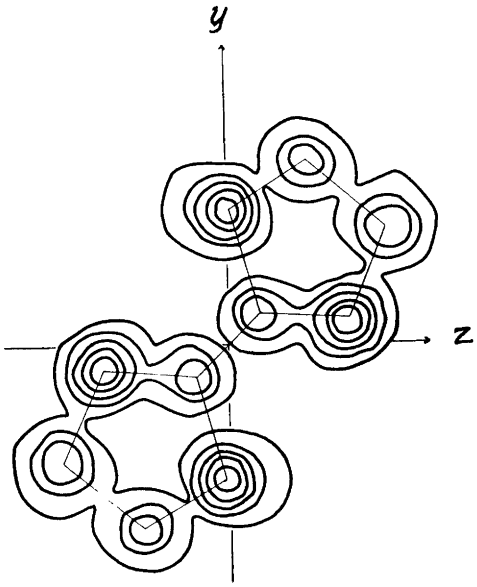
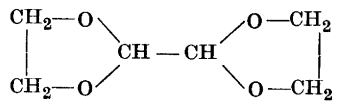


Fig. 1. Fourier projection in the direction of the a-axis.

The higher melting isomer. A few measurements of the dielectric properties of this substance indicate a lower value of the dipole moment than reported by Henriquez [1]. It seems possible that the moment may in fact be zero. This would seem to indicate that the molecule may correspond to *trans* or *cis* decalin, the former demanding a zero moment, the latter probably a very small one.

The crystals of this isomer are monoclinic with $a = 11.55$ Å, $b = 7.04$ Å, $c = 15.88$ Å and $\beta = 92.5°$. Density: 1.46 g/cm^3 (calc. 1.50 g/cm^3). The unit cell contains eight molecules and the space group is either $A2/a$ or Aa. The crystals do not appear to be piezoelectric (Blomgren [3]), and the former is therefore the most probable one. A complete structure determination would in all cases demand a considerable amount of work, as the space groups in question do not require any symmetry of the individual molecules.

The lower melting isomer. This compound proved to be much better suited for an X-ray structure analysis. The crystals are monoclinic with the following lattice constants: $a = 4.46$ Å, $b = 7.76$ Å, $c = 11.64$ Å and $\beta = 122°$. The observed density is 1.40 g/cm^3 (calc. 1.42 g/cm^3), and there are only *two* molecules in the unit cell. The space group is $P2_1/c$, from which follows that the molecules must be centrosymmetrical. Although the dipole moment observed in solution would seem to exclude the possibility of molecules corresponding to those of *trans* decalin, a structure determination based on such a model was attempted. We found, however, that it was impossible to obtain a satisfactory agreement between observed and calculated intensities in this way. The assumption was therefore made that the structural formula



might be the correct one. This possibility was discussed also by Böeseken *et al.*, but rejected mainly on chemical grounds. However, good agreement with the experimental intensities was soon obtained when this formula was assumed. In Fig. 1 the electron density map corresponding to a projection along the short axis is reproduced, and it shows, we think, with certainty that the compound actually has the formula given above, and should be described not as "naphtodioxane", but as bi-1,3-dioxacyclopentyl (2). The dipole moment observed in solution is thus satisfactorily accounted for.

Full details of the crystal structure determination will be given later.

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Received July 4, 1950.

The Potentiometric Titration of Phenolic Hydroxyl Groups. I. Description of a "Calomel Electrode"

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In the last decades, water-free solvents have been widely used in the titrations of weak acids and bases. One of the main difficulties encountered in such determinations has been to find electrodes with sufficiently high stability. Moss, Elliot, and Hall [1] have used antimony electrodes, and have stated that platinum electrodes do not give good results. In connection with investigations on the possibility of determining phenolic hydroxyl groups by titration in water-free solvents, the present authors were not able to obtain satisfactory results with either platinum or antimony electrodes arranged according to Moss, Elliott, and Hall [1]. Later, however a "calomel electrode" was developed, which has given very good results [2]. Investigations on the solvent effect have shown ethylene diamine to be the most satisfactory solvent for use with the above-mentioned "calomel electrode".

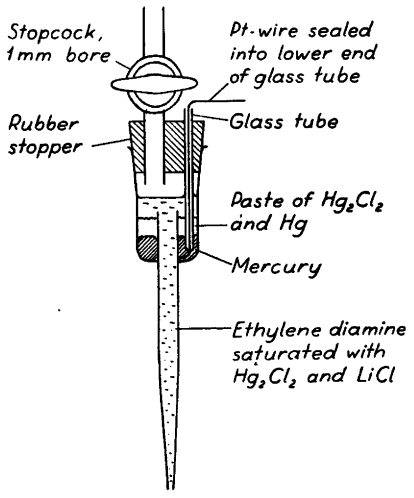

Fig. 1. Schematic drawing of the "calomel electrode".

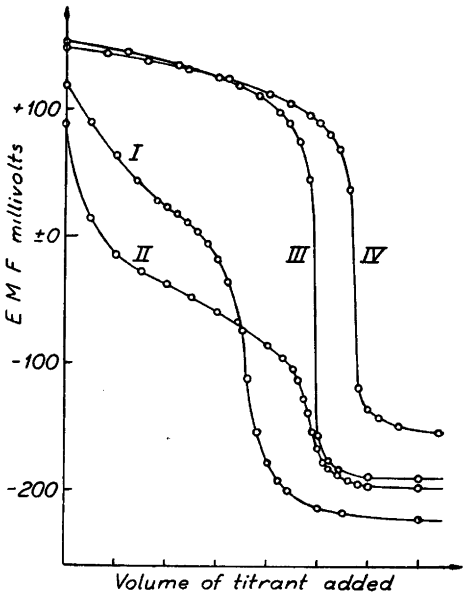

Fig. 2. Titration curves obtained from titrations of various acidic substanses using the electrode described.

I Lignin derivative
II Phenol
III Benzoic acid
VI Methyl p-hydroxybenzoate

Description of the "calomel electrode". The electrode is shown in Fig. 1. It is made of Pyrex glass and consists of a wide, upper tube with a capillary tube (2.0 to 2.5 mm bore) sealed through the bottom. The lower part of the capillary is drawn out to a smaller diameter. The upper tube has a ring-formed bottom, where mercury is placed. On the mercury is placed a paste consisting of mercurous chloride and mercury. The upper tube is closed by a rubber stopper with two holes, through which lead a glass stopcock (1 mm bore) and a glass tube with a sealed in platinum wire, which gives contact with the mercury. The electrode vessel is filled with a saturated solution of mercur-

Exchange of Isotopic Ions in Cation Exchangers

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In theories published on the mechanism of cation exchange it has been postulated that there is a dynamic equilibrium between the cations sorbed and the cations of the same element in the surrounding solution. No direct experimental proof has been given, although it seems very probable in view of the many successful separations of different cation species [1] and especially Taylor and Urey's classical separations of isotopes [2].

In 1947 one of the authors [3] observed that a cation exchanger (Wofatit KS) saturated with thorium ions did not seem to be in dynamic equilibrium with a solution containing the thorium isotope UX_1. The possibility that a dynamic equilibrium really exists even in this case, is not excluded because of the high affinity of the thorium ions caused by their high charge. Another conceivable explanation is that thorium forms radiocolloids which are physically adsorbed on the exchanger and removable only by complexing agents and not by ion exchange alone.

In order to get a better understanding of this problem an investigation was started concerning the dynamic equilibrium in ion exchange systems. In this paper is reported a study of the exchange between silver ions using Ag^{111}, which is a pure beta-emitter with a maximum energy of 1.0 Mev. and a half-life of 7.5 days. A commercial quality of the synthetic cation exchange resin, Dowex 50, was used, the spherical particles of which had an average size of 10—30 mesh. Experiments were carried out both as single-stage operations and as column operations.

Single-stage experiments. A beaker containing "labelled" silver nitrate solution was supplied with a proper amount of Dowex 50 previously saturated with inactive silver ions. During the experiment the solution was stirred vigorously and samples of the solution were withdrawn at suitable time intervals for radioactivity measurements. Another single-stage experiment was made with the labelled silver saturated exchanger obtained in the experiment just mentioned. Its performance was the same except that in this case the outer solution was originally inactive. In both experiments the volume of the silver nitrate solution was 30 ml and its concentration 0.080 *N*. The amount of ion exchanger was chosen so as to have about as many exchanging groups as there were cations in the outer solution. If there is

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ous chloride and lithium chloride in ethylene diamine by gentle suction through the stopcock tube. When the vessel is filled the stopcock is closed, and the electrode is left for at least one day to reach equilibrium. When in use, the platinum wire is connected to the potentiometer and the drawn out capillary tube is put in the reaction solution. An ignited, bright platinum electrode is used as indicator electrode.

In Fig. 2 are shown some titration curves obtained from titrations of various substances of different acidity. An ethylene diamine solution of sodium aminoethoxide was used for the titrations of benzoic acid and methyl p-hydroxybenzoate, while sodium methoxide, in ethylene diamine, was used for the lignin derivative and phenol. A *Radiometer* potentiometer model PHM 3f was used for measuring the potential differences.

1. Moss, M. L., Elliott, J. H., and Hall. R.T. *Anal. Chem.* **20** (1948) 784—788.
2. Gran, G. *Acta Chem. Scand.* **4** (1950) 559—577.

Received July 6, 1950,

Exchange of Isotopic Ions in Cation Exchangers

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In theories published on the mechanism of cation exchange it has been postulated that there is a dynamic equilibrium between the cations sorbed and the cations of the same element in the surrounding solution. No direct experimental proof has been given, although it seems very probable in view of the many successful separations of different cation species [1] and especially Taylor and Urey's classical separations of isotopes [2].

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any exchange between isotopic ions, this will result in the first experiment in a total decrease of about 50 % in the specific activity when equilibrium is reached; a magnitude of decrease which gives the best experimental accuracy.

Two different types of *column experiments* were carried out. In the first case the column was saturated with hydrogen ions and after washing with distilled water a labelled silver nitrate solution was percolated at a rate of 2.5 ml/cm^2/min. The silver content of this solution corresponded only to a small fraction of the total exchanger capacity. After washing the activity of the column was measured by means of a "scanning" device (previously shortly described [3]) containing an end-window Geiger-Müller counting tube. Then a solution of inactive silver nitrate was allowed to flow through the exchanger. The silver content of this solution was chosen to displace the active silver ions previously sorbed for a distance of a few centimetres provided that there is an exchange. After washing the column activity was measured again. Another portion of inactive silver nitrate solution was percolated and so on. The other type of column experiment was similar to the one just described except that in this case the exchanger originally was completely loaded with inactive silver ions. All experiments were carried out at room temperature.

All experiments showed clearly that there is a rapid exchange between ions bound to the exchanger and their isotopic ions in the surrounding solution. Thus, in the single-stage experiments the specific activity of the outer solutions changed as expected from the exchanger capacity and the cation content of the outer solution. As would be anticipated this change was exponential with a half-time of the exchange of the order of 1—2 minutes under the conditions here employed. In the column experiments the maxima of the sorption bands of the active silver moved a distance, the length of which wa sequivalent to the cation content of the displacing silver nitrate solution. As the displacement progressed, the band width increased with a corresponding decrease of the maximum.

In a paper, which appeared after this work was completed, Borland and Reitemeier [4] reported the exchange of calcium ions between a calcium solution labelled with Ca^{45} and a number of clays, saturated with inactive calcium ions. They state that the equilibration is virtually complete after ½ to 1 hour, their techniques preventing the study of shorter periods. Apparently this calcium exchange is slower than the silver exchange studied by us. The chemical and structural differences, however, between clays and synthetic sulphonic acid cation exchange resins plays probably a much more important rôle in this rate difference, than the higher charge of the calcium ions.

The work will be continued with isotopic ions of higher charge and the single-stage techniques improved by using immersion Geiger-Müller counters to make possible the study of rather fast exchanges. Together with the column experiments this may be of fundamental interest for processes involving ion exchange. A more detailed report will be published later.

The authors wish to thank Professor Ole Lamm for his encouragement and kind interest in this work; Mr. C. G. Carlström for the purification of the active silver, which was purchased from the A. E. C., Oak Ridge; The Dow Chemical Co. for samples of Dowex 50. The work was supported by the Swedish Atomic Committee.

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Received July 27, 1950.

Oxidation of Glucosamine and Galactosamine with Ninhydrin to Arabinose and Lyxose and their Identification with Paper Chromatography

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The identification of the natural amino-hexoses was formerly a difficult task. While the hydrochloride of glucosamine yielded characteristic crystals that of galactosamine, because of its high solubility, was very difficult to obtain. The first way to identify small quantities of them was given with the use of paper chromatography as worked out by Partridge [1] and Aminoff and Morgan [2]. Collidine cf the highest degree of purity is necessary.

We found this technique reliable, and at the same time we tried to apply paper chromatography to the oxidation products of glucosamine and galactosamine.

The fact that the hexosamines under the same conditions as the amino acids react with ninhydrin to give blue coloured compounds has been known for a considerable time [3]. This reaction has been used for the detection of the sugars in paper chromatography by Aminoff and Morgan [2], Pratt and Auclair [4] and others.

When ninhydrin is allowed to act upon amino acids, an aldehyde with one carbon atom less than the original acid is formed. If the same type of reaction is assumed to take place with the hexosamines the aldehyde formed must be a pentose, for D-glucosamine it must be D-arabinose and for D-galactosamine (chondrosamine) D-lyxose.

These two pentoses can easily be separated by means of paper chromatography, if a mixture of butanol and ethanol in proportions 9 : 1 is used as solvent. Thus it would be very simple to identify the pentoses from a reaction mixture. This also proved to be the case.

200 mg of glucosamine and 180 mg of ninhydrin were dissolved in 150 ml of water and hydrochloric acid was added to pH 4.7. The mixture was placed in a boiling water bath for half an hour. The blue coloured solution was filtered to remove a brownish precipitate and the mother liquor was evaporated *in vacuo* to dryness. The residue was taken up in 1 ml of water and the solution was subjected to chromatography in the following way. Spots containing 0.002 – 0.005 ml were placed on the starting line of a chromatographic paper (Munktell OB). On each side of the testspot there were placed spots containing 100 γ of arabinose. The chromatogram was run in butanol-ethanol 9 : 1 mixture for 18 hours at 22° C. After drying, the paper was sprayed with an aniline trichloroacetate reagent, suggested by Dr Erik Vasseur [5]. In the test a strong spot appeared at the same distance from the starting line as the spots of arabinose. If the experiment was carried out in the same way with galactosamine and if lyxose was used instead of arabinose, a spot corresponding to the lyxose spots was obtained.

The technique of using this principle for the identification of aminosugars in mucopolysaccharides will be dealt with in another communication.

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Received August 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 971–977

Studies on the Sulphonation of Lignin *

II. Sulphonation of Ethoxylated Lignin Sulphonic Acids of low Sulphur Content **

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In 1939 Kullgren made the important observation that strong pulp which had been successively washed with dilute hydrochloric acid and with distilled water on heating with distilled water yielded soluble lignin sulphonic acids [1]. If the heating was carried out with methanol instead of water, soluble lignin sulphonic acids were also obtained which, however, showed an increased methoxyl content.

Strong sulphite pulp contains a small amount of solid, insoluble lignin sulphonic acids. When heated with water or methanol hydrolysis or methanolysis takes place with formation of soluble lignin sulphonic acids. Schematically the process may be represented in the following way:

$$\text{Lig}-\text{OX} + \text{HOH (or } CH_3OH) \rightarrow \text{Lig}-\text{OH (or Lig}-OCH_3) + \text{HX}$$

(Lig-OX is the solid lignin sulphonic acid, X a lignin residue = Lig or a carbohydrate. Lig-OH and Lig-OCH_3 are the resulting soluble acids.)

The yield of soluble acids obtained in Kullgren's experiments was low.

Hägglund and Johnson applied the Kullgren process to sulphonated wood, obtained by heating wood with strong sodium sulphite solutions, and much larger yields of "low-sulphonated, soluble lignin sulphonic acids" were obtained [2]. Such acids prepared by heating with water have been subjected recently to stepwise sulphonations with ordinary sulphite cooking acid [3]. It

* Part I. This journal 4 (1950) 228.

** Part 47 on the chemistry of the sulphite process by E. Hägglund and co-workers.

was found that for each sulphonic acid group introduced approximately one hydroxyl group capable of acetylation disappears.

We have refluxed sulphonated wood in "the hydrogen state" with absolute ethanol. The soluble lignin sulphonic acids obtained by this treatment were isolated by shaking the alcoholic solution with barium carbonate and they were obtained directly in a state of high "purity". As expected these acids were low-sulphonated and contained ethoxyl groups which could be split off by heating with mineral acids.

On heating with strong sodium sulphite solution of pH 5.45 (measured in the cold) some sulphonation took place but no ethoxyl groups were eliminated.

Heating with normal sulphite cooking acid furnished highly sulphonated products containing no ethoxyl groups. A corresponding amount of ethanol was found in the liquor. The "low-sulphonated, ethoxylated lignin sulphonic acids" apparently react in a way which is closely analogous to that of sulphonated wood and they may be regarded as simple, soluble models for the low-sulphonated, insoluble lignin sulphonic acids of sulphonated wood:

$$(SO_3HLig)OC_2H_5 + H_2O \text{ (or } H_2SO_3) \rightarrow (SO_3HLig)OH \text{ [or } (SO_3HLig)\,SO_3H] + HOC_2H_5$$

It should be noted that experiments closely analogous to those described in this paper were carried out fifteen years ago by Holmberg [4]. "Ethyl lignin" ("ethanol lignin") was subjected to the action of thioglycolic acid when normal lignin thioglycolic acids were obtained. Holmberg interpreted this result by assuming that ethanol lignin is a mixed ethyl-benzyl-ether which is cleaved by thioglycolic acid with the formation of ethanol and S-substituted thioglycolic acids. He also found that several ethyl ethers (ethyl α-phenethyl ether, ethyl benzhydryl ether) reacted similarly.

EXPERIMENTAL

Preparation of low-sulphonated, ethoxylated lignin sulphonic acids

In a rotating autoclave of stainless steel finely disintegrated, acetone-extracted wood (1.1 kg = 1.0 kg dry) was heated with a cooking acid as described in an earlier paper [5]. The acid was prepared in the following manner. Sulphur dioxide was passed into a solution of sodium hydroxide (583 g) in water (5 l) until pH 5.23 (20°) was reached. The sulphonated wood was washed very carefully with large amounts of distilled water and finally dried in the air. (Yield 910 g = 839 g dry).

Air-dry sulphonated wood (100 g) was slowly washed with 0.1 *N* hydrochloric acid (2.5 l) and finally with distilled water until the filtrate contained no chlorine ions. The material was then transferred into a wide glass tube and alcohol (95 %) slowly passed through from below in order to remove water. Finally absolute alcohol was passed in

until determinations of the specific gravity indicated that absolute alcohol was leaving the tube.

The material was immediately plunged into absolute alcohol (1.5 kg) and the mixture refluxed for two hours (calcium chloride tube). The alcoholic solution was rapidly removed by suction and the residue again heated with absolute alcohol (1 kg). The process was repeated twice. In this way three alcohol extracts (I—III) were obtained. They were immediately shaken with an excess of pure barium carbonate, suspended in a little water, in order to neutralise and precipitate the lignin sulphonic acids. The precipitates were separated by centrifuging and then shaken with water. The resulting solution was again centrifuged and then poured into eight volumes of methanol. The precipitate was collected and the dissolution-precipitation process repeated. The product was stirred with methanol and centrifuged, repeatedly stirred with ether and centrifuged, and finally spread on an inclined glass plate to ensure rapid drying. Yield from extract I 4.1 g (3.9 g dry), from II 2.5 g (2.4 g dry) and from III 2.0 g (1.9 g dry) barium lignin sulphonate. (Preparations I, II and III.) For the analyses, *cf.* Table 1.

Similarly extracted and sulphonated spruce wood (700 g air-dry) was treated in the same way with hydrochloric acid, distilled water and alcohol, and refluxed with absolute ethanol (4 hours). The precipitated barium salts were dissolved in water (1500 ml) and the solution concentrated to 300 ml by vacuum distillation. The solution was then freed from barium ions by a cation exchanger. The sulphonic acids were purified by two precipitations with 4,4′-bis-dimethylamino-diphenylmethane and converted into the barium salt as described earlier[5,6]. Yield 27.2 g (25.5 g dry). Preparation IV. For analyses, *cf.* Table 1.

Sulphonation of low-sulphonated, ethoxylated lignin sulphonic acids

The barium salts I—IV (air dry, corresponding to 1.0 g dry salts) were dissolved in a small volume of water and the solutions freed from barium ions by passing through a cation exchanger. Calcium carbonate (0.715 g) was added to the filtrate (40 ml) and sulphur dioxide passed into the mixture until the "total amount of SO_2" was 5 g per 100 ml solution ("CaO" = 1.0, "SO_2" = 5). The solutions were heated in sealed tubes of Pyrex glass for 6 hours at 135°. After cooling, carbon dioxide was passed through the solutions to remove excess sulphur dioxide. The calcium ions were removed by passing through a cation exchanger, and the solutions were again treated with carbon dioxide and finally neutralised with barium carbonate. After filtration the solutions were concentrated to a small volume by vacuum distillation, centrifuged and precipitated by pouring into five volumes of alcohol. The precipitates were collected by centrifuging, carefully washed with alcohol and with ether as already described, and dried in the air.

The preparations are designated I S, II S, III S and IV S respectively. Yield of I S 0.77 g (0.74 g dry), of II S 0.80 g (0.77 g dry), of III S 0.82 g (0.78 g dry), and of IV S 0.79 g (0.75 g dry). For analyses, *cf.* Table 1.

Estimation of hydrolysable ethoxyl in the preparations I—IV

The preparations (250 mg) were dissolved in 10 ml water. 40 ml of 10 % sulphuric acid were added and the mixture refluxed for 30 minutes. 25 ml were then distilled off and potassium bichromate (3 g), followed by 98 % phosphoric acid (10 ml), was added to

Table 1. Analyses of the various lignin sulphonic acids.

Preparation	C	H	O	S	Ba	$\frac{Ba}{2S}$	O-alk[a]	$(C)CH_3$	OCH_3[b] calc	OC_2H_5[c] calc	OC_2H_5[d] found	$\frac{S}{OCH_3}$ calc	$\frac{OC_2H_5}{OCH_3}$ calc
I	50.65	4.88	28.65	4.72	11.1	1.10	15.28	1.96	12.04	4.70	4.47 4.62	$\frac{1}{2.63}$	$\frac{1}{3.7}$
I S	43.45	4.01	30.47	6.55 6.20	15.7	1.15	11.01	0.35	11.01	—	0.25	$\frac{1}{1.78}$	—
II	51.84	4.89	28.78	4.29	10.2	1.11	15.94	2.02	12.58	4.88	4.93	$\frac{1}{3.03}$	$\frac{1}{3.7}$
II S	45.20	4.07	29.18	6.15	15.4	1.17	11.56	0.30	11.56	—	0.23	$\frac{1}{1.94}$	—
III	52.86	5.03	28.68	4.25	9.18	1.01	15.63	2.05	12.22	4.95	5.28	$\frac{1}{2.97}$	$\frac{1}{3.6}$
III S	46.33	4.17	28.52	6.38	14.6	1.07	11.54	0.30	11.54	—	0.20	$\frac{1}{1.87}$	—
IV	51.35	4.96	29.41	4.28	10.00	1.09	16.02	1.65	13.30	3.95	4.12 3.93 3.68	$\frac{1}{3.21}$	$\frac{1}{4.9}$
IV S	46.56 46.37	4.68 4.54	27.57	6.20	15.15	1.14	11.43 11.54	0.27	11.43 11.54	—	0.16	$\frac{1}{1.91}$	—

a) Estimated according to the Vieböck method.
b) Calculated by subtracting OC_2H_5 calc. from O-alk.
c) Calculated by subtracting $(C)CH_3$ of resulphonated material from $(C)CH_3$ of the original ethoxyl-containing sulphonic acid salt.
d) By acid hydrolysis and oxidation of liberated alcohol with chromic acid (see experimental part).

the distillate. The mixture was refluxed for 5—10 minutes, 20 ml $MgSO_4$-solution added (*cf.* Clark [7]) and the acetic acid formed isolated by steam distillation and estimated by titration with 0.04 *N* barium hydroxide. Indicator: phenol red (0.1 % in 90 % ethanol). For analyses, *cf.* Table 1.

Table 2. Composition of the preparations on the C_9-basis.

Preparation	C	H	O	OCH_3	OC_2H_5[a)]	SO_3	Ba	$\frac{OC_2H_5}{SO_3}$[b)]
I	9	7.84	2.1	0.96	0.3	0.37	0.20	1.4
I S	9	8.04	2.6	0.98	—	0.55	0.32	
II	9	7.52	2.2	0.99	0.3	0.33	0.18	1.5
II S	9	7.75	2.3	0.99	—	0.51	0.30	
III	9	7.75	2.1	0.93	0.3	0.31	0.16	1.1
III S	9	7.78	2.0	0.96	—	0.55	0.28	
IV	9	7.83	2.3	1.05	0.2	0.33	0.18	1.3
IV S	9	8.89	2.0	0.96	—	0.50	0.28	

a) Calculated on the basis of OC_2H_5 calc. in Table 1.

b) Quotient of OC_2H_5 in this table and the increase in SO_3 content caused by resulphonation.

Drying of the preparations prior to analysis

All preparations were dried at room temperature for three to four weeks in evacuated desiccators charged with sodium hydroxide, concentrated sulphuric acid and phosphorous pentoxide. As a check, it was shown that further drying at 50° over phosphorous pentoxide and paraffin did not result in any further loss of weight.

Sulphonation of preparation IV with cooking liquor of low acidity

An amount of preparation IV corresponding to 3.0 g "lignin" ($Ba(SO_2)_2$ subtracted) was dissolved in water and transformed into the free acid by passage through a cation exchanger. A solution of sodium hydroxide (6 g) in water (about 20 ml) was added to the filtrate and sulphur dioxide passed in until the solution showed a pH of 5.45. The volume of the solution was 50 ml. It was now heated in a sealed Pyrex tube for 6 hours at 135°. On cooling a jelly was formed, due to the salting out of the sodium salt of the lignin sulphonic acid. This was separated from the liquor by centrifuging. The jelly and the supernatant solution were investigated separately.

The clear solution was examined for free ethanol (steam distillation of the alkaline solution) but only traces were found. The jelly was dissolved in water and transformed into the barium salt in the usual way. The yield was 1.5 g (containing about 5 per cent moisture). The analysis of the dry preparation gave the following results:

Ba 11.49 (10.00), S 4.78 (4.28), O-alk 15.93 (16.02)
"OC_2H_5 found" (*cf.* Table 1) 2.88 (3.95), (C)CH_3 1.7 (1.65).

The figures in parentheses correspond to the analytical figures for preparation IV (*cf.* Table 1).

DISCUSSION

Table 1, column (C)CH_3, shows that all the ethoxylated low-sulphonated acids (I—IV) contain ethoxyl groups which are removed on resulphonation (I S—IV S). The small amount of (C)CH_3 remaining in the resulphonated preparations belongs to lignin. Column "OC_2H_5 found" shows that within the limits of experimental error all the ethoxyl groups introduced during the Kullgren process are hydrolysable with hot, dilute sulphuric acid. The small amounts found in the resulphonated products probably do not originate from ethoxyl groups.

The fact that the methoxyl and ethoxyl contents must be calculated by difference makes the relation between ethoxyl and methoxyl uncertain, but the last column of Table 1 shows that at any rate there is in the preparations I—IV about one ethoxyl group per four methoxyl groups.

In the resulphonation experiments a high sulphonation was not aimed at. A certain excess of "base" was present in the cooking liquors. As seen from the last column of Table 2 the amount of ethoxyl groups which has been split off apparently exceeds that of sulphonic acid groups which has been introduced during the resulphonation process. This may indicate that the hydrolysis of ethoxyl groups proceeds at a higher rate than the sulphonation under the conditions of acidity employed in the experiments. The result of the sulphonation of preparation IV at a higher pH (5.45 at room temperature) shows that scarcely any ethoxyl groups are removed when sulphonation is attempted under these conditions.

The results are quite consistent with the view that "genuine lignin" contains groups characterised by different reactivity. One type of group ("A") is directly available in the wood and may be sulphonated by hot cooking liquor of low acidity and high buffering capacity. This is accomplished more or less completely depending on the experimental conditions, *e. g.* the duration of the cook. Another type of group ("B") is not attacked under these conditions, or at least is attacked very slowly. The groups B are sulphonated only under more acid conditions and are presumably the groups connecting "lignin molecules" of the molecular size occurring in normal waste liquors with other similar lignin molecules (or with carbohydrates) to form the so-called "genuine lignin" of wood. (In stating this, of course, we do not overlook the possibility of secondary condensations with formation of lignin sulphonic acids of higher molecular weight.)

Much evidence can be brought forward in favour of the view that the groups A are of the benzyl alcohol type [8], as first suggested by Holmberg [9]. The groups B may be of the benzyl ether type, as also indicated by the same

author [10]. It has been suggested that, alternatively, the groups B may possess a modified ether structure being of acetal or ketal [11] type. Experiments on the sulphonation of various benzyl ethers and compounds containing acetal or ketal groups with sulphite cooking acids of varying acidity are being carried out in order to throw more light on the nature of the groups B.

SUMMARY

Low-sulphonated, ethoxylated lignin sulphonic acids have been obtained from sulphonated wood containing free sulphonic acid groups by refluxing with ethanol. These acids have been subjected to resulphonation with sulphite cooking acid of varying acidity. If the acidity is low no elimination of ethoxyl groups takes place. If, however, the acidity is high the ethoxyl groups are split off and apparently more or less completely replaced by sulphonic acid groups. This provides a new indication of the presence of different groups in lignin characterised by varying reactivity to sulphite.

This work was originally planned during numerous discussions with Professor Erik Hägglund. It is a privilege that this paper is also included in his distinguished series of memoirs on the chemistry of the sulphite process.

The generous financial support of *Statens Tekniska Forskningsråd* is gratefully acknowledged.

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Received June 10, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 978–989

Studies on Liver Arginase

III. Its Activation and Inhibition Reactions

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The properties of arginase especially these pertaining to its activation and inhibition represent an interesting aspect in the field of enzymes. They have been the subject of numerous investigations which, unfortunately, have yielded quite conflicting results. In the following is a brief summary of some of the various results previously reported.

The activation of arginase was first attributed specifically to sulfhydryl compounds [1–3]. This view was modified later, as Edlbacher, Kraus and Walter [4], and Klein and Ziese [5] found that the effect of the sulfhydryl group was not absolute, but depended on the purity of the enzyme preparation as well as on the hydrogen ion concentration. Further work, by Waldschmidt-Leitz, Scharikova and Schäffner [6], revealed that sulfhydryl compounds, combined with heavy metals (Fe or Cu), could function as specific activators of arginase. Waldschmidt-Leitz and Kocholaty [7] visualized the activated arginase as only an oxidized form of the enzyme. This was confirmed by Purr and Weil [8] who added that many products of the intermediary metabolism when combined with Fe, such as alloxan-Fe, ascorbate-Fe, and methyl glyoxal-Fe, could also activate arginase. The conclusions reached by these authors were that the activation process required a certain definite oxidation-reduction potential. Quite contradictory results were reported by Leuthardt and Koller [9], as they stated that all activators of arginase must be reducing agents, whose action was actually a protection of the enzyme against oxidizing agents, and mainly oxygen. Using partially purified arginase, Hellerman and Perkins [10], found that neither mercaptide forming organomercurials (*e. g.* C_6H_5HgOH), nor such potent reducing agents as bisulfite, cysteine, cyanide or hydrogen sulfide had any effect on its action. On the other hand Weil [11] observed activation by cysteine-Fe under several conditions.

The first reference to the activation of arginase by metallic ions was made by Hellerman and Perkins [10]. They visualized the arginase molecule as being a metal-protein complex from which the metal ion could be easily dissociated. Klein and Ziese [12] observed that Mn^{++} ions were essential for obtaining maximum activity only in the further stages

of purification, and that they were without effect in crude liver extracts. The mechanism of activation was explained by Hellerman [13], as due to formation of enzyme-metal-substrate coordination complex. Edlbacher and Zeller [14], however, proposed that arginase consists of a metal with a protein carrier. The latter could be digested by trypsin except in the presence of an excess of Mn^{++} ions. This was later modified by Edlbacher and Pinösch [15] who assumed arginase to be a conjugated protein with a Mn-containing prosthetic group.

Rossi [16] rejected the idea of manganese being an actual constituent of arginase. The same author and Pontecorno [17] suggested the presence of a coenzyme, coarginase, that contains no manganese. This was followed later by a report made by Rossi and Ruffo [18] claiming that arginase, completely inactivated by prolonged dialysis, could not be reactivated by addition of Mn ions alone or by readmixture with Mn free diffusate, but only by both added together. Recently, Thompson [19] described a procedure for obtaining a coarginase which could activate his purified arginase a further 50 per cent.

The effect of manganese, cobalt and nickel was interpreted by Mohamed and Greenberg [20, 21], as a catalytic reaction, influenced by time, temperature, pH, and type and concentration of the activating ion. Bach [22], using a partially purified arginase, reported it to be unaffected by such metal activators. This was later disproved by Mohamed [23] who obtained 2—3 fold activation of the same preparation if incubated with Mn^{++} ions at 40° C for one hour.

The activation of arginase by the metal ions was observed by Hellerman and Stock [24] to be accompanied by a shift in the pH optimum. The same results were obtained by Mohamed and Greenberg [20].

Edlbacher and Bauer [25] observed that dialysis of both yeast and liver arginase resulted in complete inactivation, but it could be restored by adding manganese, cobalt, nickel, cadmium, and vanadium ions, the manganese ions being the most effective. Similar results were reported by Kocholaty and Kocholaty [26] who found that maximum activation by Mn^{++} ions required about fifteen minutes and that other ions, such as Fe^{++}, Ni^{++}, and Co^{++} also activated arginase but to a less extent than Mn^{++}. The activating action of cobalt was studied by Hunter and Downs [27] who found it a slow reaction at 20° and 37° but proceeding quite rapidly at 50° reaching completion after twenty minutes. This difference in ability of activation by the different metals was also observed by Anderson [28] on Jack bean arginase.

Arginase is quite stable even in the purified state. It is unaffected by a variety of agents, such as heat, urea, most heavy metals with the exception of Ag and Hg [20]. However, the enzyme is very sensitive to pH values lower than 5 and the effect is irreversible.

Lenti [29] reported that the activity of guinea pig and rat liver arginase was inhibited by F^- ions. The effect was reversible, *i. e.* on dialysis and addition of a trace of $MnSO_4$ the activity was restored.

Karrer and Zehender [30] found that cysteine and lactoflavin inhibited arginase and that the addition of F^{++} or Fe^{++} ions in small concentration protected the enzyme against this inhibition. Other inhibitors, namely quinone, Fe^{++}, iodine, and H_2S were reported by Hellerman and Perkins [10]. In all cases they could restore the lost activity by Mn^{++}, Co^{++} and Ni^{++} ions.

Kitagawa [31] studied the action of various compounds on arginase. He found *d,l*-α-amino valeric acid the most inhibitory. Fatty acids, acyl derivatives and esters of α-amino acids and urea had no effect. He concluded that the inhibition was due to a combination of arginase with the amino acids. Hunter and Downs [27] investigated the inhibitory effect

of a large number of amino acids and their derivatives and of some proteins. The enzyme was only inhibited in varying degrees, by the α-amino acids having the l-configuration, but not by the d-isomers, or by mono amino acids having the amino group in positions other than the α-position. The inhibition was non-competitive except in the cases of ornithine and lysine which exhibited a clearly competitive type of inhibition.

MATERIALS AND METHODS

Horse liver was used as the source for arginase throughout the work. The liver was obtained fresh from the sacrificed animal, chopped and mixed with several volumes of cold acetone (at 3° C). The mixture was stirred several times during the period of one hour. The acetone was then decanted and more fresh acetone was added. This procedure was repeated several times and then the mixture was filtered on a large Büchner funnel by suction. Several washings with fresh cold acetone were applied to the liver residue on the filter. The suction was continued until the residue became dry. It was then put in a desiccator under vacuum and stored in the cold room (at 3° C).

Arginase activity was measured according to a procedure previously published by Mohamed and Greenberg [20]. The activity was always reported as mg urea produced in 30 minutes.

EXPERIMENTAL

The effect of borate ion on arginase

Arginase has been shown to be partially depressed when the activity measurement was made using borate buffer [20]. This was explained as due to the borate forming a complex with the metal activator thus rendering it unavailable for the enzyme. A further attempt to study this effect was made in the following manner:

Two 100 ml volumes of a 2.5 % sodium acetate extract of the acetone dried liver (prepared by extracting 100 g liver powder in 1 liter sodium acetate) were treated each with 120 ml cold acetone at 3° C. Each mixture was centrifuged and one of the precipitates was taken up in 20 ml borate buffer (pH 8.0) and the other precipitate was taken up in the same volume of phosphate buffer at the same pH. The resulting extracts were centrifuged and the residues were discarded. The pH of the extracts were checked and adjusted to 7.2. A portion of each extract was dialyzed against running distilled water for 48 hours. Another portion of each was dialyzed for 24 hours against KCN solution (.05 M) adjusted to pH 8.0.

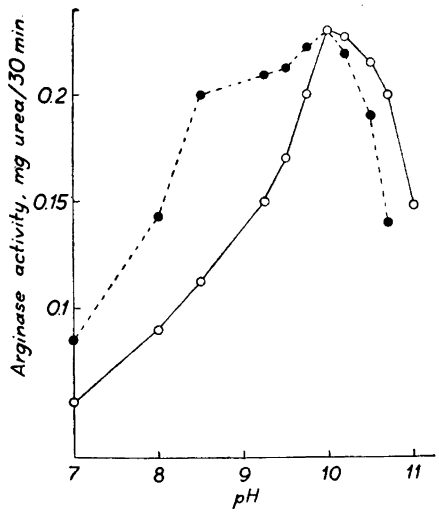

Fig. 1. pH-Activity curves of arginase extracted in borate before and after dialysis vs. water.

—○— *Borate extract*
— —●— — *Dialyzed borate extract*

The pH-activity curve of the borate and water-dialyzed borate extract are shown in Fig. 1. Only phosphate and glycine buffers were used in determining the pH-activity relationship. The pH-activity curve of borate extract essentially resembles the curve for Mn treated arginase [20] except for the difference in the extent of activity. On dialysing the borate out an extension of the activity to the less alkaline side is observed thus becoming similar to the Co-activated enzyme. In the following communication this is given as an evidence for the presence of two enzymes with arginase activity.

Comparison of the arginase activity of the borate, phosphate, water dialyzed and CN^--dialyzed extracts of both was made at both pH 7.2 (phosphate) and 9.2 (glycine) in presence and absence of Mn^{++} and Co^{++} ions. The results of these tests are recorded in Table 1.

The results show almost a complete inhibition of activity in the less alkaline pH 7.2 in the borate extract while the phosphate extract shows a good deal of activity. This inhibition is not released by Co^{++} ions which, in the phosphate extract, show high activating power. Manganous ions activate in the more alkaline side even in presence of borate.

On dialysis the borate ions are removed and we find that practically all the activity in the less alkaline region is restored to the normal and Co^{++} exerts again its activating effect.

Table 1. Comparison between borate and phosphate extracted arginase and the effect of dialysis and activating metals thereon.

	Arginase activity * (mg urea / 30 min)											
	Borate extract						Phosphate extract					
	no metal		Mn^{++}		Co^{++}		no metal		Mn^{++}		Co^{++}	
	pH 7.2	pH 9.2	pH 7.2	pH 9.2	pH 7.2	pH 9.2	pH 7.2	pH 9.2	pH 7.2	pH 9.2	pH 7.2	pH 9.2
Undial. extract	.08	.14	.08	.26	.08	.15	.17	.28	.21	.33	.23	.28
Ext.dial vs. H_2O	.14	.20	.16	.29	.23	.30	.17	.27	.19	.32	.22	.30
Ext. dial. vs. CN^-	.15	.22	—	.28	.21	.28	.15	.26	—	.29	—	—

* Arginase activity was measured in the usual manner at 40° C for 30 minutes, .05 ml of the extract was used.

When Mn++ or Co++ ions were used, incubation of the extract with the metal (conc. 2.5×10^{-3} *M*) at 40° C for half an hour preceded the activity determination.

Dialysis was carried out in cellophane bags at room temperature for 24 hours.

The effect of high temperature on arginase under different conditions

Arginase is quite stable towards high temperatures, a property which has been utilized as a means of purification by several workers [33, 22, 19, 34].

A study was conducted to see the effect of heating different arginase extracts at different temperatures. Crude extracts were prepared by suspending 25 g portions of acetone dried liver in 250 ml aliquots of the extracting solution. The mixtures were adjusted to pH 7.0 and placed in a shaker for two hours, after which they were centrifuged discarding the residues. The extractants used were as follows: 2.5 % sodium acetate, .01 *N* KCN, .005 *N* KCN, and 0.1 *M* cysteine hydrochloride.

Aliquots of each extract (10 ml) were heated at 30°, 40°, 50°, 55°, 60°, 65° and 75° C each for exactly 5 minutes, with continuous mixing. Each extract was then cooled at once under running water, centrifuged and arginase activity of the supernatent was determined. The same extracts were stored at 3° C and the enzyme activity was again measured after seven and fifteen days of

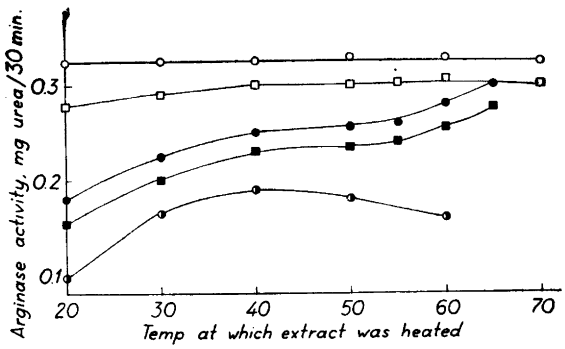

Fig. 2. The effect of high temperatures on arginase activity in different extracts.

- ● *Fresh 0.01 N KCN extract.*
- ○ *0.01 N KCN extract after 15 days at 3° C.*
- ■ *Fresh 0.005 N KCN extract.*
- □ *0.005 N KCN extract after 15 days at 3° C.*
- ◑ *Fresh 0.1 M cysteine-HCl extract.*

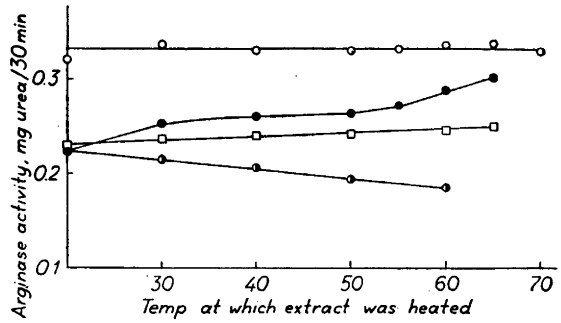

Fig. 3. The effect of high temperatures on arginase activity in different extracts.

- ● *Fresh KCN extract.*
- ◑ *Dialysed KCN extract.*
- ○ *KCN extract stored at 3°C for 15 days.*
- □ *NaAc extract stored at 3°C for 15 days.*

storage at 3° C. Plots of the activities against the temperatures at which the extracts were heated are shown in Figs. 2 and 3.

Portions of the .01 CN^- extract were heated at 65° C for various periods; namely 2, 4, 6, 8, 10, and 12 minutes. This was followed by measurement of arginase activity in the resulting supernatents. Plots of activity vs. time of heating are shown in Fig. 4.

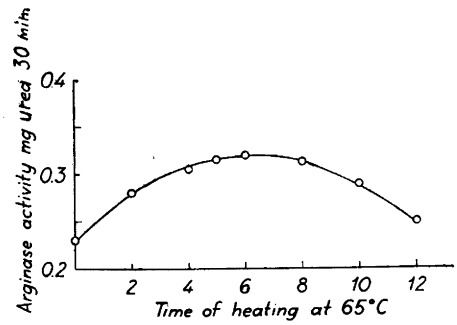

Fig. 4. The effect of duration of heating on arginase activity in 0.01 N CN^-.

Table 2. Arginase activity in cyanide and acetate buffers, as affected by heating, storage at 3° C, dialysis and treatment with Mn^{++} ions.

	Total N	Arginase activity *	
	mg/ml	day of extraction	after 2 weeks at 3° C
Sodium acetate extract	1.9	0.195	0.23
» » » + Mn^{++}	1.9	0.26	0.30
» » » + H_2S	1.92	0.25	0.29
» » » heated at 40° C for one hour	1.8	0.26	0.27
» » » heated at 65° C for 5 minutes	1.4	0.24	0.28
cyanide extract (.01 *N*)	2.1	0.22	0.34
» » + Mn^{++}	2.0	0.32	0.38
» » heated at 40° C 1 h	2.0	0.30	0.35
» » » » 65° C 5 min.	1.3	0.30	0.38
» » » » 65° C 5 min. + Mn added	1.35	0.30	0.38
» » dialysed vs. water	2.0	0.23	0.20
» » » » » and heated at 65° C for 5 minutes	1.4	0.19	0.14
Cyanide extr. heated at 40° C for 1 h and dialyzed vs. H_2O	1.0	0.16	0.195
Same as above + Mn^{++}	1.0	0.34	—

* Reported as mg urea produced in 30 minutes. Detns. were conducted at 40° C, pH 9.9. Incubation with Mn++ (2.5×10^{-3} *M*) was made at pH 7.0, and 40° C for one hour prior to the addition of substrate.

In Tables 2, 3 and 4 are recorded the activities of the forementioned extracts under various conditions and treatments with H_2S, Mn^{++}, Co^{++}, and Ni^{++} ions.

The common feature about Figs. 2 and 3 is that heating of freshly undialyzed arginase extracts results in enhancing the enzyme activity. In cyanide extracts a peculiar sort of an S-shaped curve results that is difficult to explain without assuming the complicated nature of arginase and probably because of an interaction with CN^- ions, and also with other components of the extract.

If, however, the extracts are stored at 3° C for two weeks their activity seems to rise to the maximum and heating has no further effect except for the denaturing of other foreign nonarginase proteins.

Table 3. The effect of heating and dialysis on cyanide extracts of arginase in presence and absence of Mn^{++}, Co^{++}, and Ni^{++} ions.

Extract	Arginase activity mg urea/30 min.						
	No activator	30 min incubation with activ.			24 h incubation with activ.		
		Mn	Co	Ni	Mn	Co	Ni
I .01 *N* CN^- (after 7 days at 3° C)	.26	.35	.28	.26	.38	.30	.29
II .005 *N* CN^- extr. (after 7 days at 3° C)	.23	.36	.30	.26	.38	.24	—
III Treatment I + heat at 65 C 5 min	.28	.35	.27	.25	.37	.29	—
IV » I + dialysis vs. H_2O	.21	.34	.25	.24	.38	.30	.28
V » III + » » H_2O	.13	.28	.20	.21	.34	.23	.21
VI » II + heat at 65° C 5 min. followed by dialysis vs. CN^-	.19	.34	—	—	.37	.27	—

Arginase activity measurement was run at pH 9.9 and 40° C. Conc. of Mn++ ion was 2.5×10^{-3}, conc. of Co++ and Ni++ ions was 17×10^{-3}. Incubation was made at 40° C, pH 7.0.

Table 4. The effect of Co^{++} and Fe^{++} on cysteine extracts of arginase.

Treatment	Arginase activity mg urea / 30 min.
0.1 *M* cysteine extract	0.08
0.1 » » » + Co^{++}	0.18
0.1 » » » + Fe^{++}	0.08
Dialyzed 0.1 » » »	0.10
» 0.1 » » » + Co^{++}	0.17
» 0.1 » » » + Fe^{++}	0.06

Arginase activity was determined at pH 9.9 and 40° C. Concn. of Co++ and Fe++ was 1.7×10^{-3}. Incubation with the metal was made at pH 7.0 and 40° C.

If the crude extract is first dialyzed against water and then heated, a loss in its activity is gradual depending upon the temperature at which the heating was performed. Thus it seems that there is an element, most probably one of the metal activators that, although present in minute amounts, not only protects the enzyme against inactivation by heat but also causes further activation (2-fold approximately). Removal by dialysis, heating or even

storage at room temperature leads to loss of the arginase activity of the extract.

A crude extract can be heated to 70° or 80° C without any damage if the duration of heating does not exceed 5—7 minutes. If heated for longer periods a gradual loss in activity ensues as is shown in Fig. 4.

Cysteine extracts show much lower activity, and the latter is enhanced by heating to 40° C but is lowered again if the temperature exceeds 40°—50° C.

If an extract heated to 65° C for 5 minutes, thus reaching its maximum activity, then is dialyzed, it is observed to lose most of the activity. However, it can be regained by incubating the extract with Mn^{++} ions.

The activities recorded in Table 3, which have been run at pH 9.9, show definitely a response to Mn^{++} activation but not to either Co^{++} or Ni^{++} ions.

Contrary to reports by other workers neither cysteine alone nor cysteine — Fe^{++} can activate arginase.

The effect of prolonged dialysis

It has been reported that arginase activity is decreased after dialysis of the preparation. An experiment was performed to study the effect of prolonged dialysis and to correlate the loss in activity with the duration of dialysis. The effect of Mn^{++} ions, H_2S—Mn, the dialysate, and H_2S treated dialysate in restoring the lost activity and in further activation were also investigated.

An extract was prepared by mixing 40 g of acetone dried liver with 400 ml of water and the pH was adjusted to 7.0. After mixing for two hours on a shaker a clear extract was obtained by centrifugation.

A 100-ml portion of this extract was dialyzed against the same volume of distilled water. The dialysis water was not changed in order to test the dialysate for its ability to restore the activity and its power of activation. This dialysis was continued for 5 days.

The rest of the extract was placed in another bag which was suspended in 4 liters of distilled water which was changed twice daily. Samples of the extract were withdrawn at intervals and tested for their arginase activity as such and after incubation with Mn^{++}, H_2S saturated Mn, phosphate buffer, dialysate, and H_2S treated dialysate.

Dialysis was performed at room temperature and with continuous stirring by hanging the bag on the stirrer. A layer of toluene was added to the water of dialysis for preservation. The results are recorded in Table 5.

The results recorded in Table 5 show that prolonged dialysis has very little effect on arginase activity measured at pH 9.9.

Table 5. The effect of prolonged dialysis and some activators on arginase activity.

	Arginase activity *, mg urea / 30 min.										
	Untreated extr.	Incubated with Mn		Incubated with H_2S-Mn		Incubated with phosphate bf.		Incubated with dialysate		Incubated with H_2S-dialysate	
		½ h	24 h	½ h	24 h	½ h	24 h	½ h	24 h	½ h	24 h
Water extract	0.18	0.28	0.45	—	—	0.22	0.31	0.2	0.2	0.18	0.18
Water extract, stored at 3° C for 192 h	0.22	0.29	0.43	0.28	0.44	0.22	0.30	—	—	0.21	—
Extr. dial. 20 h	0.17	0.27	0.38	0.23	—	—	0.22	0.18	0.11	0.19	0.03
» » 72 »	0.20	0.29	0.42	0.26	0.44	0.20	0.10	0.18	0.10	0.17	0
» » 144 »	0.18	0.26	0.41	0.25	0.43	0.18	—	0.18	0.07	—	0.04
» » 264 »	0.17	0.28	0.42	0.27	0.43	0.17	0.02	0.18	0	0.17	0.02
» » 360 »	0.17	0.26	0.41	0.25	0.44	0.15	0.01	0.14	0	0.15	0

* pH 9.9 and 40° C.

Incubation of dialyzed arginase with Mn^{++} ions alone or H_2S treated Mn^{++} ions caused a rise in the activity. This shows the importance and specificity of Mn^{++} ions in activation of arginase when the measurement is made as pH 9.9. Incubation for ½ h with other solutions such as phosphate buffer (pH 7.0), dialysate, and H_2S-treated dialysate showed no activation. If the time of incubation was extended to 24 h the activity was measurably diminished or completely lost particularly in extracts previously dialyzed for a long time (144—360 h). This may be attributed to exposure to the high temperature (40° C) for a long time in absence of the protecting metal activator.

SUMMARY

1. Arginase activity is reversibly lost in the pH region between 7—8.5 in presence of borate ion. This effect is partially removed by dialysis. The activity in the forementioned region still remains low owing to the effect of dialysis itself in diminishing action in this pH range.

2. Mn^{++} ions have no effect whatsoever on the activity of borate extracted arginase in the pH region of 7—8.5, yet it considerably enhances the activity at pH 9.9 even in presence of borate.

3. Co^{++} ions have no effect on arginase in both pH ranges; namely, 7.0—8.5 and 9.9 if borate is present. Only when borate ions are completely removed does cobalt exert a distinct activation in the first pH range (7.0—8.5) with no effect in the second.

4. Heating fresh extracts of arginase to different temperatures results in enhancing its activity. This effect can also be reached if the extracts are merely stored at 3° C for 15 days. Any subsequent heating only exerts a denaturing action on other nonarginase proteins without any untoward effect on arginase itself.

5. The effect of duration of heating at 65° C was studied. The activation is gradual up to 7 minutes where the activity reaches maximum. If the duration is prolonged a gradual loss results.

Heating of dialyzed extracts is detrimental to the activity. The loss of activity is dependent on the temperature and duration of heating.

6. Prolonged dialysis has no deleterious action on arginase activity with a maximum at pH 9.9. Only Mn^{++} and H_2S-treated Mn^{++} ions strongly activate the extract.

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Received April 29, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 990–992

Studies on Liver Arginase

IV. Evidence for the Presence of Two Enzymes with Arginase Activity

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The conflicting reports in the literature and the peculiar and unexplained properties ascribed to arginase have been reviewed and studied by the author. It is the aim of this communication to put some end to these conflictions by proving that liver extracts contain two enzymes with arginase activity. These two enzymes have quite different and rather interesting properties.

If one studies the pH-activity curves of arginase preparations [1–3], one is confronted with a sharp optimum at pH 9.8—10. In the less alkaline region, however, the activity extends, although at a lower level, but nevertheless, it forms a distinct and persistent bulge between pH 7.0 and 8.0. The curve can actually be resolved into two separate curves partially superimposed (Fig. 1). One curve would have its maximum at around pH 7.4—7.6 (curve I) and the second at pH 9.9—10 (curve II). If one measures the activity at pH 8.0 or 9.0 it would show a combination of activities of the two enzymes; thus giving no true picture of either enzyme at all. This will explain many peculiarities such as, the variation of the Michaelis-Menten constant (K_s) with the change of pH of the determination, as was reported by Greenberg and Mohamed [4].

When a liver extract is dialyzed against water, and the pH activity relationship of the dialyzed extract is plotted, a curve very much similar to curve II alone is obtained. The characteristic bulge (pH 7.0—8.0) is completely absent. This is taken to mean that the enzyme represented by curve I (may be called arginase *a*) must be inhibited by dialysis while the enzyme (arginase *b*) represented by curve II remains practically unchanged. Experiments on prolonged dialysis reported by Mohamed [5] showed that arginase activity,

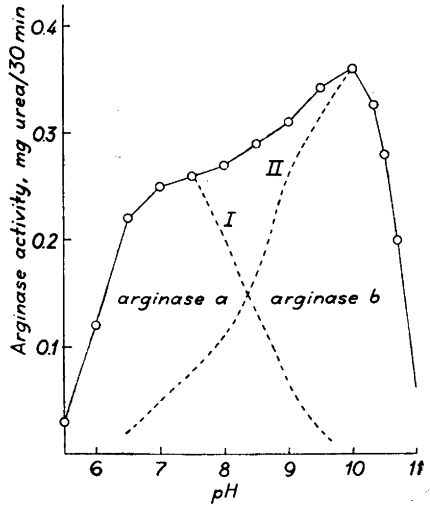

Fig. 1. pH-activity curve of liver arginase resolved into two separate curves.

measured at pH 9.9 remained practically the same throughout a dialysis period of 360 hours at room temperature.

A further means of differentiating between the two enzymes is by making use of the metal activators, namely, Mn^{++}, Co^{++}, and Ni^{++} ions. Manganous ions definitely activate arginase *b* alone, while Co^{++} and Ni^{++} ions activate arginase *a* only. Water dialyzed extracts tested at pH 9.9 did not respond when incubated with Co^{++} or Ni^{++} ions, but were distinctly activated by Mn^{++} ions [5]. In fact, Mn^{++} ions may have a definite inhibitory action on arginase *a*.

This difference in specificity of these metal ions in activating either one of the two enzymes clears up a great deal of the controversy in the literature concerning the part these ions play. It is clear when dealing with crude or partially purified extracts (containing the two enzymes), how important it is to select the right pH for measurements of the activity in the presence of the different ions in order to get the right information.

The effect of borate ion on arginase activity [4, 5] clearly adds more evidence to the presence of two enzymes. The pH-activity curve of borate extracted arginase has a shape very similar to a water dialysed extract, *i. e.*, arginase *a* is completly absent in both. The activity at pH 7.0 is very low amounting only to the activity of arginase *b* at that pH. The inhibiting effect of borate cannot be released by Co^{++} ions [5]. Only when the borate ions are removed does arginase *a* activity reappear and then it can be activated by cobalt or nickel.

When a liver extract is heated at 65—80° C, cooled and then treated with lead ions (2 mg/ml) a precipitate is obtained leaving a supernatent with bluish color. Both precipitate and supernatent contain strong arginase activity.

The above procedure was utilized in separating a pure arginase (electrophoretically) from the supernatent [6]. It represents arginase *b*, and thus far, it has been shown to be strongly activated by Mn^{++} but not by Co^{++} ions and is not affected by dialysis. The precipitate obtained by treatment with lead contains arginase *a*, although not in a pure state. Work is in progress to purify it and to further elucidate the properties of both enzymes.

The purified arginase *b* was erronuously reported as resulting from splitting of a large molecule of arginase under the effect of heat and lead ions. However, after studying its properties and reviewing the forementioned points of evidence the only conclusion reached is that there are two arginases with the following properties:

Arginase *a*	Arginase *b*
1. pH optimum: 7.4—7.6.	1. pH optimum 9.9—10.
2. Activated by Co^{++} and Ni^{++} ions and inhibited by Mn^{++} ions.	2. Activated by Mn^{++} ions. Unaffected by Co^{++} or Ni^{++} ions.
3. Inactivated by dialysis; activity restored by Co^{++} and Ni^{++} ions.	3. Dialysis has little effect.
4. Reversibly inhibited by borate, inhibition not released by Co^{++} ions.	4. Borate has no effect.
5. Easily precipitated by heat and Pb^{++} ions.	5. Remains in solution when heated and mixed with Pb^{++} ions.

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Received April 29, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 993–996

Über die Umsetzung von Formaldehyd mit Allylcarbinol *

Eine neue Synthese des Pentantriols-(1,3,5) und des 4-Oxytetrahydropyrans

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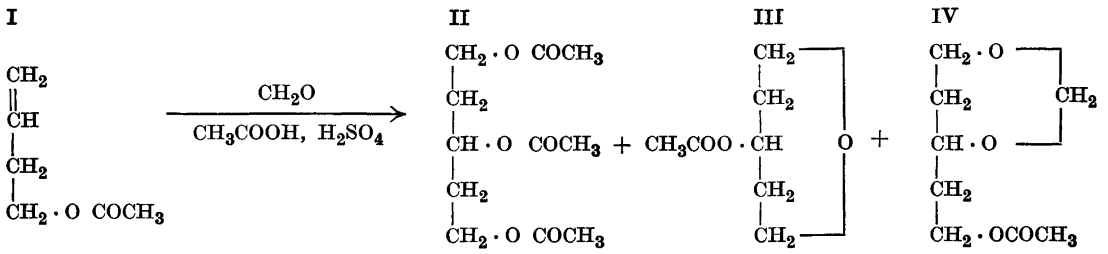
Die vorliegende Arbeit gehört zu einer Untersuchungsreihe, die den allgemein aufbauenden Charakter der Formaldehyd-Olefin-Reaktion zeigen soll. Nachdem in der aliphatischen Reihe bisher der stufenweise Aufbau vom Äthylen [1] über das Allylacetat bis zum Butantriol-(1,2,4) [2] und kürzlich dessen Überführung in Allylcarbinol, Erythrol und Crotonaldehyd [3] gelungen ist, war die Grundlage vorhanden, von diesen Olefinverbindungen aus den weiteren Glykolaufbau zur Fünfkohlenstoffkette in Angriff zu nehmen. Die Umsetzung des Allylcarbinols, von der hier die Rede sein soll, erschien uns wichtig, weil man auf diesem Wege eine neue Synthese des symmetrischen Pentantriols und des 4-Oxytetrahydropyrans erhoffen konnte — Verbindungen, die wir als Ausgangssubstanzen für synthetische Arbeiten auf dem Gebiete antibiotisch wirksamer Stoffe in Aussicht genommen haben.

Das 4-Oxy-tetrahydropyran wurde erstmalig von Borsche und Frank [4], später von Blanchard und Paul [5], Prelog und Mitarbeitern [6] und Mozingo und Adkins [7] durch Hydrierung von γ-Pyron erhalten. Baker [8] gewann 4-Acetoxytetrahydropyran bei der Umsetzung von Propylen mit Formaldehyd nach dem Verfahren von Prins. Blanchard und Paul (*l. c.*) haben den Ring des 4-Oxytetrahydropyrans mit Bromwasserstoff geöffnet und das entstehende Gemisch von Mono- und Dibromhydrinen mit Kaliumacetat in Acetine übergeführt, die beim Verseifen Pentantriol-(1,3,5) ergaben — bisher die einzige Synthese des symmetrischen Pentantriols.

Das aus Allylalkohol und Formaldehyd nach dem früher [3] angegebenen Verfahren hergestellte Allylcarbinol wurde durch Essigsäureanhydrid zu-

* 8. Mitt. über Formaldehyd-Olefin-Reaktionen.

nächst in das [Allylcarbin]-acetat (I) übergeführt und das entstandene Acetat-Essigsäuregemisch unter Zusatz von Eisessig und Schwefelsäure ohne weiteres mit Paraformaldehyd umgesetzt. Die Reaktion verlief kräftig exotherm. Wie aus der Jodzahl hervorgeht, war schon bei der ersten Umsetzung fast alles [Allylcarbin]-acetat in Reaktion getreten. Dieses Ergebnis erscheint bemerkenswert, da sowohl Olefin-Kohlenwasserstoffe als auch Allylacetat nur unvollständig reagieren. Bei der wiederholten Destillation des Reaktionsgemisches erhielt man, wie aus der Zusammenstellung im experimentellen Teil ersichtlich ist, eine Reihe von Fraktionen, deren Natur bisher noch unaufgeklärt blieb. Aus den Jodzahlen ersieht man, dass auch in diesem Falle ungesättigte Stoffe entstanden sind. Die Fraktionen 2) (Sdpkt.$_9$ 66—67°), 7) (Sdpkt.$_9$ 106—109°) und 13) (Sdpkt.$_9$ 154—155°) sind rein bzw. nahezu rein. Die nähere Untersuchung ergab, dass es sich bei der Fraktion 2) um *4-Acetoxy-tetrahydropyran* (III) handelt. Dieses wurde nach Baker mit Salpetersäure zu *β*-[Carboxymethoxy]-propionsäure oxydiert. Mittels methylalkoholischer Salzsäure erhielt man das 4-Oxy-tetrahydropyran, das als p-Nitrobenzoat identifiziert wurde. Die Fraktion 7) enthält, nach der Verseifungszahl und dem Siedepunkt zu urteilen, wahrscheinlich dem *1,3-Methylenäther des Pentantriol-(1,3,5)-monoacetates* (IV). Die Fraktion 13), die gemäss der gefundenen Verseifungszahl aus ziemlich reinem *Pentantriol-(1,3,5)-triacetat* [II] besteht, liess sich zum freien Pentantriol-(1,3,5) umestern. Dieses stimmte im Siedepunkt und in der Bildung eines bei 151—154° schmelzenden Trisphenylurethans mit der von Blanchard und Paul beschriebenen Verbindung überein.



EXPERIMENTELLER TEIL

Umsetzung des [Allylcarbin]-acetates mit Formaldehyd

I. *Veresterung des Allylcarbinols*

166 g Allylcarbinol (Spkt.$_{748}$ 111—113°) wurden mit 250 g Essigsäureanhydrid vorsichtig auf 85° erwärmt. Zwecks Mässigung der nun einsetzenden exothermen Veresterungsreaktion wurde bis zu deren Abklingen gelegentlich mit Wasser gekühlt und dann ½ Stde. unter Rückflusskühlung gekocht (Estergemisch A).

II. *Umsetzung mit Formaldehyd*

Ein Gemisch aus 105 g Paraformaldehyd, 280 g Essigsäure und 10 ml konz. Schwefelsäure wurde bis zur vollständigen Auflösung des Paraformaldehyds unter Rückflusskühlung erwärmt. Nach mässigem Erkalten wurde das Estergemisch A zugefügt und die entstandene Mischung bis zum Einsetzen der unter Selbsterwärmung und Dunkelfärbung erfolgenden Reaktion erhitzt. Während der Spontanreaktion (nötigenfalls kühlen!) stieg die in der Flüssigkeit gemessene Temperatur bis auf 117°, um dann langsam abzufallen. Das Reaktionsgemisch kochte man nun 2 Stden., nach welcher Zeit die Temperatur der siedenden braunschwarzen Flüssigkeit bei 124,5° konstant geworden war. Nach dem Erkalten fügte man zur Neutralisation der Schwefelsäure 20 g wasserfreier Soda hinzu und destillierte an der Brücke überschüssige Essigsäure neben geringen Mengen nicht umgesetztes [Allylcarbin]-acetat bei gewöhnlichem Druck zwischen 104—127° ab: 325 g, J. Z. 2,97. Der Rückstand wurde unter vermindertem Druck zuerst an der Brücke, dann mehrmals an der Widmerspirale destilliert. In der folgenden Tabelle 1 sind die Siedepunkte, Ausbeuten, Jodzahlen und Verseifungszahlen der einzelnen Fraktionen angegeben.

Tabelle 1. Destillation des [Allylcarbin]-acetat-Formaldehyd-Ansatzes.

Frakt. Nr.	Sdpkt. °C/9 mm	g	J. Z.	V. Z.	
1)	60—66	5,2	3,6	450,4	
2)	66—67	68,0	1,9	372,7	4-Acetoxy-tetrahydropyran
3)	67—72	14,1	1,3	356,5	
4)	72—(87)	8,5	5,2	270,4	
5)	87—88—(92)	17,1	9,2	405,9	
6)	92—106	3,2	15,0	322,2	
7)	106—108—(109)	37,3	9,1	315,8	1,3-Methylenäther des Pentantriol-(1,3,5)-monoacetates
8)	109—112	15,0	16,5	359,8	
9)	112—120	11,6	32,1	415,9	
10)	120—134	8,4	36,0	476,0	
11)	134—150	27,5	10,9	454,8	
12)	150—154	8,0	9,0	533,5	
13)	154—155	19,6	5,1	605,3	Pentantriol-(1,3,5)-triacetat

III. *Identifizierung*

a) *Fraktion 2)* (Sdpkt.$_9$ 66—67°) erwies sich nach ihrem Siedepunkt, ihrer Verseifungszahl und der Elementaranalyse als reines *4-Acetoxy-tetrahydropyran*

$C_7H_{12}O_3$ (144,2)	Ber.	C	58,32	H	8,39
	Gef.	»	57,96	»	8,25

Die Substanz wurde nach Baker mit Salpetersäure zu *β-[Carboxymethoxy]-propionsäure* oxydiert und aus Äther-Ligroin umkrystallisiert. Schmp. 93—94° (Baker gibt Schmp. 97° an).

$C_5H_8O_5$ (148,1)	Ber.	C	40,50	H	5,45	
	Gef.	C	40.67	H	5.47	

Durch Umesterung mit methylalkoholischer Salzsäure (2 %) erhielt man *4-Oxy-tetrahydropyran* vom Sdpkt.$_{11}$ 87,5—88°. Dieses wurde mit p-Nitrobenzoylchlorid in das *p-Nritobenzoat* vom Schmp. 68—70° übergeführt. (Baker gibt Schmp. 69° an)

$C_{12}H_{13}O_5N$ (251,2)	Ber.	C	57,36	H	5,22	N	5,58
	Gef.	»	57,38	»	5,27	»	5,67

b) *Fraktion 7)* (Sdpkt.$_9$ 106—109°) dürfte gemäss der gefundenen V. Z. den *1,3-Methylenäther des Pentantriol-(1,3,5)-monoacetates* enthalten.

c) *Fraktion 13)* (Sdpkt.$_9$ 154—155°) besteht nach der V. Z. aus ziemlich reinem *Pentantriol-(1,3,5)-triacetat.* Die Substanz wurde mit methylalkoholischer Salzsäure zum freien *Pentantriol-(1,3,5)* vom Sdpkt.$_9$ 185—187° umgeestert und dieses als *Trisphenylurethan* vom Schmp. 151—154° identifiziert (Blanchard u. Paul geben für das Pentantriol-(1,3,5) den Sdpkt.$_{11}$ 188—189°, für dessen Trisphenylurethan den Schmp. 154° an).

$C_{26}H_{27}O_6N_3$ (477,5)	Ber.	C	65,40	H	5,70	N	8,80
	Gef.	»	65,32	»	5,84	»	8,72

ZUSAMMENFASSUNG

[Allylcarbin]-acetat liess sich durch Umsetzung mit Formaldehyd in Eisessig-Schwefelsäure in das Pentantriol-(1,3,5)-triacetat, den 1,3-Methylenäther des Pentantriol-(1,3,5)-monoacetates und das 4-Acetoxy-tetrahydropyran überführen. Mit dieser Reaktion wurde der beim Äthylen begonnene stufenweise Formaldehyd-Olefin-Aufbau bis zur Fünfkohlenstoffkette verwirklicht.

Der Eine von uns dankt dem *Fridtjof Nansen-Fond* und *Norges Almenvitenskapelige Forskningsråd* für finanzielle Unterstützung. Frl. Birgit Sanne sind wir für die Ausführung der chemischen Kennzahlanalysen zu Dank verpflichtet.

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Eingegangen am 3. Juli, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 997–1004

Metal Ammine Formation in Solution

VII. Cupric-Pentammine Formation with *n*-Butylamine

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Complexes with three typical chelate ligands bound to the central atom normally have octahedral configuration, but in a paper by J. Bjerrum and Nielsen [1] it has been made evident that the tri-ethylenediamine cupric complex is a pentammine complex in which two of the ethylenediamine molecules are chelate-bound to the cupric ion, while the third molecule is bound only with one of its amino groups. This suggestion was supported by a number of facts, *e. g.* the similarity of the absorption curves of the tri-ethylenediamine cupric ion and the previously [2–4] studied pentammine complexes: $Cu(NH_3)_5^{++}$ and $Cu\ en_2NH_3^{++}$.

As the cupric ion has the characteristic coordination number 4, and cupric complexes with 4 bound ligand groups have been shown by X-ray data to have a planar square configuration, Bjerrum [5] assumed that the 4 strongly bound amine groups were also planar arranged in the pentammine complex, and that the fifth and much more loosely bound ligand molecule is placed perpendicular to this plane. It deserves notice that such a configuration, in which the bound ligand groups are placed at the corners of a tetragonal pyramid with the central atom at the base, was postulated in 1945 on quantum-mechanical basis by Daudel and Bucher [6]. These investigators were not thinking of the pentammine complex in this connection, and they searched for cases where the configuration mentioned might be found. In the best known configurations with the coordination number 5, such as in the pentahalogen compounds of phosphorus and in iron pentacarbonyl, it has been shown (*cf.* ref. 7) that the five ligands are placed in a trigonal bipyramid around the central atom, but quite recently Jensen and Nygaard [7] have prepared a tervalent nickel complex with triethylphosphine $NiBr_3(Et_3P)_2$, which like the cupric-

pentamine complex seems to exemplify the configuration theoretically treated by Daudel and Bucher.

In this paper is reported some extinction measurements of cupric-butylamine solutions. These measurements show that it is also possible with aliphatic primary amines to follow the amine formation beyond the tetrammine step. However, it is only possible to determine the order of magnitude of the affinity constant for taking up the fifth butylamine molecule owing to an extremely high medium effect, which for example is shown by the fact that the absorption curves at high amine concentrations are not going through a common point of intersection as in the analogous ammonia- and ethylenediamine systems (see Fig. 1).

Aliphatic monamines have an essentially lesser affinity to metal ions than ammonia, coincident with their greater affinity to the hydrogen ion (*cf.* Tables 1 and 2). These two facts combined necessitate very great amine concentrations in order to prevent the complex solutions separating out as basic precipitates. Thus, in the case of cupric-butylamine solutions, it is only possible to make measurements at high amine concentrations > 7 N, and even in 1 N butylammonium salt solutions rather high concentrations of amine are required.

GLASS ELECTRODE MEASUREMENTS

The hydrogen ion affinity constants

$$k_{\mathrm{am,H}^+} = \frac{[\mathrm{am\ H^+}]}{[\mathrm{am}]\,[\mathrm{H^+}]}$$

for n-butylamine and methylamine in 1 N (or 0.5 N) alkylammonium salt solution at 25° were determined by glass electrode measurements in the same way as previously described for ammonia (*cf.* ref. 5, p. 122). The values found are recorded in Table 1 and for comparison the previously determined values for ammonia, re-calculated under similar conditions and at the same temperature, are given.

The concentration of free amine in some cupric-n-butylamine and methylamine * solutions were determined in the limited range where the concentration values were suitable for the calculation of the formation function:

* For the making of the measurements with methylamine the authors are indebted to Mr. Bent Morsing.

Table 1. The hydrogen ion affinity constant $k_{am,H}^+$ of n-butylamine, methylamine and ammonia at 25°.

Amine	C_{amHNO_3}	C_{KNO_3}	C_{am}	log $k_{am,H}^+$
n-butyl	0.50		0.01—0.2	10.70 ± 0.02
»	1.00		0.02	10.75 ± 0.02
»	0.50	0.50	0.01	10.75 ± 0.02
methyl	0.50		0.004—0.08	10.72 ± 0.02
ammonia	0.50		0.01	9.30 ± 0.02
»	1.00		0.01	9.36 ± 0.02

$$\bar{n} = \frac{C_{am} - [am]}{C_{Cu(NO_3)_2}}$$

(C_x = total molar concentration of X).

In order to correct for the salt effect and the deviation of the glass electrode from theoretical pH-dependence in the rather basic range (pH $\gtrsim$ 10), the solutions were measured against standard solutions with the same alkylammonium and very nearly the same amine concentration as in the complex solutions.

The solutions were made from stock solutions of cupric nitrate, alkylammonium nitrate and amine. The stock solutions of ammonium nitrate were 2.5 *N* and were prepared by careful neutralization of the amine with the calculated amount of 5 *N* HNO_3. The *n*-butylamine (Heyl & Co., gereinigt) was carefully dried over KOH and distilled through a column. The methylamine was a Kahlbaum product.

The results of glass electrode measurements are shown in Table 2. In Table 2 are given, for comparison, the concentrations of free ammonia for the corresponding values of the formation function in the cupric ammonia system. These values are calculated by using the known complexity constants in this system:

$$\log k_n = \log k_n^0 + 0.080\ C_{NH_4NO_3} + 0.013\ (30°—t°)$$
$$\log k_1^0 = 3.99;\ \log k_2^0 = 3.34;\ \log k_3^0 = 2.73;\ \log k_4^0 = 1.97$$
$$\log k_5 = -0.52 + 0.008\ (18°—t°).$$

From the figures in the table it may be estimated that

$$k_4 = \frac{[Cu(am)_4^{++}]}{[Cu(am)_3^{++}]\ [am]}$$

Table 2. Free amine concentration [am] and formation function $\bar{n}$ of some cupric-amine solutions at 25°.

Amine	C_{amHNO_3}	$C_{Cu(NO_3)_2}$	C_{am}	p[am]	[am]	$\bar{n}$
n-butyl	1.00	0.0816	0.4298	0.791	0.162	3.29
»	1.00	0.03238	0.2676	0.796	0.160	3.32
»	1.00	0.0816	0.5076	0.635	0.232	3.38
methyl	0.50	0.1229	0.5714	0.823	0.150	3.42
»	0.50	0.1148	0.5358	0.823	0.150	3.36
ammonia	1.00			2.193	0.0064	3.30
»	1.00			2.093	0.0081	3.40
»	0.50			2.054	0.0088	3.40

the fourth consecutive constant in the methylamine and *n*-butylamine system is approximately 20—25 times smaller than in the ammonia system.

EXTINCTION MEASUREMENTS

The extinction measurements were made at 25° with a König-Martens spectrophotometer, but some control experiments were made with a Beckman spectrophotometer. Figs. 1a and 1b give a compilation of the experimental data. As abscissa is plotted the wavelengths in mμ and as ordinate the extinction coefficient

$$\varepsilon = \frac{\log I_0/I}{C_{Cu(NO_3)_2} \cdot d}$$

where I_0 and I denote the intensity of the incident and the transmitted light, respectively, and d the thickness of the absorbing layer in cm. The composition of the solutions in mols per liter and some of the extinction coefficients measured are given in Table 3. The solutions were all about 0.01 molar with respect to cupric nitrate and contained varying concentrations of butylamine (C_{bu}). Fig. 1a gives the ε,λ-curves for solutions nos. I—IV without added ammonium salt, and Fig. 1b the curves for solutions nos. V—X, which were 0.5 *N* with respect to butylammonium salt. Curves V and VII were repeated with a Beckman spectrophotometer (o-points), and, as the figure shows, with very nearly the same results.

The cupric nitrate and the amine were added by weight either pure or from stock solutions; in the case of the "waterfree" solutions nos. I and V, a weighed amount of cupric nitrate stock solution, respectively cupric nitrate + butyl-

Table 3. Composition and molar extinction coefficients of the solutions measured.

No.	I	II	III	IV	V	VI	VII	VIII	IX	X
C_{buHNO_3}	0	0	0	0	0.50	0.50	0.50	0.50	0.50	0.50
$C_{Cu} \cdot 10^2$	1.20	0.95	1.12	1.00	1.10	1.20	1.05	1.12	1.15	0.98
C_{bu}	9.90	9.89	9.68	8.62	9.39	8.15	3.95	1.013	0.508	0.264
C_{H_2O}	~0	0.52	2.27	9.49	~0	8.53	30.3	47.2	49.5	50.8
690 mμ	117.5	116.3	104.5	79.7	123.5	102.3	66.6	53.7	46.3	43.2
670	125.4	125.9	110.6	83.6	136.0	112.2	75.6	61.2	52.6	47.8
650	131.4	129.8	114.8	88.1	143.6	118.6	82.9	68.3	58.3	53.1
630	133.2	130.0	111.8	87.5	146.2	122.3	89.6	74.5	64.1	58.8
610	127.6	122.5	104.8	84.1	140.2	118.4	92.4	77.5	67.0	62.4
590	114.6	108.0	90.3	75.2	122.3	106.0	92.1	78.0	66.8	62.1
570	91.1	87.3	70.1	62.8	96.9	87.2	83.7	72.5	63.7	58.4
550	66.1	62.6	49.3	48.6	67.0	64.1	70.2	63.8	54.0	51.9

ammonium stock solution, was evaporated in a graduated flask in a vacuum desiccator with concentrated sulphuric acid, and the residue dissolved in waterfree amine. The water content and molar water concentration C_{H_2O} of the solutions was calculated as the difference between the total weight of the contents of the graduated flask filled to the mark and the weight of the added equivalents of cupric nitrate, butylammonium nitrate and butylamine. For butylamine was used the equivalent weight 73.7 found by titration of the product used, instead of the theoretical value 73.1.

DISCUSSION

The extinction measurements give strong evidence of the existence of a tetrammine-pentammine equilibrium in cupric-butylamine solutions. This is easily seen by comparing the absorption curves especially in Fig. 1b with corresponding curves in the cupric ammonia and ethylenediamine systems (Fig. 1c and d); the fact, however, that the curves in the butylamine solutions have no common point of intersection, requires an explanation.

At the lowest amine concentrations examined, it is evident that the formation of the tetra-butylamine complex is not completed. The fourth consecutive constant in the butylamine system is, according to the glass electrode measurements already mentioned, ~ 25 times smaller than the corresponding constant in the copper ammonia system. In the last mentioned system is $\bar{n} = 4$ for $[NH_3] \simeq 0.1$, and it is therefore reasonable to conclude that the absorption curve for the tetra-butylamine copper complex is situated somewhere between

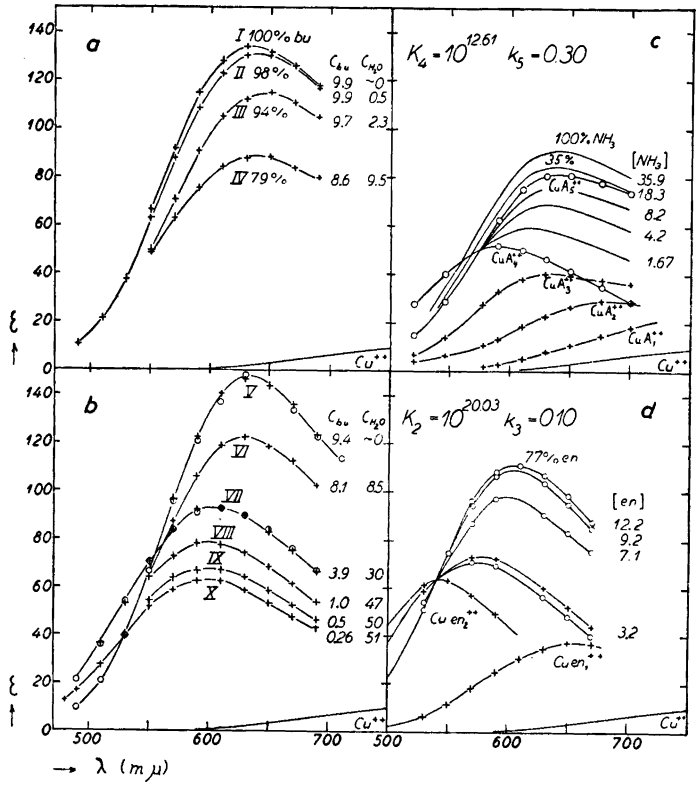

Fig. 1. Extinction curves for cupric complexes with n-butylamine, ammonia and ethylenediamine.

a: Curves for aqueous cupric nitrate butylamine solutions.

b: The same in 0.5 N butylammonium nitrate solutions. Measurements with König-Martens (+) and Beckman spectrophotometer (○).

c: Curves [2,3] for cupric ammonia solutions and tetrammine and pentammine ions (○) in water and for the lower ammine ions (+) in 2 N NH_4NO_3.

d: Curves [1] for cupric ethylenediamine solutions and complexes in water (○) and in 1 N KNO_3 (+).

the curves VIII and VII corresponding to $C_{bu} = 1.01$ and 3.95. Fig. 1b shows that sol. VIII has maximum of absorption at a little shorter wavelength than the solutions with higher as well as lower butylamine concentration (comp. Fig. 1c and d), and this makes it probable that curve VIII very nearly represents the curve for the tetra-amine complex.

If we assume that the waterfree solution V represents the curve for the penta-butylamine complex, Fig. 1b shows that the pentammine formation is about two thirds complete in sol. VI with $C_{bu} = 8.15$ and $C_{H_2O} = 8.53$. Weget

in this way a rough estimate of the very slight stability of the penta-butylamine complex, but cannot treat the data quantatively due to the unknown influence of the great change in medium.

If one compares the absorption curves of the cupric-butylamine solutions in Fig. 1b with curves with same butylamine concentration in Fig. 1a, it will be seen (comp. *e. g.* sols. VI and IV) that the presence of butylammonium salt brings about a rather strong increase in the extinction coefficient. A corresponding increase in absorption by salt addition is shown to exist in ammoniacal copper salt solutions (see ref. 2, Fig. 7) as well as in ethylenediamine-containing copper salt solutions (see Fig. 1d), but the effect in these solutions is much smaller. When addition of butylammonium salt has so great an influence on the extinction coefficients of the complexes, it is reasonable to assume that a change in the butylamine concentration also has a considerable influence, which explains that the curves have no common point of intersection. In the ethylenediamine system it is noteworthy that the curves in the whole range from nearly pure water to 77 weight per cent ethylenediamine behave very nearly as if they are belonging to a system of only two absorbing components, whilst in the ammonia system one has a raise in absorption and a not negligible shift of the pentammine curve towards blue, when the solvent is changed from about 20 to 100 per cent ammonia: an effect which is particularly manifest in the butylamine system (comp. Fig. 1a and c). But why do changes in concentration in the butylamine system have a stronger influence on the extinction coefficients of the complexes than corresponding changes in the composition of the medium in the ammonia and ethylenediamine systems?

It is probable that the cause of this is that we have a lower dielectric constant in butylamine solutions and, therefore, we must reckon with a stronger interaction between cations, anions and solvent molecules [8] than in corresponding ammonia and ethylenediamine solutions.

In the literature the dielectric constant of liquid ammonia is determined to 15.6 at 20°[9], but no data are available for butylamine and ethylenediamine hydrate. We therefore express our thanks to Professor Kai Arne Jensen, who has kindly determined for us the order of magnitude of the dielectric constant of the following solutions:

n-Butylamine	Dielectric constant
~ 100 weight per cent (corresp. to sol. I)	4.9 ± 0.2
94 » » » (» » » III)	6.8 ± 0.3
79 » » » (» » » IV)	17.5 ± 0.7
Ethylenediamine	
77 weight per cent (ethylenediamine hydrate)	~ 30

It will be seen from these figures that we have actually in butylamine solutions a much smaller dielectric constant than in corresponding ammonia solutions: 80 per cent butylamine corresponds about to 100 per cent ammonia, which again has a much smaller dielectric constant than ethylenediamine hydrate. This agrees well with the observations made in this paper and supports the already earlier maintained assumption [3,5], that the deviations from additivity are caused by the changes of the solvent and not by formation of hexammine.

As the cupric ion is able to bind five amino groups, it is reasonable to assume that the tetrammine ion contains a complex-bound water molecule [2,3,5], and we had originally hoped that a study of the light absorption of cupric butylamine solutions would throw some light on this problem. But due to lack of knowledge of the activity coefficients of amine, water, etc. and the great influence of the medium on the light absorption in the range where the change in water concentration is relatively highest (*cf.* the molar concentrations of butylamine and water in the solutions examined), we cannot say with certainty that the tetrammine complex contains a complex-bound water molecule. On the other hand, the removal of the last traces of water from the solvent (comp. sols. I and II in Fig. 1a) has no influence on the light absorption, which can be taken as a proof that the pentammine complex does not contain any water of constitution.

SUMMARY

The complex formation between cupric ion, methylamine and especially *n*-butylamine is studied by means of glass electrode and spectrophotometer measurements. The glass electrode measurements show that k_4, the fourth consecutive complexity constant, is about 20—25 times smaller than in the corresponding ammonia system. The extinction measurements show that a pentammine complex is formed at high amine concentrations, but due to a strong medium effect on the light absorption much higher than in corresponding ammonia and ethylenediamine solutions, it was not possible to give a quantitative treatment of the measurements.

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Received June 20, 1950.

ACTA CHEMICA SCANDIBAVICA 4 (1950) 1005–1013

The Iodine-Azide Reaction

V. The Catalytic Effect of Trithionate Ions, and Their Decomposition in Aqueous Solution

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Kinetic investigations of the iodine-azide reaction catalyzed by tetrathionate ions[1, 2], pentathionate ions[3], and carbon disulphide[4] have shown that the rate of reaction is proportional to the concentrations of catalyst and of azide ions, but independent of the concentration of iodine. Therefore, it would have seemed natural if the trithionate catalyzed iodine-azide reaction had shown a similar kinetic picture, but this is not the case. According to Kurtenacker *et al.*[5] trithionate decomposes in aqueous solution to thiosulphate and sulphate:

$$S_3O_6^{--} + H_2O \longrightarrow S_2O_3^{--} + SO_4^{--} + 2\ H^+ \qquad (1)$$

The rate of reaction is, within a pH-range of 9—5, independent of the pH. The thiosulphate ions formed according to (1) seem to be responsible for the iodine-azide reaction of trithionate.

EXPERIMENTAL

Potassium trithionate, $K_2S_3O_6$, was prepared according to the method of Kurtenacker and Matejka[6]. A polythionate analysis, as outlined by Kurtenacker[7], was carried out. A double analysis gave the results: 99.8 and 100.0 % potassium trithionate, and no detectable impurities.

In case of the tetra[2]- and pentathionate[3] catalyzed iodine-azide reaction, kinetic experiments could be carried out in a very simple way by determining the time of consumption of a definite amount of iodine with starch as the indicator, because the rate of reaction was independent of the concentration of iodine. The same method was used for trithionate, and although the rate of

reaction was proportional to the iodine concentration to a varying power of α, where $0 < \alpha < 1$, there was a clear and abrupt colour change when all the iodine was consumed. In other words, the rate of reaction did not become very small when the iodine concentration was very small.

Into a 300 ml Erlenmeyer flask were pipetted 30 ml of a solution which was 4.013 *M* with respect to sodium nitrate and 0.1 *M* to potassium iodide, 10 ml freshly prepared 0.05 *M* potassium trithionate solution, and 0.2 ml 0.5 % starch solution. For the sake of temperature adjustment the flask was left in the thermostat water for 15 min. 0.00982 *N* iodine solution was now added dropwise until a faint blue colour was visible (1 or 2 drops were enough). The purpose of this addition of iodine was to convert the thiosulphate formed during the temperature adjustment, to tetrathionate. Thereafter 10 ml 0.00982

Table 1. Reaction between sodium azide and iodine at 25° C, catalyzed by potassium trithionate.

In all four experiments — besides the solutions mentioned in the table — were added 0.2 ml starch solution, and 30 ml solution which was 4.013 *M* with respect to sodium nitrate and 0.1 *M* to potassium iodide. The concentrations of the stock solutions used were: Potassium trithionate: 0.05 *M;* sodium azide: 0.5 *M;* iodine: 0.00982 *N* with respect to iodine and 0.02 *M* to potassium iodide; sodium acetate: 0.5 *M*.

Expt. no.		*t* Time of reaction in min
1	10 ml trithionate 10 ml iodine 10 ml sodium azide	8.65
2	10 ml trithionate 5 ml iodine 5 ml water 10 ml sodium azide	5.67
3	5 ml trithionate 5 ml iodine 10 ml water 10 ml sodium azide	12.28
4	10 ml trithionate 5 ml iodine 5 ml water 5 ml sodium acetate 5 ml sodium azide	6.95

N iodine, and after an additional 1 min. 10 ml 0.5 *M* sodium azide solution were added and the time of reaction determined. Carried out in this way the experiments were reproducible.

Expt. nos. 2 and 3 in Table 1 show that the time of reaction (t) was approx. inversely proportional to the concentration of trithionate, *i. e.* that the rate of reaction was directly proportional to the concentration of the catalyst. Expt. nos. 2 and 4 show that when the sodium azide concentration was halved, the time of reaction increased only by about 20 %.

When similar experiments were carried out in such a way that iodine solution was added *after* sodium azide solution, it was unmistakable that a considerable part of the iodine was consumed immediately with a brisk evolution of nitrogen. The only reasonable explanation is that the very small amounts of thiosulphate formed when the solution of trithionate, neutral salt, and starch was left for temperature adjustment, caused an instantaneous thiosulphate catalyzed iodine-azide reaction. As the presence of sodium azide during the temperature adjustment did not alter the amount of thiosulphate formed, it seems evident that the trithionate catalyzed iodine-azide reaction actually is a thiosulphate catalyzed iodine-azide reaction, and that the thiosulphate is formed by spontaneous decay of trithionate ions.

ENERGY OF ACTIVATION OF THE TRITHIONATE CATALYZED IODINE-AZIDE REACTION

The energy of activation was determined by experiments analogous to those previously described. In each experiment were used: 30 ml of a solution which was 4.013 *M* with respect to sodium nitrate and 0.1 *M* to potassium iodide, 10 ml 0.05 *M* potassium trithionate, 0.2 ml starch indicator, 10 ml 0.00982 *N* iodine which was 0.02 *M* with respect to potassium iodide, and 10 ml 0.5 *M* sodium azide. The reacting solution was 2 *M* with respect to sodium nitrate.

The reciprocal of the time of reaction (t) was used as a relative rate constant ($k_{exp.}$). Using the method of least squares, the numerical values of H and A were calculated according to the equation:

$$\log k_{exp.} = H - \frac{A}{T}$$

where T is the absolute temperature. The result is

$$\log k = 13.060 - \frac{4173}{T}$$

From this equation values of k were calculated by substituting T with the experimental temperatures. These values ($k_{calc.}$) are, together with the experimental ones, recorded in Table 2. The energy of activation is:

$$A \times 4.571 = 4173 \times 4.571 = 19\,070 \text{ cal/mole}$$

The frequency exponent H, of course, is not a true one, because the rate constants are only relative.

Table 2. Energy of activation of the trithionate catalyzed iodine-azide reaction.

In all the experiments were used: 30 ml of a solution which was 4.013 M with respect to sodium nitrate and 0.1 M to potassium iodide, 10 ml 0.05 M potassium trithionate, 0.2 ml starch indicator, 10 ml 0.00982 N iodine which was 0.02 M with respect to potassium iodide, and 10 ml 0.5 M sodium azide. The reacting solution was 2 M with respect to sodium nitrate. $k_{exp.}$ is the reciprocal of the time of reaction (t). $k_{calc.}$ was calculated from the equation $\log k = H - \frac{A}{T}$, which is a straight line fitted to the experimental k and T values.

Expt. no.	Temp. °C	t Time of reaction min	$k_{exp.}$	$k_{calc.}$
1	25	8.65	0.1156	0.1152
2	25	8.58	0.1165	
3	30	5.15	0.1942	0.1961
4	30	5.08	0.1969	
5	35	3.13	0.3195	0.3278
6	35	3.12	0.3205	
7	40	1.83	0.5464	0.5394
8	40	1.82	0.5494	

DECOMPOSITION OF TRITHIONATE IN AQUEOUS SOLUTION

According to Kurtenacker *et al.*[5], trithionate — as already mentioned — decomposes in aqueous solution to thiosulphate and sulphate (eq. (1)). Within a pH-range of 9—5 no side reactions take place, and the rate of reaction is independent of the pH. Kurtenacker writes that the reaction probably is unimolecular when autocatalytic phenomena can be excluded. As thiosulphate ions usually catalyze decomposition of polythionates, it is to be expected that the rate of (1) will be proportional to the concentration of trithionate ions only when the degree of decomposition is small. Concerning the iodine-azide reaction, formation of only a small amount of thiosulphate is of interest, because just after formation the thiosulphate ions react rapidly with azide ions and iodine.

It was chosen to measure the rate of decomposition of trithionate ions in sodium acetate solutions because they yield a pH nearly the same as that of sodium azide solutions. According to Hughes [8] the ionization constant of hydrazoic acid is about 2×10^{-5} at 25° C. Of course, sodium acetate solutions are not buffer solutions, and when the decomposition of trithionate ions takes place, the pH will be slightly altered due to the formation of hydrogen ions according to (1). But as the same would be the case in sodium azide solutions, and as experiments showed that the pH in the range 9—5 was of no influence, it was not necessary to use buffered solutions.

EXPERIMENTAL

100 ml aqueous solution, which was 0.1 *M* with respect to potassium trithionate and 0.5 *M* to sodium acetate, was placed in a water thermostat. Every 15 minutes 10.00 ml solution was taken out, and the thiosulphate formed was titrated with y ml 0.00978 *N* iodine solution. The degree of decomposition of trithionate was in none of the experiments larger than 1 per cent, so the concentration of trithionate ions could be considered constant.

From Table 3 it can be seen that the number of ml of 0.00978 *N* iodine used in the titration is directly proportional to the time of reaction (t). Using the method of least squares, the number of ml iodine solution per one minute time of reaction, were calculated. In Expt. no. 1 the concentration of trithionate was 0.2 *M*, while in no. 2 it was 0.1 *M*. The relation between ml iodine per minute time of reaction is seen to be as 2 to 1. Using the method of least squares, the relation between y and t in Expt. no. 1 was found to be: $y = 0.104 + 0.00888 \times t$. From this equation values of y were calculated by substituting t with the experimental times of reaction. These values ($y_{\text{calc.}}$) are, together with the

experimental ones, recorded in Table 3. The concentrations in Expt. no. 3 were the same as those in no. 2, except that acetic acid was added, so that pH was 5.1. This is about the pH at which reaction (1) no longer is the only one taking place. As was to be expected, the number of ml iodine per minute time of reaction was somewhat different from that in Expt. no. 2.

Table 3. Decomposition of potassium trithionate in aqueous solution at 25° C.

Concentrations in the experiments: Potassium trithionate: 0.2 M in no. 1, and 0.1 M in nos. 2 and 3. Sodium acetate: 0.5 M in all 3 experiments. In Expt. no. 3 was added acetic acid, so that the pH was 5.1. y is the number of ml 0.00978 N iodine used for titration of the thiosulphate formed in 10 ml of the reacting solution. $y_{calc.}$ (Expt. no. 1) was calculated from the equation: $y = 0.104 + 0.00888 \times t$, which is a straight line fitted to the experimental y and t values.

t Time of reaction min	Expt. no. 1		Expt. no. 2	Expt. no. 3
	y	$y_{calc.}$	y	y
15	0.21	0.24	0.14	0.12
30	0.40	0.37	0.19	0.18
45	0.51	0.50	0.26	0.22
60	0.64	0.64	0.34	0.28
75	0.76	0.77	0.41	0.32
90	0.90	0.90	0.47	0.37
105			0.53	0.44
Ml iodine per minute time of reaction	0.00888		0.00448	0.00343
pH	~ 9		~ 9	5.1

ENERGY OF ACTIVATION OF THE DECOMPOSITION OF TRITHIONATE IONS

The rate of decomposition of trithionate ions in aqueous sodium acetate solution was determined at different temperatures by experiments analogous to those described above. The concentrations used were the same as in Expt. no. 2, Table 3.

Table 4. Energy of activation of the decomposition of trithionate ions into sulphate and thiosulphate ions.

Concentrations in the experiments: Potassium trithionate: 0.1 M; sodium acetate: 0.5 M. y is the number of ml 0.00978 N iodine used for titration of the thiosulphate formed in 10 ml of the reacting solution. $k_{exp.}$ is the rate constant calculated when assuming that the decomposition is a bimolecular reaction between trithionate ions and water molecules.

t Time of reaction min	Expt. 1. 25° C	Expt. 2. 30° C	Expt. 3. 35° C	Expt. 4. 40° C
	y	y	y	y
15	0.14	0.21	0.50	0.12
30	0.19	0.33	0.71	0.47
45	0.26	0.47	0.90	0.85
60	0.34	0.61	1.08	1.22
75	0.41	0.70	1.28	1.61
90	0.47	0.85	1.48	
105	0.53	0.97	1.66	
120		1.08		
Ml iodine per minute time of reaction	0.00448	0.00835	0.01286	0.02487
$k_{exp.} \times 10^7$	8.27	15.41	23.73	45.89
$k_{calc.} \times 10^7$	8.28	14.73	25.70	44.06

In Table 4 are given the number of ml 0.00978 N iodine (y) used for the titration of 10 ml of the reacting solution at different times of reaction. Using the method of least squares, the number of ml iodine solution per one minute time of reaction, were calculated for all 4 experiments. From these values the rate constants ($k_{exp.}$) were derived by assuming that the stoichiometric scheme (1) was also the kinetic equation of the reaction, so that:

$$\frac{da}{dt} = k_{exp.} \cdot a \cdot b$$

where a is the concentration of trithionate, b is the concentration of water, and $\frac{da}{dt}$ is the change in trithionate concentration per minute. For b was inserted the value 53, which is about the molar concentration of water in an

aqueous solution, which is 0.1 M with respect to potassium trithionate and 0.5 M to sodium acetate. Of course, it is most likely that the activity coefficient of water has not the value 1, but it is difficult to tell what the exact value is.

Using the method of least squares, the numerical values of H and A were calculated according to the equation:

$$\log k_{\text{exp.}} = H - \frac{A}{T}$$

where T is the absolute temperature. The result was:

$$\log k = 9.074 - \frac{4518}{T}$$

From this equation values of k were calculated by substituting T with the experimental temperatures. These values ($k_{\text{calc.}}$) are, together with the experimental ones, recorded in Table 4. The energy of activation is:

$$A \times 4.571 = 4518 \times 4.571 = 20650 \text{ cal/mole.}$$

DISCUSSION

The experimental evidence for thiosulphate ions (formed according to (1)) being responsible for the trithionate iodine-azide reaction is as follows. When sodium azide was added to an aqueous solution of potassium trithionate, which had been kept for x minutes in a thermostat, titration with iodine could be carried out with starch as the indicator. The consumed amount of iodine was of the same order of magnitude as the amount that was consumed when a comparable solution of trithionate, azide and iodine was allowed to react for x minutes.

The rate of reaction of the trithionate catalyzed iodine-azide reaction was not proportional to the azide ion concentration, as was the case with carbon disulphide [4], tetrathionate [1, 2], and pentathionate [3] ions. The energy of activation was found to be 19 070 cal. This value was surprisingly high keeping in mind that the energies of activation of the penta [3]- and tetrathionate [2] catalyzed iodine-azide reactions were found to be respectively about 11 000 and 13 000 cal. When two kinetic experiments were carried out respectively with trithionate and tetrathionate as the catalysts, so that all concentrations were identical, the two rates of consumption of iodine were of the same order of

magnitude irrespective of the actual concentrations. This fact, together with the large difference in energies of activation, also indicated a different reaction mechanism.

The energy of activation of the decomposition of trithionate ions to thiosulphate and sulphate ions was found to be 20 650 cal. This value is 1580 cal. larger than that of the iodine-azide reaction (19 070 cal.). Assuming that the thiosulphate formed by the decomposition of trithionate was responsible for the iodine-azide reaction, this difference could be accounted for by an observation of Dodd and Griffith[1, p. 548]: If thiosulphate acts as catalyst on the iodine-azide reaction, a considerable number of iodine molecules will be consumed per molecule thiosulphate consumed. The thiosulphate will be converted partly to tetrathionate and partly to sulphate. The ratio R (moles I_2 consumed/moles $Na_2S_2O_3$ consumed) is somewhat dependent on temperature. A decrease of temperature from 18° to 1° C increases R by about 20 %. This temperature dependency, found by Dodd and Griffith, could be calculated to correspond to an apparent, negative energy of activation of 1700 cal., which agrees very well with the found difference of 1580 cal.

SUMMARY

Kinetic investigations have been carried out on the iodine-azide reaction catalyzed by trithionate ions. The energy of activation was determined to be 19 070 cal/mole. The mechanism of reaction was definitely different from that of the tetra[2]- and pentathionate[3] catalyzed. Determination of the energy of activation of the spontaneous decomposition of trithionate ions to sulphate and thiosulphate ions, seemed to show that the thiosulphate ions formed by this decomposition were responsible for the trithionate catalyzed iodine-azide reaction.

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Received June 20, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1014–1019

Electrophoresis of Leghemoglobin

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Electrophoresis has generally been used to determine the purity and homogeneity of proteins. Up to the present time the purity of leghemoglobin has been estimated by comparing its analyses with those of blood hemoglobin. In the work to be reported we have used the electrophoretic technique for determination of the homogeneity and the isoelectric point of the purified leghemoglobin preparations.

EXPERIMENTAL

Leghemoglobin preparations

The leghemoglobin was prepared essentially in the same manner as previously described [1]. Nodules of soya bean were crushed in a mortar at 0° C and the leghemoglobin was precipitated at 58–75 per cent saturation of ammonium sulphate. The precipitate was dissolved in a small quantity of water and dialyzed against running water until free from ammonium sulphate and then diluted with distilled water to contain 0.3–0.5 % hemoglobin. The iron in this prepraation is always trivalent. Hence, all our findings concern leghemiglobin.

Other samples used in this work were prepared by extracting the uncrushed nodules with distilled water at room temperature for 48 hrs. (10 % toluene as an antiseptic.)

Apparatus

The apparatus employed by us in the mobility determinations and in the analytical work was the Tiselius electrophoresis apparatus (Perkin Elmer). The principle of this apparatus has been described by Moore and White [2].

In the preparative electrophoretic experiments a Klett electrophoresis apparatus was used. Separation was carried out in 150 ml cells.

Measurements

For the homogeneity studies 0.3 to 0.4 per cent leghemiglobin solutions were used. Before the protein solutions were placed into the electrophoretic cell they were dialyzed against 2 litres of buffer in a cellophane tube at 4° C until there was no difference in the

pH and the conductance between the dialysate and the buffer. To eliminate a possible denaturation of protein within the acid region of the pH scale we used a mechanical dialyzer according to Reiner and Fenichel [3], whereby the equilibrium between the protein solution and the buffer was established within 2—3 hrs. The outside buffer solutions were then used to fill the upper compartments of the electrophoresis apparatus.

The usual Wheatstone bridge type of circuit and a conductivity cell were employed in measuring the specific conductance of the solutions at the same temperature as the electrophoretic experiments were performed. The movement of the boundaries during electrophoresis was followed at intervals of 1 hr. The average displacement of the boundary per second represents the apparent speed of the boundary because the refractive index gradients in the cell are recorded on films at a unit magnification. The hydrogen ion concentration of the protein and the buffer solutions were determined with a glass electrode.

The spectrum of the leghemiglobin was determined with a Beckman spectrophotometer.

The buffer solutions used in all experiments were prepared by mixtures of sodium acetate-acetic acid and sodium dihydrogen phosphate-sodium monohydrogen phosphate. Sodium chloride was added to both mixtures to obtain a ionic strength of $\mu = 0.1$.

Protein-N was determined by the Kjeldahl procedure. Distillation was carried out according to Kirk [4].

Mobility calculations

The mobility is calculated by a simple equation: $u = S/F$ where S is the apparent speed of the boundary as defined above and F the potential gradient $F = i/q \cdot \varkappa$ where i is the current in ampere, q cross-section of the electrophoretic cell in sq. cm and $\varkappa$ the specific conductance of the solutions.

RESULTS

The concentration of the protein solutions used for homogeneity studies was 0.4—0.5 per cent. The studies were carried out at pH values between 4 and 7.2. At all pH values two boundaries were present (Fig. 1). Only at pH 4.0 was a single sharp ascending boundary with a very diffuse descending boundary observed. At this acidity both of the components migrated to the negative pole and obviously were denatured. By magnifying the electrophoretic patterns 5 times and measuring the areas with a planimeter we found out that the concentrations of the two components were practically equal (Table 1). These leghemiglobin solutions were then diluted to 0.2 per cent for the mobility

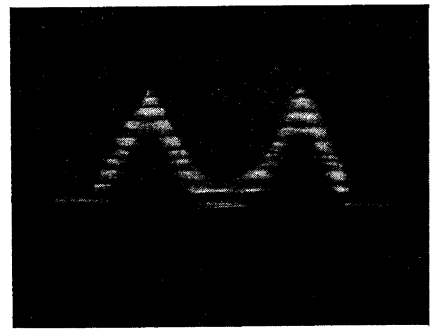

Fig. 1. Electrophoresis of leghemoglobin at pH 7.15. Ascending limb.

Table 1. Electrophoretical analyses.

No.	Faster comp.	Slower comp.
1	55.5	44.5
2	54.5	45.5
3	50.5	49.5
4	51.5	48.5
5	50.8	49.2
Average:	52.6	47.4

studies. The potential gradients were kept practically constant in all experiments (ca 8 volt/cm). The results are summarized in Fig. 2 which represents the mobilities of the sharper boundaries as a function of the pH.

The plus and minus signs refer to the charges on the protein. It will be seen from Fig. 2 that the isoelectric points of the two components are pH $\sim$ 4.4 and $\sim$ 4.7.

To test whether the leghemiglobin can be split into components by precipitation with ammonium sulphate, experiments were carried out with preparations which were obtained by extracting the uncrushed nodules with distilled water. The electrophoretic picture in this case showed the two components mentioned above, and furthermore a possible big salt component with a very small mobility.

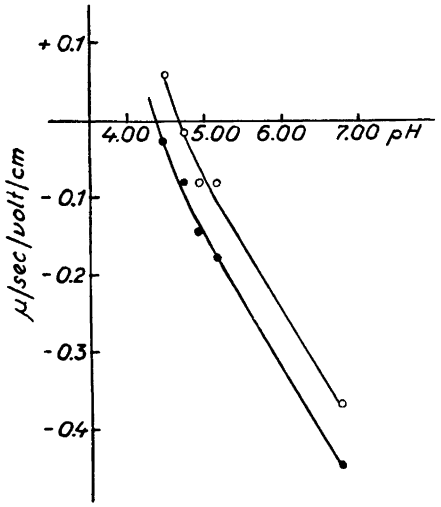

Fig. 2. Electrophoretic mobilities of the two components of leghemiglobin at 4° C in acetate buffer at varying pH and constant ionic strength (0.1).

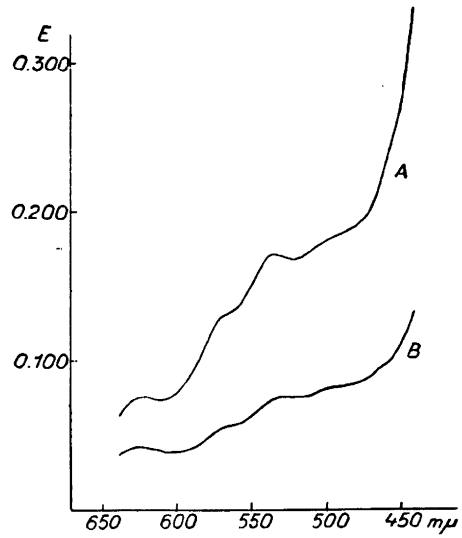

Fig. 3. The spectra of the two components of leghemiglobin calculated at the same protein concentration (~ 0.07 mg/ml) at pH 7.10.

A: spectrum of the faster component (I. P. 4.4).
B: spectrum of the slower component (I. P. 4.7).

Because the region between the two ascending boundaries will contain only the faster component and that between the two descending boundaries in the other limb only the slower component, the two components can be electrophoretically separated as pure proteins. This separation was carried out at pH 7.0 and $\mu = 0.1$ in a phosphate buffer. Both components proved to be hemin proteins.

From the spectra of these components we could also see a quantitative difference in the hemin content in respect to protein-N. The component with I. P. 4.4 has twice as high hemin content as that with I. P. 4.7 (Fig. 3).

A re-electrophoresis of the faster component at pH 3.40 and 7.25 indicated that the protein in question is electrophoretically homogeneous at these reactions (Fig. 4).

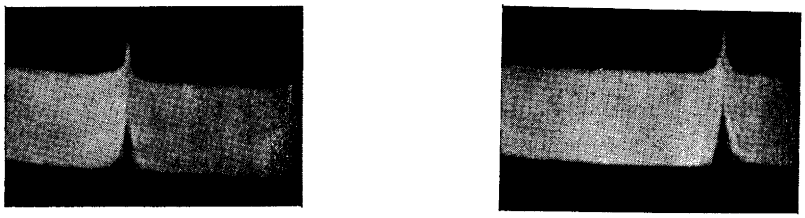

Fig. 4. Electrophoresis of the faster component.

A: ascending limb, pH 5.40, $\mu = 0.1$, $t = 210$ min.
B: ascending limb, pH 7.25, $\mu = 0.1$, $t = 180$ min.

DISCUSSION

The electrophoretic picture of the leghemiglobin indicates the existence of two components in this hemin protein. The anomality at pH 4.0 must be interpreted as a denaturation of globin. The two components of leghemiglobin are not splitting products caused by the high ammonium sulphate concentration of the preparation. The spectrum of the two components of leghemiglobin show that both of them are hemin proteins. The hemin content of the faster component seems to be about twice that of the slower one.

The leghemiglobin purified by ammonium sulphate precipitations contains usually about 0.27 % iron. In one preparation precipitated 5 times the percentage was 0.34 Fe[1], hence, its iron content was equal to that of crystalline blood hemoglobin. On the basis of this it seems that even by means of ammonium sulphate precipitations it may be possible to separate a leghemiglobin which corresponds to the faster component in the electrophoretic separation. The iron content less than 0.3 % found in most of the leghemiglobin preparations is evidently due to the fact that the preparations contained both of the electrophoretically separable components. Whether this slower fraction with a smaller hemin content represents a homogeneous substance or whether it possibly is a mixture or an adsorption compound of leghemiglobin and globin (possibly denatured) remains still to be solved. Besides, the possibility must not be overlooked that the quantitative difference in the hemin content of the two components may be due to the splitting of one hemin group from the leghemiglobin during electrophoresis. Hence, the separation method employed would cause the differences observed.

SUMMARY

Leghemiglobin isolated from the crushed nodules of soya bean and precipitated at 58—75 per cent saturation of ammonium sulphate contains two components with the isoelectric points pH 4.4 and 4.7.

Both of the components are hemin proteins.

The component with the I. P. 4.4 seems to be homogeneous between pH 5.4 and 7.20.

We wish to express our thanks to Dr. E. Uroma, Director of the State Serum Institute, for allowing us to use the Klett electrophoresis apparatus and especially to Phil. mag. Antti Louhivuori, Head of the Chemical Department of that institute, for kindly assisting us in the use of the apparatus.

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Received May 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1020–1023

Remarks on the Crystal Structure of Asymmetric Molecules. — The β-Phenylglyceric Acids

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Molecules capable of existing in the liquid or gaseous state or in solution in different configurations which do not differ appreciably in internal energy would in general be expected to be able to be present in more than *one* configuration even in a defined crystalline modification. In the crystals of pentaerythritol esters $C(CH_2X)_4$ for example (X = chlorine, bromine or iodine) which have large unit cells containing a great number of molecules it has been suggested [1] that molecules of different configurations are present. In such cases a complete crystal structure determination will in general be very difficult or even impossible with our present means of investigation. It would appear to be of interest, however, to approach such problems even in cases where unit cells and space groups only are being determined.

Amongst the substances which would seem to deserve the attention of the crystallographers a certain class contains molecules which in the gaseous state may exist in *two* relatively stable forms with the same energy, one form being the mirror image of the other. If we assume that the mutual conversion of the two forms do not imply a breaking of chemical bonds and that the activation energy of the process is sufficiently small, the substance will of course always correspond to a "racemic mixture" as long as we are concerned with vapours, liquids or solutions. Examples are provided by amines of the type $N\begin{smallmatrix}H\\R_1\\R_2\end{smallmatrix}$ and certain *cyclo*hexane derivatives [2]. When such substances crystallize we will in general expect the crystals to be of the common "racemic compound" type in which an equal number of *d* and *l* molecules are present. It has been pointed out [2], however, that cases will probably also be found in which substances of the kind just described will yield enantiomorphous crystals, some containing *d* molecules only, others *l* molecules. In order to make such a

mixture of crystals thermodynamically more stable than a corresponding "racemic" system, special conditions regarding the lattice energy of the two types of crystals must of course be fulfilled.

In certain cases even molecules considered to be individually "symmetric" (the symmetry corresponding for example to a symmetry plane) may yield enantiomorphous crystals containing either *d* or *l* molecules: According to X-ray analysis β-phloroglucitol crystallizes in the space group $P2_12_12_1$ with four molecules in the unit cell. It seems obvious that the compound must be the *trans* ($\varkappa\varepsilon\varkappa \rightleftharpoons \varepsilon\varkappa\varepsilon$) form of 1,3,5-*cyclo*-hexanetriol [3]. The free rotation about the C—O bonds and the almost self-evident presence of hydrogen bonds in these crystals makes it very probable that the individual molecules are asymmetric in the lattice. If this is so, it follows that only one form (*d* or *l*) is present in one single crystal.

From a chemical point of view it does not appear justified to use expressions like "racemic compounds" or even "racemic crystal" in cases where it would not be possible — at least in principle — to separate *d* and *l* molecules in the liquid or gaseous state (*e. g.* 1,2-*cyclo*hexanediol $\varkappa\varepsilon \rightleftharpoons \varepsilon\varkappa$). Another view expressed by some crystallographers which cannot be accepted by chemists is that *d* and *l* molecules in crystals of true racemic compounds are necessarily related by certain elements of symmetry. We may for instance refer to the crystals of the inactive phenylglyceric acid of m. p. 122° described later on in this paper in which the *d* and *l* molecules are crystallographically independent and probably not true mirror images.

Phenylglyceric acid $C_6H_5CHOH\ CHOH\ COOH$ occurs in two stereoisomeric forms, each of which may be resolved into its two optically active components, and these isomers would appear to provide an interesting object for crystallographic research. Macroscopic examinations have been carried out by Goldschmidt [4] and by Berner [5]. Thanks to the kindness of professor Berner who placed crystals at our disposal we have been able to carry out X-ray examinations of both the inactive and active forms of these two substances.

β-PHENYLGLYCERIC ACID OF M. P. 141°

According to the literature the resolution of the inactive acid has been very difficult to accomplish, but finally Riiber [6] succeeded in resolving it into optical antipodes of m. p. 164°. Goldschmidt [4] carried out a careful crystallographic examination of both the active and the inactive crystals, and was able to prove that the inactive compound of m. p. 141° was no true racemate, but consisted of thin submicroscopic lamellae of alternating *d* and *l* crystals, with (100) as a twin plane.

We have confirmed this conclusion by X-ray examinations of the active and the inactive crystals. In both cases identical cell dimensions were found, namely $a = 12.49$ A, $b = 5.70$ A, $c = 5.90$ A, and $\beta = 94°$. The space group is $P2_1$, and there are two molecules in the unit cell. Weissenberg photographs about the b-axis recording the $h0l$-reflexions show very clearly the relationship between the active and the inactive crystals, the latter giving a diagram which corresponds in all respects to that of a twin crystal of the former, with (100) as a twin plane. Equivalent reflexions from d and l lamellae have the same intensity, as was to be anticipated.

The original inactive preparation offered us by professor Berner was expected to have a melting point of 141°. As, however, a crystal fragment gave a Weissenberg photograph corresponding to an untwinned crystal, the melting point of the fragment was tested and found to be 164°. In another experiment submicroscopically twinned crystals of m. p. 141° were obtained by rather rapid recrystallisation from water. Professor Berner, who was informed about these results, then performed a very slow crystallisation from water which yielded large single crystals, having dimensions some times as great as $10 \times 10 \times 3$ mm, and which proved to be pure active d and l crystals of m. p. 164°. The resolution of the inactive acid may thus be performed simply by very slow recrystallisation from water.

The occurence of twinning of the type described above seems to be more frequent than generally expected, and may in certain cases, *e. g.* for orthorhombic crystals belonging to the class 222, escape the attention of the crystallographer.

β-PHENYLGLYCERIC ACID OF M. P. 122°

Berner[5] examined the crystals of the inactive acid, and found that they were monoclinic sphenoidal. We have determined the lattice constants to be as follows: $a = 15.35$ A, $b = 5.81$ A, $c = 10.07$ A and $\beta = 101.5°$. The unit cell contains four molecules. The only systematic absences occur in the $0k0$-reflexions for odd values of k. Using X-ray data only, the space group may be either $P2_1$ or $P2_1/m$, but Berners crystal class determination excludes the latter possibility. The two right-hand molecules in the unit cell are thus crystallographically equivalent, and the same holds for the left-hand molecules. However, the d and l molecules are crystallographically independent, as mentioned above, and it appears very probable that the configuration of say the d molecule is different from the mirror image of the l molecule — taking the many "movable" links in the molecule into consideration. A complete crystal structure determination will certainly be extremely difficult.

The *active* forms of this acid have been isolated by Berner and Riiber [7]. We have carried out X-ray measurements of the crystals, and found them to be orthorhombic with the lattice constants $a = 13.58$ A, $b = 5.35$ A and $c = 23.65$ A. The unit cell contains eight molecules, and the space group is $P2_12_12_1$. Two crystallographically inequivalent groups of molecules are present, and it seems reasonable to assume that molecules of one group differ in shape from those of the other.

In his publication Berner [5] favours the view that the crystals are monoclinic with a β angle of 90° 40′. However, the diffraction symmetry and the systematic absences characteristic of the space group $P2_12_12_1$ makes it seem far more probable that the crystals are in fact orthorhombic.

SUMMARY

The geometrical arrangement of non-rigid molecules in crystals has been discussed, special reference being made to the different stereoisomers of β-phenylglyceric acid. For these substances new experimental material has been collected.

We wish to express our sincere gratitude to professor Berner both for his kindness in placing the interesting material at our disposal and for his interest in our experimental work. One of us (S. F.) is indebted to *Norges Teknisk Naturvitenskapelige Forskningsråd* for a grant.

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Received July 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1024–1030

Preparation of Cytochrome c with the Aid of Ion Exchange Resin

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A certain phase of the research in our cytochrome c program called for the preparation, from different sources, of relatively large quantities of this pigment in a form as pure as possible. We found, in following the previously published procedures for the electrophoretic purification of cytochrome c (Theorell and Åkeson [1]) that the project would soon develop into a rather formidable task. Therefore, in the course of our preparative work, we have been particularly interested in the introduction of any new technique which might augment the effectiveness of the original method.

The first highly purified cytochrome c was obtained in 1935 by Theorell [2]. In this and in subsequent methods (Theorell [3]; Keilin and Hartree [4]) advantage was taken of the extreme stability of the enzyme. These methods gave preparations containing 0.34 % iron. Another outstanding property of the enzyme, the very basic character of its protein (I. P. = 10.05)[1], was finally used by Theorell and Åkeson [5] in 1939 when they employed electrophoresis at different pH levels to obtain a preparation containing 0.43 % iron. Keilin and Hartree [6] later obtained cytochrome of the same order of purity by precipitation with ammonium sulfate at pH 10.

In our experiences with cytochromes from different animal sources, the application of the electrophoretic technique as the final step always leads to a higher iron content but cannot be depended upon, except possibly for horse cytochrome, to give material containing as much iron as that originally described by Theorell and Åkeson [1]. In addition, electrophoresis is very time consuming and not well adapted for large-scale isolation work.

It was considered possible that the exceedingly high isoelectric point of the cytochrome might render its separation possible on a cation exchange resin. This technique has been tested and developed with cytochrome prepa-

rations from various animal sources. In all cases it has led to the rejection of a golden-colored impurity, protein in nature, and the separation of the original cytochrome into several fractions, each of which contain higher levels of iron.

PREPARATION METHODS

The first cytochrome c preparation tried was one from salmon hearts, prepared according to Keilin and Hartree [6]. It contained 0.24 % iron as determined by the sulfosalicylic acid method of Lorber [7]. As cation exchanger we chose the new, white, bead-form, carboxylic acid Amberlite IRC—50 *. The resin was dried at room temperature and pulverized in a mortar to give a fine, white powder. The powder was made into a slurry with water and poured into a glass tube of inner diameter 0.7 cm to a height of 20 cm. The column was then washed with liberal quantities of 5 % H_2SO_4, water and 5 % ammonia. Finally, the resin was conditioned with 0.1 M ammonium hydroxide-ammonium acetate solution, pH 9.0, until both influent and effluent had the same pH and the same optical density at 280 mμ. Ammonium acetate was chosen as the agent for controlling the pH on the column since this salt quickly evaporates during the dry weight determinations which are required for finding the per cent. iron in aliquots of the effluent.

The cytochrome sample was introduced in a 0.15 ml volume in aqueous solution containing about 15 mg of dry weight, allowed to flow in to the column and washed with the acetate solution. The cytochrome c lodged at the surface of the resin, where it formed a brick-red layer. A golden-colored material quickly appeared in the effluent. The latter fraction could be precipitated with either trichloroacetic acid or four volumes of acetone and had a very high optical density at 280 mμ. Prolonged washing of the column with 0.1 *M* ammonium hydroxide-ammonium acetate solutionof pH 9.0 did not elute the cytochrome nor cause any 280 mμ-absorbing material to appear in the effluent. When the pH of the ammonium acetate solution was adjusted to 10.8 by the addition of more ammonium hydroxide, the cytochrome migrated down the column. The elution rate appeared to be much higher for the smaller particles of the resin. Consequently, in all subsequent work, the Amberlite was first pulverized to pass a 0.075 mm mesh screen.

The colored band was collected in a few ml and dry weight and iron determinations carried out. The iron content was 0.420 %. A repeat experiment starting with the same crude cytochrome from salmon gave the figures 0.447 %

* Obtained from the Rohm and Haas Co., Philadelphia, U.S.A., kindly supplied to us by J. Troedsson, Nordiska Armaturfabrikerna AB, Linköping.

and 0.461 % iron respectively for the front and tail fractions of the colored zone in the effluent.

It was then of considerable interest to determine the precise elution-point pH of cytochrome. Such information might: 1) enable one to refine the method so as to increase its resolving power and 2) indicate whether or not the elution-point pH coincides with the isoelectric point of the protein.

A column was prepared as above and after loading with a sample of salmon cytochrome the alkalinity of the ammonium hydroxide-ammonium acetate solution was increased in intervals of 0.1 pH unit, starting with pH 9.0. The golden-colored impurity soon appeared in the effluent and was saved for future investigation. At pH 9.9 the lower portion of the cytochrome band increased in color and on continued washing a new red band could be seen to leave the original red zone at the top of the column. The latter was easily eluted with 0.5 % ammonium hydroxide. Chromatography of dog-heart and chicken heart cytochromes at pH 9.0 also gave a three component mixture. In all these experiments the first band to appear in the effluent had the spectrum of reduced cytochrome while the second band had the spectrum of the oxidized form. The small amount of pigment which remained at the top of the column was eluted with 0.5% ammonium hydroxide and had the oxidized-type spectrum.

When it became apparent that the use of ion exchange resin in the above way for the purification of cytochrome c would resolve the pigment into several fractions, we decided to next study cow heart cytochrome. This is generally considered to be the most readily available source of cytochrome c. The run with cow heart cytochrome is described below in detail.

The primary product was obtained by the method of Keilin and Hartree and had an iron content of 0.34 %. After it had been obtained as a dry, salt-free powder by lyophilization, the material was dissolved in 0.1 *M* ammonium hydroxide-ammonium acetate solution, pH 9.0, to give a clear solution containing 112.7 mg per ml. At first some difficulty was experienced in washing the column free from very finely divided particles of resin. However, if the dry, powdered resin was first suspended in the ammonium acetate solution in a beaker, stirred, the supernatant liquid decanted after several minutes and this process repeated several times, this difficulty disappeared. The bottom of the chromatography tube was closed with a one-hole rubber stopper; on the top of the stopper a layer of glass wool and a layer of powdered asbestos prevented the particles of Amberlite from escaping from the tube. The resin, a column 2.0 × 15 cm, was prepared by washing for several hours each with 5 % H_2SO_4, water, 5 % ammonium hydroxide and 0.1 *M* ammonium hydroxide-ammonium acetate solution, pH 9.0. The effluent was checked for correct pH and the absence of any appreciable optical density at 280 mμ.

Exactly 4.5 ml (dry weight 507 mg) of the above cytochrome solution was placed on the resin and the column developed with 0.1 *M* ammonium hydroxide-ammonium acetate solution, pH 9.0. The golden-colored impurity, which was brownish in more concentrated solution, could be almost exclusively confined between the 22 and 37 ml marks in the effluent. This fraction contained a dry weight of 101 mg. The per cent. iron was 0.040 and the optical density ratio $\frac{550\ m\mu}{280\ m\mu}$ was 0.072. These figures plus the fact that in the reduced solution the handspectroscope showed only a very faint band at 550 mμ shows that the amount of cytochrome in this material must have been very small. As mentioned above, the addition of trichloroacetic acid or four volumes of acetone to this fraction produced a heavy precipitate. The first cytochrome band to emerge was collected between the 182 ml and 482 ml marks in the effluent. It had the spectrum of reduced cytochrome and was labelled Fraction I. This band was immediately followed by another (Fraction II) of oxidized cytochrome and was collected in a volume of 500 ml. Finally, Fraction III could be stripped off the top of the column with 0.5 % ammonium hydroxide to give a 50 ml solution of oxidized cytochrome.

The next problem was the concentration of the cytochrome from these rather dilute solutions. Direct lyophilization was ruled out as impractical. Attempts to remove the buffer by evaporation under reduced pressure always led to the formation of a certain amount of insoluble cytochrome, possibly foam denatured. Some success was had by re-adsorbing the material on a fresh column of Amberlite IRC-50. A more effective process proved to be the addition of 20 % trichloroacetic acid to pH 3.5 in the cold followed by centrifugation after the solutions had stood at 4° C for several hours. This isolated all the color and yielded the cytochrome as a red precipitate which dissolved at once in water. Concentrated solutions of each fraction were thus obtained and dialyzed overnight against flowing 0.1 % ammonium hydroxide.

The three fractions were then analyzed for dry weight and iron. The results are given in Table 1.

Table 1. Yield and iron content of three cytochrome c fractions separated with ion exchange resin.

Fraction	Dry weight mg	Relative % of each fraction	Fe content %	Fe content mg
I	66.2	27.2	0.401	0.27
II	155.4	63.9	0.466	0.72
III	21.8	8.9	0.353	0.08
Total	243.4	100.0		1.07

The dry weight of the impurity, 101 mg, when added to the 243.4 mg of cytochrome, gives a recovery of $\left(\frac{344.4}{507} \times 100\right)$ or 67 %. All three fractions reported in Table 1 gave different iron analyses. An admixture of the three fractions would have an iron content of $\left(\frac{1.07}{243.4} \times 100\right)$ or 0.44 %. The iron content of the impurity fraction added to the cytochrome iron, 1.07 mg, leads to a total iron recovery of 11.1 mg or $\left(\frac{1.11}{1.75} \times 100\right) = 63$ %. Much of the unrecovered dry weight and iron was probably contained in the effluent which emerged between the impure fraction and the reduced cytochrome band. This fraction had a certain small amount of density at 280mμ and 550 mu (after reduction).

Fraction II of Table 1 appeared, from the iron content, to be relatively pure cytochrome. Therefore, the optical density of this material was measured in a Beckman Model DU spectrophotometer at various wavelengths for the reduced pigment (carried out according to Tint and Reiss[8]) and at 280 mμ for the oxidized form. This enabled us to calculate the light absorption constants recorded in Table 2. It is obvious that Fraction II, the main component, is a very highly purified substance. A short run in the electrophoresis apparatus of Tiselius at pH 10.5 did not reveal the presence of any contaminating material.

Table 2. Some physical constants for fraction II of beef cytochrome c prepared with ion exchange resin.

Cytochrome c (form)	Wavelength (mμ)	$E^{1\%}_{1cm}$	e_{1cm} *	Extinction ratios and MW
Oxidized	280	19.15	22 940	$\frac{e_{550}}{e_{280}} = 1.17$ MW from Fe analysis (0.466 %) = 11 980
				$\frac{e_{550}}{e_{535}} = 3.82$
Reduced	520	12.91	15 466	
»	535	5.87	7 035	$\frac{e_{520}}{e_{535}} = 2.20$
»	550	22.41	26 882	

* Molar extinctions per gram atom of iron.

DISCUSSION

The separation of cytochrome into several fractions by chromatography on cation exchange resin could be most easily explained if there should exist cytochromes with different degrees of basicity. Or, on the other hand, a single cytochrome might enter into complex formation with other constituents of the medium. In the future it would be of interest to study the comparative electrophoretic behaviour of all the different fractions and their reconstituted mixtures. Zeile and Reuter [10] claimed that oxidized, but not reduced cytochrome, could be adsorbed on kaolin.

There is a rather striking agreement between the constants reported for Fraction II and those calculated by Tint and Reiss [8] for a beef preparation which they believed to be 81 % pure. These authors reported, per gram atom of iron, $e^{1\text{ cm}}_{550\text{ m}\mu} = 26.400 \pm 560$; $e^{1\text{ cm}}_{535\text{ m}\mu} = 7\,030 \pm 140$; $\frac{e_{550\text{ m}\mu}}{e_{535\text{ m}\mu}} = 3.76 \pm 0.11$. Corrected for purity the $E^{1\text{ cm }550\text{ m}\mu}_{1\%}$ was 21.38 $\pm$ 0.34 and the $E^{1\text{ cm }535\text{ m}\mu}_{1\%} = 5.69 \pm 0.08$. However, we do not feel that we should claim the isolation of pure beef cytochrome c. This is partly because we have not made a sufficiently large number of preparations and partly because of the fact that such claims should be substantiated by several criteria of purity. This would certainly include analyses for several elements, such as Fe, N and S; electrophoresis at different pH levels; a maximum extinction coefficient at 550 mμ and maximum ratio $\frac{e_{550\text{ m}\mu}}{e_{535\text{ m}\mu}}$; and maximum activity in an enzyme assay. Since reduced cytochrome c has such sharp maxima and minima, accurate spectrophotometric constants could only be derived with an apparatus having a narrow spectral interval.

While making cytochrome preparations from different animals, Tint and Reiss [9] observed that the same or similar impurities always contaminated the different cytochromes. As mentioned above, we have made a similar observation. Since this impurity has such a low ratio $\frac{e_{550\text{ m}\mu}}{e_{280\text{ m}\mu}}$ this figure should be of value as a general check on the freedom of cytochrome preparations from this impurity.

Chromatography on the ion exchange resin is a method which can be easily scaled up to handle any desired quantity of material at a negligible cost. The same column can be washed and used for successive preparations.

SUMMARY

Preparation of cytochrome c by a new method, chromatography on ion exchange resin at controlled pH, has been described. The method has been tested with salmon, dog, chicken and beef-heart cytochromes. A separation

of the cytochrome into different fractions has been observed. A preparation of beef heart cytochrome has been obtained which gave the following constants: Fe = 0.466 %, $E^{1\ \%}_{1\ cm}{}^{550\ m\mu} = 22.41$; $e^{1\ gram\ atom\ Fe/liter}_{1\ cm}{}^{550\ m\mu} = 26\,882$; $\frac{e_{550\ m\mu}}{e_{535 m\mu}} = 3.82$. The measurements for light absorbtion were carried out with a Beckman model DU spectrophotometer.

The authors wish to express their appreciation for the interest and encouragement obtained during the course of their work from Professor Hugo Theorell. Miss Inger Agerberg and Mr B. Holmström assisted with various technical phases of this research. One of use (JBN) holds a fellowship from the National Research Council. *Stiftelsen Therese och Johan Anderssons minne* kindly provided funds.

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Received July 11, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1031–1041

Studies in the Tropolone Series

I. Thujaplicins and Nootkatin

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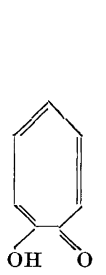
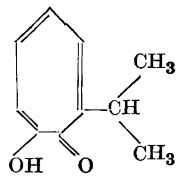
The cycloheptatrienolone (I), for which Dewar [1] proposed the name tropolone, was unknown until quite recently. However, two different syntheses of the substance have now been reported [2,3] as well as some of its properties, *e. g.* ultra-violet absorption maxima.

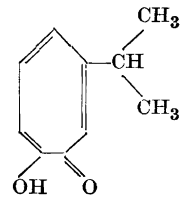


(I)



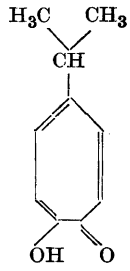
(II)

α-



(III)

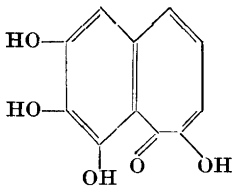
β-



(IV)

γ-thujaplicin

Tropolone forms part of the structure of several compounds of established constitution: the thujaplicins (II—IV) [4–6], stipitatic and puberulic acid (*cf.* Part II), purpurogallin (V) [7] and colchicine (VI, R = CH_3, R′ = $COCH_3$) [7–8], as well as derivatives and degradation products of these compounds, *e. g.* *β*-methyl-tropolone (III, — CH_3 instead of — $CH(CH_3)_2$) [9]. Recent members of the series which have been synthesised are 3,4-benz-tropolone [10] and 4,5-benz-tropolone [11].



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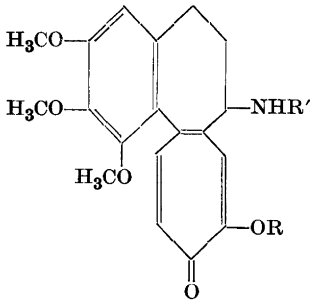

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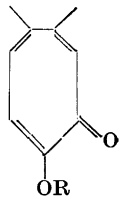
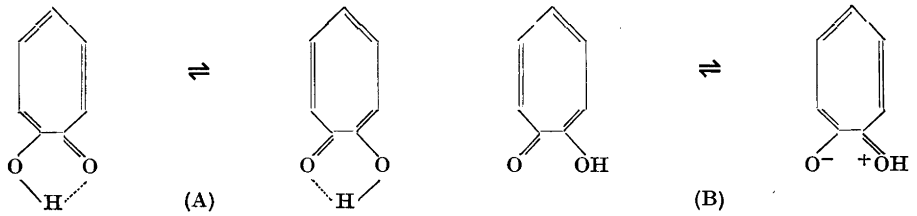


(VI)

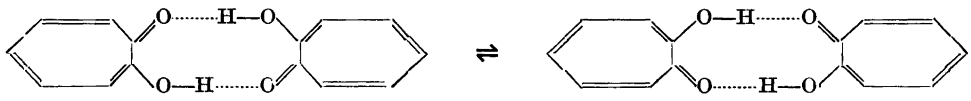
As Dewar pointed out [1], the ring system (I) represents a new type of "aromatic" structure of considerable theoretical interest. Its character was originally [1] explained by Dewar as depending upon resonance chelation (A). Later, however, he considered [12] that a hydrogen bridge is improbable in this case because it would mean a stretching of the O—H bond. Instead of the



(A) (B)

mesomerism (A) he therefore suggested a system of easily interconvertible tautomeric forms, each of which shows an ionic type of resonance (B). However, the latter explanation is also somewhat unsatisfactory, because the high water solubility of colchicine in contrast to for example colchiceine (VI, R = H, R′ = $COCH_3$) "is better explained if in it (colchicine) alone an ionic resonance (implying a semi-zwitterion structure) occurs"[12].

In an interesting study of the infra-red absorption properties of some tropolones Scott and Tarbell [13] found a considerable displacement of the CO bond of these substances as compared with simple ketones. This displacement was explained as an effect of conjugation with the unsaturated ring rather than of chelation through a hydrogen bond. However, a decision between these alternatives could not be made.



(C)

A double molecule mesomerism (C), therefore, appeared to be worth consideration, even though the ring formed would be very wide. The molecular weight has not been determined — with one or very few exceptions — for any tropolone derivatives possessing the intact, "sterically unhindered" "ol-one" grouping. Abnormally high values were found by Zeisel and von Stockert [14] in 1913 for trimethyl colchicic acid (VI, R = R′ = H) in *acetic acid* solution (found 427—468, calc. 343). (The high molecular weights found by the same authors for colchicine and colchiceine were explained as depending upon associations involving the —$NHCOCH_3$ group.)

The molecular weight of β-thujaplicin has now been determined cryoscopically in benzene solutions of two different concentrations. In both cases, however, the values obtained coincide within a few per cent with the value calculated for the monomer (0.623 molal solution, depression 2.85°, mol. wt. 176; 0.155 molal solution, depression 0.72°, mol. wt. 173; calc. 164). It should be noted that, in the case of *e. g.* benzoic acid in benzene, these concentrations would have been high enough to cause nearly complete association as double molecules.

It is clear, therefore, that tropolone units do not associate in solution to any considerable extent. Some other explanation must be found for the high molecular weight reported for trimethyl colchicic acid in acetic acid solution.

As already mentioned, the question whether an intramolecular hydrogen bond occurs in the tropolone structure has not yet been definitely answered. Various properties of tropolones, *e. g.* the steam volatility of the thujaplicins [6], point more or less strongly towards the existence of a hydrogen bridge. It is a well-known fact that hydrogen involved in chelate rings dissociates less easily than would be expected in the case of no hydrogen bonding. A good example is found in the pK value of 8.8 for 1-hydroxy-anthraquinone, and 5.7 for the 2-hydroxy compound in 96 per cent ethanol [15].

With regard to this question it is of interest to note that the acidity of tropolone itself corresponds to pK 6.7 [2] or 6.95 [3]. A potentiometric titration curve for β-thujaplicin is shown in Fig. 1, giving the pK value of approximately 7.0. However, in the case of the tropolones it is difficult to find suitable model substances for a comparison of the acidity. The following may serve for this purpose:

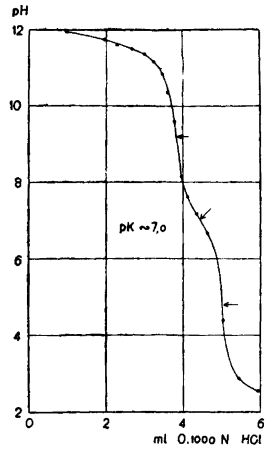


Fig. 1. Potentiometric titration of β-thujaplicin, dissolved in excess sodium hydroxide solution.

		pK
Substances with hydrogen bonds:	Salicylaldehyde [16]	8—9
	CH_3—C(OH) = CH—CO—CH_3 [17]	8
Substances without hydrogen bonds:	Benzoic acid	5
	2,5-dihydroxy-benzoquinone [18]	3 and 5
	(Phenol, catechol	10)

It is seen by comparison that the tropolones — with pK around 7 — take an intermediate position between the two groups.

Besides the ultra-violet absorption maxima given for tropolone itself [2,3], spectra in the ultra-violet have been reported for the thujaplicins by Erdtman and Gripenberg [4], for stipitatic and puberulic acid by Todd and co-workers (*cf.* Part II), and for purpurogallin and some of its derivatives by Haworth *et al.*[9], neutral solvents being used in all cases. The tropolone colchiceine (VI, R = H, R′ = $COCH_3$) has been investigated both in neutral and alkaline solutions [19]. The acidic — "semi-phenolic" — properties of the molecule give rise to a bathochromic shift of the absorption spectrum on proceeding from a neutral to an alkaline medium.

The main purpose of these ultra-violet absorption studies was to demonstrate structural similarities between the members of each group of substances. A discussion of the tropolone system itself from the point of view of U. V. spectrochemistry, however, is still missing although highly desirable on account of the general analogy between phenols and tropolones.

In the present paper spectra are given for the thujaplicins in *n*-heptane and alkaline water solutions. In a separate note a similar investigation of a new natural compound, nootkatin, is described.*

* *Cf.* second note on page 1035.

EXPERIMENTAL

The α-thujaplicin specimen * used was purified by distillation *in vacuo*, recrystallisation from *n*-heptane and redistillation, m. p. 33—33.5° (literature [5] 34°). The β- and γ-thujaplicin samples were distilled *in vacuo* and recrystallised from *n*-heptane and light petroleum respectively. M. p. for the β-compound 51—51.5° (lit. [6] 52—52.5°); for the γ-compound 79—79.5° (lit.[4] 82°).

Solvents: *n*-heptane, purified for spectrophotometric purposes [20], and an aqueous phosphate buffer solution with pH 12.

The measurements were carried out with a Beckman Model DU spectrophotometer, using calibrated cells. The alkaline solutions of the thujaplicins are somewhat unstable, the absorption in the long wave portion of the spectra (round 400 mμ and above) tending to increase. In these cases, therefore, the absorption was determined soon after preparing the solutions (within an hour or less).

RESULTS AND DISCUSSION

The absorption spectra for the thujaplicins in heptane solution are shown in Fig. 2. Their mutual resemblance is great, except for slight differences in the ε-value for the symmetrical γ-compound, and the long wave part of the β-compound curve occurring at somewhat shorter wavelengths than the others. It could be expected that tropolone itself would show a spectrum similar to those of the thujaplicins. By analogy with the known difference between the absorption of benzene and its alkyl derivatives, however, the tropolone spectrum must be expected to occur at somewhat shorter wavelengths, the maxima being a little lower and more distinct than those of the thujaplicins. No spectrum of tropolone in a saturated hydrocarbon solvent has yet been published. von E. Doering and Knox [2] did not state which solvent they employed, and Cook and co-workers [3] used ethanol. The data of the latter authors, however, fit in rather well with the values expected on the basis of the ethanol spectra of the thujaplicins [4]. **

* My thanks are due to Professor H. Erdtman and Dr. J. Gripenberg for providing samples of the thujaplicins.

** *Note added in the proof* (September 8th, 1950):

Thanks to the courtesy of Professor J. W. Cook, Glasgow, the author is able to include the absorption spectrum of tropolone in cyclohexane (Fig. 2) as determined in Glasgow by Mr. A. R. M. Gibb. The solvent used was "cyclohexane for spectroscopic purposes", supplied by British Drug Houses. Regions above 250 mμ were examined with the Hilger "Spekker" Quartz Spectrophotometer, slit width 0.03 m. m., match points being found with a microphotometer. For the region below 250 mμ, the Unicam spectrometer was used.

The tropolone spectrum is in good agreement with the expected curve, keeping in mind the greater resolving power of the "Spekker" spectrophotometer as compared with the Beckman instrument.

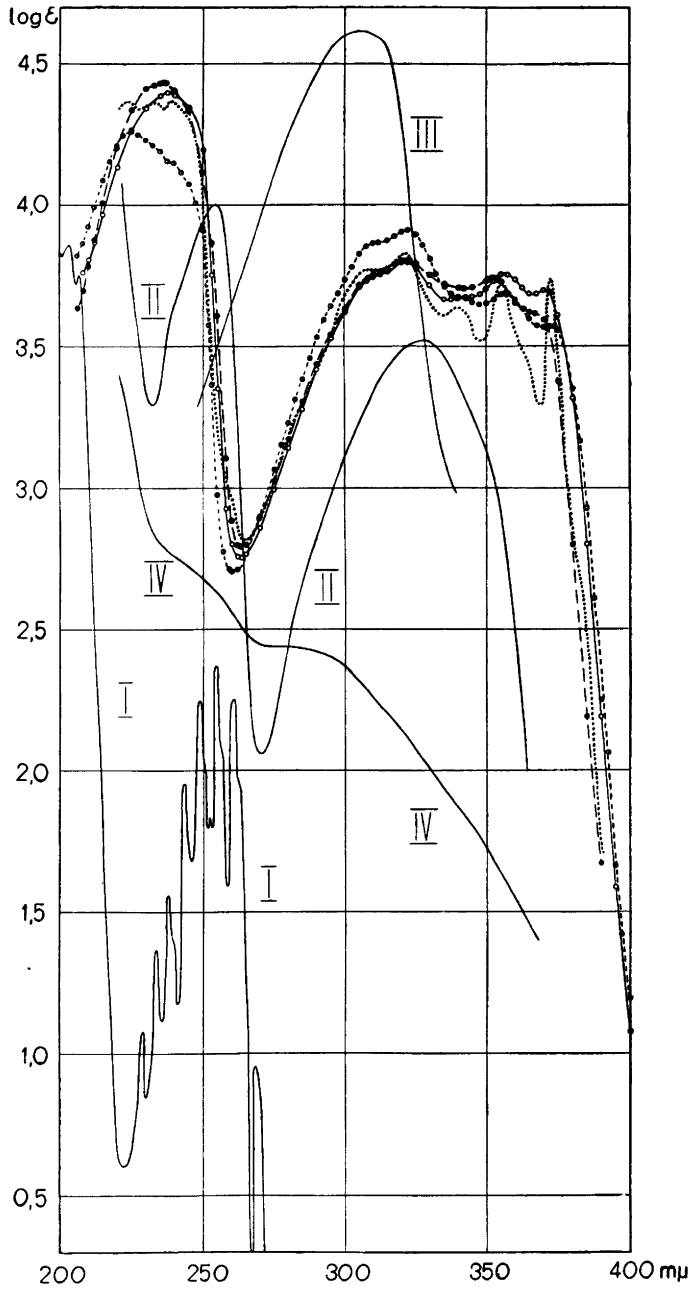


Fig. 2. Ultra-violet absorption spectra for α-thujaplicin (○——○——○), β-thujaplicin (●-·-·-●—●—), and γ-thujaplicin (◑– – –◑———◑), all in n-heptane, benzene in pentane [21] (I), salicylaldehyde in hexane [22] (II), octatrienal in hexane [23] (III), and cyclooctatetraene in cyclohexane [24] (IV). Dotted line: tropolone in cyclohexane (cf. note on page 1035).

The absorption of the thujaplicins in heptane differs only slightly from that in ethanol. Being a polar solvent, ethanol causes a smoothing of the curves and also a slight increase of the ε-values, trends that are characteristic for phenols and other polar solutes. In analogy with for example salicylaldehyde [22] a small hypsochromic shift of the curves takes place on changing from the saturated hydrocarbon to ethanol. This may be taken as an indication of the existence of a hydrogen bond in the tropolone structure [22] *. The shoulders around 400 mμ in the spectra of β- and γ-thujaplicin in ethanol [4] are not found in heptane solution.

Two main absorption ranges are exhibited by the thujaplicins, one around 225—240 mμ, the other at approximately 310—375 mμ. The latter region is occupied by a most characteristic group of maxima of high and comparatively similar intensities. Both absorption ranges are remarkably wide. Moreover, in the long wave one the central part of the curve is somewhat depressed. Evidently, at least the long wave group of maxima is built up by two distinct sub-groups. This view is supported by the shape of the alkali curves (Fig. 3), which possess two separate long wave maxima. These curves also show an indication of a subdivision of the short wave band.

The strong absorption of the thujaplicins in the near ultra-violet (log $\varepsilon = 3.7$—3.9) shows beyond doubt that the double bonds are conjugated, in other words, that the molecules are planar. Cycloöctatetraene, on the contrary, exhibits a low and sloping absorption curve in this region (Fig. 2) due to the predominating non-planar structure of the molecule.

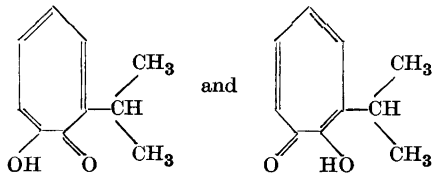
A definite interpretation of the absorption curve of tropolone will require a quantum mechanical treatment. However, a few more conclusions can be drawn empirically.

In benzene, the long wave maxima are the result of "forbidden transitions". A characteristic property of benzeneoid compounds, therefore, is a depressed absorption in the long wave region as compared with non-cyclic substances containing the same number of conjugated double bonds. The absorption intensity of the thujaplicins in this region is intermediate between that of octatrienal and salicylaldehyde although closer to the latter (Fig. 2). This conforms with the aromatic character of the tropolone derivatives.

---

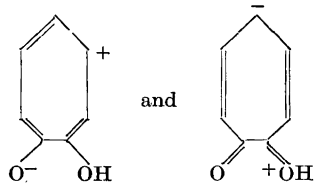
* Morton and Stubbs [22] found a difference of nearly 20 mμ between the long wave λ_{max} for salicylaldehyde and its methyl ether in hexane solution. The higher value of λ_{max} for the former substance was ascribed to the hydrogen bond. An analogous comparison of a simple tropolone and its methyl ether might throw some light upon the question of the existence of a hydrogen bond in tropolone.

Assuming no hydrogen bridge in the tropolone molecule, two different structures are possible for the unsymmetrical tropolone derivatives, *e. g.* for *α*-thujaplicin:



There is no reason why, in solution, either form should be greatly predominant. In tropolone and *γ*-thujaplicin the two forms are identical. The close spectral resemblance between *γ*-thujaplicin and the *α*- and *β*-compound shows that the above tautomerides, if existing side by side, must possess very similar absorption properties. Otherwise, the *α*- and *β*-curves would be levelled as compared with the *γ*-curve.

At present it is difficult to understand why the thujaplicins exhibit two groups of long wave maxima. Due to the complexity of the bands it is impossible to determine precisely the frequency corresponding to each electronic transition, but the ratio of them is approximately 1.1—1.2. Assuming the same ground state in each case, this implies that the difference in energy between the excited states is remarkably small. Several types of zwitterion structures can be constructed which may contribute to the excited states, *e. g.*:



Apart from the characteristics already discussed, the vibrational fine structure is the most prominent feature of the thujaplicin spectra. Although not very marked, it is clear enough to indicate a rigid ring structure. Among benzene derivatives, phenol exhibits a similar degree of subdivision of electronic bands. With salicylaldehyde, on the other hand, the long wave maximum is completely smoothed (Fig. 2), which can be explained as a "loose bolt" effect [25] of the aldehyde group.

The spectra of the anions of the thujaplicins were determined in aqueous buffer solutions of pH 12. It is true that strong steric hindrance exhibited by

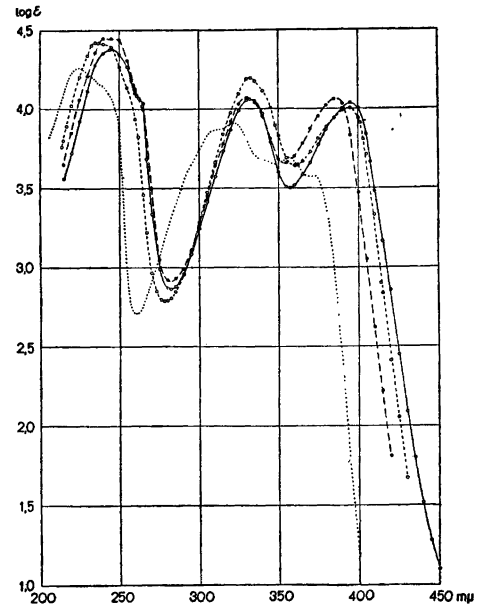


Fig. 3. Ultra-violet absorption spectra for α-thujaplicin (○———○———○), β-thujaplicin (●— —●— —●), and γ-thujaplicin (○– – –○– – –○), all at pH 12. Dotted line: γ-thujaplicin in n-heptane.

ortho-substituents can prevent dissociation of phenols to such an extent that ionisation is not nearly complete at pH 12 or even higher [26]. Therefore, α-thujaplicin, being the only one that might show this effect, was submitted to a control investigation at pH 14. No spectral difference was found, however, between pH 12 and 14.

The alkaline solutions of the thujaplicins are all yellow. Their absorption spectra are shown in Fig. 3. As compared with the spectra in heptane the entire curves are shifted bathochromically to an extent which would be about normal for a phenol. Moreover, the fine structure disappears due to electrostatic interaction between solvent and solute, so that two distinct maxima — at 330—332 mμ and 385—396 mμ respectively — remain instead of the groups of long wave maxima of the non-ionised molecules. A marked increase of the absorption coefficients in this range is also brought about by alkalisation, a clear analogy with the normal phenol-phenolate ion shift. Tropolone itself will no doubt show an alkali spectrum very similar to those of the thujaplicins.

NOTE ON A NEW NATURAL PRODUCT, NOOTKATIN, ISOLATED FROM CHAMAECYPARIS NOOTKATENSIS

According to a private communication from Professor H. Erdtman, a new compound with m. p. 91.5—92° and corresponding to the formula $C_{15}H_{20}O_2$ has been isolated from the heart wood of *Chamaecyparis nootkatensis*. As the

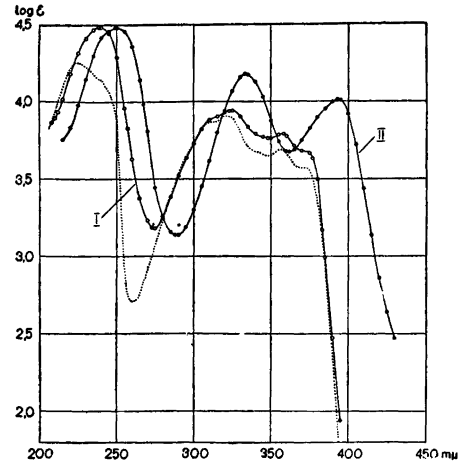


Fig. 4. Ultra-violet absorption spectra for nootkatin in n-heptane (I) and at pH 12 (II). Dotted line: γ-thujaplicin in n-heptane.

substance, which has been named nootkatin, bears a close chemical resemblance to the thujaplicins, it has been subjected to a physico-chemical investigation in connection with the thujaplicin work just reported *.

The molecular weight was determined cryoscopically in a benzene solution (0.907 g nootkatin, 12.68 g benzene, depression 1.41°). Found: mol. wt. 249, calculated for $C_{15}H_{20}O_2$: 232.

Using a Beckman Model DU spectrophotometer, the ultra-violet absorption curves of nootkatin were determined in *n*-heptane and in a phosphate buffer solution of pH 12 (Fig. 4). The spectra resemble those of all three thujaplicins, the curves of the γ-compound being slightly more similar than the others. Thus, nootkatin is evidently a tropolone derivative. If an extra double bond exists in the molecule it will not be conjugated with the ring chromophore. Assuming a single alkyl substituent in the ring, the γ-position is to be slightly preferred to the others.

SUMMARY

Ultra-violet absorption spectra are given for the thujaplicins and a new natural compound, nootkatin, in heptane and alkaline water solutions as well as for tropolone in cyclohexane solution (added in the proofs). A potentiometric titration curve for β-thujaplicin and molecular weight determinations for the latter substance and nootkatin are presented.

* The providing of a specimen by Professor Erdtman is gratefully acknowledged.

These data are discussed in connection with known properties of the tropolones. It is shown that the molecule is planar and does not associate with other tropolone molecules in solution. The problem of an intramolecular hydrogen bond is discussed on the basis of acidity and spectro-chemical evidence. The "aromatic" nature of the tropolone ring system is confirmed and some analogies with phenols are pointed out.

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Received July 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1042–1046

Constituents of Pine Heartwood

XXII.*) The Isolation of Pinostrobin and 3,5-Dihydroxy-7-methoxyflavanone from the Heartwood of *Pinus clausa* Vasey

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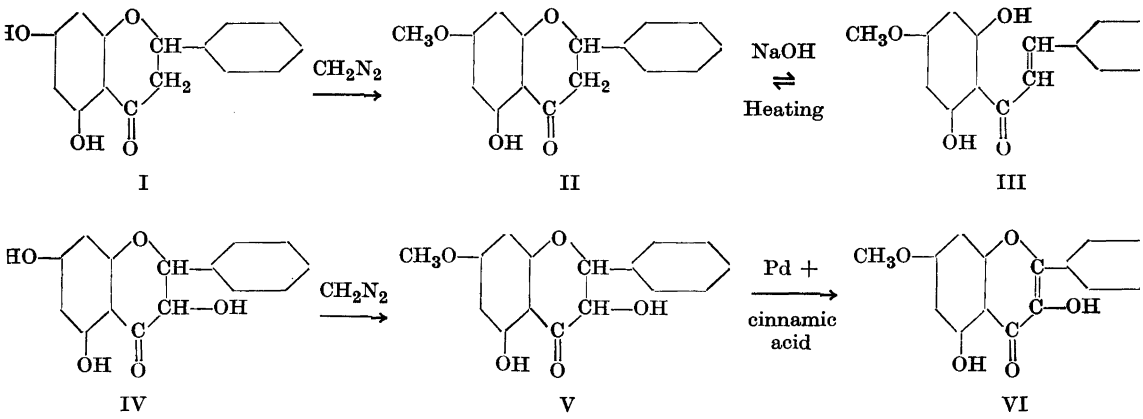
In an earlier paper, the usefulness of paper partition chromatography for analysing pine heartwood extracts was demonstrated [1]. This method has now been applied to a great number of pine species, and a detailed account of this work will appear later. The heartwood extracts from pines belonging to the section *Diploxylon* (Hard Pines) usually contain four phenolic substances, pinosylvin and its monomethyl ether, pinobanksin, and pinocembrin. The acetone extract from one sample of *Pinus clausa*, however, gave a paper chromatogram which contained two additional spots with R_F 0.84 and 0.93 respectively. (The standard solvent and benzidine spraying reagent described in Part XX[1] were used.) The colour and position of one of these spots (R_F 0.93) coincide with that of pinostrobin (5-hydroxy-7-methoxyflavanone), previously isolated from the heartwood of *P. strobus* [2], but the second spot must evidently be due to some new constituent.

In order to isolate the two substances, a large sample of heartwood was extracted with ether and then with acetone, and the acetone extract divided into fractions in the usual manner [3]. Each fraction was analysed by the aid of paper chromatography. The 4 % sodium hydroxide extracts contained the main part of the two new compounds. This extract deposited a colourless sodium salt, from which a crystalline product, melting at 112—113°** was isolated after acidification and recrystallisation. It was optically active, $[\alpha]_D^{20}$ —56° (chloroform). This compound proved to be identical with pinocembrin monomethyl ether (II), which is obtained by treatment of this flavan-

* XXI. *Acta Chem. Scand.* 4 (1950) 772.

** All melting points uncorrected.



I II III

IV V VI

one (I) with diazomethane. The pinostrobin isolated by Erdtman [2] melted at 90—91°. He identified the compound as pinocembrin monomethyl ether by mixed m. p. determination. The difference in melting points may perhaps be due to dimorphism. Due to lack of material Erdtman could not determine the specific rotation of his pinostrobin, but his pinocembrin monomethyl ether had $[\alpha]_D^{20}$—52.7° (chloroform) which is in good agreement with the figure obtained by the present author. The m. p. of racemic 5-hydroxy-7-methoxyflavanone is 101°[4].

Pinostrobin, m. p. 109—112°, has since been isolated from the heartwood of *P. strobus* and of *P. Lambertiana* (not published).

The second unknown substance (R_F 0.84) was isolated from the liquid part of the strong alkali extract, along with additional amounts of pinostrobin. It crystallises from ethanol in colourless needles, m. p. 179—181°, $[\alpha]_D^{20}$—20° (chloroform). The analyses agreed with the composition $C_{15}H_{11}O_4(OCH_3)$, and the compound proved to be identical with pinobanksin 7-methyl ether (V), which has previously been synthesised from pinobanksin (IV)[5]. An independent proof of this structure was furnished by catalytic dehydrogenation to izalpinin (VI = galangin 7-methyl ether) with palladium in the presence of cinnamic acid. Pinobanksin and its 5,7-dimethyl ether can also be converted into the corresponding galangin derivatives by the same method [5].

A very small quantity of a third compound was also isolated from subsequent extracts with 8 and 20 % sodium hydroxide. This compound crystallises in orange red needles, m. p. 152—154°. When dried in a vacuum at 100°, it rearranged to racemic 5-hydroxy-7-methoxyflavanone. Hence, the red compound was apparently 2′,6′-dihydroxy-4′-methoxychalkone (III), formed from pinostrobin under the influence of strong alkali. The presence of

two hydroxyl groups in *o*-position to the carbonyl may facilitate its rearrangement to the flavanone.

When some other heartwood samples from *P. clausa* were investigated, no traces of pinostrobin or pinobanksin monomethyl ether could be found *, nor have these two compounds been observed in any other pine from the section *Diploxylon*. (33 such species have now been investigated.) In *Haploxylon* pines, however, pinostrobin is a common heartwood constituent.

EXPERIMENTAL

The sample of wood came from a pine which had grown at Lake City Branch Station, Florida, USA.

3.2 kg of air-dry heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract was concentrated to a dark brown syrup (167 g = 5.5 % of the heartwood). A paper chromatogram of this extract did not give any visible spots on spraying with benzidine reagent. The ether extract was not further investigated.

A paper chromatogram of the acetone extract showed six spots, four of which were due to pinobanksin, pinocembrin (very strong spots), pinosylvin and its monomethyl ether (very weak spots). The two other spots were orange red and developed rather slowly (5—10 minutes after spraying). Their R_F values were approximately 0.84 and 0.93. (See Part XX[1] for the technique of the paper chromatography.) The extract was concentrated to a brown syrup and an aqueous solution containing *l*-arabinose. This solution was decanted off, and "membrane substances" precipitated by addition of ether. The ether solution (700 ml) was shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide, 4 % sodium hydroxide and 8 % sodium hydroxide (3 × 200 ml of each), and, finally, with 20 % sodium hydroxide (200 ml). A colourless, jelly-like precipitate was deposited in the 4 % and stronger sodium hydroxide fractions. The sodium carbonate fraction contained a yellow crystalline precipitate (pinobanksin sodium salt). Each fraction was investigated by paper chromatography. The two new substances accumulated in the 4—20 % alkali fractions, which were further investigated.

Isolation of pinostrobin

All jelly-like precipitates were separated by filtration, combined, and acidified with dilute sulphuric acid, and the suspension extracted with ether. The ether solution was dried over anhydrous sodium sulphate and concentrated, leaving a crystalline residue. After three recrystallisations from methanol, colourless crystals, m. p. 112—113° were obtained. $[\alpha]_D^{20} -56° \pm 2°$ (chloroform, $c = 2.1$). Yield, 0.75 g. A mixture with pinostrobin from *P. Lambertiana* (m. p. 109—112°) melted at 109—111°.

$C_{15}H_{11}O_3(OCH_3)$ (270.3)	Calc.	C 71.1	H 5.22	OCH_3 11.5
	Found	» 71.0	» 5.28	» 11.5

* The identification of the wood samples was carried out in the USA and thus was completely outside the author's control. A confusion with some other species is of course possible, but is rather unlikely, since all the species growing in the south-eastern part of the USA have now been investigated without finding any traces of either compound.

Methylation of pinocembrin

An ether solution, containing about 0.18 g of diazomethane and pinocembrin from *P. Banksiana* (0.7 g, m. p. 194—195°, $[\alpha]_D^{20}$ —54°) was left overnight. On evaporation of the ether, this solution left a colourless crystalline residue, which was recrystallised twice from methanol. Colourless leaflets, m.p. 110—111°, (0.56 g) were obtained. $[\alpha]_D^{20}$ —53°±1° (chloroform, c = 3.9). The m. p. was not depressed on admixture with pinostrobin from *P. clausa* or from *P. Lambertiana*.

Isolation of 3,5-dihydroxy-7-methoxy-flavanone

The filtrate from the 4 % alkali extract was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and evaporated to dryness, leaving a crystalline residue, which was washed with a few ml of ether. The ether-insoluble part melted at 163—169° and the soluble part (after evaporation of the ether) at 111—112°. This fraction consisted of pinostrobin.

The substance melting at 163—169° (0.85 g) was recrystallised twice from methanol, yielding pale yellow needles, m. p. 170—174°. Coloured impurities were removed by filtration of a solution of the substance in ether through aluminium oxide. The ether was then evaporated and the residue recrystallised from benzene and, finally, from dilute ethanol. Colourless needles, m. p. 179—181°. $[\alpha]_D^{20}$ —20° ± 1° (chloroform, c = 2.7). These data lie very close to those of pinobanksin monomethyl ether (m. p. 180—182°, $[\alpha]_D^{20}$ — 19°), and the m. p. was not depressed on admixture with that substance. The two substances also gave identical spots on the paper chromatogram (R_F 0.84).

$C_{15}H_{11}O_4(OCH_3)$ (286.3)	Calc.	C 67.1	H 4.93	OCH_3 10.8
	Found	» 67.0	» 4.80	» 10.9

Dehydrogenation to izalpinin

The flavanone (0.25 g), cinnamic acid (0.6 g), palladium-charcoal catalyst [6] (0.15 g) and water (15 ml) were reacted in a stainless-steel bomb, rotating in an oil bath at 180° for 80 minutes. The reaction mixture was extracted with ether, and the ether washed with saturated sodium bicarbonate solution three times to remove cinnamic acid. It was then dried over anhydrous sodium sulphate and filtered through aluminium oxide, which adsorbed brownish impurities. The filtrate was concentrated to a yellow crystalline product, which was recrystallised from ethanol. Yellow needles, m. p. 194—195°, were obtained. Yield, 0.08 g. The m. p. was unchanged on admixture with an authentic sample of izalpinin *.

Isolation of 2',6'-dihydroxy-4'-methoxychalkone

The 8 and 20 % alkali extracts were filtered, and the precipitates combined with the precipitate from the 4 % alkali extract (see above). The filtrates were combined, acidified, and extracted with ether. On concentration, the ether extract yielded a yellow syrup, which deposited colourless crystals. These crystals were isolated after trituration with a

* This sample was kindly supplied by Prof. T. R. Seshadri, Delhi, India.

little methanol, and a paper chromatogram of the crystals revealed the presence of pinostrobin and pinosylvin monomethyl ether.

The methanol-soluble portion was concentrated to a syrup. From this, red crystals slowly deposited. The crystals were separated and recrystallised several times from methanol, benzene and from chloroform-light petroleum. Orange red crystals, m. p. 146—147°, were obtained. Further recrystallisation from methanol-water raised the m. p. to 152—154°. Yield, 30 mg. The substance gives a reddish-brown colour with ferric chloride in ethanol solution. It travels a little faster than pinocembrin on the paper chromatogram ($R_F \sim 0.5$). The spot is clearly visible in ultraviolet light by its brownish-violet fluorescence. After spraying with benzidine reagent, it acquires a reddish-brown colour, developing immediately, just as in the case of pinosylvin derivatives.

The red crystals were dried for analysis at 100° in a vacuum for 45 hours. During this time, they transformed into a pale yellow syrup, which crystallised when the tube was scratched with a spatula. The colourless crystalline mass was recrystallised from methanol-water, yielding colourless crystals, m. p. 99—100° alone or on admixture with racemic 5-hydroxy-7-methoxyflavanone [4].

SUMMARY

From one sample of heartwood from *Pinus clausa* Vasey, pinostrobin (5-hydroxy-7-methoxyflavanone) and a new substance have been isolated. The latter substance is identical with pinobanksin 7- methyl ether (3,5-dihydroxy-7-methoxyflavanone) and can be dehydrogenated to izalpinin (3,5-dihydroxy-7-methoxyflavone). The two substances were detected by the aid of paper chromatography.

Other samples of *Pinus clausa* did not contain these substances.

This investigation has been financially supported by *Fonden för Skoglig Forskning*. The author is indebted to Mr. A. Misiorny for skilful experimental assistance and to Dr. I. T. Haigh, Southeastern Forest Experimental Station, Asheville, North Carolina, USA, and Mr K. B. Pomeroy, Lake City, Florida, USA, for the samples of wood.

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Received July 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1047–1053

Preparation of Desoxyribonucleosides from Thymonucleic Acid

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The preparation of desoxyribonucleosides from tymonucleic acid (DNA) has been tried by different authors after enzymatic hydrolysis of the polynucleotide. The used enzymes were usually crude mixtures of phosphatases. Levene [1] and coworkers and Klein [2] have especially worked on this problem, and the latter has worked out a detailed method which allows the preparation of each nucleoside. This method is rather laborious and it seems, at least in one case [3], to have been impossible to reproduce the yields obtained by Klein. Especially the preparation of pyrimidine desoxyribosides seems to be difficult. For these in particular Schindler [4] recently has worked out a new method in which, after a preliminary separation according to Klein's principles, he separates the pyrimidine desoxyribosides on an adsorption chromatogram of alumina. But even in this case the yield of desoxycytidine, in particular, is rather low.

Our interest in these problems arose when we wanted to study the utilisation of isotopically marked desoxyribonucleosides in the rat. It was thought that the simplest method for the preparation of the marked nucleosides would be first the preparation of biologically marked DNA and then, from that, the isolation of the constituent nucleosides. For this reason it was first necessary to work out a method which allows the preparation of the desoxyribosides in a reasonably good yield from rather small amounts of DNA. Such a method is described in the present paper. The application of the method to the metabolic problem will be described in a following paper.

It was thought that, after a preliminary preparation of the nucleosides according to Klein's principles, the use of ion exchange chromatography with Dowex and starch chromatography might make it possible to obtain better yields. Chromatography on Dowex has been successfully used for the first

time by Cohn [5,6] for the separation of purine and pyrimidine bases and ribo mononucleotides. Cohn used for these separations both the anion exchanger Dowex 1 and the cation exchanger Dowex 50.

The nucleosides formed by the enzymatic hydrolysis of DNA are the desoxyribosides of hypoxanthine, guanine, thymine and cytosine. From a theoretical point of view it was first considered better to use an anion exchanger for their separation. At this time, however, it was impossible to obtain the Dowex anion exchanger and so the separation was tried with the cation exchanger (Dowex 50). The pyrimidine desoxyribosides were easily separated from each other by the use of 0.5 *N* HCl as eluting agent. A complete separation of all nucleosides could be achieved by running the chromatogram first with 0.1 *M* ammonium acetate buffer of pH 3.9 and then with 5 % ammonia. The buffer could be completely removed from the different fractions of the effluent by evaporation *in vacuo* at 40°, and the nucleosides further purified by crystallisation. In the case of desoxycytidine before crystallisation one more chromatogram was run on Dowex 50 with 0.5 *N* HCl as eluting agent. This method allowed the preparation of all four nucleosides in a pure crystalline state in gram amounts from about 20 g of DNA.

When the amount of DNA available was much less, a chromatogram on starch developed with butanol-water could be used after the Dowex chromatogram as a further step of purification instead of crystallisation. Chromatography on starch has been successfully used by Reichard [7] for the separation of the ribonucleosides. In the case of the desoxyribonucleosides the separation was not quite as good, since desoxyguanosine and desoxyhypoxanthosine did not separate completely. However, in the present case, this was of minor importance as the starch chromatogram was only used after a preliminary complete separation of the desoxyribosides on Dowex 50. This type of purification was used in the isotope experiments. Freedom of the starch chromatogram-prepared desoxyribonucleosides from foreign nitrogen was established by controlling the ratio between light absorption and amount of nitrogen for each component. Standard values for these ratios had been previously obtained on pure crystallized substances.

EXPERIMENTAL

DNA. The method of isolation of the DNA chosen for the enzymatic preparation of desoxyribosides proved to be of great importance. The DNA's prepared by the two different methods of Hammarsten [8,9] did not give any appreciable amount of free phosphate during the digestion. When a 10 % solution of this type of sodium nucleate in *N*/10 NaOH was boiled for one

hour still only between 10—20 % of its phosphorus was split off by a later enzymatic digestion. Much better results were, however, obtained when a sample of Feulgen's b-nucleic acid [10] was tried. This "DNA" was obtained by the action of unpure pancreas desoxyribonuclease on a higher polymer form of DNA. It thus consisted of oligonucleotides, probably of about the size of trinucleotides [11]. This mixture of oligonucleotides was readily attacked by the intestinal phophatases and as in the case of Klein more than 90 % of its phosphorus was set free during the course of digestion.

Preparation of crude desoxyguanosine and hypoxanthosine. For obtaining the crude desoxyribosides the methods of Klein [2] and Schindler [4] were used with some slight modifications.

Twenty g of b-nucleic acid according to Feulgen [10] (wet weight % P = 6.60) were suspended in 200 ml water and dissolved with the aid of conc. ammonia. The pH of the solution was adjusted to 8.8. Two hundred and fifty ml of ammonia-ammonium sulfate buffer (1 molar with respect to NH_4) of pH 8.8 and 100 ml 0.3 *M* magnesium sulfate solution were added. To this mixture was added the enzyme dissolved in 75 ml water with the aid of ammonia (final pH 8.8). The enzyme was prepared and once purified by precipitation at pH 4.7 as described by Klein [12], the only difference being that pig intestine was used instead of beef, since the former material was easy to obtain in a frozen state from the slaughter house. The amount of enzyme that was used in the present experiment corresponded to 50 g of mucosa. The mixture was allowed to stand for two days at 37°.

Purification of the nucleosides from most salts and the separation of the crude desoxyguanosine and desoxyhypoxanthosine from the pyrimidine desoxyribosides were carried out as described by Schindler [4]. One fifth of all the volumes used by him was taken.

Preparation of desoxyguanosine. The first two desoxyriboside precipitates, obtained by the method of Schindler, were combined, suspended and almost completely dissolved in 20 ml of warm water. The solution was extracted with 100 ml of a chloroform : ethanol (2 : 1) mixture as described by Schindler. Three layers were formed on standing in a separatory funnel and the lowest removed. This procedure was repeated 20 times. Once or twice during the procedure, when the separation of the layers became incomplete a few ml of water were added to the mixture. The combined chloroform extracts were evaporated to dryness *in vacuo*, giving a white dry residue weighing 1.36 g. A chromatogram on Dowex 50 (see below) revealed that this fraction contained besides smaller amounts of pyrimidine desoxyribosides almost as much desoxyhypoxanthosine as desoxyguanosine. This seems not to be in accordance with Schindler's statement that chloroform : ethanol extracts hardly any

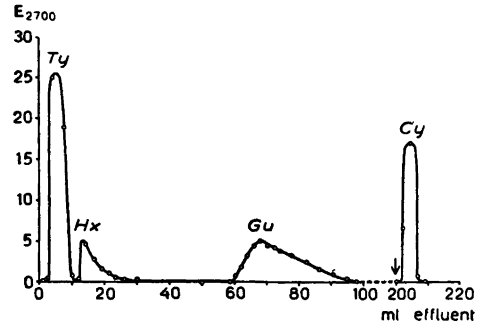

Fig. 1. Chromatogram on Dowex 50 of approximatively 3 mg of each desoxyriboside of thymine, hypoxanthine, guanine and cytosine. Length of column 140 mm, diameter 9 mm. Solvent: first, 0.1 M ammonium acetate buffer pH 3.9; after the arrow, 5 % ammonia.

desoxyhypoxanthosine. Even after three recrystallisations this fraction contained about 20 % desoxyhypoxanthosine. Therefore, the whole amount was dissolved in 100 ml warm ammonium acetate buffer and fractionated on Dowex 50 as described below. The desoxyguanosine fraction was combined with the corresponding fractions from the desoxyhypoxanthosine and pyrimidine chromatograms. Water and ammonium acetate were practically completely removed from the combined fractions by evaporation in an oil pump vacuum at 40° C. The dry white residue weighed 0.91 g. It was easily crystallizable from water and gave after two crystallisations 0.71 g of a product with the following analytical values: C = 42.1 %, H = 5.21 %, N = 24.8 %. Desoxyguanosine. H_2O gives theoretical: C = 42.1 %, H = 5.30 %, N = 24.6 %.

Preparation of desoxyhypoxanthosine. The crude desoxyhypoxanthosine was washed twice with 10 ml of 90 % ethanol and dried with ethanol and ether. It weighed 1.15 g. It was dissolved in 50 ml warm ammonium acetate buffer and after running a chromatogram on Dowex 50 as described below the main fraction was combined with the desoxyhypoxanthosine fractions of the other Dowex chromatograms. The water and buffer were removed *in vacuo*. The dry residue weighed 2.51 g. After three crystallisations from water 1.63 g of desoxyhypoxanthosine were obtained with the following analytical values: C = 47.9 %, H = 4.64 %, N = 22.4 %. Theoretical: C = 47.6 %, H = 4.79 %, N = 22.2 %.

Preparation of pyrimidine desoxyribosides. The mother liquor after the first crystallisation of desoxyhypoxanthosine according to Schindler was evaporated *in vacuo* until an oily residue was left. This contained the pyrimidine desoxyribosides together with a considerable amount of desoxyhypoxanthosine. Fractionation experiments with chloroform : ethanol as suggested by Schindler did not meet with success as all desoxyribosides showed considerable solubility

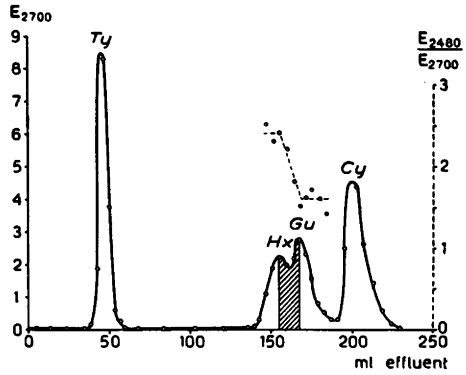

Fig. 2. Chromatogram on starch of ca. 2 mg of each desoxyriboside of thymine, hypoxanthine, guanine and cytosine. Length of column 290 mm, diameter 19 mm. The dash line gives the ratio E_{2480}/E_{2700} and indicates where desoxyhypoxanthosine starts to become contaminated with desoxyguanosine. The area under the diagonal lines indicates the mixed fractions.

in this medium. It was thought that extraction with propanol as used by this author would not be of any greater advantage. Instead, after dissolving the residue in 20 ml of ammonium acetate buffer a chromatogram on Dowex as described below was run directly. The purinedesoxyribosides obtained from the chromatogram were combined with the corresponding main fractions. From the fractions containing thymidine water and buffer were removed and the dry residue was three times crystallized from methanol: ethanol. The crude product which weighed 2.12 g gave 1.31 g crystalline thymidine with the following analytical values: C = 49.9 %, H = 5.70 %, N = 11.5 %. Theoretical: C = 49.6 %, H = 5.83 %, N = 11.6 %.

The desoxycytidine fractions, which were obtained from the column by elution with 5 % ammonia were the most impure. After removal of water and ammonia by evaporation *in vacuo* it proved to be of great value to rerun the crude product on another Dowex 50 column (diameter 50 mm, length 100 mm) with 0.5 *N* HCl as solvent. The fractions containing desoxycytidine were located by their characteristic ultra violet light absorption which was measured in the Beckman quartz spectrophotometer. From the combined fractions the excess HCl was carefully removed by repeated evaporation *in vacuo*. The white residue proved to be the hydrochloride of desoxycytidine. It weighed 1.37 g. It was easily crystallized from methanol-ethanol. After two crystallisations 0.91 g desoxycytidine. HCl were obtained with the following analytical values: C = 40.8 %, H = 5.24 %, N = 15.6 %, Cl = 13.7 %. Theoretically: C = 41.0 %, H = 5.35 %, N = 15.9 %, Cl = 13.5 %.

Chromatography of desoxyribosides on Dowex 50. For each chromatogram of the three crude desoxyriboside fractions a column of Dowex 50 (mesh size ca 300) of 50 mm diameter and 140 mm length was used. The commercial ion exchanger was first washed with 4 *N* HCl, then water, 5 % ammonia and at last 0.1 *M* ammonium acetate buffer. The buffer was prepared by titrating

1 *M* ammonia with glacial acetic acid to pH 3.9, the pH of the solution being determined with the aid of a glass electrode. The buffer was diluted ten times before use.

When the effluent after the last washing reached a pH of 3.9 the crude desoxyriboside fraction was dissolved in 20—100 ml of the buffer, placed on the top of the column and run in by air pressure. The chromatogram was then run with the buffer. Ten ml fractions were taken from the beginning. After the appearance of thymidine the fraction size was approximately 50 ml. The fractions were analyzed by measuring their light absorption in the ultra violet with the Beckman quartz spectrophotometer. Fractions containing the same type of light absorption were combined. When the desoxyguanosine had come out from the column the solvent was shifted to 5 % ammonia. This caused desoxycytidine to come out immediately.

Fig. 1 shows a model chromatogram of this type run on a smaller scale.

Chromatography of desoxyribosides on starch. Chromatography on starch was used as a method of purification when only very small amounts of desoxyribosides were available. It was used instead of crystallisation and was always preceeded by a chromatogram on Dowex 50. Thus the desoxyribosides had already been separated and freed from most salts.

Fig. 2 shows a model experiment in which a mixture of all four desoxyribosides was subjected to chromatography. The starch column was prepared in the way described by Stein and Moore [13]. The chromatogram was run with butanol-water (86.5 parts butanol + 13.5 parts water). Two ml fractions were taken and analyzed by their ultra violet absorption in the usual way. The separation of desoxyhypoxanthosine and desoxyguanosine was incomplete. The mixed fractions containing both desoxyribosides could be indentified by determining the ratio of the light absorption at 2480 Å and 2 700 Å for each fraction. This ratio differs for the two desoxyribosides. The procedure was thus principally the same as that used by Reichard [14] for guanine and xanthine.

Criteria of purity of components. When large amounts of crystallized substances were available elementary analyses were carried out. After starch chromatograms, however, the desired degree of purity was only a relative one. This type of purification was used when the material contained the stable isotope N^{15}. The only desired purity was thus the purity from foreign nitrogen. This was determined in each case by reference to a ratio used previously [15], a comparison between the maximum light absorption and the amount of nitrogen present in the sample $\frac{E_{max}}{\gamma N/ml}$. These ratios were first determined on crystalline standard samples and found to be: thymidine (E_{max} at 2670 Å)

= 0.341; desoxyhypoxanthosine (E_{max} at 2490 Å) = 0.201; desoxyguanosine (E_{max} at 2550 Å) = 0.176; desoxycytidine (E_{max} at 2790 Å) = 0.303. All values were determined in $N/10$ HCl.

SUMMARY

A method is described for the preparation of desoxyribosides, after enzymatic hydrolysis of thymonucleic acid, by chromatography on Dowex 50 and on starch.

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Received July 14, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1054–1065

The Crystal Structure of Cobaltpyroborate

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X-ray studies on preparations, belonging to the CoO-rich region of the system CoO—B_2O_3, indicate ortho- and pyroborate as the only compounds formed. In a previous communication from this Institute [1] the crystal structure of the orthoborate is described. The present paper gives an account of the crystallographic arrangement of the pyroborate $Co_2B_2O_5$. As a result of this study the existence of the composite ion $B_2O_5^{4-}$ was proved.

Crystals of this borate have been mentioned by Ebelmen [2], Mallard [3], Burgess and Holt [4], Ouvrard [5], Guertler [6] among others. They are reported to be violet, double refracting, triclinic needles.

The crystals used in the present investigation were prepared by melting together the pure oxides in the molar proportions 2 : 1. They form very hard, reddish-violet prisms and needles. In this case well-shaped, prismatic crystals were picked out and a prism, suitable for taking single crystal photographs, was examined by the rotation and Weissenberg methods. Mo-K-radiation was used.

UNIT CELL AND SPACE GROUP

The external shape of the crystal speaks in favour of the triclinic symmetry, which was also proved by the Weissenberg photographs. Rotation and Weissenberg photographs were taken about the shortest axis, which was chosen as [100], and about [211]. Thereby the layer lines 0—4 and 0—8, respectively, were obtained. The connection between intensities of reflections from different layer lines of the former photographs were brought about by means of the reflections $0kl$ of the latter. The intensities were estimated visually, 4 : 2 : 1 being adopted as the intensity ratio of Mo-Kα_1, Mo-Kα_2 and Mo-Kβ radiation. Relative values of F^2_{hkl} were obtained by multiplying the intensity values with the factor $\cos^2\mu \cdot \sin\Upsilon / (1 + \cos^2 2\Theta)$ utilizing the curves given by Chia-Si Lu [7]. The temperature factor was neglected.

Fig. 1. Projection of the Patterson-function on the bc-plane, p(yz). The dotted line corresponds to the 0-level.

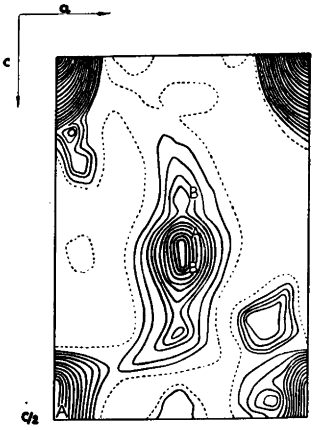


Fig. 2. Projection of the Patterson-function on the ac-plane, p(xz).

The linear and angular constants as calculated from the single crystal photographs are found to be:

$a = 3.16$ Å	$\alpha = 103.9°$
$b = 5.94$ Å	$\beta = 91.0°$
$c = 8.95$ Å	$\gamma = 92.0°$

These values correspond to the axial ratios 0.35 : 0.66 : 1. The unit cell containing 2 $Co_2B_2O_5$ has the volume 162.8 Å^3 and the calculated density 4.48. The observed density is 4.40.

Since there are no regular extinctions observed and no piezoelectric effect detected, C_i^1—$P\,\bar{1}$ must be the most probable space group.

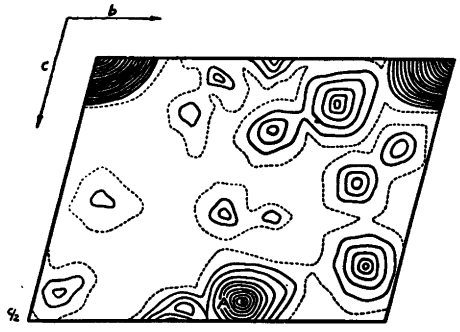


Fig. 3. The Harker-section P(0yz).

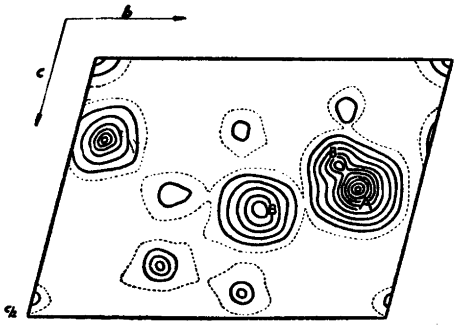


Fig. 4. The Harker-section P(½yz).

THE ATOMIC ARRANGEMENT OF COBALT AND OXYGEN

The fact that in the space group $P\bar{1}$ four heavy atoms, the contribution of which to the structure factor is dominating compared with that of the other atoms in the cell, give rise to two main peaks and two of half the size in the projections of the Patterson function on the axial planes, made it possible to determine the present approximate parameters of the Co atoms from $p(yz)$ and $p(xz)$. The map $p(yz)$ — Fig. 1 — shows two strong maxima (A) and four, which are about half as large — two of which, belonging to Co—Co vectors, are called (B). The projection $p(xz)$ — Fig. 2 — also contains two strong maxima and a few minor peaks, but here vector-overlapping takes place, so that it was impossible to make any distinctions at all between the weaker maxima. After having developed the function $p(xyz)$ into a set of sections, however, the different (A) and (B) maxima could be determined from $P(0yz)$ and $P(1/2\ yz)$ — Figs. 3 and 4. In that way the following arrangement of the Co atoms was obtained:

	x	y	z
2 Co^{2+} in (2i) = Co I	0.74	0.218	0.351
2 Co^{2+} in (2i) = Co II	0.25	0.375	0.103

By means of these values the more accurate ones, given below, could be obtained from a few successive calculations of the electron density projections $\varrho\ (xy)$ and $\varrho\ (yz)$:

	x	y	z
Co I	0.744	0.214	0.358
Co II	0.245	0.374	0.100

Simultaneously, the density distribution gave the following approximate parameters of the oxygen atoms:

	x	y	z
2 O^{2-} in (2i) = O I	0.25	0.706	0.052
2 O^{2-} in (2i) = O II	0.23	0.092	0.190
2 O^{2-} in (2i) = O III	0.75	0.483	0.253
2 O^{2-} in (2i) = O IV	0.58	0.853	0.284
2 O^{2-} in (2i) = O V	0.75	0.704	0.492

It was then possible to obtain the sign of the structure factor of most reflections with a high degree of certainty, especially as the influence of the oxygen atoms in general being too small to be of any importance compared with the contribution of the metal atoms.

FOURIER-REFINEMENT OF THE PARAMETERS

Instead of using threedimensional calculations of the electron density function to determine the final Co parameters, the values settled above were refined by means of "differential synthesis" according to Booth [8]. In making the calculations leading to the necessary system of equations, utilizing the about 1 100 reflections, two aids, devised by Hägg [9], have highly facilitated the work. The corrections were found to be:

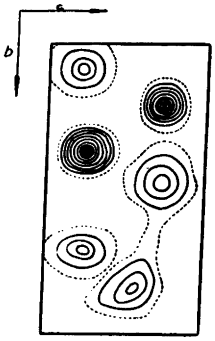
	Co I	Co II
ε_x	− 0.001 Å	0.000 Å
ε_y	− 0.001 Å	0.000 Å
ε_z	+ 0.002 Å	0.000 Å

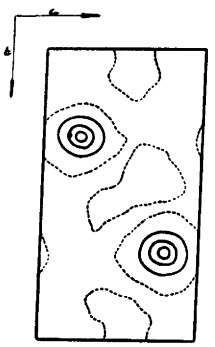
As a consequence of this result the coordinates of the Co atoms given above may be considered rather satisfactory.

The x-coordinates of the oxygen lattice derived above are not of the same accuracy as are y and z, owing to the fact that $\varrho\ (xy)$ is not free from overlapping, $\varrho\ (yz)$, however, is. To get final parameters the "bounded projection" method, devised by Booth [8], was applied for the xy-coordinates. The z-parameters were obtained from the Bragg projection $\varrho\ (yz)$, where consideration was taken to the influence on the sign of the structure factor of both the Co and oxygen atoms.

It is clear from $\varrho\ (yz)$ that a section through $z = 0.440$ passes a region with the electron density practically zero. On account of this, two "bounded projections" on the ab-plane were chosen, namely between the limits $z = 0$—0.440 and $z = 0.440$—0.560, respectively. These gave maps without overlapping, as is evident from the Figs. 5 and 6. The final z-parameters settled from $\varrho\ (yz)$ — Fig. 7 — were found to be nearly the same as those given without taking consideration of the influence of the oxygen atoms on the sign of the structure factor. The final oxygen parameters were found to be:

	x	y	z
O I	0.244	0.709	0.054
O II	0.208	0.092	0.190
O III	0.735	0.476	0.249
O IV	0.562	0.842	0.288
O V	0.770	0.698	0.489





The "bounded projections".

Fig. 5. $B^{z_2}_{z_1}$, *when* $z_1 = 0$ *and* $z_2 = 0.440$. *Fig. 6.* $B^{z_2}_{z_1}$, *when* $z_1 = 0.440$ *and* $z_2 = 0.560$.

THE ATOMIC ARRANGEMENT OF BORON

Pyroborates have not been investigated by x-ray methods earlier and, consequently, experimental data of the configuration of the pyroborate ion are lacking. Owing to the well known tendency of the boron to aquire a plane, triangular coordination of oxygen, one must expect the pyroborate ion to consist of two such triangles with a corner in common. From earlier investigations [10] on different borates it is known that the oxygen-oxygen distances within the boron-oxygen triangles are of about the length 2.2—2.4 Å. It can therefore be taken as granted that the four boron atoms must be placed in, or very near, the centres of gravity of the four nearly equilateral oxygen triangles with edges of about these dimensions, which exist in the cell. If the boron atoms are placed exactly in the centres of gravity they obtain the following parameters:

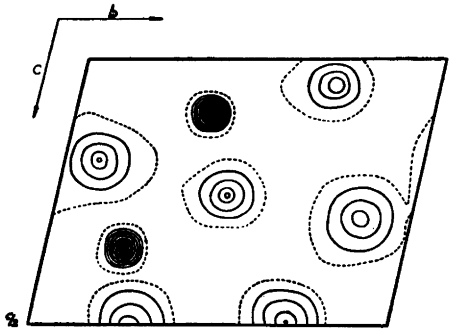


Fig. 7. Projection of the electron density parallel to [100].

	x	y	z
2 B^{3+} in (2i) = B I	0.67	0.67	0.34_5
2 B^{3+} in (2i) = B II	0.36	0.88	0.17

These parameters give boron positions corresponding to $B_2O_5^{4-}$-ions with the expected configuration.

DESCRIPTION OF THE STRUCTURE

The following structure is thus arrived at:
Cell content: 2 $Co_2B_2O_5$
Space group: C_i^1—$P\,\bar{1}$

	x	y	z
2 Co^{2+} in (2i) = Co I	0.743	0.213	0.360
2 Co^{2+} in (2i) = Co II	0.245	0.374	0.100
2 O^{2-} in (2i) = O I	0.244	0.709	0.054
2 O^{2-} in (2i) = O II	0.208	0.092	0.190
2 O^{2-} in (2i) = O III	0.735	0.476	0.249
2 O^{2-} in (2i) = O IV	0.562	0.842	0.288
2 O^{2-} in (2i) = O V	0.770	0.698	0.489
2 B^{3+} in (2i) = B I	0.67	0.67	0.34_5
2 B^{3+} in (2i) = B II	0.36	0.88	0.17

Projections of the structure on $(\bar{3}\bar{2}4)$ and parallel to [021] and $[2\bar{1}1]$ are given in the Figs. 8, 9 and 10.

By using the above parameters and atomic scattering factors according to *International tables*, the following agreement between calculated and observed $|F|$-values was obtained:

DISCUSSION OF THE STRUCTURE

The structure of $Co_2B_2O_5$ is built up by $B_2O_5^{4-}$-groups and Co^{2+}-ions, the latter surrounded by six oxygen atoms forming a deformed octahedron. The average space available per oxygen atom, given by the quotient between the volume of the unit cell and the number per cell of oxygen atoms, is 16.3 Å^3. Within the composite ion $B_2O_5^{4-}$, formed by two BO_3^{3-}-triangles, linked by sharing one oxygen atom, there exist very short interatomic distances. When calculated from the above parameters they are found to be:

hkl	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$	*hkl*	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$	*hkl*	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$
100	64	58	$24\bar{0}$	27	26	$20\bar{2}$	75	76
200	513	412	$25\bar{0}$	103	84	$20\bar{4}$	269	294
			$26\bar{0}$	24	24	$20\bar{7}$	230	200
010	122	102	$27\bar{0}$	140	112	$20\bar{8}$	145	190
020	243	252	$28\bar{0}$	74	48	$20\ \bar{11}$	147	172
040	76	66	320	199	184	302	328	316
050	108	92	330	215	202	303	154	134
070	159	142	340	171	148	305	165	145
0 12 0	128	105	350	140	143	306	148	102
			$32\bar{0}$	218	200	307	91	52
002	73	40	$33\bar{0}$	247	218	309	208	170
003	73	56	$34\bar{0}$	132	90	30 11	121	88
004	206	218	$35\bar{0}$	145	106	30 13	96	36
005	162	162	$37\bar{0}$	89	46	$30\bar{2}$	366	352
007	227	224	3 $\bar{10}$ 0	97	56	$30\bar{3}$	48	24
008	108	120	440	30	28	$30\bar{5}$	114	70
00 11	188	172	470	170	142	$30\bar{6}$	192	164
00 13	110	108	$42\bar{0}$	202	193	$30\bar{9}$	228	178
00 15	140	125	$45\bar{0}$	145	106	30 $\bar{10}$	101	82
00 17	63	56				30 $\bar{13}$	91	48
			101	76	86	402	54	32
110	18	28	102	440	504	403	106	84
120	248	268	103	222	252	404	181	114
130	214	216	105	110	144	405	158	120
140	145	164	106	126	158	407	202	146
150	169	160	109	260	260	409	96	72
170	56	40	10 11	116	111	40 11	110	82
180	81	48	10 13	131	160	$40\bar{3}$	25	26
190	68	46	$10\bar{2}$	567	536	$40\bar{4}$	172	116
1 10 0	135	105	$10\bar{3}$	118	92	$40\bar{8}$	181	124
1 11 0	53	68	$10\bar{5}$	156	162			
$1\bar{1}0$	33	40	$10\bar{6}$	145	196	$0\bar{1}1$	69	52
$1\bar{2}0$	204	243	$10\bar{9}$	223	204	$0\bar{2}1$	70	76
$1\bar{3}0$	306	372	10 $\bar{11}$	86	40	$0\bar{3}1$	174	196
$1\bar{4}0$	182	192	10 $\bar{13}$	131	114	$0\bar{4}1$	279	304
$1\bar{5}0$	185	168	10 $\bar{15}$	84	46	$0\bar{6}1$	158	184
$1\bar{7}0$	81	92	10 $\bar{17}$	119	92	0 $\bar{11}$ 1	178	106
$1\bar{8}0$	76	48	201	135	166	0 $\bar{12}$ 1	31	28
1 $\bar{10}$ 0	106	60	202	18	44	011	317	328
220	155	192	203	21	24	021	194	252
230	83	60	204	179	236	031	146	176
250	130	148	205	102	126	041	93	120
270	209	216	207	251	324	051	64	44
$2\bar{1}0$	163	192	208	98	80	061	179	156
$2\bar{2}0$	179	176	20 11	164	180	081	223	194
$2\bar{3}0$	37	16	20 12	63	32	091	76	56
			20 13	127	98			

hkl	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$	*hkl*	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$	*hkl*	$\lvert F\rvert_{Calc.}$	$\lvert F\rvert_{Obs.}$
$0\bar{1}2$	140	128	$0\bar{4}5$	213	188	$0\bar{2}9$	115	126
$0\bar{2}2$	325	328	$0\bar{5}5$	119	100	$0\bar{3}9$	65	56
$0\bar{3}2$	399	496	$0\bar{6}5$	154	156	$0\bar{4}9$	196	184
$0\bar{4}2$	100	140	$0\bar{7}5$	27	32	$0\bar{5}9$	210	195
$0\bar{5}2$	177	164	$0\bar{8}5$	32	50	019	132	140
0 $\bar{1}0$ 2	123	106	$0\bar{9}5$	166	128	029	114	94
012	28	45	015	355	348	039	190	174
022	142	124	025	101	88	079	157	90
032	79	92	035	286	242	$0\bar{1}$ 10	82	100
042	153	152	045	59	26	$0\bar{3}$ 10	155	156
052	164	172	055	6	vw	$0\bar{4}$ 10	146	160
072	81	32	065	51	44	$0\bar{8}$ 10	145	164
092	132	98	085	113	108	02 10	92	77
0 10 2	146	86	$0\bar{1}6$	219	232	03 10	90	64
$0\bar{1}3$	234	200	$0\bar{2}6$	57	81	04 10	220	184
$0\bar{2}3$	252	208	$0\bar{3}6$	201	208	$0\bar{2}$ 11	138	152
$0\bar{3}3$	87	96	$0\bar{5}6$	51	40	$0\bar{3}$ 11	42	20
$0\bar{4}3$	60	36	$0\bar{7}6$	46	24	$0\bar{7}$ 11	193	165
$0\bar{5}3$	34	32	$0\bar{8}6$	161	140	03 11	104	78
$0\bar{6}3$	205	224	0 $\bar{1}0$ 6	121	104	05 11	184	134
$0\bar{7}3$	142	116	016	84	99	$0\bar{1}$ 12	122	128
$0\bar{8}3$	80	76	026	299	316	$0\bar{4}$ 12	69	56
$0\bar{9}3$	106	84	046	204	192	$0\bar{5}$ 12	44	24
013	136	156	056	104	88	$0\bar{6}$ 12	139	118
023	88	72	066	51	32	01 12	224	204
033	187	180	096	158	81	06 12	114	75
043	236	208	$0\bar{1}7$	114	124	$0\bar{3}$ 13	100	67
053	221	196	$0\bar{2}7$	305	324	$0\bar{5}$ 13	101	80
063	176	143	$0\bar{3}7$	78	44	0 $\bar{1}0$ 13	110	92
$0\bar{1}4$	80	52	$0\bar{4}7$	59	52	02 13	172	142
$0\bar{2}4$	44	28	$0\bar{7}7$	142	126	03 13	122	98
$0\bar{3}4$	21	12	$0\bar{8}7$	21	20	$0\bar{1}$ 14	132	108
$0\bar{5}4$	224	224	$0\bar{9}7$	141	134	$0\bar{2}$ 14	196	163
$0\bar{6}4$	162	144	017	171	152	$0\bar{3}$ 14	87	56
$0\bar{7}4$	113	116	037	53	36	$0\bar{4}$ 14	122	104
024	238	252	047	53	52	$0\bar{2}$ 15	69	56
034	34	48	057	154	144	$0\bar{3}$ 15	70	40
044	137	144	$0\bar{1}8$	259	276	$0\bar{4}$ 16	75	52
054	174	168	$0\bar{4}8$	138	120	$0\bar{6}$ 16	96	88
064	90	64	$0\bar{5}8$	142	164	01 16	94	70
074	224	196	$0\bar{6}8$	133	136	02 17	151	86
094	89	46	018	132	122			
$0\bar{2}5$	52	60	028	87	104			
$0\bar{3}5$	7	vw	068	165	96			
			078	158	94			

O I –O II_1	2.31 Å
O I –O IV	2.25 Å
O II_1–O IV	2.22 Å
O III –O IV	2.20 Å
O III –O V	2.21 Å
O IV –O V	2.26 Å
B I –O III	1.28 Å
B I –O IV	1.30 Å
B I –O V	1.28_5 Å
B II –O I	1.30 Å
B II –O II′	1.33 Å
B II –O IV	1.29 Å

In the deformed octahedron, formed by the oxygen atoms, surrounding the Co^{2+}-ions, the interatomic distances calculated are:

Co I –O II	2.22 Å	Co II –O I	2.12 Å
Co I –O II_2	2.13 Å	Co II –O I′	2.02 Å
Co I –O III	2.02 Å	Co II –O I_2'	2.13 Å
Co I –O IV_1	2.20 Å	Co II –O II	2.02 Å
Co I –O V′	2.14 Å	Co II –O III	2.00 Å
Co I –O V_2'	2.01 Å	Co II –O III_2	2.12 Å
O II –O III	2.71 Å	O I –O I′	2.98 Å
O II –O II_2	3.16 Å	O I –O I_2'	2.82 Å
O II_2–O IV_1	2.75 Å	O II –O I′	3.19 Å
O II –O V′	2.84 Å	O II –O I_2'	3.18 Å
O III –O II_2	2.68 Å	O II –O III	2.71 Å
O III –O V_2'	3.18 Å	O II –O III_2	2.68 Å
O III –O V′	3.19 Å	O III –O I	2.92 Å
O V′–O IV_1	3.18 Å	O III –O I′	2.67 Å
O V_2'–O IV_1	3.01 Å	O III –O I_2	2.95 Å
		O III –O III_2	3.16 Å

The denomination of the oxygen atoms in the one half-cell is OI—OV and in the other OI′—OV′. The subscripts 1 and 2 refer to equivalent positions in the surrounding cells in horizontal and vertical direction, respectively.

From the interatomic distances given above it is seen that the connecting forces within the $B_2O_5^{4-}$-groups undoubtedly must be much stronger than the other bonds. Accordingly, there is direct evidence for the existence of these groups in the lattice, and for the chemical formula $Co_2(B_2O_5)$.

Formally the structure may be described as constructed of two-dimensional layers formed by the $B_2O_5^{4-}$-groups, as is illustrated by the Figs. 8, 9 and 10. These layers are extended in $(\bar{1}\bar{1}1)$ — Fig. 10 — and are a little rough because of the fact that the separate groups included are not parallel to $(\bar{1}\bar{1}1)$ but

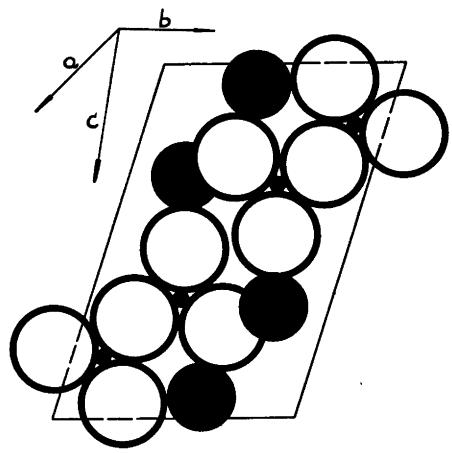


Fig. 8. Projection of the structure on $(\bar{3}\bar{2}4)$.

The large black spheres represent the cobalt atoms,
» *small* » » » » *boron* »
» *large white* » » » *oxygen* »

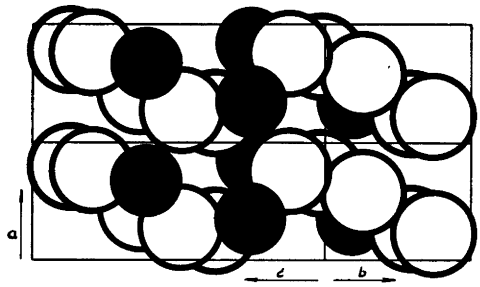


Fig. 9. Projection of the structure parallel to [021].

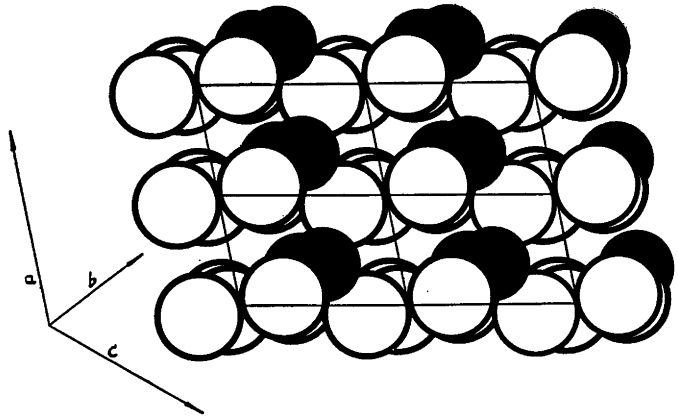


Fig. 10. Projection of the structure parallel to $[2\bar{1}1]$.

nearly to $(\bar{3}\bar{2}4)$ — Fig. 8. The connection between the groups within one layer and the binding between the different layers is maintained by the Co^{2+}-ions. From the figures it is also visible that a line through the centres of gravity of the two triangles in a group and the corner in common, is not a straight one. The angle is measured to be about 153°. The planes of these triangles are furthermore slightly screwed in opposite directions to each other related to $(\bar{3}\bar{2}4)$ — Figs. 9, 10 and 11.

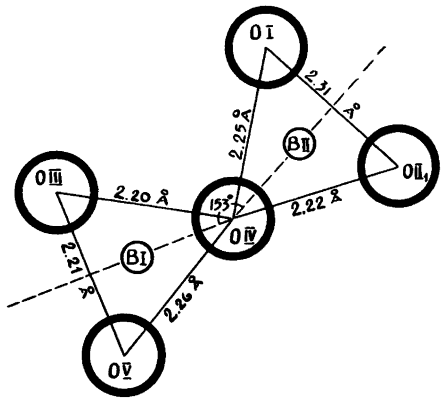


Fig. 11. $B_2O_5^{4-}$-ion projected on $(\bar{3}\bar{2}4)$. This plane contains the two boron atoms and the central oxygen atom. The planes of the two triangles are screwed about the indicated axial lines in opposed directions about 7 degrees from the plane of projection.

SUMMARY

The crystal structure of cobaltpyroborate is triclinic (space group C_i^1—$P\bar{1}$) with 2 $Co_2(B_2O_5)$ in the unit cell. Dimensions and atomic positions have been determined. The structure contains pyroborate groups $B_2O_5^{4-}$, formed by two BO_3^{3-}-triangles with one oxygen atom in common — Fig. 11.

The present study was carried out at the Institute of Inorganic Chemistry of the University of Uppsala. To the Head of this Institute, Professor G. Hägg, I wish to express my sincere gratitude for valuable discussions and never failing support. I am also very grateful to his co-workers, especially Dr I. Lindqvist, for many valuable conversations.

To the Head of the Institute of Silicate Research at Chalmers Techn. Univ., Professor J. Arvid Hedvall, I wish to form my special gratitude for the kind interest he always has devoted to my work.

The research has been financially supported by the Foundation of the Member of the magistrates' count and Mrs Ernst Colliander, which support is gratefully acknowledged.

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Received August 29, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1066–1074

Crystal Structure Studies on Anhydrous Sodium Molybdates and Tungstates

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In a thorough thermal investigation of the systems Na_2MoO_4—MoO_3 and Na_2WO_4—WO_3, Hoermann[1] has established the existence of *i. a.* Na_2MoO_4, $Na_2Mo_2O_7$, Na_2WO_4 and $Na_2W_2O_7$, the crystal structures of which will be given in this paper. All crystals have been obtained from melts in accordance with Hoermann[1].

SODIUM MOLYBDATE AND TUNGSTATE

These two compounds (in the notation of Hoermann[1], the isotropic δ-phases), which are isomorphous, have been studied by powder photographs obtained with monochromatized Cu—K_α radiation. Both compounds were easily shown to be regular with a cube edge of $a = 8.99$ Å. Na_2MoO_4 and Na_2WO_4, thus seem to be isomorphous with Ag_2MoO_4, which has a spinel structure[2] with $a = 9.26$ Å. In agreement with Ag_2MoO_4 only $h\,k\,l$ with h, k and l all odd or all even appear on the photographs. A calculation of I ($h\,k\,l$), for Na_2WO_4 under this assumption gives values in agreement with the observed ones (Table 1).

Na_2MoO_4 and Na_2WO_4 thus have a spinel structure quite as Ag_2MoO_4, while Li_2MoO_4 has a phenacite structure[3]. This fact may give a new insight in the morphotropy of A_2BX_4 structures, which has been discussed by Goldschmidt[4]. (A review is given by Evans[5].) The theory has been put forward, that the transition from phenacite to spinel structure is brought about by the polarizing power of Ag^+. As Na_2MoO_4 also has a spinel structure and Na^+ undoubtedly has a smaller polarizing power than Li^+, this is obviously not correct. The most natural way to treat this problem seems to be a consideration of the ionic radii. Ag^+ and Na^+ have larger ionic radii than Li^+, and therefore may require a 6-fold octahedral coordination instead of the 4-fold

tetrahedral in the phenacite structure. This type of spinel structure A_2BX_4, where A has a larger ionic radius than B, appears more natural than the classical one with B > A, although B has a lower oxygen coordination. The spinel structure should thus be considered as related to the olivine structure (*cf.* 5), both having the same types of coordination around A and B. The olivine structure gives, however, a more well defined MoO_4^{2-} ion, while the spinel structure has more double oxide character.

With an idealized spinel structure ($x_0 = 3/8$) the Mo—O (W—O) distances will be 2.0 Å, Na—O = 2.3 Å and O—O = 3.2 Å.

Table 1. Powder photograph of Na_2WO_4 taken with CuK_α radiation.

h k l	$\sin^2\Theta$		intensities		*h k l*	$\sin^2\Theta$		intensities	
	obs.	calc.	obs.	calc.		obs.	calc.	obs.	calc.
1 1 1	0.0219	0.0219	v st	193	5 3 1	0.2568	0.2566	m	54
2 0 0	0.0587	0.0586	v st	209	6 0 0 4 4 2	—	—	—	0
2 2 0	—	—	—	0	6 2 0	0.2933	0.2933	m	53
3 1 1	0.0799	0.0806	v st	135	5 3 3	0.3145	0.3152	w	31
2 2 2	—	—	—	1	6 2 2	—	—	—	1
4 0 0	0.1165	0.1173	v w	16	4 4 4	0.3510	0.3518	v w	5
3 3 1	0.1387	0.1393	m	59	7 1 1 5 5 1	0.3733	0.3738	w	38
4 2 0	—	—	—	0	6 4 0	—	—	—	0
4 2 2	0.1760	0.1759	v st	104	6 4 2	0.4104	0.4105	st	88
5 1 1 3 3 3	0.1981	0.1979	st	71	7 3 1 5 5 3	0.4321	0.4323	st	79
4 4 0	0.2347	0.2346	st	79					

SODIUM DIMOLYBDATE AND DITUNGSTATE

Unit cell. Space group

The crystal structure of these two isomorphous compounds, which have identical unit dimensions, has been determined from an X-ray crystallographic investigation of a needle-shaped dimolybdate single crystal. Rotation and Weissenberg photographs were taken around the *a*-axis with Cu-*K* radiation. The crystal was found to be orthorhombic with $a = 7.17$ Å, $b = 11.83$ Å and $c = 14.70$ Å. The density is 3.6, corresponding to eight formula units $Na_2Mo_2O_7$ in the cell. Reflections *h k l* are missing if $h + k$ is odd, *h k* 0 if *h* or *k* is odd,

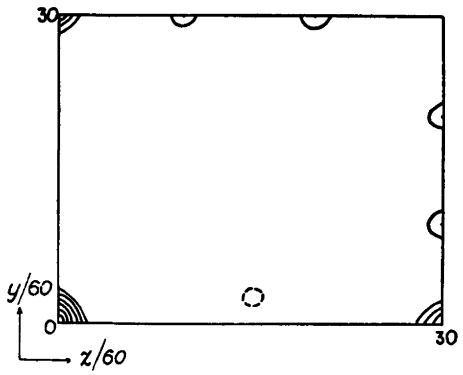

Fig. 1. Patterson function P (0, y, z) of the sodium dimolybdate.

$h\ 0\ l$ if h or l is odd, and $0\ k\ l$ if k is odd. As an investigation with a dynamic method showed no signs of piezo-electricity, the most probable space group is D_{2h}^{18}—*Cmca*. The |F| values were calculated from the estimated intensities according to Lu[6].

Molybdenum and sodium positions

The first problem is to determine the positions of 16 Mo atoms in the unit cell. The coordinates of the general position (16 *g*), which indicates the most interesting Patterson cuts P (x, y, z) to calculate, are the following:

(x, y, z) $(x, \bar{y}, \bar{z})$ $(x, \frac{1}{2}+y, \frac{1}{2}-z)$ $(x, \frac{1}{2}-y, \frac{1}{2}+z)$
$(\bar{x}, \bar{y}, \bar{z})$ $(\bar{x}, y, z)$ $(\bar{x}, \frac{1}{2}-y, \frac{1}{2}+z)$ $(\bar{x}, \frac{1}{2}+y, \frac{1}{2}-z)$
$(\frac{1}{2}+x, \frac{1}{2}+y, z)$ $(\frac{1}{2}+x, \frac{1}{2}-y, \bar{z})$ $(\frac{1}{2}+x, y, \frac{1}{2}-z)$ $(\frac{1}{2}+x, \bar{y}, \frac{1}{2}+z)$
$(\frac{1}{2}-x, \frac{1}{2}-y, \bar{z})$ $(\frac{1}{2}-x, \frac{1}{2}+y, z)$ $(\frac{1}{2}-x, \bar{y}, \frac{1}{2}+z)$ $(\frac{1}{2}-x, y, \frac{1}{2}-z)$

P(0, y, z) will give all equivalent Mo—Mo vectors [2 y_{Mo}, 2 z_{Mo}], [$\frac{1}{2},\frac{1}{2}$—2 z_{Mo}], and [$\frac{1}{2}$—2 y_{Mo},$\frac{1}{2}$], (*cf.* the more complete discussion in ref. 7), and was therefore calculated. (Fig. 1.)

The two largest maxima have the (y, z) coordinates ($0,\frac{1}{2}$) and ($\frac{1}{2}$, 0). Then there are four maxima at (1/6, 1/2), (1/3, 1/2), (1/2, 1/6), and (1/2, 1/3). Finally there is one still smaller maximum at (1/12, 1/4).

The three maxima ($y,\frac{1}{2}$) may correspond to $y_{Mo} = 1/12$, 1/6 or 1/4 (in $0 \leq y \leq 1/4$) and the three maxima (1/2, z) to $z_{Mo} = 1/12$, 1/6 or 1/4 (in $0 \leq z \leq 1/4$). These two triplets can be combined to give the following [$2y_{Mo}$, $2z_{Mo}$] values: (1/6, 1/6) (1/6, 1/3) (1/6, 1/2) (1/3, 1/6) (1/3, 1/3) (1/3, 1/2) (1/2, 1/6) (1/2, 1/3) and (1/2, 1/2), of which (1/6, 1/2) (1/3, 1/2) (1/2, 1/6) and (1/2, 1/3)

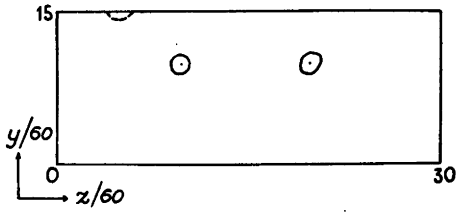

Fig. 2. Patterson function P (¼, y, z) of the sodium dimolybdate.

are found in P (0, y, z). The only combinations possible are evidently $y_{Mo} = 1/12$ or $1/6$ with $z_{Mo} = 1/4$, and $y_{Mo} = 1/4$ with $z_{Mo} = 1/12$ or $1/6$, which may correspond to positions 8 (e) and 8 (f):

8 (e): (1/4, y, 1/4) (3/4, $\bar{y}$, 3/4) (3/4, y, 1/4) (1/4, $\bar{y}$, 3/4)
(3/4, ½ + y, 1/4) (1/4, ½—y, 3/4) (1/4, ½ + y, 1/4) (3/4, ½—y, 3/4)
with $y = 1/12$ or $1/6$

8 (f): (0, y, z) (0, $\bar{y}$, $\bar{z}$) (½, y, ½—z) (½, $\bar{y}$, ½ + z)
(½, ½ + y, z) (½, ½—y, $\bar{z}$) (0, ½ + y, ½—z) (0, ½—y, ½ + z)
with $y = 1/4$ and $z = 1/12$ or $1/6$.

In order to confirm this assumption and to get a choice between the two alternatives for each position, P (1/4, y, z) was calculated (Fig. 2). The largest maxima obtained are situated at (1/6, 1/6) and (1/6, 1/3). Besides there is a smaller maximum at (1/4, 1/12).

In the following discussion we treat the interatomic vectors between Mo atoms in $x = 0$ and $x = 1/4$. In $x = 0$ there are four Mo atoms in 8 (f), having the (y, z) coordinates (1/4, 1/12) (3/4, 11/12) (3/4, 5/12) (1/4, 7/12) or (1/4, 1/6) (3/4, 5/6) (3/4, 1/3) (1/4, 2/3) and in $x = 1/4$ there are four Mo atoms in 8 (e): (1/12, 1/4) (11/12, 3/4) (5/12, 3/4) (7/12, 1/4) or (1/6, 1/4) (5/6, 3/4) (1/3, 3/4) (2/3, 1/4).

The sets derived from (1/4, 1/12) in $x = 0$ and (1/12, 1/4) in $x = 1/4$ should give the following maxima in P (1/4, y, z): (1/6, 1/6) (1/6, 1/3) quite in agreement with the results obtained.

(1/4, 1/12) and (1/6, 1/4) should require *e. g.* (1/12, 1/6) which is not obtained,

(1/4, 1/6) and (1/12, 1/4) should require *e. g.* (1/6, 1/12) which is not obtained,

(1/4, 1/6) and (1/6, 1/4) should require *e. g.* (1/12, 1/12) which is not obtained.

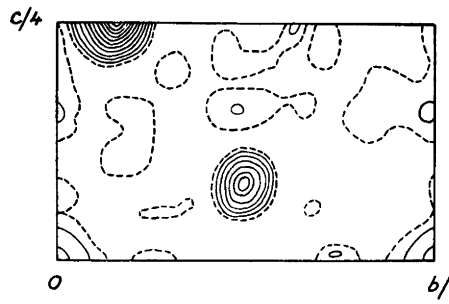

Fig. 3. Electron density projection ϱ (y, z) of the sodium dimolybdate.

A calculation of the electron density projection ϱ (y, z) definitely confirmed that the Mo atoms are placed in 8 (e) with $y = 1/12$, and in 8 (f) with $y = 1/4$ and $z = 1/12$. (Fig. 3.)

On the electron density projection map, ϱ (y, z), there appeared some small maxima (with a height of about 1/10 of the Mo peaks), which possibly could be interpreted as Na or O atoms. To test the reliability of ϱ (y, z), a new ϱ (y, z) was calculated from the theoretical intensities of only those reflections which were used in the actual ϱ (y, z) summation. It then was evident that almost all the small maxima were due to termination effects of a type also found by Magnéli [8]. The only clearly real maximum was situated at (0, 0). Three-dimensional electron density calculations ϱ $(0, y, z)$ and ϱ $(\frac{1}{4}, y, z)$ confirmed the Mo positions, but indicated that there was not any chance to determine the Na and O positions in this way.

In P $(0, y, z)$ and P $(\frac{1}{4}, y, z)$, however, there remain to interpret two obvious peaks at (0, 1/12, 1/4) and (1/4, 1/4, 1/12), corresponding to (0, $\pm$ 1/12, $\pm$ 1/4) and ($\pm$ 1/4, $\pm$ 1/4, $\pm$ 1/12) if care is taken of the whole cell. If now the vector [0, 1/12, 1/4] is added to the Mo position (0, 1/4, 1/12), the point (0, 1/3, 1/3) is reached. The vector [$\overline{1/4}$, 1/4, 1/12] added to the Mo position (1/4, 1/12, 1/4) will also give (0, 1/3, 1/3). In the same way [1/4, $\overline{1/4}$, $\overline{1/12}$] added to (0, 1/4, 1/12) will give (1/4, 0, 0), which point is also reached by adding [0, $\overline{1/12}$, $\overline{1/4}$] to (1/4, 1/12, 1/4). Just that point was found in ϱ (y, z). The two vectors cannot be combined with the Mo atoms to give any other new point. If the Na atoms are placed in 8 (d) with $x = 1/4$ and in 8 (f) with $y = 1/3$ and $z = 1/3$, the multiplicity of the Mo—Na vectors accounts for the appearance of the peaks in P (x, y, z). The correctness of this assumption is confirmed below.

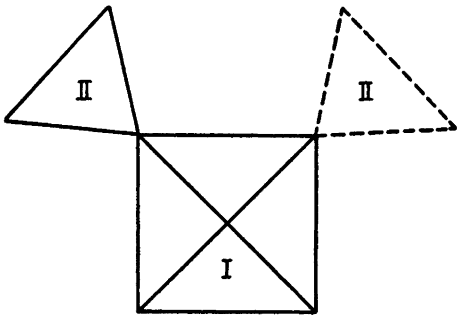

Fig. 4. The Structure of $Mo_2O_7^{2-}$.

I: MoO_6 *octahedra forming chains perpendicular to the plane of the figure.*

II: MoO_4 *tetrahedra sharing corners with each of two* MoO_6 *octahedra in the chain, with Mo in the planes* $x = 0$ *(full lines) and* $x = 0.5$ *(dotted lines).*

Oxygen positions. Crystal structure

In some other crystal structure studies on polymolybdates, the oxygen coordination has been worked out by considering the Mo-Mo distances corresponding to MoO_6 octahedra sharing edges or corners [7,9,10]. The Mo-Mo distances in $Na_2Mo_2O_7$ were therefore calculated. The Mo atoms in 8 (*e*) (I in fig. 4, dotted circles in fig. 5) are placed in straight lines parallel to the *a*-axis ($\perp$ the *b c* plane) with a distance of $1/2\ a = 3.62$ Å. Each Mo_I atom has two Mo_{II} neighbours from 8 (*f*) (II in fig. 4, full drawn circles in fig. 5), 3.63 Å apart. Distances of this magnitude are found as corresponding to $MoO_6(WO_6)$ octahedra sharing corners in infinite zig-zag chains in several compounds: 3.69 Å in MoO_3 [11], 3.64 Å in WO_3 [11], 3.71 Å in $MoO_3 \cdot 2H_2O$ [9]. It may also correspond to MoO_6 octahedra and MoO_4 tetrahedra sharing corners: 3.73—3.79 Å in Mo_4O_{11} [12]. Finally it might correspond to MoO_4 tetrahedra sharing corners: Mo-O is 1.8 Å in some normal molybdates containing MoO_4 complexes.

The problem of the oxygen coordination around Mo can now be approached from this point of view, investigating all possibilities in agreement with the Mo-Mo distances obtained. At first we regard the infinite 3.62 Å Mo-Mo chain in the direction of the *a*-axis. If all these distances correspond to Mo-O-Mo, it is easy to see that MoO_4 tetrahedra cannot build up the straight chains. A sharing of corners within groups of each two MoO_4 tetrahedra should in itself give Mo_2O_7 complexes, making it impossible to get a coordination around Mo_{II} giving the net formula $Mo_2O_7^{2-}$ for the compound. Finally one has the possibility of Mo_2O_7 complexes being formed by couples of Mo_IO_4 and $Mo_{II}O_4$ tetrahedra. This implies that there is no sharing of oxygen between the Mo_I atoms in the chain. Due to the mirror plane in $x = 0$, however, every Mo-O polyhedron around Mo_{II} must share oxygens with two Mo_I polyhedra (in $x = 0.25$ and $x = 0.75$), which definitely excludes the possibility of having

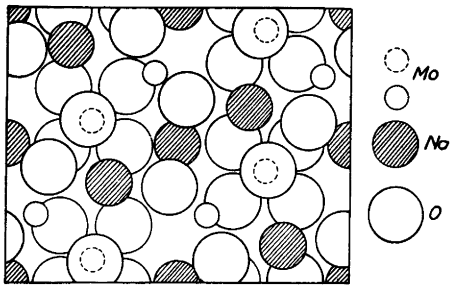

Fig. 5. Structure of sodium dimolybdate showing atoms with $x = 0$ (upper layer) and $x = 0.25$ (lower layer). The hidden atoms in the lower layer are denoted by dotted circles.

tetrahedra in I. (Tetrahedra in I and tetrahedra in II will give Mo_2O_6, tetrahedra in I and octahedra in II will give $Mo_2O_8^{4-}$.) It can thus be stated that there are MoO_6 octahedra in I. Further it seems most reasonable to assume that there are infinite Mo-O-Mo chains in the direction of the a-axis in agreement with earlier data on interatomic distances, *v. supra.* (If the octahedra shared edges in the chain direction they would be extremely distorted.) The only possibility to obtain a formula $Mo_2O_7^{2-}$ is then to place MoO_4 tetrahedra in 8 (*f*) (II), sharing corners with each of two MoO_6 octahedra in the chain (Fig. 4 and 6). This structure requires some distortion of the MoO_6 octahedra in order to get reasonable O—O distances, so the four oxygens in the plane of Mo_I can not be placed in $x = 0.25$ but in $x = 0.21$ and 0.29.

If we consider the sodium positions earlier arrived at as correct we can attack the problem in another way. Putting the Mo-O distances to at least 1.7 Å and Na-O to at least 2.3 Å, and taking care of the symmetry of the space group, the space available for oxygen atoms can be investigated. Such an investigation gives only two possible arrangements for the oxygen atoms, one of which is in agreement with the structure in Fig. 4. (The other arrangement corresponds to a 90° rotation of the MoO_6 octahedra, but gives no possible coordination around Mo_{II}.) After adjustments of the O and Na parameters in order to get the most reasonable interatomic distances, the structure shown in fig. 5 was obtained. The parameters are:

8 Mo_I	in	8(*e*) :	$x = 0.25$	$y = 0.08$	$z = 0.25$
8 Mo_{II}	in	8(*f*) :	$x = 0.00$	$y = 0.25$	$z = 0.08$
8 Na_I	in	8(*d*) :	$x = 0.25$	$y = 0.00$	$z = 0.00$
8 Na_{II}	in	8(*f*) :	$x = 0.00$	$y = 0.36$	$z = 0.30$
16 O_I	in	16(*g*) :	$x = 0.21$	$y = 0.21$	$z = 0.16$
16 O_{II}	in	16(*g*) :	$x = 0.21$	$y = 0.49$	$z = 0.34$
8 O_{III}	in	8(*f*) :	$x = 0.00$	$y = 0.08$	$z = 0.27$
8 O_{IV}	in	8(*f*) :	$x = 0.00$	$y = 0.41$	$z = 0.13$
8 O_V	in	8(*f*) :	$x = 0.00$	$y = 0.35$	$z = 0.49$

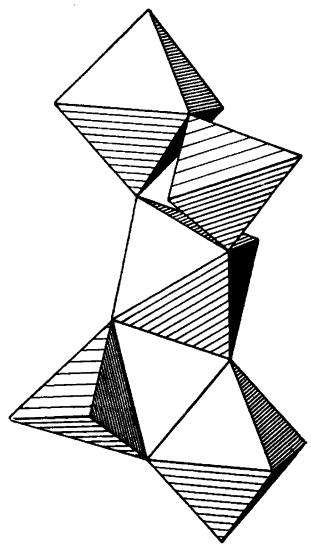

Fig. 6. Part of the infinite dimolybdate or ditungstate chain $Mo_2O_7^{2-}$ or $W_2O_7^{2-}$. Idealized drawing with regular polyhedra.

Each Mo_I atom is surrounded by 6 oxygen atoms at the distances 1.80 Å, 1.81 Å and 2.06 Å; each Mo_{II} atom has four oxygen neighbours, 1.78 Å and 1.93 Å apart.

The sodium atoms each have six oxygen neighbours (distorted octahedra) at distances of 2.4—2.6 Å. The O—O distances vary between 2.4 and 3.6 Å (*cf.* 2.7—3.5 Å in MoO_3[11] and 3.2 Å in Na_2MoO_4).

Discussion of the structure

The ion $Mo_2O_7^{2-}(W_2O_7^{2-})$ is evidently not a discrete ion built up by double tetrahedra, but it is an infinite complex ion forming chains in the crystals parallel with the *a*-axis. This is in agreement with the physical evidence that this compound always crystallizes as long needles with the *a*-axis in the needle direction. The structure of one chain is shown in fig. 4 and 6, where it is seen how the tetrahedra are attached to the chain of octahedra.

A comparison of this structure with the discrete $Mo_7O_{24}^{6-}$ and $Mo_8O_{26}^{4-}$ ions found in crystals obtained from water solutions [7, 10], clearly shows the quite different states of molybdates in melts and water solutions.

In an earlier paper [7], it has been proposed that in the molybdates crystallizing from melts, the ratio Mo:O determines whether the coordination around Mo is tetrahedral (as for Mo:O = 1 : 4) or octahedral (as for Mo:O = 1 : 3). This salt (with Mo:O = 1 : 3.5) is evidently a limiting case as it contains both tetrahedra and octahedra.

It is also interesting to compare the molybdates and tungstates with complex oxygen salts of related elements. The structure of *e.g.* $K_2Cr_2O_7$ has not been determined, but as it crystallizes from acid water solutions of chromates, where probably $Cr_2O_7^{2-}$ ions exist, it seems reasonable to assume that the dichromates contain discrete $Cr_2O_7^{2-}$ ions built up as double tetrahedra. If we on the other hand consider the uranates, it is known that even normal uranates, *e.g.* $BaUO_4$, contain UO_6 octahedra forming two-dimensional infinite UO_4^{2-} ions [13].

SUMMARY

Na_2MoO_4 and Na_2WO_4 have been shown to have spinel structure. The crystal structure of $Na_2Mo_2O_7$ and $Na_2W_2O_7$ has been determined. It is built up by infinite $Mo_2O_7^{2-}$ ions, formed by MoO_4 tetrahedra, each sharing two oxygens with a chain of MoO_6 octahedra sharing corners.

I wish to thank Mr Bengt Lindqvist for valuable aid with the calculations. The work has been supported financially by the Swedish Natural Science Research Council, which support is here gratefully acknowledged. Finally I wish to thank Professor G. Hägg for the facilities put at my disposal.

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Received July 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1075–1079

Investigations on Plasmin

IV. Some Experiments on Activation

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The activation mechanism of plasmin, the proteolytic enzyme in blood, is still rather obscure in spite of several investigations. On the basis of recent work carried out by Christensen and MacLeod [1], Loomis *et al.*[2], Permin [3], and Macfarlane and Pilling [4], the following nomenclature now seems to be generally accepted.

Plasminogen is the inactive precursor present in the euglobin fraction.

Antiplasmin is the plasmin inhibitor, present in the albumin fraction and removable with chloroform.

Antiplasminogen — the naturally occurring plasminogen inhibitor that prevents the conversion of plasminogen to plasmin.

Streptokinase is the exotoxin produced by certain strains of hemolytic streptococci, catalysing the conversion of plasminogen to plasmin.

Fibrinokinase — activating factor(s) in certain tissue cells.

Among the investigations on activating and inhibiting factors the following works may be mentioned. Streptokinase and fibrinokinase are considered to promote the conversion of plasminogen to plasmin directly, while chloroform destroys the plasmin inhibitor after which plasminogen autocatalytically is transformed slowly to plasmin (Christensen [5]). Ungar and Mist [6] observed an increase in fibrinolytic activity in serum from normal guinea pigs by mixing either with peptone, agar, hyaluronic acid, chondroitin sulfuric acid, glycogen or small amounts of heparin. This activation of plasmin required the presence of some serum factor which is non-precipitable with the euglobin fraction and is destroyed by heating at 56° C. Guillaumie [7] found a reduction of the proteolytic inhibiting power in serum after heating to 56° C, and Grob [8] found that the heating of diluted serum at 80° C resulted in a better medium for bacterial

growth. It is believed that this is at least partly due to the destruction of the serum antiprotease. Herberts [9] has reported an increase in proteolytic activity in lung extracts from shocked guinea pigs on heating to 60° C. In all these cases the activation seems to occur through the destruction of the plasmin inhibitor. Grob [10] found that both reducing and oxidizing agents decreased the proteolytic activity. Hultin and Lundblad [11] probably proved the existence of antiplasminogen, which is assumed by Loomis *et al.*[2].

This paper will report some experiments on activation by oxidizing agents together with some results obtained from experiments with plasmin inhibitor.

EXPERIMENTAL

The plasmin was prepared from bovine serum, according to Loomis *et al.*[2], treated with chloroform and lyophilized. The proteolytic activity was measured viscosimetrically on gelatin at 35.5° and calculated according to Hultin's formula as reported earlier by the present writer [12]. The dry plasmin preparation was stored at — 20° C and dissolved in physiological saline immediately before use. A one per cent solution of plasmin in saline was used. The N-content of this solution, which was called P500, was 0.075 per cent. The plasmin inhibitor was prepared according to Permin [3] and from the same serum as the plasmin. The original activity of the plasmin solution P500 was 4.25×10^{-9} per ml and after treatment with streptokinase 6.15×10^{-9} per ml, which is about 37 per cent activation. As oxidizing agents were used 3.0 per cent hydrogenperoxide and 0.002 *N* iodine solution. In the activation experiments the solution P500 was not treated with streptokinase. 1.00 ml P500 and 0.50 ml of either oxidizing solution or water were incubated 90 minutes at 20° C and then mixed with 3.0 ml of 4.0 per cent gelatin of pH 7.20. Table 1 shows the results from two different series.

Table 1. Activation by oxidation.

Expt. no.	Added	pH in mixture	Activity
1	H_2O	7.10	1.85
2	H_2O_2	7.05	4.13
3	I_2-KI	7.10	4.41
4	H_2O	7.12	1.61
5	H_2O_2	7.07	2.81
6	I_2-KI	7.08	3.11

Thus the plasmin is activated ca. 1.7—2.2 times by hydrogen peroxide and 1.9—2.4 times by iodine. Now, in order to see if this activation is due

to the destruction of the remaining plasmin inhibitor, this was prepared according to Permin [3] as shown in table 2, and its influence upon P500 was investigated. This is shown in table 3.

Table 2. Preparation of plasmin inhibitor.

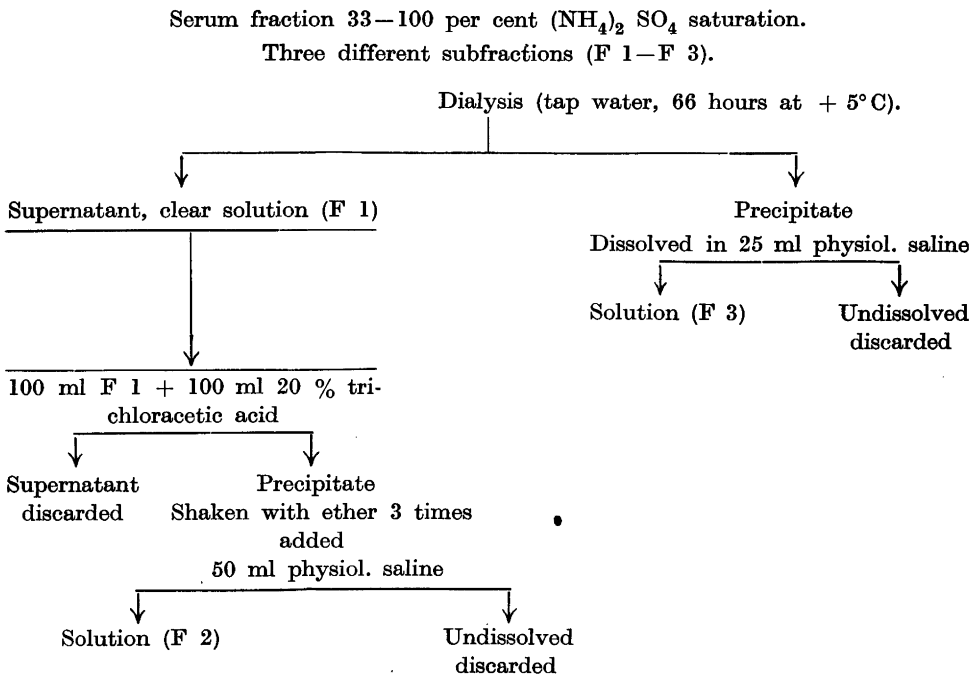

Table 3. Influence of the fractions on plasmin.

Each sample which consisted of 1.00 ml P500 and 0.50 ml of added substance was incubated 90 min. at 20° C and then mixed with 3.00 ml 4.0 per cent gelatin.

Expt. no.	Added	pH in mixture	Activity
7	H_2O	7.12	1.98
8	F 1	7.10	0.00
9	F 2	7.01	1.51
10	F 3	7.11	5.34

These results show the complete inhibition by the untreated plasmin inhibitor in F 1, the power of which is strongly deminished when precipitated with trichloracetic acid. It is astonishing to see the great plasmin content in

F 3, which is the remaining globin fraction evidently not precipitated at the plasmin preparation, and which perhaps explains the low plasmin activity of several other preparations.

If the activating power of the oxidants used in Table 1 is due to the destruction of plasmin inhibitor present, the treatment of F 1 with the oxidants should take away the inhibiting power of the plasmin inhibitor. The experiments shown in Table 4 were therefore performed.

Table 4. Treatment of plasmin inhibitor.

1.00 ml P500 and added solution were mixed after incubation with 3.00 ml of 4.0 per cent gelatin. In exp. 14 and 15 F 1 was treated for 1 hour at 20° C, before addition to P500 and incubation at 20° C.

Expt. no.	Added solution	Incubation time	pH in mixt.	Activity
11	0.50 ml H_2O	90 min.	7.04	4.8
12	0.50 ml H_2O + 0.50 ml H_2O_2	90 »	6.90	26.7
13	0.50 ml F 1	90 »	7.02	0.0
14	0.50 ml F 1 + 0.50 ml H_2O_2	30 »	6.82	1.0
15	0.50 » F 1 + 0.50 » I_2-KI	30 »	7.04	0.2

We notice the strong activation by hydrogenperoxide in this series and we see that the treatment of F 1 abolishes its inhibiting power only to a low extent, which shows that the activation of plasmin by oxidizing agents is not caused by the destruction of the plasmin inhibitor.

Whether the activation partly depends on the influence upon the formation of plasmin from plasminogen, and partly upon destruction of the anti-plasminogen or yet another factor, cannot be judged until further investigations have been carried out.

SUMMARY

The proteolytic activity of a plasmin solution is increased by oxidizing agents such as hydrogenperoxide and iodine.

The inactivating power of the plasmin inhibitor does not considerably diminish on the treatment of the plasmin inhibitor with these agents.

Thus the activation of the plasmin by these oxidizing agents is not obtained by the destruction of the plasmin inhibitor, but by another factor.

I wish to express my thanks to Prof. J. Runnström for the privilege of carring out this work in his laboratory. I thank Mr. P.-E. Andersson and Miss G. Berleen for technical assistance.

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Received July 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1080–1084

A Hexahydro Matricaria Ester-"Composit-Cumulene I"- from Scentless Mayweed (*Matricaria inodora* L.)

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In 1940 Jörgine Stene and N. A. Sörensen [1] investigated the essential oil from the flowers of the very widely distributed weed, scentless mayweed (*Matricaria inodora* L.). To about 4/5 the oil from the flowers consisted of a new very highly unsaturated derivative of methylcaprate the matricaria ester (I) $C_{11}H_{10}O_2$

$$CH_3-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3 \qquad (I)$$

In a study of the occurrence of the matricaria ester (I) in the essential oil from Norwegian compositae plants we got indications in the U. V.-spectrograms of its occurrence in oils prepared from the leaves of some plants but absence from the corresponding flower oils. These observations gave rise to an investigation of the essential oil of the non floral parts of scentless may-weed. Whereas the flowers of the fresh plant have only a very faint odour, the tiny leaves of the fresh plant has a rather strong and not very pleasant odour. As is the case when the flowers are steam distilled, the note is strikingly altered; the steam distilled oil possesses a rather mild and sweet smell. The oil from the stems and leaves proved as rich in matricaria ester as the flower oil, the yield of essential oil however amounted only to ½ of that from the flowering part of scentless mayweed.

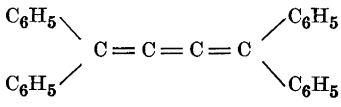
Whereas the mother liquor from the matricaria ester crystallizations of the flower oils has a faint straw-yellow tint remaining approximately constant as the mother liquors are concentrated, the mother liquor from the leave oil got remarkably orange in colour. U. V.-spectrograms indicated dominating amounts of matricaria ester compounds contaminated with small amounts of a compound with rather strong absorption about 3500—3700 ÅU. By chromatography of a petrol solution on Al_2O_3 all unsaturated compounds was

adsorbed, pure benzene eluted the matricaria ester and the contaminant was eluted with alcohol.

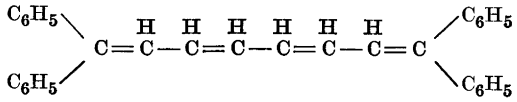
The new compound was a yellow oil, distilling at the same temperature — air bath about 50° at 0.0001 mm — as the matricaria ester itself. It analysed $C_{11}H_{16}O_2$, that is a hexahydro matricaria ester. Since the oil was yellow, quite pure matricaria ester, however, colourless, the possibility of a hexahydro matricaria ester of yellow colour was at the first instant rather improbable. On micro-hydrogenation, however, the yellow oil consumed exactly 3 moles hydrogen and gave a colourless oil of the very characteristic "strawberry"-odour of methylcaprate. Unfortunately the amount of hydrogenation product available was insufficient for a more exact identification.

Fig. 1 shows the U. V.-spetctra of matricaria ester and of the new compound for which we propose the name "Composit-cumulene I". Then in our opinion, the scarce facts given above seems to necessitate the assumption of a cumulene structure for the new compound. As stated elsewhere the long-wave U. V.-spectrum of matricaria ester lies in the same region as the spectrum of decatetraenoic acid, and the hydrogenation of one of the double bonds in decatetraenoic acid to get the composition of a hexahydro matricaria ester should displace the U. V.-spectrum at least 200 ÅU towards shorter wavelengths. Only the cumulation of the three remaining double bonds and a conjugation of the cumulene system with the carboxyl group may explain the observed composition and U. V. spectrum.

Aliphatic cumulenes (higher than the allenes) are unknown and so there is some lack of suitable comparison substances. The excellent synthetic work of Kuhn *et. al.*[2] has, however, presented a series of aromatic cumulenes the simplest member being tetraphenyl-butatriene (II)



(II)



(III)

The spectra of these aromatic cumulenes are characterized through one broad and intense absorption band at the long wave end of their electronic spectra. Kuhn and Wallenfels [2] state that the yellow colour of (II) corresponds to that of the 1,1,8,8-tetraphenyl-octatetraene (III) of Wittig and Klein [3]. So with identical end substituents the colour of Kuhns cumulene with three double

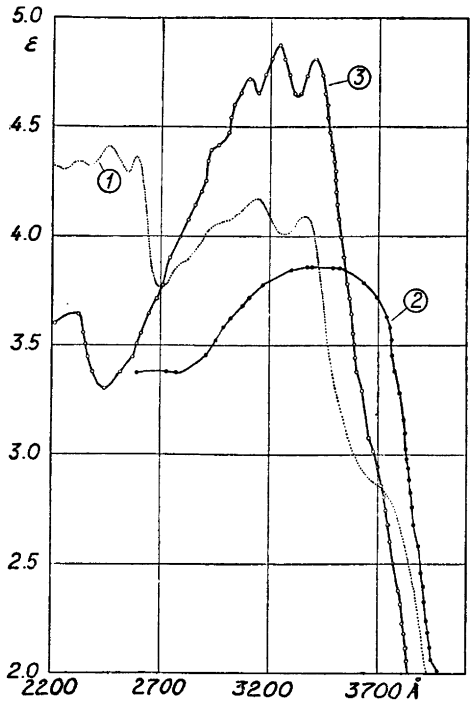


Fig. 1. Ultraviolet absorption of hexane solutions of:
Curve 1 (·····) Matricaria ester, see R. Holman and N. A. Sørensen, Acta Chem. Scand. **4** *(1950) 416.*
Curve 2 (—●—●—) Composit-cumulene I
Curve 3 (—○—○—) methyl decatetraenoate
$(\varepsilon = \frac{\lg I_0/I}{c \cdot d}$, c in Mol/l)

bonds corresponds to Wittigs conjugated polyene with four double bonds. Since the comparison of the colour shade of substances with selective absorption in the outskirts of the visible region may introduce considerable uncertainty, we have tried to get all colour comparisons on a spectral curve basis. The U. V.-spectrum of tetraphenyl-butatriene (II) in benzene is given by Kuhn and Wallenfels [2], presented in Fig. 2, curve I.

We have been unable to find the spectrum of the 1,1,8,8-tetraphenyl-octatetraene (III) in literature. We are greatly indebted to professor Dr. G. Wittig, Tübingen, who put this polyene at our disposal. The U. V.-spectrum of (III), also in benzene, is presented in Fig. 2, curve II.

The U. V.-spectra are, as will be seen, of a rather similar general character; conclusive for the physiological accordance is the nearly parallel course of the two curves in the blue region.

It is obvious that if the accordance in colour observed by Kuhn and Wallenfels between tetraphenylbutatriene and 1,1,8,8-tetraphenyl-octatetraene is a general one then the U. V.-absorption spectrum of the new compound $C_{11}H_{16}O_2$ (IV) from scentless mayweed as a cumulene with 3 /= ought

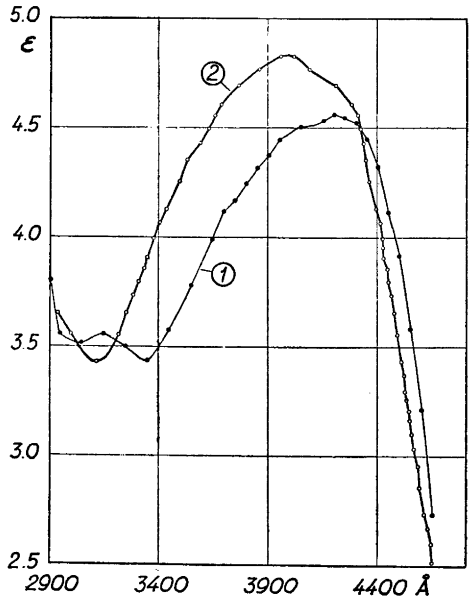
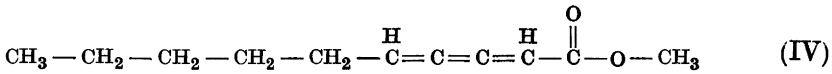
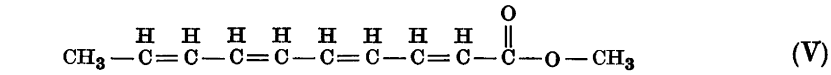


Fig. 2. Ultraviolet absorption of benzene solutions of:
Curve 1 (—●—●—●—) Tetraphenyl butatriene, see R. Kuhn and K. Wallenfels [2] recalculated to lg.
Curve 2 (—○—○—○—) 1,1,8,8-Tetraphenyl octatetraene
$(\varepsilon = \frac{\lg I_0/I}{c \cdot d}$, c in Mol/l)

to show the same relationship to methyl-decatetraen-oate (V) the polyene with corresponding end groups and 4 /=.



(IV)



(V)

(V) was synthesized according to Kuhn and Hoffer [4]. The U. V.-spectrum of (V) in hexane is given in Fig. 1, curve III. As will be seen the analogy between the U. V.-spectra of our supposed natural cumulene — "Composit-cumulene I" — and methyl-decatetraen-oate and the two spectra given in Fig. 2 is as close as would be expected. These spectral analogies thus support our assumption that the new compound ought to have the cumulene structure (IV).

EXPERIMENTAL

9 kg scentless mayweed (devoid of all floral parts) was steam-distilled. The essential oil which crystallized when the condensate was cooled, was extracted with petroleum ether, yield 7.4 g oil. Crystallisations from petroleum ether gave 3.9 g matricaria ester followed by further 0.25 + 0.25 g on successive concentrations of the mother liquor. The final, orange coloured mother liquor was separated through chromatography on activated

alumina. With petroleum ether as solvent there was eluted only small amounts of a liquid sesquiterpene. Benzene eluted some further amounts of matricaria ester. Benzene-methanol followed by pure methanol removed a yellow oil total amount 140 mg. This oil distilled at 0.0001 mm at air bath temperature about 50° leaving only a small, brown residue. The destillate, which was somewhat more lemon-yellow gave in hexane the U. V.-spectrum given in Fig. 1, curve II.

$C_{11}H_{16}O_2$ (180.2)	Calc.	C	73.31	H	8.95
	Found	»	73.82	»	8.48

17.4 mg was hydrogenated with H_2/2 % Pd-catalyst in acetic acid. H_2-consumed 7.5 ml (20°, 728 mm Hg) which corresponds to 3.1 mol H_2. The perhydrocompound was a neutral compound extractable with ether after neutralisation of the solvent, acetic acid. The colourless liquid distilled at 0.001 mm at air bath temperature 50°, its pleasant "strawberry" odour was the same as that of methyl-caprate.

SUMMARY

From the essential oil of scentless mayweed (*Matricaria inodora* L.) there has been isolated a new compound, $C_{11}H_{16}O_2$, which ought to be a hexahydro matricaria ester with the cumulene structure (IV).

One of the authors (N. A. S.) wish to thank *Norges Tekniske Högskoles Fond* for financial support which has provided his institute with a pilot plant steam distillation apparatus.

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Received May 31, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1085–1098

The Distribution of Organic Compounds Between *iso*-Butanol and Water

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In the course of an investigation into the distribution of solutes between organic solvents and water (*cf.* Collander [1, 2]) it was thought of interest to study the solvent system *iso*-butanol/water, partly in order to have a monohydric alcohol included amongst the solvents studied, but also because the *iso*-butanol is a fairly good solvent even for decidedly hydrophilic substances, such as sugars and amino-acids.

Hitherto, the distribution of solutes between *iso*-butanol and water seems to have been studied scarcely at all. The distribution in the solvent system *n*-butanol/water, too, has been studied with regard to a few substances only, *viz.*, amino-acids by England and Cohn [3], some fatty acids by Archibald [4], and some organic dyes by Reinders [5].

METHODS AND EXPERIMENTAL RESULTS

Table 1 contains the distribution coefficients of some solutes in the *iso*-butanol/water system as found by the author. These distribution coefficients have been determined in about the same manner as those in the previously studied ether/water system (*cf.* Collander [2]). The *iso*-butanol used in almost all determinations was from Dr. Theodor Schuchardt, Görlitz, Germany (quality: "purum"). Most of the solutes were ordinary reagent grade. A suitable amount of the solute was shaken vigorously for about 5 minutes with butanol and water in a glass-stoppered separatory-funnel. After complete separation of the layers the concentration of the solute in each of them was determined using some of the following analytical methods: — 1. Acidimetric or alkalimetric titration in the case of almost all the acids and bases. — 2. Kjeldahl determination in the case of many compounds containing nitrogen.

— 3. Evaporation of the solvent and weighing of the non-volatile residue. — 4. If the methods 1—3 were not applicable, more specific methods were used.

The determinations do not pretend a very high degree of accuracy. Errors of about 10—20 per cent might frequently occur. The most uncertain results are given in brackets.

The first column of Table 1 gives the brutto formulae and the names of the solutes studied. The order of the compounds is determined, in the first place, by the number of carbon atoms in the molecule, in the second place, by that of nitrogen atoms, in the third and fourth place by the numbers of oxygen and hydrogen atoms. Sulphur compounds are listed immediately after the corresponding oxygen compounds, halogen compounds after the corresponding halogen-free compounds.

The second column gives the temperature in degrees centigrade.

The third column gives the concentration of the solute in the aqueous phase. The concentrations are expressed as millimols per liter solution.

The fourth column gives the distribution coefficients k_{butanol} defined as the ratio of the total concentration of the solute in the *iso*-butanol phase to its total concentration in the aqueous phase. Also the distribution coefficients referred to in the text always mean *concentration in the organic phase/concentration in the aqueous phase*, never the inverse value. In general only gross distribution ratios are given. However, in the case of acids and bases with a dissociation constant greater than 10^{-3} the distribution of the undissociated molecules has been calculated assuming that only undissociated molecules are soluble in the butanol phase. The values arrived at in this way are marked with *M*. In the case of the very strongest electrolytes, *viz.*, tetraethylammonium hydroxide, trichloroacetic acid, the sulfonic acids, and the aliphatic amino-acids, it was not, however, possible to calculate the distribution of the molecules alone.

In order to get an idea of the dependence of the distribution coefficient on the concentration, the distribution of 26 acids was studied at two different concentrations, one being roughly 10 times greater than the other. The results of these experiments are included in Table 1. (The first figure in the fourth column of this table always refers to the more dilute solution while the second refers to the more concentrated one.) It is seen that in 23 cases out of 26 the two distribution coefficients found did not differ by more than about 20 per cent. Moreover, the three acids — trichloroacetic acid, oxalic acid, and salicylic acid — showing a greater difference are all very strong acids and their aberrant behaviour is thus easily explicable as a consequence of their varying degree of dissociation.

Table 1. Distribution coefficients iso-butanol/water as compared with the distribution coefficients ether/water.

Solute	°C	c_{water}	$k_{butanol}$	k_{ether}
CH_3I Methyl iodide	20	20.8	35	84
CH_4O Methanol	20	(2 600)	(0.6)	0.14
CH_2O_2 Formic acid	20	54.6—583	0.85—0.84	0.42
CH_5N Methylamine	20	670	0.62	0.023
CH_3ON Formamide	19	1 738	0.22	0.0014
CH_4ON_2 Urea	19	3 090	0.13	0.0_347
C_2H_5I Ethyl iodide	20	6.17	74	280
C_2H_4O Acetaldehyde	19	818	1.8	0.41 *
C_2H_6O Ethanol	20	(2 000)	(1.0)	0.26
$C_2H_4O_2$ Acetic acid	19	30.3—296	1.2—1.2	0.52
$C_2H_3ClO_2$ Chloroacetic acid	20	50.8—420	2.5—2.5 (*M* 2.6)	*M* 2.9
$C_2HCl_3O_2$ Trichloroacetic acid	18	22.0—126	2.5—5.7 (*M* ?)	4.2—10 (*M* ?)
$C_2H_3BrO_2$ Bromoacetic acid	20	81.3—263	3.7—3.5 (*M* 3.8)	*M* 4.4
$C_2H_3JO_2$ Iodoacetic acid	21	150	5.9	7.2
$C_2H_4O_3$ Glycolic acid	21	78.3—748	0.35—0.33	0.028
$C_2H_2O_4$ Oxalic acid	23	71.4—581	0.39—0.61(*M* 0.75)	*M* 0.12
C_2H_7N Ethylamine	18	44.0	1.2	0.060
» Dimethylamine	21	47.9	1.2	0.055
C_2H_7ON Ethanolamine	20	165	0.24	0.0013
$C_2H_5O_2N$ Acetamide	19	1 750	0.33	0.0025
$C_2H_4N_2$ Dicyandiamide	19	170	0.43	0.0029
$C_2H_8N_2$ Ethylenediamine	22	85.6	0.23	0.0_333
$C_2H_6ON_2$ Methyl-urea	19	982	0.24	0.0012
C_3H_6O Propionaldehyde	18	290	6.7	2.0
$C_3H_4O_2$ Acrylic acid	19	12.2—240	3.9—3.3	2.3
$C_3H_6O_2$ Methyl acetate	20	239	2.6	2.7
» Propionic acid	20	23.9—239	3.3—3.1	1.8
$C_3H_5ClO_2$ α-Chloropropionic acid	19	114	7.6 (*M* 8.4)	*M* 11*
$C_3H_5BrO_2$ α-Bromopropionic acid	20	77.7	10 (*M* 11)	*M* 15*
$C_3H_6O_3$ Lactic acid	21	60.5—612	0.66—0.72	0.091
» β-Hydroxypropionic acid	20	57.3	0.74	0.084
» Methoxyacetic acid	19	314	0.81	0.18*
$C_3H_8O_3$ Glycerol	19	2 170	0.10	0.0_366
$C_3H_4O_4$ Malonic acid	21	60.6—613	0.69—0.70(*M* 0.73)	*M* 0.10
$C_3H_6O_4$ Glycerinic acid	20	87.0—697	0.18—0.22	0.0090
C_3H_9N Propylamine	19	84.6	3.7	0.29
» Trimethylamine	21	25.5	3.1	0.46
C_3H_7ON Propionamide	19	1 020	0.69	0.013
C_3H_9ON *iso*-Propanolamine	21	385	0.43	0.0043
$C_3H_7O_2N$ α-Alanine	19	2 080	0.0069	0.0_514
$C_3H_{10}N_2$ 1,2-Propylenediamine	20	590	0.34	0.0011
» Trimethylenediamine	22	76.1	0.36	(0.0_37)

Solute	°C	c_{water}	$k_{butanol}$	k_{ether}
$C_3H_6O_2N_2$ Malonamide	19	911	0.086	0.0_330
$C_3H_{10}O_2N_2$ 1,3-Diaminopropanol-2	21	820	0.12	0.0_320
C_4H_8O Butyraldehyde	20	113	16	
$C_4H_{10}O$ *iso*-Butanol-1	20	1 124	8.5	6.9
$C_4H_8O_2$ Ethyl acetate	20	166	7.2	8.5
» *n*-Butyric acid	—	10.3—97.6	9.4—8.1	6.5
$C_4H_7BrO_2$ α-Bromo-*n*-butyric acid	19	30.3	25 (*M* 29)	*M* 45
$C_4H_6O_3$ α-Hydroxy-*iso*-butyric acid	20	46.0—452	1.2—1.2	0.26
$C_4H_4O_4$ Fumaric acid	20	18.4	4.6 (*M* 5.8)	*M* 1.5
» Maleic acid	19	54.7—247	0.88—0.98 (*M* 1.3)	*M* 0.15
$C_4H_6O_4$ Succinic acid	23	50.8	0.96	0.15
$C_4H_5BrO_4$ Bromosuccinic acid	20	39.2	4.4 (*M* 5.6)	*M* 2.9
$C_4H_{10}O_4$ Erythritol	20	(2 300)	0.037	0.0_311
$C_4H_6O_5$ Diglycolic acid	20	345	0.49	0.030
» Malic acid	22	75.4—746	0.35—0.37	0.015
$C_4H_6O_6$ Tartaric acid	21	79.7—792	0.15—0.17	0.0034
$C_4H_{11}N$ *n*-Butylamine	19	72.8	9.2	1.1 *
» Diethylamine	21	19.1	4.4	0.53
C_4H_9ON *n*-Butyramide	18	690	1.5	0.058
$C_4H_9O_2N$ α-Amino-*n*-butyric acid	20	1 286	0.016	0.0_526
$C_4H_{11}O_2N$ Diethanolamine	18	173	0.19	0.0_354
$C_4H_7O_4N$ Aspartic acid	20	36.6	(0.010)	—
$C_4H_{10}N_2$ Piperazine	18	77.8	0.24	0.0_352
$C_4H_{12}N_2$ Tetramethylenediamine	22	62.8	0.69	0.0013
$C_5H_{10}O_2$ *iso*-Valeric acid	20	4.6—51	21—19	20
» Trimethylacetic acid	21	15.6	32	32
$C_5H_8O_3$ Levulinic acid	20	287	1.2	0.26
$C_5H_6O_4$ Itaconic acid	22	34.4	1.8 (*M* 1.9)	*M* 0.35
$C_5H_8O_4$ Glutaric acid	19	34.4	2.0	0.27
» Dimethylmalonic acid	19	16.3	4.9	1.6
$C_5H_{12}O_4$ Pentaerythritol	19	411	0.14	0.0_330
$C_5H_{10}O_5$ Arabinose	20	(2 000)	0.019	(0.0_438)
C_5H_5N Pyridine	19	22.5	7.3	1.2
$C_5H_{11}N$ Piperidine	19	19	4.6	0.57
$C_5H_6N_2$ 2-Aminopyridine	—	93.2	4.5	0.77
$C_5H_{14}N_2$ Pentamethylenediamine	22	42.1	1.5	0.0025
$C_6H_{12}O_2$ Caproic acid	19	11.9	75	93
$C_6H_6O_2S$ Benzenesulfonic acid	19	73.0	0.40 (*M* ?)	0.0013
$C_6H_{10}O_4$ Adipic acid	22	22.9	3.5	0.54
$C_6H_{10}O_4$ Ethylene diacetate	20	117	2.7	2.0
$C_6H_{14}O_4$ Triethylene glycol	19	939	0.26	0.0031
$C_6H_{12}O_5$ Rhamnose	20	(1 000)	0.057	0.0_319
$C_6H_6O_6$ Aconitic acid	20	15.6—162	2.2—1.9 (*M* 2.1)	*M* 0.50
$C_6H_8O_6$ Tricarballylic acid	20	38.9—232	0.95—0.94	0.060

Solute	°C	c_{water}	$k_{butanol}$	k_{ether}
$C_6H_{12}O_6$ Glucose	18	1 911	0.011	0.0_545
» Fructose	19	1 960	0.017	—
$C_6H_{14}O_6$ Mannitol	19	962	0.014	—
$C_6H_8O_7$ Citric acid	21	79.6—788	0.28—0.32	0.0086
$C_6H_{12}O_7$ Gluconic acid (+ lactone)	20	950	0.034	(0.0_32)
$C_6H_{15}N$ Hexylamine	19	8.2	83	16 *
» Dipropylamine	20	18.6	33 (*M* 41)	*M* 8.9
» Triethylamine	20	27	21	5.9
$C_6H_{15}ON$ Diethylethanolamine	23	23.3	3.1	0.35
$C_6H_{13}O_2N$ Leucine	18		0.062	0.0_412
$C_6H_{15}O_2N$ Di-*iso*-propanolamine	21	515	0.70	0.0059
$C_6H_5O_3N$ *o*-Nitrophenol	18	—	40	150
» *m*-Nitrophenol	18	—	62	160
» *p*-Nitrophenol	18	—	58	110
$C_6H_{15}O_3N$ Triethanolamine	23	167	0.26	0.0011
$C_6H_{12}N_4$ Hexamethylenetetramine	18	252	0.067	0.0_326
$C_6H_{18}N_4$ Triethylenetetramine	20	735	0.15	0.0_468
$C_7H_6O_2$ Benzoic acid	21	19.4—171	49—54	78
$C_7H_6O_3$ *o*-Hydroxybenzoic acid	22	1.6—11.1	61—85 (*M* 117)	*M* 236
» *m*- » »	20	13.1	25	21
» *p*- » »	20	11.6	27	26
$C_7H_{12}O_4$ Pimelic acid	20	40.7	7.3	1.5
» Diethylmalonic acid	19	8.4—60.1	11—12 (*M* 15)	*M* 11
$C_7H_{12}O_6$ Quinic acid	21	89.9—378	0.081—0.084	0.0_331
$C_7H_{14}O_6$ α-Methylglucoside	20	1 603	0.039	0.0_45
C_7H_9N Benzylamine	21	9.7	(9.5)	1.9
$C_7H_7O_2N$ *o*-Aminobenzoic acid	20	23.7	15	27
» *m*- » »	20	33.0	2.9	1.5
» *p*- » »	20	17.9	7.7	7.6
$C_8H_8O_2$ Phenylacetic acid	18	3.7—33.3	27—28	37
$C_8H_8O_3$ Mandelic acid	20	15.9—138	5.3—5.1	3.2
$C_8H_6O_4$ *o*-Phthalic acid	23	15.5	5.5 (*M* 7.2)	*M* 1.6
$C_8H_{18}O_5$ Tetraethylene glycol	19	732	0.24	0.0024
$C_8H_{17}N$ Coniine	18	1.75	51	—
$C_8H_{19}N$ Octylamine	19	(2.5)	(176)	—
» Di-*iso*-butylamine	21	5.05	179	151
$C_8H_{15}ON$ Tropin	19	19.2	3.1	0.053
$C_8H_{21}ON$ Tetraethylammonium hydroxide	19	129	0.015	0.0_52
$C_8H_{10}O_2N_4$ Caffeine	—	17.8	1.2	0.060
$C_9H_{16}O_4$ Azelaic acid	20	4.35	43 (*M* 46)	*M* 16
$C_9H_6O_6$ Trimesic acid	21	3.70	30	11
$C_9H_{22}N_2$ 2-Amino-5-diethylaminopentane	20	25.8	11	0.58

Solute	°C	c_{water}	$k_{butanol}$	k_{ether}
$C_{10}H_3O_3S$ *a*-Naphthalenesulfonic acid	23	42.6	1.4 (*M* ?)	0.0051
$C_{10}H_{10}O_4$ Benzylmalonic acid	—	11.0	22	15
$C_{10}H_{15}ON$ Ephedrine	18	1.96	15	2.0
$C_{11}H_{12}ON_2$ Antipyrine	20	127	3.2	0.073
$C_{12}H_{16}O_7$ Arbutine	22	201	0.28	0.0_374
$C_{12}H_{22}O_{11}$ Sucrose	18	1 300	0.0056	(0.0_511)
» Maltose	19	853	0.0040	—
$C_{13}H_{18}O_7$ Salicine	22	94.9	0.40	0.0_349
$C_{13}H_{20}O_8$ Pentaerythritol tetra-acetate	20	23.1	9.4	9.3
$C_{13}H_{20}O_2N_2$ Novocaine	19	1.69	63	64
$C_{15}H_{26}N_2$ Sparteine	—	1.2	(110) (*M* ?)	65
$C_{15}H_{16}N_4$ Neutral red (base)	20	(0.03)	110	5.0
$C_{17}H_{23}O_2N$ Atropine	17	(0.69)	(67)	(4.1)
$C_{17}H_{19}O_3N$ Morphine	19	(2.1)	(7.4)	(0.21)
$C_{17}H_{21}O_4N$ Cocaine	20	(0.40)	(107)	(138)
$C_{18}H_{21}O_3N$ Codeine	19	(2.0)	(16)	(0.80)
$C_{19}H_{21}O_3N$ Thebaine	—	(0.41)	(104)	(16)
$C_{20}H_{19}O_5N$ Berberine	18	(10)	(0.071)	< 0.005
$C_{32}H_{26}O_4N_2$ Brucine	18	(0.57)	(21)	(0.18)
$C_{32}H_{49}O_9N$ Cevadine	19	(0.37)	(146)	(280)

The distribution coefficients used in the following chapters are always those referring to the highest concentration studied or those referring to the undissociated molecules.

In the last column of Table 1 the distribution coefficients ethyl ether/water are given for the sake of comparison. These values are taken from Collander [2]. Only those values marked with an asterisk are based on later determinations not yet published.

THE DISTRIBUTION AS INFLUENCED BY THE CHEMICAL CONSTITUTION OF THE SOLUTE

On the whole the distribution of organic compounds in the solvent system *iso*-butanol/water depends on the constitution of the solutes in much the same way as their distribution in the previously studied solvent system ethyl ether/water (cf.[2]) except that the differences in the distribution coefficients are smaller in the butanol/water than in the ether/water system.

Table 2. Increase of the distribution coefficient within homologous series.

Solutes	$q_{butanol}$		q_{ether}
Erythritol — Pentaerythritol	0.14 : 0.037	= 3.8	2.7
α-Chloroacetic acid — α-Chloropropionic acid	8.4 : 2.6	= 3.2	3.8
Acetaldehyde — Butyraldehyde	$\sqrt{16 : 1.8}$	= 3.0	
Arabinose — Rhamnose	0.057 : 0.019	= 3.0	(5.0)
Acetic acid — Caproic acid	$\sqrt[4]{75 : 1.2}$	= 2.8	3.7
Methyl acetate — Ethyl acetate	7.2 : 2.6	= 2.8	3.1
α-Bromoacetic acid — α-Bromo-n-butyric acid	$\sqrt{29 : 3.8}$	= 2.8	3.2
Methanol — *iso*-Butanol	$\sqrt[3]{8.5 : (0.6)}$	= (2.4)	3.7
Methylamine — Di-*iso*-butylamine	$\sqrt[7]{179 : 0.62}$	= 2.2	3.5
α-Alanine — Leucine	$\sqrt[3]{0.062 : 0.0069}$	= 2.1	(2.0)
Methyl iodide — Ethyl iodide	74 : 35	= 2.1	3.3
Malonic acid — Diethylmalonic acid	$\sqrt[4]{15 : 0.73}$	= 2.1	3.2
Acetamide — *n*-Butyramide	$\sqrt{1.5 : 0.33}$	= 2.1	4.8
Malonic acid — Azelaic acid	$\sqrt[6]{46 : 0.73}$	= 2.0	2.3
Glycolic acid — α-Hydroxy-*iso*-butyric acid	$\sqrt{1.2 : 0.33}$	= 1.9	3.0
Ethylenediamine — Pentamethylenediamine	$\sqrt[3]{1.5 : 0.23}$	= 1.9	2.0
Diethanolamine — Di-*iso*-propanolamine	$\sqrt{0.70 : 0.19}$	= 1.9	3.3
Ethanolamine — *iso*-Propanolamine	0.43 : 0.24	= 1.8	3.3
Urea — Methylurea	0.24 : 0.13	= 1.8	2.6
Formamide — Acetamide	0.33 : 0.22	= 1.5	1.8
Formic acid — Acetic acid	1.2 : 0.84	= 1.4	1.2
Oxalic acid — Malonic acid	0.73 : 0.75	= 1.0	0.8

This being so it may suffice here to point out, very briefly, only some of the most conspicuous relations existing between the chemical constitution of different solutes and their distribution in the butanol/water system.

In studying the ether/water system it was found that the distribution coefficients of the members of each homologous series increase, with but a few exceptions, by a factor (q_{ether}) of about 2—4 for every new CH_2 group incorporated in the solute molecule. As seen from Table 2 the corresponding factor in the butanol/water system mostly varies between 1.8 and 3.0. Both from Table 2 and Fig. 1 it is seen that the first members of a given homologous series often differ less from each other in this respect than the following. This is especially obvious in the case of the fatty acids, the fatty acid amides, and the dicarboxylic acids. In the ether/water system the same phenomenon was observed.

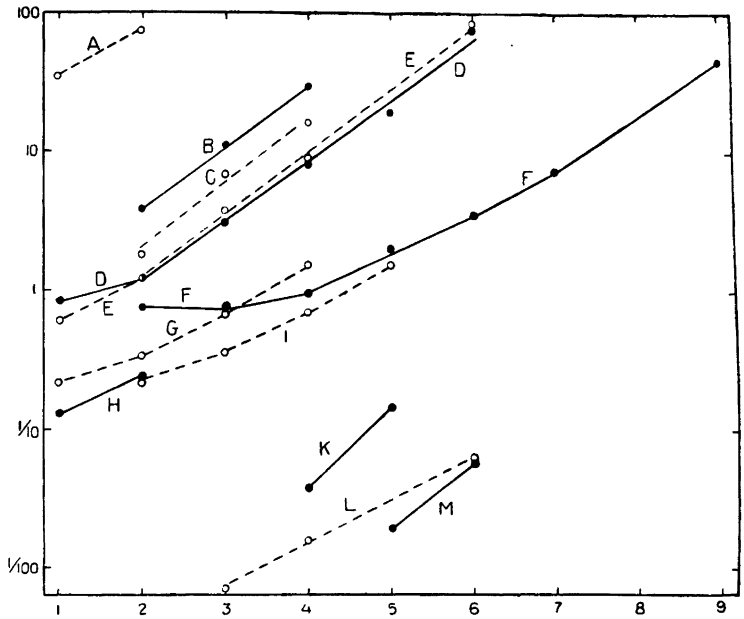

Fig. 1. Ordinate: distribution coefficient iso-butanol/water, abscissa: number of carbon atoms of the solute molecule. A alkyl iodides, B α-bromo-substituted fatty acids, C alkyl aldehydes, D fatty acids, E alkyl amines, F dicarboxylic acids, G fatty acid amides, H alkyl-ureas, I polymethylenediamines, K tetrahydric alcohols, L α-amino-acids, M pentoses.

An alcoholic hydroxyl group was found to reduce the distribution coefficient by about 5—50, sometimes even by about 190 times in the ether/water system. Correspondingly this group is now found to reduce the distribution coefficient by about 2—6 times in the butanol/water system (Table 3). More-

Table 3. Influence of an alcoholic hydroxyl group on the distribution coefficient.

Solutes	Butanol	Ether
iso-Butanol : Erythritol	$\sqrt[3]{8.5 : 0.037} = 6.1$	40
Phenylacetic acid : Mandelic acid	$28 : 5.1 = 5.5$	12
Ethylamine : Ethanolamine	$1.2 : 0.24 = 5.0$	46
Diethylamine : Diethanolamine	$\sqrt{4.4 : 0.19} = 4.5$	31
Propionic acid : α-Hydroxypropionic acid	$3.1 : 0.72 = 4.3$	20
» » β- » »	$3.1 : 0.74 = 4.2$	21
Triethylamine : Triethanolamine	$\sqrt[3]{18 : 0.26} = 4.1$	17
Acetic acid : Glycolic acid	$1.2 : 0.33 = 3.6$	17
Lactic acid : Glyceric acid	$0.72 : 0.22 = 3.3$	9.3
Tricarballylic acid : Citric acid	$0.94 : 0.32 = 2.9$	7.0
Succinic acid : Malic acid	$0.96 : 0.37 = 2.6$	10
Malic acid : Tartaric acid	$0.37 : 0.17 = 2.2$	4.4

Table 4. Influence of an amino-group on the distribution coefficient.

Solutes	Butanol	Ether
Caproic acid : Leucine	75 : 0.062 = 1 210	7.8×10^6
n-Butyric acid : α-Amino-*n*-butyric acid	8.1 : 0.016 = 506	2.5×10^6
Propionic acid : α-Alanine	3.1 : 0.0069 = 449	1.3×10^6
Benzoic acid : *m*-Aminobenzoic acid	54 : 2.9 = 19	52
Propylamine : 1,2-Propylenediamine	3.7 : 0.34 = 11	264
» Trimethylenediamine	3.7 : 0.36 = 10	(410)
Benzoic acid : *p*-Aminobenzoic acid	54 : 7.7 = 7.0	10
Ethylamine : Ethylenediamine	1.2 : 0.23 = 5.2	182
Ethanol : Ethanolamine	(1.0) : 0.24 = 4.2	200
Benzoic acid : *o*-Aminobenzoic acid	54 : 15 = 3.6	2.9
Propanolamine : Diaminopropanol	0.43 : 0.12 = 3.6	22
Pyridine : 2-Aminopyridine	7.3 : 4.5 = 1.6	1.6

Table 5. Influence of the substitution of a CH_3 group by a COOH group on the distribution coefficient.

Solutes	Butanol	Ether
Butylamine : α-Aminobutyric acid	9.2 : 0.016 = 575	4×10^5
Propylamine : α-Alanine	3.7 : 0.0069 = 536	2×10^5
Caproic acid : Adipic acid	75 : 3.5 = 21	172
Ethyl iodide : Iodoacetic acid	74 : 5.9 = 13	47
n-Butyric acid : Succinic acid	8.1 : 0.96 = 8.4	45
Propionic acid : Malonic acid	3.1 : 0.73 = 4.2	18
Ethanol : Glycolic acid	(1.0) : 0.33 = (3.0)	9.3
Acetic acid : Oxalic acid	1.2 : 0.75 = 1.6	4.4

Table 6. Influence of the substitution of a COOH group by a $CONH_2$ group on the distribution coefficient.

Solutes	Butanol	Ether
n-Butyric acid : *n*-Butyramide	8.1 : 1.5 = 5.4	110
Propionic acid : Propionamide	3.1 : 0.69 = 4.5	140
Formic acid : Formamide	0.84 : 0.22 = 3.8	300
Acetic acid : Acetamide	1.2 : 0.33 = 3.6	210
Malonic acid : Malonamide	$\sqrt{0.73 : 0.086}$ = 2.9	18

Table 7. Influence of a halogen atom on the distribution.

Solutes	Butanol	Ether
α-Chloropropionic acid : Propionic acid	8.4 : 3.1 = 2.7	6.1
Chloroacetic acid : Acetic acid	2.6 : 1.2 = 2.2	5.6
Bromosuccinic acid : Succinic acid	5.6 : 0.96 = 5.8	19
α-Bromobutyric acid : Butyric acid	29 : 8.1 = 3.6	6.9
α-Bromopropionic acid : Propionic acid	11 : 3.1 = 3.5	8.3
Bromoacetic acid : Acetic acid	3.8 : 1.2 = 3.1	8.5
Iodoacetic acid : Acetic acid	5.9 : 1.2 = 4.9	14

over, the more numerous and the more effective the hydrophilic groups that the molecule initially contains, the smaller is in general the effect of the incorporation of a new hydroxyl group. This is readily understandable, for if such a group is introduced into a molecule containing hydrophilic groups already in advance, this generally results in the formation of an intramolecular hydrogen bond between the new hydroxyl group and an initially existing hydrophilic group. Hereby the capacity of these groups to form hydrogen bonds with the solvent molecules is, of course, reduced.

The influence of an amino-group on the distribution is very similar to that of a hydroxyl group, only somewhat stronger (Table 4). If, however, the amino-group is introduced into the molecule of an aliphatic acid so that a strongly dissociated amino-acid results, the change in distribution is, of course, much greater.

If a CH_3 group is substituted by a COOH group, the distribution coefficient ether/water gets about 4—170 times smaller, while the distribution coefficient butanol/water gets 1.6—21 times smaller (Table 5). In this case, also, the formation of strongly dissociated amino-acids causes a much stronger diminution of the distribution coefficients.

When a COOH group is substituted by a $CONH_2$ group the distribution coefficient ether/water becomes 18—300 times smaller while the distribution coefficient butanol/water becomes 2.9—5.4 times smaller (Table 6).

A halogen atom, quite in contrast so the substituents hitherto mentioned, makes the distribution coefficient greater: by about 5.6—19 times in the ether/water system and by about 2.2—5.8 times in the butanol/water system (Table 7). This effect increases in the series $Cl < Br < I$.

On the whole, the distribution of organic compounds between *iso*-butanol and water, just like that between ether and water, is largely understandable if we assume that it is principally due to the formation of hydrogen bonds between solute and solvent molecules.

THE DISTRIBUTION IN THE SOLVENT SYSTEM ISO-BUTANOL/WATER AS COMPARED WITH THAT IN THE ETHER/WATER SYSTEM

A comparison of the distribution in the butanol/water system with that in the ether/water system may profitably be based on Fig. 2. In this graph all 138 solutes whose distribution in both these solvent systems has been studied are represented by points, with the only exception of those 10 solutes whose ether/water distribution coefficient is smaller than 1/10 000. If the distribution coefficients in the butanol/water system were identical with those in the ether/water system, all the points would evidently lie on the straight line

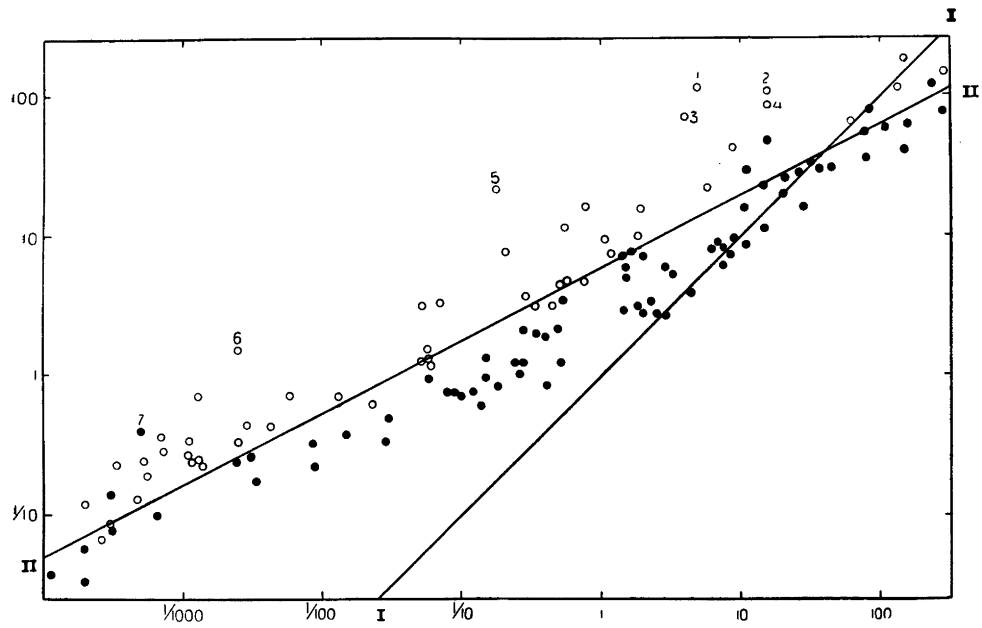

Fig. 2. Abscissa: distribution coefficient in the ether/water system, ordinate: distribution coefficient in the iso-butanol/water system. ● *N-free compounds, nitro-compounds, and amino-acids,* ○ *amines, imines, and amides. The most aberrant points are: 1 neutral red, 2 thebaine, 3 atropine, 4 di-iso-butylamine, 5 brucine, 6 pentamethylenediamine, 7 salicine.*

I whose slope is 45°. In reality, however, the points are scattered along the straight line II whose slope is much less steep. This is an expression of the already stated fact that the distribution coefficients of different solutes in the butanol/water system differ much less from each other than the corresponding distribution coefficients in the ether/water system. Thus, if the distribution coefficients of two solutes in the ether/water system are as, say, 1 : 1 000 000, then their distribution coefficients in the butanol/water system will be as about 1 : 1 000.

Mathematically the correlation existing between the distribution coefficients in these two solvent systems may be expressed by the equation

$$\log k_{\text{butanol}} = a \cdot \log k_{\text{ether}} + b.$$

In this equation a, which is an expression for the slope of the line II, has a value of about 0.5, while b, which is an expression for the height of the same line, has a value of about + 0.8. (*Cf.* Collander [1, p. 368].)

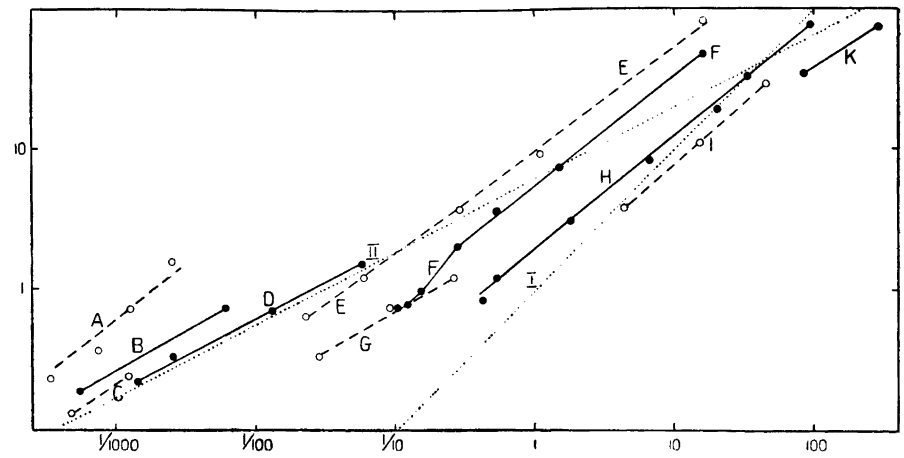

Fig. 3. Relations between ether/water distribution coefficients (abscissa) and iso-butanol/water distribution coefficients (ordinate) in some homologous series. A polymethylenediamines, B aminodiols, C alkyl-ureas, D fatty acid amides, E alkylamines, F dicarboxylic acids, G α-hydroxy-substituted fatty acids, H fatty acids, I α-bromo-substituted fatty acids, K alkyl iodides.

The relative smallness of the differences between the distribution coefficients of different solutes in the butanol/water system is a consequence of the fact that butanol is a considerably more hydrophilic, *i. e.*, a more hydrogen bonded solvent than, *e. g.*, ethyl ether. For, owing to the relative similarity of butanol and water as solvents, the work necessary to transport one mole of a solute from the aqueous phase to the butanolic phase, or vice versa, is evidently much smaller than the work necessary to transport the same amount of the same solute from the aqueous phase to the ether phase, or vice versa.

From Fig. 2 it is, however, also seen that the points representing the different solutes are not irregularly scattered on both sides of line II, but that the overwhelming majority of the points representing more or less basic compounds (amines, imines, amides) lie above this line while the points representing neutral or acidic compounds lie, for the most part, below it. Butanol is thus, *ceteris paribus*, a better solvet for basic compounds than is ether. This is almost certainly due to the fact that butanol, like other alcohols, is a more acidic compound than ether.

Fig. 3 shows more in detail how the distribution of some homologous series in the butanol/water system is correlated with their distribution in the ether/water system. For the sake of clearness solutes belonging to 10 series only are included in this graph. The lines I and II are the same as in Fig. 2. It is seen

that the lines representing different homologous series are, as was to be expected, scattered along line II, the series comprising basic compounds being situated a little above it, the series of neutral or acidic compounds a little below it. It is, however, also seen that the lines representing homologous series do not run exactly parallelly with line II but have a somewhat steeper slope so that many of them begin below line II but end above it. This means that, although all differences in the distribution coefficients of different solutes are smaller in the butanol/water than in the ether/water system, this diminution of the differences in the first-named system is less marked if we compare solutes which differ as to the length of their carbon chains than if we compare solutes differing as to their more or less hydrophilic substituents. The reason for this is not quite clear.

Finally it may be pointed out that the most aberrant points in Fig. 2 nearly all represent very large molecules, *e. g.*, alkaloids. This is probably no mere chance but a circumstance connected with the just-mentioned fact that the points representing higher homologues are situated higher in Fig. 2 than the points representing lower homologues.

SUMMARY

The distribution of 145 organic compounds between *iso*-butanol and water has been determined experimentally. The distribution coefficients thus found are given in Table 1.

An inspection of them shows that the distribution in this solvent system is influenced by the chemical constitution of the solute in much the same way as the previously studied distribution of the same solutes in the solvent system ether/water.

The distribution coefficients of the different solutes differ, however, considerably less in the butanol/water system than in the ether/water system. Thus, if the distribution coefficients of two solutes in the latter system are as 1 : 1 000 000 they will be, in the first-named system, as about 1 : 1 000. (*Cf.* Fig. 2.) This is a consequence of the fact that butanol is a much more hydrogen bonded solvent than is ether.

Basic compounds (amines, imines, amides) have *ceteris paribus* a relatively greater solubility in butanol than have neutral or acidic compounds. This is very probably due to the more acidic nature of butanol as compared with ether.

Thanks are due to Professor Eero Tommila and Dr. Veijo Wartiovaara for kind criticism of the manuscript and to Mr. Väinö Heikinheimo for help in the determination of the distribution coefficients.

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Received June 22, 1950.

ACTA CHEMICA SCANDINAVIGA 4 (1950) 1099–1108

Die thermische Analyse bei der Untersuchung der kristallographischen Umwandlung des Silbersulfates

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EINLEITUNG UND PROBLEMSTELLUNG

Die thermische Analyse ist seit langem bekannt als eins der wirkungvollsten Mittel zur Erforschung von Festkörperreaktionen [1]. Während die ersten derartigen Untersuchungen mehr qualitativen Charakter hatten, war mit der Einführung und Verbesserung der Differentialthermoanalyse [2,3] auch die Möglichkeit zu quantitativen Untersuchungen gegeben.

Wenn wir im folgenden die Differentialthermoanalyse zur Untersuchung einer kristallographischen Umwandlung verwenden, hoffen wir, Aufschluss über vier verschiedene Grössen zu bekommen. Diese sind: Umwandlungspunkt, Umwandlungswärme, Umwandlungsgeschwindigkeit und thermische Hysterisis (Unterkühlung und Überhitzung der thermodynamisch instabilen Phasen).

Das in dieser Mitteilung untersuchte System Silbersulfat Ag_2SO_4 ist von Interesse, weil es als Komponente in die sogenannten »Platzwechselreaktionen» [4] eingeht, wobei eine seinerzeit überraschende Abhängigkeit der Temperaturen messbaren Umsatzes von der Umwandlungstemperatur des Silbersulfates vorliegt.

Bevor eine eingehende Untersuchung jener Gesetzmässigkeiten begonnen werden kann, muss offenbar die Umwandlung als solche näher untersucht werden. In der Literatur findet sich eine Arbeit von Friedrich [5], der mittelst thermischer Analyse den Umwandlungspunkt zu 411° festlegt (obwohl die wiedergegebene Kurve einen höheren Wert wahrscheinlich macht).*

* Auch R. Nacken (*Jahrb. Min. Geol. Pal.* **24** (1907) 30) gibt als Umwandlungspunkt 412° an, obwohl schon hier von einer Verzögerung um 20° die Rede ist.

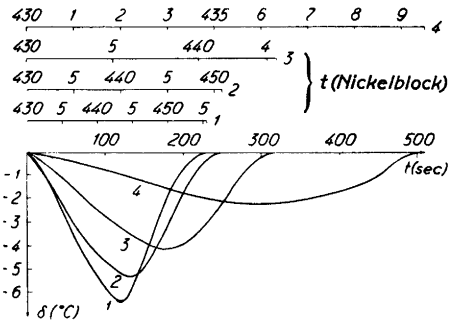

Fig. 1. Die Temperaturdifferenz δ zwischen Silbersulfat und der Bezugssubstanz als Funktion der Zeit (einheitliche Abszisse) und der Temperatur des Nickelblockes (verschiedene Abszissen für Erwärmungsgeschwindigkeit 1 : 6,7 Grad / min; 2 : 5,0 Grad/ min; 3 : 2,7 Grad / min, 4 : 1,0 Grad / min).

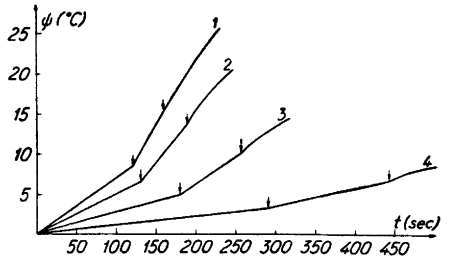

Fig. 2. Die mittlere Überhitzungstemperatur ψ als Funktion der Zeit bei verschiedenen Erwärmungsgeschwindigkeiten (wie in Fig. 1).

Jagitsch [6], der in unserem Institut zuerst die Leitfähigkeit bestimmte, untersuchte das Verhalten am Umwandlungspunkt nicht so eingehend, dass Zweifel an diesem Wert aufgetaucht wären. Neuere Versuche [7] zeigten indessen, dass die auch schon bei der Leitfähigkeit beobachtete Unstetigkeit in der Beweglichkeit der Silberionen bei etwa 430° liegt (Messung der Diffusion radioaktiven Silbers in Silbersulfat).

Daher schien eine Neubestimmung des Umwandlungspunktes nötig, wobei Augenmerk auf etwaige Unterschreitungserscheinungen gelegt — und, wenn möglich, auch etwas über den Umwandlungsmechanismus ausgesagt werden sollte.

VERSUCHSANORDNUNG

Als Wärmequelle stand uns ein Ofen nach Norton [8] zur Verfügung. Hierbei befanden sich Analysensubstanz und die thermisch inaktive Vergleichssubstanz (α-Al_2O_3) in je einer Bohrung eines Nickelblockes. In der Mitte der Bohrungen waren die Lötstellen eines Chromel-Alumel-Chromel-Differentialthermoelementes angebracht. Ausserdem wurde die Temperatur des Nickelblockes durch ein Platin/Platin-10 %-Rhodium-Thermoelement gemessen und durch ein schreibendes Millivoltmeter registriert (»Speedomax» von Leeds-Northrup).

So konnte der Verlauf der Erhitzung des Nickelblockes kontrolliert werden. Für eine annähernd konstante, beliebige Erhitzungsgeschwindigkeit wurde folgendermassen gesorgt:

An die Klemmen des Ofens wurde eine Spannung gelegt, die den Ofen auf eine Temperatur kurz unterhalb der Umwandlungstemperatur brachte (in unserem Fall etwa 60 V für etwa 400°).

Dann wurde eine von einem Drehtransformator bezogene Zusatzspannung von etwa 10 V durch einen Motor wahlweiser Geschwindigkeit mittels Untersetzung kontinuierlich eingeschaltet im Laufe von 1—5 Stunden. (Alle Primärspannungen wurden durch einen Philips-Wechselspannungstabilisator stabilisiert.) Auf diese Art wurde der Nickelblock mit den Substanzen mit einer konstanten Geschwindigkeit von 1 — 5 Grad/Min. erwärmt. Die Verfolgung der auftretenden Differentialthermospannungen geschah durch Ablesen des Ausschlages eines Moll-Spiegelgalvanometers von der Empfindlichkeit $5 \cdot 10^{-8}$ V pro mm Ausschlag in 1 m Abstand.

DIE VERSUCHE UND IHRE ERGEBNISSE

Das Platinrhodium-Thermoelement wurde in üblicher Weise gegen die Erstarrungspunkte von Blei, Zink und Antimon geeicht, das Chromel-Alumel-Differentialthermoelement nach Eintauchen der beiden Lötstellen in je ein Wasserbad durch Vergleich mit einem in Hundertstelgrade geteilten Thermometer kalibriert. Diese bei Zimmertemperatur vorgenommene Eichung wurde auf die Anwendungstemperatur korrigiert unter Anwendung des theoretischen Verlaufes der Thermokraft von Chromel-Alumel-Elementen.

1. Zunächst wurden die Temperaturdifferenz-Zeit-Kurven bei *verschiedener Erhitzungsgeschwindigkeit* aufgenommen. Die Ergebnisse sind in Fig. 1 wiedergegeben.

Wir sehen, dass die Umwandlung unabhängig von der Erhitzungsgeschwindigkeit, stets bei etwa 430° einsetzt.

Die Form der Kurven ist eine Funktion der Überhitzungstemperatur, die ihrerseits in diesem Fall fast ausschliesslich durch die Erhitzungsgeschwinditkeit gegeben ist.

Der Betrag der Überhitzungstemperatur ist aus der Figur ohne weiteres abzulesen, er ist nämlich offenbar gleich der Differenz zwischen der Abszissentemperatur und der Umwandlungstemperatur (430°), verringert um den Betrag der Ordinate, da ja durch die Umwandlung Wärme verbraucht, die Temperatur der Substanz also herabgesetzt wird.

Dies gilt offenbar für den Mittelpunkt des Probekörpers, da hier die Lötstelle des Differentialthermoelementes eingeführt ist. An der Grenzfläche Substanz-Nickelblock dagegen ist die Überhitzungstemperatur grösser, nämlich gleich der Differenz zwischen Temperatur des Nickelblockes und der Umwandlungstemperatur.

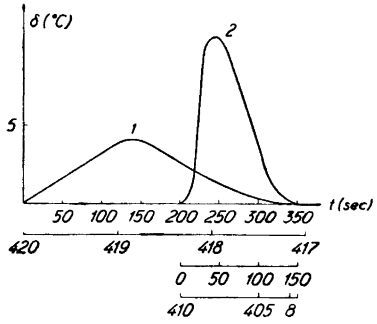

Fig. 3. Temperaturdifferenzen wie in 1, aber diesmal bei verschiedenen Abkühlungsgeschwindigkeiten (1 : 0,5 Grad / min; 2 : 3,0 Grad / min).

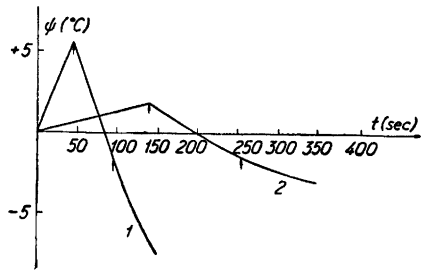

Fig. 4. Die mittlere Überhitzungstemperatur ψ als Funktion der Zeit bei verschiedenen Abkühlungsgeschwindigkeiten (wie in Fig. 3).

Nimmt man eine parabolische Temperaturverteilung innerhalb der Analysensubstanz an, so lässt sich eine »mittlere Überhitzungstemperatur definieren, die gleich 2/3 der maximalen Überhitzungstemperatur» (für den Mittelpunkt des Probekörpers gerechnet) ist.

Die Werte dieser mittleren Überhitzungstemperatur sind in Figur 2 als Funktion der Zeit wiedergegeben.

Während der Umwandlung ergeben sich so annähernd gerade Linien, wobei zwischen Nullpunkt und der ersten Marke der Wärmeeffekt der Umwandlung und zwischen den Marken der Wärmeausgleich mit dem Nickelblock überwiegt. Während des Temperaturausgleiches ergeben sich schwach gebogene Kurven. Auf diese Art lässt sich die Dauer der Umwandlung gut erkennen.

2. Die entsprechenden Kurven wurden auch bei *verschiedener Abkühlungsgeschwindigkeit* aufgenommen. Dabei lässt sich die Möglichkeit erheblicher Unterkühlung feststellen, die bei einer Abkühlungsgeschwindigkeit von 0,5°/min. 10 Grad, bei einer solchen von 3,0°/min. 20° Grad beträgt.

Die entsprechende Darstellung der über den Anfangswert 10 bzw. 20° hinaus vorhandenen Unterkühlung ist in Figur 4 gegeben.

Um einen Vergleich mit Fig. 2 zu ermöglichen, sind die Koordinaten identisch gewählt. Es zeigt sich, dass durch die schlagartig einsetzende Reaktion zunächst eine Erhitzung eintritt, wodurch die hier exotherme Umwandlung abgebremst werden sollte. Die Anfangs-Unterkühlung ist indessen so gross gewesen, dass die Reaktion fortschreiten kann (auch wenn der Abkühlungsverlauf des Ofens unterbrochen würde). Später geht die Kurve wieder ins

Negative, d. h. der Punkt der Anfangs-Unterkühlung wird mit fortschreitender Abkühlung des Ofens unterschritten.

3. Für die auf den Fig. 2 und 4 dargestellte *Zeitfunktion der relativen Überhitzung* lässt sich eine Gleichung aufstellen:

$$\psi = (b - 135\, e^{-\frac{K}{\xi}})\, \frac{t}{100}$$

mit einem Wert von 0,21 für K.

4. Die *Umwandlungswärme* lässt sich auf folgende Art ermitteln.

Nach Speil und Berkhammer [9] lässt sich die Umwandlungswärme Q entnehmen aus: $\frac{mQ}{g\lambda} = \int \triangle T \mathrm{d}T$, wo m die Masse in Gramm, g ein Geometriefaktor, λ die Wärmeleitfähigkeit in cal · grad^{-1} · cm^{-1} · sec^{-1} und das Integral die Fläche der Differentialtemperatur-Zeit-Kurve ist.

Nach einer Kalibrierung unserer Anordnung mit der α—β Umwandlung des Quarzes ($\int \triangle T \mathrm{d}t = 16$ grad sec; $Q = 3{,}5$ cal/g; $\lambda = 9{,}3 \cdot 10^{-3}$) erhielten wir für Ag_2SO_4 mit $\int \triangle T \mathrm{d}t = 700$ grad sec und $\lambda = 4{,}6 \cdot 10^{-4}$ für die Umwandlungswärme $Q = 12{,}0$ cal/g. (Der hohe Wert ist allerdings mit Zurückhaltung zu betrachten mit Rücksicht auf den Unterschied in der Wärmeleitfähigkeit von Analysen - bzw. Vergleichsubstanz.)*

Die in die Berechnung eingehende Wärmeleitfähigkeit des Silbersulfates wurde auf zwei voneinander unabhängigen Wegen bestimmt: Erstens durch Hervorrufen eines bekannten Peltier-Effektes in der Differential-Thermo-Anordnung und Messung der so zwischen beiden mit Silbersulfat gefüllten Bohrungen des Nickelblockes aufrechterhaltenen Temperaturdifferenz; zweitens nach der Methode von Christiansen [10], wobei bekanntlich die Wärmeleitfähigkeit durch Vergleich des in der Substanz sich einstellenden Temperaturgradienten mit dem einer in Serie geschalteten Eichsubstanz ermittelt wird **.

Beide Messungen lieferten übereinstimmende Werte.

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* Darüber hinaus liegen über die Umwandlungsvärme des Quarzes widersprechende Angaben vor ($Q = 3{,}1 - 7{,}8$ cal/g).

** Die hierher gehörenden Messungen wurden von Ing. F. Karnik ausgeführt.

ZUR KINETIK DER UMWANDLUNG

Beim Versuch, etwas über den Mechanismus der Umwandlung mit Hilfe thermischer Messungen zu erfahren, kann man grundsätzlich auf zwei verschiedenen Wegen vorgehen.

Entweder benutzt man die hier im experimentellen Teil beschriebene Anordnung und versucht Aufschluss über den Umwandlungsmechanismus zu erhalten durch Vergleich der so gewonnenen Kurven mit Kurven, die unter gewissen Annahmen berechnet worden sind.

Oder aber, man versucht durch Kompensation der Umwandlungswärme auf elektrischem Wege (Erzeugung einer Joule'schen Wärme im Analysen-, notfalls im Referenzgefäss) das Auftreten einer Temperaturdifferenz zwischen den beiden Gefässen überhaupt zu vermeiden. Dies hätte (bei gleicher Wärmeleitfähigkeit der beiden Substanzen) den Vorteil, dass die Wärmeleitungswirkungen wegfielen und dass das Quadrat, des Kompensationsstromes ein direktes Mass für die Umwandlungsgeschwindigkeit wäre.

Die Verwirklichung einer solchen Kompensationsanordnung erscheint indessen so schwierig (homogene oder genauer eine dem Umwandlungsprozess adäquate Entwicklung einer Joule'schen Wärme), dass vorerst davon abgesehen wurde.

Im folgenden wird also der erstgenannte Weg beschritten, wobei hier nur *das prinzipielle des Verfahrens* gezeigt werden soll an dem einfachsten denkbaren Fall, nämlich dem *konstanter Umwandlungsgeschwindigkeit* *. (Die dem wirklichen Verhalten näherkommenden Annahmen einer Abhängigkeit der Umwandlungsgeschwindigkeit von der Überschreitungstemperatur (linear, parabolisch, exponentiell) erfordern zur genauen Berechnung weitere Untersuchung der Temperaturverteilung in der Substanz, der zum Einsetzen der Umwandlung nötigen Überschreitung usw.)

Bei den behandelten Rechnungen werden folgende Vereinfachungen angenommen:

Die Substanzen befinden sich in kugelförmigen Ausbohrungen (in Wirklichkeit zylindrische Löcher, deren Höhe etwa gleich dem Durchmesser ist). Dies erlaubt die Anwendung von sphärischen Polarkoordinaten. (Angenähert kugelförmige Ausbohrungen zeigten keine prinzipielle Abweichung im Temperaturverlauf.)

Die Wärmeleitung innerhalb des Nickelblockes ist so gut, dass an der Grenzfläche Substanz/Nickelblock die im Schwerpunkt des Nickelblockes gemessene Temperatur herrscht.

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* Durchführt von N. Hartler als Teil der Examensarbeit, Göteborg 1949.

Folgende Symbole werden benutzt:

ϑ ; Temperatur in der Analysensubstanz, bezogen auf die Temperatur bei der die Umwandlung einsetzt.

λ : Wärmeleitfähigkeit der Analysensubstanz in $\mathrm{cal \cdot cm^{-1} \cdot sec^{-1} \cdot grad^{-1}}$

$\varkappa = \dfrac{\lambda}{\varrho c}$: die Temperaturleitzahl (ϱ : Dichte, c: spezifische Wärme)

$A \cdot \lambda$: die absorbierte Wärmemenge in $\mathrm{cal \cdot cm^{-3} \cdot sec^{-1}}$.

Damit erhält die Wärmeleitungsgleichung folgende Form:

$$\left\| \frac{1}{\varkappa} \cdot \frac{\partial \vartheta}{\partial t} = \nabla^2 \vartheta - A \right\| \tag{1}$$

Bei der Aufstellung der Anfangs- und der Randbedingungen ist folgendes zu bemerken:

Das Innere der Analysensubstanz zeigt bei laufender Erhitzung gegenüber dem Nickelblock eine Verzögerung des Temperaturanstieges, die sich folgendermassen berechnen lässt:

Die Wärmeleitungsgleichung hat in dieser Periode folgendes Aussehen:

$$\frac{1}{\varkappa} \cdot \frac{\partial \Theta}{\partial t} = \nabla^2 \Theta \tag{2}$$

wobei Θ die Temperatur der Analysensubstanz bezogen auf die des Nickelblockes ist. ($\Theta = 0$ im thermischen Gleichgewicht.)

In Polarkoordinaten ausgedrückt und unter Annahme kugelsymmetrischer Temperaturverteilung nimmt Gleichung (2) folgende Form an:

$$\frac{\partial^2 \Theta}{\partial r 2} + \frac{2}{r} \cdot \frac{\partial \Theta}{\partial r} - \frac{1}{\varkappa} \frac{\partial \Theta}{\partial t} = 0 \qquad \text{mit} \begin{cases} \Theta = 0 \text{ für } t = 0 \\ \Theta = bt \text{ für } r = a \end{cases} \tag{3}$$

a: Radius der Kugel

b: die Erhitzungsgeschwindigkeit.

Wird $u = RT$ geschrieben, wobei R ausschliesslich eine Funktion von r und T ausschliesslich eine Funktion von t ist, so ergibt sich

$$\frac{1}{\varkappa} \frac{1}{T} \frac{\mathrm{d}T}{\mathrm{d}t} = \frac{1}{\mathrm{R}} \frac{\mathrm{d}^2 R}{\mathrm{d}r^2} \tag{4}$$

Setzt man beide Seiten gleich einer Konstanten — m^2 so ergibt sich schliesslich als Lösung zu (3):

$$\Theta = \frac{1}{r} e^{-m^2 \varkappa t} (\sin mr + \cos mr) \tag{5}$$

Um die zu (3) gehörenden Anfangs- und Randbedingungen zu erfüllen, muss (5) umgeformt werden mit Hilfe einer Fourier-Reihenentwicklung sowie unter Anwendung von Duhamels Theorem. Die so erhaltene Lösung vereinfacht sich für den stationären Zustand durch Wegfall sämtlicher Exponentialausdrücke zu:

$$\left\| \Theta = \frac{b}{6\varkappa} (r^2 - a^2) \right\| \tag{6}$$

Nun kann Gleichung (1) weiter entwickelt werden:
Analog (3) ergibt sich:

$$\frac{\partial^2 \vartheta}{\partial r^2} + \frac{2}{r} \frac{\partial \vartheta}{\partial r} - \frac{1}{\varkappa} \frac{\partial \vartheta}{\partial t} - A = 0 \tag{7}$$

$$\text{mit} \begin{cases} \vartheta = \dfrac{b}{6\varkappa} (r^2 - a^2) & \text{für } t = 0 \\ \vartheta = bt & \text{für } r = a \end{cases}$$

Um das Partikularintegral zum Störungsterm A festzulegen, wird der Ansatz gemacht:

$$\vartheta_1 = \alpha_0 + \alpha_1 t + \alpha_2 t^2$$

Einsetzen in (7) ergibt: $\vartheta_1 = -A \cdot \varkappa t$
Daher erscheint eine Substitution $u = \vartheta r + A\varkappa t \cdot r$ zweckmässig, die, in (7) eingesetzt, ergibt:

$$\frac{\partial^2 u}{\partial r^2} - \frac{1}{\varkappa} \frac{\partial u}{\partial t} = 0$$

Dies ergibt analog (4) und (5):

$$\vartheta = -A\varkappa t + \frac{1}{r} \cdot e^{-m^2 \varkappa t} (\sin mr + \cos mr)$$

Eine entsprechende Umformung wie bei (5) ergibt:

$$\vartheta = -A\varkappa t + \frac{2}{ar} \sum_{n=1}^{\infty} e^{\frac{-\varkappa n^2 \pi^2 t}{a^2}} \sin \frac{n\pi r}{a} \cdot \left[\int_0^a \frac{b}{6\varkappa} (r^3 - a^2 r) \sin \frac{n\pi r}{a} dr - \right.$$

$$\left. - n\pi\varkappa (-1)^n \int_0^t e^{\frac{\varkappa n^2 \lambda}{a^2}} B\lambda d\lambda \right] \tag{8}$$

mit $B = b + A\varkappa$

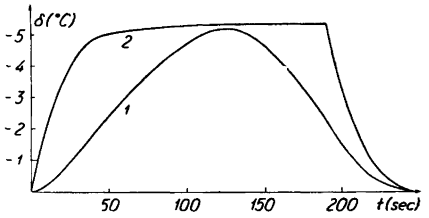

Fig. 5. Die Temperaturdifferenz zwischen der Silbersulfatprobe und der Bezugssubstanz als Funktion der Zeit: (1) Experimentell. (2) Berechnet unter der vereinfachenden Annahme konstanter Umwandlungsgeschwindigkeit nach Gl. (9).

Die registrierte Temperatur δ ist der Temperaturunterschied zwischen dem Mittelpunkt der Probesubstanz und dem der Vergleichsubstanz. Es gilt:

$$\left\| \delta = -\frac{Aa^2}{6} - \frac{2Aa^2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} e^{\frac{-\varkappa n^2 \pi^2 t}{a^2}} \right\| \tag{9}$$

Dies gilt für die Dauer der Umwandlung; später folgt ein rein exponentieller Abkühlungsverlauf.

Setzt man die zum Silbersulfat gehörenden Werte der Konstanten ein: $A = 806$; $\varkappa = 2{,}72 \cdot 10^{-4}$; $A \cdot \varkappa = 0{,}219$; $B = 0{,}0834 + 0{,}219 = 0{,}302$; $\frac{\varkappa \pi^2}{a^2} = 0{,}067$ so erhält Gleichung (9) folgendes Aussehen;

$$\delta = -5{,}38 + 6{,}53e^{-0{,}067t} - 1{,}63e^{-0{,}268t} + 0{,}73e^{-0{,}596t} - 0{,}41e^{-1{,}07t} + 0{,}26e^{-1{,}67t} \ldots\ldots$$

Diese Funktion ist in Fig. 5 wiedergegeben zusammen mit dem experimentell festgestellten Temperaturverlauf.

Der Vergleich zeigt, dass die Wärmeentwicklung und damit die Umwandlungsgeschwindigkeit nicht konstant sind, wie hier angenommen, sondern von einem kleinen Anfangswert zu einem Maximalwert steigen, um gegen Schluss wieder abzusinken.

Die Umwandlungsgeschwindigkeit ist offenbar eine Funktion der Überschreitung der Gleichgewichtstemperatur.

Ein exponentieller Zusammenhang erscheint wahrscheinlich. Das verträgt sich mit der Auffassung von Avrami [11], der den Umwandlungsprozess aufteilt in Keimbildung (oder genauer Aktivierung existierender Keime) und Keimwachstum, wobei die Keimbildung einer Exponentialfunktion der Temperatur gehorcht.

Eine Entscheidung zwischen Keimbildung und Keimwachstum als geschwindigkeitsbestimmendem Teilprozess liesse sich eventuell experimentell durch Impfung einer mässig unterkühlten Silbersulfatmenge mit der Tieftemperaturmodifikation treffen.

Eine genauere Analyse des Umwandlungsvorganges, als wir sie hier durchführen konnten, bedarf sicher einer verbesserten experimentellen Anordnung, wie z. B. der oben erwähnten Kompensationsanordnung, bei der kaum Wärmeleitungsprobleme zu berücksichtigen sind.

Auch das Studium der Umwandlung bei verschiedenen Überschreitungen als Anfangsbedingung wäre von Interesse.

Hierzu dürften sich aber nur recht langsam verlaufende Umwandlungen eignen, wobei die Wärmeeffekte so klein werden, dass eine empfindlichere Messanordnung als die hier beschriebene verwendet werden muss.

ZUSAMMENFASSUNG

Die kristallographische Umwandlung des Silbersulfates wird mittels thermischer Differentialanalyse untersucht, und der Umwandlungspunkt zu $430 \pm 3°$ C festgelegt.

Die Tieftemperaturmodifikation lässt sich nicht nennenswert überhitzen, die Hochtemperaturmodifikation dagegen lässt sich bis zu einem Betrag von 20° C unterkühlen.

Die Umwandlungsgeschwindigkeit hängt von der Überschreitung der Gleichgewichtslage ab. Berechnungen und Vergleich mit dem Experiment zeigen nämlich, dass keine konstante Umwandlungsgeschwindigkeit bei fortlaufender Erhitzung vorliegt. Die Methodik zur eingehenden Untersuchung der Umwandlungskinetik wird diskutiert.

Die Untersuchung wurde durch Mittel des *Statens Tekniska Forskningsråd* unterstützt, wofür auch an dieser Stelle bestens gedankt sei.

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Eingegangen am 29. Juni 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1109–1118

The Crystal Structure of 1,2,3,4-Tetrabromocyclohexane, M. P. 142° C

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For some time chemical investigations dealing with 1,2,3,4-tetrahalogenocyclohexanes have been carried out in this laboratory. A report of the results obtained will be published in the near future [1]. In the course of this work three different tetrabromocyclohexanes have been isolated by adding bromine to cyclohexadiene-1,3. The melting points are 89°—90° C, 142° C and 155°—156° C respectively. It would be expected that one of these is identical with the compound melting at 140°—141° C reported by Zelinsky and Gorsky [2]. Harries [3] prepared a compound of m. p. 89°—90° C and obtained also a substance melting about 140° C. By recrystallization, however, he succeeded in raising the melting point of the latter to 155°—156° C. This result indicated at that time some doubt about the existence of the compound melting at 142° C. A detailed study of this isomer was therefore regarded to be most interesting.

First of all, preliminary X-ray investigations of all three compounds have been carried out and the data are given in Table 1.

Table 1. X-ray data for isomers of 1,2,3,4-$C_6H_8Br_4$.

M. p.	a	b	c	β	Calc. density gcm^{-3}	Number of molecules per unit cell	Space group
89—90° C	14.21	10.22	7.91	121°	2.70	4	$P2/a$ or Pa
142° C	11.28	22.55	7.89		2.65	8	Fdd
155—156° C	9.91	8.52	8.06	133°	2.67	2	$P2_1$

The values of calculated density correspond well to those measured for the 1$\varkappa$, 2$\varkappa$, 4ε, 5ε [4] — and 1$\varkappa$, 2$\varkappa$, 4$\varkappa$, 5$\varkappa$ [5]-tetrabromocyclohexanes, respectively

2.66 g cm^{-3} and 2.74 g cm^{-3}. The compound melting at 142° C appeared to be the most suitable one for an X-ray analysis.

Tetrabromocyclohexane with m. p. 142° C crystallizes in two different shapes. From ligroin fine needles elongated along [001] were obtained and from methanol the crystals are orthorhombic pyramids with (010) as base. The X-ray investigation shows that both forms have the same lattice.

The unit cell dimensions, measured from rotation and Weissenberg photographs, are given in Table 1. With eight molecules in the unit cell these values give a calculated density of 2.65 g cm^{-3}. Oscillation and Weissenberg photographs show that the crystals belong to the orthorhombic system. The study of systematic extinctions gives the result that only the following reflexions are present:

(hkl) when all indices are even or all odd
($hk0$) when $h = 2n$ and $k = 2n$
($0kl$) when $k = 2n$, $l = 2n$ and $k + l = 4n$
($h00$) when $h = 4n$

This leads to a face-centred lattice and without ambiguity to the space group Fdd which has a sixteen-fold general position. The molecule must therefore possess the point symmetry, *viz.* a twofold axis associated with the eight-fold special positions.

From this follows that the molecule must possess a "chair"-like carbon ring, and the configuration for the bromine atoms must be either $1\varkappa$, $2\varkappa$, $3\varkappa$, $4\varkappa$ or $1\varkappa$, 2ε, 3ε, $4\varkappa$ respectively 1ε, $2\varkappa$, $3\varkappa$, 4ε, the two latter ones being transformed into each other by a conversion of the carbon ring.

For the determination of the structure the intensities of all reflexions ($hk0$) and ($0kl$) obtainable with Cu-K radiation were estimated visually by comparison with a standard scale and using the multiple film method. The f-values used in computing structure factors were those of the International Tables, with temperature factors to be mentioned later. No correction for absorption has been introduced, the crystals used in the investigation being very small.

In order to decide between the three possibilities mentioned above first of all a Patterson projection on (001) was computed. The resulting map is reproduced in Fig. 1, which corresponds to one sixteenth of the unit cell. The points marked with squares are the projections of terminating points of the vectors between bromine atoms in the final xy-projection.

The map shows two dominating peaks situated in ($\frac{1}{4}$, 0.114) and (0.083, $\frac{1}{4}$). These peaks are obviously due to vectors from a bromine atom in (x, y, z) to two other equivalent ones in ($\frac{1}{4} + x$, $\frac{1}{4} - y$, $\frac{1}{4} + z$) and ($\frac{1}{4} - x$, $\frac{1}{4} + y$, $\frac{1}{4} + z$) respectively, the projections of the corresponding vectors having the compo-

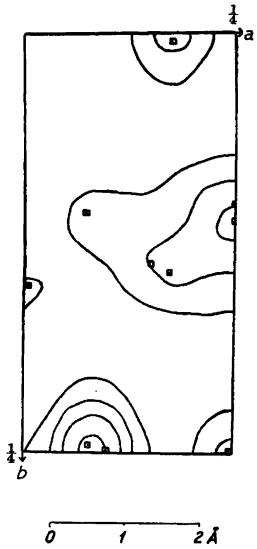

Fig. 1. Patterson projection on (001).

nents ($\frac{1}{4}$, $\frac{1}{4} - 2y$, $\frac{1}{4}$) and $\frac{1}{4} - 2x$, $\frac{1}{4}$, $\frac{1}{4}$). This leads to the coordinates $x = 0.083$, $y = 0.068$. Now in an "ideal" structure with tetrahedral angles the distance from the twofold axis to a bromine atom in $2x$-position is 1.69Å taking the C—C and C—Br bond lengths to be respectively 1.54 Å and 1,94 Å. Assuming the x- and y-coordinates found above to belong to one bromine atom, the distance of this atom from the twofold axis is 1.80 Å. It might therefore be assumed that the molecule contains a bromine atom in $2x$-position when some deformation from an "ideal" structure is taken into account. With a $2x$-bromine atom in the point thus found, the Patterson peak in (0.175, 0) may be interpreted as resulting from a vector between $1x$ and $2x$ bromine atoms. In an "ideal" structure the corresponding vector has the x- and y-components (0.150, 0.030). This preliminary investigation based on the Patterson projection should thus indicate a $1x$, $2x$, $3x$, $4x$-configuration with some deformations of the molecule from an "ideal" model.

Due to extensive overlapping of vector peaks, however, a Patterson projection is difficult to interprete uniquely. It was therefore felt that the above considerations had to be supported by a more systematic treatment of the different possibilities. Assuming an "ideal" structure of one of those having a twofold axis, we have in the xy-projection a one-parameter problem, the parameter being the angle α which a representative line in the molecule makes with the a-axis, say.

For the three different possible structures the variation of the $F(hk0)$'s with this angle was determined in the whole range of α-values. The desired

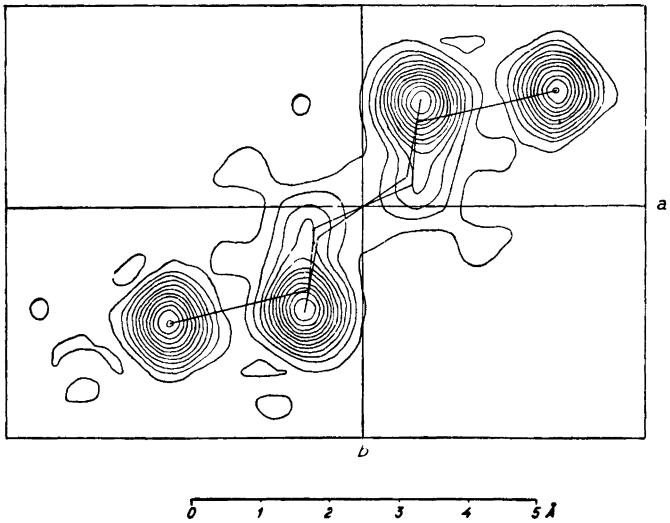

Fig. 2. Fourier projection on (001).

agreement between observed and calculated F-values was obtainable only for the $1x$, $2x$, $3x$, $4x$-configuration and for an orientation of the molecule very nearly the same as that found by means of the Patterson projection.

This preliminary structure could then serve as the base for a Fourier projection on (001). The number of $hk0$-reflexions used in the analysis was 49. With a few exceptions the signs of the structure factors were determined by the bromine atoms. In fact, in the first synthesis the contribution of the carbon atoms was omitted, but their introduction at a later stage gave a better agreement between calculated and observed intensities and a few sign changes.

It is impossible in this projection to locate the carbon atoms with any degree of certainty on account of the overlapping of the neighbouring large peak due to a bromine atom. The presence of the two carbon atoms C_2 and C_6, which in the "ideal" model have the same x- and y-coordinates, appears as an extension of this same bromine peak. The projection in the (100)-plane gives a little more information on this point. As to the hydrogen atoms their effect is so negligible that they have not been taken into account at all.

In order to improve the agreement between observed and calculated $|F|$-values for the reflexions with larger ϑ-values, a temperature factor of $B = 5.78$ Å^2 was introduced. The agreement factor ($\Sigma|\Delta F/F|$) for the final xy-projection was found to be 0.20. In Fig. 2 the electron density map is reproduced.

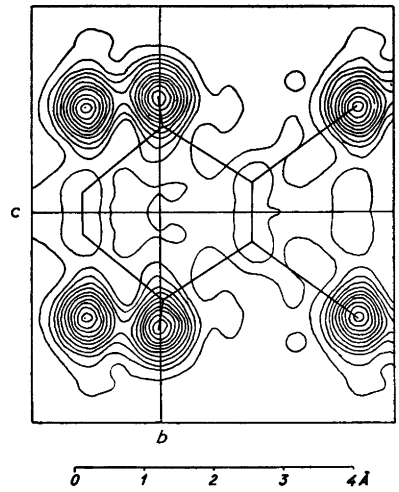

Fig. 3. Fourier projection on (100).

As to the determination of the z-parameters a projection on (100) appeared to be more convenient than one on (010) which gives larger amount of overlapping of different peaks than the former. The projection of the structure on (100) does not contain a center of symmetry and phase angles had therefore to be computed. On account of the extinction rule $k + l = 4n$ only 33 reflexions were used in this projection.

As a starting point served a structure where the y-coordinates of the bromine atoms were taken from the xy-projection while their z-coordinates as well as all the coordinates for the carbon atoms were derived from an "ideal" model of the molecule in a position approximately determined by the x- and y-coordinates of the bromine atoms. This trial structure proved to be nearly correct as to the position of the bromine atoms, the first Fourier synthesis giving no change in their coordinates. The presence of the carbon atoms C_2 and C_6 appears as small peaks well separated from those due to the bromine atoms. The ordinary procedure of successive approximations was followed until the changes in the phase angles were insignificant. As in the xy-projection the hydrogen atoms have not been taken into account. Use has been made of a temperature factor $B = 3.31$ Å^2, the value of which was determined by the curve showing the variation of $|F_{obs}/F_{calc}|$ with ϑ. For the final agreement factor $\Sigma|\Delta F/F|$ the value 0.20 was obtained for this projection too.

Fig. 3 gives the electron density map of a part of the structure on (100). In Figs. 4 and 5 are reproduced schematic projections on (001) and (100) respectively, showing the mutual arrangement of the molecules whose centres

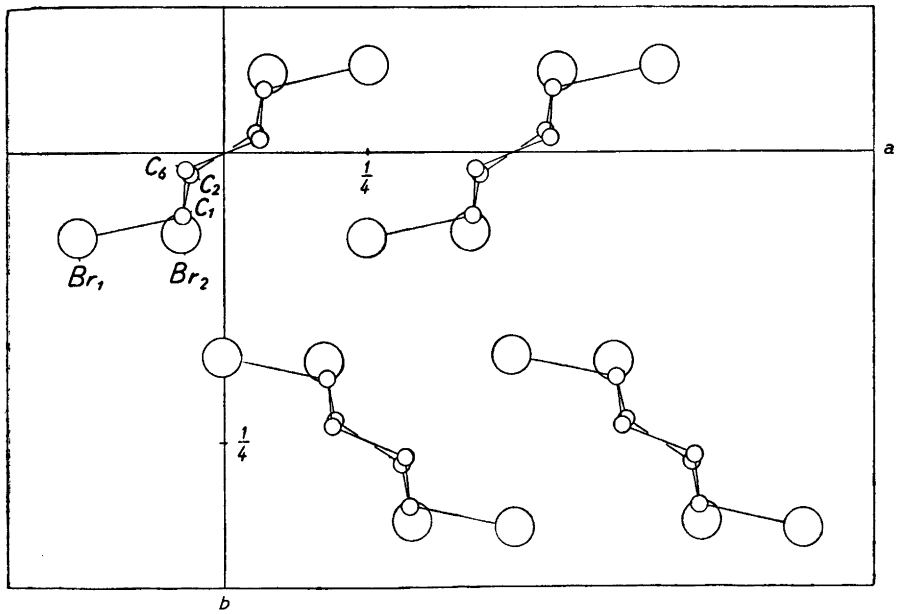

Fig. 4. 1x, 2x, 3x, 4x—$C_6H_8Br_4$. Projection of the structure on (001).

are situated in (0, 0, z_0), ($\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{4} + z_0$), ($\frac{1}{2}$, 0, $\frac{1}{2} + z_0$) and ($\frac{3}{4}$, $\frac{1}{4}$, $\frac{3}{4} + z_0$), the origin being chosen in such a way that z_0 is nearly zero.

In both projections the peaks due to bromine atoms are well defined and these atoms can thus be given rather reliable coordinates. As to the carbon atoms the situation is different. The *yz*-projection gives the *y*- and *z*-coordinates of C_2 and C_6, while their *x*-coordinate as well as the coordinates for the carbon atom C_1 are not determinable by means of the maps. The assumption that the C—C bond length is 1.54 Å allows a computation of the *x*-coordinates for C_2 and C_6, while the coordinates given to C_1 are those derived from an "ideal" carbon ring. This procedure gives a mean C—Br bond length of 1.99 Å. In Table 2 the coordinates of crystallographically independent atoms are listed. Table 4 gives the observed and calculated $|F|$-values for the (*hk*0) and (0*kl*) reflexions.

Table 2. Coordinates for bromine and carbon atoms.

	x	*y*	*z*
Br_1	— 0.252	0.074	0.002
Br_2	— 0.075	0.069	— 0.360
C_1	— 0.071	0.055	— 0.004
C_2	— 0.057	0.019	— 0.167
C_6	— 0.063	0.013	0.144

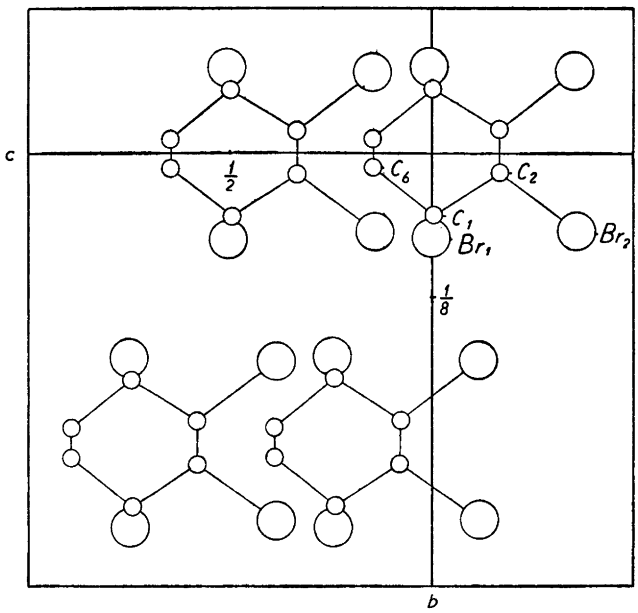

Fig. 5. 1x, 2x, 3x, 4x—$C_6H_8Br_4$. Projection of the structure on (100).

In spite of the non rigorous localization of carbon atoms there is no doubt that the configuration of bromine atoms is 1x, 2x, 3x, 4x. Fig. 6 shows a model of this tetrabromocyclohexane molecule.

As to the deviation of the molecule from an "ideal" model no statement about deformation of valency angles can be made on account of the uncertain positions of the carbon atoms. The Br—Br distances within the same molecule may nevertheless give an indication of the directions in which the deformations take place. The Br_1—Br_2 and Br_2—Br_3 distances are 3.49 Å and 3.54 Å respectively, while the "ideal" value for both is 3.38 Å when the C—C and C—Br bond lengths are taken to be 1.54 Å and 1.94 Å. The Br_1—Br_4 distance has, however, decreased from an "ideal" value of 6.62 Å to 6.59 Å and the Br_1—Br_3

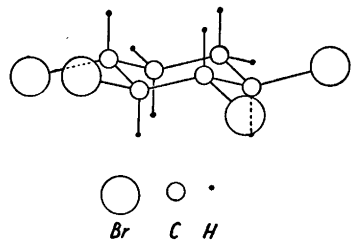

Fig. 6. Model of a molecule of 1x, 2x, 3x, 4x—$C_6H_8Br_4$.

distance correspondingly from 5.69 Å to 5.67 Å. A probable deformation is thus mainly composed of two parts:

1. In order to increase the distance between the atoms Br_2 and Br_3, these atoms are displaced oppositely in the directions of the three-fold inversion axis of the carbon ring.

2. Perpendicularly to this axis the atoms Br_1 and Br_4 are displaced in such a way that their distance from the respective neighbouring bromine atoms is increased.

A consideration of the distances between atoms belonging to neighbouring molecules allows some conclusions to be drawn about the dominating van der Waals' forces. The shortest Br—Br distance of this type has the length of 3.80 Å. It would be of interest to know the distances between hydrogen and bromine atoms belonging to different molecules, but it is impossible to locate hydrogen atoms by means of the maps. Assuming, however, tetrahedral angles between the C—C and C—H bonds and a C—H bond length of 1.08 Å, "ideal" coordinates for the hydrogen atoms may be calculated. The shortest H—Br distance thus found is 2.88 Å. In Table 3 some of the distances between atoms belonging to neighbouring molecules are listed. The first column gives the coordinates for the symmetry center of the carbon ring ($z_0 = -0.09$) in the molecules concerned. In the second column those atoms are indicated between which the distance in column three is computed.

Table 3. Intermolecular atomic distances.

$(0, 0, z_0)$ $(\frac{1}{2}, 0, \bar{\frac{1}{2}} + z_0)$	$Br_{2\varkappa}$ $Br_{1\varkappa}$	3.80 Å
$(0, 0, z_0)$ $(0, 0, 1 + z_0)$	$H_{6\varkappa}$ $Br_{2\varkappa}$	3.06 Å
$(0, 0, z_0)$ $(\frac{1}{4}, \frac{1}{4}, \frac{1}{4} + z_0)$	$H_{1\varepsilon}$ $Br_{2\varkappa}$	2.99 Å
$(0, 0, z_0)$ $(\frac{1}{4}, \frac{1}{4}, \frac{1}{4} + z_0)$	$H_{1\varepsilon}$ $Br_{1\varkappa}$	2.88 Å

These correspond reasonably well to normal van der Waals' distances. It may therefore be concluded that the cohesion of the lattice in the a-direction is due, in the main, to forces acting between neighbouring bromine atoms. In other directions the dominating forces are those between hydrogen and bromine atoms.

Table 4. Observed and calculated $|F|$-values.

(*hkl*)	$\|F\|_{obs}$	$\|F\|_{calc}$	(*hkl*)	$\|F\|_{obs}$	$\|F\|_{calc}$
0. 4.0	0	7	10. 2.0	6	7
0. 8.0	65	70	10. 4.0	8	11
0.12.0	45	37	10. 6.0	8	6
0.16.0	24	26	10. 8.0	0	2
0.20.0	24	26	10.10.0	0	0
0.24.0	0	6			
0.28.0	4	10	12. 0.0	8	9
			12. 2.0	0	2
2. 2.0	5	3	12. 4.0	0	2
2. 4.0	43	42	12. 6.0	0	2
2. 6.0	20	21	12. 8.0	5	7
2. 8.0	0	3			
2.10.0	5	4			
2.12.0	14	15	0. 2.2	37	37
2.14.0	6	7	0. 6.2	57	48
2.16.0	6	5	0.10.2	10	11
2-18.0	8	6	0.14.2	24	29
			0.18.2	9	6
4. 0.0	22	25	0.22.2	9	12
4. 2.0	31	32	0.26.2	6	6
4. 4.0	13	8			
4. 6.0	23	22	0. 0.4	10	10
4. 8.0	12	14	0. 4.4	9	8
4.10.0	14	18	0. 8.4	8	11
4.12.0	16	12	0.12.4	6	5
4.14.0	0	1	0.16.4	11	6
4.16.0	0	2	0.20.4	0	3
4.18.0	11	11	0.24.4	8	6
6. 2.0	38	35	0. 2.6	31	25
6. 4.0	10	8	0. 6.6	24	25
6. 6.0	35	36	0.10.6	6	6
6. 8.0	0	1	0.14.6	16	21
6.10.0	6	7	0.18.6	5	4
6.12.0	0	2	0.22.6	6	10
6.14.0	20	22			
6.16.0	0	2	0. 0.8	30	22
			0. 4.8	4	5
8. 0.0	0	2	0. 8.8	19	17
8. 2.0	7	6	0.12.8	7	9
8. 4.0	6	5	0.16.8	7	8
8. 6.0	6	4			
8. 8.0	0	2	0.2.10	4	2
8.10.0	0	5			
8.12.0	0	3			

SUMMARY

The crystal structure of 1,2,3,4-tetrabromocyclohexane of m. p. 142° C, obtained from cyclohexadiene-1,3 and bromine, has been determined. The bromine atoms occupy the positions: $1\varkappa$, $2\varkappa$, $3\varkappa$, $4\varkappa$. Deviations from the "ideal" structure and the intermolecular atomic distances have been considered.

I wish to thank Prof. O. Hassel for placing samples of the 1,2,3,4-$C_6H_8Br_4$ compounds at my disposal and also for his continued interest in the investigation. Further I should like to express my gratitude to *A/S Lilleborg Fabriker* for financial assistance from *A/S Lilleborg Fabrikers Jubileumsgave.*

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Received June 17, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1119–1130

Vanadium Pentoxide — a Compound with Five-Coordinated Vanadium Atoms

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In the attempts to solve the structure of CrO_3 (Byström and Wilhelmi[1]) it was found that the calculations of the atomic parameters in one direction could be considerably simplified if it was assumed that the distance O—O within the oxygen tetrahedron around the chromium atoms was not shorter than 2.4 Å. A survey of the distances in already known structures showed that in structures with tetrahedra or octahedra around the central atom the shortest O—O distances are generally 2.4 Å or longer. However, in V_2O_5 according to Ketelaar's determination [2] there are considerably shorter O—O distances (of only 2.14 Å), and therefore a recalculation of the atomic parameters in that compound was of interest.

The dimensions of the unit cell are according to Ketelaar $a = 11.48 \pm 0.01$ Å, $b = 4.36 \pm 0.005$ Å and $c = 3.55 \pm 0.005$ Å. The structure which he suggests is described in C_{2v}^7—*Pmmn* with the atoms in the following positions:

4 V in 4 (*b*): *x*, *y*, *z*	$x = 0.148$	$y = 0.097$	$z = 0$
4 O in 4 (*b*): *x*, *y*, *z*	$x = 0.148$	$y = 0.45$	$z = 0.92$
4 O in 4 (*b*): *x*, *y*, *z*	$x = 0.20$	$y = 0.03$	$z = 0.46$
2 O in 2 (*a*): 0, *y*, *z*		$y = 0.03$	$z = 0.89$

This arrangement leads, according to Ketelaar, to a structure built up by a two-dimensional network of tetrahedra in the *xz*-plane. However, Machatschki[3] has suggested that the structure is composed of double chains of the composition $(V_2O_5)_\infty$.

THE STRUCTURE DETERMINATION

From powder photographs taken in focussing cameras the following cell dimensions were calculated (Cr—K, $\lambda_\alpha = 2.2909$ Å) $a = 11.519 \pm 0.006$ Å, $b = 4.373 \pm 0.002$ Å, $c = 3.564 \pm 0.002$ Å.

Weissenberg photographs of the $hk0$, $hk1$, $h0l$, and $h1l$ reflexions (Cu-K) showed that the space group must be D_{2h}^{13} or C_{2v}^{7}, as was also found by Ketelaar.

The values of $|F_{obs}|$ were calculated according to the formula $|F_{obs}| = K\sqrt{\frac{I}{PL}}$. The value of K was adjusted so that for each layer line $\sum|F_{obs}| = \sum|F_{calc}|$. The scattering factors of V^{5+} and O^{2-} were used in the calculations of F_{calc}. No corrections for the decrement in f_{v5+}, due to the wave length of the used radiation, were made. However, the bonds in V_2O_5 are probably to a large extent covalent and thus the scattering factor of f_{v5+} is too low. Hence the decrement in the f values is, to a certain extent, allowed for.

The xy-projection has in both these space groups a centre of symmetry. A recalculation of the F values of the $hk0$ reflexions with Ketelaar's parameters showed that the agreement with the observed values was so good that the signs of the structure factor of most of the reflexions could be established. Already in the second calculation of the projection of the electron density all reflexions were used and, as the confirmatory calculation of the F-values showed, with correct signs. The final adjustment of the parameters was made from a careful trial and error process.

The parameters in the xy-plane are:

4 V :	$2\pi x_1 = 53.5° \pm 0.5°$	$x_1 = 0.1486 \pm 0.0015$	$2\pi y_1 = 38° \pm 1°$	$y_1 = 0.105 \pm 0.003$
4 O_1 :	$2\pi x_2 = 53.5° \pm 1.5°$	$x_2 = 0.149 \pm 0.004$	$2\pi y_2 = 165° \pm 5°$	$y_2 = 0.458 \pm 0.014$
4 O_2 :	$2\pi x_3 = 115° \pm 1.5°$	$x_3 = 0.320 \pm 0.004$	$2\pi y_3 = 0° \pm 5°$	$y_3 = 0.000 \pm 0.014$
2 O_3 :			$2\pi y_4 = 0° \pm 2°$	$y_4 = 0.000 \pm 0.006$

The errors in the parameters are partly deduced from the trial and error process (V and O_2) and partly from the electron density projection (O_1 and O_3). As will be seen from Table 1, the largest differences $|F_{obs}|—|F_{calc}|$ are found for two strong reflexions at low glancing angles (200 and 010). This is certainly due to the difficulty of estimating the intensities and not to parameter-errors, and therefore in the final Fourier synthesis, F_{calc} was used instead of F_{obs} for these reflexions.

As will be seen from Fig. 1 a, the maxima from V and O_2 partly overlap in the xy-projection and thus the O_2 positions are not clearly shown. Therefore a new Fourier synthesis was made using the values of $F_{obs} - F_V$ instead of F_{obs}

Table 1. V_2O_5. Comparison between observed and calculated F-values (Cu-K radiation) for hk0 and hk1 reflexions. Reflexions marked with an asterisk are observed only for Cu-K β radiation.

hkl	$\lvert F_{obs} \rvert$	F_{calc}	*hkl*	$\lvert F_{obs} \rvert$	F_{calc}
200	47	− 36	330	48	− 46
400	65	− 62	430	32	25
600	74	76	530	< 11	− 2
800	18	16	630	< 11	− 4
1000	43	− 41	730	35	40
1200	14	17	830	11	− 13
1400	33	31	930	18	− 20
1600 *	< 25	− 19	1030	29	26
010	50	70	1130	24	− 23
110	29	31	040	14	− 15
210	12	− 11	140	9	5
310	47	− 43	240	11	7
410	14	− 14	340	13	− 9
510	< 7	− 2	440	33	25
610	42	40	540	< 14	− 1
710	33	33	640	14	− 10
810	< 12	2	740	11	7
910	11	11	840	12	− 10
1010	12	− 12	940	< 11	− 4
1110	11	− 15	1040	32	30
1210	11	12	050	23	− 23
1310	16	21	150	< 13	0
1410	13	13	250	12	10
020	56	59	350	< 11	− 1
120	26	27	450	28	31
220	7	− 11	550	< 8	1
320	41	− 41	650	15	− 16
420	14	− 15	060 *	< 23	− 11
520	< 8	− 2	160 *	25	− 18
620	32	37	260 *	< 25	5
720	39	34	360 *	30	34
820	< 11	3	101	(∼ 47) **	58
920	18	− 16	301	51	− 46
1020	< 11	− 9	501	11	− 8
1120	19	− 19	701	75	67
1220	11	11	901	14	− 14
1320	30	− 26	1101	36	− 33
030	13	− 9	1301	40	45
130	31	31	011	42	44
230	< 9	6	111	19	27

**) Not registrered in this photograph.

hkl	$\lvert F_{obs} \rvert$	F_{calc}	*hkl*	$\lvert F_{obs} \rvert$	F_{calc}
211	16	− 12	131	20	− 15
311	7	4	231	16	− 14
411	45	− 32	331	45	41
511	7	− 6	431	38	− 37
611	28	26	531	< 9	− 2
711	33	30	631	24	31
811	11	11	731	17	− 15
911	< 11	0	831	14	14
1011	30	− 26	931	16	19
1111	21	− 16	1031	30	− 35
1211	< 9	5	1131	9	9
1311	22	29	041	9	11
021	52	43	141	20	− 17
121	17	17	241	< 9	− 3
221	15	− 12	341	38	38
321	< 7	− 2	441	9	− 8
421	40	− 33	541	< 9	− 2
521	7	− 5	641	7	7
621	31	28	741	17	− 20
721	24	25	841	< 9	3
821	13	12	941	16	20
921	< 9	2	051	< 8	2
1021	24	− 30	151	20	− 19
1121	9	− 13	251	< 9	0
1221	< 7	6	351	36	42
031	48	48			

(as in the former synthesis, F_{calc} was used for 200 and 010). Thus in this projection the V-atoms are eliminated, and the O_2 atoms showed up at the expected positions (Fig. 1 b).

The value of the quotient $R = \frac{\sum \| F_{obs} | - | F_{calc} \|}{\sum | F_{obs} |}$ is very low, being only 0.10 for the *hk*0 reflexions (the values for 200 and 010 are not incorporated in the value because of the estimation errors for the reflexions, see above) and 0.14 for the *hk*1 reflexions.

Ketelaar stated that the intensities of the reflexions could not be accounted for, assuming the space group to be D_{2h}^{13}, and that therefore the space group must be C_{2v}^{7}. However, we cannot confirm this statement. There are no discrepancies between observed and calculated intensities for the *hk*1, *h*0*l*, and *hll* reflexions that indicate that the atoms must be moved from the positions in D_{2h}^{13}, which are parameter-free along the *z*-axis. It is true that

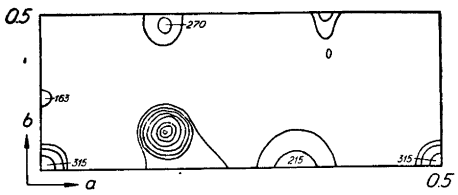

Fig. 1. a. V_2O_5. *Projection of the electron density on (001).*

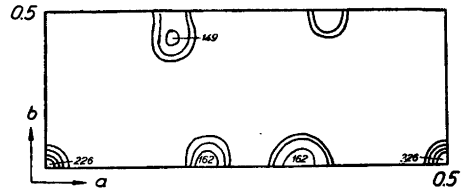

Fig. 1 b. V_2O_5. *Projection of the electron density on (001), with the V-atoms eliminated. The heights are on an arbitrary scale.*

because of the habit of the crystals (the prisms have the c-axes parallel to the prism axes) it is more difficult to estimate the intensities of the $h0l$ and hll reflexions than the intensities of the $hk0$ reflexions. The reflexions $00l$ show up too weak compared with the $h00$ reflexions. As will be seen from Fig. 2, the quotient $\frac{F_{obs}}{F_{calc}}$ increases from about 0.5 for the $00l$ reflexions to about 2 for reflexions with high h-values.

An attempt to consider the influence of the habit of the crystals (and the temp. factor) has been made. In Fig. 2 the values of $\frac{F_{obs}}{F_{calc}}$ are plotted against h. A curve has been drawn, from which a correction factor K has been taken. The values of F_{obs} have been divided by this factor. As will be seen from Table 2, the agreement between $F_{corr} = \frac{1}{K} \cdot F_{obs}$ and F_{calc} is quite satisfactory and the value of R, using F_{corr} instead of F_{obs} in the formula is only 0.14. Thus the structure determination leads to the following centrosymmetrical arrangement of the atoms:

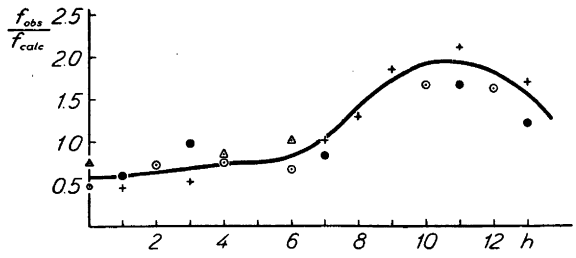

Fig. 2. $\frac{F_{obs}}{F_{calc}}$ *plotted against h for reflexions h0l. Explanations in the text p. 1123.*

Table 2. V_2O_5. *Comparison between observed and calculated intensities for h01 and h1l reflexions. Cu-K radiation.*

hkl	$\lvert F_{obs}\rvert$	F_{calc}	$\lvert F_{corr}\rvert$	*hkl*	$\lvert F_{obs}\rvert$	F_{calc}	$\lvert F_{corr}\rvert$
101	27	58	47	1202	26	16	15
301	24	− 46	38	103	18	31	28
501	< 18	− 8	< 23	303	31	− 32	42
701	70	67	70	503	< 18	− 5	< 22
901	26	− 14	15	703	38	46	38
1101	70	− 33	37	903	20	− 12	12
1301	77	45	51	1103	49	− 29	26
002	44	92	71	1303 *	49	42	33
202	15	− 21	21	004	40	53	65
402	35	− 46	46	204	< 18	− 13	< 26
602	38	59	48	404	26	− 31	34
802	18	14	14	604	40	39	47
1002	60	− 36	33				

hkl	$\lvert F_{obs}\rvert$	F_{calc}	*hkl*	$\lvert F_{obs}\rvert$	F_{calc}
011	40	44	912	< 22	− 13
111	17	27	1012	23	− 12
211	13	− 12	1112	20	− 16
311	< 13	4	1212	15	11
411	29	− 32	1312	17	20
511	< 18	− 6	013	25	29
611	26	26	113	< 20	13
711	36	30	213	< 28	− 7
811	21	11	313	< 22	− 5
911	< 22	0	413	22	− 21
1011	40	− 26	513	< 21	− 4
1111	26	− 16	613	21	17
1211	< 23	5	713	23	24
1311	34	29	813	< 18	8
1411	20	18	913	< 16	0
012	40	48	1013	19	− 21
112	17	20	014	23	30
212	< 17	− 9	114	< 20	13
312	26	− 31	214	< 20	− 7
412	18	− 15	314	20	− 21
512	< 19	− 1	414	< 18	− 13
612	26	32	514	< 16	− 1
712	21	26	614	20	24
812	< 22	3			

$D_{2h}^{13} - Pmmn$
4 V in 4 $(f): x_1, y_1, 0; \bar{x}_1, y_1, 0; \frac{1}{2} + x_1, y_1, \frac{1}{2}; \frac{1}{2} - x_1, \bar{y}_1, \frac{1}{2}$
4 O_1 in 4 $(f): x_2, y_2, 0$
4 O_2 in 4 $(f): x_3, y_3, 0$
2 O_3 in 2 $(a): 0, y_4, 0; \frac{1}{2}, \bar{y}_4, \frac{1}{2}$

$x_1 = 0.1486 \pm 0.0015$ $\quad y_1 = 0.105 \pm 0.003$
$x_2 = 0.149 \pm 0.004$ $\quad y_2 = 0.458 \pm 0.014$
$x_3 = 0.320 \pm 0.004$ $\quad y_3 = 0.000 \pm 0.014$
$y_4 = 0.000 \pm 0.006$

CRYSTAL HABIT AND ETCH FIGURES

Ketelaar's arguments for hemihedral symmetry are — besides the fact that he could not obtain agreement between observed and calculated intensities — that the habit of the crystals and the etch figures indicate a non-centrosymmetrical space group.

Crystals obtained from V_2O_5-melts grow on the walls of small cavities, and are almost exclusively bounded by crystal faces in the prism-zone and at only one termination. This applies to crystals from two melts studied by us, as well as to that measured by Nordenskjöld [4]. Thus it is impossible to conclude from the habit of the crystals, whether the symmetry is holohedral or hemimorphous. However, as has been shown by Leydolt [5], the symmetry of etch-figures corresponds to that of the face on which they are formed. This statement is not valid in some cases, *e. g.*

when the orientation of the etch-figures in relation to the symmetry elements of the crystal is influenced by the nature of the solvent [6, 7],

when some chemical or mechanical strain exists in the structure [6],

when the molecules of the solvent have only axial symmetry [8, 9]

when there exist surface films or impurities [10].

Wyckoff [11] and others have discussed various discrepancies between the symmetry derived from the crystal habit and that derived from the etch-figures, e. g. in Cu_2O, $CaWO_4$, and $PbWO_4$, and x-ray analyses of these compounds have shown that the etch figures and the atomic arrangement have the same symmetry [12—14]. Wyckoff has discussed the symmetry of the alkali-halides (KCl and NH_4Cl), derived from x-ray analysis and from etch-figures. His arguments as regards the discrepancies in symmetry that these two methods show are not valid. Later investigations show that etch-figures on pure KCl-crystals have the same symmetry as has the atomic arrangement, derived from x-ray data. The etch-figures on NH_4Cl, referred to by Wyckoff, were obtained by Tschermak on crystals, contaminated by iron [15]. This con-

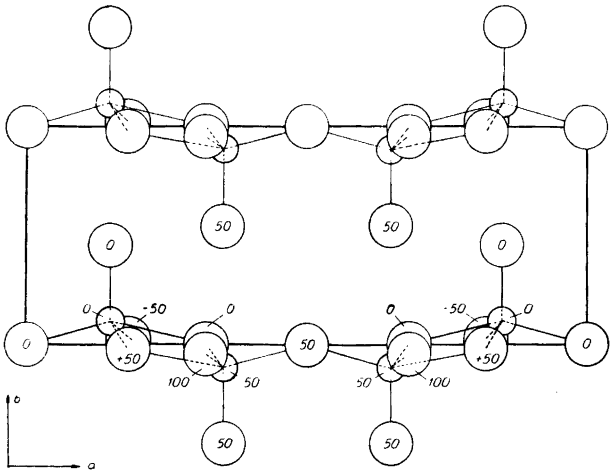

Fig. 3 a. V_2O_5. *Projection of the structure on (001). Small circles denote V-atoms, large circles O-atoms. The figures denote the height of the atoms in fraction of c. Superimposed oxygen atoms are symmetrically displaced.*

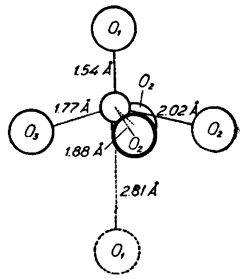

Fig. 3 b. The coordination around one vanadium-atom. Full drawn lines correspond to the five strong V—O bonds and the dotted line shows the sixth, much weaker one. Bond lengths in Å. Explanations in the text, p. 1133.

tamination seems to be necessary for the formation of large crystals[16] and has evidently some influence on the habit of the crystals.

To make certain that the solvent did not influence the symmetry of the etch-figures, three different etching agents were used, *viz.* 4 C NaOH, 0.1 C NaOH and 3 C H_2SO_4. The etch pits obtained (all lying on 010 cleavage-planes) shown in Figs 4—6, clearly demonstrated the presence of a centre of symmetry in the structure of V_2O_5. Thus the symmetry cannot be C_{2v}-*mm* or D_2-*222* and V_2O_5 must crystalize in the rhombic-holohedric (bipyramidal) class, *e. g.* D_{2h}-*Pmmn*. This is also shown by the determination of the atomic positions.

DISCUSSION OF THE STRUCTURE

The following interatomic distances are obtained:

$V - O_1 = 1.54 \pm 0.06$ Å	$O_1 - O_1 = 2.93$ Å
$V - 2O_2 = 1.88 \pm 0.04$	$O_1 - O_2 = 2.70$ and 2.80
$V - O_2 = 2.02 \pm 0.08$	$O_1 - O_3 = 2.63$
$V - O_3 = 1.77 \pm 0.03$	$O_2 - O_2 = 2.39$
$(V - O_1 = 2.81$ Å)	$O_2 - O_3 = 2.73$

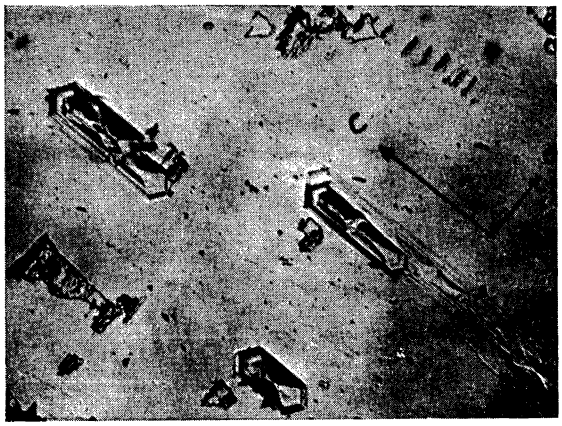

Fig. 4. Well developed, richly facetted etchpits. 3 C H_2SO_4, 8 min at 100° C. Orientation of axes indicated. Refl. light, one Nicol. magn. 360 x. (Phot. Brotzen.)

The structure is given in Fig. 3a. As will be seen, the vanadium atoms form five bonds, one with the O_1 atoms, three with the O_2 atoms and one with the O_3 atoms. These five oxygen atoms form distorted trigonal bipyramids around the vanadium atoms. The bipyramids are linked together by sharing corners in the x and z directions. In the y-direction, there can only be weak forces.

These forces between the layers may correspond to the longer V—O_1 distances of 2.81 Å. If this sixth oxygen atom is incorporated in the coordination figure, a very distorted octahedron is obtained. However, the pronounced cleavage of the substance along (010) shows that these bonds must be much weaker than the other five bonds and our description of the structure as built up by trigonal bipyramids is therefore more adequate.

In Fig. 3b the three kinds of oxygen atoms in the structure are shown. The O_1 atoms form only one strong bond (V—O = 1.54 Å). The O_3 atoms form two bonds (V—O = 1.77 Å) with the angle V—O_3—V = 125°. The O_2 atoms form three bonds (mean distance V—O = 1.93 Å) with the bond angles 104°, 104°, and 143°. The V—O_1 distance agrees closely with that found by Palmer [17] in $VOCl_3$ (1.56 Å).

Nordenskjöld observed that the cleavage along (010) was perfect and could also be obtained along (100) and (001). Ketelaar confirmed the perfect cleavage along (010) and stated that he could obtain the cleavage along (100) but only with difficulty along (001). Obviously, the suggested structure requires a perfect cleavage along (010). Because of the short distances O_2—O_2,

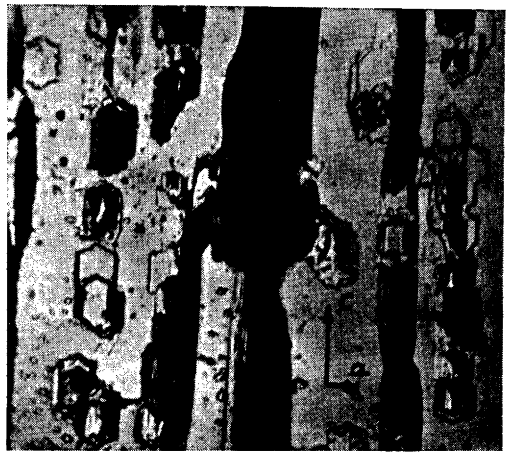

Fig. 5. Different types of etch-pits. 3 C H_2SO_4, 8 min at 100° C. Refl. light, one Nicol, magn. 230 x. (Phot. Brotzen.)

Fig. 6 a. Diluted NaOH, 16 hours, 20° C. c-axis along the length-direction of the individuals. One welldeveloped pit to the left and to the right open fishbone-like etch figures. These are due to the tapering shape of the individuals and may not be interpreted as indications of hemihedral symmetry. Magn. 3 x. (Phot. Brotzen.)

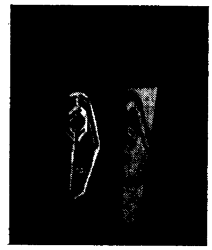

Fig. 6 b. Etch figures 0.10 N NaOH, 4 min 100° C. Schematic drawing illustrates the symmetry and the location of the small pit in the large one. c-axis in the length-direction. Cleavage plane somewhat inclined. Magn. 3 x. (Phot. Brotzen.)

which must imply very strong repulsing forces between the oxygen atoms, it can be expected that the V—O_2 bonds of 2.02 Å are those most easily broken of the V—O bonds (they correspond to the largest V—O distances) and thus the cleavage along (100) can be expected to be easier to obtain than that along (001), in agreement with Ketelaar's findings.

Ketelaar also investigated the colloid particles from a V_2O_5 hydrosole and found that the dimensions of the particles in the three lattice directions were $a \sim 20$ Å, $b \sim 10$ Å, and $c \sim 150$ Å. The structure, which we have suggested, obviously implies that the growth velocity in the b-direction must be very slow, as no primary metal-oxygen bonds operate in this direction. Further, it is probable that because of the short O—O distances (see above) the growth in the a-direction can be expected to be slower than in the c-direction, thus in agreement with the observed data.

SUMMARY

The crystal structure of V_2O_5 has been investigated. Contrary to Ketelaar [2], we found no evidence for hemihedral symmetry neither in the intensities of the x-ray reflexions nor in the etch-figures. According to our determinations the space group is D_{2h}^{13}—$Pmmn$ and the atoms occupy the following positions:

4 V in 4 (f): $x_1, y_1, 0$; $\bar{x}_1, y_1, 0$; $\frac{1}{2}+x_1, \bar{y}, \frac{1}{2}$; $\frac{1}{2}-x_1, \bar{y}_1, \frac{1}{2}$;	$x_1=0.1486$	$y_1=0.105$
4 O_1 in 4 (f): $x_2, y_2, 0$;	$x_2=0.149$	$y_2=0.458$
4 O_2 in 4 (f): $x_3, y_3, 0$;	$x_3=0.320$	$y_3=0.000$
2 O_3 in 2 (a): $0, y, 0$; $\frac{1}{2}, \bar{y}, \frac{1}{2}$		$y_4=0.000$

The vanadium atoms are surrounded by five oxygen atoms which form trigonal bipyramids around the V-atoms with V—O distances ranging from 1.54 Å to 2.02 Å. The weak cohesion of the crystals in the b-directions is probably due to a sixth much weaker V—O bond with a distance V—O of 2.81 Å. The shortest O—O distance is 2.39 Å.

This work forms part of an investigation on the structure of metal oxides, financially supported by the *Swedish Natural Science Research Council.*

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Received May 5, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1131–1141

The Crystal Structure of Chromium Trioxide

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Two suggestions have been made previously concerning the structure of chromium trioxide, CrO_3. Wooster and Wooster [1] gave the dimensions of the unit cell as $a = 8.50$ Å, $b = 4.73$ Å, and $c = 5.72$ Å, and showed that the observed density corresponded to a cell content of four formula units. The space group was assumed to be D_{2h}^{17}-*Ccmm* with the chromium atoms in the positions u, 0,1/4; $\bar{u}$, 0,1/4; $\frac{1}{2}+u$, $\frac{1}{2}$,1/4; $\frac{1}{2}-u$, $\frac{1}{2}$,1/4. Because of the twinning of the crystals, u was difficult to determine but was assumed to be about 1/9. They also stated that the observed halvings appeared to require a structure consisting of tetrahedra of CrO_4 linked together in chains parallell to the c-axis. Further work on the structure was announced but has not yet been published. Bräkken [2] obtained a unit cell with approximately the same dimensions ($a = 8.46$, $b = 4.77$, and $c = 5.70$ Å) and suggested a structure, in which the chromium atoms are octahedrally surrounded by oxygen atoms. A structure which follows Bräkkens suggestions, is given in Strukturbericht (II), and in Wyckoff [3], and is also described by Wells [4,5] as a deformation of the simple ReO_3 structure. Bräkken also promised further work on the structure but no account of this work has been published.

DETERMINATION OF THE STRUCTURE

A determination of the cell dimensions from powder photographs (Table 1), taken with a 19 cm Bradley camera with Cr-K radiation ($\lambda = 2.2909$ Å) gave the following values:

$$a = 4.789 \pm 0.005 \text{ Å}, \qquad b = 8.557 \pm 0.005 \text{ Å}, \quad c = 5.743 \pm 0.005.$$

Weissenberg photographs were taken with the crystals sealed up in thin tubes of lithiumborate glass, using Cr$-K$, Cu$-K$, and Mo$-K$ radiation. Due to an abnormally rapid decrease with increasing glancing angle in the intensity of the reflexions, the

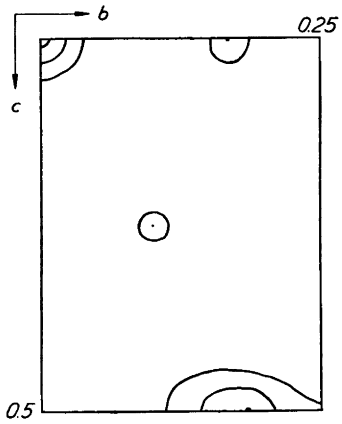

Fig. 1. CrO_3. *Patterson projection in (100).*

photographs taken with Mo-K radiation showed only a few reflexions that were not registered in the Cu-K photographs.

Photographs registering $0kl$, $1kl$, $hk0$, $hk1$ were made. The following reflexions appeared:

hkl for $h + k = 2n$
$0kl$ for $k = 2n$
$hk0$ for $h + k = 2n$
$h0l$ for $h = 2n$ and $l = 2n$ (less certain than the other halvings)

Thus the following space groups must be taken into account:

D_{2h}^{17}, C_{2v}^{12}, C_{2v}^{16}

and also (if the last criterion is in reality $h0l$ for $h = 2n$):

D_2^5, D_{2h}^{19}, D_2^6, C_{2v}^{11}, C_{2v}^{14}.

A Patterson projection in the yz-plane (Fig. 1) can only be interpreted in one way, if the space group is D_{2h}^{17}, with the atoms in the following positions:

4 Cr in $4(c) : 0_1\ y_1\ 1/4$ with $2\pi y \sim 150°$
4 O_1 in $4(b) : 0_1\ \frac{1}{2}_1\ 0$
8 O_2 in $8(g) : x_1\ y_1\ 1/4$ with $2\pi y \sim 90°$

The values of $|F_{obs}|$ were calculated from the estimated values of I according to the formula: $|F_{obs}| = \sqrt{\frac{I}{PL}}$. The scattering factors of O^{2-} and Cr^{6+} have been used in the calculations of F_{calc}.

Table 1. Weissenberg photographs of chromium trioxide, CrO_3.

Comparison between $|F_{obs}|$ and $|F_{calc}|$. Cu—K radiation and Cr—K radiation. Intensities taken from Weissenbergh photographs with Cr—K are marked with an asterisk.

hkl	$\|F_{obs}\|$	$\|F_{calc}\|$	*hkl*	$\|F_{obs}\|$	$\|F_{calc}\|$
002	90	89	110	45	74
004	96	84	130	25	38
006	45	44	150	53	49
008	44	54	170	22	27
			190	32	26
020	13	3			
021	40	63	200	68	56
022	21*	39	220	21	36
023	53	50	240	36	64
024	15	4	260	53	30
025	57	37	280	28	10
026	—	4	2 1 0 0	40	32
027	38	33			
			310	45	30
040	13	9	330	32	23
041	30	36	350	61	38
042	44	32	370	33	35
043	38	30	390	31	33
044	25	5			
045	35	24	400	31	42
046	38	23	420	—	15
			440	50	38
060	28	50	460	37	23
061	28	23			
062	77	61	510	18	25
063	30	20	530	—	10
064	35	33	550	39	48
065	25	16	600	30	46
066	44	45			
080	15	25	111	32	38
081	25	38	131	50	57
082	—	2	151	26	4
083	33	36	171	71	36
084	13	18	191	39	24
085	21	35			
			221	35	51
0 1 0 0	21	36	241	33	32
0 1 0 1	—	5	261	23	27
0 1 0 2	18	29	281	47	35

hkl	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$	hkl	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$
311	26	27	111	42*	38
331	55	43			
			112	47*	40
351	—	3	113	46*	30
371	42	33	114	70*	43
391	32	24	130	26*	38
			131	33*	57
421	45	34	132	25*	20
441	14	23	133	41*	46
461	12	17	134	24*	18
511	—	20			
531	26	34	150	36*	49
551	—	3	151	26*	4
			152	42*	66
110	50*	74	153	21*	4

The agreement between observed and calculated F-values was rather good, but some discrepancies appeared which could not be removed by a variation of the two y-parameters. However, in most cases the sign of the structure factor could be evaluated. A Fourier synthesis using all the observed $0kl$ reflexions except those very few ones with doubtful signs, showed the expected maxima for the Cr and O_1 atoms. However, the maximum corresponding to the two O_2 atoms, superimposed in the a-direction, was elongated and besides, a ghost-maximum of the same height as an oxygen peak appeared. It seemed that the suggested structure was in some way distorted, and that the symmetry must in fact be lower than D_{2h}^{17}. This was confirmed by the intensities of the reflexions $h00$, which did not correspond to an arrangement with only one parameter in the a-direction.

Then the symmetry of the space group D_2^5 was considered, but the same difficulties were met with as in D_{2h}^{17}. A survey of the other possible space groups showed that only in C_{2v}^{16} a suitable modification of the arrangement in D_{2h}^{17} could be obtained with the atoms in the following positions:

$(000;\ \frac{1}{2}\frac{1}{2}0) +$

4 Cr in $4(b): x_1\ y_1,\ 1/4;\ x_1,\ \bar{z}_1,\ 3/4;$	$2\pi y_1 \sim 150°$
4 O_1 in $4(a): x_2,\ 0,\ 0;\ x_2,\ 0,\ 0;$	
4 O_2 in $4(b): x_3,\ y_3,\ 1/4;\ x_3,\ \bar{y}_3,\ 3/4;$	$2\pi y_3 \sim 90°$
4 O_3 in $4(b): x_4,\ y_4,\ 1/4;\ x_4,\ \bar{y}_4,\ 3/4;$	$2\pi y_4 \sim 90°$

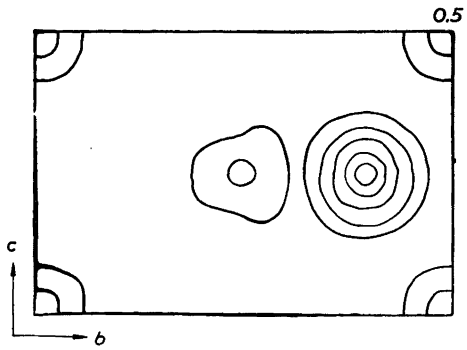

Fig. 2. CrO_3. Projection of the electron density in (100).

A variation of the y-parameters indicated that the agreement between observed and calculated F-values is satisfying for the following values:

$$2\pi y_1 = 145° \pm 1° \quad y_1 = 0.403 \pm 0.003$$
$$2\pi y_3 = 80° \pm 5° \quad y_3 = 0.222 \pm 0.013$$
$$2\pi y_4 = 100° \pm 5° \quad y_4 = 0.278 \pm 0.013$$

As will be seen from Table 1, there are still some discrepancies between observed and calculated F-values. As it was expected, that for this comparetively simple structure a better agreement between observed and calculated F-values should be possible to obtain, parameter values were also tried, which differed much more from the values obtained with Patterson and Fourier synthesis, than the probable errors in these values. However, this variation did not show improved agreement for any other values of the y-parameters, and indicated that the values we have chosen are in fact the best ones. As will be seen, the agreement is generally better for reflexions with high k-values ($k = 6$, 8, and 10) and therefor it seems reasonable to assume, that these discrepancies are at least partly due to some other factor than parameter errors.

A recalculation of the electron density projection showed that the ghost-maximum mentioned above was very much depressed, and the projection gave a parameter value for Cr agreeing with the value obtained through the trial and error process. Because of the partial super position of the O_2 and O_3 oxygen atoms, their parameters are difficult to determine from the projection. However, a comparison with the oxygen maximum, corresponding to the O_1-atom, and an estimation of the effect of the superposition shows, that the form of this elongated oxygen maximum agrees with that of a maximum composed of two partially superimposed maxima from oxygen atoms with the assumed parameters (Fig. 2).

The Weissenberg photographs, taken with [001] as rotation axis, were not as good as those with [100] as rotation axis. A lot of crystals have been

tried but the only really good one was the crystal that was used for the $0kl$ and $1kl$ photographs. In all the other cases, the crystals actually consisted of bundles of single individuals with slightly different orientation.

The Patterson projection in (001) showed only the Cr—Cr and the Cr—O_1 vectors clearly. The Cr—Cr vector agreed with the assumed Cr-position and further it could be deduced that $2\pi x_1 — 2\pi x_2 = 130°$. However, in that part of the projection where the Cr—O_2 and Cr—O_3 peaks were expected, only a very large region of about equal height was obtained. Obviously the x_3 and x_4 parameters must be deduced in another way.

With the symmetry of the space group C_{2v}^{16}—$C2cm$ one of the x-parameters can be arbitrarily chosen as 0. Therefor the x_3 parameter was taken as 0 and, to obtain reasonable O_2—O_3 distances, x_4 must be $\frac{1}{2}$. These values of x_3 and x_4 correspond to an O_2—O_3 distance of 2.44 Å. A study of previous works shows, that the shortest O—O distances in oxygen compounds of adjacent elements in the periodic system with tetrahedral or octahedral networks of oxygen atoms are 2.4—2.5 Å. (A surprisingly short value of 2.13 Å is reported [6] for O—O in V_2O_5 but as will be shown in another paper, this distance must be increased considerably [7].) As both the O_2 and O_3 atoms are linked to only one Cr-atom, it is reasonable to assume that the distances Cr—O_2 and Cr—O_3 are equal. Then the following values of the x-parameters are obtained.

$2\pi x_1 = 70°$	$x_1 = 0.194$	$2\pi x_3 = 0$	$x_3 = 0$
$2\pi x_2 = 200°$	$x_2 = 0.556$	$2\pi x_4 = 180°$	$x_4 = 0.5$

A comparison of observed and calculated F-values for the $hk0$ reflexions (Table 2) showed that these x-values must be approximately correct, but it was also found that the intensities of these reflexions are almost entirely due to the Cr and O_1 atoms, and are thus determined by the difference x_1—x_2, which is fixed from the Patterson projection (see above). The intensities of the reflexions permitted a variation of x_3—x_1 with about $\pm$ 30°. To prove the chosen x-parameters the projection of the electron density in the xy-plane was calculated. Because of the lack of a centre of symmetry in this projection, the formula for the electron density will be:

$\sigma(xy) = \sum_{-\infty}^{\infty}\sum_{-\infty}^{\infty} |F(hk)| \cdot \cos\{2\pi hx + 2\pi ky - \alpha(hk)\}$, which can be transformed into

$$\sigma(xy) = \sum_{0}^{\infty}\sum_{0}^{\infty} C(hk) \cdot \cos 2\pi hx \cos 2\pi ky + \sum_{0}^{\infty}\sum_{0}^{\infty} S(hk) \sin 2\pi hx \cos 2\pi ky \text{ with}$$

$$C(hk) = |F(hk)|_{\text{obs}} \frac{A}{|F(hk)|_{\text{calc}}} \text{ and } S(hk) = |F(hk)|_{\text{obs}} \frac{B}{|F(hk)|_{\text{calc}}}$$

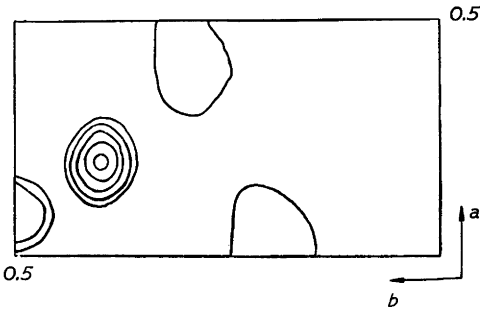

Fig. 3. CrO_3. Projection of the electron density in (001).

As will be seen from the projection, the suggested x-parameters are confirmed (Fig. 3). In Table 2, the intensities from the powder photographs are compared with those calculated. It is obvious, that the reflexions $0kl$ are enhanced compared with the reflexions with $h \neq 0$. However, if due consideration is given to this enhancement, the agreement between observed and calculated intensities is satisfying.

THE INTERATOMIC DISTANCES

The following interatomic distances are obtained.

$Cr—2O_1 = 1.79 \pm 0.05$ Å
$Cr— O_2 = 1.81 \pm 0.1$ Å
$Cr— O_3 = 1.81 \pm 0.1$ Å

$Cr—Cr = 3.32$ Å
$O_1—O_1 = 2.87$ Å
$O_1—O_2 = 2.79$ Å
$O_1—O_3 = 3.20$ Å
$O_2—O_3 = 2.44$ Å

The four bond angles O—Cr—O are:

$O_1—Cr—O_1 = 107°$
$O_1—Cr—O_2 = 104°$
$O_1—Cr—O_3 = 126°$
and $O_2—Cr—O_3 = 85°$

The angle $Cr—O_1—Cr$ is 136°.

The accuracy of the $Cr—O_1$ distance depends on the accuracy of the difference between the x_1 and x_2 parameters, all other errors ($\triangle y_1$ and the errors in the cell dimensions) being comparatively small. The maximal error in $x_1—x_2$ does not exceed 0.015 and thus the maximal error in $Cr—O_1$ is about $\pm$ 0.05 Å. As was mentioned above, the x_3 and x_4 parameters are determined with the assumption that the corresponding distances $Cr—O_2$ and $Cr—O_3$ are of equal length. If this assumption is accepted, then the maximal error in the $Cr—O_2$ and $Cr—O_3$ distances is about $\pm$ 0.1 Å, chiefly due to the inaccuracy of the y_3 and y_4 parameters ($= \pm$ 0.015).

Table 2. Powder photograph of chromium trioxide, CrO_3. Comparison between observed and calculated $\sin^2\Theta$ values and intensities. Cr—K radiation, β-reflexions omitted.

hkl	$\sin^2\Theta_{calc} \cdot 10^4$	$\sin^2\Theta_{obs} \cdot 10^4$	I_{calc}	I_{obs}
020	0716	0725	0.3	w
110	0751	0757	320	v.st.
021	1114	1117	155	v.st.
111	1149	1158	105	v.st.
002	1591	1593	100	st.
130	2183	2195	25	v.v.w.
200	2288	2288	24	st.
022	2307		26	
112	2342	2346	50	st.
131	2581	2594	86	st.
201	2685	—	0.0	—
040	2864	—	0.5	—
220	3004	—	15	—
041	3262	3272	14	m +
221	3402	3394	52	st.
132	3774	—	7	—
202	3879	3880	18	v.v.w.
023	4296	4303	20	m(diff.)
113	4331		14	
042	4455	4464	8	w.
222	4595	4595	43	w +
150	5047	5067	17	v.w.
240	5152	5162	39	v.w.
310	5327	5332	6	v.w.
151	5445	—	0.2	—
241	5550	5554	14	v.w.
311	5725	5741	10	w(diff.)
133	5763		29	
203	5868	—	0.0	—
004	6365	6356	25	m.
043	6444	6447	6	m.
060	6444		9	
223	6584	6551	26	w.
152	6638	6641	61	m.
242	6743	6740	34	w.
330	6759		4	
061	6842	6867	4	v.v.w.
312	6918	6911	40	m.
024	7081	7103	0.0	v.w.
114	7116	7146	27	m(diff.)
331	7157		27	
062	8035	8044	35	w.
134	8548	—	2	—

hkl	$\sin^2\Theta_{calc} \cdot 10^4$	$\sin^2\Theta_{obs} \cdot 10^4$	I_{calc}	I_{obs}
153	8627	8636	0.4	v.w.
204	8653		18	
243	8732	8743	19	v.v.w.

v.w. = very weak, w. = weak, m. = medium, st. = strong,
v.v.w. = very very weak, v.st. = very strong, diff. = diffuse

I_{calc} is calculated according to the formula $I_{calc} = F^2 \cdot m \cdot \frac{1 + \cos^2 2\Theta}{\sin^2\Theta\cos\Theta}$ where m = multiplicity.

DISCUSSION OF THE STRUCTURE

As will be seen from Fig. 4, the oxygen atoms form distorted tetrahedra around the Cr-atoms. These tetrahedra are linked together to chains by sharing corners in the *c*-direction in agreement with Wooster and Wooster's previous suggestion [1]. This chain direction is often marked on the crystals by a heavy striation and the crystals break up very easily into small needles parallel to the streaks. This agrees very well with the fact that the bonds between the chains both in the *a*- and *b*-direction must be rather weak. It seems that no secondary Cr—O bonds can operate in these directions between atoms in different chains,

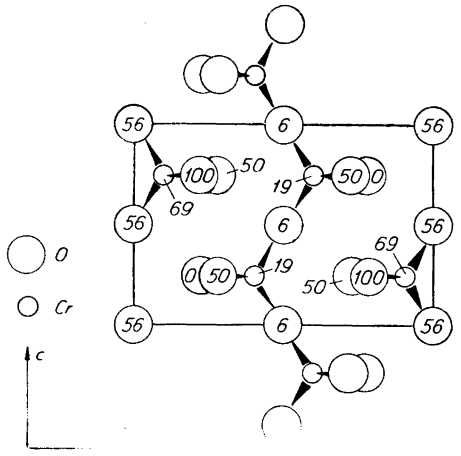

Fig. 4. CrO_3. Projection of the structure in (100). Small circles denote chromium atoms, large circles oxygen atoms. The figures denote the height of the atoms in percentages of a.

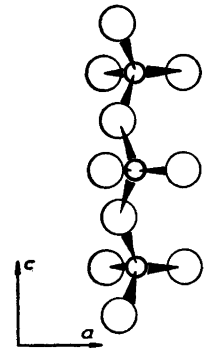

Fig. 5. CrO_3. Projection of a CrO_3-chain on (010).

because of the fact that these distances are all very long, the shortest being 3.10 Å. Thus only van der Waal's attraction between the CrO_3-chains holds the structure together in the *a*- and *b*-directions. In agreement with this assumption it was observed that the orientation in the *a*- and *b*-direction is often disturbed. (See above.) The low melting-point of the substance ($\sim 197°$ C) is also explained by this structure.

It would be expected that the O_2 and O_3 atoms which are bonded only to one chromium atom would lie closer to the Cr-atom than the O_1 atom which is bonded to two Cr-atoms. However, such a difference has not been observed, but as will be seen from the errors in the interatomic distances, it may exist but is probably < 0.15 Å.

COMPARISON WITH THE INTERATOMIC DISTANCES IN OTHER COMPOUNDS OF HEXAVALENT CHROMIUM

As will be seen from the table below, the Cr—O (resp. Cr—O,F) distances in the chromates, fluorochromates, and in CrO_2Cl_2 are shorter than in CrO_3.

Reference	Compound	Cr—O (Cr—O,F resp.)
Zachariasen and Ziegler [8]	K_2CrO_4	1.60
Clouse [9]	$CaCrO_4$	1.64
Miller [10]	Na_2CrO_4	1.60
Palmer [11]	CrO_2Cl_2	1.57
Ketelaar [12]	$KCrO_3F$	1.58
Brandt [13]	$CuCrO_4$	1.67

With the exception of the distances in $CuCrO_4$, the Cr—O distances are all very close to 1.60 Å.

These distances are thus 0.2 Å shorter than the Cr—O distances for the oxygen atoms which form one Cr-bond in CrO_3. As will be seen, this difference in length is somewhat bigger than the biggest possible difference between the Cr—O_2 and the Cr—O_3 distances (one Cr-bond) and the Cr—O_1 (two Cr-bonds) distances in CrO_3.

Pauling [14] has assigned a crystal radius of 0.52 Å to the Cr^{VI} ion and thus 1.92 Å would be expected for a six-coordinated Cr^{VI} ion, and $0.922 \cdot 1.92 = 1.77$ Å (Pauling [14] p. 346 and 368) for a four-coordinated hexavalent chromium ion. As will be seen, this value agrees very well with the observed distances in CrO_3. However, the bright colour of the substance indicates that there is probably an appreciable and maybe dominating amount of covalent character in the bonds.

SUMMARY

The crystal structure of CrO_3 has been determined. The atomic positions are:

Space group C_{2v}^{16}—$C2cm$

$(000;\ \frac{1}{2}\frac{1}{2}0) +$

4 Cr in $4(b): x_1,\ y_1,\ 1/4;\ x_1,\ \bar{z}_1,\ 3/4;$	$x_1 = 0.194$	$y_1 = 0.403 \pm 0.003$
4 O_1 in $4(a): x_2,\ 0,\ 0;\ x_2,\ 0,\ \frac{1}{2};$	$x_2 = 0.556$	
4 O_3 in $4(b): x_3,\ y_3,\ 1/4;\ x_3,\ \bar{y}_3,\ 3/4;$	$x_3 = 0$	$y_3 = 0.222 \pm 0.013$
4 O_4 in $4(b): x_4,\ y_4,\ 1/4;\ x_4,\ \bar{y}_4,\ 3/4;$	$x_4 = 0.5$	$y_4 = 0.278 \pm 0.013$

The oxygen atoms form distorted tetrahedra around the chromium atoms and the Cr—O distances are 1.79—1.81 Å. The tetrahedra are linked together to chains in the c-direction by sharing corners. Between the CrO_3 chains probably only weak van der Waal's bonds are operating.

This investigation forms part of an investigation on metal oxides, financially supported by the *Swedish Natural Science Research Council.*

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Received May 10, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1142–1154

Short Communications

On the Mechanism of the Intestinal Fat Absorption

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Two different hypotheses [1,2] and much conflicting results [3,5] have been published on the intestinal fat absorption [3, 4].

We have studied this process in cats with the aid of C-14 labelled stearic acid.

The material listed in Table 1 (A–C) was given to cats by stomach intubation. One hour later the thoracic duct was cannulated under chloralose anaestesia and the lymph collected during 5–11 hours in a flask chilled in dry ice. The frozen lymph was lyophilized, the total lipids extracted and fractionated. The different fractions were then saponified and the activity of the fatty acids determined after wet combustion.

In these experiments only 3–12 per cent of the lipids fed were recovered in the lymph, apparently due to the condition of the animal after the operation, most of the fat remaining in the intestine.

After feeding equivalent amounts of glycerides (B) or of free fatty acids alone (C) the collected lymph was pronounced milky for several hours. Fractionation showed that in all cases the composition of the total lipids was approximately the same, the glyceride fatty acids constituting 70–80 per cent of the total fatty acids.

The high specific activities of the glyceride fatty acids leave no doubt that the free fatty acids fed in case C had been

Table 1. The distribution of C^{14}-labelled stearic acid in lymph fatty acids after peroral administration to cats. Activity %, specific activity in % of specific activity of administered fatty acids.

	Form of fat administered	Collection time in hours	Lymph in g	% of fat fed recovered in lymph	Fatty acids recovered in lymph as					
					Phospholipid fatty acids		Glyceride fatty acids		Cholesterd fatty acids	
					Weight %	Activity %	Weight %	Activity %	Weight %	Activity %
A.	5 ml corn oil + 25 mg active stearic acid	10	42	12.8	13.7	58.5	79.8	83.5	6.5	7.0
		5	24	3.2	16.1	57.8	76.0	73.5	7.9	6.5
B.	5 ml corn oil + 75 mg active glyceride	11	112	8.1	16.2	40.0	78.5	65.0	5.3	—
C.	5 ml hydrolyzed corn oil + 25 mg active stearic acid	9	33	7.7	14.7	15.3	75.0	77.7	10.3	20.4
		5	42	4.3	22.3	15.9	70.0	69.5	7.7	17.5

used for the synthesis of the lymph glycerides as indicated by earlier work by Munk [3], Ivy [4] and Tidwell [5], and in contradistinction to Frazer's work [2].

Frazer [2] estimates that 10—30 per cent of the fatty acids of fed triglycerides are set free by lipolysis in the intestinal lumen. Apparently he assumes that these acids are absorbed via the portal vein and that resynthesis plays a minor rôle. From our results (A) it is evident that these free fatty acids are incorporated mainly into triglycerides and to a smaller extent into phospholipids in the intestinal mucosa and transported via the thoracic duct into the systemic circulation.

The phospholipids show another picture: Apparently the phospholipids in the lymph are diluted more than the glycerides by the phospholipids coming from other organs than the intestines. The great difference between A and C requires special comments: When only free fatty acid was given C, the fatty acids in the phospholipids had 15 per cent of the activity of the acids fed. When inactive glyceride was fed together with a small amount of free radioactive acid in amounts such that total hydrolysis would give a mixture of the same composition as in case C, the phospholipid fatty acids, however, are 4 times as active in case A then in C. A possible explanation for this is that only a partial hydrolysis of the inactive triglyceride has taken place, the isotopic acid thus becoming less diluted so that the acid available for phospholipid synthesis had a higher specific activity. That can thus be taken as an experimental support of Frazer's hypothesis of partial hydrolysis, but we do not wish to place much weight on these findings until we have obtained more results with better recoveries of the fat fed.

Recent work by Chaikoff [6] with intravenous administration of fatemulsions has shown that in fasting dogs the liver is the main supplier of blood phospholipids. Under these conditions the intestine only took up triglycerides from the blood for its own metabolic use and did not contribute significantly to the blood phospholipids.

The results reported here and other work in this laboratory show that during intestinal fat absorption the intestinal wall supplies a quantitatively important part of the blood phospholipids via the thoracic duct when feeding either glyceride or free fatty acids.

The work with radioactive phosphate (Chaikoff 1948 [7]) seems to show that the entire fat absorption does not pass via phospholipins in the sence that all the absorbed glycerine or mono- and diglycerides are phosphorylated. Another possibility, suggested by our results, is that the absorbed fatty acids are incorporated into phospholipids and then used for the resynthesis of the glycerides from mono- and diglycerides possibly by some transacylation mechanism. However, the quantitative aspects of these possibilities remain to be elucidated.

A full report will appear in this journal.

This work is part of an investigation supported by *Statens Medicinska Forskningsråd.*

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Received September 11, 1950.

L-Galactose in the jelly coat of *Echinus esculentus* eggs

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The jelly-coat substance of the sea-urchin egg contains about 80 per cent polysaccharide ethereal sulphate [1]. This polysaccharide is composed of entirely galactose residues in the jelly of *Echinus esculentus* [2]. Further studies — the first results of which are given here — using the separation technique of Hough, Jones and Wadman [3] have now revealed that the major part of the galactose is present as L-galactose. Perhaps the jelly coat acts as a protective envelope, containing "unnatural" sugars (and amino acids?) which would be resistant to the enzyme system of fungi and bacteria.

L-Galactose has been demonstrated in several instances to occur in natural polysaccharides. Especially interesting in this connection are the analyses on galactan ethereal sulphate from red algae, since these algae presumably form part in the food of the sea-urchins. Tomoo Miwa [4], for example, obtained DL-galactose (90 per cent) and D-galactose from *Porphyra tenera* (see also [5]). Recently Johnston [6], working with *Gigartina stellata* and *Chondrus crispus* galactan ethereal sulphate, prepared a fraction especially rich in L-galactose (about 60 per cent of the galactose).

The hydrolysate was first freed from amino acids by the use of ion exchange resins [7]. The eluate was concentrated to a syrup and the components separated on a cellulose column by using as solvent a mixture of *n*-butanol : water 20 : 1. The eluate was fractionated by using an automatic receiver changer [3]. The fractions were tested for reducing sugars by spraying spots of the eluate on filter paper with aniline trichloroacetate in wet butanol [8]. In this way only one sugar, galactose, could be detected. The fractions containing galactose were combined and concentrated to a small volume under reduced pressure. The optical rotation was measured and the galactose concentration estimated with an alkaline iodine method [9] and with a periodate method (oxidation in subdued daylight [10] at pH 3.5 [11] and 50° [12] for 120 mins.), giving $[\alpha]_D^{19} - 74.5°$ and $-69°$ resp. (c, 5.0 in water). The L-galactose was identified by paper partition chromatography [2] and as the methyl-phenylhydrazone [13], which however gave a low melting point (182° after one recrystallisation, 172° direct; *cf.* pure D-galactose methylphenylhydrazone 190°), probably due to the presence of some D-galactose hydrazone [6].

Analyses on the jelly substance of *Echinus esculentus* with Somogyi's method [14] indicate the presence of one reducing group in a polysaccharide of 150 galactose residues [15]. Periodate oxidation (see above) gave a consumption of one O per 60 residues. Treatment of the jelly solution with acid of different strength in an autoclave increased the reducing value, but the periodate consumption was increased to the same extent, keeping the ratio between these values approx. constant. This finding reveals that the sulphate group is either very firmly attached or — more probably, see [1] — that it is bound in such a way that the release of it does not give rise to a pair of adjacent free hydroxyl groups. The periodate oxidation indicates the presence of two or three such pairs per polysaccharide molecule. A structure similar to that preponderating in carrhageenin [16] with the galactopyranose residues linked through the 1 and 3 positions and carrying the sulphate group on C^4, is in agreement with the analytical results. Both terminal galactose residues will have only one pair of adjacent hydroxyl groups each — the chain residues will have none — and the release of the sulphate will

Hexachloro-Cyclohexane, M. P. 145° C

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Hexachloro-cyclohexane of m. p. 145° C obtained by chlorination of cyclohexane in artificial light has been found to have zero dipole moment [1]. At the time when this result was obtained nothing was known about the dipole moment of ε benzene hexachloride, and the molecular structure of this compound had not yet been determined. Bastiansen and Hassel [1] therefore suggested that the 145° substance might be the second benzene hexachloride (besides the β isomer) possessing a centro-symmetric structure.

Later on electron diffraction [2,3] and X-ray crystallographic work [4] have conclusively proved that ε benzene hexachloride has the configuration $\varepsilon\varkappa\varepsilon\varkappa\varkappa\varkappa$ and therefore it became necessary to reject the possibility that the 145° substance is a member of the series of benzene hexachlorides [2]. The 145° substance must therefore contain at least *one* carbon atom attached to *two* chlorine atoms. If the lack of a dipole moment means that the molecule is rigorously centro-symmetric only *one* structural alternative is possible, corresponding to the two interconvertible configurations (1) and (2):

(1) $1(\varkappa,\varepsilon),2\varkappa,4(\varkappa,\varepsilon),5\varkappa$ (2) $1(\varkappa,\varepsilon),2\varepsilon,4(\varkappa,\varepsilon),5\varepsilon$

The vanishing of the dipole moment might, however, also be due to a chance compensation of partial moments. Although the electron diffraction results (see below) are strongly in favour of the first alternative, with the configuration (1), the result was not decisive. Additional X-ray crystallographic measurements revealed however, that the monoclinic unit cell reported earlier [1] and containing four molecules, is B-centered. The smallest possible cell thus contains *two* molecules, its lattice constants being $a = 8.25$ Å, $b = 6.72$ Å, $c = 9{,}41$ Å with $\beta = 103.7°$. The space group is $C_{2h}{}^5$-$P2_1/n$ and the molecules are therefore strictly centrosymmetric. The substance is consequently the 1,1′, 2, 4, 4′,5-hexachloro-cyclohexane capable of existing in the two steric configurations (1) and (2) given above. Our present knowledge about the relative stability of interconvertible forms of cyclohexane derivatives would suffice, we think, to predict the configuration (1) in the crystal-

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cause an increase of the periodate consumption at the non-reducing terminal residue only.

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Received July 15, 1950.

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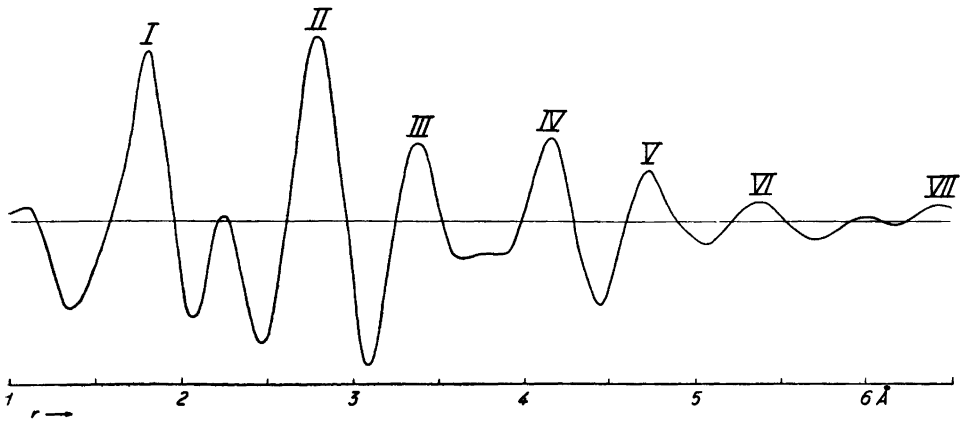

line state, and even in the vapour state quite negligible amounts of the configuration (2) would be expected.

As mentioned above this expectation has been confirmed by an electron diffraction analysis. The $\frac{\sigma(r)}{r}$-curve given in Fig. 1 corresponds to the curves reproduced in an earlier communication dealing with the benzene hexachlorides [3]. The scales have been so adjusted that both position and height of the peaks I to VII may easily be compared with those of the corresponding peaks of the benzene hexachloride curves. The pronounced peak numbered II (at about 2.8 Å) besides being considerably higher than the corresponding peaks of the hexachlorides is also somewhat displaced towards greater r-values. This observation is in excellent agreement with the formula of 1,1′,2,4,4′,5-hexachlorocyclohexane which contains *two* CCl_2-groups. An inspection of the peaks VI and VII now enables us to make a decision between the two possible configurations (1) and (2) mentioned above. The first peak (at about 5.4 Å) corresponds in height to peak VI of a and ε benzene hexachloride, thus indicating the presence of *two* $Cl_{1\varkappa}-Cl_{3\varkappa}$ distances. Moreover, the height of peak VII (6.4 Å) corresponds to that observed for δ and ε benzene hexachloride which indicates *two* $Cl_{1\varkappa}-Cl_{4\varkappa}$ distances. This observation proves, we think, that at least the greater part of the molecules have the configuration (1) in the vapour phase.

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Received September 3, 1950.

On the Composition of the Fatty Acids of Pine Wood Extract and Tall Oil. A spectrophotometric investigation

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Rather little attention has been called to the composition of the fatty acids contained in the wood of *Pinus silvestris*. Bergström and Trobeck [1] have published data obtained by chemical investigation on the fatty acids obtained by alcohol extraction of pine wood. Amongst saturated acids they found oleic, linoleic and linolenic acids.

The component fatty acids of tall oil have been more closely investigated, the same unsaturated acids having been stated to be present, among others by Sandström and Sandström [2].

The introduction of ultra-violet absorption spectrophotometry has given a more accurate and sensitive tool to substitute the chemical examination. The polyunsaturated fatty acids can be isomerized by alkali yielding more or less conjugated double bond systems which can be detected and even quantitatively calculated from the ultra-violet absorption readings at certain wavelengths. The characteristic peaks of alkali treated linoleic, linolenic and arachidonic acids appear at 2320, 2680–2800 and 3020–3160 Å respectively. Thus Brice *et al.*[3] have found small amounts of linolenic acid in American tall oil whereas Anderson and Wheeler [4] consider linolenic acid to be absent. They stated a slightly higher absorption at 3160 Å than at 2680 Å and assumed this being due to impurities.

The purpose of this investigation has been to analyze the polyunsaturated fatty acids of Finnish pine wood and tall oil and to state if the sulphate cooking process has any effect upon them.

Four specimens of pine were extracted with alcohol and ether successively and the extracts obtained were treated along four tall oil samples as follows.

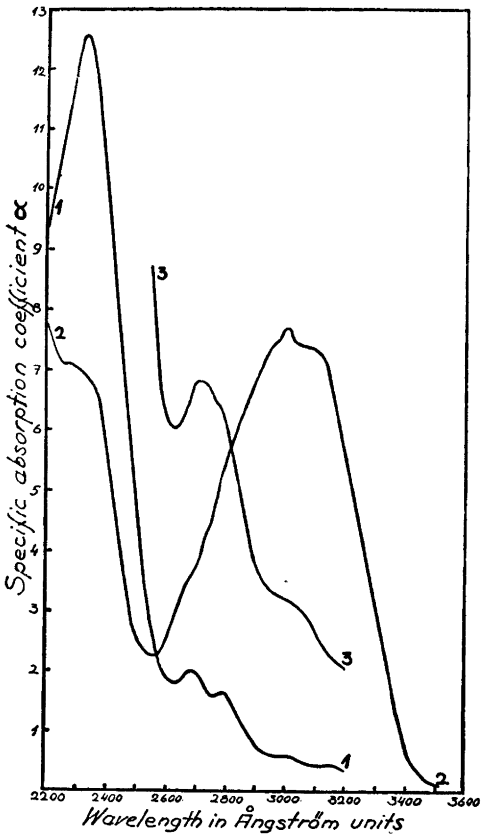
The dark and more polaric constituents were eliminated by dissolving the samples in petroleum ether. The soluble part was then saponified with alcoholic potassium hydroxide and extracted with ether until no more unsaponifiable material could be isolated. The rosin acids were thereafter separated from the fatty acids by esterifying with absolute methanol catalyzed by sulphuric acid and extracting the fatty acid methyl esters by ether from the mixture which had been made alkaninic previous to the extraction. Particular care was taken to obtain the component fractions as free from other components as possible because all of them contain systems of two or more conjugated double bonds.

As storing of the ready samples showed soon to alter the spectral characteristics, the measurements were carried out within 48 hours from the completion of the separation. The separation itself was performed with minimum delay. A Model DU Beckman spectrophotometer was used and the isomerization method of Brice *et al.*[3] employed. Throughout the investigation alcohol was used as the solvent.

The fatty acids and the rosin acids as well as the unsaponifiable materials were examined both in their original condition and after the treatment with the isomerization reagent.

The wood extract fatty acids were thereafter collected and distilled under a pressure of 3 mm and re-investigated spectrophotometrically.

The results show that the absorption curves of the rosin acids, closely resembling those published by Ritchie and McBurney [5] on abietic acid, are not materially affected by the alkali treatment. From 2420 Å



Curve 1: Tall oil fatty acids, untreated
» 2: Pine wood » » »
» 3: » » » » isomerized

the absorption rapidly decreases remaining minimal from 2 600 Å towards the longer wave lengths. The unsaponifiable materials show absorption at about 2 300 Å where a distinct peak of double conjugation appears after the alkali treatment. Another broad absorption band centered at about 3 000 Å seems to become slightly intensified by alkali.

The fatty acids of pine wood have a rather intensive band equally centered at about 3 000 Å. In the isomerization process, however, this band almost disappears and a new one at about 2 700 Å appears. This band does not have the inflexion between the peaks characteristic of conjugated triene acids. However, it becomes visible in the distilled acids thus being indicated to be only obstructed. On the other hand the absorption at 3 000 Å was strongly decreased in the distilled acids and still lessened by alkali. The possible interference of the carotenoid pigments at 3 000 Å is eliminated through the absence of any appreciable absorption at longer wave lengths where most carotenoid pigments according to Karrer and Jucker [6] have characteristic triple peak bands.

The above holds even for the rosin acids and the unsaponifiable materials of tall oil. In the fatty acids, however, there are interesting and different features. The isomerization causes a marked increase in the conjugated diene absorption but there is no change in the triene absorption, where there are distinct conjugated triene peaks already in the spectrum of the untreated fatty acids, thus indicating that there are no more isolated triene systems to be affected by alkali. A very slight absorption at 3 020 and 3 160 Å indicate minute amounts of conjugated tetraene.

Anderson and Wheeler [4] have calculated that the sulphate cooking process corresponds to less than 10 % of the reaction time in the analytical isomerization process. Thus the constancy of the conjugated triene absorption does not agree with the behaviour of linolenic acid.

In Fig. 1 some typical absorption curves are collected.

From these results following conclusions can be derived: The fatty acids of *Pinus silvestris* grown in Finland contain a fatty acid with four conjugated double bonds. During the sulphate cooking this tetraene system is converted into a triene system appearing as "linolenic acid" in the spectrum. Supposedly even conjugated diene systems are formed simultaneously. Fin-

The Enzymic Hydrolysis of Triacetin by Acetylcholine-Esterase and its Inhibition by Various Compounds

KLAS-BERTIL AUGUSTINSSON

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In the course of investigations on the mechanism of action of the inhibition of acetylcholine-esterase (human erythrocytes) by various compounds, triacetin has been used as a substrate. There are proofs for the existence of two active groups, centre I and centre II, of the acetylcholine-esterase molecule. Centre I is a negatively charged group which attracts the positive nitrogen atom of the choline ester. Centre II combines with the acyl group of the ester, acetates being split at a higher rate than other esters. Any acetates, acetylcholine as well as the acetates of a great variety of alcohols [1], are split. Centre II alone is responsible for this hydrolysis [2].

Acetylcholine protects acetylcholine-esterase against the action of tetraethylpyrophosphate (TEPP) [3-5]. Physostigmine also has this protective action against TEPP when acetylcholine is used as substrate. It has now been demonstrated that choline has a similar action.

Choline inhibits the enzymic hydrolysis of triacetin. As expected this inhibition is not competitive. At constant triacetin concentration the degree of inhibition increases with increasing choline concentration to about 70 per cent (corresponding to 0.25 *M* choline). Then the inhibition is constant in the presence of still higher choline concentrations. This inhibition may be due to steric hindrance.

It has been demonstrated recently that, in a mixture of acetylcholine, prostigmine or physostigmine, and acetylcholine-esterase, an equilibrium rate is reached in 10 to 25 minutes, depending upon the inhibitor concentration, after adding the inhibitor or substrate respectively. The inhibition is stronger than during equilibrium when the enzyme has been incubated with the inhibitor, it is less strong when substrate and inhibitor are added simultaneously. This is the result obtained with acetylcholine [3,4,5]. With triacetin the reaction curves are different from those obtained with acetylcholine. Equilibrium is attained immediately after adding triacetin to the incubated enzyme as well as in the case of simultaneous addition of substrate and inhibitor to the enzyme. Therefore, comparing the degrees of inhibition of the hydrolysis of acetylcholine and triacetin this fact must be considered.

Another important result has also been obtained with prostigmine and triacetin. If the concentration of this inhibitor is raised above 10^{-8} *M* keeping the substrate concentration constant, the degree of inhibition is no more increased; on the contrary, it decreases. Even with 10^{-6} *M* prostigmine the inhibition is less than with 10^{-8} *M*.

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nish tall oil does not contain linolenic acid.

This investigation will be published more in detail.

1. Bergström, H., and Trobeck, K. G. *Svensk Papperstidn.* **50** (1947) 215–221.
2. Sandström, G., and Sandström, M. *Svensk Papperstidn.* **47** (1944) 385.
3. Brice, B. A., Swain, M. L., Schaeffer, B. B., and Ault, W. C. *Oil and Soap* **50** (1945) 219–224.
4. Anderson, R. H., and Wheeler, D. H. *Oil and Soap* **50** (1945) 137–141.
5. Ritchie, F., and McBurney, L. F. *J. Am. Chem. Soc.* **71** (1949) 3736.
6. Karrer, P., and Jucker, E. *Karotinoide.* Basel (1948).

Received July 5, 1950.

The Enzymic Hydrolysis of Triacetin by Acetylcholine-Esterase and its Inhibition by Various Compounds

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Received July 5, 1950.

Free Amino Acids in Brewing Materials

L. LJUNGDAHL and E. SANDEGREN

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The free amino acids occurring in barley, malt, wort, beer and bottom yeast *(Saccharomyces Carlsbergensis)* have been determined by means of paper partition chromatography [1]. Finely ground, six row barley (Stella) was extracted with an 8 per cent solution of trichloroacetic acid and the same extraction fluid was used on malt and yeast. The trichloroacetic acid extracts were washed with ether and purified by precipitation with 80 per cent ethyl alcohol. After filtration, the filtrate was concentrated on a boiling water bath and the concentrate used for the chromatographic separation. Wort and beer were treated with alcohol and filtered in the same way as the trichloracetic acid extracts. The beer was fermented a second time after the evaporation of alcohol and the addition of dextrose. The amino acids found are given in Table 1.

Table 1.

	Barley	Malt	Unboiled wort	Boiled wort	Beer	Beer 2nd ferm.	Yeast
Alanine	+	+	+	+	+	+	+
γ-Amino-butyric acid	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	−	+
Asparagine	+	+	+	+	−	−	+
Aspartic acid	+	+	+	+	−	−	+
Cystine	+	+	?	?	?	?	+
Glutamic acid	+	+	+	+	+	+	+
Glutamine	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	−	+
Isoleucine	+	+	+	+	+	−	+
Leucine	+	+	+	+	+	−	+
Lysine	+	+	+	+	+	−	+
Phenylalanine	+	+	+	+	+	−	+
Proline	+	+	+	+	+	+	+
Serine	+	+	+	+	+	−	+
Threonine	+	+	+	+	−	−	+
Tyrosine	+	+	+	+	+	−	+
Valine	+	+	+	+	+	+	+
Unknown (peptides)	−	1	3	2	−	−	9

The occurrence of γ-amino-butyric acid, which has not earlier been found in brewing

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The protective action of acetylcholine, physostigmine, and choline against TEPP action (acetylcholine as substrate) lets us assume that TEPP reacts with at least one of the two active centres of the enzyme molecule. It has now been demonstrated that triacetin also protects the enzyme against TEPP action. Triacetin is attracted to centre II and, consequently, the inactivation of the enzyme by TEPP is a destruction of that centre. Furthermore, it was demonstrated that choline, reacting only with centre I, does not protect the enzyme when it is actively splitting triacetin. These results support the recent proposal [6] that the alkyl phosphates combine with the "ester-grouping" of the enzyme.

A full report will be published elsewhere in due course.

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2. Augustinsson, K.-B. *Acta Chem. Scand.* **4** (1950) 948.
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Received September 23, 1950.

Free Amino Acids in Brewing Materials

L. LJUNGDAHL and E. SANDEGREN

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The free amino acids occurring in barley, malt, wort, beer and bottom yeast *(Saccharomyces Carlsbergensis)* have been determined by means of paper partition chromatography [1]. Finely ground, six row barley (Stella) was extracted with an 8 per cent solution of trichloroacetic acid and the same extraction fluid was used on malt and yeast. The trichloroacetic acid extracts were washed with ether and purified by precipitation with 80 per cent ethyl alcohol. After filtration, the filtrate was concentrated on a boiling water bath and the concentrate used for the chromatographic separation. Wort and beer were treated with alcohol and filtered in the same way as the trichloracetic acid extracts. The beer was fermented a second time after the evaporation of alcohol and the addition of dextrose. The amino acids found are given in Table 1.

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Asparagine	+	+	+	+	−	−	+
Aspartic acid	+	+	+	+	−	−	+
Cystine	+	+	?	?	?	?	+
Glutamic acid	+	+	+	+	+	+	+
Glutamine	+	+	+	+	+	+	+
Glycine	+	+	+	+	+	+	+
Histidine	+	+	+	+	+	−	+
Isoleucine	+	+	+	+	+	−	+
Leucine	+	+	+	+	+	−	+
Lysine	+	+	+	+	+	−	+
Phenylalanine	+	+	+	+	+	−	+
Proline	+	+	+	+	+	+	+
Serine	+	+	+	+	+	−	+
Threonine	+	+	+	+	−	−	+
Tyrosine	+	+	+	+	+	−	+
Valine	+	+	+	+	+	+	+
Unknown (peptides)	−	1	3	2	−	−	9

The occurrence of γ-amino-butyric acid, which has not earlier been found in brewing

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The protective action of acetylcholine, physostigmine, and choline against TEPP action (acetylcholine as substrate) lets us assume that TEPP reacts with at least one of the two active centres of the enzyme molecule. It has now been demonstrated that triacetin also protects the enzyme against TEPP action. Triacetin is attracted to centre II and, consequently, the inactivation of the enzyme by TEPP is a destruction of that centre. Furthermore, it was demonstrated that choline, reacting only with centre I, does not protect the enzyme when it is actively splitting triacetin. These results support the recent proposal [6] that the alkyl phosphates combine with the "ester-grouping" of the enzyme.

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Received September 23, 1950.

The Degradation of Pyrimidines for Tracer Work. Bicarbonate as a Precursor for Ribonucleic Acid Pyrimidines

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The degradation of uric acid for tracer work by Buchanan *et al.*[1] revealed many interesting facts concerning the synthesis of this substance in the organism. It was thus thought that a similar degradation of pyrimidines would be of great interest. A method for the isolation of nitrogen 1 and 3 from pyrimidineribosides as methylamine and ammonia respectively has already been elaborated by the author[2]. The present paper deals with the isolation from pyrimidines of carbon atom 2 as urea, and carbon atoms 5 and 6 as oxalic acid, and with experiments using radioactive bicarbonate as a precursor for pyrimidines in the rat. The radioactivity of carbon atom 4 was obtained by difference.

The degradation method has been worked out for uracil using an amount of about 20 mg. It may also of course be applied to cytosine as this substance can easily be converted into uracil by deamination.

Uracil was converted into 5-nitro-uracil according to Johnson [3] and this substance was degraded with $KMnO_4$ in neutral solution as described by Offe [4]. After hydrolysing with alkali the oxalic acid formed was precipitated as calcium oxalate, dissolved in dilute sulfuric acid and treated with $KMnO_4$ to liberate CO_2 which was precipitated as $BaCO_3$.

The urea formed during the degradation was extracted with boiling absolute alcohol and treated with urease to liberate CO_2.

Offe [4] has shown in his work on the degradation of 4-methyluracil and its derivatives with $KMnO_4$ in neutral solution that the oxalic acid formed is derived from carbon 5 and 6. After degradation of 4-methyluracil in the cold he was able to isolate acetylurea and oxalic acid in good yields.

The experiments made by Offe make it reasonable to suppose that the oxalic acid formed during degradation of 5-nitrouracil with $KMnO_4$ in neutral solution, is entirely derived from carbon atoms 5 and 6.

Experiments with C^{14}-bicarbonate. C^{14}-bicarbonate was administered subcutanously to partially hepatectomized rats at 30 minutes intervals. Each rat received 12 doses and the animals were sacrificed 30 minutes after the last dose. Samples of the respiratory CO_2 were analyzed for

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materials, has been proven by isolation by paper chromatography. When run side by side with a synthetic preparation [2], it gives identical R_F values in phenol, *m*-cresol, butanol-acetic acid, collidine, and pyridine-amylalcohol. Spots, which do not appear after hydrolysis and thus indicate peptides, have been found in malt, unboiled and boiled wort, but not in beer. In yeast no less than 9 such peptides have been found. During the first fermentation, asparagine, aspartic acid and threonine disappeared, and a distinct decrease was observed in all basic amino acids and serine. After the second fermentation, the basic amino acids had completely disappeared together with serine, leucine, isoleucine, phenylalanine and tyrosine.

A more detailed account of this work will appear elsewhere.

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Received August 5, 1950.

Force Constants in the Hydrogen Halides and Ionic Radii

CHR. KNAKKERGAARD MØLLER

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Let us make the rather bold assumption that the bond in the hydrogen halides is suddenly disrupted when the hydrogen atom is removed to a certain distance r_2 from the centre of the halide atom. Outside this limiting distance we then have the bond order 0 while inside we reckon with a bond order 1 [1]. We further assume that the interatomic force is quasi-elastic so that the increase in potential energy on moving the proton from the equilibrium distance r_1 to $r_2 = r_1 + \Delta$ is approximately $E = \frac{1}{2}C\Delta^2$ where C is the force constant. (Outside r_2 C is 0). Identifying E with the dissociation energy ε of the molecule (— for dissociation into atoms —) we get approximately

$$\Delta = \sqrt{\frac{2\varepsilon}{C}}$$

or when ε is in e. v., r_1 in Å, C in 10^5 c. g. s. units and Δ in Å:

$$\Delta = 0.566\sqrt{\frac{\varepsilon}{C}}$$

Hence we can easily calculate the "limiting distance" from the halide centre: $r_2 = r_1 + \Delta$ because r_1 and C are known from the infrared spectra [1] and ε is also known [2].

It is remarkable that these "limiting distances" come out nearly identical with the van der Waals or ionic radii of the halides as seen from the table below. (As Pauling has shown the ionic and van der Waals radii are nearly identical [3]).

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their radioactivity in the Geiger-Müller counter.

Uracil was isolated from the PNA of the regenerating liver according to methods worked out at this laboratory [5]. The uracil was probably contaminated with cytosine that had undergone deamination to uracil during the preparation.

After the degradation procedure described above the samples obtained were analyzed in the Geiger-Müller counter. The results are given in Table 1.

Table 1.

	counts/min
Precursor	28 000
Mean value of respiratory CO_2	2 880
Uracil	35
Carbon atom 2	62
Carbon atoms 5 and 6	9
Carbon atom 4	60

These results indicate that in regenerating liver the carbon from bicarbonate enters positions 2 and 4 in the pyrimidine ring to the same degree. Wilson has previously found that bicarbonate enters position 2 (personal communication to professor Hammarsten) and his findings are confirmed by this investigation.

A more detailed report of the method and the experiment with C^{14}-bicarbonate will be published later.

1. Sonne, J. C., Buchanan, J. M., and Delluva, A. M. *J. Biol. Chem.* **173** (1948) 69.
2. Lagerkvist, U. G. *Acta Chem. Scand.* **4** (1950) 543.
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5. Reichard, P. *Acta Chem. Scand.* **3** (1949) 422.

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Received September 20, 1950.

The Absorption of Ultraviolet Light by Enterogastrone

A preliminary report

K. J. ÖBRINK and H. WINBERG

Institute of Physiology, University of Uppsala, and the Central Laboratories, AB Astra, Södertälje, Sweden

It has been found that an enterogastrone preparation from intestines of hogs, which contains no amino acids or peptides (biuret and ninhydrin reactions negative) and has a nitrogen content of 0.24 %, shows a characteristic absorption curve in the ultraviolet region. It has a maximum at 2 600 Å and a minimum at 2 350 Å. Evidence has been obtained, that this absorption curve is due to the active enterogastrone molecule. It was found that the decrease in biological activity which occured when the extract was dissolved in water and stored at room temperature (probable due to bacterial activity) was accompanied by a decrease in the extinction at 2 600 Å. A detailed description of the experiments and a full discussion of the results will shortly appear in this journal.

Received August 15, 1950.

Compound	H—X dist. r_1	Dissociat. energy	C dyne/cm	Δ	r_2	Ionic radii [4]	
						Pauling	Goldschmidt
HF	0.92 Å	6.1 e.v.	$9.7 \cdot 10^5$	0.45 Å	1.37 Å	1.36 Å	1.33 Å
HCl	1.28 »	4.43 »	$5.15 \cdot 10^5$	0.52 »	1.80 »	1.81 »	1.81 »
HBr	1.41 »	3.60 »	$4.11 \cdot 10^5$	0.53 »	1.94 »	1.95 »	1.96 »
HJ	1.60 »	2.75 »	$3.14 \cdot 10^5$	0.53 »	2.13 »	2.16 »	2.20 »
HH	0.74 »	4.48 »	$5.9 \cdot 10^5$	0.50 »	1.24 »	2.08 »	1.27 »

We may perhaps understand this by considering the distance r_2 as the boundary for interference between electrons belonging to the halogen and those belonging to another electronic system. As the proton approaches r_2 from r_1 the electron cloud is polarized and finally — at r_2 — it is just about being deprived of one electron. But if an electron belonging to a "stable" cation tries to get inside the limiting sphere of the halide ion the resulting electronic interaction will give an antibonding effect so that usually the ionic crystal will be more stable than the molecular state. (As the proton has no electrons there will be no antibonding effect when it enters the position inside the limiting sphere giving minimum of electrostatic energy.) This point of view is in essential agreement with our ideas about ionic crystals according to which the interionic distances are determined by the condition that the energy shall be a minimum and the electron clouds of the ions must not inter-penetrate. But as it is only a very rough picture to reckon with a definite limit r_2 for electronic interaction in the hydrogen halides it may, perhaps, be concluded that the ionic radii to the same extent represent idealized concepts.

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Received September 26, 1950.

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Compound	H—X dist. r_1	Dissociat. energy	C dyne/cm	Δ	r_2	Ionic radii [4]	
						Pauling	Goldschmidt
HF	0.92 Å	6.1 e.v.	$9.7 \cdot 10^5$	0.45 Å	1.37 Å	1.36 Å	1.33 Å
HCl	1.28 »	4.43 »	$5.15 \cdot 10^5$	0.52 »	1.80 »	1.81 »	1.81 »
HBr	1.41 »	3.60 »	$4.11 \cdot 10^5$	0.53 »	1.94 »	1.95 »	1.96 »
HJ	1.60 »	2.75 »	$3.14 \cdot 10^5$	0.53 »	2.13 »	2.16 »	2.20 »
HH	0.74 »	4.48 »	$5.9 \cdot 10^5$	0.50 »	1.24 »	2.08 »	1.27 »

We may perhaps understand this by considering the distance r_2 as the boundary for interference between electrons belonging to the halogen and those belonging to another electronic system. As the proton approaches r_2 from r_1 the electron cloud is polarized and finally — at r_2 — it is just about being deprived of one electron. But if an electron belonging to a "stable" cation tries to get inside the limiting sphere of the halide ion the resulting electronic interaction will give an antibonding effect so that usually the ionic crystal will be more stable than the molecular state. (As the proton has no electrons there will be no antibonding effect when it enters the position inside the limiting sphere giving minimum of electrostatic energy.) This point of view is in essential agreement with our ideas about ionic crystals according to which the interionic distances are determined by the condition that the energy shall be a minimum and the electron clouds of the ions must not inter-penetrate. But as it is only a very rough picture to reckon with a definite limit r_2 for electronic interaction in the hydrogen halides it may, perhaps, be concluded that the ionic radii to the same extent represent idealized concepts.

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Received September 26, 1950.

A Preliminary Report on the Dielectric Dispersion of Hyaluronic Acid

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Hyaluronic acid has been studied by Jorpes and co-workers in this labotory. They have obtained preparations of very high purity from umbilical cord after digestion with pancreatic enzymes (unpublished results). In connection with their investigations some dielectric measurements have been made. Fürth's ellipsoid method, adapted to determinations of dielectric constants at different frequencies within the radio frequency range, is used in the manner described for investigations of polynucleotides [1,2]. The preparations used have been kindly supplied by Prof. E. Jorpes.

The neutral sodium salt of hyaluronic acid is found to belong to those substances which have a (positive) dielectric increment. The increment is very high, about 6 DC units in 0.07 per cent aqueous solution, and shows a pronounced dependance on the concentration.

The dielectric dispersion is fairly well defined with a rather theoretical course, indicating a certain degree of monodispersity. With decreasing concentrations the anomalous dispersion curve moves considerably to longer wave-lengths. This corresponds to a higher aggregate weight in dilute solutions.

With the assumptions of high aggregate asymmetry, and the resulting dipole moment transverse to the longer axis (which are plausible assumptions), a mean molecular weight of about 80 000 is calculated. The theoretical treatment of results of such substances are by no means easy, however, and the value mentioned must be taken with reserve until more exact knowledge has been obtained.

However, the order of magnitude is interesting because the supposed monomer (glucuronido-N-acetyl-glucosamin) might have been expected to orient rather freely around the glucosidic linkages. The existence of large aggregates, orientating as a whole, with high polarity, offers a chance for further dielectric investigations and especially for studies of the enzymatic decomposition.

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Received September 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1155–1170

Gustav Komppa

Gustav Komppa wurde am 28. Juli 1867 in Wiborg, der Hauptstadt der Landschaft Karelien geboren. Seine Eltern waren David Komppa und Helena Lipponen. Seine Schulerziehung erhielt er in Wiborgs Realschule, einer Schule, die gute Lehrer hatte. Der junge Schüler studierte mit Fleiss und Fortschritt alle Fächer, aber am meisten fesselten ihn die Naturwissenschaften. Schon sehr früh wurde es ihm klar, dass er sich der Chemie widmen würde. Er assistierte dem Lehrer bei den Versuchen, die während der Chemie- und Physiklektionen ausgeführt wurden, führte selbst, zusammen mit seinen Kameraden chemische Experimente durch und baute Apparaturen für physikalische Versuche. Bei den Schulausflügen war er der selbstverständliche Führer im Botanisieren und Ausführen zoologischer Beobachtungen. Der Direktor der Schule empfahl ihn als Privatlehrer für nachgebliebene Schüler. Leider zwangen ökonomische Umstände Komppa, seinen Schulgang zu beenden, nachdem er das 5-jährige Mittelschulpensum absolviert hatte. Um aber auch weiterhin mit dem chemischen Experimentieren und Studieren fortsetzen zu können, wählte er die Apothekerlaufbahn und nahm Anstellung als Schüler in einer Apotheke seiner Vaterstadt. Die gestrenge Dienstausübung liess jedoch für Zeitvertreib oder Studium keine Zeit übrig. Glücklicherweise konnten die abgebrochenen Studien nach einem Jahre durch private Hilfe doch fortgesetzt werden. Komppa immatrikulierte sich am 1. September 1886 in der Fachschule für Chemie des Polytechnischen Instituts in Finnland.

Mit Begeisterung und Energie betrieb er seine Studien an diesem Institut, das er im Frühjahr 1890 als Ingenieur verliess, wobei ihn die Auszeichnung Zuteil wurde, das Diplom des Instituts zu erhalten. Die technische Laufbahn stand nun für Komppa offen und recht bald, nachdem er Ingenieur geworden war, wurde er zum Stellvertreter des Vorstehers der Station für Lebensmitteluntersuchung der Stadt Helsingfors gewählt. Als solcher führte er die erste Untersuchung aus, die zu einer Veröffentlichung führte und den Titel trug »Om natronkalk som torkningsmaterial vid Marsh'ska profvet» (Über Natronkalk als Trocknungsmaterial bei der Marsh'schen Probe). Neben seiner Dienstausübung setzte er seine Studien an der Universität zu Helsingfors fort. Hier legte Komppa i. J. 1891 das Philosophie-Kandidat-Examen ab und zog es nun vor, anstatt eine technische Stellung anzunehmen, die mühevolle Laufbahn des Forschers zu betreten.

Früher hatte sich Komppa hauptsächlich für anorganische Chemie interessiert, aber unter dem Einfluss seines Lehrers am Polytechnischen Institut, Prof. H. A. Wahlforss, der selbst ein kundiger und experimentell geschickter Organiker war, spezialisierte er sich in organischer Chemie, die zu jener Zeit im Zeichen bemerkenswerter Synthesen stand. Als Thema für seine Doktorsabhandlung wählte Komppa Derivate des aromatischen Kohlenwasserstoffs Styrol. Diese Arbeit wurde teils im Chemischen Laboratorium der Universität ausgeführt, das zu jener Zeit unter Prof. Edv. Hjelts Leitung stand, teils im Eidgenössischen Polytechnicum zu

Zürich, wo er unter Prof. A. Hantzsch studierte. Das Resultat dieser Studien war die akademische Dissertation »Über kernsubstituierte Styrole», die in hergebrachter Weise am 26. April 1893 verteidigt wurde. Nach dem im selben Jahre erworbenen Lizentiatgrad wurde Komppa 1894 zum Doktor der Philosophie promoviert. Ein Jahr später wurde Komppa als stellvertretender Lehrer für Chemie am Polytechnischen Institut angestellt. Komppas Unterricht gestaltete sich noch umfassender als seine Lehreranstellung voraussetzte, da er, allerdings zusammen mit anderen, den ordentlichen Lehrer während dessen langwieriger Krankheit vertreten musste. Eine Ausnahme war 1895, als Komppa Gelegenheit hatte, ein Jahr lang Studien an der Universität zu Leipzig unter deren schon damals weltberühmten Professoren der Chemie Wilhelm Ostwald, John Wislicenus und F. A. Stohmann zu betreiben. Nachdem die ordentliche Lehrerstellung für Chemie am Polytechnischen Institut durch den Abgang von H. A. Wahlforss i. J. 1898 freigeworden war, bewarb sich Komppa um diese Stellung und wurde i. J. 1899 in diese ernannt. Bei der Umorganisation des Polytechnischen Instituts (1908) zur Technischen Hochschule in Finnland, wurde Komppa Professor für Chemie an dieser. Vordem hatte er schon als Vorstand des Chemischen Laboratoriums gewirkt, und 1899 wurde er zum Vorstand der Fachschule für Chemie des Polytechnischen Instituts, 1908 zum Vorstand der Chemischen Abteilung der Technischen Hochschule ernannt. Nach vollendeten 70 Lebensjahren nahm Komppa als Professor Emeritus Abschied von der Technischen Hochschule, betrieb aber auch hiernach noch Lehr- und Forschungstätigkeit an der Hochschule bis 1946. Fügen wir noch hinzu, dass Komppa von 1898 bis 1903 Dozent der Chemie an der Universität (Helsingfors) war und i. J. 1902 eine Studienreise nach Paris unternahm, so sind die wichtigsten Daten betreffend Komppas Studien und Lehrertätigkeit in aller Kürze angegeben.

Komppa hat fast ein halbes Jahrhundert als Lehrer an dem Institut, später Hochschule gewirkt das er selbst absolviert hatte, und dazu während dem grössten Teil dieser Zeit als Vorstand der Chemischen Abteilung einen leitenden Posten eingenommen. So ist es keine Übertreibung zu behaupten, dass Komppa während fast einem halben Jahrhundert die Verantwortung für die Ausbildung der Chemikeringenieure in Finnland trug und dieser seine Prägung aufdrückte. Dies um so mehr, als er seine Lehrertätigkeit mit grossem Eifer betrieb und ständig mit Vorschlägen zu wohlbedachten Verbesserungen kam, die sowohl die Laboratorien, die Ausrüstung als auch den Unterricht betrafen. Er war der Gründer des neuen Laboratoriums, das Anfang dieses Jahrhunderts für den chemischen Unterricht des Polytechnischen Institutes errichtet und mehrmals erweitert wurde und im grossen genommen dem Bedarf genügt hat, bis ein Zubau nach Komppas Tod kürzlich fertig wurde. Die Professuren in organischer, insbesonders holzchemischer Technologie, in physikalischer Chemie, in Biochemie sowie die Aufteilung seiner eigenen Professur in anorganische und organische Chemie nach seinem Abgang von der Technischen Hochschule sind alle auf Komppas Initiative zurückzuführen. Unzählige durchgreifende Verbesserungen in den Unterrichtsplänen hat er durchgeführt.

Als Lehrer besass Komppa eine einzigartige Fähigkeit, auch verwickelte Theorien und Forschungsresultate leichtfasslich und klar unter Hervorhebung des Wesentlichen darzustellen. Infolge seiner eigenen experimentellen Gewandtheit beim chemischen Arbeiten und der Gewohnheit, mit kleinen Quantitäten zu arbeiten, war er der von allen anerkannten Meister im Leiten der Laborationen und der Forschung, wobei er eine einzigartige Befähigung hatte, auch die trägsten Schüler zu

enthusiasmieren. Als Examinator war er sachlich und gerecht, aber von vielen doch sehr gefürchtet. In diesem Zusammenhang sei erwähnt, dass Komppa i. J. 1906 eine Bearbeitung von Remsens berühmtem, elementaren »Lehrbuch der anorganischen Chemie» in finnischer Sprache herausgab, die in 4 Auflagen erschien.

Eine Lebensschilderung über Komppa wäre mangelhaft ohne alles das zu erwähnen, was er, neben seiner Tätigkeit als Lehrer und Forscher, innerhalb wissenschaftlicher Vereinigungen, für den Fortschritt der Wissenschaft, zur die Hebung des Gemeinsinnes und für das Kulturleben im allgemeinen geleistet hat. Seine Einsätze auf diesen Gebieten sind so vielfältig und so bedeutend, dass ihre Aufzählung auf die allerwichtigsten beschränkt werden muss. Komppa gehörte zu den Begründern des Finska Kemistsamfundet (die Finnische chemische Gesellschaft) i. J. 1891, und er entfaltete eine grosse Aktivität, um die Sitzungsprogramme möglichst wertvoll zu gestalten. Er war auch Begründer des Vereins Finnischer Ingenieure und der Gesellschaft Finnischer Chemiker, und in diesen Vereinigungen war er sein Leben lang einer der leitenden Persönlichkeiten. Von grösserer Bedeutung für die Wissenschaft des ganzen Landes war die Gründung der Finnischen Akademie der Wissenschaften, deren Initiative Komppa zukommt, und in der er während nicht weniger als 28 Jahren (1908—1936) mit nie versiegender Energie als ständiger Schriftführer wirkte. Komppa beteiligte sich auch an der Gründung der Turun Yliopisto (Finnische Universität zu Turku), und wirkte 1935—45 als höchster Beamter der neuen Universität. Ferner sei erwähnt, dass Komppa seit 1920 bis zu seinem Tode der Vorsitzende der Kordelinschen Stiftung war.

Komppas Initiative und Tätigkeit zur Hebung der Industrie Finnlands sind gleichfalls vielgestaltig und von weitgehender Bedeutung. Die Erwähnung dieser Tätigkeit muss hier auf eine Aufzählung der Fabriken beschränkt werden an deren Gründung er teilgenommen hat oder die er als Vorsitzender der Direktion längere Zeit geleitet hat, das sind die Staatliche Pulverfabrik, die Finnische Gummifabrik AG, die »Kemiallinen OY» sowie die Arzneifabrik Orion AG. Komppa gehört auch zu den Begründern des Finnischen Gasschutzvereins, später Finnlands Luftschutz-Organisation.

Es ist natürlich, dass ein berühmter Forscher und eine leitende Persönlichkeit der wissenschaftlichen Welt in Finnland viele Auszeichnungen nicht nur von Seiten des Staates, von Universitäten, Akademien und Hochschulen, sondern auch von Korporationen und Vereinigungen erhalten hat. Komppa ist Inhaber des Freiheitskreuzes erster Klasse. Er war Kommendeur des Grosskreuzes des Ordens der Weissen Rose Finland, Kommendeur erster Klasse des dänischen Dannebrogsordens und des schwedischen Nordsternordens und Ehrenvorstand oder Ehrenmitglied von vielen einheimischen und ausländischen Akademien, Stiftungen und Vereinigungen sowie Doctor honoris causa der Universitäten zu Kopenhagen, Uppsala und Heidelberg sowie der Technischen Hochschule. Der Verein Finnischer Ingenieure hat Komppa zu seinem 50. Geburtstage ein Heft der Teknillinen Aikakauslehti (Technische Zeitschrift) gewidmet. Die Gesellschaft Finnischer Chemiker hat eine Silberplakette zu seinem 60-Jahrestag schlagen lassen und eine Festschrift zu seiner Ehre herausgegeben. Dieselbe Gesellschaft hat ihm auch die Gadolin-Medaille verliehen. Die Deutsche Chemische Gesellschaft hat Komppa mit der A. W. v. Hofmann-Medaille ausgezeichnet. Um ihrem Ehrenvorstand zu gedenken, hat die Gesellschaft Finnischer Chemiker i J. 1949 den Gust. Komppa-Fond zur Beförderung der finnischen chemischen Forschung gestiftet.

Komppas vornehmste Liebhabereien waren Dendrologie und Hortikultur. Schon

frühzeitig begann er damit auf seiner Sommervilla Metsola am See Vesijärvi. Da aber der Boden für ausländische Baumarten karg war, beschloss er, das Gut Tammisto in Karjalohja einzukaufen, nachdem er systematisch ermittelt hatte, dass sowohl Boden als Klimat in dieser Gegend für dendrologische und Pflanzenveredlungsversuche am vorteilhaftesten wären. 100 verschiedene Nadelhölzer, 200 Laubbäume, 400 verschiedene Straucharten sowie 2 000 Pflanzenarten auf Tammisto neben besonderen Publikationen zeugen von diesem botanischen Nebeninteresse.

Mit einigen wenigen Ausnahmen war Komppa während der Zeit 1897—1948 Finnlands Repräsentant an allen internationalen und nordischen Kongressen, die reine und angewandte Chemie betrafen, wodurch er mit so ziemlich allen Koryphäen der Chemie während der ersten Hälfte des zwanzigsten Jahrhunderts persönlich bekannt wurde.

Komppa war mit Siiri Andelin verheiratet, die i. J. 1945 verschied; er wird von seiner Tochter Leila Komppa-Mylläri sowie von seinem Sohn Olavi Komppa betrauert.

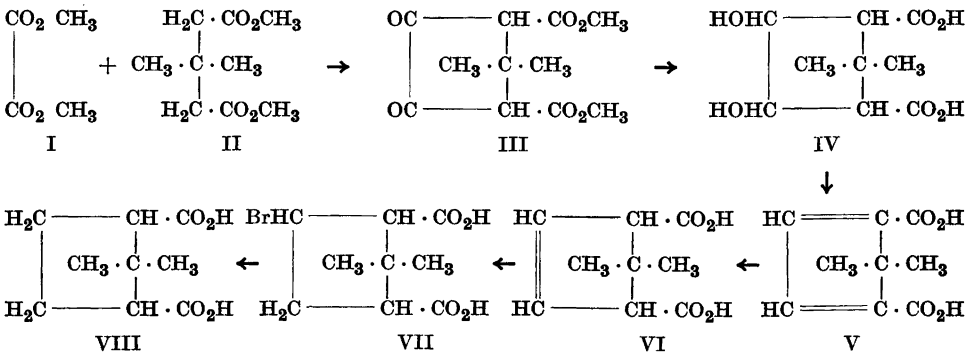
Wir stehen nun vor der Aufgabe, eine kurze Zusammenfassung über Komppas Tätigkeit als Forscher von seiner Dissertation und ersten, schon genannten Abhandlung bis zur letzten Veröffentlichung v. J. 1946 zu geben. Hierbei ist zunächst zu beachten, dass Komppa bis zu seinem 60. Jahr genötigt war, alle Laboratoriumsarbeiten mit Examensarbeiten ausführenden Schülern, Doktoranden oder zufälligen Privatassistenten durchzuführen. Eine Änderung trat erst ein, als ihm i. J. 1928 das Geselliussche Stipendium zuerteilt wurde, sowie Mittel für einen staatlich angestellten Assistenten bewilligt wurden. Diese Änderungen führten zu einer sehr gesteigerten wissenschaftlichen Produktion. Nach seiner Emeritierung setzte Komppa seine wissenschaftliche Experimentalforschung in einem von der Hochschule eingerichteten Laboratorium beharrlich fort, bis dieses beim Luftangriff auf Helsingfors am 6/7. Februar 1944 vollständig vernichtet wurde. Die Zerstörung betraf leider nicht nur das Laboratorium und seine Ausrüstung, sondern auch Komppas Handbibliothek, Laboratoriumsaufzeichnungen sowie die Präparate und Ausgangsstoffe. Nach dieser Katastrophe, die sogar einen Mann mit Komppas elastischem Sinn sehr hart traf musste er seine halbtechnischen Versuche zur Hydrierung von Torf und Teerölen in einem provisorisch eingerichteten Laboratorium fortsetzen. Auch das Alter nahm sein Recht in Anspruch, und trotzdem Komppa an seinem 80. Geburtstage am 28. Juli 1947 die Huldigung seiner Schüler, Freunde und Kollegen mit scheinbar ungebrochenen Körper- und Geisteskräften empfangen konnte, war er gezwungen, seine Arbeitsintensität zu vermindern. Ende 1948 wurde er von einem Schlagenanfall betroffen, der am 20. Januar 1949 sein an intensiver Tätigkeit reiches Leben beendete.

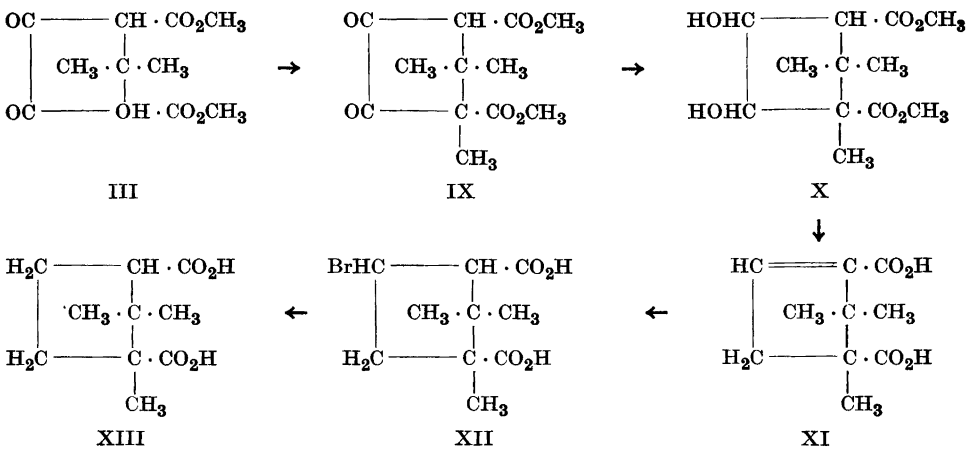
Komppas erste wissenschaftliche Veröffentlichungen in internationalen Zeitschriften leiten sich, wie natürlich, direkt von seiner Dissertation ab. Sie behandeln die Cumaronsynthese [1] und die Cyanzimtsäure [2]. Ein Versuch zur Darstellung der Camphoronsäure [3] gibt einen Hinweis, dass Komppa die Camphersynthese als ein Ziel seiner Forschung aufgestellt hatte. Doch kamen ihm bei der Camphoronsäuresynthese keine Geringeren als J. F. Thorpe und W. H. Perkin jr. zuvor, und aus diesem Grund, sowie wegen der Schwierigkeiten, die von ihm gewünschten Produkte zu gewinnen, war er gezwungen, den Plan der Synthese völlig umzulegen. Eine kurze Mitteilung über $\beta\beta$-Dimethylglutarsäure [4] markiert den veränderten Kurs. Im Übrigen behandeln Komppas Veröffentlichungen aus der Zeit vor der Jahrhundertwende recht verschiedene Gebiete, die die Synthese des Thionaphtens [5], Nitrierung von

Guajakol [6], Kondensationen mit Malon- und Cyanessigsäure [7] sowie die elektrolytische Synthese von Undekamethylen- und Dekamethylendicarbonsäure [8] betreffen.

Nach 8-jähriger, mühevoller Arbeit veröffentlicht Komppa i. J. 1901 eine vorbereitende Mitteilung in den Berichten [9], dass er Apocamphersäure, VIII, synthetisch durch Anwendung der Dieckmannschen Kondensation auf die Ester der Oxal- und $\beta\beta$-Dimethylglutarsäuren dargestellt hatte. Es war ihm nämlich gelungen, nach vielen ergebnislosen Versuchen das Dimethyldihydroresorcin nach Vorländer darzustellen und durch Oxydation mittels Natriumhypobromit aus diesem gute Ausbeuten an $\beta\beta$-Dimethylglutarsäure zu erzielen. Diese Säure wurde mit Methylalkohol zum Dimethylester, II, esterifiziert, der mit Oxalsäuredimethylester I zum Diketoapocamphersäureester III kondensiert wurde, der mittels Natriumamalgam zur Dioxyapocamphersäure, IV, reduziert wurde. Durch Wasserabspaltung erhielt Komppa eine doppelt ungesättigte Säure, V, die Wasserstoff unter Bildung von Isodehydrocamphersäure, VI, anlagerte. Die letztgenannte Säure addierte Bromwasserstoff, wonach die entstandene β-Bromapocamphersäure, VII, mit Zn in Eisessig zur erstrebten Apocamphersäure, VIII, reduziert wurde. Um aber zur Camphersäure zu gelangen, war die Einführung einer Methylgruppe erforderlich. Das einzige für diesen Zweck geeignete Zwischenstadium in der Apocamphersäuresynthese war das erste Kondensationsprodukt, der Diketoapocamphersäureester, III. Obwohl diese Methylierung eine neue Schwierigkeit bildete, da die Reaktion nicht eindeutig verlief, wurden alle Schwierigkeiten überwunden, und die Ausbeute wurde befriedigend durch Anwendung der doppelten theoretischen Menge Methyljodid und Natrium. Die Reduktion des Diketocamphersäuredimethylesters, IX, wurde analog der des entsprechenden Apocamphersäureesters ausgeführt mit der Abweichung, dass die Reduktion des Dioxyesters, X, eine einfach ungesättigte Säure, XI, ergab. Das Endprodukt, die *r*-Camphersäure, XIII, erwies sich als identisch mit der aus Naturcampher hergestellten *r*-Camphersäure.

Die erste Mitteilung über die Camphersäuresynthese [10] stammt aus dem November 1903. Damit erhielt Komppa einen Vorsprung von einigen Monaten vor J. F. Thorpe und W. H. Perkin jun., die ihre, einen anderen Weg benützende Camphersäuresynthese im Jahre 1904 veröffentlichten. Dies war für Komppa eine grosse Genugtuung, nachdem ihm dieselben Forscher bei der Camphoronsäuresynthese zuvorgekommen waren. Die Synthese des *r*-Camphers wurde danach nach einer Reaktionsfolge vollzogen, die Haller sowie Bredt und Rosenberg früher benutzt hat-

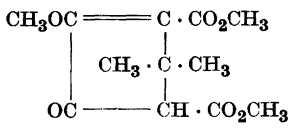




III IX X XI XII XIII

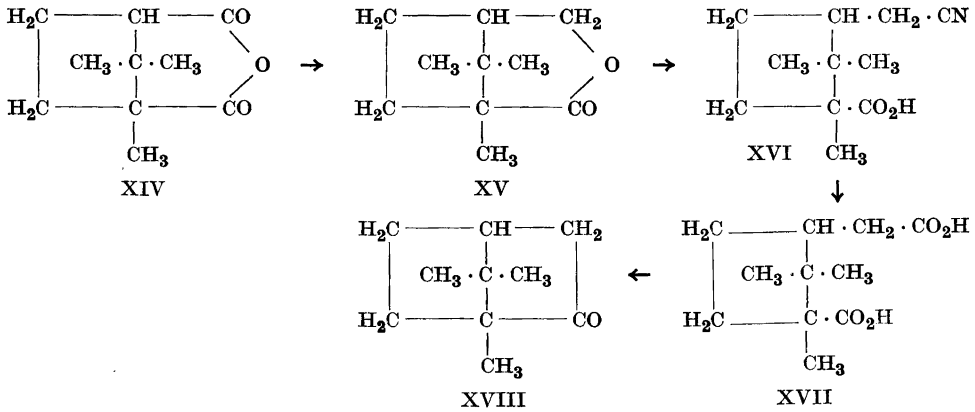
ten, und in der Sitzung der Finnischen Chemischen Gesellschaft vom 13. Oktober 1905 konnte Komppa den erhaltenen synthetischen Campher vorweisen.

Die elegant ausgeführte und übersichtliche Camphersäuresynthese weckte jedoch Zweifel bei Forschern, die ihre Wiederholung versucht hatten. G. Blanc [11] und J. F. Thorpe [12] sprachen sogar die Ansicht aus, dass Komppas Folgerungen auf einem Irrtum beruhten und behaupteten, dass ein C-methylierter Diketosäureester, IX, nach Komppas Vorschriften überhaupt nicht darstellbar wäre, sondern dass sich bei der Methylierung des Diketoapocamphersäureesters, III, nur der Monomethyläther, XIX, des enolisierten Diketoesters bilden kann.



XIX

Ehe Komppa den Angriff zurückwies, wiederholte er mit anderen Mitarbeitern seine Synthesen, wobei das Resultat das Gleiche



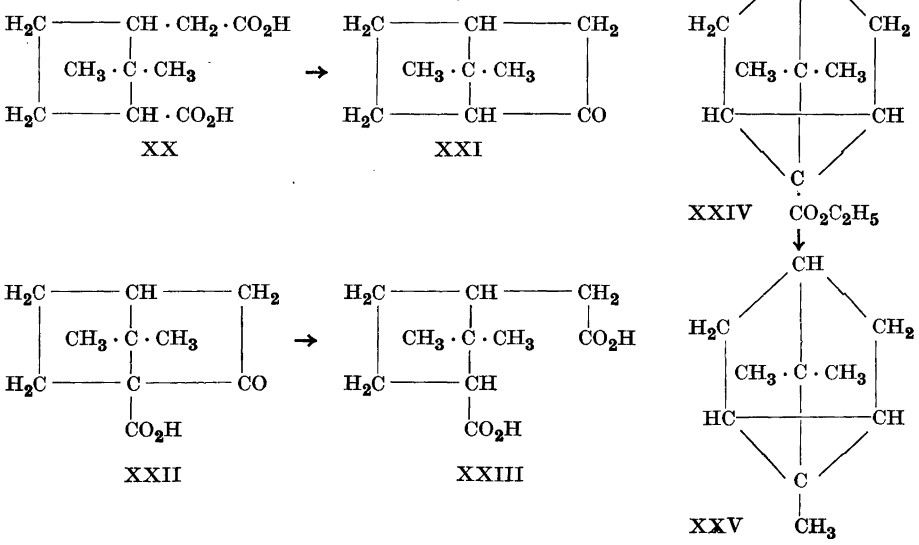
XIV XV XVI XVII XVIII

wie früher war. In seiner Erwiderung [13] wies Komppa darauf hin, dass Blanc und Thorpe es nie unternommen hatten, das Reaktionsprodukt, IX, das bei der Methylierung von Diketoapocamphersäuredimethylester, III, entsteht, zu reduzieren, sondern dasselbe nur mit Kalilauge abgebaut hatten. Bei der Reduktion des genannten Reaktionsproduktes hatte Komppa Soda oder eigentlich nur Bicarbonatlösung verwendet. Neben allen anderen Beweisen, dass es sich um C-Methylierung handelte, war die Synthese der Äthylcamphersäure[14], die Komppa zusammen mit Routala ausführte, die bindendste. Später sind die Komppasche Camphersäuresynthese oder andere experimentelle Arbeiten niemals bezweifelt worden.

Sowohl die Camphersynthese, als auch die Arbeiten, die Komppa zu ihrer Bekräftigung vornahm, führten zu weiteren Synthesen von Campher- und Terpenverbindungen. So versuchte Komppa, Dimethylcampher und Camphenilon herzustellen durch Anwendung der Hallerschen Reaktionsserie für Campholid, XV, auf Methyl- bzw. Dimethylnorcampholid, die durch Grignardierung erhalten werden. Dies gelang jedoch nicht. In der nächsten Arbeit [15], die zusammen mit Hintikka ausgeführt wurde, schlug Komppa eine verbesserte Nomenklatur für gewisse wichtige Campherderivate vor u. a. sollten Bicyclo [1,2,2]-heptanon-2 als Norcampher sowie Fenchocamphoron als Apocampher bezeichnet werden. Diese Nomenklaturvorschläge haben auch allgemein Eingang gefunden.

Für das Apocamphersäureanhydrid glückten jedoch die Hallerschen Reaktionen und führten zum Fenchocamphoron oder Apocampher, XXI [16]. Aus dieser Synthese gingen zwei wichtige Resultate hervor.

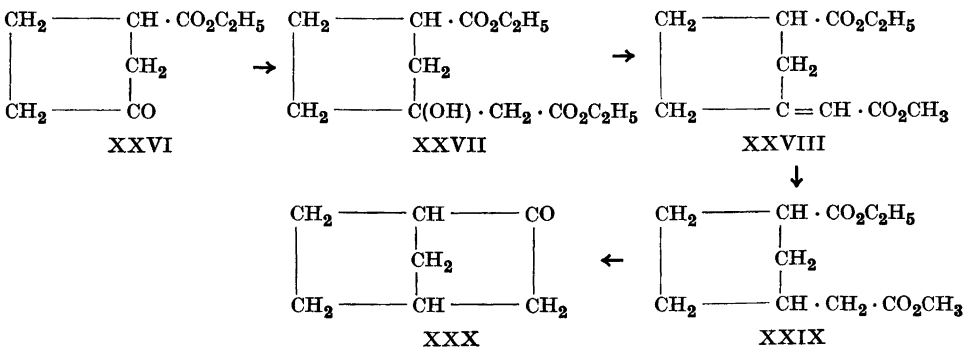
Wenn Wagners Formel für die Camphencamphersäure richtig wäre, so hätte sie identisch mit der synthetisierten Homoapocamphersäure, XX, sein sollen, was aber nicht der Fall war, sondern letztere war identisch mit der Pinophansäure, XXIII [17], die durch Säurespaltung aus der Ketopinsäure XXII entsteht. Hierdurch wurde auch die Struktur dieser Säure endgültig festgelegt.



Weiterhin wurde die Synthese des Tricyclens durchgeführt mittels Reduktion des Tricyclensäureesters, XXIV, zu Tricyclenol, welches in das Chlorid übergeführt und sodann zum Tricyclen, XXV, reduziert wurde.

Mangel an Rohmaterial hatte Komppa verhindert, auf analogem Wege, der von Camphersäure zum Campher führt, Norcampher [18] aus Pospischills Säure, d. i. *cis*-1,3-Cyclopentandicarbonsäure, darzustellen. Er wählte daher einen anderen Weg und bereitete (zusammen mit Hintikka) Oxyhomonorcamphersäureester, XXVII, mit Hilfe von Reformatzkys Reaktion durch Kondensation von Cyclopentanoncarbonsäureester, XXVI, und Bromessigsäureester mit Zn. Der erstgenannte Säureester, XXVII, spaltet Wasser ab und geht in Dehydrohomonorcamphersäureester, XXVIII, über, der leicht zu Homonorcamphersäureester, XXIX, hydriert wird. Das Bleisalz dieser Säure gibt befriedigende Ausbeute an methylfreiem Campher, Norcampher, XXX. Nach mehr als ein Jahrzehnt währenden Anstrengungen war es also Komppa gelungen, das Stammketon des Pentoceansystems darzustellen. Die Mühe war ihrem Lohn wert, da Norcampher bemerkenswerte Eigenschaften besass, Wasserlöslichkeit und grosse Flüchtigkeit und praktische Anwendbarkeit in der Chemoterapie zu versprechen schien.

Da es die Synthese des D-*d*-Fenchocamphorons oder *r*-Apocamphers wahrscheinlich gemacht hatte, dass die Camphencamphersäure de facto Dimethylhomonorcamphersäure war, wurde durch Trockendestillation des Ca-Salzes der genannten Säure das Camphenilon [20] synthetisiert. Damit haben wir jedoch früheren Camphenilon- [20] und Santenuntersuchungen [21] vorgegriffen, die ihrerseits ein Sprungbrett zur Erforschung der Fenchenserie darstellten, Arbeiten die Komppa zusammen mit Roschier, Hasselström, Nyman, S. Beckmann u. a. während mehr als drei Jahrzehnten durchführte. Der Anlass zur Bearbeitung des Camphenilons bestand in der Anhäufung von dazu geeignetem Material bei der Herstellung von Apocamphersäure durch Oxydation von Camphen. Nach Semmlers Formel für Camphenilon ist dieses Keton ein niederes Homologe zu Fenchon. Gemäss dem Reaktionsschema Fenchylalkohol, Fenchen, Isofenchylalkohol bereitete Komppa aus dem niederen Homologen Camphenilol, Camphenilen und Isocamphenilol, welches sich jedoch als identisch mit Santenol erwies. Der also doch nicht einheitliche Kohlenwasserstoff Camphenilen enthielt Santen, aus dem Santenol entstanden und somit synthetisiert worden war. In diesem Zusammenhang wurde auch Apobornylen durch Einwirkung von metallischem Natrium auf Camphenilon erhalten, und es

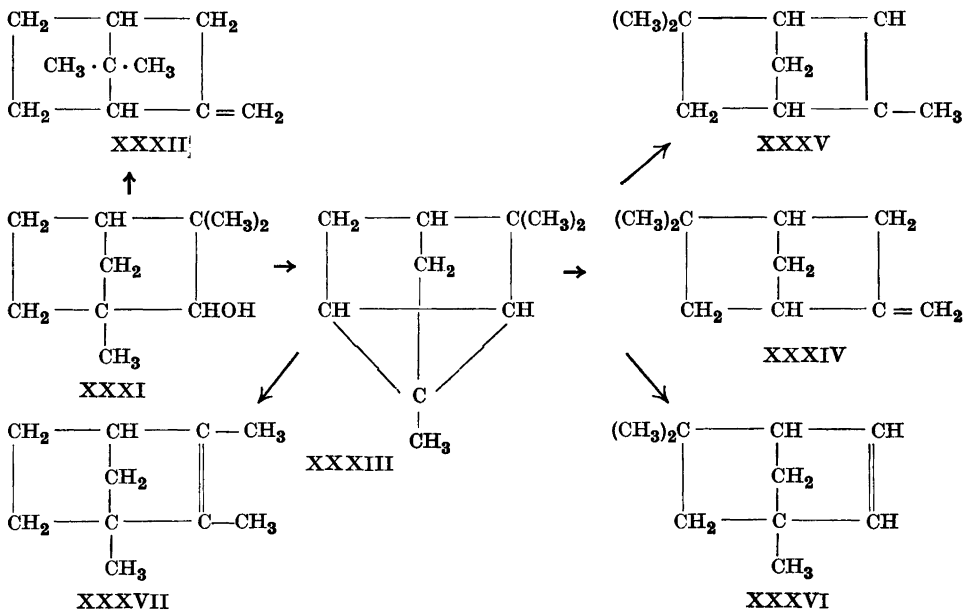


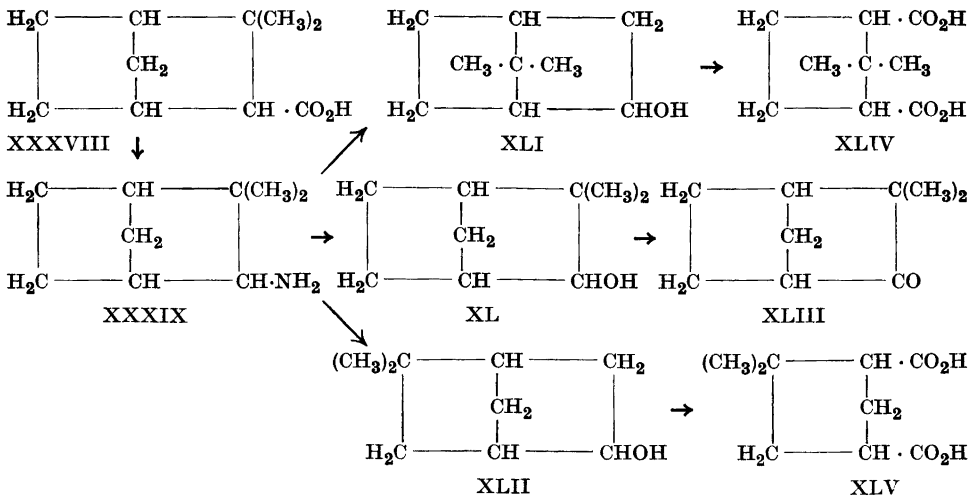
wurde angenommen, dass die Reaktion über einen neuen, tricyclischen Kohlenwasserstoff, Apocyclen verläuft. Die Konstitution des Apobornylens wurde durch Abbau mit Ozon zur Apocamphersäure bestimmt. Die Ozonisierung wurde hiernach für Komppa ein wichtiges Kontrollmittel zur Bekräftigung der Konstitution ungesättigter Terpenderivate.

Als Komppa kurz vor dem ersten Weltkrieg seine Fenchenforschungen begann, waren nur zwei Fenchene bekannt, von Wallach D-*l*- bzw. L-*d*-Fenchen sowie D-*d*- bzw. L-*l*-Fenchen genannt. Seitdem sind noch drei weitere hinzugekommen, das Methylsanten oder *ε*-Fenchen und W. Hückels *ζ*-Fenchen nicht mitgerechnet. Diese Zunahme der Anzahl der Fenchene veranlasste Komppa, Wallachs nicht zweckmässige, oben dargelegte Nomenklatur mit einer [22], der *α*- und *β*-Pinene analogen zu ersetzen.

Die Fenchene können in verschiedener Weise erhalten werden; Komppa hat hauptsächlich die Gemische untersucht, die bei der Wasserabspaltung mittels Alkalibisulfat aus Fenchylalkohol, XXXI, entstehen. Bezüglich der Dehydratisierung konstatierte Komppa mit Nyman [23], dass sie in zwei Richtungen verläuft, ohne bzw. unter Umlagerung. Im ersteren Fall bildet sich das Cyclofenchen XXXIII, im zweiten durch Wagnersche Umlagerung *α*-Fenchen XXXII, oft aber auch durch Nametkinsche Umlagerung, von Komppa Santenverschiebung genannt, Methylsanten (*ε*-Fenchen, XXXVII). Durch Einwirkung von Bisulfat auf Cyclofenchen entstehen dann sekundär *β*-, XXXIV, *γ*-, XXXV, und *δ*-Fenchen, XXXVI.

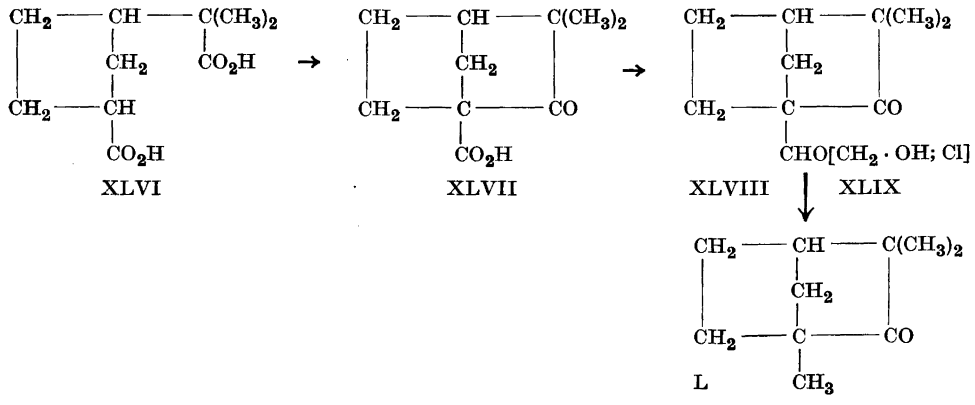
Die Konstitution der *α*-, *β*-, *γ*- und *δ*-Fenchene bewies Komppa durch Partialsynthese, indem er eine Methylgruppe nach Grignard in *α*-Fenchocamphoron oder Apocampher [24], sowie in *β*-Fenchocamphoron [25] einführte und danach Wasser abspaltete. Später erweiterte er, zusammen mit seinem Sohn Olavi Komppa [26] diese partiellen zu Totalsynthesen: Aus Cyclopentadien und Dimethylacrylsäure wurde Isocam-

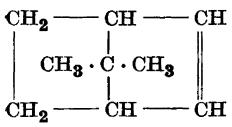




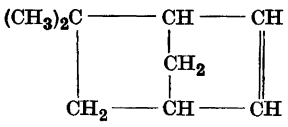
phenilansäure, XXXVIII, dargestellt, die dann in Camphenylamin, XXXIX, übergeführt wurde, wonach mittels salpetriger Säure die Amino- in die Hydroxylgruppe verwandelt wurde, wobei Camphenilol, XL, α-, XLI, und β-Fenchocamphorol, XLII, entstanden. Eine Oxydation mit Permanganat gab Camphenilon, XLIII, Apocamphersäure, XLIV, und Isofenchocamphersäure, XLV.

Das zentrale Keton der Fenchene, das Fenchon wurde schon 1917 von Ruzicka [27] synthetisch durch eine umständliche Reaktionsfolge mit l-Methylnorcampher als Ausgangssubstanz dargestellt. Um eine synthetische Brücke vom Campher zum Fenchon zu schlagen, führte Komppa eine andere Synthese [28] durch, ausgehend von der von Lipp schon synthetisierten *d,l-cis*-Camphencamphersäure, XLVI. Die Zwischenprodukte der Synthese waren Camphenonsäure, XLVII, Camphenonsäurechlorid, Oxofenchon, XLVIII (teilweise Fenchon), ω-Oxyfenchon, XLIX, und ω-Chlorfenchon, das dann zum Fenchon, L, reduziert wurde.

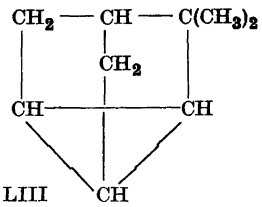




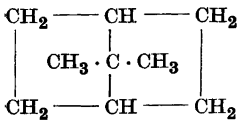
LI



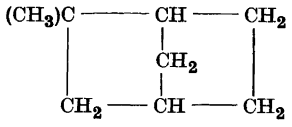
LII



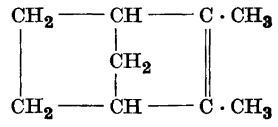
LIII



LIV



LV

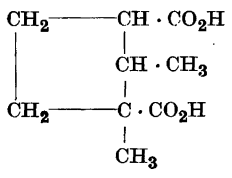


LVI

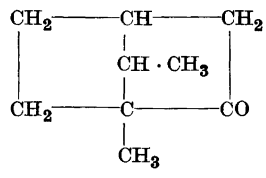
Um eingehender die Umlagerungs- und Razemisierungserscheinungen zu untersuchen, an denen die Fenchene so reich sind, hat Komppa mit Nyman[29] sowohl Methyl- und Phenylfenchene sowie Apofenchene und Apoterpene im allgemeinen dargestellt, wie z. B. Apobornylen, LI, Apoisofenchen, LII, Apocyclen, LIII, Apocamphan, LIV, β-Apofenchan, LV, und das wichtigste von allen, Santen, LVI.

Die Richtigkeit der Semmlerschen Santenformel wurde zwar durch Komppas und Hintikkas Totalsynthese 1916[30] bestätigt, hinsichtlich der Santenole und Santensäuren waren die Strukturen dessenungeachtet nicht endgültig bewiesen. Komppa führte deshalb eine Kondensation von β-Methylglutarsäureester mit Oxalester durch, um analog der Camphersäure die π-Apocampher- oder Santensäure zu synthetisieren[31]. Das Resultat dieser Arbeit war, dass die 4 stereoisomeren Säuren *cis*- und *trans*-Santen- sowie *cis*- und *trans*-Isosantensäure, synthetisch dargestellt und identifiziert wurden. Hiermit war die Struktur nicht nur der Santensäuren, LVII, sondern auch des Santenons, LVIII, und der 2 bekannten Santenole, LIX, sichergestellt.

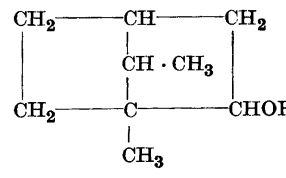
Da die π-Methylgruppe im Santenon *cis*- und *trans*-Form voraussetzt, müssen 2 Stereoisomere existieren, und es gelang Komppa[32], auch die zweite Form, β-Santenon, zu isolieren, die bei der Oxydation *cis*-Isosantensäure liefert und nicht *cis*-Santensäure wie α-Santenon. Für die entsprechenden Alkohole kommt eine endo-exo-Isomerie hinzu, weshalb 4 racemische Santenole *cis*-endo, *cis*-exo, *trans*-endo und *trans*-exo theoretisch möglich sind. Zu den zwei schon bekannten, α-Santenol und α-Santenonalkohol, stellte Komppa einen dritten dar, den er β-Santenonalkohol nennt. Die vierte stereoisomere Form, β-Santenol, ist unbekannt. Die tertiären Santenhydrate sind Strukturisomere, tragen aber dazu bei, die bunte Nomenklatur der Santenole zu verwirren. Von grosser



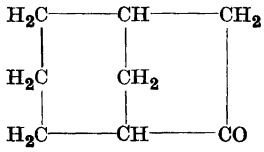
LVII



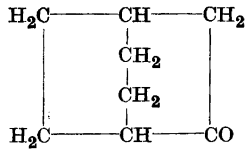
LVIII



LIX



LX



LXI

Bedeutung war auch, dass Komppa feststellte, wie die tertiären Methyl- und Dimethylsantenole Wasser abspalten[33]: Auf Grund der erhaltenen Resultate wurde die von Komppa aufgestellte Regel bekräftigt, dass die tertiären Alkohole des Pentoceansystems Wasser nur dann unter Wagner-Umlagerung abspalten, wenn sowohl das C-Atom 7 der Brücke als C-Atom 1 vom Substituenten belastet ist. In allen übrigen Fällen verläuft die Wasserabspaltung ohne Umlagerung.

Mit einer Zwischenzeit von mehr als 30 Jahren hat Komppa zuerst mit Hirn[34] 1903 das Bicyclo-[1,2,3]-octanon, LX, und dann allein das Bicyclo-[2,2,2]-octanon, LXI, dargestellt.

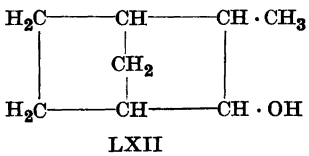
Isophtalsäure konnte leicht zur Hexahydroisophtalsäure hydriert werden, während die Terephtalsäure allen damals bekannten Hydrierungsmethoden widerstand und 6 Wasserstoffatome erst aufnahm, als Skitas Verfahren angewandt wurde[35]. Komppa hat auch die den bicyclischen Ketonen, LX und LXI, entsprechenden, gesättigten bicyclischen Kohlenwasserstoffe dargestellt.

Bei der Synthese bicyclischer Ketone führt die Abspaltung von Kohlendioxyd und Wasser aus Homocyclopentan- bzw. -hexandicarbonsäuren zum Ziele. Bei dieser Reaktion hat Komppa[36] gezeigt, dass die beste Ausbeute manchmal mit Magnesium- oder Calciumsalzen erzielt wird, während in anderen Fällen nur Bleisalze anwendbar sind.

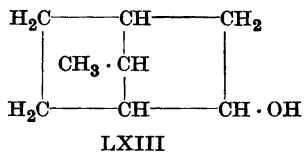
Nachdem die Diels- und Alderssche Diensynthese in vielen Fällen die Darstellung bicyclischer Verbindungen vereinfacht hat, hat Komppa dieselbe für die Synthese von 3-Methylbicyclo-[1,2,2]-heptanol, LXII, von ihm Apocamphenilol benannt, sowie der stereoisomeren 7-Methylbicycloheptanole, LXIII und LXIV, von ihm Aposantenol und Apoisosantenol[37] benannt, angewendet.

In Bezug auf die entsprechenden Ketone konstatierte Komppa, dass Apocamphenilon chemisch ebenso resistent wie Camphenilon und Fenchon ist, sowie dass die Aposantenole leicht zu Aposanten- und Apoisosantensäure oxydierbar sind.

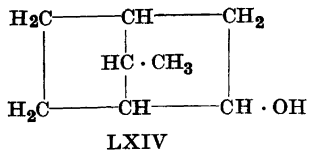
Alders und Rickerts[38] Diensynthese ermöglichte es, genügende Mengen von Norcamphern für eine eingehendere Untersuchung dieser Derivate zu erhalten. Es wurden sowohl α- als β-Norborneol[39] dargestellt, wobei es sich erwies, dass sie hinsichtlich der Wasserabspaltung dem Borneol bzw. Isoborneol entsprechen. Durch Überführung des β-Norborneols zum Chlorid und dessen Reduktion zum entsprechenden gesättigten, bicyclischen Kohlenwasserstoff war Komppa zum wirklichen Grundkohlenwasserstoff aller natürlichen Campherarten vorgedrungen, der in Beil-



LXII

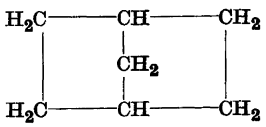


LXIII



LXIV

steins Handbuch mit Norcamphan bezeichnet wird, für den aber Komppa den Namen Norbornylan LXV vorschlägt.



LXV

Dieser symmetrisch gebaute Kohlenwasserstoff besitzt den Schmp. 87° (zugeschmolzenes Rohr), einen betäubenden Geruch, grosse Flüchtigkeit sowie vollständige Resistenz gegen Permanganat.

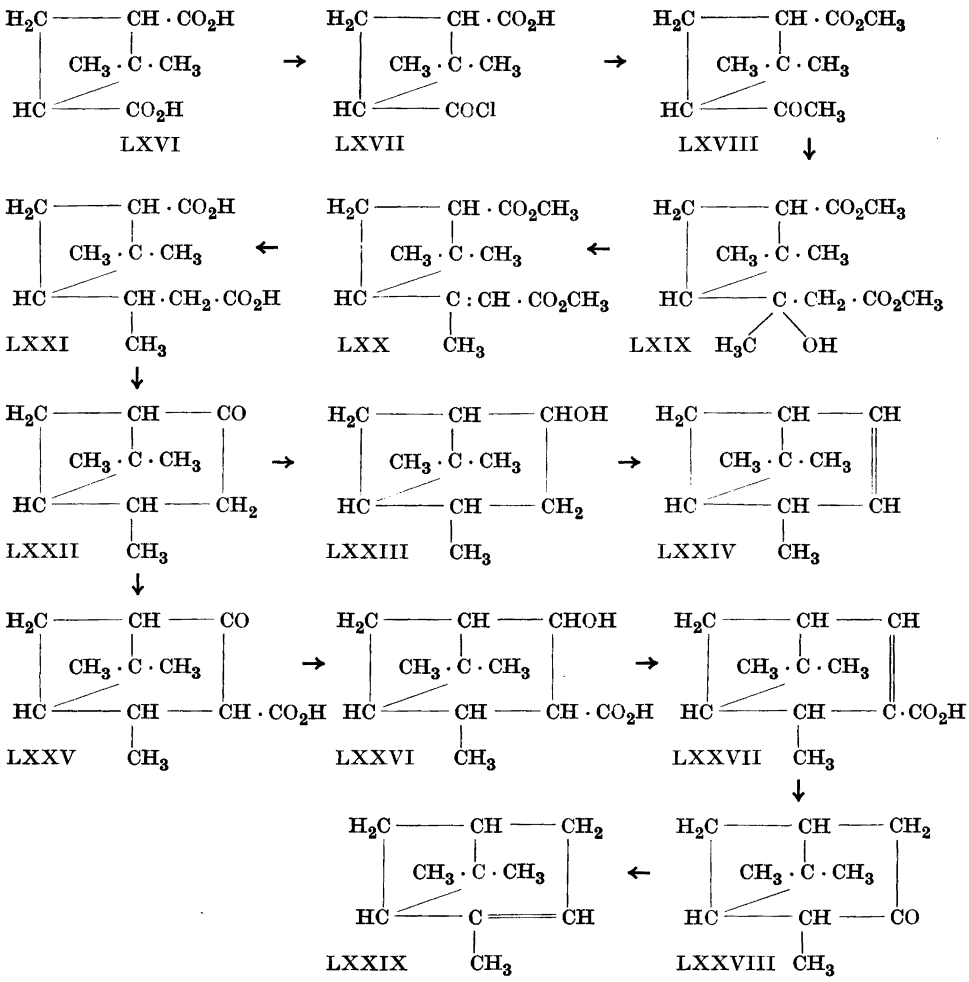
In einem Vortrag[40], den Komppa vor der Gesellschaft Finnischer Chemiker am 21. April 1942 über die Arbeiten hielt, die er nach seinem 60. Geburtstag aus- bzw. zuendegeführt hat, gestand er, dass ihm die Pinensynthese fast vier Jahrzehnte im Sinn gestanden hat. Ohne auf einige misslungene Synthesen näher einzugehen, lässt sich sagen, dass das Misslingen derselben teils auf die schwierige Entstehung von Vierringen überhaupt, teils auf die grosse Neigung des bicyclischen Systems [1,1,3] zu Umlagerung und Polymerisation zurückzuführen ist. Die Reaktionsfolge, die schliesslich zum Ziele führte, besteht in einer Synthese des Verbanons, LXXII, aus dem einerseits durch Reduktion und Wasserabspaltung δ-Pinen, LXXIV, andererseits Pinocamphon, LXXVIII, dargestellt wird[41]. Die letztgenannte Synthese komplettiert Ruzickas[42] schon 1920 ausgeführte partielle Synthese des α-Pinens, LXXIX, zu einer totalen. Komppa führte dann das α-Pinen durch Kochen mit Selendioxyd in β-Pinen über.

Ausgangssubstanz der Verbanonsynthese war die von Kerr synthetisierte Norpinsäure, LXVI, die über das Anhydrid in das Esterchlorid, LXVII, übergeführt wurde, welches nach Blaise mit Zinkmethyljodid den mit Pinonsäureester identischen Ketosäureester, LXVIII, gab. Dessen Kondensation nach Reformatzky mit Zn und Bromessigsäureester ergab Oxyisohomopinocamphersäureester, LXIX, aus dem mit Thionylchlorid ein Dehydrosäureester, LXX, entstand. Die katalytische Hydrierung des Letztgenannten gab die entsprechende gesättigte Säure Isohomopinocamphersäure, LXXI, die durch Trockendestillation ihres Bleisalzes in d,l-Verbanon, LXXII, übergeführt werden konnte. Die δ-Pinensynthese verläuft über Verbanol, LXXIII, zum δ-Pinen LXXIV. Die Pinocamphonsynthese[43] hat mehrere Zwischenstadien und wurde so ausgeführt, dass das synthetische Verbanon mittels Natriumamid und Kohlendioxyd in Verbanoncarbonsäure, LXXV, übergeführt und diese elektrolytisch zu Verbanolcarbonsäure, LXXVI, reduziert wird. Aus dieser Oxycarbonsäure wurde mittels Essigsäureanhydrids Wasser abgespaltet unter Bildung von δ-Pinencarbonsäure, LXXVII, die in Form von Säurechlorid mittels des Naegeli-Curtiusschen Abbauverfahrens in das Pinocamphon, LXXVIII, umgewandelt wird.

Gleichzeitig mit diesen letzten Synthesen führte Komppa Laboratoriumsversuche aus, betreffend die Druckhydrierung von Torf und Teerölen, deren Resultate nur teilweise veröffentlicht worden sind. Komppas Forscherwerk ist in 160 Veröffentlichungen niedergelegt, von denen der grösste Teil dem Campher- und Terpengebiet zugehört.

Es sind nicht nur die Synthesen der Camphersäuren, die schon frühzeitig Komppa internationalen Ruf schafften, sondern zahlreiche Totalsynthesen von Terpenen, Terpenkohlenwasserstoffen und anderen, mit diesen zusammenhängenden Verbindungen, die beweisen dass er die synthetischen Methoden der organischen Chemie suverän beherrschte.

Obwohl Komppas Synthesen mit der Fantasie eines schaffenden Künstlers geplant waren, wurden sie mit grosser wissenschaftlicher Präzision, mit systemati-



schem Blick und unter strenger Kontrolle aller Resultate ausgeführt. Der Stil in Komppas Veröffentlichungen ist oft knapp aber stets konzis. Mit nie versiegender finnischer Zähigkeit und Energie führte Komppa seine Synthesen trotz aller Hindernisse und Schwierigkeiten zu dem von ihm angestrebten Ziel.

Durch Gust. Komppas Ableben verlor Finnland einen in der ganzen Welt bekannten Forscher und die Chemie unseres Landes ihren »grand old man».

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John Palmén.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1171–1184

The Effect of Anionic Detergents on Collagens of Mammals and Teleostei

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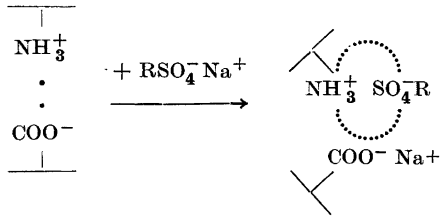
By treating globular proteins with solutions of anionic detergents, as sodium dodecyl sulphate (= SDS) or dodecylbenzene sulphonate (= DBS), the proteins are denatured [1] and acquire fiber forming properties [2]. This effect of anionic agents is of great theoretical interest and also of some technical importance. Recent reviews of Putnam [3] on the theory of the reaction and of Lundgren [2] on the problem of fiber formation give excellent expositions of this field and complete literature references.

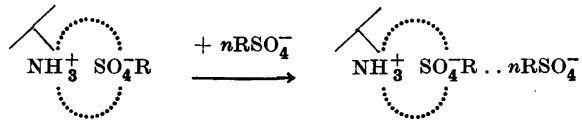
In the denaturation and unfolding of the chains of globular proteins by means of anionic detergents, the primary reaction is apparently the interaction of the anions with the cationic protein groups [4]. This attachment leads to a weakening or partial breaking of the saltlinks. In micellar systems containing a large excess of detergent over the equivalent of basic protein groups, a secondary type of reaction probably is also involved, considered by some investigators [5, 7] to be directed towards the peptide groups, *i. e.* the imide nitrogen which consequently should result in rupture of hydrogen bonds. Other investigators, notably Lundgren [6], believe that forces of the type which bind detergent ions into micelles are responsible for the secondary attachment of the amount of detergent in excess of the equivalent of ionic protein groups.

The first reaction (electrovalent) of the anionic detergent may according to Lundgren [6] be schematically illustrated as follows:



The rather long chains of the organic anion tend to wedge apart the chains; thereby impairing the strength of the saltlike crosslinks of the polypeptide chains.

The second type of reaction (non-electrostatic) may be represented by the schematic structure given by Lundgren [6]:



The unfolded protein chains with laterally attached anions of the detergent will thus build up other layers of negative charge (micelles) which tend to repel groups of similar charge on adjacent protein chains and hence, to labilize the protein structure (rupture of hydrogen bonds). It has been indicated analytically that the primary reaction is directed towards the basic protein groups; one molecule of detergent being fixed by each of the basic groups of the protein. Some investigators, *e. g.*, Pankhurst and Smith [7] consider that the maximum binding occurs when each nitrogen atom of the protein (gelatin) has combined with one molecule of dodecyl sulphate (2.88 g SDS is then fixed by 1 g gelatin). However, it has been amply demonstrated by Lundgren [6] that by extraction of the protein-detergent compound with 60 % aqueous acetone in the absence of salt, the ionically bound detergent not being removed, the composition of the final product will approximate the limiting proportion of one detergent anion for each equivalent of the basic groups of the protein. It is interesting to note that in the presence of salt the compound is completely split up in its constituents, probably due to ionic exchange [6]. According to evidences at hand, two factors are requisite for interaction: 1) the ionic group of the detergent. 2) its specific affinity which is related to the size and structure of the detergent, resulting in stabilization by the mutual affinity of the hydrocarbon portions of adjacently bound detergent ions [3].

The effect of detergents of the SDS and DBS types on collagen and related fibrous proteins has not been studied. It has been noted in a single experiment that the shrinkage temperature of collagen is considerably decreased by treatment in solutions of 1 *M* strength of SDS; the hydrothermal test being carried out in the SDS-solution [8]. Keratin resists the action of detergents; cleavage of the -S-S-bridge being required for any effect [9]. Of great interest is the finding of Steinhardt and Fugitt that pre-treatment of keratin with SDS facilitates its deamidation in acidic medium [5]. Silk is not reactive towards these reagents; a finding to be expected from our knowledge of its structure and low content of ionic groups.

In the denaturation of globular proteins, the chains are unfolded and brought nearer to the fibrous state, whereas the denaturation of collagen and other fiber proteins takes the reverse course towards the thermodynamically more probable, coiled configuration. The fibrous state is hence artificial and bound up with the presence of stabilizing crosslinks. It is to be expected that investigations of the effect of anionic detergents and related compounds on collagen will provide indication of the types of bonds which stabilize this protein and supply additional information on the molecular organization of fibrous proteins. Further, information may be forthcoming on the still unsettled problem of the nature of the combination of detergents with proteins, and the important question whether the disruption of the protein structure initiated by its combination with the detergent is completely reversible.

EXPERIMENTAL

Besides chemical pure preparation of SDS and DBS, a number of technical products ("fatty alcohol sulphonates") have been studied. The latter types consisted entirely of SDS or mixtures of homologues, containing SDS as the main component together with sulphates of C_8-C_{10}-compounds as well as those of $C_{14}-C_{16}$ chains. The technical products were employed: 1) as received and 2) after the removal of inorganic salts by extraction of the original products with ethanol. On the whole, the various preparations gave the same effect on collagen, compared on the basis of effective detergent; the alkylbenzene compounds being in some instances slightly more effective. The data given in the present paper pertain to the chemical pure SDS-compound. It is to be noted that the SDS-solutions used had pH values higher than those corresponding to the isoelectric point of the bovine collagen preparations. Hence, the anion affinity should be expected to be the driving force in systems on the alkaline side of the isoelectric point of the protein.

As collagen substrates, isoelectric calf skin pelt (limed and subsequently delimed), American standard hide powder and skins of cod (*Gadus morrhua*), only mechanically cleansed, were employed. Standard hide powder and pelt in the deaminated state were also used (method of Thomas and Foster [10]). The hide powder was denatured by 2 min. immersion in water of 70° C. The analytical methods were the standard procedures generally employed except for the determination of sulphur which was carried out according to Grote-Krekeler [11]. Further, in the determination of the shrinkage temperature (*Ts*) the freely suspended strips were introduced into water of a temperature 1—2° C below the indicated *Ts*. After 1 min. the bath was heated ("the shocking effect"). The pertinent analytical data of the collagen preparations employed are given in Table 1.

The isoelectric points of the pelt and hide powder were 5.5 and 5.2 respectively. Cod skin showed pH 7.0—7.5. The deaminated collagens were isoelectric at pH 4.0.

In comparative experiments solutions of sodium polymetaphosphate, with an average molecular weight of 7 600, determined by means of the end group method of Samuelson [12] were employed. The solutions used were adjusted to pH 2.0 (hydrochloric acid). The average molecular weight was after 4 h 6 800 and after 24 h 3 600. Since in the present instance more than 90 per cent of the total fixation of polymetaphosphate by colla-

Table 1. Composition of collagen preparations.

No.	Type	% N	% Ash	% S	HCl-binding capacity (in meq. HCl per g collagen)	*Ts* in °C
1	Pelt	18.0	0.1	(.6	0.92	67
2	Hide Powder	17.9	0.1	0.4	0.88	—
3	Deaminated pelt	17.6	0.3	0.6	0.64	66
4	Deaminated hide powder	17.5	0.2	0.4	0.60	—
5	Skin of cod	18.3	0.4	1.0	0.94	43
6	Heat-denatured hide powder	17.9	0.1	0.4	0.88	—

gen occurs in the first two h, the molecular weight of the fixed metaphosphate anion is probably not far from the average value of the sodium polymetaphosphate (7 600).

Further, β-naphthalene sulphonic acid of analytical grade was used in 0.2 *M* solution. The special technique of determining the degree of swelling of the collagen preparations will be described in the experimental part.

RESULTS AND DISCUSSION

a. The effect of SDS on various types of collagen

The effect of the concentration of solutions of SDS on their dissolution of various preparations and types of collagens of skin is shown in Fig. 1. In these series, 2.0 g collagen was treated for 96 h in 50 ml of the SDS-solutions at final pH 6.4—6.8 (covered with toluene), containing increasing amounts of SDS. In the concentrations of SDS employed (> 0.1 %), micelle formation is prominent. See ref. 3.

Evidently, mammalian collagen in the form of skin, both native and alkali-pretreated (limed) is not solubilized by SDS, even in the most concentrated solutions. A small quantity of the heat-denatured skin is brought into solution. Hide powder which is finely ground hide, thoroughly limed and then delimed, is solubilized to a marked extent by the concentrated solutions and by denaturation this tendency is greatly enhanced. Most interesting is the finding that native cod skin collagen is rather completely brought into solution by moderately concentrated solutions of SDS. This is not surprising in view of the established fact [12] of the low degree of intermolecular stabilization of collagen of *teleostei* compared to the mammalian type, as shown by its low degree of hydrothermal stability (shrinkage temperature), its great susceptibility to proteinases, acids and alkalis, its greater reactivity for coordination-active tanning agents [13] and the destructive effect of certain sulpho acids on its macro

structure [14]. The different behaviour of fish skin collagen compared to bovine skin collagen does not appear to be a problem of different amino acid composition. The recently reported, complete analysis of fish skin collagen [15] shows practically the same contents of the polar and non-polar amino acids forming the main part of the collagens; the only difference being the greater percentages of the rarer amino acids (serine, threonine and methionine) in the collagen of *teleostei*. The possibility of different sequence of residues and the presence of polypeptide units of different composition must be left open as an explanation. The accumulated evidences appear to be best explained on the basis of molecular organization; macro-structural details as revealed histologically probably also being involved.

The following working hypothesis of the structural organization appears reasonable. The mammalian skin collagen, with its intertwining fiber bundles is mainly stabilized by means of hydrogen bridges between oppositely located -CO-NH-groups; salt links of electrostatic nature supplementing the primary forces. In fish skin collagen, with parallelly grouped layers without any marked interlacing of fiber bundles, on the other hand, the low degree of stabilization of the structure is satisfactorily accounted for by the assumption that the hydrogen bonding is only weakly developed; electrovalent crosslinking on polar protein groups being responsible for the main part of the structural cohesion. The present finding of the extraordinary behaviour of this type of collagen is in harmony with such an explanation.

b. Reversible effects on collagen

Although bovine collagen is not, or only slightly so, attacked by SDS to an extent leading to solubilization, it undergoes certain changes in the treatment. Thus, it is markedly swelled and its hydrothermal stability is greatly impaired by its combination with the SDS, as shown by the considerable Ts decrease of the pelt taken *directly* from the SDS-solution. Also certain *irreversible* changes are imparted to the protein as shown by a permanent, lightly increased degree of swelling and a small permanent lowering of the *Ts* of the skin after the removal of the SDS in the subsequent washing. The following experiments illustrate these points.

10 g collagen, as calf skin pelt, was treated for 120 h in 100 ml of 5.0 % solution of SDS and further, the same quantity of protein in the form of hide powder in 150 ml of this solution. The hide powder swelled greatly and imbibed the solution almost completely. The substrate was separated from the solution by suction filtering under identical experimental conditions. From the total weight of the moist stock and analysis of part of the treated specimens for contents of dry substance and collagen, the amount of water

sorbed by the protein was ascertained. The degree of swelling or rather the degree of imbibition of water, is expressed in g water held by 1 g collagen. No determination of sorbed SDS was attempted, since such an evaluation appears meaningless under the present conditions. The residual solution was analyzed for total nitrogen and ammonia. No ammonia was found (no deamidation). The rest of the treated stock was extracted with 200 ml 60 % acetone for 2 h, three consecutive times. A part of this stock was dried and analyzed for collagen, sulphur and ash. The rest was washed further for 48 h in several changes of water (500 ml) in order to remove watersoluble matter. The *Ts* of the skin after the various treatments were determined. (*Ts* of original skin: 67° C) Tables 2 and 3 contain the data.

Table 2. Composition of substrates taken directly from the SDS-solution.

Substrate	Final pH	% Dissolved collagen	Degree of swelling	*Ts*	*ΔTs*
Hide powder	6.4	8.3	10.5	—	—
Calf skin pelt	6.6	0.9	2.8	52	− 15

The original skin contained 1.8 g water per g collagen and the treated skin after acetone extraction and subsequent hydration 2.0; the shrinkage temperature of the skin was then 65° C or 2° C lower than the *Ts* of the original skin. Hence, it is evident that the interaction of SDS with collagen brings about certain irreversible alterations of the protein. The very large *Ts* decrease, 15° C, induced by the association of SDS with collagen, determined on pelt directly from the treating bath, is probably due to the severance of saltlinks and a partial rupture of hydrogen bonds by the swelling induced by the association of anions of the detergent on the ionic protein groups, wedging them apart. The maximal disrupture of the collagen structure is generally approached in solutions containing one equivalent detergent on each equivalent of cationic protein groups or at a detergent/protein weight ratio of 0.25 (See, *e. g.*, Fig. 1). Hence, the peptide group are apparently not involved *directly* in the attachment of the SDS present in excess of the stoichiometric equivalent. The secondary effect of the additional swelling is probably caused by the electrostatic repulsion of the micellar layers as suggested by Lundgren [6].

Table 3. Composition of SDS-treated specimens after acetone-extraction.

Substrate	% Collagen (N × 5.55)	% Ash	% Organic S	% Fixed SDS
Hide powder	99.8	0.9	0.5	0.
Calf skin pelt	100.8	0.5	0.6	0

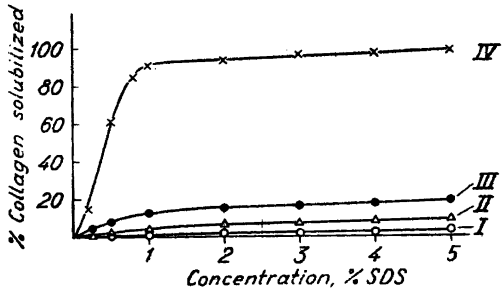


Fig. 1. The dispergation of collagen by SDS as a function of its concentration.

I = Calf skin pelt
II = Native hide powder (bovine)
III = Denatured hide powder (bovine)
IV = Collagen of cod skin

These data as well as those from a number of other series prove that no irreversible fixation of SDS by collagen takes place.

The previous data have shown that the increased degree of swelling and the lowering of the Ts of the pelt treated in moderately concentrated solutions of SDS are to some extent permanent. These changes do not affect the maximum acid binding capacity of collagen. The solubilization of the protein does not result in splitting of the protein and no deamidation takes place by the action of SDS. Hydrolysis of amide groups, however, is induced by 0.2 *M* solution of β-naphthalene sulphonic acid at pH 1.3 – 1.5.

It is illuminating in this connection to remind of an earlier finding [16] that heat denaturation of bovine collagen does not increase the binding capacity of the protein for mineral acid. However, it is important to note that denaturation leads to greater uptake of mineral acid (HCl) in the pH range 2.5 to 6, which has been explained as being due to weakening of the attraction between oppositely charged protein groups by the increased distance between these groups, forming the salt link, expected to result from the folding of the protein chains in the shrinkage. It was also shown that the main valency forces of collagen affected in the denaturation were the coordinate ones (rupture of hydrogen bonds); also a steric effect caused by the unfavourable spatial conditions created [16]. Comparing the titration curves of pelt in its original state and after SDS-treatment (washed), the latter showed on the whole a slightly greater fixation of acid in the pH range 2.5 – 6, although the values were quite irregular and hence are not included.

c. Irreversible effects on collagen

The final proof of the permanent alterations of collagen by the SDS-treatment is supplied by determination of the effect of this pretreatment on the reactivity of collagen towards tanning agents, reacting: 1) entirely with *ionic valency forces* and 2) to a large extent by means of *coordinate valency forces*, involving the groups serving as *loci* for the hydrogen bridges, primarily —CO—NH—. This method was also employed in the investigation of the mechanism of the heat denaturation of collagen [16].

The following chromium compounds were employed. The first type, mainly reacting ionically with collagen, contained only cationic chromium complexes: the 33 % basic chromic sulphate with its composition corresponding to the empirical formula:

$Cr_2(OH)_2(SO_4)_2 \cdot Na_2SO_4$ and the corresponding chromic chloride represented by the formula: $Cr_2(OH_2)Cl_4 \cdot 2NaCl$. The second type contained cationic as well as non-cationic (mainly uncharged) complexes of higher degree of aggregation than the first type. The interaction with collagen is in this instance governed by the ionic as well as by the co-ordinate potency of collagen: the 60 % basic chromic sulphate (made from the 33 % basic compound by soda-addition) and the 70 % basic chromic chloride, containing 80 % of its chromium in the form of uncharged complexes [17]. Finally a special complex chromium salt, the sulphito-sulphato-chromiate, mainly containing negatively charged complexes was studied [18].

Portions of 2.0 g collagen in the form of pelt and hide powder, both untreated and treated for 120 h in a 10 % solution of SDS, subsequently washed, were shaken for 96 h in 50 ml portions of solutions of the chromium compounds, containing 15 g/l Cr. The chromed stock was freed from uncombined electrolytes and analyzed for protein N and chromium (Table 4).

Table 4. Effect of pretreatment of collagen with 10 % solution of SDS on its affinity for chromium compounds.

Substrate	% Cr fixed by 1 g collagen from: 33 % basic Cr-sulphate	33 % basic Cr-chloride	60 % basic Cr-sulphate	70 % basic Cr-chloride	Sulphito sulphato chromiate
Hide powder	7.5	5.8	12.7	9.6	19.6
SDS-Hide powder	7.8	6.0	15.8	11.9	23.1
Calf skin pelt	7.6	5.2	10.0	6.8	—
SDS-Calf skin pelt	7.5	5.2	11.2	7.3	—

The following points are noteworthy. The treatment of collagen with SDS does only slightly affect its ionic groups as evident from the fact that the fixation of cationic chromium is not materially altered. Those agents which for their reaction with collagen require coordinate bonds and which reaction is governed by the state of the peptide groups (compensated or uncompensated intermolecularly) give considerably higher values of fixed chromium in the instance of the SDS-pretreated collagen. It is thus indicated that the main permanent changes of collagen caused by the treatment with the anionic detergent involve the coordination active groups (rupture of hydrogen bonds, indirectly by swelling).

It should also be of interest to ascertain the effect of SDS on deaminated collagen. The results of series with regular and deaminated hide powder and calf skin in the intact state and after 120 h treatment in 10 % solution of SDS (subsequently acetone and water extracted) are given in Table 5.

Table 5. Interaction of SDS with intact and deaminated collagen.

Substrate	% Collagen dissolved	Degree of swelling	Δ_0 Ts Directly	After acetone wash	After further water wash
Blank. Hide powder	9.6	9.8	—	—	—
Deaminated hide powder	1.7	4.9	—	—	—
Blank. Calf skin pelt	0.7	2.2	— 18	— 3	— 2
Deaminated calf skin pelt	0.5	2.3	— 18	— 16	— 16
Cod skin	96	—	—	destroyed	

In tanning experiments, employing deaminated hide powder in the intact state and after SDS-treatment (washed) as the substrates for the 70 % basic chromic chloride, the SDS-treated specimen showed about 30 % higher chrome fixation than the blank (untreated), proving a marked activation of the coordination *loci* of deamino-collagen as a permanent result of its interaction with the anionic detergent. This is in line with the data of the Ts of Table 5, showing that the impairment of the hydrothermal stability of deamino-collagen by SDS is *not* reversible whereas the Ts-decreasing effect of SDS on the intact skin collagen is to the largest part reversible.

d. Comparative data on the effect of other anionic agents

For a safer basis of discussion, some additional data from experiments with systems of various types of collagen and some other anionic agents will be briefly considered. The agents selected were β-naphthalene sulphonic acid (= NS) and polymetaphosphoric acid (= PMP), reacting with collagen under pH-conditions ensuring maximum fixation of these agents.

Some main points may be stressed. The strong NS is fixed by collagen entirely through its ionic groups; coordination being eliminated as a factor. The anion combines stoichiometrically with the basic groups; the combining equivalent being 1.0 meq. per g collagen (at final pH 1.3—1.4). Such solutions of NS have no swelling effect on collagen [19], which fact may be explained by the partly irreversible attachment of the anion on the cationic protein groups No Donnan effect is produced since the condition of a completely reversible system is not fulfilled. Complete inactivation of the ionic protein groups by means of NS, *i. e.*, breaking of salt links, will hence measure the extent to which the salt links contribute to the intermolecular stabilization [20]. It should also be noticed that NS is an *unifunctional* anionic agent.

The strong acid formed by acidifying a solution of sodium polymetaphosphate, 2.0 % solutions of pH values of 1.8—2.0 being employed in the present instance, is indicated to react as a *polyfunctional* electrolyte with proteins; its large anion being irreversibly fixed by the basic protein groups [21]. Also in this instance, the requirements for establishing of a Donnan effect are absent. The skin does not swell. Rather, it is dehydrated.

The results of the NS series are given in Table 6. 2.0 g portions of protein in the form of cod skin and calf skin pelt were treated two consecutive times, 24 h each time, in 50 ml. 0.2 *M* solution of NS. The experimental findings from the corresponding series of 2.0 % solutions of PMP, with pH values adjusted to pH 2.0, are contained in Table 7. Time: 24 h.

Table 6. Collagen-β-naphthalene sulphonic acid, 0.2 M.

Substrate	meq. acid fixed by 1 g collagen	*Δ Ts*	% collagen dissolved	% relative decrease of strength of treated skin
Calf skin	0.98	− 18	0.6	0
Cod skin	1.00	destroyed	1.2	90

Table 7. Collagen-polymetaphosphoric acid, pH 2.0.

Substrate	meq. acid fixed by 1 g collagen	*Δ Ts*	Degree of swelling	% collagen dissolved	% relative decrease of strength of treated skin
Calf skin	0.94	+ 1	1.6	0.4	10
Cod skin	0.96	± 0	1.8	3.2	90

The following points need to be accentuated. By complete inactivation of the cationic groups of bovine collagen by means of NS, the Ts is decreased 18° C, which figure may serve for evaluation of the degree of protein stabilization due to electrovalent crosslinks (saltlinks), since secondary effects on the other type of crosslinks (hydrogen bridges) are absent (no swelling). The character and mechanical strength of mammalian skin are not adversely affected; indicating non-ionic cohesive forces to be mainly responsible for the structural rigidity. Cod skin is not solubilized by NS. However, its tensile strength and structural features are completely lost. This behaviour fits in well with the conclusion drawn from other findings [13, 14] that the forces responsible for the stability of collagen of *teleostei* are mainly saltlinks; hydrogen bridges on —CO—NH-groups not being prominent. Cod skin is not disinte-

grated and dissolved by NS since electrostatic repulsion is lacking and also swelling. With SDS the secondary effect of the electrostatic forces promotes swelling and practically complete dispergation of fish collagen, as would be expected from the reaction mechanism postulated by Lundgren [2, 6].

The mechanism of the reaction of the PMP with the two types of collagen does also illuminate the present problem. The hydrothermal stability is not lowered, rather slightly elevated in both instances. The Ts-decrease of 18° C expected from complete rupture of the salt links by the irreversible attachment of an unifunctional agent to the cationic protein groups is not shown. Since the average molecular weight of the PMP was 7 600, about 75 PO_3-units are present in each chain. Evidently, the multifunctional PMP-anion reacts with adjacent protein chains under crosslinking. The extent of this stabilization seems about to outweigh the labilizing effect due to the breaking of the salt links of the intact collagen.

The mechanical strength of the cod skin was practically destroyed by the fixation of PMP. The strength of bovine skin was slightly lowered. Evidently, different cohesive forces control the hydrothermal stability and the mechanical strength of the collagen structures. It is indicated that the former involves the strength of the crosslinks of intramicellar units, whereas the latter is a function of the cohesive forces between the larger units (fibrils and fibers) [22]. Any marked solubilizing effect of PMP on cod skin is not shown in spite of its structural weakening. Since dehydration of collagen is effected by PMP, this fact is not surprising.

CONCLUSIONS

The results of this investigation bear upon two different aspects of protein behaviour: 1) The nature of the interaction of collagen and related proteins with anionic detergents and 2) The nature of the stabilizing forces of collagens of mammals and fishes.

It is demonstrated that the maximum dispergation of cod skin collagen, nearly complete, takes place by adsorption of SDS by collagen in amounts sufficient to allow complete interaction with the basic protein groups, or one tenth of the amount required for complete interaction of all nitrogen atoms of collagen with the detergent. Also, the degree of maximum swelling of bovine skin is approached under the conditions mentioned. These findings lend support to the view advanced by Lundgren for the explanation of the reaction mechanism of anionic detergents with globular proteins. The alterations of collagen, due to the swelling as a secondary effect of the association of SDS-anions on the cationic protein groups and the temporary destruction of the

crosslinks are to some extent permanent which is proved by the increased binding capacity of the SDS-treated collagen for agents involving coordinate valency for their reaction with proteins. The anionic detergents of the type of SDS are unifunctional, reducing the strength of the saltlinks and hence, the internal stability of the protein structure.

The interaction of fish skin with the unifunctional NS, partly irreversibly fixed and not possessing swelling power, leads to complete destruction of the polar links. However, hardly any collagen is solubilized since swelling and disorganization of the structure are not produced. Nevertheless, the mechanical strength as well as the hydrothermal stability of the collagen of *teleostei* are lost. It is pertinent to point out that solutions of hydrochloric acid of final pH values of 1.3—1.5, bring about complete discharge of the carboxyl ions of collagen, and hence a complete destruction of the saltlinks. Being a completely reversible system, the Donnan effect enters and the maximum degree of swelling is produced. In the case of cod skin, this will result in a nearly complete solubilization of collagen. In the presence of a swelling depressing neutral salt, for example sodium chloride, the acid does not dissolve fish collagen to any great extent and the Ts is unchanged or slightly elevated; proving the governing importance of swelling phenomena.

The multifunctional polymetaphosphoric acid, entirely ionic in its reaction, does not impair the heat resistance of the fish collagen. However, its tensile strength is practically lost. Since no swelling occurs, the collagen of fish skin is not turned into watersoluble products. These findings are satisfactorily explained by the supposition that *the hydrothermal stability of collagen is governed by the strength of the crosslinks of the ultimate protein units, whereas the mechanical strength concerns the forces between larger units* [22].

The various findings reported point to the importance of the anion affinity and the secondary effect of the anionic agents on the swelling of the protein. SDS is evidently such a strong competing agent, that it is capable of breaking up the internal interactions between cationic nitrogens and the other functional groups of the protein [23].

The diversity of properties and behaviour of bovine and fish collagens is probably due to different internal linking of protein chains and micellar units.

SUMMARY

The interaction of anionic detergents of the type of sodium dodecyl sulphate, in solutions of 5 % strength (micelles), with collagen of bovine skin results in: a) lowered shrinkage temperature, b) increased swelling and c) a minor solubilization. No SDS is irreversibly fixed. Permanent alteration of

the treated skin is shown by slightly lowered Ts and increased coordinate reactivity. The disruption of the protein initiated by its combination with the detergent is hence only partly reversible.

Cod skin collagen is largely solubilized. The different behaviour of the two types of collagen is in harmony with the view that mammalian collagen is stabilized by crosslinks on the peptide groups of adjacent chains (hydrogen bonds), supplemented by salt links. The latter type of cohesive forces contributes to the main organization and stability of collagen of *teleostei*.

Comparative experiments with naphthalene sulphonic acid and polymetaphosphoric acid give additional information on the organization of these collagens and the mechanism of the reaction between anionic detergents and collagens. The general concept advanced by Lundgren for the protein-detergent systems gives a satisfactory explanation of the findings.

It is indicated that the hydrothermal stability of collagen is a function of the degree of intermolecular linking of micellar units, whereas the mechanical properties are mainly governed by the cohesion between larger units, as fibrils and fibers.

A research grant from *Statens Tekniska Forskningsråd* is gratefully acknowledged.

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Received July 17, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1185–1191

Partition Chromatography of Adrenaline and Noradrenaline

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Since *nor*adrenaline has been shown to occur generally in animal organs and tissues together with adrenaline [1-8] it became desirable to work out some chemical methods to identify and separate the substances from each other and from other catechol derivatives which may occur in the tissues. Using the paper partition chromatography method of Consden, Gordon and Martin [9], James [10] demonstrated a separation of adrenaline, *nor*adrenaline and methyladrenaline with water-saturated phenol as a solvent. Euler and Hamberg [4] demonstrated *nor*adrenaline and adrenaline in suprarenal extracts of cattle, using *n*-butanol saturated with *N* HCl as mobile phase. Goldenberg *et al.*[5], Holton [3] and Engel and Euler [8] found the two catechol derivatives in paper chromatograms of adrenal medullary 'tumour extracts.

This report deals with the results of the chromatographic procedure on paper with *n*-butanol/*N* HCl as a solvent and its application on column chromatography with the purpose of separating and identifying various catechol derivatives.

EXPERIMENTAL

Paper partition chromatography

The paper used for the chromatograms was Grycksbo OB and the apparatus the one described by Consden, Gordon and Martin [9] for small paper strips of 9 × 48 cm. A bigger trough for whole filter papers of 48 × 48 cm was made after the same principles. As solvent *n*-butanol saturated with *N* HCl was used. The butanol used was *n*-butanol purum, which, when compared with the *n*-butanol pro analysi gave the same results. The temperature during the runs has been kept at 23—26° C, the papers having been dried before

at the same temperature. The resolving capacity of some other solvents has been tried. *n*-Butanol/H_2O, pyridine, *n*-amyl alcohol/0.5 *N* HCl did not give any separation of the catechols. With *n*-butanol/*N* HCl a good separation of adrenaline and *nor*adrenaline was obtained at 20—24 h in descending runs, allowing in longer runs the separation of other related compounds in addition, such as DOPA and hydroxytyramine. Addition of acetic acid shortened the separation time but tended to spread the spots. About 30 hours ascending run gave small distinct spots with *n*-butanol/*N* HCl at about 25° C, and the necessary amounts of substance could be somewhat smaller than in a descending run.

The adrenaline and *nor*adrenaline used in the experiments was pure synthetic adrenaline (Rhône-Poulenc), *dl-nor*adrenaline and *l-nor*adrenaline hydrochloride (Winthrop). The substances were dissolved in 0.1 *N* HCl and H_2O to give solutions of 5 mg pr ml and were placed on the paper with small capillary tubes to give spots of about 2—4 mm diameter. The amounts used for each spot were 5—10 μg. Hydroxytyramine and dihydroxyphenylalanine (DOPA) were used in some experiments.

When using *n*-butanol/*N* HCl it was not possible to calculate the common *R*-values because the solvent front will disappear at the developing time used. After drying for 20—30 min. at 60—80° C the papers were sprayed with a weak potassium ferricyanide solution of pH 7.7 (James [10]), which indicates the catechols by oxidizing them to coloured quinone compounds. Adrenaline gives a bright red spot and can be clearly distinguished from the bluish red spot given by *nor*adrenaline. The position of adrenaline and *nor*adrenaline are shown in Fig. 1 together with hydroxytyramine and DOPA. The latter two substances also give differently coloured spots as reported by Goldenberg *et al.*[5] with phenol as solvent. By using ninhydrin as an indicator DOPA may be distinguished from the other catechols.

Extracts of animal tissues

The extracts used in the chromatographic analysis have been prepared either by adsorption on alumina[11], or with acidulated alcohol, and suitably concentrated. The amount of catechols present in the extract was determined colorimetrically by the method of Euler and Hamberg [12], or biologically (Euler [1]). By laking the "spots" before developing and biological assay of the extracts it has been possible to verify the identity of the separated compounds. Press juice from the suprarenals may be used directly owing to its high catechol content.

If salts are present the extract may be purified by extraction with butanol in the same way as described later for the column chromatography. Ascorbic

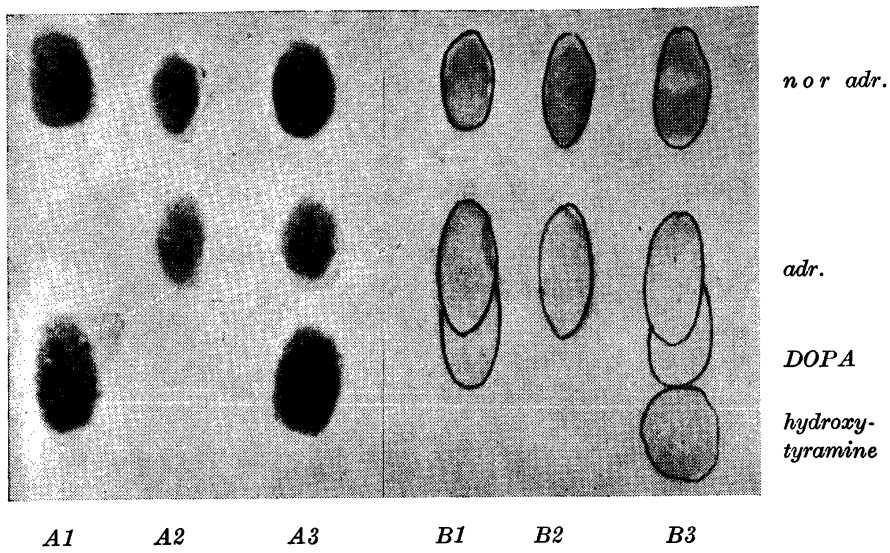

Fig. 1. Paper chromatogram showing A1 separation of n o r adrenaline and hydroxytyramine, A2 n o r adrenaline and adrenaline from suprarenal extract of cattle, A3 n o r adrenaline, adrenaline and hydroxytyramine, B1 n o r adrenaline, adrenaline and DOPA, B2 n o r adrenaline and adrenaline, B3 n o r adrenaline, adrenaline, DOPA and hydroxytyramine, n-butanol/NHCl being used as solvent. Descending run 24 hours at 25° C.

acid present in animal extracts is also adsorbed on alumina and gives spots after developing with potassium ferricyanide. The runs of animal extracts were generally made with three comparative spots: 1) the extract to be analyzed. 2) the comparating substances. 3) 1 + 2. Fig. 1 illustrates also the separation of adrenaline and *nor*adrenaline from cattle suprarenal extract.

Partition chromatography on starch column

Starch (Elsden and Synge [13]) has been used as support for the partition chromatography of adrenaline and *nor*adrenaline in the present series of experiments.

Method

The apparatus used was the one introduced by Edman [14] somewhat modified after the principles given by Moore and Stein [15]. A separatory funnel of 500 ml was connected with a stopper to a column of about 50 cm length and about 2 cm diameter containing a sintered glass filter (Pyrex X). Air pres-

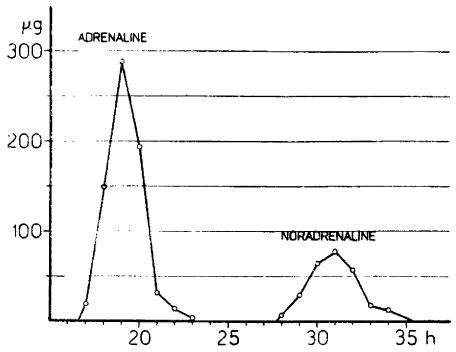

Fig. 2. Partition chromatogram of adrenaline and n o r adrenaline obtained from pure solutions on starch. R adrenaline = 0.544, R n o r adrenaline = 0.350. Amounts added 750 µg adrenaline and 250 µg n o r adrenaline (Exp. IV in Table 1).

sure, controlled by a Hg-manometer, was applied to the separatory funnel. To collect the fractions 24 bottles of 20 ml supplied with funnels (to reduce the evaporation) were placed on the round plate and automatically shifted every hour.

The starch used in the experiments was raw commercial starch which was purified from fats and alcohol-soluble matters by extracting one day in a Soxhlet with methanol. The starch was dried over night at + 60° whereafter it was sieved and kept in air.

The solvent used was *n*-butanol saturated with 0.1 *N* HCl to which one half of the volume 10 *N* HAc was added. Ascorbic acid was added in about 0.1 % to the solvent in order to protect the catechols from oxidation. The strongly acid solvent used in the paper chromatography was found to cause partial racemisation on evaporation of the extracts in vacuo, thereby reducing the biological activity of the catechols. Acetic acid was found useful in shortening the runs and in having a good resolving power.

60 g of starch was suspended in some solvent and pressed together in the column to a length of about 27 cm. Air pressure of 60—160 mm Hg was used to give fractions of about 6—10 ml per hour.

The separation of adrenaline and *nor*adrenaline was usually obtained in about 45 hours' run. The solution or extract to be analyzed, dissolved in 1—2 ml of the solvent, was pressed into the starch with a pressure of 80—160 mm Hg and followed by another 2 ml of the pure solvent. The column above the starch was filled with solvent until about 10 cm from the top. The fractionation was started immediately. The measured fractions were evaporated in a vacuum box at + 35° C. After dissolving the evaporated residue in 1—2 ml H_2O, which gave a pH of about 3, each fraction was tested colorimetrically after oxidation with iodine [4]. The fractions were also tested biologically on the cat's blood pressure and on the hen's rectal cæcum.

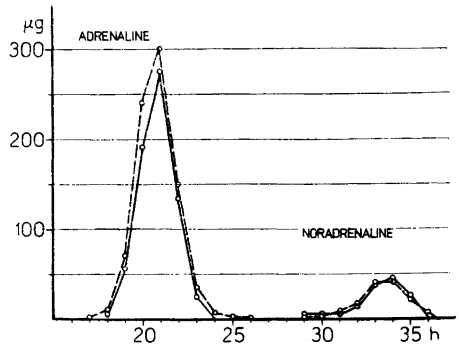

Fig. 3. Partition chromatogram of adrenaline and n o r adrenaline obtained from cattle suprarenal extract on starch. R adrenaline = 0.545, R n o r adrenaline = 0.350. Full line: colorimetrical estimation, dotted line biological assay on cat's blood pressure.

Separation of adrenaline and n o r adrenaline from pure solutions on starch.

The results of four experiments with varying amounts of adrenaline and *nor*adrenaline directly dissolved in the solvent are given in Table 1.

Table 1. Chromatographic separation of adrenaline and n o r adrenaline from pure solutions on starch column.

Expt.	µg added Adr. + *Nor*adr.	Recovered after separation µg		Percentage recovery	
		Adr.	*Nor*adr.	Adr.	*Nor*adr.
I.	400 + 500	287	389	72	80
II.	400 + 500	348	508	87	102
III.	750 + 250	634	196	84	72
IV.	750 + 250	698	265	93	106

Figures refer to the hydrochlorides. *l-nor*adrenaline was used in expts. I and II, *dl*-noradrenaline in expts. III and IV.

Fig. 2 shows a diagram of the separation.

The *R*-values calculated from expt. IV are for the synthetic hydrochlorides of adrenaline: 0.545 and *nor*adrenaline: 0.350. The results of the colorimetric assays were checked biologically and showed good agreement.

Extracts of cattle suprarenals on starch

The extracts used were prepared from fresh cattle suprarenals with acid alcohol to 5 g/ml. After colorimetric determination of the adrenaline and *nor*-adrenaline present, a suitable amount containing about 10 mg of catechols (20—30 % *nor*adrenaline) was mixed twice with *n*-butanol containing 0.1 m

N-HCl in 25 ml *n*-butanol and evaporated *in vacuo*, in order to remove water and precipitate the salts. The butanol extract thus obtained was shaken three times with 2 volumes of water which quantitatively transferred the catechols into the water phase. The butanol treatment generally entailed a loss of some 10 p. c.

The treatment with butanol did not induce any gross chemical changes of adrenaline and *nor*adrenaline as shown on the paper chromatogram. The butanol extract was filled up to 10 ml with butanol containing some drops of 0.1 *N* HCl. Of this extract 1 ml was used to the column. The extracts were run on the column in exactly the same way as the pure substances described above. The first few fractions contained some coloured matter but no catechols. The partition chromatogram obtained from cattle suprarenal extracts (expt. IV) is shown in Fig. 3. It was confirmed by colorimetrical and biological tests that the first curve represents the adrenaline and the second one the *nor*-adrenaline as indicated by the *R*-values. Moreover, the quantitative agreement between the colorimetrical estimation and the biological assay was quite satisfactory as illustrated by the Fig. 3. The total yield of adrenaline from the partition chromatograms of cattle suprarenal extracts was 83—90 p. c. of the amount added and of *nor*adrenaline 57—61 p. c.

Comment

Certain catechol derivatives such as DOPA do not easily separate from adrenaline with *n*-butanol/*N* HCl as solvent. The colour of DOPA after indicating the spots with potassium ferricyanide is not much different from adrenaline but is easy to separate from hydroxytyramine. After the drying procedure DOPA changes and gives a similar quinone colour as hydroxytyramine. In phenol the separation of the catechols gives another picture according to James [10], DOPA appearing between *nor*adrenaline and adrenaline. This difference is therefore of great use for the identification of DOPA if present in extracts. This has recently been achieved by Goodall [16] who found the characteristic spots of DOPA and hydroxytyramine with extracts of sheep suprarenals.

SUMMARY

1. With *n*-butanol/*N* hydrochloric acid as solvent adrenaline and *nor*-adrenaline and related compounds can be separated by paper chromatography.

2. By partition chromatography on starch column adrenaline and *nor*-adrenaline have been separated in cattle suprarenal extracts, *n*-butanol/0.1 *N* hydrochloric acid/acetic acid being used as solvent.

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Received July 10. 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1192–1196

On the Reactivity of 2,5- and 2,6-Dimethoxyquinone

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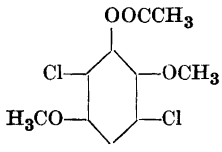
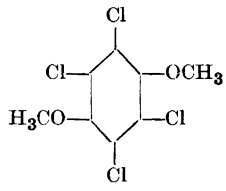
On the addition of hydrogen chloride to 4,4'-dimethoxydiquinone, Erdtman [1] obtained a substance with rather unexpected properties. Quinones generally yield stable monochloro quinols by this reaction, but in the case of 4,4[1]-dimethoxydiquinone the reaction was reversible and the substance, on attempted recrystallization, decomposed into the original materials. As mentioned in a previous paper [2], we have started a reinvestigation of the addition product.

The Thiele acetylation of 4,4'-dimethoxydiquinone is a very slow reaction [1]. Setting out from this fact, we have assumed as a hypothesis that the halogen quinols are stable when the corresponding quinones react easily with acid reagents, and vice versa. 2,5- and 2,6-dimethoxyquinone are quite stable towards both the Thiele reagent and hydrogen chloride. One might therefore expect that the corresponding monohalogen quinols would be very unstable compounds. In order to obtain the acetates of these substances we have treated the quinones with acetyl chloride and acetyl bromide.

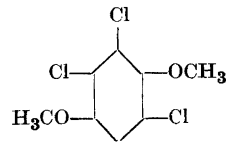
2,5-Dimethoxyquinone did not react with acetyl chloride alone. When sulfuric acid was added, however, the quinone was slowly dissolved. From the reaction mixture considerable amounts of a substance of m. p. 90—91° * could be isolated. This proved to be identical with the dimethoxydichlorophenol acetate which Oliverio [3] obtained by treating this quinone with acetyl chloride and aluminium chloride as a catalyst. Oliverio and Castelfranchi [4] have since shown that the substance is 2,5-dimethoxy-3,6-dichlorophenol acetate (I).



I



II



III

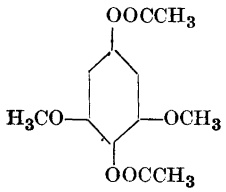
* All melting points uncorrected.

Oliverio also found tetrachloroquinol dimethyl ether (II) in a small amount in his reaction mixture. We have not been able to isolate this substance but could isolate small amounts of trichloroquinol dimethyl ether (III) from our reaction mixture. The two reactions are analogous; the aluminium chloride and the sulfuric acid both being strong acids which catalyze the reaction. Our reaction products (I) and (III) have the same degree of oxidation as the 2,5-dimethoxyquinone, but the mechanism of the reaction is difficult to interpret.

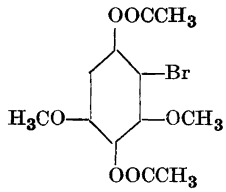
When treated with acetyl bromide, 2,5-dimethoxyquinone was reduced to 2,5-dimethoxyquinol diacetate and bromine was liberated.

2,6-Dimethoxyquinone did not react with acetyl chloride, even when sulfuric acid was added. This is in agreement with the observations of Oliverio [3], who found that this quinone did not react with acetyl chloride in the presence of aluminium chloride.

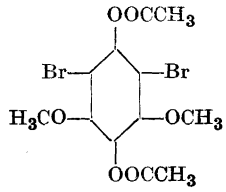
2,6-Dimethoxyquinone dissolved completely in acetyl bromide. The reaction was accelerated by small amounts of mercuric bromide. From the reaction mixture the unsubstituted (IV), the monobromo (V) and the dibromo-2,6-dimethoxyquinol diacetate (VI) could be isolated. Perhaps the primary reaction here is also reductive acetylation with formation of bromine, but this quinol diacetate is more easily brominated than the isomer mentioned above.



IV



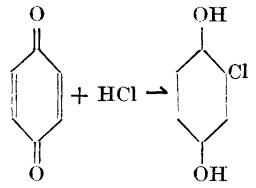
V



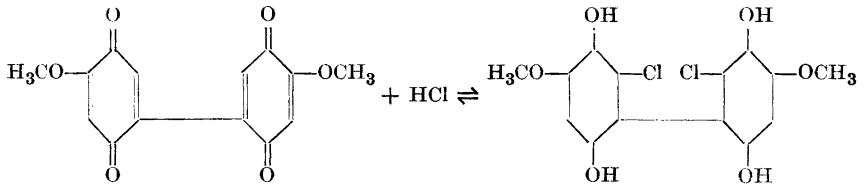
VI

The monobromo derivative, which has not been described before, was also synthesized by reductive acetylation of monobromo-2,6-dimethoxyquinone. On attempted hydrolysis with sulfuric acid in methanol, the intermediate quinol lost hydrogen bromide and gave 2,6-dimethoxyquinone. The monobromo-2,6-dimethoxyquinol could be obtained in ether solution by reducing the monobromoquinone with zinc dust in acetic acid, diluting with water and extracting with ether. (See under Experimental.) However, on concentrating, even under reduced pressure, the substance decomposed. The corresponding dibromoquinol, prepared by Aulin and Erdtman [5], is a quite stable compound.

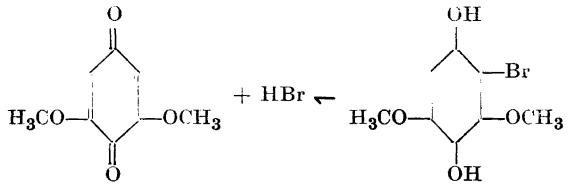
A.



B.



C.



With these experiments the existence of the reverse reaction (C) of the hydrogen halogenide addition to quinones (A) has been demonstrated. The reversibility of the reaction between 4,4′-dimethoxydiquinone and hydrogen chloride (B) is therefore not inconsistent with the assumption that the addition product is a diquinol. This assumption is rather probable when one considers that the reactivity of 4,4′-dimethoxyquinone is between that of the majority of easily reacting quinones and those completely unreactive. The fact that the addition product yields a diacetate and not a tetraacetate on acetylation, however, is still unexplained.

EXPERIMENTAL

2,5-Dimethoxyquinone and acetyl chloride

To a mixture of 2,5-dimethoxyquinone (5 g) and acetyl chloride (50 ml), concentrated sulfuric acid (5 ml) was added in portions. The quinone slowly dissolved. After three hours the brown-colored solution was poured into ice water (1500 ml) containing sodium hydrogen carbonate (50 g). An oil precipitated, which soon solidified to a crystalline mass. The crystals were collected and recrystallized from ethanol. Three recrystallizations yielded 5.2 g of a pure substance melting at 90—91°. The substance is a dimethoxydichlorophenol acetate, previously prepared by Oliverio in an analogous reaction with aluminium chloride as catalyst [3]. Oliverio reports the melting point as 92°.

$C_{10}H_{10}O_4Cl_2$ (265.1)	Calc.	OCH_3	23.6
	Found	»	23.5

The mother liquors contained another substance, which could not be obtained in a pure state by recrystallization. When the mother liquors were hydrolyzed with sodium hydroxide in ethanol, and the ethanolic solution diluted with water, this substance could be obtained in an almost pure state by steam distillation or ether extraction of the alkaline solution. The substance was recrystallized from light petroleum. M. p. 89—90°, alone, or on admixture with a specimen of trichloroquinol dimethyl ether, prepared from trichloroquinol by methylation with diazomethane.

$C_8H_7O_2Cl_3$ (241.5)	Calc.	OCH_3	25.7
	Found	»	25.5

2,5-Dimethoxyquinone and acetyl bromide

Acetyl bromide (40 ml) was added to 2,5-dimethoxyquinone (5 g). After a few minutes the quinone was dissolved. The solution had the dark red color of bromine. The solution was poured into ice water (500 ml) in which sodium acetate (40 g) had been dissolved. An oil separated which soon solidified, and was recrystallized from ethanol. Yield 6.3 g. The substance melted at 182—183°, alone, or on admixture with 2,5-dimethoxyquinol diacetate, prepared in a good yield by treating 2,5-dimethoxyquinone with zinc dust and acetic anhydride.

$C_{12}H_{14}O_6$ (254.2)	Calc.	OCH_3	24.4
	Found	»	24.0

2,6-Dimethoxyquinone and acetyl bromide

Acetyl bromide (40 ml) was slowly added to 2,6-dimethoxyquinone (10 g). A small amount of mercuric bromide (0.1 g) accelerated the reaction but was not necessary. With mercuric bromide as catalyst the reaction was vigorous, and the reaction mixture had to be cooled with ice water. After a few minutes the reaction was complete and the yellow solution was poured into ice water (1 000 ml) and sodium hydrogen carbonate (30 g) added. A yellow oil separated and solifidied after about an hour to a crystalline mass (16 g). This proved to be mixture of three substances. The chief product could be obtained in a pure state by four recrystallizations from ethanol. It melted at 108—109°, alone, or on admixture with 2,6-dimethoxybromoquinol diacetate, prepared from 2,6-dimethoxybromoquinone by reductive acetylation with zinc dust and acetic anhydride.

$C_{12}H_{13}O_6Br$ (333.1)	Calc.	OCH_3	18.6	Br	24.0
	Found	»	18.7	»	23.8

This substance, when hydrolyzed with sulfuric acid in methanol, decomposed into 2,6-dimethoxyquinone and hydrogen bromide. The mother liquors were treated with sulfuric acid in methanol, the separated quinone was removed by filtration, the solution concentrated and acetylated. By these operations the monobromo diacetate was eliminated from the mixture. The residue was fractionated by recrystallizations from methanol. Two pure substances, melting at 126—127° and 103—105° respectively were obtained. They proved to be identical with the previously known diacetates of 2,6-dimethoxyquinol and 3,5-dibromo-2,6-dimethoxyquinol respectively. We prepared those substances by reductive acetylation of the 2,6-dimethoxyquinone and its dibromo derivative.

2,6-Dimethoxymonobromoquinol

In an attempt to prepare 2,6-dimethoxymonobromoquinol, 2,6-dimethoxymonobromoquinol diacetate (2.0 g) was dissolved in methanol with 1 % sulfuric acid (5 ml) and refluxed for 15 minutes. A yellow, crystalline substance precipitated, which was collected and recrystallized from acetic acid. Yield 0.7 g. M. p. 248–249°. The melting point was not depressed on admixture with 2,6-dimethoxyquinone.

A small amount of 2,6-dimethoxymonobromoquinone was reduced with zinc dust in boiling acetic acid. The colorless solution was diluted with water and extracted with ether. The ether solution was washed with sodium hydrogen carbonate solution and water, and dried over anhydrous sodium sulfate. When this still colorless solution was concentrated, even under reduced pressure, the substance decomposed and 2,6-dimethoxyquinone was formed. The liberated acid seemed to accelerate this decomposition. The presence of the bromoquinol, which could not be isolated, was demonstrated by the following experiment. A small amount of the bromoquinone was reduced as above. To the colorless acetic acid solution, acetic anhydride and pyridine were added and the solution refluxed for some minutes. When the mixture was poured into water, a colorless substance of m. p. 108–109°, identical with the bromoquinol diacetate described above, separated.

SUMMARY

Monobromo-2,6-dimethoxyquinol, in contradiction to other halogenoquinols, is a very unstable substance, which easily decomposes into 2,6-dimethoxyquinone and hydrogen bromide.

Trichloroquinol dimethyl ether has been isolated from the reaction mixture obtained when 2,5-dimethoxyquinone was treated with acetyl chloride and sulfuric acid.

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Received August 12, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1197–1205

The Effect of *Ortho*-Substitution on the Dipole Moments of Aniline Derivatives *

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According to a suggestion by Docent N. Löfgren, Stockholm, a systematic investigation of the dipole moments of toluidines and xylidines may throw light on the special effect of substituents in the *ortho*-position, which is usually called the *ortho*-effect.

The present study includes the determination of the electric moments of some derivatives of aniline, in which one or more methyl groups are substituted at different positions in the benzene nucleus as well as at the nitrogen atom. Though some of the dipole moments now reported have been measured before by other authors we thought it advisable to measure the moments of all the substances concerned with the same apparatus and in the same solvent in order to obtain strictly comparable values. It should not be taken for certain that the new values — taken as absolute data — are more accurate than those obtained earlier, but it is assumed that the new measurements are more consistent among themselves.

PREPARATIONS

Benzene. A thiophene-free product from Baker's Ltd. was stored over sodium wire for three months. It was distilled over sodium in an atmosphere of dry nitrogen immediately before use.

Aniline. The purest Merck product was treated according to the method described by Timmermans and Hennaut-Rolland [1]. The final distillation under reduced pressure, in this as well as in all the following cases, was performed in a stream of dry nitrogen. B. p. 71° (11 mm).

N-Methyl aniline. The best commercial product was purified by conversion into the nitrosoamine. This was reduced by stannous chloride in a manner similar to that recommended by Blatt [2] for the synthesis of N-ethyl *m*-toluidine. At the final distillation the b. p. was 84° (13 mm).

* A preliminary report has been published in *Nature* **165** (1950) 239.

N,N-Dimethyl aniline. The technical product was steam-distilled, fractionally crystallised, and distilled twice under reduced pressure. B. p. 78° (12 mm).

Ortho-Toluidine. The purest Merck product was fractionally distilled at atmospheric pressure. The slightly yellow fraction boiling at 194° (uncorr.) was collected and redistilled. B. p. 84° (11 mm).

N-Methyl o-toluidine. This substance was prepared from the purified *o*-toluidine by Blatt's method [2]. B. p. 89° (11 mm).

N,N-Dimethyl o-toluidine. The methylation was performed with methyl iodide in alkaline solution in accordance with the method described by Noelting [3] for the synthesis of N,N-dimethyl 3,5-xylidine. The substance boiled at 74° (14 mm).

Meta-Toluidine. The purest commercial product was fractionally distilled at atmospheric pressure. The fraction boiling at 197° (uncorr.) was collected and redistilled under reduced pressure. B. p. 87° (11 mm).

N-Methyl m-toluidine. The substance was prepared in a similar manner to the corresponding *o*-compound. B. p. 97° (14 mm).

N,N-Dimethyl m-toluidine. The same method of preparation was used as in the case of the corresponding *o*-compound. B. p. 85° (9 mm).

Para-Toluidine. The best Merck product was purified by three recrystallisations from ligroin. M. p. 46—47°.

N-Methyl p-toluidine. The substance was synthesised from the formyl compound by the method described by Bamberger and Wulz [4]. B. p. 94° (14 mm).

N,N-Dimethyl p-toluidine. Noelting's method of preparation [3] was used. The boiling point was 90° (13 mm).

2,4-Xylidine. A pure Eastman Kodak Co. product was fractionally distilled at atmospheric pressure. The slightly yellow fraction boiling at 210° (uncorr.) was collected. After redistillation the product was almost uncoloured. B. p. 96° (12 mm).

N-Methyl 2,4-xylidine. The substance was synthesised by Blatt's method [2]. B. p. 103° (12 mm).

N,N-Dimethyl 2,4-xylidine. The preparation was carried out in the way described by Noelting [3]. B. p. 87° (13 mm).

2,6-Xylidine. A commercial product was purified by treatment with conc. sulphuric acid, ether extraction and repeated fractional distillation. B. p. 96° (12 mm).

N-Methyl 2,6-xylidine. The acetyl derivative of the primary amine was methylated with methyl iodide and converted into the nitrosoamine as described by Friedländer [5]. The nitrosoamine was then treated according to Blatt [2]. The boiling point of the final product was 87° (13 mm).

N,N-Dimethyl 2,6-xylidine. The substance was synthesised by the method of Fischer and Windaus [6]. B. p. 74° (11 mm).

METHOD AND APPARATUS

The dielectric constants and the specific volumes of dilute benzene solutions of the substances were measured at 25° $\pm$ 0.05°. In order to prepare a solution the substance was distilled into a weighing glass with ground-glass stopper and weighed, care being taken to avoid moisture. Then, benzene was added from a syringe and weighed. The same solution was used for measurements of both dielectric constant and specific volume.

The specific volumes were determined with Ostwald-Sprengel pycnometers fitted with carefully ground caps. They had a volume approximately equal to 5 cm^3.

The dielectric constants were measured with an alternating-current heterodyne beat apparatus built in this laboratory according to a design published by Hudson and Hobbs [7]. The fundamental frequency of the crystal controlled oscillator was 1 000 kilocycles. In the measuring circuit the frequency could be adjusted either to 1 000 kilocycles or to the first harmonic of the crystal, 2 000 kilocycles, by using different coils. The first harmonic was used for the present measurements. The beat frequency was adjusted to the frequency of the alternating-current, 50 cycles. The two points at each side of the zero beat frequency were determined with aid of a loud speaker and a cathode ray tube.

The measuring condenser was a modification of one described by Arrhenius [8]. The solution was placed in a cup of silver, which formed one part of the condenser. The other part was a plate of pure silver, which could be fixed at different distances from the bottom of the cup varying between 0.5 and 2 mm. The cup was filled with 5.0 cm^3 of the solution. The maximum difference in air capacity between two settings was 2 $\mu\mu$f. The variable capacity of the measuring condenser, and thus its volume, was kept small in order to make it possible to carry out a determination of the dipole moment with about 5 g of a substance, or even less. By measuring the difference between two settings instead of the total capacity of the condenser, inaccuracies due to the connections between the measuring cell and the main part of the oscillator were without influence on the capacity determination.

Two variable cylinder condensers were connected in parallel with the measuring condenser, the larger having a minimum capacity of 125 $\mu\mu$f and a variable capacity of 350 $\mu\mu$f, and the smaller a minimum capacity of 20 $\mu\mu$f and a variable capacity of 11 $\mu\mu$f. The inner cylinder of the smaller condenser could be moved with aid of a screw, each revolution allowing a movement of 0.5 mm. One revolution corresponded to a capacity change of 0.1 $\mu\mu$f and a reading could be made to 0.005 revolution, *i. e.* 0.0005 $\mu\mu$f. However, this degree of accuracy is not significant. The mechanical deficiency of the condenser made it necessary to take several readings for each complete measurement, usually eight to ten. These readings did not agree to more than 0.005 $\mu\mu$f. As the capacity changes were about 5 $\mu\mu$f the accuracy of the dielectric constant measurement would be 0.1 %. The cylinder condenser was calibrated in units of the measuring condenser. Thus, the accuracy quoted above referred to relative capacity values. In order to obtain "absolute" values we assigned to our benzene sample the value of the dielectric constant given by Hartshorn

Table 1. Measurements on benzene solutions at 25° C. The values given for the pure solvent are extrapolated ones.

$f_2 \cdot 10^5$	ε	v	$f_2 \cdot 10^5$	ε	v
Aniline			Methyl aniline		
0	2.2686	1.14457	0	2.2665	1.14445
983	2.3089	1.14268	1225	2.3259	1.14239
1417	2.3206	1.14164	2627	2.3884	1.14007
1782	2.3323	1.14092	3030	2.4042	1.13907
3073	2.3859	1.13841	5325	2.5076	1.13539
Dimethyl aniline			*Ortho*-Toluidine		
0	2.2723	1.14456	0	2.2815	1.14478
660	2.2938	1.14366	1722	2.3475	1.14143
1842	2.3402	1.14217	2733	2.3848	1.13927
2504	2.3594	1.14116	3403	2.4153	1.13813
4152	2.4175	1.13897	4304	2.4453	1.13629
Methyl *o*-toluidine			Dimethyl *o*-toluidine		
0	2.2751	1.14455	0	2.2711	1.14452
482	2.2995	1.14369	1282	2.2852	1.14332
991	2.3206	1.14274	1974	2.2931	1.14259
1694	2.3495	1.14153	2496	2.3009	1.14199
2293	2.3851	1.14041	3413	2.3102	1.14118
			3851	2.3141	1.14081
Meta-Toluidine			Methyl *m*-toluidine		
0	2.2696	1.14425	0	2.2770	1.14460
1368	2.3144	1.14247	1014	2.3140	1.14299
2165	2.3399	1.14131	1869	2.3443	1.14163
3150	2.3688	1.14014	2772	2.3844	1.14016
4004	2.4005	1.13882	3435	2.4005	1.13916
Dimethyl *m*-toluidine			*Para*-Toluidine		
0	2.2680	1.14468	0	2.2726	1.14454
726	2.2942	1.14375	1347	2.3090	1.14228
1338	2.3126	1.14304	1406	2.3140	1.14211
1387	2.3096	1.14296	2455	2.3396	1.14036
1995	2.3390	1.14213	3608	2.3742	1.13840
2393	2.3495	1.14164			
2792	2.3640	1.14118			
2838	2.3595	1.14115			

Table 1. (cont.)

$f_2 \cdot 10^5$	ε	v	$f_2 \cdot 10^5$	ε	v
Methyl *p*-toluidine			Dimethyl *p*-toluidine		
0	2.2742	1.14458	0	2.2650	1.14446
994	2.3057	1.14299	1666	2.3082	1.14259
1913	2.3272	1.14148	2667	2.3273	1.14141
2432	2.3468	1.14068	3233	2.3430	1.14076
3612	2.3820	1.13877	4281	2.3723	1.13962
2,4-Xylidine			Methyl 2,4-xylidine		
0	2.2727	1.14439	0	2.2686	1.14461
876	2.2989	1.14306	818	2.2990	1.14331
1965	2.3306	1.14137	1382	2.3149	1.14248
2289	2.3359	1.14070	2219	2.3452	1.14105
3003	2.3618	1.13963	3146	2.3794	1.13963
Dimethyl 2,4-xylidine			2,6-Xylidine		
0	2.2729	1.14456	0	2.2774	1.14449
1276	2.2828	1.14371	597	2.3004	1.14346
1973	2.2872	1.14302	1502	2.3380	1.14169
2806	2.2922	1.14258	1681	2.3396	1.14151
3503	2.2995	1.14204	2273	2.3678	1.14046
			3084	2.3943	1.13892
			3150	2.3998	1.13873
			4947	2.4698	1.13562
Methyl 2,6-xylidine			Dimethyl 2,6-xylidine		
0	2.2749	1.14452	0	2.2697	1.14458
1086	2.3018	1.14296	1359	2.2868	1.14374
1673	2.3160	1.14220	1549	2.2883	1.14358
2785	2.3380	1.14044	2148	2.2947	1.14326
4785	2.3908	1.13772	2913	2.3055	1.14274

and Oliver [9], $\varepsilon_{25°} = 2.2725$, which is in good agreement with the value obtained more recently by van der Maesen [10], $\varepsilon_{25°} = 2.2738$.

The results obtainable directly from the measurements were the dielectric constant, ε, and the specific volume, v, of the solutions. The plot of ε as well as that of v against the mole fraction of the solute, f_2, gave straight lines. The slopes, $\partial\varepsilon/\partial f_2$ and $\partial v/\partial f_2$, and the intercepts at $f_2 = 0$, ε_1 and v_1, were calculated by the method of least squares. The polarisation of the solute at infinite dilu-

Table 2. Calculated values of polarisations and dipole moments.

Substance	$\frac{\partial \varepsilon}{\partial f_2}$	$-\frac{\partial v}{\partial f_2}$	P_2^0 cm^3	MR_D cm^3	μ D
Aniline	3.77	0.202	82.4 ± 3.6	30.58 [12]	1.59 ± 0.03
Methyl aniline	4.56	0.171	99.6 1.7	35.69 [1]	1.77 .02
Dimethyl aniline	3.51	0.134	89.9 0.8	40.85 [15]	1.55 .01
o-Toluidine	3.84	0.197	88.3 2.2	34.98 [13]	1.61 .02
Methyl *o*-toluidine ..	4.66	0.180	105.5 4.5	40.43	1.78 .04
Dimethyl *o*-toluidine ..	1.14	0.098	60.5 0.8	44.62 [14]	0.88 .01
m-Toluidine	3.23	0.133	80.9 1.7	35.29 [13]	1.49 .02
Methyl *m*-toluidine ..	3.69	0.159	91.8 3.6	40.60	1.58 .03
Dimethyl *m*-toluidine..	3.36	0.125	92.5 2.7	45.68 [14]	1.51 .02
p-Toluidine	2.80	0.170	73.7 1.8	35.91 [13]	1.36 .02
Methyl *p*-toluidine ..	2.96	0.161	81.0 2.4	40.67	1.41 .02
Dimethyl *p*-toluidine ..	2.47	0.114	79.7 2.7	45.68 [14]	1.29 .02
2,4-Xylidine	2.91	0.158	80.3 3.1	40.08 [15]	1.40 .03
Methyl 2,4-xylidine ..	3.49	0.158	93.7 1.7	45.25	1.54 .02
Dimethyl 2,4-xylidine .	0.73	0.072	60.0 0.4	49.55 [15]	0.71 .01
2,6-Xylidine	3.88	0.180	94.0 1.1	39.94 [15]	1.63 .02
Methyl 2,6-xylidine ..	2.39	0.143	77.9 1.6	44.46	1.28 .01
Dimethyl 2,6-xylidine .	1.21	0.063	67.1 1.2	48.99 [15]	0.94 .01

tion, P_2^0, was calculated from the equation derived by Halverstadt and Kumler [11]:

$$P_2^0 = \frac{3\,\frac{\partial \varepsilon}{\partial f_2}\, v_1 M_1}{(\varepsilon_1 + 2)^2} + \frac{\varepsilon_1 - 1}{\varepsilon_1 + 2}\left[M_2 v_1 + M_1 \frac{\partial v}{\partial f_2}\right]$$

The sum of the electronic and the atomic polarisations was set equal to the molar refraction of the sodium line, MR_D. In cases when the value of MR_D is not known the refractive index of the pure substance was determined with an Abbe refractometer, and the density of the substance was measured with a pycnometer of volume approximately equal to 1 cm^3. As an uncertainty in this determination is not very important, an accuracy in MR_D greater than 0.1 % was not attempted. Finally, the dipole moment, μ, was calculated from:

$$\mu = 0.22118 \sqrt{P_2^0 - MR_D} \qquad \text{Debye } (D)$$

where $0.22118 \cdot 10^{-18}$ is the value of $\sqrt{4\pi N/9kT}$ at $T = 298°\,K$.

Table 3. Previously published dipole moments obtained from measurements on benzene solutions.

Substance	Dipole moment	
	This paper	Previous investigations
Aniline	1.59	1.55, [16] 1.52, [17] 1.51 [18]
Methyl aniline	1.77	1.64 [19]
Dimethyl aniline	1.55	1.58, [19, 20] 1.55 [21]
o-Toluidine	1.61	1.58, [17] 1.65 [22]
m-Toluidine	1.49	1.44, [17] 1.51 [22]
p-Toluidine	1.36	1.31, [17] 1.36 [22]
Dimethyl *p*-toluidine	1.29	1.29 [20]

RESULTS

The data of the measurements of dielectric constants and specific volumes are collected in Table 1. Table 2 contains the computed values of the slopes of the extrapolation curves, of the polarisations, and of the dipole moments.

As is seen from Table 3 the agreement between the above results and values obtained previously for dipole moments is fairly good. Table 4 shows the differences between the moments of primary, secondary, and tertiary amines.

Table 4.

Primary amine	Difference between the moments of:		
	sec.—prim.	*tert.—sec.*	*tert.—prim.*
Aniline	+ 0.18	− 0.22	− 0.04
m-Toluidine	+ 0.09	− 0.07	+ 0.02
p-Toluidine	+ 0.05	− 0.12	− 0.07
o-Toluidine	+ 0.17	− 0.90	− 0.73
2,4-Xylidine	+ 0.14	− 0.83	− 0.69
2,6-Xylidine	− 0.35	− 0.34	− 0.69

DISCUSSION

The dipole moments of the primary amines show that *ortho* substitution changes the moment less than does *meta* or *para* substitution. Thus, the dipole moment of *o*-toluidine is only 0.02 *D* larger than the moment of aniline, and

the moment of 2,4-xylidine is 0.04 *D* larger than that of *p*-toluidine. This small change of the moment is certainly the result of opposing effects, namely the finite moment of the methyl group, which tends to increase the resulting moment, and contrary moments due to induction, *cf.* Smallwood and Herzfeld [23]. Perhaps a change of valence angles is also involved.

The substitution of one methyl group at the nitrogen atom in most cases increases the resulting moment by 0.1—0.2 *D*. The only exception is 2,6-xylidine, where there are two methyl groups in the *o*-position to the amino group. In this case the resulting moment is 1.28 *D*, which is 0.35 *D smaller* than the moment of the primary amine. Moreover, the moment is only 0.1 *D* larger than that of a secondary aliphatic amine, dimethyl amine, which has a dipole moment of 1.17 *D* calculated from measurements on benzene solutions [24].

All the moments of the tertiary aniline homologues are smaller than those of the corresponding secondary amines. It is noteworthy that the substitution of *one* methyl group at the nitrogen atom *increases* the resulting moment, whereas the substitution of *two* methyl groups *reduces* the moment. When the molecules in question do not possess a methyl group in the *o*-position the moments of the tertiary amines deviate by only about 0.05 *D* from the moments of the primary amines. However, when the tertiary amine has one or two methyl groups in the *o*-position its dipole moment is 0.7 *D* smaller than the moment of the primary amine, *cf.* Table 4. The dipole moment of these tertiary aniline homologues have almost the same value as those of aliphatic tertiary amines, namely 0.86 *D* when measured in benzene solution [24].

To sum up: three or four methyl groups in the proximity of the nitrogen atom of aniline, that is substituted either at the nitrogen atom or in the *o*-position, twist the amino group around the carbon-nitrogen bond, so that one of the atoms, or groups, attached to the nitrogen lies above, and the other below, the plane of the benzene ring. This causes a change of the dipole moment from the value typical of an aromatic amine to that typical of an aliphatic amine. It has earlier been pointed out by Hampson and coworkers [25] that four methyl groups in the proximity of the nitrogen inhibit the resonance of the molecule.

It is interesting to note that the inhibition of resonance is also manifest in the ultra-violet absorption spectra of *ortho*-substituted derivatives of dimethyl aniline [26], as well as in the value of the atomic refractivity of the nitrogen of dimethyl 2,6-xylidine, calculated by Thomson [15]. This author has also measured the basic strengths of homologues of dimethyl aniline [27] and has found a clear difference between molecules with and without inhibited resonance.

SUMMARY

1. The dipole moments of 18 aniline homologues have been measured in benzene solutions at 25° C.

2. When the *ortho*-effect is not involved, the secondary amine has a larger moment than the primary one, and the tertiary amine has a smaller moment than the secondary one.

3. Three or four methyl groups in the proximity of the nitrogen atom change the moment from the value typical of an aromatic amine to that typical of an aliphatic one.

This work was made possible by a grant from *Statens Tekniska Forskningsråd*. I should also like to express my gratitude to Dr. S. Arrhenius, Stockholm, and to Dr. L. E. Sutton, Oxford, for many valuable suggestions concerning the construction of the dielectric constant apparatus.

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Received July 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1206–1215

Phosphoprotein Phosphatase in the Rat

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A phosphatase acting on the natural phosphoproteins in frog eggs was discovered by Harris [6] in 1946. On account of the important position of phosphorus in the intermediary metabolism, an enzyme involved in protein-phosphorus transfer must be of considerable interest. The properties and occurrence of such an enzyme seem, however, to have been very little investigated.

In 1949 Feinstein and Volk [3] presented a study on phosphoprotein phosphatase in mammals. The evidence brought forward by Feinstein and Volk for the existence of this hydrolase as a catalyst sui generis is, however, unsatisfactory. They only state that more phosphorus is liberated when casein is added to a tissue homogenate incubated with excess of glycerophosphate. The independence of the phosphate liberation from non-protein nitrogen formation, *i. e.* from proteolytic activities, on the other hand, seems to be well established. Furthermore, Feinstein and Volk used ascorbic acid as an activator for the enzyme, whereas ascorbic acid, as shown below, is actually an inhibitor. There are thus several reasons for further investigations of the phosphoprotein phosphatase. The object of the present study was to establish the existence of phosphoprotein phosphatase in rat tissues as an independent enzyme, and to investigate its distribution in different organs. To this end a method of assay was first developed and some properties of the enzyme studied.

EXPERIMENTAL

Estimation of the enzyme activity

In preliminary experiments the technique of Feinstein and Volk [3] was followed, although the substrate was prepared from a commercial casein according to Hammarsten. It was, however, impossible to get even and reproducible results. Isolation of the liberated phosphate before the estimation was therefore tried. This was accomplished

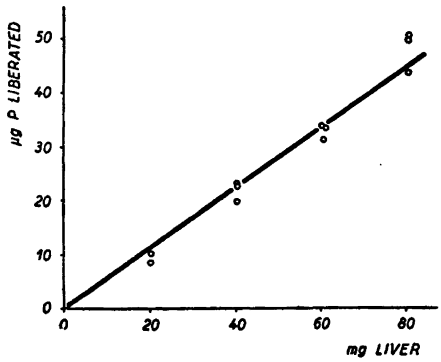

Fig. 1. Proportionality between enzyme quantity and amount of phosphorus liberated.

by precipitation with calcium (as hydroxyl apatite), slightly modified according to Fiske and Subbarow [4].

The following general technique was then used:

Four ml casein solution of pH 5.8 was preheated in a wide test-tube at the temperature of digestion, usually 37° C. One ml of the sample was added. Ten minutes later the reaction was stopped with 10 ml normal trichloracetic acid. Controls with casein and water were also made, and blank values with water and sample were run parallelly. Seven and a half ml of the protein-free filtrate was pipetted into a conical centrifuge tube. The aliquot was neutralized with 5 *N* and 0.5 *N* sodium hydroxide against brom thymol blue. Finally 2 ml 10 % calcium chloride in 0.5 *M* ammonium chloride buffer pH 9 was added. After 30 minutes the calcium phosphate was centrifuged down and washed once with 5 ml of a 1 : 5 dilution of the precipitating reagent. The precipitate was suspended in a few ml 0.05 *N* sulfuric acid and the phosphate determination with the Fiske-Subbarow reaction performed (see Norberg [11]). The amount of phosphorus was obtained from standard phosphate series precipitated in the same way.

The enzyme units are calculated as the number of micromols of phosphorus liberated per minute under the experimental conditions. The enzyme units were as a rule calculated per g of fresh tissue.

Source of enzyme. The organ to be studied was homogenized in 10 to 25 parts of 0.05 *M* acetate buffer of pH 5.8 with the technique of Potter and Elvehjem [14]. The opaque extract after centrifugation or filtration was used. All studies on the properties of the enzyme were made with fresh rat liver extracts.

Proportionality between the amount of enzyme and micrograms of phosphorus liberated was found to be satisfactory over a wide range. For liver up to 80 mg this emerges from Fig. 1. In other experiments proportionality was obtained up to 200 mg of liver. But sometimes the activities came out too low even with 80 mg of liver possibly due to influence of inorganic phosphate. It is therefore advisable to use the equivalent of about 50 mg liver or less. The standard deviation calculated from duplicates was 0.078 units per g liver. All values in this report are means of agreeing duplicates or triplicates. Thus their standard error is less than 0.055.

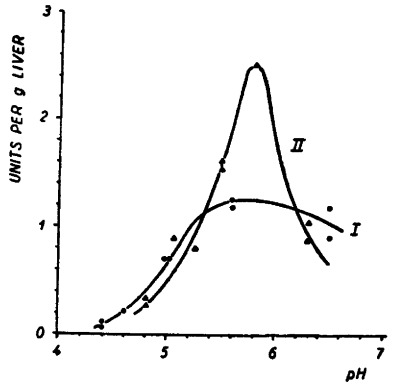

Fig. 2. Optimum pH for phosphoprotein phosphatase.

Properties of phosphoprotein phosphatase

Optimum pH was tested both with the method of Feinstein and Volk and with the new technique. The pH optimum is around pH 5.8, as is evident from Fig. 2. The peak was, however, not always so sharp as in the experiment reproduced in curve II.

Influence of the substrate. As standard substrate a commercial casein (Vitrum A. B., Stockholm) prepared according to Hammarsten was used. About 120 g of the air-dry substance was stirred with 800 ml redistilled water. About 25 ml normal sodium hydroxide was added and the pH adjusted to 5.8. This may be accomplished by dialysis against 0.05 *M* acetate buffer of pH 5.8 in the cold room, which is of advantage if the casein contains significant amounts of free phosphate. The organically bound phosphorus was estimated after precipitation with trichloracetic acid and digestion with sulfuric acid and nitric acid [13]. Finally, the substrate was diluted to an organic phosphorus content of 20 m*M*.

With this suspension the substrate concentration-velocity curve was studied. The results are shown in Fig. 3. Treatment of the values according to Lineweaver and Burk [8] gave a limiting velocity of 3 micromols of phosphorus liberated per minute per g liver and a Michealis-Menten constant of 3 m*M* casein phosphorus.

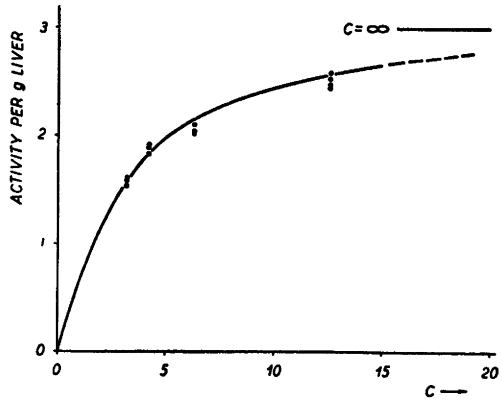

Fig. 3. Substrate concentration-velocity curve for phosphoprotein phosphatase with casein as substrate.

Table 1. Liberation of phosphorus from phosphovitin by phosphoprotein phosphatase from 50 mg liver at 37° C.

Phosphovitin phosphorus, m*M*	16	32	48	64
Phosphatase, units per g	0.715	1.15	1.16	1.15

From Fig. 3, however, it appears that the enzyme is not wholly saturated with substrate at casein concentrations corresponding to 15—20 m*M* protein phosphorus. Also the substrate is not homogeneous. It therefore appeared of interest to test phosphopeptones prepared from casein, as these are highly soluble. Calcium phosphopeptone according to Mellander [10] was dissolved in water and the calcium ion exchanged for sodium with zeocarb ion exchanger saturated with sodium. After dialysis against a 0.05 *M* acetate buffer of pH 5.8, the final concentration of phosphorus precipitable with trichloracetic acid was 7.5 m*M*. In eight experiments there was no liberation of phosphorus with phosphopeptone as substrate, whereas the activity towards casein was from 1.56 to 3.22 units per g liver. As traces of lead from the preparation or other toxic ions might disturb the enzyme activity, phenanthrolin to a final concentration of 0.4 m*M* was added in two experiments. No effect, however, was observed. Only in one experiment was phosphorus split off from phosphopeptone; but unfortunately there was a big difference between the two phosphorus values, and no conclusion can be drawn in this case. The overall results suggest that the enzyme can not attack casein after a profound proteolytic degradation.

As it seemed necessary to verify the ability of the enzyme to hydrolyze other phosphoproteins, phosphovitin was tested. Phosphovitin contains most of the protein-bound phosphorus in egg yolk. It was prepared according to Mecham and Olcott [9]. In the final dialyzed solution of phosphovitin the content of organically bound phosphorus was 54

Table 2. Activity of liver phosphoprotein phosphatase with different substrates in units per g liver. Protein phosphorus 20 mM in casein and 64 mM in phosphovitin (54 mM in preparation no. II).

Phosphovitin	Casein	Phosphovitin	Activity with casein / Do. with phosphovitin	Liver no.
No. I	2.31	1.64	1.41	25
	1.55	0.996	1.56	37
	1.94	1.20	1.62	38
	2.20	1.53	1.44	37 R
	1.91	1.16	1.65	38 R
No. II	3.03	2.66	1.14	47
	3.01	2.42	1.24	73
	3.11	2.64	1.18	74

Table 3. Course of digestion. Activity in units per g fresh liver.

Digestion time minutes	Extract 17 at 30° C	Extract 24 at 40° C
5	2.89	1.62
10	2.97	1.66
15	—	1.54
20	1.86	1.52
30	1.50	1.16

Table 4. Stability of phosphoprotein phosphatase from two liver samples at different pH values. Activities in units per g fresh liver.

Time from excision of liver, minutes	pH of homogenate			pH of extract		
	5.0	5.85	7.25	5.0	6.10	6.50
30	2.28	2.50	2.30	0	1.90	2.38
90	2.04	2.52	3.03		2.30	2.05
240	1.72	2.81	2.52	0	1.20	0

Table 5. Heat inactivation of two phosphoprotein phosphatase extracts. Assay at 37 C.°

Time of heating, minutes	At 37°		At 60°	
	Units/g	% inactivation	Units/g	% inactivation
0	2.35		2.00	
2	2.31	2	0.81	59
8	2.19	7	0.45	77
16	2.08	11.5	0.43	78
32	2.12	9.5		

and 64 m*M*. The nitrogen/phosphorus quotient should be 1.23 [9]. In the two preparations used it was 1.62 and 1.75, however, indicating a nitrogenous impurity. The solutions were tried, however, without further purification. As shown in Table 1, the enzyme seemed to be saturated with substrate at 32 m*M* of protein phosphorus. The activity was, however, always lower than with casein. This was tested on a number of different liver extracts. From table 2 it is evident that the activity was about 50 percent higher with casein as substrate than with phosphovitin for the first preparation and about 20 percent higher for the second preparation.

Table 6. Influence of different substances on the activity of phosphoprotein phosphatase. The figures denote the average percentual deviation from the activities under standard conditions.

Substance	Concentration in digest					
	0.01	0.1	1	5	10	m*M*
Ascorbic acid			− 2.5	− 12	− 32	
Iodoacetic »			− 5		− 70	
Na_2S		+ 8	+ 18		0	
Cystein. HCl			− 16		− 38	
$CuSO_4$	− 17	− 80	− 71		− 88	
Tocopheryl phosphate	+ 6	− 15	+ 6			
		+ 87	+ 40			
	0.02	0.2	2	20	m*M*	
KCN	0	+ 10	+ 7	− 40		
NaF	− 7	− 9	− 53	− 87		
$MgSO_4$			− 6	− 4		
Phenanthrolin		+ 9	+ 6			
	0.1	0.2	0.4	m*M*		
KH_2PO_4	− 8	− 27	− 40			
	1.0	6.7	10.0	26.7	mg per liter	
Heparin	− 8		+ 57			
Bayer 205	− 25		− 30			
Protamine sulfate		+ 2		+ 2		

The course of digestion was studied at two temperatures with several extracts. As examplified in Table 3, the decrease in reaction velocity was insignificant for the first ten to fifteen minutes. Digestion for ten minutes was therefore adopted as the standard period.

The stability of the enzyme was studied at different pH values. Homogenates were prepared in 0.1 *M* buffers in the cold, and samples taken for assay at different times after excision of the liver. As appears from Table 4, there is good stability between pH 5.8 and neutral reaction, whereas at pH 5 a marked drop in activity occurs after a time. When these experiments were repeated with extracts the activity was zero at pH 5 at once, at pH 6 to 7 full activity was retained for 90 minutes, but at 4 hours there was no

enzyme action in the neutral extract although about 50 % of the original activity was left in the pH 6 extract. In order to minimize spontaneous inactivation, all extracts were therefore made with cold 0.05 *M* acetate buffer of pH 5.7, and were used within half an hour. The pH of such extracts was 6.0 to 6.2.

Heat inactivation was studied at 60° and 37° C at pH 6. As appears from Table 5, the activity is little influenced at 37°. At 60°, however, the inactivation is fairly strong. After 2 minutes, when the temperature in the extract only attained 56°, as much as 50 % inactivation had occurred. After 4 minutes only about one fourth of the activity was left, but this amount of the enzyme seems to remain active even after a quarter of an hour at 60°.

The temperature coefficient for the hydrolytic action was determined through assay at 30 and 40° C or 27 and 37° C. The average value of ten experiments was 1.70 ± 0.09.

Inhibitors. Feinstein and Volk[3] found moderate activation through Mg, Ba, Ca, and Mn ions. But with 0.01 *M* ascorbic acid they had a considerable activation of + 50 to + 240 %. The ascorbic acid was, moreover, necessary to get proportionality in their assay. In my experiments, on the contrary, ascorbic acid was clearly an inhibitor, as is evident from Table 6. Also with respect to cyanide and cystein I found inhibition when Feinstein and Volk had activation. At low concentrations, however, cyanide and also sodium sulfide and hydroxylamine were slightly activating. Iodoacetate was found to be a strong inhibitor. In agreement with Feinstein and Volk, copper and fluoride ions were found to be very strong inhibitors. In conformity with the inhibitory effect of copper a slight activation resulted from the addition of complex-forming substances like phenanthrolin and diethyldithiocarbamate. Magnesium sulfate had no activating effect.

A possible inhibition through one of the split products was tested by addition of small amounts of potassium phosphate to the substrate. The inhibitory effect is obvious from the table.

Although the phosphoprotein phosphatase seems to act without any connection with proteolytic enzymes, which according to Zierler, Grob and Lilienthal[16] are inhibited by alpha tocopheryl phosphate, the latter substance was tested. As appears in the table, no effect was obtained in some series but a fairly strong activation with other extracts. This different behaviour might depend on a phenomenon similar to the balance between inhibition of alkaline phosphomonoesterase by estradiol diphosphate and restoration of its activity by tocopheryl phosphate demonstrated by Aldman, Diczfalusy, Ingelman-Sundberg, and Rosenberg[1]. If the activation, on the other hand, depends on inhibition of proteolytic activity through the tocopheryl phosphate other inhibitors of proteolysis might activate the phosphoprotein phosphatase as well. This was tested with heparin which, in fact, was activating in some experiments. Bayer 205 which as a polysulfonic acid substance has anticoagulant activity was also tested. No activation occurred, however. Finally, the heparin antagonist protamin was tested. It had no effect.

Distribution of phosphoprotein phosphatase

Liver homogenate at pH 6 was centrifuged for 5 minutes at 1400 times gravity. The opalescent extract and a suspension of the precipitate (without washing) were assayed at pH 5.8 for phosphomonoesterase with phenyl phosphate and magnesium activation, and for phosphoprotein phosphatase. The results are given in Table 7. Obviously, the phosphoprotein phosphatase is more soluble than the phosphomonoesterase, which fol-

Table 7. Distribution of phosphatases between solution (extract) and granulate matter that settles at 1 200—1 400 g in 5 minutes at pH 6—6.2 (precipitate). Units per g liver.

Date April		14	17	18	20	20 : 2
Phosphoprotein phosphatase	Precipitate	0.56	0.83	0	0	0.32
	Extract	2.60	2.91	1.87	3.52	1.84
Phosphomono-esterase	Precipitate	3.90	6.45	2.72	4.80	2.94
	Extract	5.17	4.77	3.47	3.88	2.37

lows the precipitate to a considerable extent. The phosphoprotein phosphatase activities on a homogenate and on the extract prepared therefrom were identical. Therefore the investigations on different organs were all made with extracts. The results are collected in Table 8.

The highest activities were found in the spleen. The relative activity was, however, lower than in the experiments of Feinstein and Volk [3]. Thus, as compared with the relative activity of liver taken as 100, the activity of spleen varied from 50 to 214 with a mean value of 126, whereas the values of Feinstein and Volk correspond to over 300.

Table 8. Distribution of phosphoprotein phosphatase in different organs. Digestion at 37° C for 10 minutes.

Tissue	Enzyme units per g fresh weight							Average	Relative activity
Liver	1.87	2.18	2.37	2.64	2.91	3.10	3.74	2.69	100
Adrenals	1.55	1.12	1.82	2.17			3.08	1.95	75
Blood clot	0.32			0.82			0.45	0.53	20
Bone marrow	0	1.62		1.08				0.90	36
Brain		1.55		1.16	1.88			1.53	60
Gastric mucosa			1.67	1.28			2.28	1.74	60
Heart muscle				0.71		1.42	1.70	1.28	39
Skeletal „		0		1.32	1.76			1.02	38
Kidney		1.33	1.94	2.25		4.21		2.43	96
Lungs		0.51	1.28			3.30	2.36	1.86	62
Lymph nodes	0	1.80		1.71				1.17	49
Mammary gland		0.94	0.84	1.15				0.98	41
Ovary				2.64	1.60		1.95	2.06	69
Pancreas	0.82		0.75			0.72		0.76	32
Salivary gland	0.58	0	1.83	3.86		0.47		1.35	54
Spleen	0.93	4.67	2.65	4.09	2.80	5.48	2.84	3.35	126
Testicle		2.16	1.90					2.03	90
Thymus			2.56	2.29		2.58		2.48	93

Other lymphoid organs have lower activity, although thymus is fairly high. With a view to the possible connection between phosphoprotein phosphatase and protein formation, the activity in organs which produce enzymes or other proteins is of great interest. As is evident from Table 8, the values were relatively low in salivary glands, gastric mucosa, pancreas, and mammary glands from lactating rats, whereas the liver had a high activity. Some samples of salivary glands, however, had a considerable activity. This might be due to differences in the stimulation. In two experiments, therefore, pilocarpine in a dose of 1 and 2 mg per kg was injected subcutaneously. The rats were killed 4 and 20 hours after the injection. The relative phosphoprotein phosphatase activity was about 70 for salivary glands, gastric mucosa, and pancreas, indicating an increased activity after secretory stimulation.

In brain and adrenals, as well as the sex glands and kidneys, the activity was found to be fairly high. Low activity was found in heart and skeletal muscle, as well as in red bone marrow and erythrocytes. Blood serum has practically no activity. Thus on the whole the phosphoprotein phosphatase was found to have a very wide distribution. The distribution as such therefore gives no hint as to the specific functions of this enzyme.

DISCUSSION

In the pH-optimum region of the phosphoprotein phosphatase also other enzymes are active which liberate inorganic phosphorus. The phosphomonoesterases I and II in the scheme of Folley and Kay [5], the adenylpyrophosphatase of Jacobsen [7], neutral pyrophosphatase [12], and glucose-6-phosphatase [2, 15] must be considered. The phosphoprotein phosphatase is, however, different from these enzymes in certain respects. This will be discussed presently with regard to liver, where ambiguity is most likely to occur.

Phosphomonoesterases active at pH 5.8—6 are rapidly destroyed by heat at this pH [7], whereas phosphoprotein phosphatase is comparatively resistant, Table 5. Moreover, the pH-optima are so different, and the extractability and precipitability are in such contrast (Table 7), that separate enzymes must be assumed.

Neutral pyrophosphatase is also less heat-resistant [7] than phosphoprotein phosphatase. Moreover, this enzyme is strongly activated by magnesium [12], which has no effect on the phosphoprotein phosphatase.

Adenyl pyrophosphatase has a very flat pH-optimum with the peak at pH 7.2. This enzyme is also most stable in the faintly alkaline region. But even at pH 6 it is fairly resistant, retaining about 50 % activity after 20 minutes at 55° C. The different pH-optimum and the greater stability at pH 6 than at pH 7 suggest that the phosphoprotein phosphatase is different from Jacobsen's enzyme.

Glucose-6-phosphatase has recently been investigated by de Duve, Berthet, Hers, and Dupret [2] and by Swanson [15]. They found the pH-optimum at pH 6 or possibly lower. The enzyme is, however, very labile in acid medium and

more stable at pH 8. The heat lability and pH sensitivity differentiates this enzyme from phosphoprotein phosphatase. Furthermore, glucose-6-phosphatase activity has only been found in liver, kidney and intestine [2], whereas phosphoprotein phosphatase is present in most tissues, Table 8.

The evidence put forward in the present study together with known properties of phosphatases seem to justify the assumption that the phosphoprotein phosphatase activity belongs to a specific enzyme. The wide distribution of this enzyme suggests general metabolic functions.

SUMMARY

Phosphoprotein phosphatase is an enzyme which liberates inorganic phosphorus from casein and other phosphoproteins. In the present study a method of assay has been developed. The properties and the distribution of the enzyme in rat tissues have been investigated. Comparison with known properties of other phosphatases shows that phosphoprotein phosphatase is an enzyme sui generis.

This investigation has been financially supported by the State Medical Research Council. The technical assistance of Mrs. M. Wenner and Miss I. von Francken is gratefully acknowledged. The calcium phosphopeptone was a gift of Dr. Mellander.

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Received August 10, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1216–1220

The Calculation of Velocity Constants for First- and Second-order Two-stage Reactions

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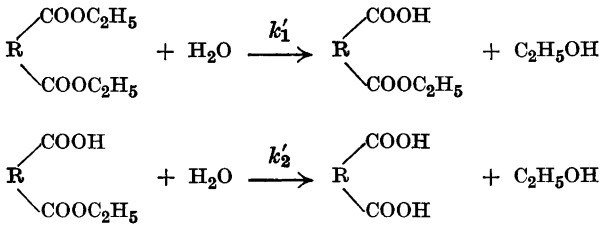
Several authors have been occupied with the problem of calculating the velocity constants for stage reactions. Among these authors should be mentioned Knoblauch [1], Meyer [2,3], Wegscheider [4], Skrabal [5], Ingold [6], Ritchie [7], and Homan [8].

As an example for a first-order two-stage reaction we may regard the catalytic hydrolysis by hydrochloric acid of symmetrical dialkyl esters. The dialkyl ester is first hydrolyzed to a monoalkyl ester, and the monoester is then hydrolyzed to a dicarboxylic acid:

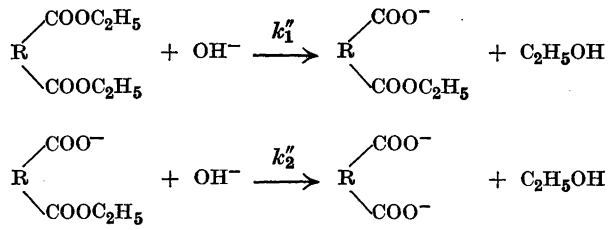


Let a be the initial concentration of the dicarboxylic ester, and let x be the fall in concentration of the same ester and y the rise in concentration of the dicarboxylic acid. Thus the concentration of the intermediate monoalkyl ester is $(x—y)$. When the first-stage reaction velocity constant is k_1' and k_2' that of the second-stage reaction, and ϑ is the time, we get the following differential equations:

$$\mathrm{d}x/\mathrm{d}\vartheta = k_1' \ (a—x) \tag{1}$$

$$\mathrm{d}y/\mathrm{d}\vartheta = k_2' \ (x—y) \tag{2}$$

A second-order two-stage reaction may be examplified by the saponification by alkali of symmetrical dicarboxylic esters:



With the same assumptions as above and if b is the initial concentration of alkali and t is the time and $z = (x + y)$, we get the following differential equations:

$$dx/dt = k_1'' \ (a—x) \ (b—z) \tag{3}$$

$$dy/dt = k_2'' \ (x—y) \ (b—z) \tag{4}$$

The second-stage velocity constant (k_2' resp. k_2'') is easily determined by the use of monoalkyl ester, when a simple single-stage reaction is obtained. The determination of the first-stage constant (k_1' resp. k_1'') has been performed in several ways by use of approximative methods, but none of the investigators who have studied the reactions above have been able to obtain an exact, explicit mathematical expression for k_1. This problem is, however, to be solved in a simple way by the following method.

The two equation systems (1,2) and (3,4) may be treated in the same manner. For that reason we shall give both systems the same mathematical form. That is possible by a formal reduction of the reaction order for the second-order reaction. To do that we put

$$(b—z) \ dt = d\vartheta \tag{5}$$

On introduction of this expression into equ. (3) and (4) we obtain

$$dx/d\vartheta = k_1'' \ (a—x) \tag{3a}$$

$$dy/d\vartheta = k_2'' \ (x—y) \tag{4a}$$

These equations are from the mathematical point of view identical with equ. (1) and (2) for the first-order reaction.

When equ. (5) is integrated, we obtain

$$\vartheta = \int_0^t (b—z)\ \mathrm{d}t \tag{5a}$$

The quantity ϑ is the area between the curve $(b—z) = f(t)$ and the t-axis. As seen from that the *area* ϑ in the second-order reaction plays the same rôle as the *time* ϑ in the first-order reaction. Thus the substitution $(b—z)\ \mathrm{d}t = \mathrm{d}\vartheta$ involves a formal reduction of the reaction order.

The equation system (3, 4) has never been integrated, but approximative solutions may be obtained.

The equation system (1, 2) resp. (3a, 4a) is easily integrated. When the indices $'$ and $''$ are discarded, we get from equ. (1)

$$k_1\vartheta = \ln\ a/(a—x) \tag{6}$$

or

$$x = a\ (1—e^{-k_1\vartheta}) \tag{7}$$

Putting this value of x into equ. (2) we get

$$\mathrm{d}y/\mathrm{d}\vartheta = k_2\ [a\ (1—e^{-k_1\vartheta})—y]$$

and

$$\mathrm{d}y/\mathrm{d}\vartheta + k_2y = k_2a\ (1—e^{-k_1\vartheta}) \tag{8}$$

On integration, we obtain

$$y = a\left[1 + \frac{k_2}{k_1—k_2}\ e^{-k_1\vartheta} — \frac{k_1}{k_1—k_2}\ e^{-k_2\vartheta}\right] \tag{9}$$

or

$$y = a\left[\frac{k_1}{k_1—k_2}\ (1—e^{-k_2\vartheta}) — \frac{k_2}{k_1—k_2}\ (1—e^{-k_1\vartheta})\right] \tag{9a}$$

During the reaction the quantity $(b—z)$ or z is measured. By addition of the equ. (7) and (9) we obtain

$$z = a\left[2— \frac{k_1}{k_1—k_2}\ e^{-k_2\vartheta} — \frac{k_1—2k_2}{k_1—k_2}\ e^{-k_1\vartheta}\right] \tag{10}$$

As the velocity constant k_2 may be determined by a separate experiment, the equ. (10) contains only one unknown quantity, namely k_1. In spite of that, k_1 cannot be explicitly expressed because of the transcendent part $e^{-k_1\vartheta}$. We

shall therefore eliminate $e^{-k_1\vartheta}$ which is easily done in the following manner. Regard the function

$$z = f\ (\vartheta)$$

The area between the curve and the ϑ-axis is

$$A = \int_0^{\vartheta} f\ (\vartheta)\ \mathrm{d}\vartheta = \int_0^{\vartheta} z\ \mathrm{d}\vartheta \tag{11}$$

Combining equ. (10) and (11) we get

$$A = a\int_0^{\vartheta}\left[2 - \frac{k_1}{k_1 - k_2}\ e^{-k_2\vartheta} - \frac{k_1 - 2k_2}{k_1 - k_2}\ e^{-k_1\vartheta}\right]\mathrm{d}\vartheta \tag{12}$$

On integration, we obtain

$$A = a\left[2\ t + \frac{k_1}{k_2\ (k_1 - k_2)}\ e^{-k_2\vartheta} + \frac{k_1 - 2k_2}{k_1\ (k_1 - k_2)}\ e^{-k_1\vartheta} - \frac{2k_2 + k_1}{k_1 k_2}\right] \tag{13}$$

On elimination of $e^{-k_1\vartheta}$ between equ. (12) and (13) we get after simplification

$$k_1\ [2k_2 a\vartheta - k_2 A - a\ (1 - e^{-k_2\vartheta})] = k_2 z$$

or

$$k_1 = \frac{z}{2\ a\vartheta - \int_0^{\vartheta} z\mathrm{d}\vartheta - \frac{a}{k_2}\ (1 - e^{-k_1\vartheta})} \tag{14}$$

Equ. (14) is an exact, explicit expression for the velocity constant k_1. This expression is valid for both first- and second-order two-stage reactions. In a first-order reaction ϑ is identical with the time; in a second-order reaction ϑ is the area between the curve $(b - z) = f\ (t)$ and the t-axis, when t is the time. A and ϑ may be evaluated by means of graphical integration.

Example

The data of Ingold [6] for the saponification of dimethyl glutarate by sodium hydroxide at 20.30° have been treated by the method above. The initial concentrations for both dimethyl glutarate (a) and sodium hydroxide (b) are 0.001999 mole/l, and the second-stage velocity constant $k_2 = 1.12$. The results are reported in Table I, where t = time in minutes, $A = \int_0^{\vartheta} z\mathrm{d}\vartheta$ and $B = \frac{a}{k_2}\ (1 - e^{-k_1\vartheta})$.

Table 1. The saponification of dimethyl glutarate by sodium hydroxide according to Ingold [6].

t	$(b-z)\cdot 10^3$	$z\cdot 10^3$	$\vartheta\cdot 10^3$	$A\cdot 10^6$	$2a\vartheta\cdot 10^6$	$B\cdot 10^6$	k_1
2.5	1.929	0.070	4.91	0.17	19.63	9.82	7.26
4.2	1.883	.116	8.15	0.48	32.58	16.08	7.24
6.0	1.839	.160	11.50	0.95	45.98	23.22	7.33
10.0	1.743	.256	18.66	2.45	74.59	36.61	7.21
12.0	1.699	.300	22.10	3.40	88.35	43.75	7.28
14.1	1.655	.344	25.62	4.54	102.43	50.90	7.32
16.0	1.619	.380	28.73	5.66	114.86	56.25	7.18
18.0	1.577	.422	31.93	6.94	127.64	62.50	7.25
20.0	1.539	.460	35.04	8.32	140.10	68.75	7.30
23.0	1.487	.512	39.58	10.52	158.24	77.68	7.31
26.0	1.435	.564	43.96	12.88	175.77	85.71	7.31
29.0	1.387	.612	48.20	15.37	192.69	93.76	7.32
32.0	1.345	.654	52.30	17.97	209.08	101.8	7.32
35.0	1.307	.692	56.27	20.64	224.90	109.0	7.25
						Mean value	7.277

For the first-stage reaction velocity constant Ingold reported the value $k_1 = 7.24$.

SUMMARY

A formula for the calculating of the first-stage velocity constant for a two-stage first- or second-order reaction has been deduced. The formula which makes use of graphical integration has been tested against the data of Ingold [6] for the saponification by alkali of dimethyl glutarate at 20.30°, whereby the value $k_1 = 7.277$ was obtained which is in good agreement with that of Ingold who reported $k_1 = 7.24$.

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Received July 3, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1221–1232

The Conversion of Ferri-porphyrins ("Hemins") to Their Corresponding Porphyrins by Means of Pyruvic Acid

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Several procedures have been tried for the removal of iron from its complexes with porphyrins. Thus metallic iron and formic acid convert protohemin to protoporphyrin [1]. In this reaction substitution of iron by palladium in a hydrogen atmosphere, under certain conditions leads to the formation of mesoporphyrin [1] in theoretical yield [2]. Hydrogen bromide in glacial acetic acid effects de-ironization; if vinyl side chains are present, the hydrogen bromide adduct is obtained [3–5]. Stannous chloride [6] or sodium amalgam [7, 8] have been suggested as reducing agents. Occasionally other methods have been used [*Cf.* 3, 9], but none of them lends itself to convenient microscale work or, if it does, is sufficiently harmless. The de-ironization with ferrous acetate [10] is unsuitable for routine work.

For certain purposes we needed a mild method, suitable for small scale, routine work. We have therefore tried some new procedures. Thiols release iron from its porphyrin complexes, but in the presence of air there is a simultaneous opening of the porphyrin ring [11]. In a few experiments we obtained from protohemin and thioglycolic acid in carbon dioxide atmosphere a mixture of protoporphyrin, small amounts of a sulphur containing porphyrin and considerable quantities of a water soluble, orange coloured pigment. The latter had no absorption bands in the visible region except for a flat maximum at about 490 mμ.

The observation of Schumm and Mertens [12] that pyruvic acid will effectively release iron from hemins seemed to be more promising.

PROCEDURE

After some trials the following procedure was found to be the best. The hemin is dissolved in 0.25 % ammonia (or weaker) and filtered if necessary. 0.6 ml pyruvic acid are added per ml of filtrate to give a final concentration of about 5 *M* acid. The suspension is then placed in a boiling water bath, where porphyrin formation starts within a few minutes. After about 30 min the reaction is finished (*cf.*, however, page 1206) (Table 1). A lower concentration of acid (2.5 *M* final concentration) gives no porphyrin formation within a reasonable time. If the hemins are not initially dissolved in ammonia, the porphyrin formation will take place nevertheless but much more slowly, and stronger acid must be used (Table 2).

Table 1. Yield of protoporphyrin from protohemin. Two series of samples of 0.2—1.0 ml of a solution, containing 375 μg protohemin per ml were diluted to 1.0 ml with 0.25 % ammonia and acidified with 0.62 ml 13 M pyruvic acid and heated for 30 and 60 min. respectively at 100°. The solutions were then diluted to 15 ml with 5 % hydrochloric acid, left for 4 h and centrifuged. No unreacted sedimented hemin could be detected as pyridine hemochrome. Calculated extinctions are based on the value $\beta_{555} = 3.30 \times 10^7\ cm^2 \times mole^{-1}$ *for protoporphyrin in 5 % hydrochloric acid * (Beckman spectrophotometer type DU, slit 0.01 mm, 1 cm cuvettes). The complete curves 380—620 mμ were determined and the wavelengths for the maxima and minima were found to agree with those for pure protoporphyrin. The addition of ferric chloride to the blanks did not influence their optical densities.*

Protohemin μg	Log I_0/I at 555 mμ		
	Reaction time in min 30	60	Calc. for 100 % conversion to protoporphyrin. Blank values not included
0	—	0.002	—
75	0.092	0.092	0.110
150	0.192	0.197	0.221
225	0.283	0.297	0.331
375	0.452	0.532	0.551

* $\beta = \frac{1}{c} \times \frac{1}{d} \times ln \frac{I_0}{I}$;

where c = concentration in moles per ml of the solution.
d = optical depth in centimeters.
I_0 = intensity of incident light.
I = intensity of transmitted light.

Table 2. De-ironization of protohemin with pyruvic acid without previous solution in ammonia. The recalculations of optical densities to μg are based on $\beta_{408} = 61.1 \times 10^7\ cm^2 \times mole^{-1}$ *for protoporphyrin in 5 % HCl, slit 0.025 mm* *.

Protohemin Dry subst. in μg	Pyruvic acid	Time min.	Dil. to ml with 5 %HCl	log I_0/I at 408 mμ	Porphyrin in μg	
					Found	Calc.
135	3 ml 9 M	150	50	0.908	96	116
183	4 » 10 »	150	50	1.330	141	158
112	4 » 10 »	150	50	0.790	84	97
115	4 » 10 »	60	50	0.636	67	100
987	4 » 10 »	210	330	0.650	460	850

The pyruvic acid should be distilled *in vacuo* without fractionation not more than one month before its use, and kept in a dark bottle. The distillate from the commercial preparation is generally about 13 *M*. The pyruvic acid could also be purified by the crystallization of its sodium salt [13]. Air exclusion with a stream of carbon dioxide has no influence, nor has the addition of sodium pyrophosphate. We have made our experiments, unless otherwise stated, in open test tubes. Since pyruvic acid is fairly strong (p*K* 2.5) it can hardly be used for the isoelectric flocculation of the porphyrins. On the other hand, if the porphyrins are extracted with ether after their precipitation with acetate, the major part of the pyruvate will remain in the aqueous phase. Pyruvic acid can be traced by its typical odour, if the ether residues after their extraction with hydrochloric acid are evaporated and the "empty" flasks slightly heated.

The method may be illustrated by the following examples.

The isolation of di-methyl esters from small quantities of ferri-porphyrins

Mesoporphyrin. 6.68 mg mesohemin in 4.1 ml 0.25 % ammonia were acidified with 2.54 ml 13 *M* pyruvic acid and heated for 60 min on the water bath. After dilution with one volume of water and the addition of ammonium acetate, the porphyrin was extracted with ether, and the ether solution washed with 5 × 100 ml water. The first washings contained some porphyrin, which was re-extracted with ether. From the combined ether solutions the porphyrin was extracted with 3 × 25 ml 1 % hydrochloric acid. The acid solution was washed with ether without loss of porphyrin. In this ether the last traces of pyruvic acid were found.

Spectrum in 1 % HCl **:	592,	570,	547				mμ
Mesoporphyrin in 5 % HCl[1]:	591.7,	570.4,	547.8				mμ
Spectrum in ether:	625,	610,	598,	580,	570,	528,	496 mμ
Mesoporphyrin in ether [1]: ***	623.5,	611.0,	596.8,	577.4,	568.2,	527.7,	496.6 mμ

The porphyrin was forced into ether by acetate, and the ether washed, dried and left overnight. Next day the porphyrin had crystallized in nice flocks, which in the microscope showed the typical mesoporphyrin shape. They were not collected, but esterified with diazomethane and crystallized from chloroform-methanol. After one recrystallization from the same system 4.9 mg were obtained (= 81 % yield), m. p. 218° **** (216° [3], 216.5° [2]).

Deuteroporphyrin. 10.72 mg deuterohemin in 4.6 ml 0.25 % ammonia were acidified with 2.85 ml pyruvic acid and treated step by step in the same way as the mesoporphyrin.

Spectrum in 1% HCl: 590, 567, 546 mμ
Deuteroporphyrin in 5 % HCl [3]: 589.5, 569.0, 546.6 mμ
Spectrum in ether: 624, 610, 597, 568, 558, 526, 492 mμ
Deuteroporphyrin in ether[3]: 621.7, 610.8, 595.7, 576.8, 566.8, 557.7, 525.9, 494.1 mμ

The crystals from the ether showed double refraction but were too small to permit any observations of their shape. Yield of twice crystallized dimethyl ester 3.6 mg (37 % yield). M. p. 219° (218°—220° [3]).

Protoporphyrin. 8.2 mg protohemin in 10 ml 0.25 % ammonia + 6.2 ml 13 *M* pyruvic acid were heated in a boiling water bath. Already after 3 min a considerable porphyrin formation had taken place. After 30 min the heating was interrupted and the solution cooled in tap water, diluted with one volume of water and filtered. No unreacted hemin remained on the filter. Ammonium acetate was added and the porphyrin was extracted with 4 × 30 ml ether. The ether was washed with 3 × 1 vol. of water. Hydrochloric acid in a con-

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** Spectroscopic date given in this paper were obtained with a Zeiss prism pocket spectroscope unless otherwise stated.

*** This spectrum is probably not the true spectrum of mesoporphyrin in dry, neutral ether, where only four bands should be seen. Our observation was made on the "combined ether solutions" before the extraction with 1 % HCl. (cf. deuteroporphyrin).

**** Melting points were determined with a Kofler-block and the values are thus to be regarded as corrected.

centration of 0.12 % or 0.18 % extracted no porphyrin from the ether, but with 0.36 % acid a small amount of porphyrin migrated over into the acid. The ether was therefore extracted with 6 × 50 ml 0.5 % hydrochloric acid, the last portion being almost colourless. By far the major amount of porphyrin remained in the ether. The porphyrin, which had been extracted with 0.5 % acid (= fraction I) was forced into ether (50 ml) by ammonium acetate.

Spectrum in ether	631,	605,	572,	535,	500	mμ

A small amount of 1 was taken into 5 % hydrochloric acid, where it gave the spectrum 600, 576, 554 mμ. The residue of I in ether was esterified with diazomethane after washing with water and drying. From chloroform-methanol shapeless, double refracting masses appeared, which melted at 205—220°.

The main bulk of the porphyrin (= fraction II) in the ether was extracted with 3 × 20 ml 5 % acid. After washings as for fraction I this portion gave the spectrum

in ether:	633,	605,	575,	538,	506	mμ
in 5 % HCl	600,	580,	555	mμ		

When standing overnight in dry ether the porphyrin crystallized in nice rhombohedra. They were not collected but esterified with diazomethane. The ester crystallized in the typical rhombohedral form from chloroform-methanol. After one recrystallization from the same system 2.4 mg (= 32 % yield) were obtained. M. p. 226—227°, (228°[2], 230°[3]).

The interposition of the spectrum of fraction I between the spectra of proto- and mesoporphyrins as well as the indefinite melting point indicated a mixture of protoporphyrin and some porphyrin with saturation of the side chains 2 and 4. Since it would be of interest for the understanding of the reaction mechanism to find out whether that porphyrin with meso-spectrum really was mesoporphyrin or not, the following experiment was made.

84 mg twice crystallized[14] protohemin in 40 ml 0.25 % ammonia were acidified with 25 ml acid. After 30 min at 100° no porphyrin could be seen, but after another 30 min the whole preparation had gone into solution and showed a strong cherry-red colour. It was diluted with 1 vol. of water, cooled and left for one hour. The material, which had settled, was washed with 2 × 50 ml 5 % hydrochloric acid. The black residue gave a pure pyridine hemochrome spectrum, corresponding to 19.2 mg unreacted protohemin.

The combined porphyrin solutions were buffered with ammonium acetate and extracted with ether, which was subsequently washed with 6 × 1 vol. of water. No porphyrin could be extracted with 0.1 % hydrochloric acid but with 3 × 1 vol. of 0.4 % acid a considerable amount (fraction I) was withdrawn. After extraction with 4 % acid (fraction II), the ether had a slight yellow colour. The yellow substance was extracted from the ether with 13 % acid in which it gave a green solution with a broad, diffuse band at 570 mμ. This last fraction was rejected. Fractions I and II were separately forced in to ether, washed with water, dried and esterified with diazomethane.

Fraction I. Since mesoporphyrin has the hydrochloric acid number 0.5 [3], it should be found in this fraction if it had been formed. The whole esterified preparation was dissolved in chloroform and put on a 5 cm alumina column from chloroform: petrol ether 1 : 20. With those solvents down to the proportion 1 : 2 nothing could be eluted, but with the ratio 1 : 1.5 a red material came out, which seemed to be homogenous (= IA).

IA. This gave a pure proto-spectrum but could not be crystallized, and was re-chromatographed as above. The first fraction to come out with pure chloroform was red, gave a pure proto-spectrum and crystallized easily from chloroform-methanol with m. p. 230°. Weight 5.3 mg (= IA1). Two small grey fractions migrated down after IA1, but showed no spectrum and were rejected. After them an emerald green compound descended slowly. It absorbed strongly at 675 mμ with weak bands at 510, 540 and 580 mμ.

Nothing more could be eluted with the above mentioned mixture or with pure chloroform, but with chloroform: glacial acetic acid 30 : 1 another red fraction left the column (= IB). Its spectrum was shifted about two mμ towards the blue as compared to protoporphyrin, but it did not crystallize. After IB an almost brown fraction was eluted with chloroform: glacial acetic acid 2 : 1. It showed a mixture of spectra with numerous bands, most of which were diffuse. It did not contain proto- or mesoporphyrin, and was discarded.

Fraction II. As a pilot experiment a part of this fraction was chromatographed on alumina as above with chloroform as eluant. Since the above mentioned green and brown regions were seen, the whole preparation, dissolved in chloroform, was washed with 7.5 % ammonia, which removed a faint brown colour. Upon the addition of methanol to the dried residual solution crystals appeared, which melted indefinitely at 218—223° and were of no regular shape (= IIA). By chromatography on alumina 7 mg of easily crystallizable material of m. p. 230° and with a pure proto-spectrum could be obtained from the mother liquor from IIA (= IIB). IIA was washed with methanol and chromatographed on alumina with chloroform as eluant. The following fractions were obtained:

IIA1. Pure proto-spectrum. Crystallized easily from chloroform-methanol with m. p. 230°. Weight 13 mg.

IIA2. A small yellowish fraction, the diffuse bands of which could not with certainty be attributed to a meso- or proto-spectrum. Rejected.

IIA3. A green fraction with a strong band at 675 mμ, weak bands at 510, 540 and 580 mμ, and a very weak band at 620 mμ.

IIA4. A green region remained at the top of the column and did not move at all until chloroform: glacial acetic acid 4 : 1 was employed. Its spectrum was the same as that of IIA3.

Fraction IIA*1*, which seemed to be pure, was recrystallized very slowly from a dilute chloroform-methanol solution. The very large crystals, which were formed in a week, gave a proto-spectrum, which was free from any band at 675 mμ, and they melted sharply at 230°. They were dissolved in 3 ml of chloroform and illuminated by a common 40-watt tungsten lamp for two hours. Afterwards the solution absorbed strongly at 675 mμ, and chromatography on alumina with chloroform as eluant gave two fractions:

IIA1a. Red, with pure proto-spectrum. Crystals, obtained from chloroform-methanol, melted at 230°. This portion was thus unchanged proto-ester.

IIA1b. Green pigment which descended very slowly with chloroform. It showed bands at 540 and 580 mμ plus the strong band at 675 mμ. Since it appeared on the column as one discrete strip and lacked the band at 510 mμ, which had been found in the other green fractions, it was believed to be pure, and attempts were made to crystallize it from chloroform-methanol and from chloroform-petrol ether, but without success.

Hematoporphyrin. Hematoporphyrin was prepared from protohemin according to the method of Nencki and Zaleski[4] as modified by Fischer[3] and crystallized as the di-hydrochloride. The free porphyrin was obtained, when the crystals were dissolved in 0.1 *M* sodium hydroxide and the porphyrin precipitated with glacial acetic acid. Since the ordinary method for the introduction of iron in glacial acetic acid could be suspected partly to dehydrate the α-hydroxy groups, a modified procedure was used. The hematoporphyrin was dissolved in a mixture of equal parts of sodium chloride saturated 90 % acetic acid and water and boiled for 1 ½ min. with one volume freshly prepared ferrous acetate solution in 50 % ($^{v}/_{v}$) acetic acid. The hematohemin, which crystallized during the cooling, was washed twice with water, twice with dilute hydrochloric acid and once again with water. It was dried overnight at 40° and then to complete dryness in a desiccator. Its pyridine hemochrome seemed to be free of any band at 557 mμ.

31.3 mg of the preparation thus obtained were dissolved in 28 ml 0.25 % ammonia, acidified with 17.5 ml pyruvic acid, and heated for 50 min at 100°.

After that time every trace of the hemin spectrum had disappeared. A muddy bottom layer settled when the mixture was cooled. After centrifugation the settled material was washed with 4 × 200 ml. 2 *M* hydrochloric acid. The combined extracts were filtered and washed with 2 × 1 vol. of ether. The porphyrin was brought into ether with ammonium acetate, the ether washed with water and subjected to fractional extraction with hydrochloric acid.

Fraction I. The major part of the porphyrin could be extracted with 5 × 200 ml of 0.12 % acid. The last portion was almost colourless. In this fraction the hematoporphyrin should be found. The preparation was again taken up into ether, where it showed a meso-spectrum, washed, dried and esterified with diazomethane. A large amount of crystals appeared from chloroform-methanol. After one recrystallization from the same system 17 mg of rhombohedral crystals of uniform size were obtained. They showed a step-ladder spectrum with maxima at 624, 571, 537 and 504 mμ (determined spectrophotometrically). The 537 band, however, was a little too low to give the perfect step-ladder appearance. The Soret band was found at 403 mμ. No band was found in the region 260—290 mμ, where carbonyl groups generally exert a band. However, since the crystals did not melt below 300°, they could not be the hematoporphyrin di-methyl ester.

Chromatography of the mother liquor on alumina with chloroform-petrol ether mixtures revealed five different red compounds in about equal quantities. In addition to these a brown region remained at the top. No green pigment was found in fraction I. Common to all these red pigments was their slower descent with chloroform as compared to the porphyrins from protohemin.

Since the fractions II (0.4 % acid) and III (4 % acid) could contain no hematoporphyrin, they were not examined in more detail. It would not have been very surprising if protoporphyrin had been formed from the dihydric secondary alcohol hematohemin, but neither proto- nor mesoporphyrin could be isolated. Both fractions contained several green pigments, all of which migrated more slowly down the alumina column with chloroform and had the absorption band in the red shifted about 5 mμ towards the blue as compared to the green pigments from protohemin.

DISCUSSION

To facilitate the conclusive identification of the porphyrins we isolated them as their di-methyl esters. The reported yields are therefore minimal values for the yields of porphyrins. Since the dimethyl esters are more sensitive to light than the corresponding free porphyrins, it is reasonable to

assume that the substances which crystallized from the ether solutions before the addition of diazomethane were pure porphyrins.

Fischer and Dürr [15] illuminated a chloroform solution of protoporphyrin dimethyl ester with a light source of about the same intensity as we used in the experiment with fraction IIA1. From the solution they were able to isolate in the crystalline state a green pigment with a sharp band at about 670 mμ. The formation of this pigment was strongly inhibited, in slightly different degrees by acids, alcohol and benzene, and the protoporphyrin spectrum reappeared when a chloroform solution of it was acidified. We could observe the same properties for our preparation (IIA1b).

From these similarities and from the formation of fraction IIA1b from protoporphyrin di-methyl ester we conclude that the green pigments were derived from protoporphyrin di-methyl ester, though we were unable to crystallize them. In agreement with the results in Table 1, the yield of the free protoporphyrin should therefore be much higher than 33—35 %.

According to Schumm [16] there is a reversible equilibrium between proto- and hematoporphyrin in hot hydrochloric acid. Since 0.12 % hydrochloric acid failed to extract any porphyrin from ether (*cf.* p. 1209) in the experiment with protohemin, there is no equilibrium of that kind in pyruvic acid, or, if it exists, it is shifted completely in favour of protoporphyrin when the mixture is cooled.

The procedure which is described in this paper, is unsuitable for large scale preparations unless large quantities of pyruvic acid be used. When about 1 g of protohemin was heated in 30 ml ammonia + 18.6 ml 13 *M* pyruvic acid, no porphyrin formation took place in 60 min and the yield after heating for 2 h was about 16 %. On the contrary, when 10—40 μg protohemin in 1.62 ml solution were warmed in the hand, strong porphyrin bands appeared after a few seconds.

The unsuccessful result with hematohemin is difficult to explain. As already mentioned, no protoporphyrin was formed. They crystals with m. p. above 300° were not analysed. An attempt was made to recrystallize them from chloroform-methanol, when the solution suddenly became brown, the pigment gave diffuse absorption bands, and could not be brought to crystallize again. Bouveault [17] identified some lower alcohols by means of the melting points of the semicarbazones of their pyruvic acid esters. He found a reaction time of 2—3 h at 140—150° to be necessary for the complete esterification. This information, the relatively large water content of the reaction mixture and the absence of any absorption at 260—290 mμ exclude the possibility that the hydroxylic groups in hematohemin could have been esterified by pyruvic acid.

Schumm and Mertens [12] had examined several organic acids for their abilities to de-ironize protohemin. Pyruvic, formic, oxalic and lactic acid were found to be active, while acetic, propionic, malonic, succinic, tartaric and citric acid were inactive. We have confirmed their results except for lactic acid, which we found to give no porphyrin. Acrylic acid as well as glycine and alanine were also inactive. Gompel, Mayer and Wurmser [18] investigated the oxidisability of some organic acids in the presence of activated blood charcoal. Samples of 125 ml of solutions, containing 1 % carbon from the compound to be investigated, were shaken with 5 g charcoal at 40° until carbon dioxide formation ceased. Pyruvic, formic and oxalic acid gave 0.40—0.62 ml gas, while acetic, propionic, lactic, succinic and citric acid, glycine and alanine gave 0.07—0.30 ml. If the concentrations of the first three mentioned acids are recalculated to normalities, it is obvious that there is a linear relationship between carbon dioxide evolution and concentration. The formation of carbon dioxide increased when pH was lowered within the region examined (pH 1—10). In our experiments we found that no porphyrin formation took place when 90 % of the pyruvic acid was neutralized by ammonia but the concentration of (pyruvic acid + pyruvate) still kept at 5 *M*.

The fact that those acids de-iron hemins which give the highest carbon dioxide formation, suggests a relationship between those two properties. In other experiments we found that no porphyrin was formed when protohemin was heated for 2 h with either pure acetaldehyde or with acetaldehyde-water-glacial acetic acid 1 : 1 : 1 in sealed test tubes. There is but little hope of success in attempts to isolate the reaction products from pyruvic acid. On the assumption that the removal of one mole of iron from hemin corresponds to the liberation of one mole of carbon dioxide, the conversion of 260 μg of protohemin to porphyrin would correspond to 10 mm^3 carbon dioxide. This quantity is impossible to determine with any accuracy under our experimental conditions.

A feature which is common to the methods of de-ironization of hemins, is the reduction of the hemin in the presence of an acid. Dilute hydrochloric acid converts pyridine hemochrome to porphyrin very easily [3]. Zeile and Meyer [19] prepared porphyrin c from cytochrome c by hydrolysis with sulphuric acid after the previous reduction of the cytochrome by dithionite. We may therefore suggest as a hypothesis, that the function of the above-mentioned acids is to act as electron donors to the trivalent iron, and that this reaction, perhaps, is in some way coupled to their decarboxylation. The excess of acid will then remove the bi-valent iron. We found that pyruvic acid gave no porphyrin from the copperprotoporphyrin complex, prepared according to Fischer and Pützer [1]. However, there must be some other factors involved in

the reaction; though the pyruvic acid must always be present in large excess of the hemin, nevertheless the ratio pyruvic acid/hemin as we have shown, exerts a profound influence upon the reaction velocity. No porphyrin formation took place when 80 % of the pyruvic acid was replaced by acetic acid, the total concentration of acid still being 5 *M*.

SUMMARY

1. A procedure is described for the conversion of small quantities of iron-porphyrins to their corresponding porphyrins.

2. The over-all yields (removal of iron, isolation of the crude porphyrin, esterification, two crystallizations of the ester) were from 6.68 mg mesohemin 81 %, from 10.72 mg deuterohemin 37 %, from 8.2 mg protohemin > 32 % and from 31.3 mg hematohemin unknown but low.

3. With amounts of hemin less than 100 μg, the reaction seems to give better yields and can conveniently be carried out under mild conditions.

4. The procedure is inconvenient and expensive for large quantities of hemins.

5. If the procedure is to be applied to a small, limited quantity of a valuable material, *e. g.* from an isotope experiment, a pilot experiment should if possible be made with quantities large enough to permit the conclusive identification of the porphyrin which may be formed. This is suggested because we failed to isolate any hematoporphyrin ester from hematohemin.

Inger Agerberg has given her valuable assistance during the work.

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Received August 10, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1233–1240

The Preparation of 2,5-Diacetoxy-2,5-Dihydrofuran, 2,5-Diacetoxytetrahydrofuran and Pyridazine

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2,5-DIACETOXY-2,5-DIHYDROFURAN

It has previously been reported [1], that 2,5-diacetoxy-2,5-dihydrofuran may be obtained in a 70 per cent yield by oxidizing furan with bromine in acetic acid in the presence of potassium acetate. We have since observed that this method of preparation now and then fails totally because the diacetoxydihydrofuran, formed by the reaction, occasionally changes into a black insoluble mass. At the same time acetic acid is formed. This conversion either takes place during the final distillation, or on standing after the distillation has been completed. Sometimes it takes several days before the distillate turns black, but usually the black mass is formed during the distillation or immediately after, with the evolution of heat and with almost explosive violence. If the preparation of larger batches is attempted, decomposition *always* occurs. It thus became clear that the directions given previously for the synthesis of diacetoxydihydrofuran were insufficient, and that much more minute information than is usually necessary in synthetic organic chemistry, was required.

If a small amount of a strong acid (H_2SO_4, HCl, $SnCl_4$, BF_3, $AlCl_3$, $ZnCl_2$) is added to diacetoxydihydrofuran a transformation similar to the one described above takes place. We therefore believe, that the occasional destruction of diacetoxydihydrofuran during its preparation is due to the liberation of hydrobromic acid from certain bromo compounds, which are formed in small amounts together with diacetoxydihydrofuran by the action of bromine on furan. This was substantiated by the fact that all our samples of diacetoxydihydrofuran contained some bromine, usually 1—3 per cent.

After the sensitivity of diacetoxydihydrofuran towards acids had been observed, we modified the original procedure for its preparation. According

to the new method, a mixture of acetic acid and acetic anhydride is used as a solvent. The bromine is added first (at — 17°) and, immediately after, all the furan at once. Before the final distillation the reaction product is refluxed in vacuum over potassium acetate. In this way 0.8 mole of diacetoxydihydrofuran is obtained from 1.1 moles of furan and 2.0 moles of bromine. The product, which contains 0.5—2.0 per cent of bromine, is stable and sufficiently pure for most purposes.

Samples of diacetoxydihydrofuran, prepared according to the new method, as well as stable samples prepared after the original procedure, gradually turn yellowish-brown when kept at room temperature without any special precautions with regard to light, moisture or oxygen; but the refractive index remains almost constant and even two year old samples are apparently practically unchanged. If, however, such samples are redistilled, they decompose during the distillation or shortly after, which indicates that some hydrogen bromide has again been liberated.

This can be explained by assuming, that two different types of bromo-compounds are present as impurities in the diacetoxydihydrofuran. The first type are bromo-dihydro- or tetrahydrofurans, *e. g.* tetrabromotetrahydrofuran, which split off hydrogen bromide spontaneously on heating under regeneration of the double bonds of the furan nucleus. These compounds react when heated with potassium acetate, and they are thus not present in the stable samples of diacetoxydihydrofuran. The other type of bromo compounds are bromofurans like 2,5-dibromofuran or tetrabromofuran. Such compounds are autoxidized by atmospheric oxygen to substances, which either split off hydrogen bromide spontaneously or, like acyl bromides, by the action of water (*cf.* Hill and Hartshorn [2]; Torrey [3]). We think that these autoxidized bromofurans cause the decomposition of old samples of diacetoxydihydrofuran, as soon as a redistillation is attempted.

In agreement herewith we have found that bromine-free diacetoxydihydrofuran is obtained by redistilling old samples of ordinary diacetoxydihydrofuran from potassium or lithium acetate. Presumably the autoxidized bromofurans, when heated with the alkali acetate, react in such a way, that no bromo-compounds distil together with the diacetoxydihydrofuran. It was later found, that a bromine-free product could also be prepared by passing oxygen through the crude diacetoxydihydrofuran for one week and then refluxing and distilling from potassium acetate. Like the crude product, bromine-free diacetoxydihydrofuran slowly turns yellow on standing at room temperature for several months, but it can at any time be redistilled without decomposition.

The crude, as well as the purified diacetoxydihydrofuran, usually crystallizes after standing for some weeks at — 20°. At room temperature, only about 2/3 of the samples remain crystalline. This may be due to the presence of impurities, but it is also possible, that the diacetoxydihydrofuran is a mixture of the *cis* and the *trans* isomer.

2,5-DIACETOXYTETRAHYDROFURAN

Diacetoxydihydrofuran may, similar to 2,5-dialkoxy-2,5-dihydrofurans [4,5], be hydrogenated catalytically to the corresponding tetrahydrofuran with Raney nickel. The rate of hydrogenation is much lower than the rate of

hydrogenation of the dialkoxycompounds so there is no sudden evolution of heat when the reaction is started. We also found, that the course of the reaction depends largely on the purity of the diacetoxydihydrofuran. With the crude, bromine-containing diacetoxydihydrofuran good yields of diacetoxytetrahydrofuran were obtained in some experiments, while other experiments, which were carried out under what one would normally call identical conditions, only led to the formation of lower boiling compounds such as acetic acid and succinaldehyde. Sometimes no hydrogenation at all took place. It was evident that during the hydrogenation varying amounts of hydrogen bromide were liberated, which interfered with the hydrogenation reaction as well as with the isolation of the tetrahydrofuran. We therefore tried to change the hydrogen pressure, the solvent, the time of hydrogenation and the amount of catalyst. We also tried adding substances such as pyridine or potassium acetate before or after the hydrogenation, but it was not possible to find any way of preparation, which gave reproducible results.

This was first achieved, when bromine-free diacetoxydihydrofuran was used for the hydrogenation. With alcohol as a solvent about 80 per cent of diacetoxytetrahydrofuran may then be obtained regularly. Hydrogenation also takes place in acetic acid but not in ethyl acetate.

Diacetoxytetrahydrofuran is a new compound. After hydrolysis with dilute hydrochloric acid and addition of phenylhydrazine to the hydrolysate a perfectly white precipitate (succinaldehyde bis-phenylhydrazone) is formed. This indicates, that no traces of the original dihydrofuran are present, since diacetoxydihydrofuran gives a yellow precipitate (malealdehyde bis-phenylhydrazone) after hydrolysis.

PYRIDAZINE

The easiest way to prepare pyridazine is by condensing malealdehyde or a derivative of malealdehyde with hydrazine. This method was first employed by Marquis [6], who added a large excess of hydrazine to nitroacetin (2-nitro-5-acetoxy-2,5-dihydrofuran) in methanol (yield of pyridazine 60 %). Later Wohl and Bernreuther [7] used malealdehyde, obtained by hydrolysis of the tetraethylacetal, and an aqueous solution of hydrazine (yield 80 %). Recently malealdehyde has become easily accessible through hydrolysis of 2,5-dialkoxy-2,5-dihydrofurans or 2,5-diacetoxy-2,5-dihydrofuran [8,9]. Of these derivatives of malealdehyde, diacetoxydihydrofuran is the easiest to prepare in large amounts in the laboratory. We have therefore worked out directions for a synthesis of pyridazine from diacetoxydihydrofuran. The diacetoxydihydrofuran is first hydrolyzed at room temperature with $N/10$ sulfuric acid, then the

acetic acid formed is neutralized and an aqueous solution of hydrazine added. The yield of pyridazine is about 85 per cent.

EXPERIMENTAL

(Microanalyses by G. Cornali)

Preparation of 2,5-diacetoxy-2,5-dihydrofuran*

205 g of anhydrous potassium acetate (2.09 moles) are placed in a 2 liter three-necked flask fitted with a calcium chloride tube, a thermometer and a stirrer of the Hershberg type [10]. 600 ml of acetic anhydride and 400 ml of acetic acid are added, the mixture is heated until the potassium acetate has dissolved, and then cooled with stirring to about − 17° in a cooling-bath of − 22°. Part of the potassium acetate precipitates, and care should be taken that no cakes of potassium acetate are formed on the walls of the flask. It is desirable that some acetic acid crystallizes, but not so much as to slow down the speed of the stirrer. Now 160 g of bromine (2.00 moles) are added and − as soon as the solution has become homogenous − 80 ml of furan (1.10 moles) (note 1) are quickly poured into the mixture with efficient stirring. The addition of furan should take 6—7 seconds. The temperature rises to about 12° and then after 1 minute begins to fall again. The major part of the bromine has disappeared after 20—30 seconds and after 2—5 minutes the reaction mixture is perfectly white. When the temperature has fallen to zero, the flask is placed in an ice-water-bath and kept there for 30 minutes. Then the mixture is heated in a water-bath to 80° in the course of 15 minutes, kept at 80° for 10 minutes and cooled with water to room temperature. During these operations stirring is continued. The potassium bromide is removed by filtration and washed with 200 ml of acetic acid containing 10 per cent of acetic anhydride. The yield of potassium bromide may, if desired, be determined by washing with ether and drying. Yield: 224 g = 94 %.

KBr (119.0) Calc. Br 67.2 Found Br 67.2

The filtrate is evaporated from a water-bath of 70°, first at 10—15 mm and then, after the removal of about 900 ml of the solvent, at 1—2 mm. When the distillation is complete, a dark, almost black oil, remains in the flask. This consists of diacetoxydihydrofuran and a small amount of acetic anhydride together with a precipitate of a mixture of potassium bromide and an acid potassium acetate. 200 ml of dry ether are added, and the precipitate (15—20 g) is removed by filtration and washed twice with 50 ml of ether. The etheral solution of diacetoxydihydrofuran is evaporated from a water-bath of 50° and the residue poured into a 250 ml distillation flask, which has previously been coated on the inside with 5 g of potassium acetate by melting the potassium acetate in the flask and letting it crystallize while the flask is swirled. The flask is now fitted to the distillation apparatus by a 40 cm long vertical air-condenser and the product refluxed in vacuum (10—15 mm) by heating with an infrared lamp. The temperature at the top of the condenser hereby rises to 60—80° and a fore-run (15—20 ml) is collected. After ½ hour the air-condenser is removed and the remaining diacetoxydihydrofuran is distilled under 10—15 mm. Distillation is continued until the temperature begins to fall in spite of continous heating. At this point the distillation flask is almost dry and only contains

a black mass. The boiling range from the first to the last drop is 4—5° at about 135°. Yield of diacetoxydihydrofuran 155 g = 83 %, $n_D^{25} = 1.4534 \pm 0.0005$. The product is not perfectly colourless, but has a yellowish-brown tinge. The time required for the synthesis is 6—7 hours.

$C_4H_4O_3$ $(CH_3CO)_2$ (186.2)	Calc.	CH_3CO	46.2	Br	0
	Found	»	45.4	»	0.9

After standing for one month at room temperature without any special precautions with regard to light, moisture or oxygen the colour is somewhat darker yellowish-brown, but the refractive index remains constant within 0.0005. If the product is stored at — 20° it usually crystallizes to a solid mass after some weeks. When these crystals are used to inocculate liquid samples kept at room temperature, about 2/3 of the sample will crystallize in the course of a few days with the formation of very large rhombic plates. After being filtered off and washed with petroleum ether, the melting point of the crystals is 29°—35° (Kofler hot stage), 35°—38° (Hershberg apparatus[10]). The refractive index of the crystals, when molten, as well as of the mother liquid is the same as of the original sample.

Note 1. The purity of the reagents does not seem to be essential. We used good technical grades of furan, bromine, acetic acid and potassium acetate and an analytical grade of acetic anhydride. The reagents were not purified in any way before use.

Purification of crude diacetoxydihydrofuran

Bromine-free diacetoxydihydrofuran was obtained in two ways. In one experiment various 6 months old samples of crude diacetoxydihydrofuran, containing 1—3 per cent of bromine, were redistilled in vacuum from potassium acetate and then from lithium acetate. In another experiment oxygen was passed through a sintered glass disk into the diacetoxydihydrofuran for one week at room temperature. Then the product was refluxed over potassium acetate with an air condenser and distilled as described above for the synthesis of diacetoxydihydrofuran. Both procedures yielded a distillate, which gave a negative Beilstein test for halogen during the major part of the distillation, while the last fractions gave a weak positive Beilstein test. The refractive index remained almost constant during the distillation, ($n_D^{25} = 1.4537—1.4541$).

When attempting the purification of a large amount of diacetoxydihydrofuran the product may decompose to some extent during the distillation with the formation of acetic acid. Probably the traces of hydrogen bromide formed on heating are not neutralized by the alkali acetate on the walls of the flask as rapidly in a large as in a small flask. Usually a second distillation from alkali acetate proceeds without decomposition.

Preparation of 2,5-diacetoxytetrahydrofuran*

50 g of bromine-free diacetoxydihydrofuran and 200 ml of absolute ethanol (technical product) are hydrogenated with 4 g of Raney nickel under 100 atmospheres for 2 hours in a 1 liter flask. After hydrogenation the solvent is removed in vacuum and the reddish brown residue distilled under 10 mm. About 12 g of fore-run are collected, then

* Patent pending.

the main fraction distils at 125°—128°. There is no residue in the flask. Yield 39 g = 77 %; $n_D^{25} = 1.4390$.

$C_4H_6O_3$ $(CH_3CO)_2$ (188.2)	Calc.	C 51.1	H 6.43	CH_3CO	45.6
	Found	» 51.4	» 6.92	»	44.7

The product gives a pure white precipitate with phenylhydrazine after hydrolysis with N/10 hydrochloric acid.

Hydrogenation of 2,5-dialkoxy-2,5-dihydrofurans

For the sake of comparison we have hydrogenated dimethoxydihydrofuran and diethoxydihydrofuran with Raney nickel. The hydrogenation of dimethoxydihydrofuran to the tetrahydro compound has previously been reported in a patent by Jones [4] (yield 85 %) and the reaction conditions given below are almost identical with those of Jones. The hydrogenation of diethoxydihydrofuran was first described by Fakstorp, Raleigh and Schniepp [5] (yield 85 %).

50.0 g of analytically pure dimethoxydihydrofuran (commercial product from *Kemisk Værk Køge A/S*, Copenhagen) and 60 ml of methanol (technical product) were shaken with 1.5 g of Raney nickel under 100 atmospheres for 1 hour in a 1 liter flask. The temperature rose to about 80° during the first few minutes of shaking. After hydrogenation the methanol was removed through a packed column, and the residue distilled under ordinary pressure without the column.

Fractions	g	b. p.	n_D^{25}
1	2.6	145.2°—147.2°	1.4138
2	40.4	147.2°—149.6°	1.4160
3	3.3	148.0°—152.0°	1.4160
4	1.2	151.3°—160.2°	1.4160

There was no residue in the flask. All fractions gave a pure white precipitate with phenylhydrazine after hydrolysis with N/10 hydrochloric acid.

It will be seen, that the boiling point is not constant during the distillation, while the refractive index remains constant. We have in separate experiments found, that this is due to overheating, which always occurs, when the substance is distilled in the usual way without a column.

Fractions 2—4 (44.9 g) were mixed and analyzed.

C_4H_6O $(OCH_3)_2$ (132.2)	Calc.	C 54.6	H 9.16	CH_3O	47.0
	Found	» 55.4	» 9.57	»	46.1

The yield of analytically pure dimethoxytetrahydrofuran is thus 89 %.

50.0 g of analytically pure diethoxydihydrofuran (commercial product from *Kemisk Værk Køge A/S*, Copenhagen) and 60 ml of absolute ethanol were shaken with 1.5 g of Raney nickel under 50 atmospheres for 3 hours in a 1 liter flask. The temperature rose to about 60° during the first 10 minutes of shaking. After hydrogenation the ethanol was removed through a column and the residue distilled under ordinary pressure without the column. There was no residue. Since both fractions are analytically pure, the yield

Fractions	g	b. p.	n_D^{25}	C_2H_5O (calc. 56.2)
1	46.0	167°—174°	1.4170	56.6
2	3.0	174°—178°	1.4171	56.9

of pure diethoxytetrahydrofuran is 49.0 g = 97 %. The product gave a pure white precipitate with phenylhydrazine after hydrolysis with *N*/10 hydrochloric acid. The value for n_D^{25} given by Fakstorp, Raleigh and Schniepp[5] (1.4164) agrees with our values.

The rise in the boiling point towards the end of the distillation is due to overheating (see above).

Preparation of pyridazine

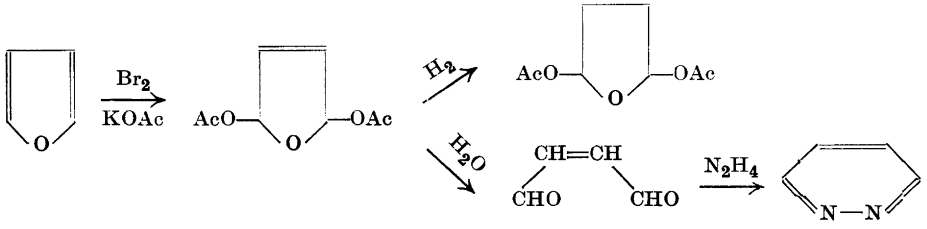
93 g of diacetoxydihydrofuran (0.50 mole) and 400 ml of N/10 sulfuric acid are placed in a 2 liter three-necked flask fitted with a separatory funnel, a thermometer and a stirrer of the Hershberg type. The mixture is stirred efficiently at room temperature until a homogeneous solution is obtained, which usually requires 20—30 minutes. After standing further for 30 minutes, the flask is placed in an ice-salt-bath, the solution cooled to zero and neutralized by adding a solution of 42 g of sodium hydroxide in 800 ml of water in the course of 15—20 minutes. When the major part of the sodium hydroxide has been added, the solution turns yellow. The addition is continued until the solution reacts neutral towards litmus paper. During the neutralization the temperature rises to 5°. Now a previously cooled solution of 98 g of hydrazine sulfate (note 1) (0.75 mole) and the equivalent amount of sodium hydroxide (61 g) in 300 ml of water is added within 2 minutes. The temperature of the reaction mixture hereby rises to 13°. Stirring is discontinued and the flask removed from the cooling-bath and left standing overnight at room temperature. In the first half hour after the addition of hydrazine the colour becomes a little darker. The next day the reaction mixture is refluxed for 30 minutes, then 168 ml of concentrated hydrochloric acid are added with stirring and the dark brown reaction mixture is evaporated in vacuum in a 3 liter round-bottomed flask until 1500 ml of water have been removed. Hereby a large amount of sodium chloride and sodium sulfate precipitates. The residue is cooled with ice-water, filtered and the precipitate washed with 500—600 ml of 60 % aqueous ethanol, which has first been cooled to zero. The filtrate is further evaporated in vacuum in a 2 liter flask till about 200 ml and another batch of salts is removed by filtration. After renewed evaporation to 200 ml 195 g of anhydrous potassium carbonate are added to liberate the pyridazine, and the reaction mixture is stirred or shaken until all the potassium carbonate has reacted or dissolved (30—60 minutes). Now the pasty mixture is shaken vigorously in the flask with 100 ml of ether and the etheral extract decanted. This is repeated about 10 times until no black oil drops remain. The combined etheral extracts are shaken for ½ hour with 30 g of

potassium carbonate and the ether is removed through a small packed column. The oily yellowish-brown residue is then distilled in vacuum. After 0.5—1 ml of fore-run, the fraction boiling within 1° at about 85° under 13 mm is collected. About 2 g of a dark red residue remains in the flask. Yield of pyridazine 34 g = 85 %. The product is slightly yellow. $n_D^{23,5} = 1.5231$ (Brühl [11] = 1.5231).

Note 1. The hydrazine sulfate, as well as the sodium hydroxide used in this synthesis were good technical grades.

SUMMARY

Detailed directions are given for the preparation of 2,5-diacetoxy-2,5-dihydrofuran, 2,5-diacetoxytetrahydrofuran and pyridazine by the following sequence of reactions.



During this investigation one of us (Si-Oh Li) received financial aid from *The Danish Committee on Training of Foreign Scientists in Danish Laboratories.*

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Received July 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1241–1245

The Action of Cyanide on Selenopentathionate and Telluropentathionate

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In aqueous solutions, cyanide reacts with pentathionate ions to give thiosulphate, sulphate and thiocyanate [1, 2]:

$$S(S_2O_3)_2^{--} + 2\ CN^- + 2\ OH^- = S_2O_3^{--} + 2\ SCN^- + SO_4^{--} + H_2O \qquad (1)$$

We have found that selenopentathionate *[3] reacts with cyanide in an analogous way, as follows:

$$Se(S_2O_3)_2^{--} + 2\ CN^- + 2\ OH^- = S_2O_3^{--} + SeCN^- + SCN^- + SO_4^{--} + H_2O \qquad (2)$$

In presence of an excess of potassium cyanide, both reactions are rapid and quantitative. The first of the reactions forms the basis for the cyanide method [2, 4] for the iodometric analysis of pentathionate. Correspondingly, the second one may, in dilute solutions, be used for the iodometric analysis of selenopentathionate.

The mechanism of the reaction of cyanide with pentathionate has been discussed elsewhere [4], on the basis of the kinetic results of Foresti [5] and the general reactivity of pentathionate as a monosulphur di(thiosulphate). So far, no kinetic experiments have been made on the reaction of cyanide with selenopentathionate. However, in view of the similarity of the two reactions, it seems reasonable to assume that the mechanism is the same in both cases. Thus, the first step is probably a nucleophilic displacement, on divalent selenium, of thiosulphate by cyanide.

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* The prefix "mono" is omitted, since the monoseleno and monotelluro compounds are the only selenium- or tellurium-substituted pentathionates known.

Heuer [6], in his studies on the Norris and Fay reaction between selenious acid and potassium thiosulphate, isolated crystalline mixtures which perhaps contained potassium selenopentathionate, beside other selenopolythionates and polythionates. He said (Ref. 6 p. 31) that selenopentathionate seemed to react with cyanide to give selenosulphate. The salt did not (Ref. 6 p. 35) react to give thiosulphate, and thus (Ref. 6 p. 44) not according to Eq. (2). However, Heuer did not investigate the pure salt. Actually, no selenosulphate is formed, as can be shown by means of formaldehyde, since this reagent immediately liberates selenium from selenosulphate [7].

Telluropentathionate * [8] does not react with potassium cyanide. Instead, due to the alkalinity of the cyanide solutions, a rapid and complete hydrolysis takes place, as in presence of hydroxyl ions alone [8]:

$$2\ Te(S_2O_3)_2^{--} + 6\ OH^- = 4\ S_2O_3^{--} + Te + TeO_3^{--} + 3\ H_2O \qquad (3)$$

In this respect, the tellurium derivative differs markedly from the corresponding sulphur and selenium compounds.

Experimental

The behaviour of selenocyanate towards iodine. In sodium hydrogencarbonate buffer, potassium selenocyanate is oxidized by an excess of iodine, to give selenite and iodine cyanide [9]:

$$SeCN^- + 3\ I_2 + 6\ OH^- = SeO_3^{--} + ICN + 5\ I^- + 3\ H_2O$$

In neutral solutions, selenocyanate is only partly oxidized by iodine [10], to give selenocyanogen, $(SeCN)_2$, or the brown-coloured triselenocyanate, $(SeCN)_3^-$.

The following experiment shows that in dilute aqueous solutions, in presence of acetic acid and sufficient amounts of potassium iodide, potassium selenocyanate is not attacked by small amounts of iodine: To a mixture of 10 ml of 0.01 *M* potassium selenocyanate, 10 ml of 5 % potassium iodide, 10 ml of 10 % acetic acid, and starch, was added one drop of 0.01 *N* iodine. A distinct and stable starch-iodine colour appeared. In absence of potassium iodide, more than 1 ml of 0.01 *N* iodine was consumed, to produce, in the solution, a brownish yellow colour, probably due to the triselenocyanate ion, $(SeCN)_3^-$. On addition of potassium iodide to the reaction mixture, iodine was regenerated, in accordance with an equilibrium which, tentatively, may be formulated thus:

$$3\ SeCN^- + I_3^- \rightleftharpoons (SeCN)_3^- + 3\ I^-$$

The finding that a species is indifferent to iodine in presence of potassium iodide, does not, however, exclude the possibility that an oxidation of the compound may be induced through the simultaneous oxidation of thiosulphate by iodine, as has been demonstrated earlier [4] in the case of thiosulphonates, sulphinates, and dialkylthiophosphates. In fact, larger amounts of potassium iodide and acetic acid are required in order to prevent such

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* See footnote on the foregoing page.

an induced oxidation of selenocyanate. Some control titrations, performed under the same conditions as later used for the iodometric analysis of selenopentathionate by means of cyanide, are reported in the following paragraph.

(1) To a mixture of 10 ml of approx. 0.01 *N* sodium thiosulphate and 10 ml of 0.01 *M* potassium selenocyanate, volume 40 ml, were added 2 ml of 1 *M* potassium cyanide, 1 ml of 40 % formaldehyde, 2 g of potassium iodide, 50 ml of water, 20 ml of 10 % acetic acid, and 10 ml of starch solution, and the mixture was titrated with 0.01094 *N* iodine: 9.30 ml, 9.29 ml. (2) Without selenocyanate and potassium iodide: 9.29 ml, 9.30 ml. (3) With 20 ml of 0.01 *N* sodium thiosulphate and 20 ml of 0.01 *M* potassium selenocyanate, otherwise as (1): 18.59 ml, 18.61 ml. (4) With 10 ml of selenocyanate: 18.59 ml, 18.57 ml. (5) Without selenocyanate and potassium iodide, otherwise as (3) and (4): 18.58 ml, 18.59 ml of 0.01094 *N* iodine.

In the above experiments, in presence of 2 g of potassium iodide, the iodine-thiosulphate reaction was not complete unless 50 ml of water were added, as described, before the addition of acetic acid. Thus, Exp. (1) performed without 50 ml of water: 9.20 ml of 0.01094 *N* iodine were consumed. Exp. (2) without 50 ml of water, and in presence of 2 g of potassium iodide: 9.21 ml, 9.20 ml. Even when the solutions were thus diluted, the presence of 2 g of potassium iodide (containing no iodate) seemed to depress the thiosulphate-iodine titer to a slight degree. *E. g.*, Exp. (5) in presence of 2 g of potassium iodide: 18.54 ml, 18.54 ml. Also, as has been noted earlier [4], formaldehyde and acetic acid, especially when potassium iodide is present, markedly retard the reaction between thiosulphate and iodine. The fact that concordant results are obtained in Exps. (1)—(5) may thus be due to a compensation of small errors.

In more concentrated solutions, *e. g.*, when using 0.1 *N* thiosulphate and 0.1 *N* iodine, a simultaneous oxidation of selenocyanate cannot be prevented by use of potassium iodide and acetic acid.

Selenopentathionate and cyanide. The following procedure was accordingly adopted for the iodometric analysis of selenopentathionate by means of cyanide, Eq. (2).

10—20 ml of approx. 0.01 *M* selenopentathionate are diluted to 40 ml. 2 ml of 1 *M* potassium cyanide are added, and the solution is allowed to stand for 5 minutes. Then 1 ml of 40 % formaldehyde, 2 g of potassium iodide, 50 ml of water, and 20 ml of 10 % acetic acid are added, and the solution is titrated with 0.01 *N* iodine (using starch as an indicator).

The following example illustrates the method. 1.0980 g (approx. 2.5 millimole) of potassium selenopentathionate, $K_2Se(S_2O_3)_2 \cdot 1\frac{1}{2} H_2O$, were dissolved to 250 ml in a volumetric flask. 10 ml samples were pipetted out and analyzed as described above: 9.81 ml, 9.84 ml, 9.82 ml of 0.01094 *N* iodine. 20 ml samples: 19.69 ml, 19.64 ml, 19.66 ml of 0.01094 *N* iodine. Average values, 9.82 ml and 19.66 ml; theoretically, according to Eq. (2): 9.83 ml and 19.65 ml, respectively. A 10 ml sample, analyzed for selenious acid according to Norris and Fay after oxidation by means of potassium bromate [3]: 46.54 ml—7.24 ml = 39.30 ml of 0.01094 *N* iodine, which corresponds to 9.83 ml (one fourth of the Norris and Fay value) per 10 ml after Eq. (2), or to 19.65 ml per 20 ml sample.

Some experiments were made in more concentrated solutions. 4.2618 g (approx. 10 millimole) of potassium selenopentathionate hemitrihydrate were dissolved to 100 ml in a volumetric flask. 10 ml samples were pipetted out and diluted to approx. 200 ml, and 10 ml of 1 *M* potassium cyanide were added. After 5 minutes, 5 ml of 40 % formaldehyde, 2 g of potassium iodide, and 20 ml of 10 % acetic acid were added, and the mixture was

titrated with 0.1020 *N* iodine: 11.14 ml, 11.12 ml. Using 4 g of potassium iodide: 10.44 ml, 10.50 ml, 10.40 ml. Theoretically, 10.23 ml of 0.1020 *N* iodine. The values are thus too high. On standing, the titrated solutions became red (selenium being liberated from the selenocyanate). To the three last samples, before selenium appeared, was added barium chloride, and the barium sulphate thus precipitated was filtered off, dried and weighed: 0.2436 g, 0.2407 g, 0.2501 g; theoretically, according to Eq. (2): 0.2435 g $BaSO_4$.

Telluropentathionate and cyanide. 0.4376 g (approx. 1 millimole) of sodium telluropentathionate, $Na_2Te(S_2O_3)_2 \cdot 2\ H_2O$, was dissolved to 250 ml in a volumetric flask. 25 ml samples were pipetted out, diluted to 40 ml, and 2 ml of 1 *M* potassium cyanide were added. Tellurium immediately appeared. After 5 minutes, 1 ml of 40 % formaldehyde was added, and the tellurium was filtered off (the filtrate remained clear and colourless, also if the formaldehyde was added after the filtration, instead of before). 10 ml of 10 % acetic acid and a few crystals of potassium iodide were added to the filtrate, and the mixture was titrated with 0.01132 *N* iodine: 17.78 ml, 17.77 ml, 17.79 ml; theoretically, according to Eq. (3): 17.81 ml. A 25 ml sample of the sodium telluropentathionate solution was titrated directly with iodine as described earlier [8]: 35.60 ml and (the formed tetrathionate being analyzed by means of the sulphite method) 8.91 ml of 0.01132 *N* iodine; theoretically, 35.62 ml and one fourth of this value, respectively.

Similar results were obtained in more concentrated solutions, using 0.1 *N* iodine. In one case, the tellurium, appearing on addition of potassium cyanide, was dried and weighed. Thus, 0.4156 g of sodium telluropentathionate dihydrate, dissolved in 200 ml of water, and 10 ml of 1 *M* potassium cyanide added, gave, when filtered after 5 minutes, 62.1 mg of tellurium; theoretically, according to Eq. (3): 61.1 mg. On addition of acetic acid to the filtrate, tellurous acid separated out.

NOTE ON TELLUROCYANATE AND ANALOGOUS TELLURO ANIONS

The fact that the cyanide ion does not react with telluropentathionate reflects upon the poor ability of tellurium, as compared with sulphur and selenium, to give compounds with the cyanide group, and with analogous sulphur- and selenium-bonding groups or ions like sulphite and phosphite.

A survey of the literature fails to reveal descriptions of salts of tellurocyanic acid. Inferring from the lower stability of selenocyanates as compared with thiocyanates, tellurocyanates should, even in solutions, be fairly unstable. Nor are such solutions easily prepared. *E. g.*, hot aqueous solutions of potassium cyanide attack tellurium only to a slight degree [11]. Tellurium dissolves very slowly in solutions of potassium cyanide in liquid ammonia; however, no definite compound could be isolated [12]. Solutions of potassium tellurocyanate have been employed by Birckenbach and Kellermann [13] for measurement of the decomposition potential of the tellurocyanate ion, but the salt itself is not described in the article. According to patents [14], potassium tellurocyanate reacts with aliphatic halides to give the corresponding organic tellurocyanates; however, no such examples are included in the patents. In fact, the only com-

pound of tellurium with the cyanide group, yet described in literature, appears to be the tellurium dicyanide, $Te(CN)_2$, of Cocksedge [15].

No tellurosulphates, whether inorganic or organic, are known. Thus, tellurium is not affected by sodium sulphite solutions. As for tellurophosphates, no such compounds are described in literature. However, according to unpublished experiments by the author, finely divided tellurium does dissolve, on gentle heating, in ethanol solutions of potassium diethylphosphite, to give potassium diethyltellurophosphate, $(C_2H_5O)_2OPTeK$. It is of interest to note that the diethylphosphite takes up tellurium more readily than does potassium cyanide under the same conditions. Potassium diethyltellurophosphate, which may be isolated as colourless, hygroscopic needles, rapidly darkening in moist air, is less stable than the corresponding thio and seleno salts [16], *e. g.*, dilute acids liberate tellurium from its solutions, and so does iodine.

SUMMARY

Selenopentathionate reacts with potassium cyanide to give one equivalent of thiosulphate, as does pentathionate.

Telluropentathionate does not react with potassium cyanide; instead, a hydrolysis takes place, to give two equivalents of thiosulphate.

The author is indebted to *Styret for Nansenfondet og de dermed forbundne fond* for a grant.

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Received August 31, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1246–1249

Constituents of Pine Heartwood

XXIII *. Isolation of Dihydropinosylvin Monomethyl Ether from the Heartwood of *Pinus albicaulis* Engelm.

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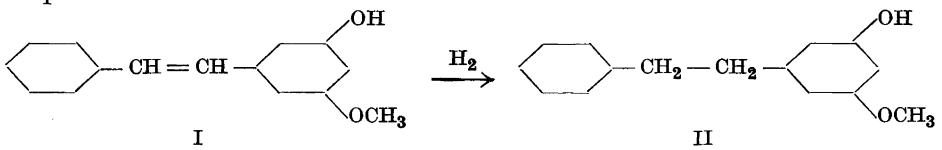
In Part XX of this series [1], the general methods for analysing pine heartwood extracts by paper partition chromatography were described. In the same paper it was mentioned that heartwood extracts of pines belonging to the section *Haploxylon* give rather complicated chromatograms, indicating the presence of some unknown phenolic compounds in addition to those already isolated. The most distinct one of these unknown spots has R_F about 0.80 in the standard solvent and is rather sharp. When sprayed with diazotised benzidine solution, it acquires a brownish red colour, which becomes visible immediately. The coloured spots of flavones and flavanones generally require some time (0.5—10 min.) to develop, but phenols of the pinosylvin type give visible spots at the first moment of contact with the reagent. From the R_F values given in Part XX [1], it is evident that hydrogenation of a double bond causes a small increase in R_F. Since pinosylvin monomethyl ether has R_F = 0.71, one might suspect the new spot to be due to a dihydro derivative of this compound.



I II

Dihydropinosylvin monomethyl ether (II = 3-hydroxy-5-methoxy-dibenzyl), which has not been synthesised before, was easily prepared by catalytic hydrogenation of pinosylvin monomethyl ether (I). The reaction product,

* XXII. *Acta Chem. Scand.* **4** (1950) 1042.

when purified by vacuum distillation, slowly crystallised on cooling, m. p. 50—52°*. It was characterised as its phenylurethan, m. p. 124—125°. When investigated on the paper chromatogram, dihydropinosylvin monomethyl ether gave a spot which was identical with that found on chromatograms of heartwood extracts.

The spot of dihydropinosylvin monomethyl ether has been found on chromatograms from most pines belonging to the section *Haploxylon* hitherto investigated, but never in the section *Diploxylon*. However, the spot generally is rather weak, except for three species, *P. koraiensis*, *P. cembra* and *P. albicaulis*, which, according to many botanists [2], constitute a separate group, *Cembrae*. These three pines give strong spots for the new compound, and they would thus be the most suitable source for its isolation. Since a large sample of heartwood from *P. albicaulis* was available, this wood was chosen as the starting material.

When the heartwood extract was divided into fractions in the usual manner, the new compound accumulated in the 4 % sodium hydroxide fraction, just as does pinosylvin monomethyl ether. The two compounds were separated by repeated distillation at low pressure, after the main part of the pinosylvin monomethyl ether had been deposited as crystals. The dihydropinosylvin monomethyl ether thus obtained proved to be identical with the synthetic sample in every respect. The yield was comparatively high, 3.2 g of the pure product being isolated from 3.7 kg of air-dry heartwood (0.09 %). Since only a part of the total quantity could be separated from pinosylvin monomethyl ether, the content of dihydropinosylvin monomethyl ether in the heartwood must be estimated to be at least 0.2 %.

EXPERIMENTAL

Hydrogenation of pinosylvin monomethyl ether

Pinosylvin monomethyl ether from *P. excelsa* (4 g) was dissolved in absolute ethanol and hydrogenated at room temperature with platinic oxide (0.2 g) as catalyst. The hydrogen uptake corresponded to 1.0 moles of hydrogen per mole of pinosylvin monomethyl ether. The reaction product, after evaporation of the solvent, consisted of a greenish brown oil, which boiled at 195—200° when distilled at 5 mm pressure. The distillate (2.7 g) was a thick, pale yellow oil, which slowly crystallised in the refrigerator. After further purification by vacuum distillation, it melted at 50—52°. A faint brownish-violet colour was obtained with ferric chloride in ethanol solution, and diazotised benzidine gave a strong brownish red colour.

* All melting points uncorrected.

$C_{15}H_{16}O_2$ (228.3)	Calc.	C 78.9	H 7.07	OCH_3	13.6
	Found	» 79.0	» 6.96	»	13.8

The phenylurethan was prepared by heating the phenol with phenyl isocyanate and one drop of pyridine. The product thus obtained was recrystallised from ligroin and from ethanol. Colourless needles, m. p. 124—125°.

$C_{22}H_{21}O_3N$ (347.4)	Calc.	OCH_3	8.95
	Found	»	8.93

Isolation of dihydropinosylvin monomethyl ether from *P. albicaulis*

The wood sample came from a tree which had grown on Mount Hood, Oregon, U. S. A.

The air-dried heartwood (3.7 kg) was extracted in the usual way with ether and then with acetone. The ether extract, which, according to the paper chromatogram, contained considerable amounts of phenols, was concentrated and treated with light petroleum, which precipitates the phenols but dissolves resin acids. The sticky brown precipitate was added to the acetone extract, which was concentrated to a small volume, and then dissolved in ether, which precipitates "membrane substances". The ether solution was then extracted with saturated sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide. Each fraction was investigated by paper chromatography. Since the 4 % sodium hydroxide extract seemed to contain the greatest quantity of the compound desired, this extract was further investigated.

The alkaline solution was acidified and extracted with ether, and the ether solution dried and concentrated to a brown syrup. This syrup soon deposited considerable amounts of crystals of pinosylvin monomethyl ether. These crystals were separated by suction. The filtrate, on standing, deposited additional crystals, which were removed in the same way. When no more crystals could be separated, the dark brown syrup (18.5 g) was distilled at 2 mm pressure. The distillate was divided into three fractions, boiling at 179—185°, 185—191°, and 191—200°, respectively. All fractions were viscous, pale yellow oils. Total weight of distillate = 11 g. Each fraction was investigated on the paper chromatogram. Fractions 1 and 2 consisted mainly of dihydropinosylvin monomethyl ether with small amounts of pinosylvin and its monomethyl ether, but fraction 3 gave almost equally strong spots of pinosylvin monomethyl ether and its dihydro derivative.

Fractions 1 and 2 (7.2 g) were once again distilled at 2 mm pressure. The first fraction (b. p. 189—198°) contained almost pure dihydro derivative, with traces of pinosylvin, which were completely removed by washing an ether solution of the distillate with 0.3 % sodium hydroxide. On concentration of the ether solution, an almost colourless oil (3.2 g) was obtained, which soon solidified in the refrigerator, forming a colourless crystalline cake, melting between 48° and 53°. The melting range was unchanged on admixture with synthetic dihydropinosylvin monomethyl ether.

The phenylurethan, prepared as described above, melted at 124—125° alone or on admixture with the corresponding derivative of the synthetic phenol.

SUMMARY

From the heartwood of *Pinus albicaulis* Engelm., dihydropinosylvin monomethyl ether (3-hydroxy-5-methoxy-dibenzyl) has been isolated. This compound has also been prepared by catalytic hydrogenation of pinosylvin monomethyl ether. The new substance was detected by the aid of paper chromatography. It seems to be present in the heartwood of most pines from the section *Haploxylon*.

The author is indebted to *Fonden för Skoglig Forskning* for financial support, to Mr. A. Misiorny for skilful experimental assistance and to Dr. E. G. Locke, Pacific Northwest Forest and Range Experiment Station, Portland, Oregon, U.S.A., for the sample of wood.

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Received October 5, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1250–1260

Electrolytic Reduction of Zinc Ions and of Zinc Cyanide Complex Ions from Aqueous Solutions of Zinc Perchlorate and Zinc Perchlorate with Potassium Cyanide

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Solutions of complex zinc cyanides have been investigated earlier in different ways. They have inter alia been the object of polarographic examination by Pines [1]. He was chiefly interested in finding an explanation of the fact that no zinc is electro-deposited from solutions of zinc salts if they contain a surplus of alkali cyanide, whereas cadmium is deposited under corresponding conditions. Pines concluded that slowness of dissociation of the potassium-zinc cyanide complexes hinders the deposition of zinc, whereas in cadmium-alkali-cyanide solutions the free ions are in reversible equilibrium with the complex ions [2]. Glasstone [3] on the other hand, in consequence of his researches on electrolytic polarization states that there is no reason to believe that any of the complex cyanides studied hitherto dissociate slowly. Glasstone studied the solutions by means of cathode-potential measurements and electrometric titrations. The shape of his titration curve suggests that $Zn(CN)_4^{--}$ is the chief complex ion present in solution. One of the earliest determinations of the instability constant for the $Zn(CN)_4^{--}$ ion and for the $Cd(CN)_4^{--}$ ion was made by v. Euler [4]. He found about the same values for both complexes. Britton and Dodd [5] also came to the conclusion that they are of the same order of magnitude. This seems strange in view of the fact that the zinc ion is a stronger acid than the cadmium ion. We must therefore, according to the Lewis conception of acids and bases expect the stability of the zinc cyanide complexes to be greater than that of the corresponding cadmium cyanide complexes. Pines (*l. c.*) concludes from his polarographic investigations that in solutions of cadmium chloride with potassium cyanide the greater part of the cadmium exists as $Cd(CN)_3^-$, whereas the corresponding complex is excluded

from his reflections on zinc cyanide solutions. Kunschert[6] on the other hand maintains as a result of measurements with a silver electrode in potassium zinc cyanide solutions that the zinc in cyanide solutions is present partly in the form of $Zn(CN)_4^{--}$ ions and partly in the form of $Zn(CN)_3^{-}$ ions.

The aim of the present investigation is primarily to ascertain whether the "prewaves" which we formerly have recorded by the electro-reduction of divers metal ions as for instance the Zn^{++} ions and the Cd^{++} ions[7] can be reproduced also when complex forming anions, such as the CN^- ion are present. We chose first to examine the Zn^{++} ion, which ought to form the most stable cyanide complex ions of the two. An eventual impeding effect to the phenomenon might therefore be expected to be more pronounced in zinc salt solutions than in cadmium salt solutions.

EXPERIMENTAL PROCEDURE

For our experiments we employed a polarograph from Radiometer in Copenhagen with a photo-electric amplifier and automatic registrator. As by former investigations we made use of its counter current arrangement in order to vary the slopes of the curves.

We took extra precautions to secure against eventual oxygen waves. Not only were the solutions in the electrolytic vessels treated for a whole night before the readings were taken with hydrogen or nitrogen that had been purified over red hot copper; but the gas was also passed through solutions of chromous chloride. If the solutions were no longer sky-blue the copper as well as the solutions were renewed. As the slightest amount of oxygen changes the blue colour of chromous chloride to green, we can guarantee the purity of our gas. The solutions in the electrolytic vessel were kept under gas also during the readings.

The zinc perchlorate was prepared as usual by precipitation of zinc sulphate solutions with equivalent amounts of barium perchlorate solutions. The potassium cyanide solutions were freshly prepared for each series of measurements as it is well known that a solution of alkali cyanide decomposes on standing.

The molar concentration of the zinc ion varied between the order of magnitude of 10^{-4} and 10^{-3}. The molar concentration of the cyanide ion was increased from zero to six hundred times that of the zinc ion.

Some solutions of pure zinc cyanide were prepared by shaking a small amount with conductivity water under hydrogen in an electric shaker. The solubility of zinc cyanide has been determined by Masaki[8] by means of electrometric measurements. He found the molar solubility of $Zn(CN)_2$ to be $4.49 \cdot 10^{-5}$. Pines (*l. c.*) states that it is of the order of magnitude of 10^{-4}.

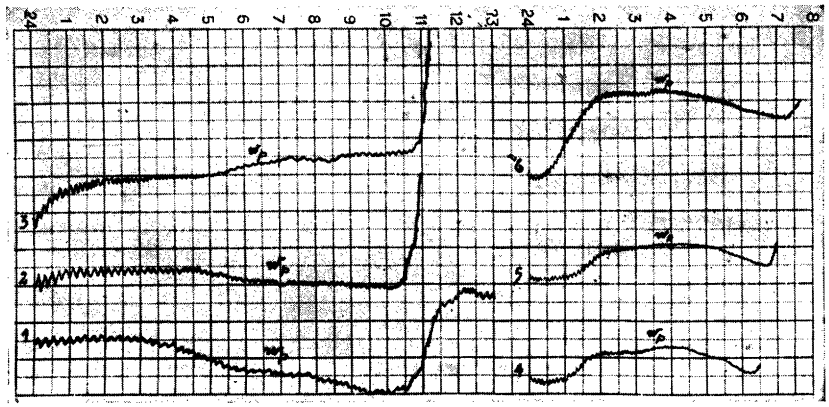

Fig. 1. Electro-reduction of zinc perchlorate in aqueous solutions.

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc.	
				$NaClO_4$	$Zn(ClO_4)_2$
1	2	2	1 × 1	0	0.0001
2	2	2	1 × 1	0	0.001
3	2	2	1 × 1	0	0.005
4	3	3	1 × 1	0.1	0.0006
5	3	3	1 × 1	0.1	0.0012
6	3	3	1 × 1	0.1	0.004

EXPERIMENTAL RESULTS

In the first instance we devoted ourselves to the checking of earlier results as regards stepwise reduction of zinc ions in pure zinc perchlorate solutions and in solutions of zinc perchlorate with 0.1 *M* sodium perchlorate as supporting electrolyte. Our experimental material is for these as for the following types of solutions too great to publish as a whole; but we have given several curves for each type. As will be seen from Fig. 1 the curves for pure zinc perchlorate solutions fully confirm earlier results, the "prewaves" being distinctly registered.

Next we tested some solutions of pure zinc cyanide. As will be seen from the height of the zinc wave the concentration of Zn^{++} ions is not very great considering that we have used maximum of sensitivity, *viz.* 1 × 1 as in Fig. 1. The half wave potential for the zinc ion lies, as might be expected, at less negative values than when perchlorate ions are present, and the position of the pre-wave is correspondingly found between — 0.5 and — 0.6 volts.

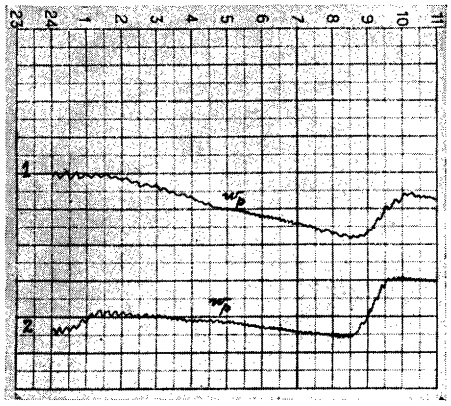

Fig. 2. Electro-reduction of saturated aqueous solutions of zinc cyanide.

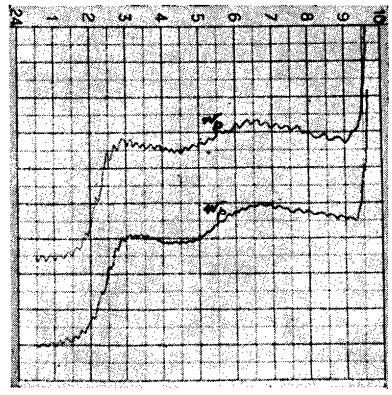

Fig. 3. Electro-reduction of solutions of zinc perchlorate with equivalent amounts of potassium cyanide.

No.	E. M. F. in volts	Counter current	1/sens	Gas stream in hours
1	2	2	1 × 1	1
2	2	2	1 × 1	20

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	2	2	1 × 1	0.001	0.002	0.1
2	2	2	1 × 1	0.001	0.002	0.1

The principal part of our investigation was taken up with measurements of solutions of zinc perchlorate with gradually increasing amounts of potassium cyanide. We first examined solutions with equivalent amounts of $Zn(ClO_4)_2$ and KCN. One might expect to get curves of the same shape as those for solutions of $Zn(CN)_2$. The curves in Fig. 3, however, show more analogy to curves 4, 5 and 6 of Fig. 1 than to the curves in Fig. 2. It seems as if the Zn^{++} ions and the CN^- ions have not as yet had time to combine to $Zn(CN)_2$ in these dilute solutions, the zinc wave being much higher than those observed in a solution prepared from solid $Zn(CN)_2$. The influence of the CN^- ions is only seen in a lowering of the value for the half wave potential of Zn^{++}. These effects will have to be the object of a separate investigation as regards the influence of ageing on solutions of Zn^{++} ions with equivalent amounts of CN^- ions.

When we have added as much as two equivalents of CN^- per equivalent of Zn^{++} conditions for formation of $Zn(CN)_4^{--}$ ions ought to be present. We get, as will be seen from Fig. 4,1 a small wave at greater negative potential than the Zn wave. This wave is still more pronounced at curve 4,2. Its half-wave potential lies about — 1.3 volts.

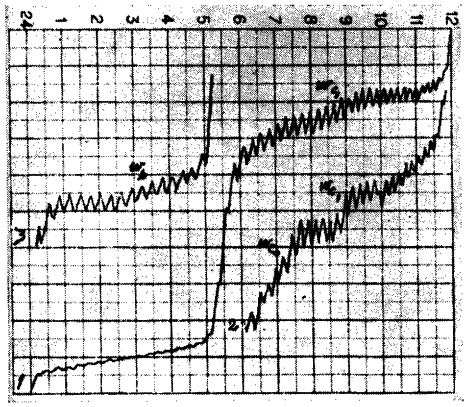

Fig. 4. Electro-reduction of solutions of zinc perchlorate with two equivalents of potassium cyanide per equivalent of zinc.

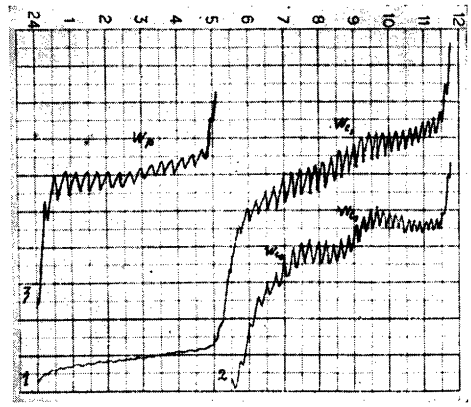

Fig. 5. Electro-reduction of solutions of zinc perchlorate with three equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc. $Zn(ClO_4)_2$	Mol. conc. KCN	Mol. conc. $NaClO_4$
1	3	0	10 × 1	0.001	0.004	0.1
2	3	0	3 × 1	0.001	0.004	0.1
3	3	2	1 × 1	0.001	0.004	0.1

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc. $Zn(ClO_4)_2$	Mol. conc. KCN	Mol. conc. $NaClO_4$
1	3	0	10 × 1	0.001	0.006	0.1
2	3	1	3 × 1	0.001	0.006	0.1
3	3	2	1 × 1	0.001	0.006	0.1

The Zn^{++} wave has shifted even more towards less negative values, but is still quite high, the pre-wave being less pronounced.

With three equivalents of CN^- per equivalent of Zn^{++} we should expect the Zn^{++} wave to be less pronounced and the complex ion waves to be correspondingly more conspicuous. As will be seen from Fig. 5, 1 and 2 this is also the case. At this concentration, however, a complex ion wave unmistakably begins to appear at lower negative potential than the one marked w_{c_1}. One might accordingly assume the formation of the complex ion $Zn(CN)_3^-$, although Britton and Dodd (*l. c.*) in their investigation of the progressive formation in dilute solutions of the complex cyanides of zinc (and other metals) find no evidence for the existence of a $Zn(CN)_3^-$ ion. Kunschert [9], however, found it necessary to postulate the existence of the zinc complex cyanide ion, $Zn(CN)_3^-$ in order to account for the potentials of silver electrodes in solotions containing both silver and zinc complex cyanides. This disputed ion is evidently not so easily formed as the well known $Zn(CN)_4^{--}$ ion. The prewave is still observable.

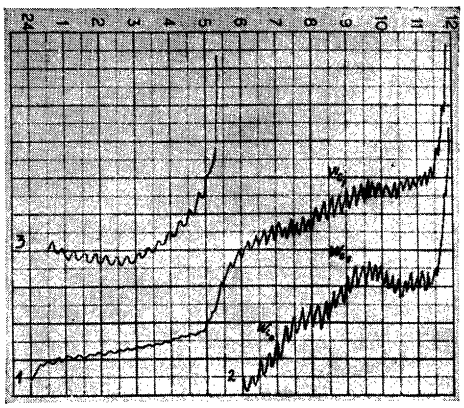

Fig. 6. Electro-reduction of solutions of zinc perchlorate with four equivalents of potassium cyanide per equivalent of zinc.

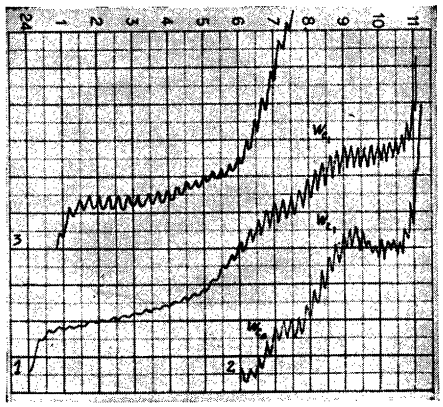

Fig. 7. Electro-reduction of solutions of zinc perchlorate with six equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	l/sens	Mol. conc. $Zn(ClO_4)_2$	Mol. conc. KCN	Mol. conc. $NaClO_4$
1	3	0	10 × 1	0.001	0.008	0.1
2	3	2	3 × 1	0.001	0.008	0.1
3	3	3	1 × 1	0.001	0.008	0.1

No.	E. M. F. in volts	Counter current	l/sens	Mol. conc. $Zn(ClO_4)_2$	Mol. conc. KCN	Mol. conc. $NaClO_4$
1	3	0	10 × 1	0.001	0.012	0.1
2	3	3	3 × 1	0.001	0.012	0.1
3	3	2	1 × 1	0.001	0.012	0.1

With the molar ratio 1 : 8 a complex ion wave also begins to appear at higher negative values than the one marked w_{c_1}. It has a half wave potential of about — 1.5 volts as against the mercury anode. This wave may be attributed to the complex ion $Zn(CN)_5^{---}$. Pines (*l. c.*) calculates with this ion on the assumption that the $Zn(CN)_6^{----}$ ion is not polarographically reduced at lower negative values than — 2 volts.

The only difference which the curves of Fig. 6 show from those of Fig. 5 is that the height of the Zn^{++} wave has decreasedperceptibly while that of the complex waves has increased.

An increase of CN^- to six equivalents of CN^- ion per equivalent of Zn^{++} ions only augments the effects just mentioned, as will be seen from Fig. 7.

An increase of CN^- ions to twelve equivalents of CN^- ions per equivalent of Zn^{++} ions brings w_{c_2} into prominence as seen from Fig. 8.

Increasing the amount of CN^- ions to sixty equivalents of CN^- ions per equivalent of Zn^{++} ions, we obtain a new polarographic wave at about — 1.65 volts as against

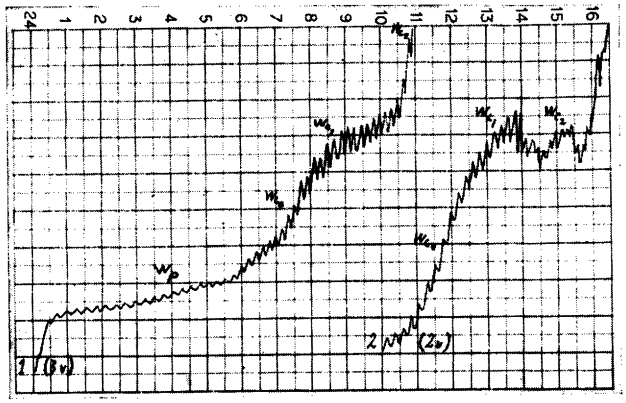

Fig. 8. Electro-reduction of solutions of zinc perchlorate with twelve equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	l/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	3	0	10 × 1	0.001	0.024	0.1
2	2	4	3 × 1	0.001	0.024	0.1

the mercury anode. We have called it w_{c_3} and we may assume that wave to be due to the presence of $Zn(CN)_6^{----}$ ions. The assumption that this ion has a more negative reduction potential than the potassium ion, (Pines, *l. c.*) does evidently not tally with our experimental results as given in Fig. 9.

The last and greatest concentration of CN^- ions that we used was three hundred equivalents to one equivalent of zinc. With the usual concentration of Zn^{++}, *viz.* 0.001, we did not get any distinct complex waves, though the pre-wave appeared, as shown in Fig. 10 a.

To test the effect of an increased dissociation of the complexes we next repeated the polarographic treatment of solutions in the same equivalent proportions but a hundred times more diluted. The most conspicuous result was a very great rise of the Z^{++} wave as will be seen from Fig. 10b; w_{c_0} and w_{c_1} are also observable.

Lastly we wanted to try out the effect of lesser ionic strength, that is, to dispense with the supporting electrolyte. We have earlier found that many effects that otherwise hardly may be traceable seem to appear in such solutions. As will be seen from Fig. 11 both the prewave, the Zn^{++} wave, and the complex ion waves are unmistakable. We even seem to get a w_{c_4} that lies at about, — 1.8 volts, just before the potassium wave.

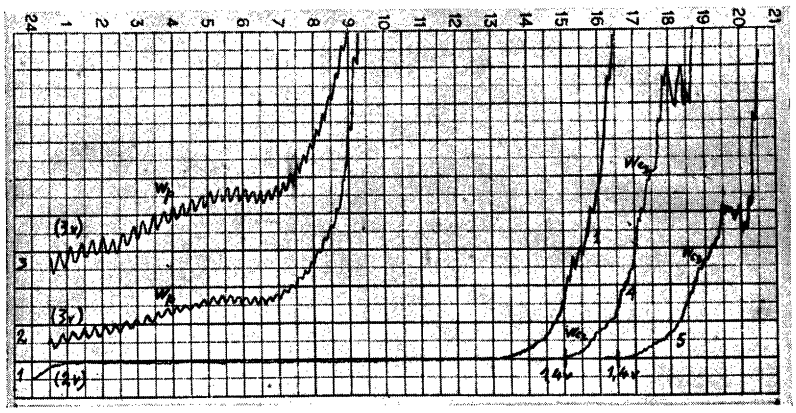

Fig. 9. Electro-reduction of solutions of zinc perchlorate with sixty equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	3	0	100 × 1	0.001	0.120	0.1
2	3	0	3 × 1	0.001	0.120	0.1
3	3	0	2 × 1	0.001	0.120	0.1
4	2	0	100 × 3	0.001	0.120	0.1
5	2	0	100 × 5	0.001	0.120	0.1

DISCUSSION

The chief experimental data as regards the polarographic behaviour of zinc in cyanide medium have been summarized in Table 1.

As one might expect, the prewave for Zn^{++} comes out most strongly in those solutions where the complex ions have not as yet been formed and the zinc wave is still the dominant one. With increasing concentration of CN^- ions, complex formation takes place and the Zn^{++} wave gets correspondingly smaller.

A complex wave, w_{c_1}, with a half wave potential of about — 1.3 volts as against the mercury anode gets very distinct at the molar ratio $Zn^{++}:CN^- = 1:4$, and does not disappear till the ratio has reached 1 : 120. It is natural to

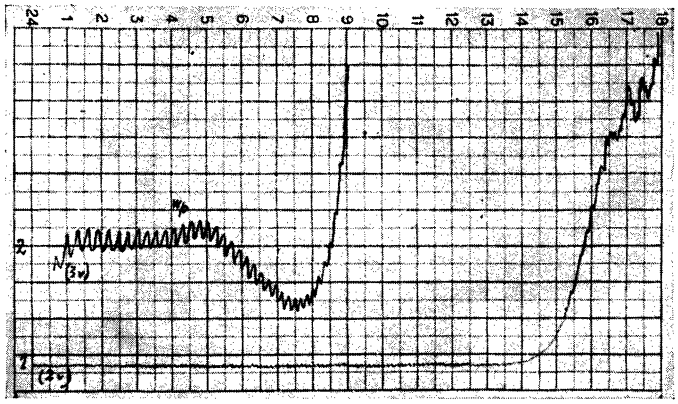

Fig. 10 a. Electro-reduction of solutions of zinc perchlorate with three hundred equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	1/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	2	0	100 × 5	0.001	0.600	0.1
2	3	1	1 × 1	0. 001	0.600	0.1

attribute this to the complex ion $Zn(CN)_4^{--}$ inasmuch as it is usually considered the dominant complex ion, the zinc ion readily forming tetracoordinated complexes. At the same ratio however a small wave, w_{c_0}, with a somewhat lower half wave potential just begins to appear. At the ratio 1 : 8 it is fairly distinct and does not disappear as long as the w_{c_1} is present. Not until the molar ratio has become 1 : 8 does the third complex wave, w_{c_2}, make its appearance. Its half wave potential lies about 1.5 volts. It gets distinct at the ratio 1 : 24, and is still apparent at the ratio 1 : 120, when both w_{c_0} and w_{c_1} have disappeared. At that ratio however a fourth half wave, w_{c_3}, appears with a half wave potential of about — 1.65 volts. as against the mercury anode. It may be natural to attribute those last two waves to the complex ions $Zn(CN)_5^{---}$ and $Zn(CN)_6^{----}$ although Pines (*l. c.*) with the concentrations and the sensitivity he disposed of, did not observe any wave corresponding to the last ion.

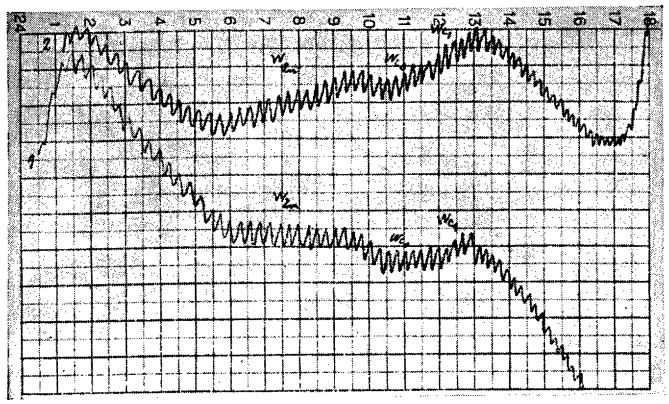

Fig. 10 b. Electro-reduction of highly dilute solutions of zinc perchlorate with three hundred equivalents of potassium cyanide per equivalent of zinc.

No.	E. M. F. in volts	Counter current	l/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	2	6	1 × 1	0.00001	0.006	0.001
2	2	5	1 × 1	0.00001	0.006	0.001

When we used extremely dilute solutions, and when we lowered the ionic strength by dispensing with supporting electrolyte, the complex waves were still more plainly seen, and in the solutions that did not contain $NaClO_4$ we even obtained a complex wave with a half wave potential of about — 1.8 volts. One might perhaps attribute this wave to the forming of still higher complexes with this extremely high excess of CN^- ions. The observation of Pines that the complex waves disappear when the CN^- excess is very great seems only to hold good for relatively concentrated solutions, but dilution evidently brings about sufficient dissociation of the complexes for reaching equilibrium. From these observations we may conclude that the $Zn(CN)_4^{--}$ complex ion is predominant in dilute solutions of zinc salts on addition of CN^- ions, and is perhaps even formed before the $Zn(CN)_3^-$ ion. With great excess of CN^- ion the coordination number 4 is seemingly exceeded.

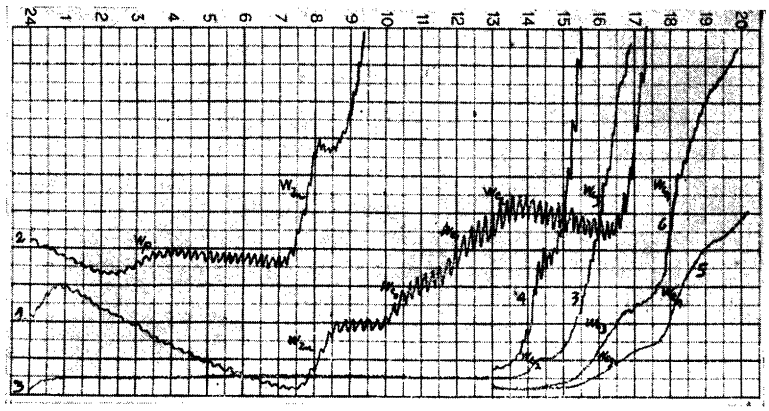

Fig. 11. Electro-reduction of zinc perchlorate with gradually increasing amounts of potassium cyanide, but without supporting electrolyte.

No.	E. M. F. in volts	Counter current	l/sens	Mol. conc.		
				$Zn(ClO_4)_2$	KCN	$NaClO_4$
1	2	2	5 × 1	0.001	0.0147	0
2	2	1	10 × 1.5	0.001	0.048	0
3	2	0	10 × 10	0.001	0.240	0
4	2	0	10 × 2	0.001	0.240	0
5	2	0	100 × 10	0.001	0.240	0
6	2	0	100 × 5	0.001	0.240	0

SUMMARY

1. The polarographic behaviour of the system $Zn(ClO_4)_2$—KCN—H_2O has been investigated.

2. As previously found, the Zn^{++} ions in very dilute solutions show a stepwise polarographic reduction when a sufficiently sensitive apparatus is used. The double waves previously obtained were repeatedly reproduced.

3. As the CN^- ion concentration is increased beyond the point $Zn^{++} : CN^- = 1 : 4$ complex waves appear. At first only a wave with $w_{1/2}$ = ca. — 1.3 volts as against the mercury anode is manifest. This is attributed to the ion $Zn(CN)_4^{--}$. With increasing concentration of CN^-, however, a wave appears at lesser $w_{1/2}$.

Table 1. Main points in the polarographic behaviour of the system $Zn(ClO_4)_2 - NaClO_4 - KCN - H_2O$.

Fig. no.	Mol.ratio Z++: CN-	Pre-wave	Zn++ wave	w_{c_0}	w_{c_1}	w_{c_2}	w_{c_3}
1	1 : 0	Distinct	Dominant	Non existent	Non existent	Non existent	Non existent
2	1 : 2 $Zn(CN)_2$	Observable	Low	Not considered	Not considered	Not considered	Not considered
3	1 : 2 $Zn(ClO_4)_2$ + KCN	Distinct	Dominant	Not considered	Not considered	Not considered	Not considered
4	1 : 4	Still observable	Still dominant	Just appeared	Distinct	Not appeared	Not appeared
5	1 : 6	Still observable	Still dominant	Unmistake-ably appeared	Distinct	Not appeared	Not appeared
6	1 : 8	Dis-appeared	Distinct	Fairly distinct	Distinct	Just appeared	Not appeared
7	1 : 12	Just reappeared	Not dis-tinguishable	Distinct	Distinct	Just appeared	Not appeared
8	1 : 24	Reappeared	Not dis-tinguishable	Distinct	Distinct	Distinct	Not considered
9	1 : 120	Reappeared	Disappeared	Disappeared	Disappeared	Distinct	Distinct
10 a	1 : 600	Reappeared	Distinct	Disappeared	Disappeared	Disappeared	Indistinct
10 b	1 : 600	Not observable	Distinct	Observable	Observable	Not observable	Not observable

This may be owing to the formation of $Zn(CN)_3^-$ ions, which are evidently not as dominant as the $Zn(CN)_4^{--}$ ions. With gradually increasing amounts of CN^- a wave is obtained with $w_{1/2} = -1.5$ volts, which may be due to $Zn(CN)_5^{---}$ ions, and still another wave with $w_{1/2} = -1.65$ volts. The last wave seems to indicate that the $Zn(CN)_6^{----}$ ion is polarographically reduced before the K^+ ion, and not as formerly supposed at negative potentials greater than 2 volts. In dilute solutions without any supporting electrolyte we even got a separate wave with $w_{1/2} = -1.8$ volts.

4. The assumption made by earlier polarographic investigators, that no complex wave is to be observed when a large excess of potassium cyanide is added to a zinc solution, seems not to hold when the solutions are sufficiently dilute and the apparatus correspondingly sensitive.

The authors are indebted to Anne Lise Kallevig for her careful and reliable work as our assistant.

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Received June 28, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1263–1274

Corrosion of Steel in Laminar Flowing Water

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When studying corrosion from tap water in galvanized and non-galvanized iron pipes, the author attempted to investigate the formation of the rust layer upon an iron surface overflown by water. For this purpose a test cell as shown in Fig. 1 was built.

THE TEST CELL

The test cell consisted of a circular iron disk with a machined surface and a glass disk secured at a distance of 2 mm from the iron surface by means of a 2 mm thick circular rubber ring. Above the glass there was another rubber ring, and on the top of that a metal ring, connected to the iron disk by means of screws and nuts. The long cylindrical heads of the screws formed four legs supporting the cell.

The iron disk had an outside diameter of 140 mm and the openings in the metal ring and the rubber rings were 90 mm in diameter.

The space between the iron disk and the glass was filled with water, flowing in a steady stream from a hole in the disk, placed 39 mm from the centre, to another in the diametrically opposite position. To avoid the entrapping of air bubbles the iron disk was supported in an inclined position so that the outlet was located at a higher position than the inlet.

The water was taken from the tap to a reservoir with an overflow giving it a head of approximately 350 mm above the cell (Fig. 2). From the reservoir the water was led through a rubber hose to a stainless steel tube screwed into the bottom of the disk.

An air vent was arranged just before the inlet to the cell. The hole in the iron disk which formed the outlet for the water flow had a smaller cross section than any other part of the circuit, and consequently it controlled the discharge of water. Most of the tests were made with disks having a 2 mm

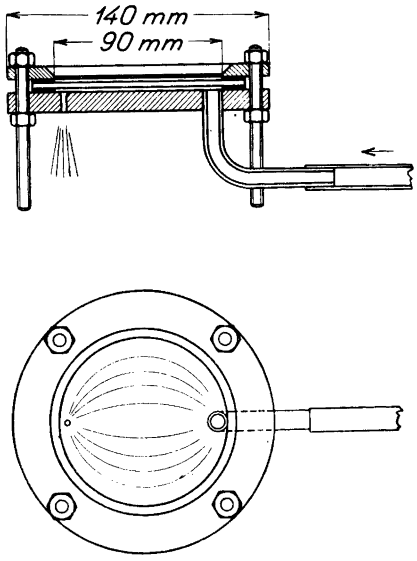

Fig. 1. The test cell.

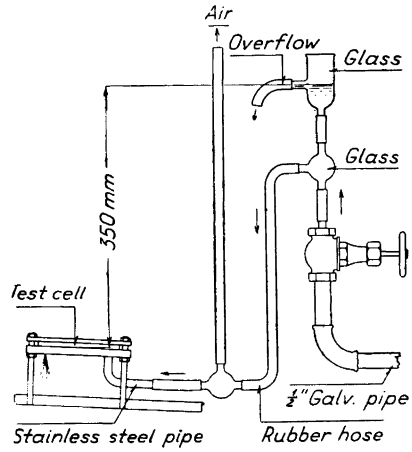

Fig. 2. The connection of the tap to the test cell.

slightly countersunk outlet hole, corresponding to a discharge of 5 ml per second or 18 litres per hour. The flow pattern in the cell was purely laminar except for an area around the inlet. Here a three-dimensional flow with local eddies prevailed.

PREPARATION OF A TEST CELL

The test cells were prepared in the following manner. The iron disk was burned out of a plate, and holes for the inlet, outlet and screws were drilled. As the last operation, the front surface of the disk was machined by turning without using oil or any other coolants on the tool. The final cut was made with a feed of 0.025 mm, equal to one thousandth of an inch, per revolution. The tool was well sharpened, and the surface of the finished disk appeared bright and uniform with a fine spiral groove from the last cut. Only in special cases the surface was afterwards polished with emery cloth. Without being touched by hand, the surface was then washed in absolute alcohol with a stiff non-metallic brush in order to remove all chips. The rubber ring and the glass cover were placed on the disk, on top of that the second rubber ring and the metal ring, all parts being kept together by the four screws. The inlet pipe of stainless steel was screwed into its place. When the test was to begin the cell was connected to the water reservoir by means of a rubber hose. The

water flow to the reservoir was started in advance in order to remove air from the pipings.

If, in spite of all precautions, air bubbles collected on the inside of the glass, they could be washed away by a blow dealt to the cell by hand. It was very important, during the preparations, to keep the iron surface absolutely free from any grease. Even a slight touch with a finger would easily be traceable later.

THE DEVELOPMENT OF RUST DURING A NORMAL TEST

28 tests were made under what may be described as normal conditions:

1. The test disk was cut out of a piece of rolled steel containing 0.10—0.40 % C.

2. The outlet hole had a diameter of 2 mm, giving a flow of 5 ml per second or 18 litres per hour.

3. The water was Copenhagen tap water. Its composition varied daily, and some examples are given in Table 1. (The variations were due to the water being a mixture drawn from several groups of wells, to which the city mains are connected through a number of feed pipes. Several water reservoirs are also connected to the mains. Therefore, the composition of water delivered to the consumers will depend upon the flow condition in the city mains.)

The water temperature was 12—16° C.

The following description gives the general trend of the development of rust. The individual test disks showed variations in the rapidity of rust formation and in the ratio of areas of rust and bright iron. It was not possible to relate these variations to differences in the carbon contents of the steel or to any known characteristics of the water.

In the beginning of a test the appearance of the iron surface changed very quickly; later on the changes were developing more slowly. Therefore, the description is referred to a series of stages considered with increasing time intervals. Each stage applies to the appearance of an "average" test disk.

1. stage. 3-5 minutes after the start of the water flow. Faint green lines become visible on the upper half of the test piece (the half next to the discharge opening). The lines evidently follow the stream lines and their spacing varies between a fraction of a millimetre and several millimetres.

2. stage. 30 minutes after start. The lines have become very clear and some finer lines have combined into broader ones. Their colour is green-blue, and their starting points are located at a distance of 15— 30 mm from the inlet hole.

3. stage. 3—4 hours after start. The lines show a faint sepia colour at their sources. Short green—blue lines are to be seen near the inlet hole; they are direc-

Table 1. Analyses of Copenhagen water (examples).

		$^{9}/_{7}$ 1949	$^{22}/_{9}$ 1949	$^{23}/_{11}$ 1949	$^{31}/_{1}$ 1950	$^{20}/_{3}$ 1950	$^{24}/_{5}$ 1950
Hardness (German)	total	18.2	19.6	18.6	16.6	18.6	17.8
	permanent	2.2	3.1	2.6	.6	2.4	2.7
	temporary	16.0	16.5	16.0	16.0	16.2	15.1
pH		7.7	7.75	7.8	7.65	7.75	7.6
Total solids	p.p.m.	520	584	588	524	520	480
Calcium (Ca^{++})	»	97	97	97	94	97	91
Magnesia (Mg^{++})	»	20	26	22	15	22	22
Iron (Fe^{++})	»	.06	.02	.02	.04	.03	.04
Manganese (Mn^{++})	»	0	0	0	0	0	.022
Sodium (Na^{+})	»	55	67	69	69	61	44
Bicarbonate (HCO_3^-)	»	348	360	348	348	354	329
Chlorid (Cl^-)	»	78	92	85	71	76	56
Sulphate (SO_4^{--})	»	49	66	74	58	66	62
Nitrate (NO_3^-)	»	traces	traces	0	0	0	0
Phosphate (PO_4^{---})	»	0	0	0	0	0	0
Iodine (I^-)	»	.02	< .01	.04	.02	.01	.02
Sulphuretted hydrogen (H_2S)	»	0	0	0	0	0	0
Silica (SiO_2)	»	16	18	18	18	16	16
Free carbondioxide (CO_2)	»	8	9	9	13	11	11
Dissolved oxygen (O_2)	»	9	8.1	8.7	9	9	8.7
Alkaline bicarbonates ($NaHCO_3$)	»	0	0	0	0	0	0

ted towards the inlet and are traces of the eddies set up by the incoming water. Their configuration is similar to a bouquet of flowers in a vase (Fig. 3).

4. stage. One day after start. All lines have become sepia-coloured. It can be observed through a magnifying glass that they are of a certain height, while the green and blue lines only consist in a stain of the iron surface.

5. stage. One week after start. All lines have become more dark and have grown in width and height, now forming a system of hedges. In some cases one or two tubercles (sepia in colour) have grown up at the edge of the inlet hole (Fig. 4).

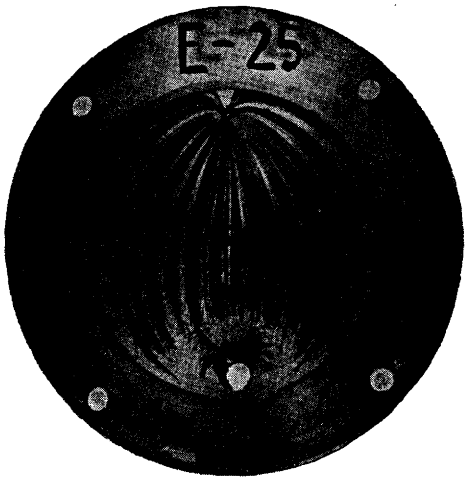

Fig. 3. Disk of SM-steel, 3 hours after the start.

Fig. 4. Disk of the SM-steel, 7 days after the start.

6. stage. One or two months after start. The lines have grown in width and height and in some places show a kind of dark brown fractures with fresh growth, light sepia in colour, in the middle of the fracture.

When a disk was to be preserved for later reference, it was carefully dipped in a bath of absolute alcohol, dried in the air for some minutes and then dipped in a bath of nitro—cellulose lacquer (if the dipping was not done carefully, loose rust would be washed away). When the lacquer had dried, the plate could be touched without impairing the rust.

If the abrasion of the disk itself was to be studied, the corrosion products were removed by dipping the disk in diluted and inhibited hydrochloric acid. The disk showed corroded grooves and caves where rust had collected, and lanes of untouched iron between the grooves.

THE MICROSCOPICAL DEVELOPMENT

Most observations in microscope are made with a 25 time magnification; 100 times are used for a few checks.

The green and blue colours to be seen shortly after the start of a test are, as mentioned, only a stain of the iron surface. All details in the surface, such as turning grooves, small burrs *etc.*, are equally visible after the staining.

The sepia colouring can in the microscope be seen to originate in small pins of the said colour, standing on ends in contact with the metal surface. Their diameter ranges from less than 0.01 mm to 0.02 mm, their height is about 0.03—0.05 mm. Observing the same spot at time intervals their number is steadily increasing. It is characteristic that they seem to prefer to settle to the lee side of any obstacle. Any collection of pins is therefore followed downstream by a tail of less concentrated pins, the tail growing with time in concentration and length. At any spot where pins have once begun to settle, they will after one or two days have covered the iron surface completely, hiding all marks and grooves once to be seen around the spot.

At this stage, the mass of pins take the shape of diminutive coral reefs, growing in height and width for some weeks. Then the growth seems to decrease in speed, and some day deep fractures may appear in the reefs; they disclose a dark brown colour behind the lighter exterior. A day or two later, light brown regions are growing in the fractures, thereby increasing the height of the reef until the growth again is retarded.

Through the microscope pins can be seen also on the bright iron stripes between the coral reefs, their number is increasing very slowly and the stripes appear macroscopically to be bright for weeks or even months.

THE DEVELOPMENT OF RUST DURING TESTS DIFFERING FROM THE NORMAL CONDITIONS

Each of the conditions described in the following section has been tested only a few times; the results must therefore be taken as rather preliminary.

On a disk of the same material as the normal ones but *polished* with fine emery-cloth, the number of lines was smaller and their spacing larger, depending upon the smoothness of the surface. Lines once started developed in the same way as previously described, but it seemed to be more difficult to get a line started here than upon an unpolished surface of the same material.

Cast iron, machined but not polished, did not show any lines for the first half hour. The first traces appeared in the eddy region around the inlet. When the lines became visible also in the laminar flow region, they were very closely spaced and darker in colour than upon steel. One day after the start the whole disk was dark brown with darkgreen stripes. Bright iron was only visible in a ring-shaped area around the inlet hole, where iron was seen in short streaks arranged in a featherlike design.

At *smaller velocities* (discharge hole 0.5 mm) the test disk of ordinary steel quickly became almost completely covered by lines. The green-blue colour

changed within 2—3 hours to sepia, but in addition to the fixed rust on the disk fine brown rust flakes collected under the glass and in the discharge hole. The cells had to be cleaned several times every day to prevent complete obstruction of the discharge and to allow inspection of the disk.

At *larger velocities* (discharge hole 4 mm) the formation of lines was less distinctive but the difference in colours between the beginning and the end of the lines was after some time more pronounced than for the 2 mm discharge. After two days the colours of the stripes were golden-yellow near the inlet and dark-brown, almost black, near the discharge.

Chips not removed from the iron disk invariably caused the formation of distinct lines beginning in each chip. The lines were visible 3—5 minutes after the start of the water flow. The question arose whether the lines were in reality caused by small chips in all cases, also after the most careful cleaning with alcohol and a non-metallic brush. A microscopic study of the lines revealed no chips on thoroughly cleaned disks, not even at the sources of the lines. Furthermore, after some experience. a method was found by which deficiencies in removing chips could be detected during the cleaning procedure: About half a minute after the surface had been flushed with alcohol, any chips not removed could be seen as small projections from the half-wet surface.

Experiments showed that not only metallic chips but also small pieces of rubber or pats of glue got a tail of rust. It was concluded that any obstruction to the true laminar flow will cause a line of rust downstream.

Disks with a galvanized zone were made by shaping a flat groove 19 mm wide diametrically across the iron disk, hot galvanizing the total of the disk and then turning away so much of the surface that the zinc coating disappeared except in the groove. The depth of the groove was chosen so that iron and zinc constituted a smooth surface after the turning. A ring of zinc was also left outside the test surface, to avoid rusting under the rubber ring. Incidentally this ring of zinc also protected a part of the iron surface inside the rubber ring.

The iron did not show any rust for a week but one day after the start the zinc had dark lines, following the stream lines and being more intense at the edges near the iron than at the inner part of the belt. After a week a tubercle had formed on the iron at the inlet hole (Fig. 5). The picture shows protected bright iron surfaces in the following three places:

1) Between the zinc belt and the outlet.
2) Along the circumference.
3) In a narrow zone upstream of the zinc belt.

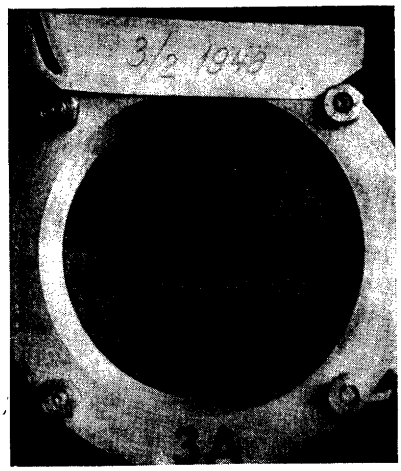

Fig. 5. Disk of SM-steel with a galvanized zone, 9 days after the start.

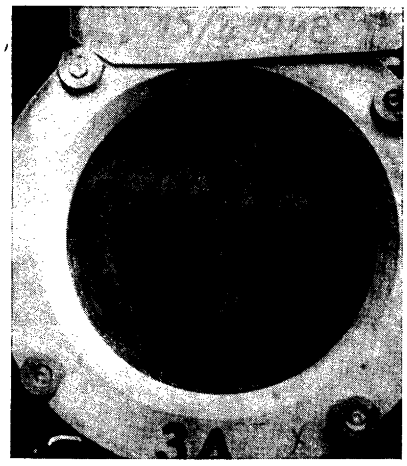

Fig. 6. Same disk as Fig. 5, almost three months after the start.

The average velocity of the water crossing the zinc belt was 28 millimetres per second, higher in the centre and lower at the edges. The protected zone had a width of 5 millimetres on an average, less in the centre (4 millimetres) and more at the edges (6 millimetres).

The dark coloured area between the inlet and the protected zone in front of the zinc belt could in the microscope be seen to be covered with small vertical pins. They were not arranged in stripes but evenly spread; in part of the area they could be seen with the naked eye.

The dark stripes on the zinc which appeared after one day grew in length and width, and after two weeks the zinc surface was dark with bright stripes only to be seen in the central part of the zinc belt. After a month, all zinc was dark, without any brightness.

At this time the tubercle caused a stripe of rust to be formed from the inlet to the outlet. It crossed the zinc belt but its adhesion to the zinc was of a much looser nature than that to the iron.

The continued flow of the water produced rust both in front of and behind the zinc belt, and after almost three months the picture Fig. 6 was taken. The great mass of rust in the upper right section was formed around an air bubble, which was not removed but continued growing by increments from air dissolved in the water.

Finger-prints and other traces of grease either protected iron or gave rise to other kinds of corrosion than those seen on a clean iron surface. In all cases, any greasy contamination of the surface could easily be detected after the start of the water flow.

Water with a small amount of Calgon (a hexameta phosphate, the strength of the solution being about 50 parts per million) gave no coloured lines or rust even after weeks of water flow. The bright surface showed some interference colours, and after some weeks dark shades could be observed, which in a microscope were seen to consist of a great number of very fine dark points. The shades followed the streamlines, but each shade was much wider than the lines appearing when no calgon was added.

ATTEMPTS AT QUANTITATIVE MEASUREMENTS

It was attempted to standardize the preparation of the disks in such a manner that possible differences in the ability of the water to form a protective layer upon the steel could be detected as differences in the proportion of bright and rust-covered iron after some fixed time. However, it was found, that some undetermined factors had as much influence upon the formation of lines as the differences in water, which could be tapped at different times of the day (Table 1). It is supposed that a very slight difference in the roughness of the surface after the machining was one such disturbing factor. It is realised that the way in which the roughness governs the initial formation of lines is of a very complex nature and that even the smallest departures from some selected type of roughness might lead to large differences in the configuration of lines. A finely polished surface is perhaps easier to reproduce, but experience has shown that the formation of lines on such a surface is even more casual than on an unpolished surface. The attempts to develop the cell into an instrument for quantitative measurements must therefore be postponed until a method is found of making a perfectly reproduceable rough iron surface.

THE ELECTRO-CHEMICAL EXPLANATION

The growth of rust on an iron surface submerged in water containing oxygen has been explained by McKay [1] and Baylis [2]. On page 377 Baylis says (see also Evans [3,p.3]).

"When a fresh iron surface is exposed to the water of any of our public water supplies, corrosion starts immediately. The dissolved oxygen within a certain zone next to the metal surface is soon used up, and precipitation of ferric hydroxide, or certain hydrous oxides of iron, usually takes place at a point a slight distance from the metal surface. When the precipitate has formed, part of it is attracted back towards the metal surface. This starts the formation of deposits of rust on the metal surface. Since ferrous hydroxide, or certain other ferrous salts, is not ordinarily precipitated within the pores of rust, but diffuses outside to where it is oxidized before being precipitated, the building up of the precipitate is from outside. In other words, iron goes into solution at the metal surface, diffuses to the dissolved oxygen zone, is precipitated probably as a hydrous oxide of iron, and the precipitate is attracted back to the surface of the metal, or precipitate already formed. In this way the precipitate builds up porous and somewhat fibrous."

The process is autocatalytic (McKay p. 24) because the oxygen concentration is smaller inside the mass of the precipitate than outside, where the water flow immediately replenishes the consumed oxygen. Thereby a differential cell is set up, the covered areas being anodic and the non-covered ones being cathodic. A pit is formed under the precipitate, while the uncovered surface is protected. A concentration of precipitate is commonly called a tubercle because of its fibrous natur.

Baylis explains the retardation of the growth in this way:

"In following the progress of pitting and tuberculation it has been found that there are active and dormant periods, and that the character of the water determines largely whether a pit is active or dormant most of the time. When the precipitate overlying an area where the iron is going into solution reaches a certain thickness, which is quite variable for different conditions, the diffusion of soluble iron from the surface to where there is dissolved oxygen present slows up to where the oxygen in the water reaches the hydroxide precipitate already adhering to iron. Precipitation then begins to take place within but very near the surface of this precipitate. In other words, the soluble iron is oxidized and precipitated before it reaches the outside solution."

Baylis proceeds by explaining how the iron precipitated within the older precipitate forms a diaphragm which stops further action. After some time the diaphragm in some cases breaks, and the process continues for some time again. Inside the tubercles, some of the precipitated ferric hydroxide will be reduced to black magnetic oxide Fe_3O_4.

The explanation agrees very well with the observations made, though the reason why the precipitated ferric hydroxide is attracted back to the iron

surface or the older precipitations, has not been given definitely. Baylis has seen particles of rust suddenly move at considerable speed from a point as far as 0.2 mm from an iron surface towards the surface.

The very first reason why a rust formation starts must be quite incidental. The difference between the oxygen concentration on the windward and that on the leeward side of a turning groove, small differences of structure in adjoining iron grains *etc.* give rise to the first faint potential differential and corrosion process. The oxygen consumption in connexion with this process immediately reduces the local oxygen concentration and thereby makes the spot more anodic.

If the water flow is turbulent, the oxygen deficiency is quickly replenished from all sides. Consequently, the first incident for forming a tubercle must be rather strong; when formed, it will grow in a concentrated way with only a weak tendency for the growth to follow the direction of flow.

If the water flow is laminar, the oxygen deficiency will spread downstream in a well defined line. Therefore, a differential concentration cell is formed, making the particular streamline anodic and the adjoining streamlines cathodic. The attracted precipitate of $Fe_2O_3\ (H_2O)_n$ reduces the flow velocity and thereby increases the oxygen deficiency and the potential difference.

A PROTECTIVE LAYER

It had been expected that a chalky rust layer would be formed upon the cathodic iron surfaces. No such layer could be seen, however. If, after all, there was a layer it did not give a lasting protection. It was observed that any alteration of the direction of the flow was followed by a formation of rust in areas which had previously been cathodic.

Under the conditions considered, consequently no natural absolute protection seems to occur. The continous growth of rust, and corresponding corrosion of iron, will however be hampered when the whole area is covered with an evenly thick layer of rust. The formation of such layer is counteracted by the auto-catholytic effect of rust-formation but might probably be supported by a turbulent flow of the water and by such chemical properties of the water as make the diaphragm on the surface of the rust more durable.

SUMMARY

The effect of flowing water containing oxygen upon an iron surface has often been described (Evans [3,p.317]), but no references have been found in the literature to the well defined effect of laminar flow of water. A cell with

laminar flow has been found a useful help in the study of the mechanism of rust formation on submerged iron surfaces.

LITERATURE

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2. Baylis, J. R. *Ind. Eng. Chem.* **18** (1926) 370.
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Received August 9, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1275–1292

Corrosion due to Tuberculation in Water Systems

I. The Effect of Calgon on the Potential of Iron Electrodes in Differential Aeration Cells with Running Tap Water

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It was the purpose of this work to show in what way Calgon interferes with the mechanism of corrosion due to tuberculation in water pipes. Primary experiments [1] showed a definite effect of minute concentrations of Calgon on the avoidance of rusting of an iron surface exposed to laminar flow of water. In the absence of Calgon we got rusting in the form of well pronounced patterns. When plates had been run with tap water containing increasing amounts of Calgon, the "lines" of rust gradually became less pronounced, and at a concentration of about 20 parts per million (ppm.), the lines did not appear at all.

So far, in our preliminary, short-term experiments, we had been able to show that only the Calgon addition simultaneous with the start of the experiment, completely inhibited the formation of the rust patterns. The addition of Calgon after the patterns had been formed did not remove them, and only high Calgon concentrations (over 100 ppm.) seemed to inhibit the further development of rust lines (streaks).

A surface of iron run with water containing Calgon remained bright for rather a long time, showing only some interference colours indicating the formation of a very thin protective layer.

All these facts together with indications in literature [2] show that the inhibitive effect of Calgon is due to the adsorption of this compound on the iron surface, and that Calgon belongs to the class of adsorption inhibitors [3].

But we were interested in the details of the inhibitive effect of Calgon, and especially in the effect of Calgon on the electrochemical potentials of electrodes in the differential aeration cell (DA-cell) with running tap water.

There exists a rather comprehensive literature[4] about measurements of the potentials of corroding metals and about the influence of inhibitors on the potential of electrodes in bimetallic cells. The measurement of the potentials of the corroding metals has mostly been made either in solutions with well defined, rather high concentrations of the respective metallic ions, or in strong acid, alcaline or salt solutions. In many cases in measurements of the potentials of iron rather little attention has been paid to the influence of oxygen on the potential. Some potential measurements have also been made in natural waters, but either at rather short intervals or without taking into account the division of the metallic surface into anodic and cathodic zones.

Beginning with the experiments of Chyżewski and Evans[5] many measurements have been made of the changes of potentials due to inhibitors. Much literature is quoted by Shih-Jen Ch'iao and Mann[6]. But as far as we know, no experiments have been made on the influence of the inhibitors on the potentials of the electrodes in a DA-cell. Herzog and Chaudron[7] have studied the influence of some inhibitors but only on the current generated by a DA-cell.

DA-cells have been studied mostly in the form first built by Evans[8] *. The form of DA-cell that naturally occurs (tubercles in water pipes — Fig. 1a) or its laboratory modifications (Fig. 1b) have as far as we know never been studied in laboratory until the appearance of the work of Olsen and Szybalski[9].

For this reason we thought that it would be of some interest to study the influence of the inhibitors on the potentials of the anode and cathode of the DA-cell of a shape normally met in tuberculation of water pipes. In this case the concentration of oxygen in the cathodic space will be constant, because the water is saturated with oxygen and the water flow is kept constant; the oxygen concentration on the anodic side will be automatically controlled by the corrosion process. The influence of the atmospheric oxygen is in this way eliminated. This arrangement makes it possible to test natural waters directly from the municipal supply. Also the pressure and temperature of the tested water might be controlled. In order to get well defined potential-time curves we used an automatically recording multipoint instrument, and the potentials of the electrodes of all the cells, simultaneously tested and run with water from a 30 ton storage tank, were measured against one calomel standard electrode. For the determination of the influence of Calgon we have registered simultaneously the potentials of at least four cells *i. e.* two with Calgon and two without Calgon, and the reproducibility of the parallel experiment was within

* Dr. F.L. LaQue drove kindly our attention to two early papers of Adie[21] and Viard[22], describing the differential aeration effect.

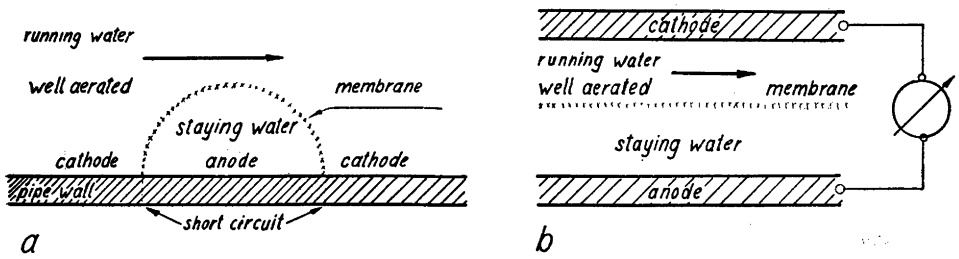

Fig. 1. Schematic comparison of the conditions (a) during pitting with tubercle formation and (b) in laboratory model of DA-cell (after Olsen and Szybalski).

the limits of 1—5 mV. The equipment used allowed us to extend our measurements on a single cell to some months.

EXPERIMENTAL

Three types of DA-cells were tested: cell I with laminar water flow and cells II and III with turbulent water flow (Fig. 2). In all the cells we had the metal surface divided into two regions insulated from each other: anode and cathode. Between the anode and cathode we always had a filter-paper membrane (Schleicher Schuell No. 589—2) to protect the anode from direct access of the water stream. In cell I the membrane was attached with synthetic resin glue to the insulation i between the electrodes. In cells II and III filter paper was fixed between parts b and c. In this way the cathodic surface of

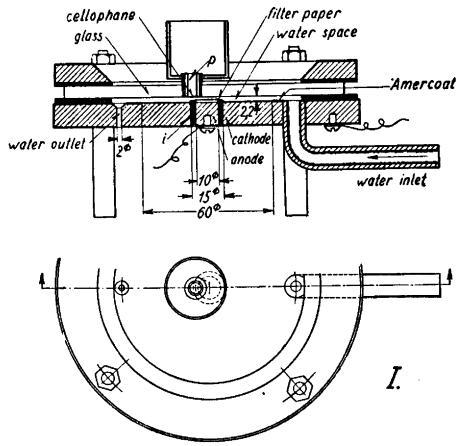

Fig. 2 I. DA-cells for experiments with running tap water. Cell type I.

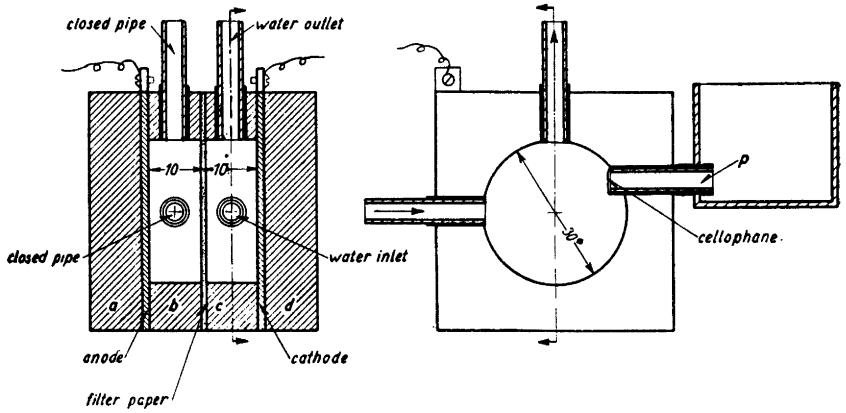

Fig. 2 II. Cell type II.

the metal was always violently washed with aerated water; in the anodic space water was stagnant, and consequently we had a strongly reduced access of oxygen, which moreover was quickly used up for (i) the oxidation of ferrous ions, for (ii) the depolarization of the micro-cathodic regions of the anode (due to the micro-cell structure of the anodic surface) and for (iii) direct oxidation of metallic iron.

For cell I (with the dimensions in mm shown in Fig. 2) we worked with a water flow of 300 ml/min, which gave a laminar flow pattern over the main part of the metal surface (Reynolds number — Re = 100). The water flow was the same in cells II and III, but due to the design of the cells the flow pattern was turbulent, which could be directly observed. As electrode material we used S.M.-steel with approximately 0.15—0.18 % C.

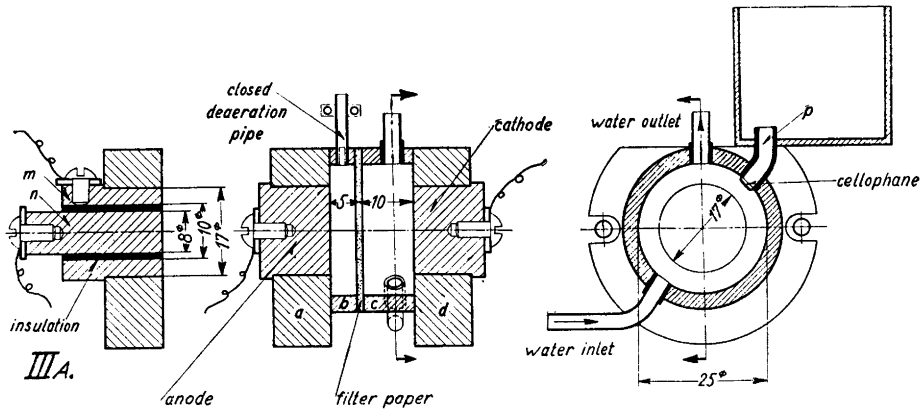

Fig. 2 III. Cells type III A and type III.

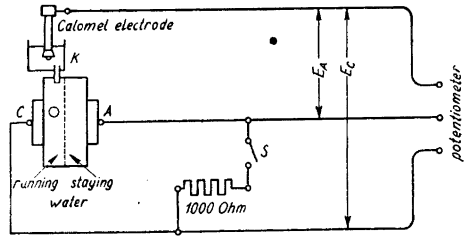

Fig. 3. The diagram of electric connections for automatic potential registration in a DA-cell.

In order to have a rather even water flow over the whole metal surface in cell I we worked only with the central part of the plate; the periphery was covered with a highly water-resistant coat of low electrical conductivity (Amercoat No. 23 — South Gate, Calif.). For the same reason we later changed from cell II to III, because, in cell II on the aerated side in the corners between "Etronax"-wall (c) and the metal plate, the water moved very slowly in comparison with the central part of the cathodic surface. In cell III the surface of the cathode did not reach these dead corners. Another difference between cell II and III was that the central part (b, c) of cell III consisted of a plexiglass tube permitting the experimenter to observe changes in the cell during the experiment. The distance between the electrodes was reduced in cell III in order to get lower internal resistance, and the symmetry of the cell shape was sacrified. In all the cells the metal surfaces (excluding the anodic and cathodic surfaces) which might get in contact with water and could have some influence on the measured potentials were covered with Amercoat. Amercoat was also used for the tightening of cells II and III. The electrolytic connection between running water and the standard calomel electrode (saturated KCl) was made by a saturated KCl solution; the water and the KCl-solution were separated from each other by a thick cellophane membrane. Air from the glass pipe p had been sucked out with a thin plastic pipe so as not to damage the membrane. The insulation i (Fig. 2 I) and the parts a, b, c, d (Fig. 2 II) a, d (Fig. 2 III) consisted of linen-bakelite laminate "Etronax".

The first experiments were made by continuous measurement of the current between the cathode and anode and, only occasionally, by measurement of the potential of the electrodes against a standard calomel half cell. But it was soon found that it would be necessary and more convenient to register the potentials automatically. For this purpose we used the "Multipoint Potentiometric Recorder — SPEEDOMAX" of Leeds and Northrup Cat. No. 60358 with the scale range extended to 1000 mV and registration frequency 6 times per minute. The diagram of connections is shown in Fig. 3. We were able to measure the potentials of the anode and cathode either when they were con-

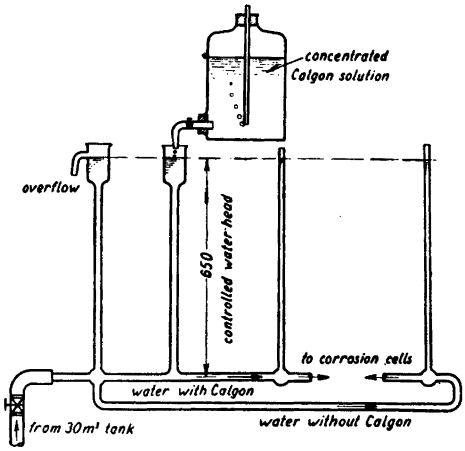

Fig. 4. The diagram of water-connection arrangement for experiments with DA-cells.

nected through a 1 000 ohm resistance or when disconnected. If many cells were used, connections between the calomel electrode and the respective cell cups (K — Fig. 3) were made with saturated KCl-agar bridges, rather thick and as short as possible, because the resistance in the potentiometric circuit must not exceed 10 000 ohms. This feature of the potentiometer used was rather inconvenient for our work. The diagram of the arrangement of the water-connection and the feeding of Calgon is given in Fig. 4.

The used tap-water shows the following average analysis:

Saturation index ($pH-pH_s$)	$-0.1-+0.1$	pH	7.5	SiO_2	15—17 ppm
Total hardness	18.3—18.8° H	Cl^-	81—93 ppm	Fe^{++}	0.01—0.03 ppm
Permanent hardness	1.2— 2.0° H	SO_4^{--}	71—72 ppm		
Dissolved solids	560—571 ppm				
Water was air-saturated at atmospheric pressure (8° C).					

EXPERIMENTAL RESULTS

1. The measurement of the corrosion current and internal resistance of the DA-cell

Measurement was made with cell I. Only in this experiment we used "The Electroflo Recording Instrument" No. 10306 and the scheme of connections is shown in Fig. 5. By the reading of v_1 and v_2 (switch s in position 1 or 2, respectively) we could calculate the internal resistance of the cell:

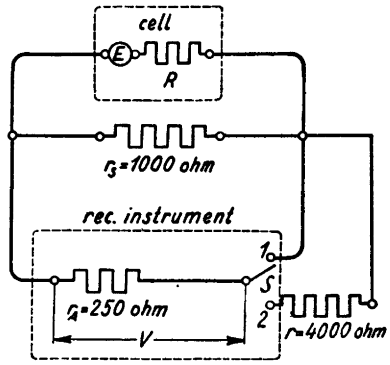

Fig. 5. The diagram of electric connections for automatic measurement of the corrosion current and internal DC-resistance in a DA-cell.

$$R = \frac{v_2 r - (v_1 - v_2)\, r_A}{(v_1 - v_2)\,\left(\frac{r_A}{r_S} + 1\right) - v_2 \frac{r}{r_S}}.$$

All the time the cathode and anode were connected through a resistance of 1 000 ohms. The calculated internal D. C. resistance of the cell was at the beginning of the experiments of the order of 1 000 ohms. Curves R and R_c (Fig. 6) show the changes with the time of the internal resistance of the cell. The curves i and i_c show the corrosion current. We see that if the Calgon is added just from the beginning of the experiment, when the iron surface is still uniformly bright, it has a very strong influence on the increase of the internal resistance of the cell and on the decrease of the corrosion current. It is obvious that the decrease of the corrosion current cannot be explained only on the basis of the increase of the resistance. The current not only decreases but also changes its direction. That means that Calgon must change the potentials

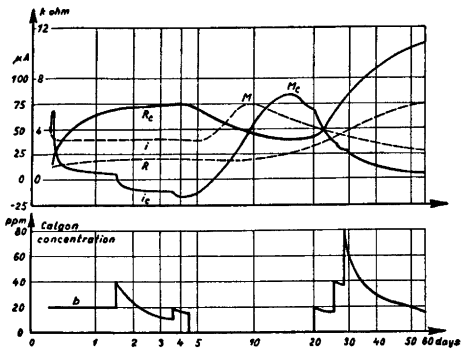

Fig. 6. Time-corrosion current (i, i_c), time-internal DC-resistance (R, R_c) and time-Calgon concentration (b) curves for DA-cells type I with (i_c R_c) and without (i, R) addition of Calgon. Rate of water flow 300 ml/min.

of the electrodes. The peaks M and M_c also indicate some changes in potentials. For this reason, in further experiments we have measured the potentials, trying on this basis to explain the mechanism of the action of Calgon.

Curve i_c in its beginning shows very characteristic sudden falls at the points of the increase of the Calgon addition. Curve R_c shows continuous changes. After the discontinuation of the Calgon addition on the fifth day we see that the inhibitive effect of Calgon is fully reversible. The next addition of Calgon for a 20 days old cell shows the same changes as in the beginning but much slower and smaller (the apparently great slope of i_c and R_c curves is due only to the logarithmic time scale). The reversal of the corrosion current was unobtainable in the case of old cells. Curves R and i show that also without the addition of Calgon we get an increase of the resistance and decrease of the corrosion current in the cell probably due to the formation of some compact layers on the cathode, as has been shown by capacity and A. C. and D. C. resistance measurement (Olsen and Szybalski [9]).

After finishing the experiment we opened the cell, removed the filter-paper membrane and examined the anodic and cathodic surfaces. The anodic space under the membrane was filled with rust which could easily be washed off with a water stream; then appeared the iron surface evenly corroded and of a dull metallic lustre. The cathodic surface outside the anode was covered almost completely with a thin, strongly adherent layer, but in some places elevated rust paths appeared, which it was possible to remove by washing and gently brushing. They then showed an underlying iron surface just as under the filter-paper membrane. These rustpaths appeared in places where there was a slower stream of water and consequently smaller aeration than in the neighbouring areas. This tubercle formation on the cathodic surface certainly interfered with the reproducibility of our experiments, but with this shape of the cell and with laminar water flow it was impracticable to get a quite even water flow over the whole surface and consequently an even aeration. For this reason, and because when using a slow stream of water we have had rather small measured potential differences between the anode and cathode, we decided to change over to cells of another shape using turbulent water flow.

2. The measurement of potentials

In the measurement of the potentials of the electrodes we had two alternatives: 1) The anode and cathode short-circuited through a low resistance; the situation would then be just the same as in a natural "tubercle", but it would be impossible independently to measure true potentials of the cathode and anode. 2) The anode and cathode disconnected; we were then able to measure

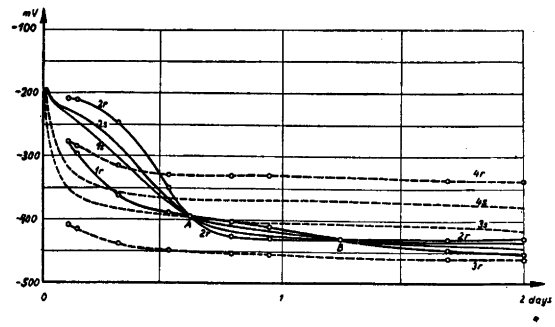

Fig. 7. Time-potential curves for the aerated (2, 4) and non-aerated (1, 3) electrodes of DA-cells type I with (1, 2) and without (3, 4) addition of Calgon. Electrodes connected through a 1 000 ohm resistance (s) or disconnected (r). Rate of water flow 300 ml/min.

the true potentials independently, but the conditions in such a cell are different from those in a real tubercle. We chosed a combination of both. During most of the time of the experiment the electrodes were connected through a 1 000 ohm resistance and only from time to time the circuit was opened so that the true potentials against the calomel electrode were registered.

Diagram (Fig. 7) shows the potential-time curves of two cells of type I with a water flow of 300 ml/min. The electrodes of both cells were connected through a 1 000 ohm resistance (switch S closed — Fig. 3). Curves 3s and 4s show that the potential of iron in contact with water is falling from about — 200 mV to — 370 mV for an aerated and to — 405 mV for an unaerated electrode (normal hydrogen-electrode scale) in the course of 24 hours. The decrease after that time is much slower. The potential difference between the anode and cathode is rather pronounced from the first hours of experiment. From curves 1s, 2s (water with 10 ppm. Calgon-product of Albright and Wilson, Birmingham) it is seen that the potential first falls slower, then quicker and finally reaches a level more negative than curves 3s, 4s. Moreover in the latter half of the first day the potential of the aerated electrode was more negative than of the unaerated one. That is in good agreement with the reversal of the corrosion current on the diagram in Fig. 6. In experiments with higher Calgon concentrations it was possible to get reversed and still greater potential differences and keep them for many days.

Curves 1r, 2r, 3r, 4r show the real potentials of the electrodes which were registered by means of a potentiometer when opening the switch S (Fig. 3) for a short period of time at the points marked on the diagram (Fig. 7). The features shown by curves 1s, 2s, 3s, 4s are here still more pronounced.

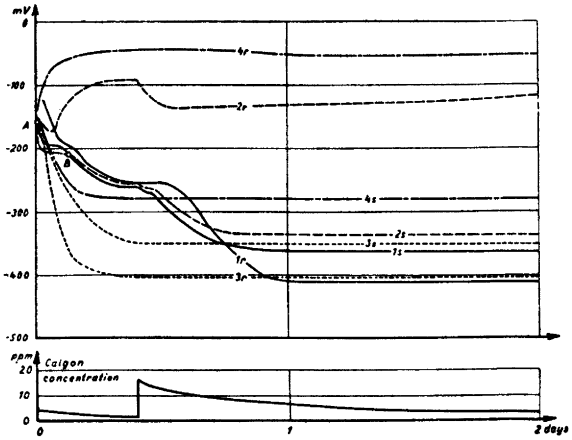

Fig. 8. Time-potential and time-Calgon concentration curves for DA-cells type III A with (1, 2) and without (3, 4) addition of of Calgon. Aerated (2, 4) and unaerated (1, 3) electrodes connected through a 1 000 ohm resistance (s) or dis-connected (r). Rate of water flow 300 ml/min.

The diagram in Fig. 8 shows the continuous time-potential curves for two cells of type III with double electrodes (fig. 2, III A). Electrodes m were connected through a 1 000 ohm resistance (curves 1s and 2s with Calgon and 3s and 4s without). Internal electrodes n were not connected so that they show their true potentials (curves 1r, 2r with Calgon; 3r, 4r without). It can be seen that a cell of type III shows much greater potential difference and a more noble cathode due to the turbulent flow of water and consequently to

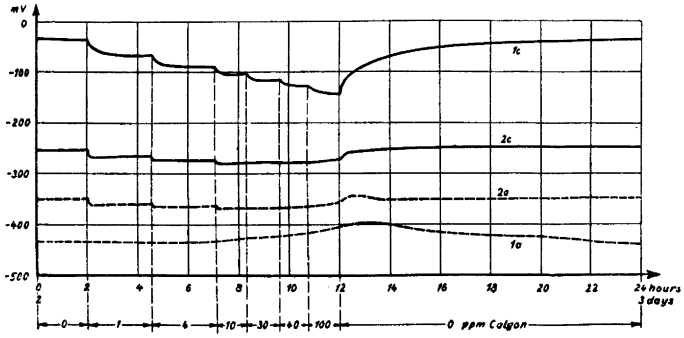

Fig. 9. The effect of various Calgon concentrations on the potentials of an aerated (c) and non-aerated (a) electrode of a two-day-old DA-cell. Electrodes connected through a 1 000 ohm resistance (2) or disconnected (1). Rate of water flow 300 ml/min.

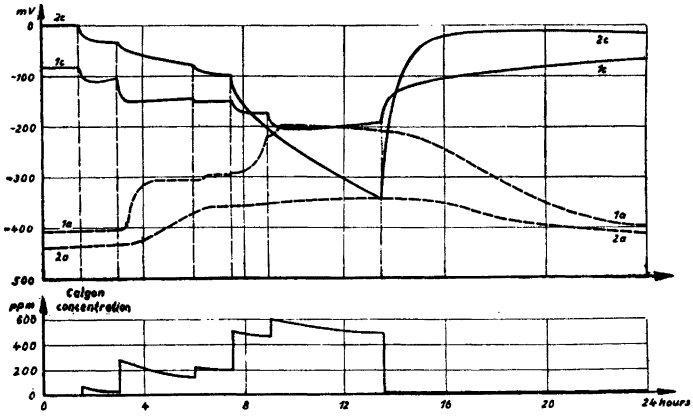

Fig. 10. The effect of various Calgon concentrations on the true potentials of an aerated (c) and a non-aerated (a) electrode in a one-day-(1) and a six-day-old (2) DA-cell. The electrodes disconnected 8 hours before each start of the addition of Calgon. Rate of water flow 300 ml/min.

the greater aeration of the cathodic surface. At this low Calgon concentration it was possible to get the potential difference reversed only during the first hour of the experiment. The potential difference for a cell run with Calgon-treated water is smaller than for a cell without Calgon. The increase of the addition of Calgon causes a very distinct decrease of the cathode potential (2r). A decrease of the addition of Calgon then slowly restores the more noble potential. The influence on the anode is difficult to discuss because the potentials of the electrodes have not yet reached a steady state.

The influence of Calgon on two-day-old cells, where the cathode and anode have reached some rather stable balanced potentials, is shown on the diagram Fig. 9. Curves 2a and 2c show the potentials of the anode and cathode connected through a 1 000 ohm resistance of a cell of type III. The upper curves 1a and 1c show the true potentials of a parallel cell where the electrodes have been disconnected 8 hours before the start of the addition of Calgon. Every increase of the addition of Calgon gives a decrease of the cathode potential, but low concentrations of Calgon show a relatively greater effect. The effect on the anode is slower and changes the potential in the noble direction. That is the reason why at concentrations higher than 40 p.p.m. the curves 2a and 2c cease to move in the negative direction but change to the noble one.

The diagram in Fig. 10 shows the influence of the age of the cell on the potential-time curves. On both electrodes the calgon-effected changes are slower with an increasing age of the cell. On the anode, moreover, the changes are smaller, but not on the cathode.

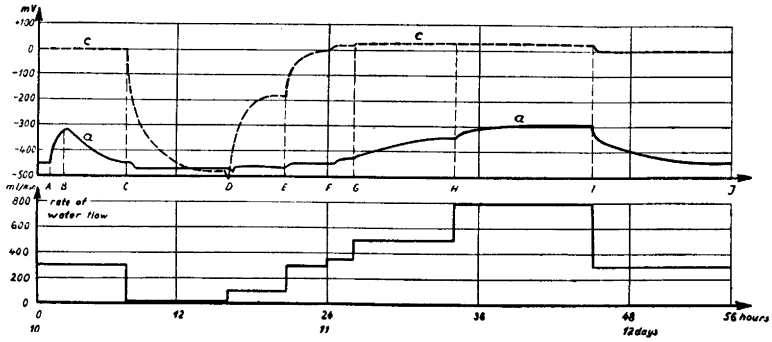

Fig. 11. The effect of the rate of water flow on the potentials of an aerated (dotted line) and a non-aerated (full line) electrode in a six-day-old cell. Explanations in text.

The diagram in Fig. 11 shows the influence of changes of water-flow velocity. The flow of water through the anodic space (the deaeration pipe under the filter-paper membrane (Fig. 2, III) is opened, rate of water flow through it = 2 ml/min.) increases the potential of the anode (A—B); the interruption of this percolation (the deaeration pipe closed) restores the original state but more slowly (B—C). — The interruption of the whole water flow through the cell causes the potentials of the electrodes to be equalized (C—D). The return to the previous velocity of the water flow ennobles the potential of the cathode to the former value (D—E—F). The increase of the water flow first has a great effect on the increase of the cathodic potential and almost none on the anodic potential (D—E—F). The further increase of the water velocity brings the cathodic potential to a constant value and only causes a further increase of the anodic potential (F—G—H). A reduction of the water flow to 300 ml/min. restores the first observed potentials (I—J).

CONCLUSIONS

The most striking feature observed in all experiments was the great range of different potentials shown by iron. The ennoblement of the iron potential is referred to by the term p a s s i v a t i o n. The nature of passivity is still an object of controversy in the literature (*e. g.* Evans [3], Uhlig [10]), but for our purpose we could regard it as a result of some factor which impedes the passage of metal ions from the surface of the crystal lattice to the water solution. Oxygen adsorbed or chemically bound in a reversible manner on the metal surface stabilizes its lattice and diminishes the tendency of the metal to pass into the solution, which means that the potential of the metal becomes more

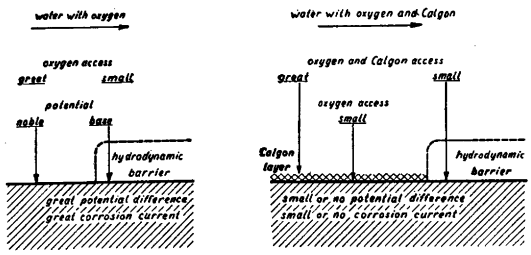

Fig. 12. Diagram of the influence of Calgon on the corrosion due to the action of DA-cells.

noble. The passivity and ennoblement of the iron potential * depend on the saturation of the metal surface with oxygen, but because the adsorbtion process is reversible, and because the oxygen is used all the time for the oxidation of hydrogen and ferro-ions, we must have a constant supply of oxygen to the metal surface. The covering of the metal surface with some porous layer impedes oxygen diffusion to the true iron surface. The saturation of the metal surface with oxygen decreases and consequently the potential is shifted in the base direction. This porous layer could be of various kinds. It could be some deposits of rust or of a microbiological character or any other hydrodynamic barrier which diminishes the water velocity in some area and consequently the oxygen transport towards iron surface. But also the adsorbtion layer of large molecules of Calgon or its complexes with other substances found in natural waters, seems to have the same depressing effect on the potential of iron due to the hindrance of oxygen access. But there is some fundamental difference between the action of Calgon-layers and other hydrodynamic barriers. The situation is shown on the following diagram (Fig. 12). In cathodic places where water carrying oxygen and Calgon has an easy access to the iron surface, the Calgon adsorbtion layers are rapidly well developed and consequently do not allow the oxygen diffusion to the true metal surface. In this way in stead of getting an easier oxygen access to the true iron surface in places of greater water flow the oxygen access becomes more difficult and the electro-chemical potential decreases in these places. At a small concentration of Calgon its transport to surfaces covered by hydrodynamic barriers will be

* The potential difference between two identical electrodes immersed, the one in a staying and the other in a running "oxygen-free" water solution, has been called Motor-Electric Potential [11]. But there are various opinions in the literature as to the nature of this effect [12]. Many authorities believe it could be referred to the action of the omnipresent oxygen. Apart from the nature of the motor-electric potential, its reported values are negligible in comparison with the fluctuation of potential observed in our case and caused by the differential aeration effect.

very small and no great changes are supposed to occur there. Consequently on a fresh iron surface, after the addition of Calgon, there will be no great changes in the potential of the anodic areas (we leave out of account the secondary influence of Calgon due to the interference of Calgon with the reaction of the oxygen depletion) and the higher cathodic potential will decrease with a resulting decrease of potential difference and corrosion current.

This smaller corrosion current causes the decrease of the production of ferro-ions at the anode, which allows a greater oxygen diffusion to the anodic surface with a resulting decrease of potential difference and corrosion current. That induces a further reduction of the production of ferro-ions on the anode, etc.

This picture of the influence of Calgon is very clear in the case of rather good differentiation of the iron surface into regions with an exposed clean metal surface and regions covered by some hydrodynamic barriers. The covering of the metal surface with some protective layers or a rather strong development of tubercles change the speed and effectivity of the action of Calgon.

The reported peptization power of Calgon [13] causing the solution and removal of the tubercles must also be taken into consideration, because it means the removal of the well developed hydrodynamic barriers and consequent equalization of the aeration and the potentials of the metal surface.

We can extend the explanation of the cathodic Calgon action in order to explain the tuberculation faculty of natural waters. We can be sure that the aerated water running over an iron surface will always in the beginning cause the formation of differential aeration cells, because the velocity of the water will change from point to point owing to the hydrodynamic properties of the surface. In this way in the places where we have a higher rate of water flow we shall have a better oxygen access and a more noble potential. Non-corrosive waters (Fig. 13b) are able to form quickly some impervious layers in the area where the water velocity and consequently the aeration are greater, which diminishes the oxygen access and turns the potential in the base direction in the same places. In places of a more rapid water exchange the non-corrosive water must show the ability of a so strong protective layer formation that the oxygen access to the true metal surface will be more difficult than in places with lower water flow. That will cause the equalisation or even reversal of the difference of potentials between more and less washed areas, and consequently there will be no tuberculation. This cathodic formation of a protective layer must be quick, before, owing to the primary differential aeration effect, we get well developed rust tubercles in the anodic areas with a consequent very great decrease of potential in comparison with the rather

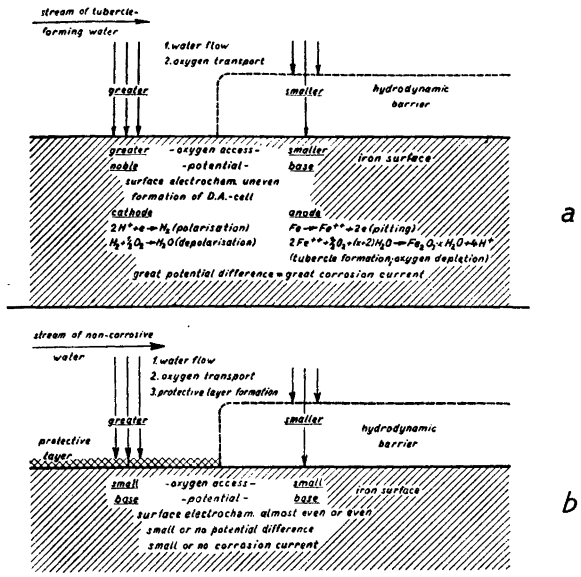

Fig. 13. Diagram of the influence of tubercle-forming (a) and non-corrosive (b) waters on the corrosion due to the action of DA-cells.

small primary potential differences due to the irregularities of the metal surface.

The tubercle-forming water (Fig. 13a) has no ability to form quickly some protective layers in the places of primary better oxygen access. That means that in every anodic area of the primary DA-cells due to the uneven water flow, we get, in a border case, the formation of tubercles. This case is not so very dangerous, because the ratio of the cathodic to the anodic areas is then rather high and because there is a possibility that anodic rust deposits, always extending beyond the anodic surfaces, will all join together, causing an even, small oxygen access to the whole surface. More dangerous is the case when the water has a limited ability to form protective layers. In this case only a few primarily stronger DA-cells will develop tubercles on the anodic surfaces, but because of the high ratio of the cathodic to the anodic surface the pitting might be rather serious.

Biological deposits can be especially active in causing tuberculation either by interference with the cathodic formation of the protective layers or by very quick establishment of highly anaerobic conditions in primarily anodic areas with a so rapid decrease of the potential that the slower cathodic decrease due to the formation of a protective layer cannot equalize the potentials and stop

the tubercle formation. In the literature we very often see that together with tuberculation a growth of iron bacteria has been found [9, 14, 15].

The non-corrosive water must show not only the ability to form protective layers, but this ability must also be 1) quick and energetic 2) differential — *i. e.* stronger where we have a higher rate of water flow and primary oxygen access, and weaker in the other places.

The nature of the protective layers built by natural waters and acting in the same way as Calgon will not be discussed here.

On this theoretical basis we can now discuss some aspects of the experiments described above.

The decrease of the cathodic potential caused by the increase of the Calgon concentration can be seen in Figs. 9 and 10 curves 1c and 2c. We see that small additions of Calgon to a 1-day-old cell give more rapid changes, which can be explained by an easier Calgon access to the fresh iron surface. A large and a long-term addition of Calgon shows a greater effect on the older cell. It seems that the joint effect of the cathodic covering of the surface by Calgon and by some natural protective layers gives a better protection against the oxygen access, but a somewhat longer time is necessary for the development of such layers.

At the anode a higher Calgon concentration has an influence on the potential opposite to the influence on the cathode. That is more pronounced in young than in old cells, because in old cells the diffusion of Calgon through the membrane toward the anodic surface is strongly impeded by the development of rust in the whole anodic space. This ennoblement of the anode potential by the addition of Calgon we may try to explain in two ways:

1) Calgon has the same effect on the potential as oxygen 2) Calgon interfers with the process of consuming the oxygen for the anodic oxidation processes. Explanation 1) is not very probable, because of the quite different Calgon action on the cathode. Explanation 2) sounds reasonable because if the consumption of oxygen in the anodic space slows down or stops, then the difference in the oxygen concentrations in the anodic and the cathodic space will be slowly equalized with a consequent increase of the anodic potential. The oxygen diffusing to the anodic space is mostly used for (i) oxidation of ferro to ferri ions, (ii) oxidation of hydrogen on the cathodic surfaces of micro-corrosion cells due to the uneven structure of the anodic metal surface and (iii) direct oxidation of metallic iron. We have not investigated if the auto-oxidation of ferro ions is retarded by the presence of Calgon. Nor can we say if Calgon interferes with the two other reactions.

The curve i_c on the diagram in Fig. 6 shows the corrosion current when Calgon is added. The decrease of the current is the result of the decrease of

the potential difference and the increase of the internal resistance of the cell due to the formation of the Calgon layer on the metal surface (curve R_c). At the outset of the experiment it was possible to get a reversal of the current also in the case of a low Calgon concentration, because the highly anaerobic conditions in the anodic space have not yet been established. It is obvious that this reversal of the current is due to the reversal of potentials, which could be seen in Fig. 7 and 8 between points A and B. If the hydrodynamic barrier between the anodic and cathodic surfaces is less developed, and we have a smaller water stream (cell I, Fig. 2), then it is easier to bring about the reversal of potentials by a smaller addition of Calgon than in the better developed DA-cell (cell III, Fig. 3).

Diagram in Fig. 11 shows the great influence of the changes of the water flow (aeration) on the potential of a DA-cell. We see that the ennoblement of the iron potential due to the oxygen content of water is fully reversible. After the interruption of the water flow, when oxygen will be used up in some oxidation processes, we get almost equally base potentials of both electrodes (C—D). First the increase (D—E—F) and afterwards the decrease (F—G—H—I) of the potential difference by the increase of water flow are in good agreement with the phenomenon often reported in the literature of an increase of the corrosion rate and tuberculation with increasing rates of flow to some critical value, after which the corrosion decreases (for inst. Hayn and Bauer [16], Friend [17], Pallo [18] — the apparently different results of Speller and Kendall [19] are discussed by Russell, Chapell and White [20]).

SUMMARY

The influence of Calgon on the potentials of the electrodes in a DA-cell with running tap water has been studied. Calgon decreases the potential of the cathode by the formation of an adsorbtion layer on its surface, which inhibits the oxygen access to the iron surface with a consequent decrease of its passivating action. The potential of the anode rises because Calgon interferes with the anodic depletion of oxygen. Both these effects cause the decrease of the potential difference and corrosion current. The decrease of the corrosion current is also caused by the increase of the internal resistance of the cell due to the formation of protective layers of low electrical conductivity. The peptization power of Calgon must also be taken into consideration.

The attempt at a general theory of tubercle-forming and non-corrosive waters is based on the property of non corrosive waters of the d i f f e r e n t i a l formation of protective layers in the areas of greater water flow, which decreases the oxygen access in these primary cathodic zones, equalizes the

potentials and causes the corrosion and tuberculation to cease. Some general aspects of the DA-cell are discussed and the phenomenon of critical corrosion rate with a successive increase of water flow is explained.

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Received February 24, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1293–1299

Corrosion due to Tuberculation in Water Systems

II. The Effect of Calgon on the Potential of Zinc and Copper Electrodes in Differential Aeration Cells with Running Tap Water

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In the previous paper [1] we have studied the time-potential curves of iron electrodes in differential aeration cells (DA-cells) with running tap water. We have tried to advance a general theory for the explanation of the tuberculation properties of corrosive and non-corrosive waters.

In domestic water supply systems we very often meet galvanized *i. e.* zinc coated pipes, besides black iron pipes. Therefore it was interesting to study the electrochemical behaviour of zinc and zinc coatings in the zinc-zinc and zinc-iron DA-cells.

The construction of cells, electric and other equipment, have been described in the previous paper [1]. In the experiments described below similar cells (type III) were used except that the iron electrodes were replaced by zinc, zinc-coated or copper electrodes of the same construction. All values of potentials are given in millivolts in the normal hydrogen-electrode scale.

ZINC-ZINC DA-CELLS

Curves 3 and 4 (Fig. 1) show the potentials of aerated and unaerated zinc-electrodes connected through a 1000 ohm resistance and measured against a

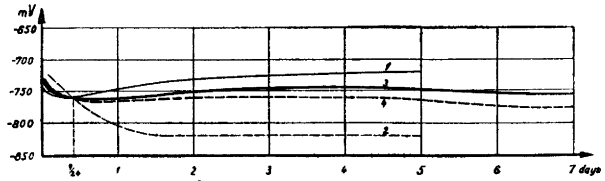


Fig. 1. Time-potential curves for the aerated (1,3) and non-aerated (2,4) zinc electrodes of DA-cell type III. Electrodes connected (3,4) through a 1 000 ohm resistance or disconnected (1,2). Rate of water flow 300 ml/min.

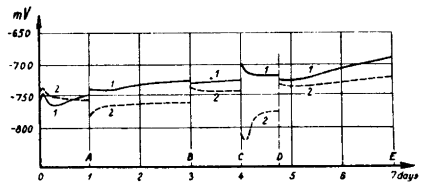


Fig. 2. Time-potential curves for the aerated (1) and non-aerated (2) zinc-coated (galvanized) electrodes of a DA-cell. Electrodes connected (O—A, B—C, D—E) through a 1000 ohm resistance or disconnected (A—B, C—D). Rate of water flow 300 ml/min.

calomel reference electrode (saturated KCl). Only in the beginning of the experiment the aerated electrode shows a slightly more base potential than the unaerated one. After about 9 hours the potentials change. The potential difference slightly increases during the first days of the experiment. Curves 1 and 2 show the true potentials of the same electrodes disconnected.

Fig. 2 shows the potentials of the galvanized iron electrodes. In the intervals OA, BC, DE the electrodes are connected through a 1000 ohm resistance. In the intervals AB, CD electrodes are disconnected. We can see that after every disconnection the potential difference between the electrodes is discreasing. After the connection the EMF of the DA-cell rises slowly.

The diagrams in Figs. 3 and 4 show the effect of the addition of Calgon on the potentials of the disconnected electrodes of the zinc-zinc DA-cell. We see that the addition of Calgon diminishes the potentials, both the cathode and anode. The effect on the easier accessible cathode is the greater one. The sufficient addition of Calgon may change the direction of the potential difference.

Conclusions: The DA-cell with zinc electrodes shows a qualitative behaviour rather similar to that of a cell with iron electrodes. Quantitative results are more different: The potentials in the beginning of the experiments are much more base (about — 750 mV) but show the continuous increase especially with galvanized electrodes. The potential difference in the beginning near to zero or slightly negative increases much slower and remains lower than with an iron cell. That seems to indicate a much smaller oxygen concentration

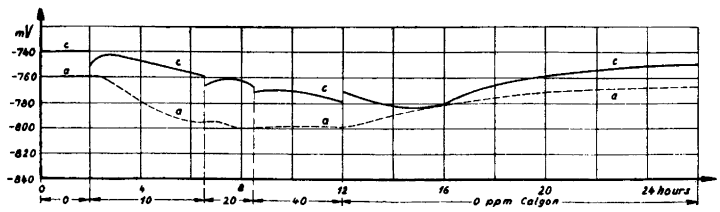


Fig. 3. The effect of various Calgon concentrations on the true potentials of the aerated (c) and the non-aerated (a) zinc-coated electrode, disconnected 8 hours before the start of the experiment. DA-cell type III, one day old. Rate of water flow 300 ml/min.

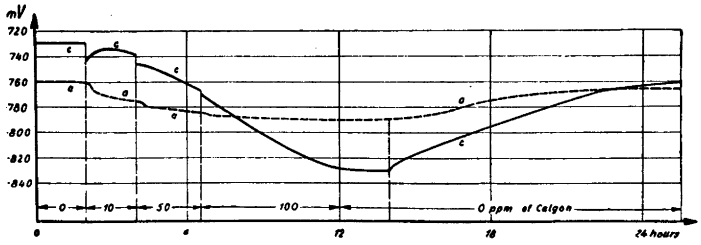


Fig. 4. The effect of various Calgon concentrations on the true potentials of the aerated (c) and the non-aerated (a) zinc electrode disconnected 8 hours before the start of the experiment. DA-cell type III, three days old. Rate of water flow 300 ml/min.

gradient between the cathodic and anodic surfaces which could be accounted for by the lack of the anodic secondary reaction consuming oxygen as in the case of the oxidation of ferro to ferri ions.

The quick decrease of the potential difference after disconnecting the electrodes (Fig. 2; C—D) indicates that the reactions going on at the electrodes during the passage of the current play an important role in establishing the potentials. The continuous renewal of the anodic zinc surface due to the dissolution of the anode could be the cause of the decrease of its potential owing to the diminishing of the chemo-adsorbtion of oxygen.

The potential-depressing effect of Calgon, both on the cathode and anode, could be explained on the basis of the film-forming property of Calgon and consequent decrease of the oxygen transport to the true metal surface. In the zinc cell the effect of Calgon on both electrodes has the same direction (Figs. 3 and 4) because we have not here the secondary reaction of the anodic oxidation as in the case of iron, where the interference of Calgon with this reaction causes the increase of the potential of the anode.

ZINC-IRON DA-CELLS

It is a rather common belief that zinc is always electronegative against iron in the natural water environments. This belief is based on the normal potential series of metals and on short experimental observations of iron-zinc couples. But pitting with overlying tubercles and rusting of galvanized iron pipes indicate the possible changes in potentials, which has been emphasized by Davis [2], Haase [3] and others. Schikorr [4] has been able to show the cathodic behaviour of zinc versus iron in hot tap water after some hours of measurement. Roters and Eisenstecken [5] show such a change of the current direction in zinc-iron couples in cold staying tap water after about two months.

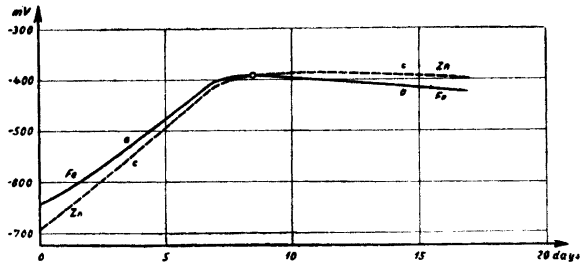


Fig. 5. Time-potential curves for the aerated (c) zinc electrode and the non-aerated (a) iron electrode of a DA-cell. Electrodes connected through a 1 000 ohm resistance. Iron electrode 25 days old (staying water); zinc electrode 15 days old (running aerated water). Rate of water flow 300 ml/min.

Gilbert [7], Hoxeng and Prutton [8] have also experimentally shown reversals of the zinc-iron potential in hot, respectively in cold water.

In our above mentioned experiments with iron and zinc DA-cells we have seen that the potentials of both metals can vary markedly, but we have not observed any overlapping of the zinc and iron time-potential curves. But as the measurement with zinc was a rather short one and the potentials still showed an increasing tendency (Figs. 1 and 2), we built a cell with an aerated zinc electrode and a non-aerated iron electrode. The diagram in Fig. 5 shows that after 8 ½ days the aerated zinc electrode, violently washed by running water, has become cathodic against the iron electrode in stagnant water. After 17 days the cell connected through a 1000 ohm resistance shows EMF equal to 30 mV. In the disconnected 18-day-old cell zinc shows a potential as

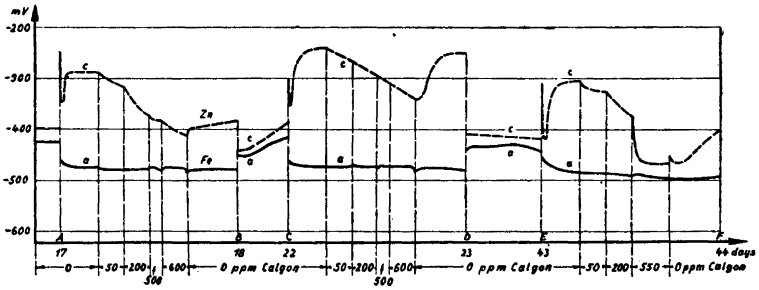


Fig. 6. The effect of various concentrations of Calgon on the potentials of the aerated (c) zinc electrode and the nonaerated (a) iron electrode of a 17 days old DA-cell. Electrodes connected through a 1 000 ohm resistance (O—A, B—C, D—E) or disconnected (A—B, C—D, E—F). Rate of water flow 300 ml/min.

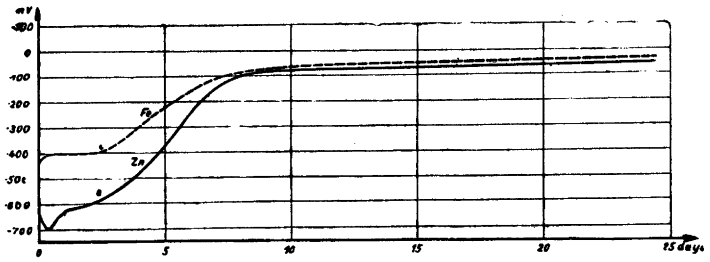


Fig. 7. Time-potential curves for the aerated (c) iron electrode and the non-aerated (a) zinc electrode of a DA-cell. Electrodes connected through a 1 000 ohm resistance. Rate of water flow 300 ml/min.

high as — 300 mV and iron — 490 mV *i. e.* the potential difference was 190 mV. After 23 days the potential difference of 230 mV was observed.

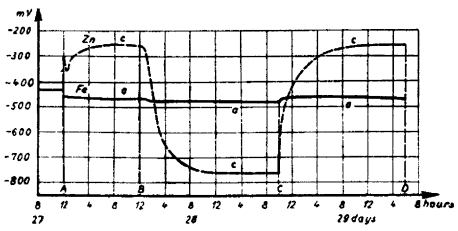
These facts seem to be the adequate explanation for the pitting and tuberculation in galvanized pipes where we have zinc-coated cathodic surfaces against the iron surface at the bottom of pits.

The addition of Calgon (Fig. 6) causes the marked decrease of the cathodic potential. But almost no changes are seen in the potential of the iron anode, which we may explain by the age of iron electrode (18 + 25 = 43 days) and a consequently large accumulation of rust in the anodic space that inhibits the diffusion of Calgon into the anodic space.

The cathodic behaviour of zinc against iron seems to be reversible, and after the discontinuation of the water flow across the zinc surface (B, Fig. 8) the potential of zinc falls to a value lower than the value of the potential of the iron electrode. The restoring of the former flow of water (C) brings the zinc potential to the former noble value.

For the sake of comparison we have measured the time-potential curves for the cells with the aerated iron cathode and the zinc anode. The potentials of the electrodes connected through a 1000 ohm resistance are shown on the diagram (Fig. 7). In the course of the first fortnight of the experiment the potentials increase markedly and the EMF decreases. Afterwards the poten-

Fig. 8. The effect of the rate of water flow on the potentials of an aerated (c) zinc electrode and a non-aerated (a) iron electrode of the 27 days old DA-cell. Electrodes connected through a 1 000 ohm resistance (O—A) or disconnected (A—D). Rate of water flow 300 ml/min (O—B, C—D) or O ml/min (B—C).



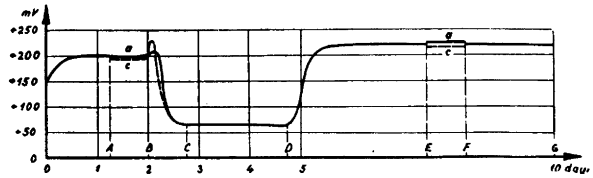


Fig. 9. Time-potential curves for the aerated (c) and the non-aerated (a) copper electrodes of a DA-cell. Electrodes connected through a 1 000 ohm resistance (O—A, C—E, F—G) or disconnected (A—C, E—F). Rate of water flow 300 ml/min (O—B, D—G) or O ml/min (B—D).

tials and EMF become rather stable. No exchange of the cathodic and anodic functions of the electrodes occurs and after 25 days the true potential difference of the disconnected electrodes is still very great (about 600 mV). After 40 days the true potentials are — 570 mV (zinc electrode) and — 160 mV (aerated iron electrode).

COPPER-COPPER DA-CELL

The time-potential curves of a DA-cell with copper electrodes are shown on the diagram (Fig. 9). When the electrodes had been connected through a 1000 ohm resistance, the potential difference was not detectable with the potentiometer used. The disconnected electrodes show a potential difference less than 5 mV with a higher potential of the non-aerated electrode. This phenomenon has been described and explained by Evans [5].

The interruption of the water flow has a pronounced diminishing effect on the potentials of both electrodes. The reproducibility of the measurements was not very good, and the cell shows a high internal resistance which in our case renders the long automatic recording impossible.

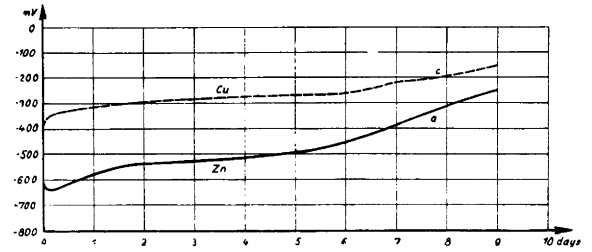


Fig. 10. Time-potential curves for the aerated (c) copper electrode and the non-aerated (a) zinc electrode of a DA-cell. Electrodes connected through a 1 000 ohm resistance. Rate of water flow 300 ml/min.

COPPER-ZINC DA-CELLS

The diagram in Fig. 10 shows the potentials of a DA-cell with a Cu-cathode and Zn-anode connected through a 1000 ohm resistance. The EMF of such a cell is rather high but shows a decreasing tendency. The increase of potentials is due to the ennoblement of the potential of zinc electrode.

SUMMARY

The behaviour of zinc in a DA-cell indicates that the corrosion of the zinc could also be due to the action of the differential aeration currents as in the case of iron. The range of obtained potential differences is smaller than with iron. The addition of Calgon depresses the potentials of both the cathode and anode due to the formation of adsorbtion layers which inhibit the access of oxygen to the metal surface. Calgon has a greater effect on the decrease of the potential of the cathodic, easier accessible surface and in this way depresses the potential difference or even changes it to negative values, thus eliminating the corrosion.

After some weeks zinc becomes cathodic against iron in the DA-cell where the zinc surface is exposed to an aerated water stream and the iron electrode placed in stagnant water. That explains the tuberculation and pitting in galvanized iron pipes in the same way as described in our previous paper for iron.

The ennoblement of the potential of zinc is reversible when the water flow decreases.

Some other aspects of the DA-cells with zinc, iron and copper electrodes are discussed.

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Received February 24, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1300–1325

The Preparation from Baker's Yeast of Yeast Nucleic Acid Containing a High-Polymer Fraction

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Dielectric studies of a number of ribose nucleotides (RNA) have led to attempts to produce more highly polymerized specimens with a view to establishing the dielectric characteristics of the RNA molecule.

In the preparation of RNA from yeast, difficulties are encountered both in the extraction and in the separation of the protein impurities. The methods applied are based, as a rule, on the use of alkalis, heat, or mechanical disintegration followed by extraction with salts; or on combinations of these processes. RNA is sensitive to such treatment, however, and rarely yields highly polymerized specimens. Earlier methods have usually involved treatment with alkali in order to split the very firmly bound nucleic acid-protein complex. If heat is used in addition, the specimens obtained are very pure as judged by their low protein content, but they show evidence of depolymerization. Treatment with salts, which may appear to be very mild, also has a disintegrating effect [1].

A number of relatively highly polymerized components are found in commercial specimens, despite the fact that their production is based on treatment with alkali under heat. We varied the concentration of alkali and the heat treatment and found that it was possible to produce specimens with a higher content of highly polymerized RNA than the commercial specimens. By varying the alkali concentrations (between 0.01 and 0.05 N NaOH) and using different boiling times, from just bringing to the boil to one hour or more boiling, fractions were obtained with a molecular weight of up to 15 000 to 20 000. Such fractions can be obtained in several ways. The nature of the specimens varies chiefly in respect of the protein content, since milder treatment results in considerably greater contamination with protein. This, how-

ever, can be reduced by subsequent treatment with chloroform and amyl alcohol according to the method introduced by Sevag, Lackman and Smolens [2].

The specimens obtained after heating are considerably more polydisperse. We found it most satisfactory to use relatively strong alkali at low temperature, although this method could be questioned. In principle it leads to the procedure indicated by Johnson and Harkins [3], which has also been used by Chantrenne [4]. In addition, it has been found suitable for the production of pentose polynucleotides of other origins [5]. By adopting a modified procedure we obtained a specimen containing a relatively well-defined high-polymer fraction of RNA with a molecular weight of 60 000—70 000. In view of this fraction's high molecular weight, considerably above that found earlier in specimens from yeast, it can be studied dielectrically. The high-polymer part of the specimen represents only some 10 per cent. Nevertheless its properties — which interest us mainly in respect of its structure from a dielectric point of view — are so outstanding that we have hitherto confined ourselves to characterizing this fraction.

It should be stated, however, that the attempts to separate and isolate the high-polymer molecules with the acid precipitation indicated by Levene [6] only resulted in the elimination of very low components.

Another procedure, which also gives better results than the methods used for commercial production, involves alkali treatment and extraction with picric acid (Levene and La Forge [7]). It gives a fairly well-defined fraction with a molecular weight of approximately 30 000.

There is no reason to describe our study of the different methods of production, since it was found that an essential point in the preparation of high-polymer specimens is to proceed rapidly. Generally it may be said that irrespective of the variations of method, the results depend essentially on the length of time to which the specimen is exposed to the possibility of decomposition. It has therefore been a matter of weighing the respective advantages of a specimen with a high degree of polymerization and a moderate contamination by proteins and of a purer specimen having a lower degree of polymerization. Even the mild method of Sevag *et al.*[2] takes up so much time that the specimen becomes depolymerized.

A very good analogy to the pronounced instability of RNA is furnished by the investigations made by Cohen and Stanley [8] on polynucleotides isolated from the tobacco mosaic virus. The limited stability which they reported agrees very well with our experience of the high-polymer fraction in yeast, although the latter appears to be even more labile. It is surprising that even in a relatively dry condition, with a 10—15 per cent moisture content, the high-polymer fraction of the specimen is destroyed in a few weeks at room tem-

perature. Such depolymerization also takes place at low temperatures and with specimens as free from moisture as possible. In fact, we found it impossible to work with any but freshly prepared specimens.

It is not possible to determine the extent to which the instability is due to an enzymatic action or to a spontaneous tendency to depolymerization. It appears probable that enzyme action is greater during extraction but that later the specimen is itself unstable, possibly after it has ceased to be bound to the proteins.

We therefore tried to find a simple and reproducible method. The following modification of Johnson and Harkins' method [3] was found serviceable. Growing baker's yeast, in portions of 100 g, with a moisture content of approximately 70 per cent, is washed by suspension in distilled water and centrifuged until a colourless supernatant is obtained. The yeast suspension is cooled to approximately 0° C and treated for two hours with NaOH in 5 per cent solution. The suspension is then neutralized with acetic acid to pH 6. Hyflo Super Cel, 5 per cent by weight, is suspended in the solution which is then suction-filtered. After acidifying with HCl (Congo), the filtrate is precipitated with 4 volumes of ethanol. The precipitate is centrifuged off and dissolved by addition of 0.1 *N* NaOH. The solution is again treated with Hyflo and filtered. The pH is adjusted to 6.0 and the solution precipitated with 4 volumes of ethanol, after which the preparation is dried with ethanol and ether. Freezing-drying is sometimes used. The entire procedure is carried out at a low temperature, around 0° C, and is completed in the course of 12 hrs.

The specimens obtained by this procedure are not entirely protein-free but appears from analysis and the biuret test to contain approximately 5 per cent of protein. In some experiments, the specimens were further treated according to the procedure of Sevag *et al.*[2] but it was found extremely difficult to remove all the proteins. Destruction of part of the high-polymer fraction generally occurs at the same time, and in any event the specimens are less homogeneous. On the other hand, it is profitable to submit the specimen to dialysis for a short time. Using cellophane it is found that a number of low-polymer nucleotides are removed even in a few hours.

The method suggested involves the use of strong alkali, and the question arises as to how far this affects the high-polymer fraction. A report on this point will be submitted in another paper . For the present, we would merely stress that the high-polymer fraction has a high polarity. It is readily digestible with crystalline ribonuclease prepared according to Kunitz and is entirely unaffected by desoxyribonuclease.

SUMMARY

A comparative study was made of different earlier methods for the production of yeast nucleic acid from baker's yeast.

Specimens with a small proportion of relatively highly polymerized fractions can be obtained in several ways. The most advantageous method involves short treatment at low temperature with relatively strong alkali.

A specimen with a relatively monodisperse, highly polymerized fraction having a dielectric molecular weight of approximately 65 000 is obtained by a modification of Johnson and Harkins' method.

The high-polymer fraction is very unstable even when in air-dry condition with a low moisture content. The preparation must be performed rapidly and the investigations made on freshly prepared specimens.

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Received September 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1304–1325

Short Communications

1,3-Dimercaptoacetone and Some Derivatives

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In 1924 Groth [1] prepared phenacylmercaptan and two years later the first aliphatic α-ketomercaptan, namely acetonylmercaptan [2]. Hromatka and Engel [3] as late as 1948 independently investigated the same substance with some differing results, which will be discussed in another communication.

No substance known to the author, however, is described in the literature which contains two mercapto groups in α-position to the keto group. Now experiments to prepare 1,3-dimercaptoacetone have been carried out.

Groth [2] treated monochloro-acetone with dithiocarboxy-glycolic acid (prepared from carbon disulfide and glycolic acid in an alkaline medium) and from the product he obtained acetonylmercaptan in good yield. When the analogous reaction was performed with 1,3-dichloroacetone, however, some derivatives of 1,3-dimercaptoacetone (I) could be obtained, but every attempt to prepare the mercaptan from these failed. Experiments with potassium xantogenate and "Benders salt", $C_2H_5O \cdot CO \cdot SK$, led to similar results. The derivatives, obtained in these reactions, will be discussed in a later communication.

Analogously to the reaction, described by Hromatka and Engel [3], sodium sulfhydrate was treated with 1,3-dichloroacetone, and thus 1,3-dimercaptoacetone could be prepared in about a 64 % yield. The substance is soluble in ordinary organic solvents in the hot but it is rather difficult to recover it from the solutions.

1,3 - Bis - (2,4 - dinitrophenyl - mercapto)-acetone (II) and 1,3-bis-(carboxy-methylmercapto)-acetone (III) could be prepared from the mercaptan by reaction with 2,4-dinitro-chloro-benzene and monobromoacetic acid resp. The dicarboxylic acid (III) could also be obtained from 1,3-dichloroacetone and thioglycolic acid. The two substances behaved in a similar manner, and the mixture showed no melting-point depression. In some of these reactions pyridine has been used as a solvent with advantage.

The equivalent weight was determined by titration with an 0.1 *N* iodine solution, each mercapto group corresponding to one atom of iodine. A qualitative test to the mercapto group could be obtained by Rheinboldt's reaction [4]. Characteristic precipitates were obtained with *e. g.* lead acetate and zink sulfate in alcoholic solutions of 1,3-dimercaptoacetone. Experiments with the mercaptan indicate that the

(I) $HS \cdot CH_2 \cdot CO \cdot CH_2 \cdot SH$

(II) $(NO_2)_2 \cdot C_6H_3 \cdot S \cdot CH_2 \cdot CO \cdot CH_2 \cdot S \cdot C_6H_3 \cdot (NO_2)_2$

(III) $COOH \cdot CH_2 \cdot S \cdot CH_2 \cdot CO \cdot CH_2 \cdot S \cdot CH_2 \cdot COOH$

substance may be dimeric. Investigations are being pursued.

Experimental. *1,3-Dimercaptoacetone.* A solution of 4.0 g (0.10 mole) of sodium hydroxide in 40 ml of water was saturated with hydrogen sulfide and cooled to about 5° by means of an ice bath, 6.4 g (0.05 moles) of 1,3-dichloroacetone, dissolved in 30 ml of alcohol, being introduced dropwise with stirring, while the temperature was kept low. The dimercaptan precipitated during the addition and was separated by filtration with suction, and then washed with about 200 ml of water. The product was dried in a vacuum desiccator over concentrated sulfuric acid. Melting point 85—86°. Yield 3.9 g (64 %).

$C_3H_6OS_2$ (122.17)	C	H	S
Calc.	29.47	4.95	52.48
Found	29.71	4.74	52.50

Equiv. wt. Calc. 61.1 Found 63.0; 62.9

1,3-Bis-(2,4-dinitrophenyl-mercapto)-acetone. According to the general process [5] 1.22 g (0.01 mole) of 1,3-dimercaptoacetone was treated with 4.06 g (0.02 moles) of 2,4-dinitro-chloro-benzene. However, pyridine was used with advantage as a solvent instead of sodium hydroxide solution. A yellow product precipitated, was filtered and washed with some water and acetone. The crude product was recrystallized from acetone and was finally obtained as small, yellow, glistening crystals. Melting point 198—200°. Yield 3.5 g (78 %).

$C_{15}H_{10}O_9N_4S_2$ (454.23) Calc. N 12.33
Found » 12.21

1,3-Bis-(carboxy-methyl-mercapto)-acetone. A. A solution of 13.9 g (0.10 mole) of monobromo-acetic acid in 25 ml of water was neutralized with 4.0 g (0.10 mole) of sodium hydroxide, dissolved in 10 ml of water and added drop by drop with cooling and vigorous stirring to 6.1 g (0.05 moles) of 1,3-dimercaptoacetone, dissolved in a solution of 4.0 g (0.10 mole) of sodium hydroxide in 30 ml of water. The stirring was continued for half an hour. The solution was then diluted with water and dropped into a mixture of concentrated hydrochloric acid and ice. The dicarboxylic acid precipitated as small crystals, was filtered and washed with some water. The acid could be recrystallized from water or ligroin (b. p. 60—70°). Melting point 148—150°. Yield 7.9 g (66.5 %).

Equiv. wt. Calc. 119.1 Found 119.7

B. 9.2 g (0.10 mole) of thioglycolic acid, dissolved in 25 ml of alcohol, was neutralized with a solution of 11.2 g (0.20 moles) of potassium hydroxide in 60 ml of diluted alcohol (1 : 1). The dipotassium salt separated as an oil; 6.4 g (0.05 moles) of 1,3-dichloroacetone, dissolved in 35 ml of alcohol, was added dropwise with cooling and stirring, and after a while the oily phase disappeared. After some hours the solution was dropped into hydrochloric acid and ice, and the acid was separated as in A. Melting point 148—150°. Yield 7.1 g (59 %).

$C_7H_{10}O_5S_2$ (238.20)

	Equiv. wt.	C	H	S
Calc.	119.1	35.26	4.23	26.91
Found	120.0	35.38	4.10	26.73

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Received October 5, 1950.

Optically Active Thiophene Compounds. II. α-Thenyl α-Thienylacetic Acid and α-Thenylphenylacetic Acid

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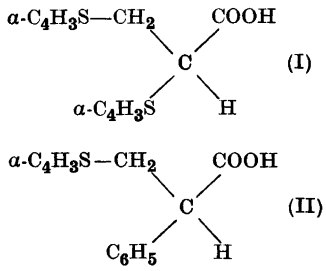
In connection with work in progress on optically active thiophene derivatives [1] we have prepared α-thenyl α-thienylacetic acid (I) and α-thenylphenylacetic acid (II). The end in view is to resolve the acids into the optical antipodes and compare them with the antipodes of benzyl α-thienylacetic acid (III) and benzylphenylacetic acid (IV).



(I)

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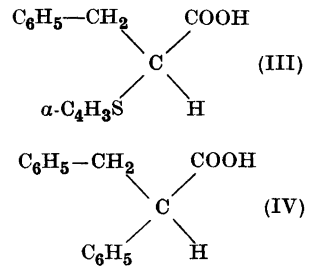

(III)

(IV)

For the optical resolution of the acids, *l*-ephedrine seems to be a satisfying operating agent. These experiments will be published later. Rupe and Kerkovins had previously resolved the benzylphenylacetic acid (IV) by means of strychnine [2]. Our results indicate that this resolution was not complete.

Thenylthienylacetic acid has been prepared from ethyl thienylcyanoacetate and thenyl chloride, using anhydrous potassium carbonate as the condensing agent and dry acetone as solvent [3]. The ethyl thienylcyanoacetate thus obtained was decarboxylated and hydrolyzed to give thenylthienylacetic acid.

Thenylphenylacetic acid has been prepared from ethyl phenylmalonate and thenyl chloride analogously to the preparation of benzylphenylacetic acid [4], but the preparation from ethyl phenylcyanoacetate and thenyl chloride will probably give a better yield.

Ethyl thenylthienylcyanoacetate: In a round-bottomed, three-necked flask, fitted with a mercury-sealed stirrer and reflux condenser, were placed 12 g (0.061 moles) of ethyl thienylcyanoacetate, 10 g (0.075 moles) of thenyl chloride, 25 g (0.180 moles) of anhydrous potassium carbonate, and 200 ml of acetone. The mixture was stirred and refluxed for 20 hours, the acetone removed by distillation, and the residue treated with 100 ml of water. The upper oily layer was separated, the water layer extracted three times with ether, the ether combined with the oily layer, the mixture dried with anhydrous sodium sulphate and distilled. 14 g (0.048 moles) of ethyl thenylthienylcyanoacetate were obtained, boiling at 195–205° C/9 mm Hg. Yield 79 %.

Thenylthienylacetic acid: 14 g (0.048 moles) of ethyl thenylthienylcyanoacetate and 6.5 g (0.116 moles) of potassium hydroxide, dissolved in 100 ml of alcohol, were heated on a water bath for 10 minutes. The potassium carbonate obtained was filtered off and washed with alcohol. The filtrate and 6.5 g (0.116 moles) of potassium hydroxide, dissolved in dilute alcohol, were placed in a round-bottomed flask, fitted with a reflux condenser, and boiled in an oil bath until no more ammonia escaped (12 hours). The alcohol was

removed by distillation, the residue acidified with dilute hydrochloric acid, and the precipitated acid dissolved in ether. After removing the ether 11.2 g (0.047 moles) of thenylthienylacetic acid were obtained. Yield 98 %. The crude acid was recrystallized from dilute formic acid and finally from petrol (b. p. 60—70° C). M. p. 90.5—92.5° C.

	Equiv. wt.	C	H	S
$C_{11}H_{10}O_2S_2$ Calc.	238.3	55.44	4.23	26.91
Found	238.8	55.19	4.12	26.78

Ethyl thenylphenylmalonate: In a round-bottomed, two-necked flask, one neck being fitted with a reflux condenser, 50 ml of absolute alcohol were placed, and then, through the other neck, 4.6 g (0.20 moles) of sodium, cut in pieces. When the sodium had reacted (at the end of the reaction, the flask had to be warmed in an oil bath), 47.0 g (0.20 moles) of ethyl phenylmalonate was added through a dropping funnel. Then 26.5 g (0.20 moles) of thenyl chloride were added, and the mixture heated in an oil bath, until the reaction had ceased. The alcohol was removed by distillation, and water was added to dissolve the sodium chloride, the oily layer separated and the water layer extracted three times with ether. The ether and oily layers were combined, dried with anhydrous calcium chloride and distilled. 36.0 g (0.11 moles) of thenylphenylmalonate were obtained as a light yellow oil, boiling at 185—195° C/9 mm Hg. Yield 55 %.

Thenylphenylacetic acid: In a round-bottomed flask, fitted with a reflux condenser, were placed 36.0 g (0.11 moles) of ethyl thenylphenylmalonate and 10.0 g (0.44 moles) of sodium hydroxide, dissolved in dilute alcohol. The mixture was boiled for one hour in an oil bath. The alcohol was removed by distillation, the residue acidified with dilute hydrochloric acid, the precipitated oily acid dissolved in ether and the ether removed. 22 g (0.095 moles) of thenylphenylacetic acid were obtained. Yield 86 %. The crude acid was recrystallized from petrol (b. p. 60—70° C). M. p. 71.5—72.0° C.

	Equiv. wt.	C	H	S
$C_{13}H_{12}O_2S$ Calc.	232.1	67.21	5.21	13.49
Found	231.5	66.89	5.07	13.58

The authors are indebted to the Royal Swedish Academy of Science for financial support from the Nobel Fund of Chemistry (*Kemiska prisgruppens särskilda fond*).

1. Fredga, A., and Palm, O. *Arkiv Kemi, Mineral. Geol.* **A 26** (1949) no. 26.
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Received October 7, 1950.

The High Rotatory Power of Cystine

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In a recent publication[1] Fieser has drawn attention to the high rotatory power of cystine already pointed out by van't Hoff. While the other acyclic amino acids, including cysteine, are characterized by low specific rotations (about 10°), cystine shows the remarkable value $[\alpha]_D = -214°$ (acid solution). The rotation is greatly dependent on the pH of the medium and in alkaline solution much lower values are reported. According to Fieser, the rotation of cystine appears as extraordinary today as it did to van't Hoff in 1898. As a possible explanation he suggests the formation of hydrogen bonds between the carboxyles and the amino groups, resulting in an endocyclic ring structure.

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It is well known that ring formation often exerts a great influence on the rotatory power. In the case of cystine, however, there is another factor to take into consideration, *viz.* the presence of a disulphide group, —S—S—. The specific rotations of some disulphide acids are given in Table 1. In all cases, the activity is greatly influenced by the nature of the solvent. The disulphide-di-*α*-propionic (dithiodilactic) acid (I) has a remarkably high rotation, especially in dilute hydrochloric acid. The rotation of the dithian-dicarboxylic acid (II) and the disulphide-di-*α*-phenylacetic acid (III) are of the same order of magnitude. Thus, in this case neither the ring formation nor the presence of aromatic groups produces any marked increase in the activity. In all these cases, the disulphide group is directly attached to the asymmetric carbon atoms.

In cystine the disulphide group is one carbon atom removed from the centres of asymmetry, and its influence would thus be expected to be less pronounced. In the course of investigations on steric relationships, the author has recently prepared the optically active disulphide-di-*β*-*iso*butyric acid (IV), where the position of the sulphur atoms is the same as in cystine. This compound has a lower activity than acids I—III, but it is quite comparable to that of cystine. In homocystine, described by du Vigneaud and collaborators [2], the disulphide group is two carbon atoms removed from the centres of asymmetry. Here we have $[\alpha]_D = -77°$ (acid solution). The value is lower than for cystine, but obviously higher than for the common acyclic amino acids.

It can thus be said that the high optical activity of cystine is only what could be expected with regard to the presence of a disulphide group and its position in the molecule.

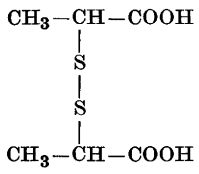
Experimental. The activity values for *disulphide-di-α-propionic acid* (I) are quoted from Bernton [3] and those for *dithian-di-carboxylic acid* from an earlier publication by the author [4].

The (−)-*disulphide-di-α-phenylacetic acid* (III) was prepared from (−)-ethylxanthogene-phenylacetic acid [5]. The particulars will be described in a later publication.

0.2308 g acid: 12.98 ml 0.1065 *N* NaOH: $C_{16}H_{14}O_4S_2$ (334.4)

Equiv. wt. Calc. 167.2 Found 167.0

0.1099 g acid dissolved in dilute ammonia to 10.00 ml; $2\alpha = -7.025°$. $[\alpha]_D^{25} = -319.6°$. — 0.1171 g acid dissolved in ethanol to 10.00 ml: $2\alpha = -5.945°$.



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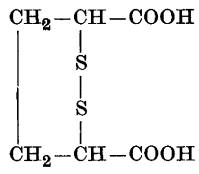

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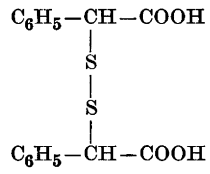

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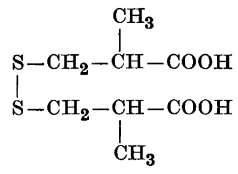

(IV)

Tab. 1. $[\alpha]_D^{25}$ *for the acids I—IV in different media.*

	I	II	III	IV
Dilute hydrochloric acid	+ 430.2°	+ 126°	—	— 151°
Neutr. water solution	+ 152.9°	— 335.8°	— 319.6°	— 219.7°
Ethanol (abs.)	+ 236.6°	— 194.8°	— 253.8°	— 40.2°

$[\alpha]_D^{25} = -253.8°$. The acid is practically insoluble in water and dilute hydrochloric acid.

The preparation of optically active *disulphide-di-β-isobutyric acid* (IV) met with some difficulties. The inactive acid described by Larsson [6] is probably a mixture of racemic and *meso*-acid, and experiments with different alkaloids gave no resolution. The ethylxanthogene-*β-iso*butyric acid is liquid at room temperature and difficult to obtain in a state of purity [7], but the methylxanthogene derivative is crystallised and can be resolved by means of cinchonidine. After ten crystallisations of the cinchonidine salt from dilute (55 %) acetone, the acid showed $[\alpha]_D^{25} = -71.5°$ (acetone solution). The rotation did not change on further recrystallisation of the salt. The xanthogenic acid was decomposed with ammonia, and the resulting mercapto acid oxidised with iodine. The disulphide acid was recrystallised twice from water, in which it is sparingly soluble at room temperature, and obtained as glistening plates. M. p. 124—125°.

0.1227 g acid: 9.68 ml 0.1065 *N* NaOH:
$C_8H_{14}O_4S_2$ (238.3)
Equiv. wt. Calc. 119.2 Found 119.6

0.0220 g acid dissolved in 0.1 N hydrochloric acid to 10.00 ml: $2\alpha = -0.665°$. $[\alpha]_D^{25} = -151°$ — 0.1022 g acid dissolved in dilute ammonia to 10.00 ml: $2\alpha = -4.49°$. $[\alpha]_D^{25} = -219.7°$ — 0.1125 g acid dissolved in ethanol to 10.00 ml: $2\alpha = -0.905°$. $[\alpha]_D^{25} = -40.2°$.

1. Fieser, L. M. *Rec. trav. chim.* **69** (1950) 410.
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3. Bernton, A. *Beiträge zur Kenntnis der optisch aktiven Thiomilchsäuren, ihrer Mercaptide und Disulfide.* Diss. Uppsala (1932).
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6. Larsson, E. *Svensk Kem. Tid.* **55** (1943) 168.
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Received October 7, 1950.

On the Sulphur Metabolism of *Rhodotorula gracilis*. I. The Importance of Sulphur and Iron for the Formation of Protein and Fat

NIELS NIELSEN and P. ROJOWSKI

Division of Food Chemistry, Royal Institute of Technology, Stockholm, Sweden

It is well known that various microorganisms are able to form considerable quantities of fat if cultivated in nutrient solutions whose nitrogen content is low in comparison with their sugar content. In *Rhodotorula gracilis* [1] the fat content may thus vary between 8 % and 65 %, depending on the composition of the nutrient solution in respect of nitrogen. Parallel with the increase in the fat content there occurs a decrease in the protein content. The latter may vary between 50 % for a normal yeast poor in fat and 12 % for a pronouncedly fat yeast. Probably it is the reduction of the protein content caused by the decrease of the nitrogen content of the nutrient solution which induces fat production.

It seemed of interest to investigate whether a reduction of the protein content of the yeast might be brought about in any other way than by cultivating it in a substrate with a low nitrogen content, obtaining an increased production of fat at the same time. For this purpose we have carried out experiments in which the yeast was cultivated in nutrient solutions containing such small quantities of sulphur or iron in relation to the other nutrient substances as to be insufficient for normal metabolism.

For the cultivation of normal, not strongly fat-producing Rhodotorula gracilis yeast we use a nutrient solution of the following composition: 15 g asparagine — 4.7 g KH_2PO_4 — 3 g $MgSO_4$, 7 H_2O — 1.5 g NaCl — 1.5 g $CaCl_2$, 6

$[\alpha]_D^{25} = -253.8°$. The acid is practically insoluble in water and dilute hydrochloric acid.

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H_2O — 0.015 g $FeCl_3$, 6 H_2O — 60 g glucose per liter. In the experiments described below we used nutrient solutions containing amounts of asparagine, KH_2PO_4, NaCl, $CaCl_2$ and glucose as stated above, but varying amounts of $MgSO_4$ or $FeCl_3$. 750 ml Erlenmeyer flasks containing 300 ml nutrient solution were inoculated with *Rhodotorula gracilis*. Subsequently the flasks were shaken for 5—7 days at 25° C. Then the amount of yeast formed was determined. The yeast was analyzed for fat and nitrogen content. For further details see Nielsen and Nilsson [2].

In the first experiment the sulphur content of the nutrient solution was varied. Six different solutions were used, containing per liter 3 g, 1 g, 0.3 g, 0.1 g, 0.03 g, and 0 g $MgSO_4$, 7 H_2O. Such amounts of $MgCl_2$ were added that the content of magnesium was constant in all solutions.

Table 1. Cultivation of Rhodotorula gracilis in nutrient solutions with varying contents of $MgSO_4$. Time of cultivation 5 days

$MgSO_4$, 7H_2O g/l nutrient solution	Yeast formed g/100 ml	Yeast fat %	Yeast protein %
3	1.32	11.8	42.5
1	1.40	10.5	44.2
0.3	1.11	17.7	36·3
0.1	0.86	37.6	13.9
0.03	0.74	36.5	13.0

Table 1 shows that if the sulphur content of the nutrient solution was reduced sufficiently, the yeast entered a fattening phase while simultaneously the protein content of the yeast declined. The changes in the fat and protein content of the yeast which took place as a consequence of the reduced sulphur content, corresponded to the changes which occur when yeast is cultivated in nutrient solutions with reduced nitrogen content.

Four other experiments with nutrient solutions of varying sulphur content gave essentially the same results. It is quite evident that an increased fat production of the yeast with a corresponding reduction of its protein content can be induced by a reduction of the sulphur content of the nutrient solution in the same way as by a reduction of its nitrogen content.

In other experiments the effect of reducing the iron content of the nutrient solution was investigated. The nutrient solutions which we used contained per liter: 0.015 g, 0.005 g, 0.0015 g, 0.0005 g, and 0 g $FeCl_3$, 6 H_2O.

Table 2. Cultivation of Rhodotorula gracilis in nutrient solutions with varying contents of $FeCl_3$. Time of cultivation 5 days

$FeCl_3$, 6 H_2O g/l nutrient solution	Yeast formed g/100 ml	Yeast fat %	Yeast protein %
0.015	1.40	12.3	44.6
0.005	1.49	13.2	45.2
0.0015	1.24	13.9	43.9
0.0005	1.01	19.4	33.5
0	0.71	27.8	21.9

Table 2 shows that if the iron content of the nutrient solution is greatly reduced, there is also formed a yeast rich in fat and poor in nitrogen. But in this case the fat content was not so high nor the nitrogen content so low as in the case of a nitrogen or sulphur defiency. As it, however, is a question of very small amounts of iron, it is probable that impurities containing traces of iron may be of some importance, and that with a nutrient solution extremely poor in iron it may be possible to obtain a higher fat content and a correspondingly lower protein content of the yeast.

Rhodotorula yeast rich in fat is red in colour. However, the colour tint of the yeast formed with a deficiency of iron is somewhat different from the tint of such fat yeast as has been formed with a deficiency of nitrogen or sulphur.

Three other experiments with varying amounts of iron gave the same result as the above experiments.

An increased fat production of *Rhodotorula gracilis* yeast with a simultaneous reduction of the protein content may thus be induced not only by a deficiency of nitrogen in the nutrient solution but also by a deficiency of sulphur or iron. The agreement in composition found in yeast formed with a deficiency of nitrogen, sulphur or iron indicates that the decisive factor in the increased fat production is the reduction of the protein content of the yeast. Certain experiments which have not yet been completed show that it is possible to inhibit the growth and protein production of yeast by the addition of certain substances, in which case a simultaneous increased fat production takes place. Every decrease of the protein synthesis of the yeast seems thus to result in an increased production of fat, provided that sugar is present in sufficient amounts.

This investigation was supported by a grant from The National Council of Technical Research, Stockholm, Sweden.

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Received October 26, 1950.

On the Sulphur Metabolism of *Rhodotorula gracilis*. II. The Ratio between SH and SS groups *

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It is known that the protein content of *Rhodotorula gracilis*, when cultivated in a substrate poor in nitrogen and rich in sugar, decreases while the fat content increases [1,2]. The protein content may vary between 12—50 %. Thus this yeast is very suitable for investigations regarding the influence of different factors on the synthesis of proteins. It seems probable that the composition of the protein differs in yeasts with low and high protein content. The object of this communication is to describe the results of some investigations on the proportion between protein SH and SS groups in *Rhodotorula* yeast containing different amounts of protein.

The cultivation of the yeast was carried out according to Nielsen and Nilsson [3] in Erlenmeyer flasks of 750 ml with 300 ml nutritient solution.

The nutrient solution for obtaining protein rich yeast (experiments a in Table 1) contained per liter:

7.5 g	Asparagine
4.7 g	KH_2PO_4
3.0 g	$MgSO_4 + 7\ H_2O$
1.5 g	NaCl
1.5 g	$CaCl_2 + 6\ H_2O$
0.015 g	$FeCl_3 + 6\ H_2O$
60 g	Dextrose

The pH was 4.8 and the shaking time varied between 2 and 6 days. For the production of protein poor yeast (experiments b in Table 1) the quantity of asparagine was reduced to 1 g per liter and that of dextrose to 40 g per liter.

The proportion of protein SH and SS groups was determined polarographically using the technic developed by Brdicka [4]. After cultivation the yeast was centrifuged off, washed with water and plasmolyzed by mixing with 5 % solid KCl. From the plasmolysate the following two mixtures were prepared.

Sample	1	2
Plasmolysate, g	0.30	0.30
1 *N* KOH, ml	0.15	0.15
H_2O, ml	0.30	—
0.2 *M* ICH_2COOK, ml	—	0.30

* This investigation was supported by a grant from The National Council of Technical Research, Stockholm, Sweden.

An increased fat production of *Rhodotorula gracilis* yeast with a simultaneous reduction of the protein content may thus be induced not only by a deficiency of nitrogen in the nutrient solution but also by a deficiency of sulphur or iron. The agreement in composition found in yeast formed with a deficiency of nitrogen, sulphur or iron indicates that the decisive factor in the increased fat production is the reduction of the protein content of the yeast. Certain experiments which have not yet been completed show that it is possible to inhibit the growth and protein production of yeast by the addition of certain substances, in which case a simultaneous increased fat production takes place. Every decrease of the protein synthesis of the yeast seems thus to result in an increased production of fat, provided that sugar is present in sufficient amounts.

This investigation was supported by a grant from The National Council of Technical Research, Stockholm, Sweden.

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Received October 26, 1950.

On the Sulphur Metabolism of *Rhodotorula gracilis*. II. The Ratio between SH and SS groups *

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It is known that the protein content of *Rhodotorula gracilis*, when cultivated in a substrate poor in nitrogen and rich in sugar, decreases while the fat content increases [1,2]. The protein content may vary between 12—50 %. Thus this yeast is very suitable for investigations regarding the influence of different factors on the synthesis of proteins. It seems probable that the composition of the protein differs in yeasts with low and high protein content. The object of this communication is to describe the results of some investigations on the proportion between protein SH and SS groups in *Rhodotorula* yeast containing different amounts of protein.

The cultivation of the yeast was carried out according to Nielsen and Nilsson [3] in Erlenmeyer flasks of 750 ml with 300 ml nutritient solution.

The nutrient solution for obtaining protein rich yeast (experiments a in Table 1) contained per liter:

7.5 g	Asparagine
4.7 g	KH_2PO_4
3.0 g	$MgSO_4 + 7\ H_2O$
1.5 g	NaCl
1.5 g	$CaCl_2 + 6\ H_2O$
0.015 g	$FeCl_3 + 6\ H_2O$
60 g	Dextrose

The pH was 4.8 and the shaking time varied between 2 and 6 days. For the production of protein poor yeast (experiments b in Table 1) the quantity of asparagine was reduced to 1 g per liter and that of dextrose to 40 g per liter.

The proportion of protein SH and SS groups was determined polarographically using the technic developed by Brdicka [4]. After cultivation the yeast was centrifuged off, washed with water and plasmolyzed by mixing with 5 % solid KCl. From the plasmolysate the following two mixtures were prepared.

Sample	1	2
Plasmolysate, g	0.30	0.30
1 *N* KOH, ml	0.15	0.15
H_2O, ml	0.30	—
0.2 *M* ICH_2COOK, ml	—	0.30

* This investigation was supported by a grant from The National Council of Technical Research, Stockholm, Sweden.

Table 1.

Experiment	Time of cultivation	Yield of yeast, dry basis	Composition of yeast: Protein dry basis	Composition of yeast: Fat dry basis	Ratio * $\frac{SH}{SH + SS}$
	days	g	%	%	
I a	2	0.69	53.0	6.9	0.71
Protein rich yeast	4	1.01	37.4	18.0	0.72
	6	1.13	38.0	18.3	0.66
I b	2	0.52	39.8	16.1	0.66
Protein poor yeast	4	0.68	20.7	36.1	0.53
	6	0.88	20.0	41.7	0.50
II a	2	0.57	54.3	7.8	0.70
Protein rich yeast	4	0.75	36.3	18.3	0.68
	6	1.12	33.6	18.1	0.73
II b	2	0.52	50.7	17.3	0.64
Protein poor yeast	4	0.60	19.0	37.8	0.52
	6	0.79	16.8	37.9	0.42

* The ratio between the polarographic currents representing SH and SH+SS groups respectively.

The samples were allowed to stand for 45 minutes. ICH_2COOK inhibits the polarographic effect of SH groups. Thus the polarographic analysis of sample 1 gives the combined effects of SH and SS groups and that of sample 2 the effect of the SS groups only.

The results of two experiments with protein rich and protein poor yeast are given in Table 1.

The protein content is reduced when the yeast is cultivated in the nitrogen poor medium of the experiments b. It is interesting that the ratio between the polarographic currents representing the SH and SH + SS groups respectively decreased when the protein content of the yeast decreased. Thus when the protein content of the yeast was varied by varying the supply of nitrogen a low protein content was accompanied by a lower value of the ratio $\frac{SH}{SH + SS}$. The metabolism of the protein poor yeast is strongly reduced compared with that of the protein rich yeast as may be seen from the reduced yield. Parallell with the decrease of protein content of the yeast a decrease of the content of the sulphydryl groups occured.

Nielsen and Rojowski [5] found that the synthesis of proteins was reduced even in a medium rich in nitrogen if the supply of sulphur was sufficiently reduced. Cultivations were carried out with the substrate containing ample quantities of nitrogen (mentioned above) for the production of protein rich yeast and further with a substrate of the same composition except for the content of $MgSO_4 + 7\ H_2O$ being 0.15 instead of 3.00 g per liter for the production of a yeast poor in protein according to Nielsen and Rojowski [5]. The results are given in Table 2.

Table 2.

Experiment	Time of cultivation days	Yield of yeast, dry basis g	Composition of yeast Protein dry basis %	Composition of yeast Fat dry basis %	Ratio * $\frac{SH}{SH + SS}$
III a	2	1.05	55.9	16.0	0.72
substrate containing	4	1.29	48.0	16.8	0.72
3 g $MgSO_4 + 7\ H_2O$	6	1.72	46.2	15.1	0.77
per lit.					
III b	2	0.78	41.8	21.0	0.73
substrate containing	4	0.81	21.8	27.3	0.92
0,15 g $MgSO_4 + 7\ H_2O$	6	1.08	17.8	28.7	1.00
per lit.					

* The ratio between the polarographic currents representing SH and SH + SS groups respectively.

In agreement with Nielsen's and Rojowski's findings the reduction of sulphur in the substrate caused a similar decrease of the protein content and increase of the fat content of the yeast as lack of nitrogen in the substrate did in the experiments of Table 1. The ratio $\frac{SH}{SH + SS}$ for the yeast cultivated on the substrate poor in sulphur was, however, after 6 days cultivation 1, *i. e.* the polarographically active sulphur existed only as SH groups.

Further experiments with different quantities of sulphur in the substrate have confirmed the results given above.

When the protein synthesis is reduced through a reduction of the supply of nitrogen in the substrate the value of the ratio $\frac{SH}{SH + SS}$ of the proteins of the yeast decreases with decreasing protein content. On the other hand when the protein synthesis is reduced through a reduction of the supply of sulphur in the substrate (the supply of nitrogen being abundant) the value of the ratio increases with decreasing protein content. If the sulphur supply is very low all the sulphur is transformed to SH groups and the value of the ratio becomes 1. The sulphydryl groups are assumed to be active parts of the protein synthesizing enzyme system of the yeast. If the nitrogen supply is low there is little need for SH groups for protein synthesis. Then the value of the ratio is low. If the supply of nitrogen and sulphur is ample there is need for sulphydryl groups for protein synthesis and the ratio is high. If the supply of sulphur (but not of nitrogen) is reduced causing a reduction of the content of protein a greater part and finally all of the sulphur is transformed into SH groups and the value of the ratio is high, finally 1, in order to promote protein synthesis from the nitrogen present and counteract the drop of the protein content of the yeast.

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Received October 26, 1950.

X-Ray Diagram of Plastein

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The investigations, which have been carried out in the Biochemical Institute in Helsinki on the structure of the so-called plastein, have shown that high-molecular polypeptides are in question. The average peptide size of different plastein preparations, calculated on the basis of amino nitrogen, has varied corresponding to about 30—100-peptides, or to mol. weights of about 3 500—12 000. The average peptide size of the protein hydrolysates used for the synthesis has usually corresponded to 5—6-peptides.

In order to be able to compare the structures of plastein and protein, from the hydrolysate of which plastein was precipitated by means of pepsin, we took X-ray photographs of pulverized plastein and zein. In the present paper these diagrams are given. The plastein used was prepared as follows.

Zein was hydrolyzed for 50 days with pepsin in a water suspension acidified with formic acid to pH 4 (150 g zein in 6 l water, + formic acid, 750 mg cryst. pepsin, 40 ml toluene, temperature 37° C). After heating for 20 min at 85° C to inactivate pepsin the insoluble residue was separated and the clear filtrate concentrated in vacuo. The solution then contained 40.0 mg N per ml. Amino-N of the solution estimated with the Cu-method (coef. 0.14) [1] was 16.3 % of total N after subtraction of ammonium and free amino acids. The average peptide size corresponded thus to about 5-peptides. 1 000 mg of cryst. pepsin was added to 400 ml of the concentrated solution (0.35 mg pepsin-N/ml), the pH of which was adjusted to 4. The precipitate was filtered with sinter 1 G2 after 20 h and washed thoroughly with water. The air-dry precipitate contained 13.0 % N. Its amino-N was 1.7 % and amide-N 12.8 % of total N. The amino-N of the initial zein was 0.25 % and amide-N 18.4 % of total N. The average peptide size of plastein thus corresponded to about 50-peptides and molecular weight to about 6 000.

For diffraction diagrams samples were prepared from zein and plastein by pressing. Their thickness varied from 1 to 2 mm. The diagrams were obtained by CuK_α-radiation filtered through Ni-filter. The ordinary transmission method (pinhole Ø = 1 mm) with the Unicam X-ray goniometer was employed. The distance between the sample and the flat film cassette was 50 mm.

The diagrams display the two rings typical of proteins and polypeptides.

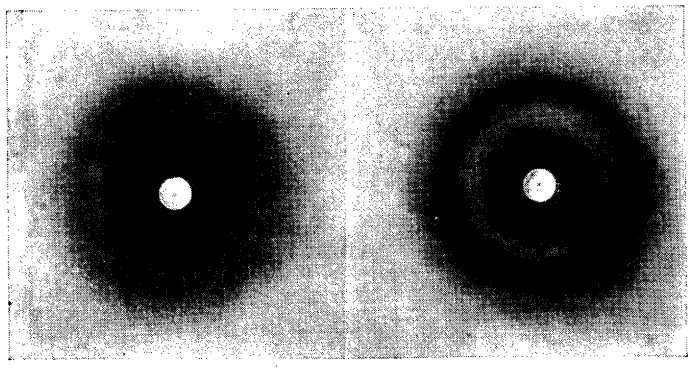

Fig. 1. X-ray diagram of zein (left) and plastein (right) precipitated by pepsin from the pepsin hydrolysate of zein. The average peptide size of plastein about 50-peptides.

Astbury interprets in general that the outer ring represents the backbone distance of a polypeptide chain and the inner ring the average side-chain distance. Both rings are much sharper in the X-ray diagram of plastein than in that of zein. The Bragg-spacings corresponding with the rings are about 4.5 Å and 10 Å in zein and about 4.7 Å and 11.1 Å in plastein in which more accurate measurements can be made. From the interpretations of Astbury regarding his X-ray studies we concluded that our X-ray diagrams are in agreement with the idea of Virtanen and his collaborators [2], based on chemical facts, that plasteins are polypeptides. One of us (V.) asked Prof. Astbury's opinion of our photograph. Prof. Astbury kindly replied (5. IV. 1950) and by his consent we publish below an excerpt from his letter.

"*Plastein from zein.* — This is the common type of diagram from a protein after it has been denatured and aggregated. It results from the unfolding or other disorganisation of the specific configuration, the liberated chains now forming more or less regular bundles in the β-configuration. The two rings are now much sharper because the chains (or portions of chains) are trying to lie as parallel as possible, linked more precisely by the sidechain and backbone linkages. The sharpness of the rings is a measure of the extent to which they succeed in doing this, the two main controlling factors being (1) the degree to which the original specific configuration has been unfolded and the chains have been freed, and (2) the average length of the chains (or portions of chains).

In my view, it follows from this line of argument that the sharpness of your plastein diagram indicates that the plastein chains must be relatively long — — certainly much longer than the 6-peptides of the pepsin hydrolysate. I am afraid it is not possible to give a more exact estimate, but I can tell you that the plastein diagram is quite as sharp as many of the diagrams I have obtained recently from synthetic polypeptides made by the Leuchs reaction. The molecular weight of these preparations is believed to be of the order of 5 000, say about 50 residues or perhaps rather fewer, which would correspond roughly to the chain-length you estimate for your plastein preparation.

To summarise, I should say that your plastein diagram is pretty good independent evidence of the presence of fairly long peptides, and is consistent both with your own estimate of the probable chain-length and with my own observations on denatured and aggregated proteins in general and on synthetic polypeptides in particular."

The X-ray diagrams thus confirm the picture obtained from plastein in the light of chemical studies as high-molecular polypeptides which are formed from comparatively low-molecular peptides (*e. g.* 4—6-peptides) by the action of proteolytic enzyme (pepsin). The result supports the reaction scheme introduced earlier [2].

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Received October 20, 1950.

An Improved Method for Carbethoxylations with Ethyl Carbonate

ARNE BRÄNDSTRÖM

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A very important method for the preparation of β-keto esters, malonic esters, and α-cyano esters is the carbethoxylation of a ketone, an ester, or a nitrile with the aid of ethyl carbonate and sodium ethoxide, as described by Wallingford and his co-workers [1-3].

The disadvantages of this method is that the preparation of dry sodium ethoxide from sodium and alcohol is a rather cumbersome process, and the reaction

Astbury interprets in general that the outer ring represents the backbone distance of a polypeptide chain and the inner ring the average side-chain distance. Both rings are much sharper in the X-ray diagram of plastein than in that of zein. The Bragg-spacings corresponding with the rings are about 4.5 Å and 10 Å in zein and about 4.7 Å and 11.1 Å in plastein in which more accurate measurements can be made. From the interpretations of Astbury regarding his X-ray studies we concluded that our X-ray diagrams are in agreement with the idea of Virtanen and his collaborators [2], based on chemical facts, that plasteins are polypeptides. One of us (V.) asked Prof. Astbury's opinion of our photograph. Prof. Astbury kindly replied (5. IV. 1950) and by his consent we publish below an excerpt from his letter.

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The X-ray diagrams thus confirm the picture obtained from plastein in the light of chemical studies as high-molecular polypeptides which are formed from comparatively low-molecular peptides (*e. g.* 4—6-peptides) by the action of proteolytic enzyme (pepsin). The result supports the reaction scheme introduced earlier [2].

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ARNE BRÄNDSTRÖM

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A very important method for the preparation of β-keto esters, malonic esters, and α-cyano esters is the carbethoxylation of a ketone, an ester, or a nitrile with the aid of ethyl carbonate and sodium ethoxide, as described by Wallingford and his co-workers [1-3].

The disadvantages of this method is that the preparation of dry sodium ethoxide from sodium and alcohol is a rather cumbersome process, and the reaction

in most cases has to be carried out under reduced pressure. This can easily be avoided, however, by the preparation of sodium ethoxide from ethyl carbonate and sodium, a method that has been known for a long time [4], but which does not appear to have been used for preparative purposes. Wallingford and his co-workers [1] stated: "Any procedure employing sodium or potassium metal suffers from the fact that these metals readily decompose alkyl carbonates." Evidently they thought that the reaction did not stop with the formation of sodium ethoxide, as is shown by the statement [2]: "The reaction of metal alcoholates with alkyl carbonates to form ethers and metal alkyl carbonates increases in rate rapidly at temperatures above about 100° limiting the extent to which one may force the carbalkylation." That this reaction does not proceed to a serious extent is shown by the fact that Widequist [5] has obtained ethyl naphtylcyanoacetate and ethyl phenylcyanoacetate in excellent yields, by adding sodium in pieces to a mixture of ethyl carbonate and a nitrile, with simultaneous removal of the alcohol formed by distillation at atmospheric pressure.

In the present investigation, sodium ethoxide was first prepared from sodium and ethyl carbonate, and a ketone was then added slowly with simultaneous removal of the alcohol formed. In the cases studied, equal or better yields of the β-keto esters than those reported by Wallingford and his co-workers [2] were obtained, indicating that the cleavage of ethyl carbonate by sodium ethoxide is not a very serious side reaction under these condensation conditions.

Experimental: In a 1-l three-necked, round-bottomed flask, fitted with a dropping funnel, a mercury sealed stirrer, and a 30-cm Widmer column connected to a distillation condenser, was placed 400 ml of dry ethyl carbonate. The flask was placed in an oil bath and heated until gently boiling. The oil bath was then removed and 11.5 g (0.5 mole) of sodium was added in pieces to the hot, stirred ethyl carbonate at such a rate that gentle refluxing was obtained. When all the sodium had dissolved, which was a few minutes after the addition of the last piece, the flask was placed in an oil bath at 140°. The ketone (0.5 mole) was added from the dropping funnel over a period of about one hour. The alcohol began to distill after about one third of the ketone had been added and the theoretical quantity 60 ml (1 mole) was collected. The temperature of the oil bath was then raised until the temperature in the top of of the column increased rapidly to over 120°. The oil bath was then removed and the flask allowed to cool to room temperature. The contents were poured over 35 ml of glacial acetic acid and ice. The ethyl carbonate layer was separated, and the water layer extracted with ether. The combined ether and ethyl carbonate layers were washed with water and then dried over anhydrous sodium sulphate. The ether and ethyl carbonate were removed at reduced pressure and the residue fractionated *in vacuo*.

By this method the following β-keto esters were prepared: *Ethyl* iso*valeryl-acetate* b. p. 90°/10 mm in 75 % yield, *ethyl caproylacetate* b. p. 114°/10 mm in 65 % yield, and *ethyl benzoylacetate* b. p. 144 – 147°/8 mm in 76 % yield.

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Received October 28, 1950.

Some Improvements in Flame Photometric Determination of Potassium

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In flame photometric determinations of metals the curves obtained plotting brightness against concentration are usually approximated by straight lines within certain regions. When potassium is determined in hot flames — air-acetylene or butane-oxygen — the graph is slightly S-shaped (Figs. 1 and 2, *Cf.*[1]), and an inappropriate approximation of the curve by a straight line may lead to a small but systematic error, especially when the determination of a reagent blank correction is involved in the analysis.

An accurate determination of the working curve in each series of determinations means a great number of standard solution readings. On the other hand small drifts in gas and air pressure always occur, which make it undesirable to include too many test samples or standard solutions in each series, if high accuracy is needed. If a formula could be found to fit the working curve and, if possible, transform it into a straight line, the number of standard solution readings could be reduced and much labour saved without loss of accuracy.

On account of interferences of different kinds it is strongly recommended to work in very dilute solutions [1, 2]. Thus the most important part of the curve is that with the upward curvature shown in Fig. 2 (up to about 40 mg K/l).

If x is the concentration of potassium and y the corresponding photometer reading, the working curve may be fitted within the range 0.5 — 40 mg K/l by the equation

$$y = a \cdot x^b; \text{ or } \quad \log y = a^1 + b \cdot \log x;$$

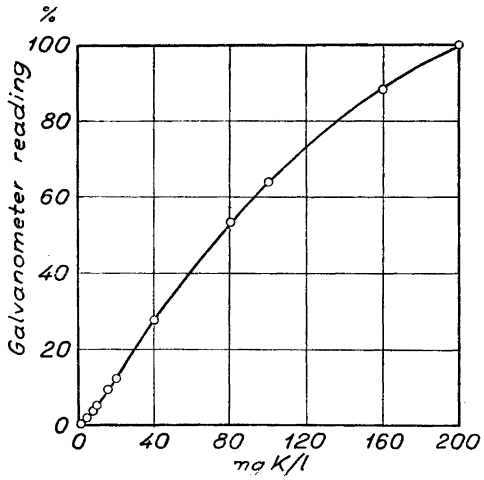

Fig. 1. Calibration curve for 0—200 mg K/l in 0.02—C HCl. Galvanometer readings as per cent of the reading for 200 mg K/l.

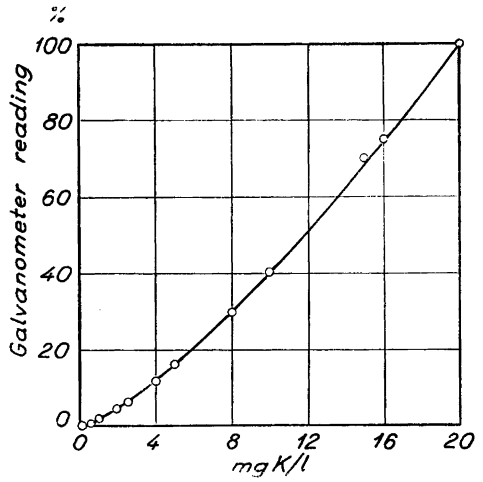

Fig. 2. Calibration curve for 0—20 mg K/l in 0.02—C HCl. Galvanometer readings as per cent of the reading for 20 mg K/l.

Technical data: air-acetylene flame; atomizer according to Rauterberg and Knippenberg (3); Jena metal interference filter S. J. F. 768 a; red sensitive phototube (Pressler 125 T), voltage 120 V; Multiflex-galvanometer MG 4.

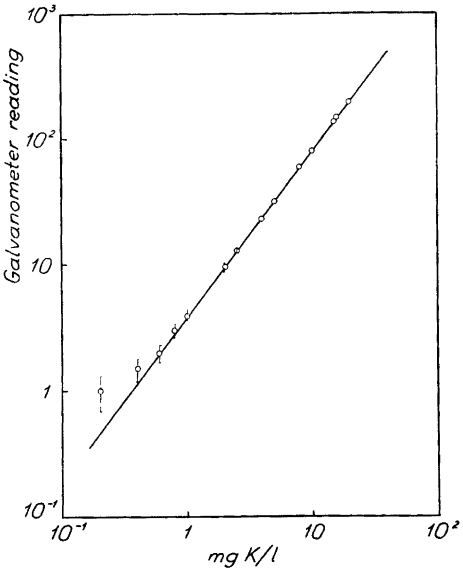

Fig. 3. Logarithmic calibration curve for 0—20 mg K/l in 0.02—C HCl. Data from Fig. 2.

The perpendicular lines indicate the standard deviations of the photometer readings.

a, b and a^1 being constants. In Fig. 3, where log y is plotted against log x, the constants are $a^1 = 0.60$; $b = 1.30$;

In ordinary routine work the photometer readings for only three standard solutions (*e. g.* 5, 10, 15 mg K/l) are plotted against the concentration on commercially available log/log paper. The concentrations of the test samples are then found by interpolation.

All measurements in a series are always referred to the same moment by reading the solutions twice (or more), the second time in the reversed order. This simple method to compensate for errors due to continous drifts in gas and air pressure seems to be usually overlooked in flame photometry. To determine deviations in the air pressure we have used a simplified model of the very sensitive pressure constance indicator described by Westin *et al.* [5]

Before correcting for the reagent blanks, the corrections must of course be converted into concentrations (mg K/l). One scale division corresponds to different amounts of potassium within different concentration ranges. In most cases the reagent blank concentration can be found by extrapolation on the same calibration curve as was used for the samples. If the reagent blank concentration is very low (less than about 0.5 mg K/l in the example in Fig. 3) and high accuracy is needed, it is advisable to make a special calibration curve with standard solutions of similar concentrations, if possible with the instrument adjusted to higher sensitivity. Such curves have been made for the range 0—5 mg K/l. They have shown the same upward curvature as the curve on Fig. 2 and a straight line on log/log paper, but the value of the constant b has been lower than that found with the stronger solutions.

Most potassium determinations at the Forest Research Institute have been made with an interference filter photometer with air-acetylene flame. The calibration curves obtained with this instrument for concentrations lower than 40 mg K/l have all been straight lines plotted on log/log paper. Use of barrier-layer cells instead of alkali phototubes, or colour filters instead of interference filters did not alter the shape of the curve. Variations in the shape of the burner outlet altered the slope of the log/log curve slightly; and so did variations in the air-acetylene ratio, probably by changing the flame temperature.

A few curves have also been made with the Beckman Spectrophotometer Model DU, using a very hot butane-oxygen flame. These curves had a form similar to that in Figs. 1 and 2, but the upward curvature continued to a higher level than with the filter instrument. When plotted

on log/log paper the curves were straight lines up to at least 100 mg K/l (770 mμ) or 1 000 mg K/l (405 mμ).

When only potassium determinations are concerned, it may be advantageous to use a cooler flame than the air-acetylene one, *e. g.* air-butane. The sensitivity for potassium is about the same, or even higher, and the presence of sodium effects the potassium reading much less *Cf.*[4]. The potassium calibration curves appear to be more linear in this case. However, such an instrument is not sensitive enough for most other elements.

My sincere thanks are due to Fil.kand. Mrs Karin Knutsson and Fil.lic. Carl Olof Tamm, Forest Research Institute of Sweden, for their invaluable help and advice.

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Received October 14, 1950.

α-Thienylsuccinic Acid

KURT PETTERSSON

Chemical Institute, University of Uppsala, Uppsala, Sweden

In the course of the present work on optically active thiophene compounds, ethyl thienylcyanoacetate has been prepared [1] and then allowed to react with halogen compounds [2]. A new example of this reaction is the preparation of thienylsuccinic acid from ethyl thienylcyanoacetate and ethyl chloroacetate, yielding ethyl thienylcyanosuccinate, which has then been decarboxylated and hydrolyzed to give thienylsuccinic acid.

Thienylsuccinic acid crystallizes from ethyl acetate as a colourless, microcrystalline powder, melting with decomposition at 156—157° C. From water it is obtained in hemi-spherical crystal aggregates. The difficulty of obtaining well-shaped crystals may indicate that the acid, like the analogous phenylsuccinic acid, is dimorphic [3].

The acid will be resolved in the optical antipodes and the result published later.

Ethyl thienylcyanosuccinate: In a round-bottomed, two-necked flask, fitted with a mercury-sealed stirrer and a reflux condenser, were placed 15.0 g (0.077 moles) of ethyl thienylcyanoacetate, 25 g (0.2 moles) of anhydrous potassium carbonate, 30 ml of ethyl chloroacetate and 100 ml of dry acetone. The mixture was refluxed with stirring for 20 hours in an oil bath, the acetone removed by distillation and the residue treated with 50 ml of water to dissolve the inorganic salts. The oily layer was separated, and the water layer extracted three times with ether. The oily and ether layers were then combined, dried with sodium sulphate and distilled *in vacuo*. 13.0 g (0.046 moles) of ethyl thienylcyanosuccinate was obtained. B. p. 200—205° C/12 mm Hg. Yield: 60 %.

Thienylsuccinic acid: 13.0 g (0.046 moles) of ethyl thienylcyanosuccinate and 12 g (0.2 moles) of potassium hydroxide, dissolved in alcohol, were warmed on a water bath for five minutes, the potassium carbonate filtered off and washed with alcohol. The filtrate was treated with 20 ml of water and refluxed in an oil bath, until no more ammonia escaped (24 hours). The alcohol was removed by distillation and the residue acidified with dilute hydrochloric acid. The thienylsuccinic acid was obtained as a yellow oil, which dissolved in warm water. The water solution was boiled with charcoal and filtered. After cooling, 6.0 g (0.030 moles) of thienylsuccinic acid was obtained as light

on log/log paper the curves were straight lines up to at least 100 mg K/l (770 mμ) or 1 000 mg K/l (405 mμ).

When only potassium determinations are concerned, it may be advantageous to use a cooler flame than the air-acetylene one, *e. g.* air-butane. The sensitivity for potassium is about the same, or even higher, and the presence of sodium effects the potassium reading much less *Cf.*[4]. The potassium calibration curves appear to be more linear in this case. However, such an instrument is not sensitive enough for most other elements.

My sincere thanks are due to Fil.kand. Mrs Karin Knutsson and Fil.lic. Carl Olof Tamm, Forest Research Institute of Sweden, for their invaluable help and advice.

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yellow, hard crystal aggregates. Yield: 65 %. The acid was recrystallized from a small amount of ethyl acetate. M. p. 156—157° C with decomposition.

$C_8H_8O_4S$

	Equiv. wt.	C	H	S
Calc.	100.1	47.99	4.03	16.01
Found	100.6	48.05	4.08	16.08

A grant from the Nobel Fund of Chemistry (*Kemiska prisgruppens särskilda fond*) is gratefully acknowledged.

1. Pettersson, K. *Acta Chem. Scand.* **4** (1950) 395.
2. Fredga, A., and Pettersson, K. *Acta Chem. Scand.* **4**. In print.
3. Schaum, K., Schaeling, K., and Klausing, F. *Ann.* **411** (1916) 193.

Received October 19, 1950.

A Spraying Reagent for Paper Chromatograms Which is Apparently Specific for Ketoheptoses

ROLF KLEVSTRAND and ARNOLD NORDAL

Department of Pharmacognosy, Pharmaceutical Institute of the University, Oslo, Norway

Pentoses, glucuronic acid and certain heptoses are known to give a bluish green colour with Bial's reagent (orcinol dissolved in hydrochloric acid, to which $FeCl_3$ is added as catalyst [1]). Attempts to use this reagent for the detection of ketoheptoses (sedoheptulose and D-mannoheptulose) on paper chromatograms were unsuccessful, as the mineral acid attacked the paper. By using trichloroacetic acid instead of hydrochloric acid, however, we obtained serviceable results. When an aqueous solution of orcinol and trichloroacetic acid was used the resulting spots were diffuse as the sugars migrate from wet to dry regions on the filter paper [2]. If on the other hand, the reagents are dissolved in water-saturated *n*-butanol [2], well-defined spots are obtained. After a number of experiments we have arrived at the following reagent:

Orcinol	0.5 g
Trichloroacetic acid	15 »
n-Butanol (water-saturated)	100 ml

Owing to rapid esterification the reagent it rather instable. It ougth to be kept in a cold place and not to be used for more than 6—8 days after preparation.

After the paper has been dried, it is sprayed with the reagent and heated at 105° C for 15—20 minutes.

Table 1 shows how the various sugars behave under this treatment. For carrying out the reaction we used about 0.1 mg of each sugar.

As we have not had any aldoheptoses at our disposal, we have not yet tested whether the reagent gives any colour with these. Of the sugars we have tested it is, however, only the ketoses which react. The two heptoses which have hitherto been found in Nature are both ketoheptoses.

Table 1.

Sugar	Colour produced in the reaction
Sedoheptulose	Bluish green
Mannoheptulose	» »
Fructose	Yellow
Sucrose	»
Glucose	No colour
Galactose	» »
Arabinose	» »
Xylose	» »
Ribose	» »
Rhamnose	» »
Glucuronic acid	» »

The reagent is useful for, *inter alia*, paper chromatographic studies of the distribution of ketoheptoses in the vegetable kingdom, a research in which we ourselves are at present engaged.

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Received November 1, 1950.

Phosphoamidase Activity of some Proteolytic Enzymes and Rennin

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In the course of an investigation of the properties and distribution of phosphoamidase we found that certain crystalline proteolytic enzymes will hydrolyze N(*p*-chlorophenyl)-amidophosphoric acid [1] a substrate of phosphoamidase (Fig. 1.)

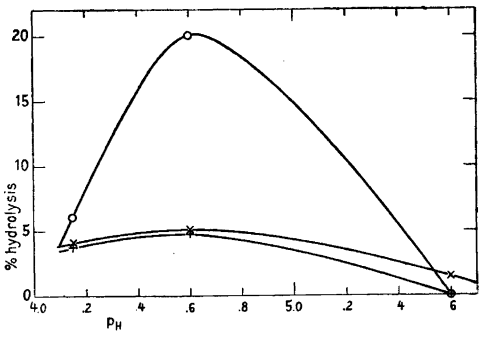

Fig. 1.

The substrate was a 2.52 m*M* solution of N(*p*-chlorophenyl)-amidophosphoric acid in 0.2 *M* acetate buffer. 2 volumes of this solution were incubated with 1 vol. of the enzyme solution for 2 hours at 40°. The enzyme solutions were: 1) 0.1 % (dry weight) Armour crystalline pepsin (0.129 mg N/ml), 2) 0.1 % (wet weight) crystalline trypsin, prepared according to Kunitz (0.0655 mg N/ml); 3) 0.1 % (wet weight) crystalline rennin, prepared by Berridge [2] (0.0535 mg N/ml) * In Fig. 1 the values for rennin are the original ones; the other values have been reduced to the nitrogen value of the rennin solution.

We cannot claim that Fig. 1 represents the correct proportion between the activities of the three enzymes since the samples, although crystalline, were not freshly prepared. However, even if the specific activities shown may be slightly falsified by the presence of inactivated enzyme nitrogen, the graph still indicates that the phosphoamidase activity of rennin far surpasses that of pepsin and trypsin. The optimum pH of 4.6 is the same that we have found for the phosphoamidase activity of various tissue homogenates against the same substrate (unpublished experiments).

To answer the question whether the phosphoamidase activity of the crystalline enzyme preparations might be due to an impurity, we have tried whether the trypsin activity can be inhibited by soybean inhibitor, in analogy to the inhibition of the proteolytic activity of trypsin. This proved to be the case, and therefore it is unlikely, at least in the case of trypsin, that the phosphoamidase activity is an impurity. Schwert, Neurath, Kaufman and Snoke [3, 4] have found that proteolytic enzymes, among them trypsin, exert esterase activity. A similar situation might exist in the present case.

A 2.52 m*M* solution of the phosphoamidase substrate was incubated at pH 4.6 with trypsin in the concentration given above, for 3 hours at 40°. In this time 11.8 % of the substrate was split. The same reagents in the presence of crystalline soybean inhibitor (twice the weight of trypsin) gave a hydrolysis of only 1.3 %.

The fact that phosphoamidase activity is especially high in the case of rennin may help in clarifying the old and unsettled question of the primary action of rennin upon casein. Several investigators [5, 6] have felt that it was by no means certain that the initial chemical change of the casein which induces the process of coagulation is of a proteolytic nature. In 1932 one of us suggested [7] that the initial rennin action might consist in the splitting of P-N bonds in casein. We have tried to study this question by

* The rennin sample was given to us by Dr. H. Nitschmann, Berne, to whom we express our sincere thanks.

comparing the milk-clotting activity of various preparations with their phosphoamidase activity. The result is given in Table 1.

Table 1.

Enzyme prep.	*a* Phosphoamidase activity % hydrol	*b* coagulation R. U.	*b/a*
Rennin	20.7	453	21.9
Pepsin	12.4	262	21.2
Chymotrypsin	8.26	184	22.3
Calf stomach	27.8	189	6.8
Cow stomach	14.7	35.4	2.4

The phosphoamidase activity is given as percentage hydrolysis under the conditions described under Fig. 1. The milk coagulation was measured according to Jacobsen [8] and the activity given in "rennin units" (R. U., according to Berridge [2], only using minutes instead of seconds to designate coagulation time). The rennin and pepsin solutions were the same as employed in the experiment of Fig. 1. Chymotrypsin was a crystalline preparation in 1 % (dry weight) solution *. The calf and cow enzymes were crude homogenates of the mucosa of the 4th stomach, applied in a concentration of approximately 10 % of wet weight.

The remarkable equality of the ratio between coagulating and phosphoamidase activities in the case of the three crystalline enzymes supports the view that there really is a connection between the splitting of P-N bonds and the coagulation of casein. It also supports the view that both activities are inherent properties of the enzyme protein molecule. Regarding the homogenates it must be remembered that in a number of other tissues (spleen, brain, liver etc.) there exists considerable phosphoamidase activity without any milk-clotting power. The same is true of crystalline trypsin. We therefore have to consider the existence of several phosphoamidases of which only one induces milk coagulation. The admixture of non-coagulating enzyme makes itself felt in the mucosa homogenates of calf and cow, in spite of their high content of rennin and pepsin. In trypsin and the tissues mentioned above only non-coagulating phosphoamidases are present and the ratio b/a correspondingly drops to zero. Of course, this phenomenon could also be explained by the assumption that the considerable proteolytic activity of the tissues complicates matters by preventing the milk coagulation which otherwise might be induced.

Finally we should like to mention the possible similarity of the enzyme here investigated and the phosphoprotein phosphatase described by Harris [9] and further investigated by Feinstein and Volk [10]. Their results regarding the distribution of the enzyme in various mammalian tissues correspond roughly to our findings in a similar survey.

This investigation is continued and will be published in greater detail in the *Comptes Rendus du Laboratoire Carlsberg.*

* Worthington Biochemical Laboratory, Freehold, N. J.

One of the authors (S.-O. Li) has received support from "The Danish Committee on Training of Foreign Scientists in Danish Laboratories".

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Received November 13, 1950.

On the Coprecipitation of Tetrapositive Ions with Lanthanum Fluoride

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Cunningham and Werner [1] in their first isolation of plutonium made use of the fact, first discovered by Seaborg, Wahl and Kennedy [2], that the lower oxidation states of Pu are coprecipitated with LaF_3 whereas the plutonyl ion PuO_2^{2+} is not. Pu could thus first be separated from the large amount of UO_2^{2+} originally present using LaF_3 as a carrier; then the Pu of the precipitate could be oxidized to PuO_2^{2+} and the La removed by a new precipitation. — The coprecipitation of Pu with La is believed to have also been used in the industrial production of Pu [3].

If Pu^{3+} coprecipitates with LaF_3 this is as could be expected. The ionic radii are very similar (La^{3+} 1.04 Å, Pu^{3+} 1.01 Å using Zachariasen's values [4] so we can expect a partial or even total replacement of the La^{3+} ions in LaF_3 by Pu^{3+}.

What is remarkable is, however, that *tetrapositive* plutonium is also carried almost quantitatively by LaF_3 under circumstances where PuF_4 would not precipitate in the absence of La. This coprecipitation is generally ascribed to adsorption [5]. On the other hand Cefola and Smith [6] analyzed a number of LaF_3—PuF_4 precipitates, prepared with an excess of Pu in the solution, and found that the ratio La^{III}: Pu^{IV} varied between 1.81 and 3.4. Their conclusion was that a compound $La_2PuF_{10}(H_2O)_X$ is formed. They do not seem to have used X-ray photographs.

We shall now give evidence supporting a third explanation, namely that solid solutions are formed, which are in this case anomalous mixed crystals.

One of us (KS) has made an extensive investigation of the coprecipitation with LaF_3 of trivalent lanthanide ions and of some tetravalent ions. Sodium fluoride solution was added to an acid solution containing La^{3+}, the other ion in question, and nitrate or sulfate ions. The precipitate was kept at 100° C for some time in order to get larger crystal grains. In some cases the samples were kept with the supernatant solution in an autoclave at 220° C. Finally the preparations were investigated by means of powder photographs using focussing cameras.

For the tripositive ions the results were qualitatively as expected. The ions of radii close to La^{3+} (La^{3+} 1.04 Å, Ce^{3+} 1.02 Å, Pr^{3+} 1.01 Å, Nd^{3+} 0.99 Å)[4] form fluorides MF_3 isomorphous with LaF_3; for these ions mixed crystals with the LaF_3 structure were obtained for all ratios $LaF_3 : MF_3$.

For lanthanides with shorter ionic radii, MF_3 is not isomorphous with LaF_3. However, a range of solid solutions were found extending from pure LaF_3 to a certain percentage of MF_3, decreasing with the radius of M^{3+}.

Experiments were also made with the tetrapositive ions Th^{4+} (0.95 Å), U^{4+} (0.89 Å) and Ce^{4+} (0.87 Å). The two latter ions have radii close to that of Pu^{4+} (0.86 Å) whereas Th^{4+} is larger [4].

From both Ce^{4+}—La^{3+} and U^{4+}—La^{3+} solutions predipitates could be obtained with the LaF_3 structure but with lattice dimensions considerably smaller than those of pure LaF_3. (For high percentages of MF_4, other phases appeared as well.) Some preliminary figures for the hexagonal a and c axes of these preparations are given in Fig. 1, and compared with those of pure LaF_3 and CeF_3. The circles give

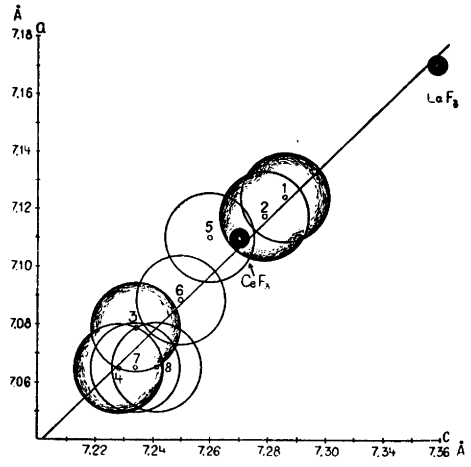


Fig. 1. a and c axes of phases with the pure LaF_3 structure: pure LaF_3 and pure CeF_3 (small circles), $La^{3+}-U^{4+}$ fluorides (large open circles 5—8) and $La^{4+}-Ce^{4+}$ fluorides (large shaded circles 1—4). The original mole percentage in the solution increases from 11.0 % Ce^{4+} in 1 to 44.0 % Ce^{4+} in 4 and from 14.7 % U^{4+} in 5 to 35.5 in 7 and 44.9 in 8. These figures, however, do not give the real percentage of U^{4+} in the fluoride crystals since there has been some transformation to Ce^{3+} and UO_2^{2+} and some M^{3+} may remain in the solution. In samples 4, 7, and 8 other phases were also present.

the estimated limits of error, which are much wider for the mixed crystals than for the pure compounds. This is because the latter could be recrystallized, CeF_3 by heating with the supernatant liquid to 220° C in an autoclave and LaF_3 even by heating in air to 400° C, so that their powder photographs gave very sharp lines. The same treatment of the $La^{3+}-Ce^{4+}$ and $La^{3+}-U^{4+}$ precipitates led to decomposition with the formation of Ce^{3+}, and of UO_2 and other products. Thus the measurements must be made on specimens recrystallized at only 100° C, which had slightly broader lines.

Obviously Ce and U must have entered the lattice in varying amounts. One might suggest that Ce had been reduced to Ce^{3+} and then entered the lattice; however the shrinking of the lattice is certainly larger than could be explained merely by the formation of mixed crystals LaF_3-CeF_3.

We thus conclude that the LaF_3 lattice really contains Ce^{4+} and U^{4+}.

The most plausible mechanism for the formation of the solid solutions seems to be a random replacement of La^{3+} by M^{4+} and "holes" in the ratio 4 La^{3+}: (3 M^{4+} + 1 hole), whereas the fluoride framework is not changed. (The replacement of 4 La^{3+} by 4 M^{4+} + 4 F^- in interstices of the lattice would require a widening rather than a shrinking of the cell dimentions.)

La^{3+} has previously been shown to enter anomalous mixed crystals with the CaF_2 structure, *e. g.* 1 La^{3+} + 1 F^- replacing 2 Sr^{2+} [7–11]. As far as we know, however, anomalous mixed crystals with the LaF_3 structure have not been demonstrated previously.

It seems overwhelmingly probable that Pu^{4+} with a radius close to those of Ce^{4+} and U^{4+} will behave in the same way as these ions and that the coprecipitation with LaF_3 is due to the formation fo solid solutions. Then the reaction used in the laboratory and technical process tends towards a real thermodynamic equilibrium and is not due to the somewhat capricious phenomenon of surface adsorption.

A more detailed account of these experiments will be published later on.

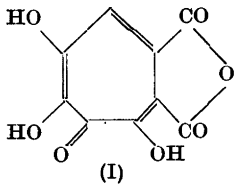
There are many other cases where precipitation on a carrier is used for practical preparation and purification purposes. It seems to us that it would also be desirable in other cases to investigate whether the process is due to surface adsorption, solid solution or compound formation since such knowledge would be a good guide in the choice of the best experimental conditions. Perhaps the formation

Studies in the Tropolone Series
II. Puberulonic Acid

GUNHILD AULIN-ERDTMAN

Cellulosaindustriens Centrallaboratorium and Organisk-Kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

At the symposium on the chemistry of tropolones held by the Chemical Society in London on November 2nd 1950, various structural formulae for puberulonic acid [1] were discussed. The author wishes to record that she proposed, on that occasion, the anhydride structure (I) for this acid.



(I)

This suggestion was based upon a new potentiometric titration curve for the substance in combination with a detailed study of its ultra-violet absorption properties in various solvents and in aqueous solutions of different pH-values, and also upon the comparison with corresponding data for stipitatic and puberulic acids.

An investigation of the infra-red spectra of puberulonic acid as well as of several related compounds, carried out in collaboration with Professor H. Theorell at Medicinska Nobelinstitutet, Stockholm, strongly supports the above formula for puberulonic acid.

Full details of these investigations will be published shortly in this journal.

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Received December 8, 1950.

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of anomalous mixed crystals will prove to be a more common phenomenon than has hitherto been believed.

This investigation has been supported by the Swedish Atomic Commission.

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Received November 14, 1950.

Studies in the Tropolone Series
II. Puberulonic Acid

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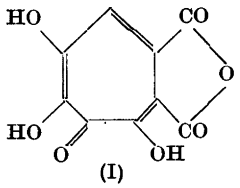
At the symposium on the chemistry of tropolones held by the Chemical Society in London on November 2nd 1950, various structural formulae for puberulonic acid [1] were discussed. The author wishes to record that she proposed, on that occasion, the anhydride structure (I) for this acid.



(I)

This suggestion was based upon a new potentiometric titration curve for the substance in combination with a detailed study of its ultra-violet absorption properties in various solvents and in aqueous solutions of different pH-values, and also upon the comparison with corresponding data for stipitatic and puberulic acids.

An investigation of the infra-red spectra of puberulonic acid as well as of several related compounds, carried out in collaboration with Professor H. Theorell at Medicinska Nobelinstitutet, Stockholm, strongly supports the above formula for puberulonic acid.

Full details of these investigations will be published shortly in this journal.

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Received December 8, 1950.

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of anomalous mixed crystals will prove to be a more common phenomenon than has hitherto been believed.

This investigation has been supported by the Swedish Atomic Commission.

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Received November 14, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1326–1328

New Books

E. A. Guggenheim. *Thermodynamics, An advanced treatment for chemists and physicists.* North Holland Publ., 1949. – 395 pp. fl. 20. ≃ £2.

Readers and admirers – which may cover just about identical assemblies – of Prof. E. A. Guggenheim's "Modern Thermodynamics" from 1931 will be eager to see his new book, and they will find that nothing has been lost of the lucidity and exactness which made that book one of outstanding merit. Several new fields are now covered with equal clarity, and many of the interventions, with which Guggenheim has in later years cut through inexact usage are now worked into the text.

As might be expected of a modern treatise, the book includes also a "Digression on Statistical Thermodynamics". The presentation is essentially that from the paper by Guggenheim at the "Kemikermøde" in Copenhagen 1939. Extensive use is made of the "Massieu-function" $J = -F/T$ and the "Planckfunction" $Y = -G/T$, convenient for statistical work.

It is an "advanced treatment" also in the respect that the reader should have some knowledge of statistics in order to understand this chapter, and as a whole the book is rather more "advanced" than "Modern Thermodynamics". The question is whether or not this is an indispensable consequence of the clarity and exactness. In other words: Do inexactness and obscurities really make a text easier or more elementary. The reviewer thinks not and would welcome an elementary text on the same lines as the present book, although it would necessarily be either more restricted or rather much bigger. But as it is, this book as a matter of course will take its place on the shelves of any physical chemist who want an easy source of information on how to elucidate a thermodynamic problem without bringing in any kind of nonsense.

The book is well produced but has rather too many misprints. An index would have made it more convenient as a reference book, but an extensive table of contents makes up for it to some degree.

J. Koefoed

Walter Hückel: *Anorganische Structure Chemie.* Enke, Stuttgart, 1948. Price DM 68: –, bound DM 71: 20. 1013 pages, 170 figures.

The title of this book reminds one of two other works: "*Structural inorganic chemistry*" by Wells, and the "*Strukturbericht*" of the *Zeitschrift für Kristallographie.* However, on reading it we find that it cannot be compared with either. It is not a text-book like Wells' book, where the reader is supposed to start on page 1 and read on, steadily increasing his wisdom, but rather a collection of quite independent essays, sometimes giving elementary information, but in other parts demanding considerable knowledge on the part of the reader. Nor does Hückel try, like Strukturbericht, to give a complete list of all inorganic crystal structures

determined; however, in the course of his discussion he happens to cover a very great number of inorganic structures. So, if you are interested in some particular structure, you have a good chance of finding a reference to it in the index of Hückel's book.

The common theme in all these essays is the way the atoms bind each other in inorganic compounds. In the preface the well-known author of the *Theoretische Grundlagen der organischen Chemie* gives an account of the various paths that led him to this theme, and how he began to feel the need for a comprehensive treatment from the stand-point of a chemist, as distinguished from that of a theoretical physicist or a crystallographer. The field is certainly wide and far from completely mapped, so that when a man of intelligence and wide experience tries to make a survey of it, there will inevitably be much to learn.

The first two chapters are historical reviews. Hückel has made a great effort really to penetrate into the ways of chemical thinking at the various times. This is very inspiring; the reader of many other histories of chemistry may be tempted to judge the scientists of past centuries by the standards of our own time. It is a common mistake to think that many of our present concepts were known even in former days and that the only thing needed was the genius to find out experiments to prove the concepts. The truth is, of course, that even the concepts were not yet conceived. In comparision with the usual panegyrics, Hückel's revelations of the shortcomings and mistakes of great scientists may seem heartless; however, they bear out this point very well.

The subsequent chapters discuss special topics. In several places the reader is reminded of one difficulty which is not always clearly understood, namely that of the accuracy of interatomic distances. In a crystal structure determination it is usually easy to determine very accurately the positions of the heavy atoms. However, the positions of the light atoms have a rather small influence on the X-ray intensities and thus cannot be determined very accurately from intensity data alone. Generally one has to use other arguments too, such as reasonable assumptions on the interatomic distances or on the type of coordination. If, for instance, an author states the distance A — X to be 1.67 Å it does not always mean as it should that in the compound studied this distance is certainly within the limits 1.67 ± 0.01 Å. Very often the author would readily admit that his experimental material alone would not permit a higher accuracy than, say, 1.7 ± 0.2 Å, but that he has arrived at the figure 1.67 Å by certain theoretical assumptions about the coordination and distances. If, now, the author of an article surveying the field uses such an "accurate" figure as a proof of those theoretical assumptions, he is obviously running around in a *circulus vitiosus*. Admittedly it is very hard for the author of the survey to check up such things, so one must wish that the original authors would state the real accuracy of the interatomic distances more explicitly than is now the practice.

The third chapter treats the theory of coordination compounds. A large number of facts from the "classical" complex chemistry are recorded, but seen from a modern stand-point. For instance, the differentiation between primary and secondary bonds (Haupt- und Nebenvalenzen), which — perhaps because of mental inertia — is often found even in recent literature, is here done without.

Chapters 4—6 treat the periodic system, the structure of atoms, experimental methods for investigating the chemical bond, and the different types of chemical bonds. Some readers may find these chapters a useful repetition. Others may

prefer to read some more elementary treatment.

Chapter 7 is on volatile inorganic compounds such as hydrides, halogenides, and carbonyls, and on what makes some compounds volatile, and others not.

Chapter 8, on crystal chemistry, treats among other things the causes of polymorphy, and phase diagrams. It is good to find that Hückel is sceptical on many points where others are cock-sure.

The next three chapters treat silicate chemistry, glasses (even non-silicate glasses), metals and alloys, and rates of reaction; in the last, of course, Hedvall's school is quoted repeatedly. Chapter 12, the last one, tries to map the trends of chemical research through the centuries.

Of course, when a number of originally independent essays are compiled into a book, it is hard to get a really homogeneous product. Sometimes one is surprised at the choice of details, *e.g.* the very detailed prescriptions for the preparation of certain compounds. In some parts of the text we find the wordiness that characterizes a successful and even inspiring lecturer, but which can be considerably cut down in a written text.

In writing this book, Hückel has not had access to a vast amount of work done in other parts of the world after 1939. It is also quite natural that he has happened to overlook a few older papers. There would be no point in making a list of such cases. However, if we consider that the book was written in Germany soon after the end of World War II, we must admit that it is an admirable one.

Certainly those interested in structural chemistry will find in it many facts and many new view-points.

Lars Gunnar Sillén

ACTA CHEMICA SCANDINAVICA 4 (1950) 1329–1346

An Interferometric Method for Recording the Refractive Index Derivative in Concentration Gradients. II. Arrangement for and Theory of the Purely Optical Differentiation of the Refractive Index Function

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The author [1] described recently an interferometric method for recording the refractive index derivative in cells with concentration gradients. This method made use of the Rayleigh interferometer as modified by Philpot and Cook [2] in combination with a twin diffusion cell. In the two compartments of the latter, two identical diffusion boundaries were formed and slightly shifted with respect to each other. Two interfering rays, passing the twin cell at the same level, thus had slightly different paths with respect to the two identical refractive index gradients. It was mentioned in the same article that this interferometric differentiation could also be carried out in one single cell by shifting the two interfering rays from each other in the vertical direction by purely optical means. In the present article, this method of optical differentiation will be described together with the theory of the method.

OPTICAL ARRANGEMENT

The optical system of the differential refractometer is shown in Fig. 1. A is a vertical slit illuminated by monochromatic light. It stands in the focal plane of the lens B. The light is collected again by the lens D to an image of the slit in the plane F. The cell is situated at C between the two lenses. The lens E is a cylindrical lens with a horizontal axis. It throws, in elevation, an image of the cell C on the photographic plate F. In plan, it acts like a plate and does not disturb the image formation of the slit A in the same plane F. The components now mentioned form together the optical system of the Ray-

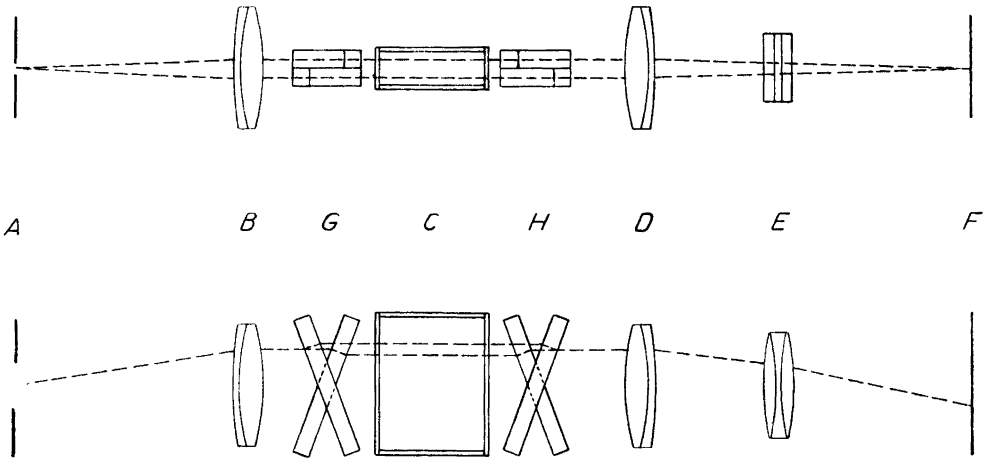

Fig. 1. The optical system. Upper figure: plan. Lower figure: elevation.

leigh-Philpot-Cook interferometer. It is characterized by the simultaneous image formation of the cell and the illumination slit. The vertical coordinate on the plate is related to the cell coordinate, the horizontal coordinate to the slit.

The components to be added for obtaining the refractive index derivative instead of the function itself are the four plano-parallel glass plates at G and H. They all make the same angle with the vertical line, but they tilt in opposite directions. Every light pencil that passes a plate with positive inclination before entering the cell will pass a plate with the same negative inclination after leaving the cell, and *vice versa*. The inclined plates, giving rise to vertical shifts of the light pencils, will cause two coherent and interfering rays to pass the cell at two different levels. The difference between these levels can be chosen arbitrarily by varying the inclination of the plates.

Notation. The following symbols will be used in the theory to be presented.

a = the thickness of the cell in the direction of the optical axis.
b = the angle of refraction in the inclined plates.
d = the thickness of the inclined plates.
D = diffusion coefficient.
e = the base of the natural logarithms.
f = the phase shift of a light pencil produced by the introduction in its path of an inclined plate; it is a function of the angle of incidence.

F = the parallel shift of a light pencil produced by the introduction in its path of an inclined plate; it is a function of the angle of incidence.
i = the angle of incidence at an inclined plate.
m = the refractive index of the inclined plates relative to their surroundings.
n = the variable refractive index of the solution in the cell.
s = optical path length = the product of distance and refractive index.
t = time.
v = the angle of inclination of the plates; it is defined as positive if the plate has been turned clock-wise from its vertical position.
x = the vertical coordinate in the cell; the direction of the positive x-axis is upwards.
X = the vertical coordinate on the plate.
z = the coordinate along the optical axis; the direction of the positive z-axis is towards the light source.
δ = the angle enclosed between a light pencil and the optical axis; it is defined as positive if x and z increase simultaneously along the direction of the pencil.
ε = that fraction of a fringe displacement which can still be measured.
φ = the difference in F between the inclined and vertical positions of the plates.
λ = the wave-length of the light.
ϱ = the relative error.

With this notation, a light pencil is shifted in the positive direction of the x-axis on passage of a plate with positive inclination if the pencil is traced in the direction of the positive z-axis.

THE PARALLEL SHIFT OF A LIGHT PENCIL PASSING THROUGH A PLATE

From Fig. 2 it is easily realized that the shift of the ray perpendicularly to itself is:

$$F(i) = \frac{d \sin (i - b)}{\cos b} \tag{1}$$

the relation between i and b being given by Snell's law:

$$\sin i = m \sin b \tag{2}$$

Elimination of b, however, gives a very complicated expression. Consequently we prefer to use the symbol $F(i)$ as long as possible. For small and moderate

angles, one can use the first and third order approximations obtained by developing $F(i)$ into powers of i. If b is first developed into powers of i, one gets:

$$b = \frac{i}{m} - \frac{i^3(m^2-1)}{m^3} + \ldots\ldots \tag{3}$$

Development of the sine and cosine functions in equation (1) into powers of the respective angles, and introduction of b according to (3) gives the following expression for the parallel shift of the ray:

$$F(i) = \frac{di\ (m-1)}{m}\left[1 + \frac{i^2\,(3+3m-m^2)}{6\,m^2}\right] \tag{4}$$

which is the third-order approximation. The angle of incidence is in our case:

$$i = v - \delta. \tag{5}$$

Thus light pencils having an inclination towards the optical axis are shifted parallel to themselves even for the vertical position of the glass plates. This shift, however, is taken into account by focusing the cylindrical lens on the middle of the cell in the presence of the plates in their vertical positions. The parallel shift which we have to take into account in this theory is only the difference:

$$\varphi\ (v,\ \delta) = F(v-\delta) - F(-\delta) \tag{6}$$

We will now regard the angle δ as a small angle, whereas v may assume fairly high values. It is then permissible to use the first-order approximation in δ and write:

$$\varphi(v,\ \delta) = F(v) - \delta\ F'(v) - F(0) + \delta\ F'(0) \tag{7}$$

The term $F(0)$, however, is the parallel shift of a ray falling perpendicularly on the plate and is thus $= 0$. Hence we have:

$$\varphi(v,\ \delta) = F(v) - \delta\ [F'(v) - F'(0)] \tag{8}$$

Similarly, we get for the plate with opposite inclination:

$$\varphi(-v,\ \delta) = F(-v) - \delta\ [F'(-v) - F'(0)] \tag{9}$$

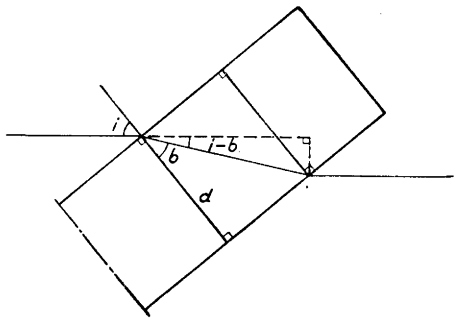

Fig. 2. Optical refraction in a plano-parallel glass plate.

The function F is an odd function of the variable, thus we have:

$$F(-v) = -F(v) \tag{10}$$

On the other hand, the function F' is an even function, and the relation:

$$F'(-v) = F'(\mathrm{v}) \tag{11}$$

holds. Consequently, equation (9) can be written:

$$\varphi(-v,\ \delta) = -F(v) - \delta\ [F'(v) - F'(0)] \tag{12}$$

The relations (10) and (11) are easily realized by inspecting the series development (4). $F(i)$ contains only odd powers of i, consequently $F'(i)$ contains only even powers.

THE PHASE SHIFT OF A LIGHT PENCIL PASSING THROUGH A PLATE

Inspection once more of Fig. 2 reveals that the optical path through the plate is:

$$\frac{md}{\cos b} \tag{13}$$

whereas the projection of this path along the direction of the ray is:

$$\frac{d\cos(i-b)}{\cos b} \tag{14}$$

The phase shift on introduction of the plate is thus:

$$f(i) = \frac{d[m - \cos(i-b)]}{\cos b} \tag{15}$$

This expression can also be developed into powers of i in the same way as was mentioned for the parallel shift. The fourth-order approximation is:

$$f(i) = d(m-1)\left[1 + \frac{i^2}{2m} + \frac{i^4(3 + 3m - m^2)}{24m^3}\right] \tag{16}$$

The angle of incidence i is again given by equation (5), hence $f(i)$ has a considerable value even for the upright position of the glass plates. This is of no importance, however, since we are only interested in the difference in phase between the two interfering rays.

The function $f(i)$ may be written as a first order approximation in δ:

$$f(i) = f(v-\delta) = f(v) - \delta\ f'(v) \tag{17}$$

Similarly, we have for the plate with negative inclination:

$$f(-v-\delta) = f(-v) - \delta\ f'(-v) \tag{18}$$

Equation (16) shows that the following relations hold for the function $f(i)$:

$$f(-v) = f(v) \tag{19}$$

$$f'(-v) = -f'(v) \tag{20}$$

Hence equation (18) can be written:

$$f(-v-\delta) = f(v) + \delta\ f'(v) \tag{21}$$

THE RELATION BETWEEN THE PARALLEL SHIFT AND THE PHASE SHIFT

The similarity between the expressions (4) and (16) indicates that there is some simple relation between these two functions. In fact, differentiation of equation (15) with respect to i gives:

$$f'(i) = \frac{d \sin(i-b)}{\cos b} \tag{22}$$

which yields, with the aid of (1):

$$F(i) = f'(i) \tag{23}$$

THE CONDITIONS FOR INTERFERENCE

The necessary and sufficient condition for interference is that two coherent rays shall be directed to the same spot on the photographic plate.

Coherent are all light pencils coming from the same point of the light source slit. In the optical arrangement depicted in Fig. 1, with this slit in the focal plane of the lens B, such rays are all parallel between the lens B and the cell. Consequently, every two rays having the same angle of incidence δ towards the cell on the light source side are coherent.

The condition that two interfering rays must be directed to the same spot on the photographic plate can of course be subdivided into two conditions, one concerning the horizontal, the other concerning the vertical coordinate on the plate.

Since the light source is a vertical slit which can be imagined as infinitely thin, and since this slit is brought to focus on the plate (in plan), there is but one coordinate in the horizontal direction on the plate as far as geometrical optics is concerned. Light falling outside the vertical line with this coordinate originates from diffraction and interference phenomena.

In order to interfere, two coherent rays must also fall upon the same vertical coordinate on the plate. Since, in elevation, an image of the cell is formed on the plate, this condition can be reformulated in terms of coordinates in the cell. We will assume that the cylindrical lens E is focused on the cell in the presence of the glass plates in their vertical positions. The condition can then be put in the form that the two interfering rays, if traced backwards from the plate through the cylindrical lens and through the vertical plates, shall pass the middle of the cell on the same x-coordinate.

The two interfering rays, having the same inclination to the left of the cell and suffering, in general, unequal deflections in the cell, will in general have different inclinations to the right of the cell. By definition, we will thus state that the angle of inclination of both rays is δ to the left of the cell and δ_1 and δ_2, respectively, to the right of the cell. Further, we will arbitrarily state that the first pencil (angle δ_1) will strike a plate with positive inclination to the right and a plate with negative inclination to the left of the cell, and *vice versa* for the second pencil (angle δ_2).

The condition for interference mentioned above makes it preferable to compute the total phase shift between the rays backwards from the plate to the light source slit. With this mode of approach, we know from the beginning which x-coordinate we are studying. This is the reason why we have defined the direction of the positive z-axis towards the light source.

The paths of two interfering pencils between the cell and the cylindrical lens are given in Fig. 3. Starting from the same X-coordinate on the plate, and tracing the rays backwards, they are in phase in points lying on the same circle with X on the plate as the centre. To the left of the cylindrical lens E, the two rays are in phase in points lying on the same circle with the point O as the centre, where O has the coordinate x corresponding to X and is situated in that plane F which is in focus in the absence of the glass plates. In the presence of a plate in the vertical position (plate P, for better visibility drawn away from the two inclined plates), the point O in the plane F is displaced to O′ in the plane F′, which is the plane of the middle of the cell. It retains the coordinate x. The first pencil (angle δ_1) is broken in the inclined plate P_1 at B_1 and C_1, emerges from it with a certain parallel shift and a certain phase shift, and strikes the plane of the cell in the point E_1. Similarly, the second pencil (angle δ_2) is broken in the inclined plate P_2 at B_2 and C_2, emerges from it with a certain parallel shift and a certain phase shift, and strikes the plane of the cell in the point E_2.

THE PHASE SHIFT BETWEEN THE TWO INTERFERING RAYS TO THE RIGHT OF THE CELL

We will now calculate the difference in phase between the two rays in the points E_1 and E_2, Fig. 3, using the equations derived earlier and the fact that the imagined rays through the vertical plate ($A_1B_1M_1N_1O'$ and $A_2B_2M_2N_2O'$) are exactly in phase in the point O′ (it will be postulated that the cylindrical lens is perfect).

The phase shift of the ray C_1D_1 relative to the ray B_1O is given by equation (17) with δ_1 instead of δ:

$$f(v-\delta_1) = f(v) - \delta_1 \, f'(v) \tag{24}$$

The phase shift of the imagined ray N_1O' relative to the ray M_1O is, according to the same equation:

$$f(0-\delta_1) = f(0) - \delta_1 \, f'(0) = f(0) \tag{25}$$

since $f'(0) = 0$. Consequently, the difference in phase between the ray C_1D_1 and the ray N_1O' is the difference between (24) and (25):

$$f(v) - f(0) - \delta_1 \, f'(v) \tag{26}$$

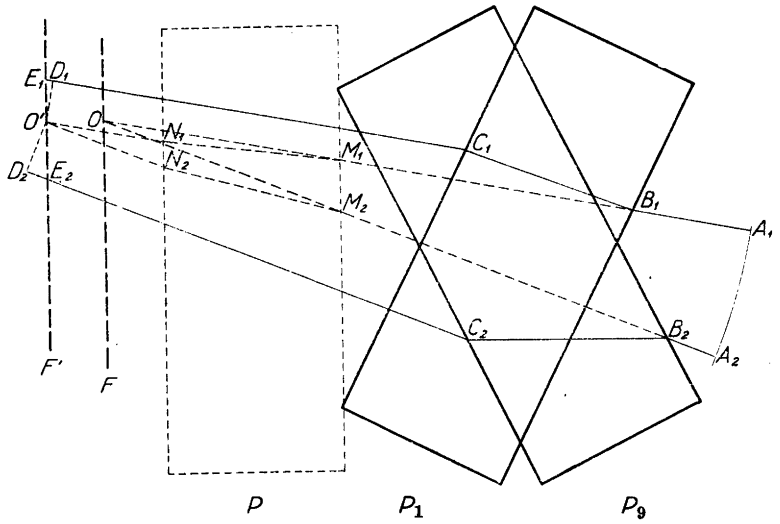

Fig. 3. The optical paths of two interfering rays to the right of the cell.

This phase difference prevails in points on the pencils lying in the same plane perpendicular to them, *e. g.* in the points D_1 and O'.

In the same way, the difference in phase between the points D_2 and O' is derived. The result is the expression (26) with $-v$ instead of $+ v$ and with δ_2 instead of δ_1:

$$f(v) - f(0) + \delta_2 f'(v) \tag{27}$$

By subtracting (27) from (26), we get the phase difference between the points D_1 and D_2:

$$-(\delta_1 + \delta_2) f'(v) \tag{28}$$

Although this is the difference in phase caused by the inclined plates, it is not the phase difference prevailing when the two pencils strike the cell wall. We must also take into account the difference in phase between the points D_1 and E_1 and between D_2 and E_2.

The distance D_1O' is given by equation (6) with δ_1 instead of δ:

$$D_1O' = F(v) - \delta_1 [F'(v) - F'(0)] \tag{29}$$

The distance D_1E_1 is obtained by multiplying this distance by δ_1:

$$D_1E_1 = \delta_1 F(v) - \delta_1^2 [F'(v) - F'(0)] \tag{30}$$

which, however, will be abbreviated to the first term since we are only considering the first power of δ_1:

$$D_1E_1 = \delta_1 \; F(v) \tag{31}$$

In the same way, we find that

$$D_2E_2 = \delta_2 \; F(-v) = -\delta_2 \; F(v) \tag{32}$$

and consequently the difference between the two distances is:

$$(\delta_1 + \delta_2) \; F(v) \tag{33}$$

Remembering the equation (23), we thus find that the phase difference (28) caused by the inclined plates is exactly compensated by the phase difference (33) due to the distances D_1E_1 and D_2E_2. Our conclusion is that the two interfering rays do not undergo any phase shift between the cell and the plate.

THE PHASE SHIFT IN THE CELL

The distance E_1O', Fig. 3, is nearly equal to the distance D_1O' given by equation (29), the difference between them being a correction term containing δ_1^2. The coordinate of the first pencil in the cell is thus $x + \Delta x_1$, where Δx_1 is given by the equation:

$$\Delta x_1 = F(v) - \delta_1 \; [F'(v) - F'(0)] \tag{34}$$

For the second pencil, we get the same expression with $-v$ instead of v and with δ_2 instead of δ_1:

$$\Delta x_2 = -F(v) - \delta_2 \; [F'(v) - F'(0)] \tag{35}$$

The difference between (34) and (35) is:

$$\Delta x = \Delta x_1 - \Delta x_2 = 2 \; F(v) - (\delta_1 - \delta_2) \; [F'(v) - F'(0)] \tag{36}$$

The second term in the above expression is small compared with the first for two reasons. First, $F(v)$ contains the first power of v, whereas $F'(v) - F'(0)$ only contains the second power (equation (4)). Second, δ_1 and δ_2 are nearly equal since the two rays must pass the cell att slightly different levels. As

long as we are not considering the error terms and the limitation of the method, therefore, we can write instead of (36):

$$\Delta x = 2\ F(v) \tag{37}$$

The phase shift between the two rays in the cell thus becomes, to the first approximation:

$$\Delta s_c = a\ n(x + \Delta x_1) - a\ n(x + \Delta x_2) = a\ \Delta x\ n'(x) = 2\ a\ F(v)\ \mathrm{n}'(x) \tag{38}$$

THE PHASE SHIFT TO THE LEFT OF THE CELL

The calculation of the phase difference in the left-hand glass plates and at the left-hand cell wall is very similar to that to the right of the cell, the only difference being that the two interfering pencils are exactly parallel with each other. This difference does of course not invalidate the result arrived at to the right of the cell: even to the left of the cell, the total phase shift between the rays is zero. The phase difference introduced by the inclined plates is found by replacing δ_1 and δ_2 by δ and by replacing v by $-v$ in equation (28):

$$2\ \delta\ f'(v) \tag{39}$$

and exactly the same amount of opposite sign is found at the cell wall.

THE TOTAL PHASE DIFFERENCE

The total phase difference in the whole optical system is thus found to be:

$$2\ a\ F(v)\ n'(x) \tag{40}$$

Since a and $F(v)$ can be measured ($F(v)$ can also be computed from equation (1) if the refractive index is known), $n'(x)$ can be derived if the fringe displacement is measured. We have made the calculation of the fringe displacement for an arbitrary coordinate x in the cell and X on the plate, thus it is valid along the whole x-axis (X-axis), *i. e.*, the interference fringes will take the form of the refractive index derivative in the cell.

The angle δ does not appear in our final expression. This means that the fringe displacement on the plate is independent of the vertical position of the light source if this is a point source. All point sources on the same vertical line will give the same interference pattern, consequently a vertical slit source can be used.

Since all light pencils of the same angle of inclination to the left of the cell are coherent, not only those going side by side on the same level, the left-hand pair of plates is not necessary to get the derivative interference pattern. However, if these plates are omitted, we have to subtract the phase difference introduced by them, expression (39), from the final expression (40). The additive term (39) is independent of x and does not introduce any errors in the interferogram, but since it depends upon δ, a point source will be necessary. The author has verified this conclusion experimentally. If the left-hand pair of plates is removed, and if a point source is applied, the interferogram remains unchanged. On gradually extending the light source in the vertical direction, the pattern becomes more and more blurred and soon disappears. If the point source is moved up and down, the interference fringes are seen to move laterally without changing shape. It is of course preferred to use both pairs of plates because a point source gives only a small fraction of the light intensity given by a slit source. No derivative interference fringes can be obtained if the right-hand pair of plates is omitted. Even in this respect the theory is in agreement with experimental evidence.

THE ERROR TERMS AND THE LIMITATION OF THE METHOD

Several approximations have been used in the above deduction. First, we used the first-order approximations in δ in the equations (7) and (17); the errors introduced thereby are exceedingly small and will not be considered. Second, in equation (34) the quantity Δx_1 was put equal to the distance D_1O' (Fig. 3) although the latter is the parallel shift perpendicular to the rays and the former should be the vertical shift. The correction factor which should have been applied is $1/\cos \delta_1$. It is insignificant and will not be considered. Third, the angles δ_1 and δ_2 were said to be approximately equal, which gave rise to the simplified equation (37). The errors introduced thereby may be important and will be investigated further, especially since they are characteristic of this method of observation. Fourth, the optical path length through the cell was put equal to $a\ n(x)$ although the ray describes a certain curve within the cell along which $n(x)$ varies. This error is inherent in all interferometric methods for the study of refractive index gradients and has been considered by Kegeles and Gosting [3]. It will therefore not be considered here. Fifth, also in equation (38), the series development was stopped with the first power of the increment. Since the second- and third-degree terms of this series are important and constitute the limiting factors in the differential interferometric method, they will be computed in this section.

Let us first consider the third-order approximation in Δx of the length of the optical path through the cell, assuming $a\ n(x+\Delta x_{1,2})$ to be sufficiently good approximations for these path lengths. We then have for the first ray in the cell:

$$s_{c1} = a\ n(x) + a\ \Delta x_1\ n'(x) + a\ \frac{\Delta x_1^2}{2}\ n''(x) + a\ \frac{\Delta x_1^3}{6}\ n'''(x) \tag{41}$$

We obtain the powers of Δx_1 from equation (34), still remembering that we only take into account terms of the first power in δ_1:

$$\Delta x_1^2 = F^2(v) - 2\ F(v)\ \delta_1[F'(v) - F'(0)] \tag{42}$$

$$\Delta x_1^3 = F^3(v) - 3\ F^2(v)\ \delta_1[F'(v) - F'(0)] \tag{43}$$

Introduction of these values into (41) gives:

$$s_{c1} = a\ n(x) + a\ F(v)\ n'(x) + \frac{a}{2}\ F^2(v)\ n''(x) + \frac{a}{6}\ F^3(v)\ n'''(x) - \tag{44}$$
$$- \delta_1[F'(v) - F'(0)][a\ n'(x) + a\ F(v)\ n''(x) + \frac{a}{2}\ F^2(v)\ n'''(x)]$$

For the calculation of δ_1, we have:

$$\delta = \delta_1 + a\ n'(x + \Delta x_1) = \delta_1 + a\ n'(x) + a\ \Delta x_1\ n''(x) + \ldots\ldots \tag{45}$$

and with the aid of (34) (where the second term can now be neglected):

$$\delta_1 = \delta - a\ n'(x) - a\ F(v)\ n''(x) \tag{46}$$

Introduction of this value into (44) gives:

$$s_{c1} = an(x) + aF(v)n'(x) + \frac{a}{2}F^2(v)n''(x) + \frac{a}{6}F^3(v)n'''(x) - [\delta - an'(x) \tag{47}$$
$$- aF(v)n''(x)][F'(v) - F'(0)][an'(x) + aF(v)n''(x) + \frac{a}{2}F^2(v)n'''(x)]$$

The optical path of the other ray, s_{c2}, becomes of course the same expression with $-v$ instead of v, *i. e.*, with $-F(v)$ instead of $F(v)$. Consequently, when we take the difference between s_{c1} and s_{c2}, all terms containing even powers of

$F(v)$ disappear, and those containing odd powers remain with the factor 2. If this is carried out, and if insignificant terms are omitted, there remains:

$$\Delta s = 2\ a\ F(v)\ n'(x) + \frac{a}{3} F^3(v)\ n'''(x) - \tag{48}$$

$$- 2\ a\ F(v)\ n''(x)[F'(v) - F'(0)][\delta - 2\ a\ n'(x)]$$

This is our final expression for the total phase difference between the two interfering rays. We will now investigate the order of magnitude of the two error terms and the conditions under which they can be neglected.

THE RESOLVING POWER

It will now be assumed that the refractive index function under observation is that given by an ideal diffusion. Thus we have:

$$n'(x) = \frac{\Delta n}{2\sqrt{\pi D t}} \exp.\left(-\frac{x^2}{4Dt}\right) \tag{49}$$

where Δn is the total refractive index change across the boundary, D the diffusion constant, and t the time. We also have the relations:

$$n''(x) = -\frac{x\ n'(x)}{2Dt} \text{ and } n'''(x) = \frac{x^2 - 2Dt}{(2Dt)^2} n'(x) \tag{50}$$

A fringe displacement can be measured to within a certain fraction of a fringe. If we call this fraction ε, it should thus be required that

$$\frac{a}{3} F^3(v)\ n'''(x) < \varepsilon\lambda \tag{51}$$

This condition is satisfied everywhere if it is satisfied in the top of the Gaussian curve. Introduction of $n'''(x)$ from (50) and putting $x = 0$ gives:

$$F^3(v) < \frac{12\,\varepsilon\,\lambda\,\sqrt{\pi}\,(Dt)^{3/2}}{a\Delta n} \tag{52}$$

Introduction of this value in the main term in (48) divided by λ gives the maximum fringe displacement in the top of the curve compatible with the precision ε:

$$\Delta s_{\max.} = \left(\frac{12\,\varepsilon}{\pi}\right)^{1/3} \left(\frac{a\Delta n}{\lambda}\right)^{2/3} \tag{53}$$

Neither the time, nor the diffusion constant appears in this expression, hence the maximum fringe displacement that can be used without introducing systematical errors is the same throughout a diffusion experiment. Antweiler [4] and Longsworth [5] claim that it is possible to localize a fringe to within 0.02 of the distance between fringes, and the author has the same experience. Putting $\varepsilon = 0.02$, $a = 2.5$ cm, $\Delta n = 0.00200$, and $\lambda = 5460$ Å, we find a fringe displacement in the top of the curve of 8.6 fringes, *i. e.* a relative precision of 2.3 parts in 1000. A general expression for the relative error can be obtained if the second term in (48) is divided by the main term and if the value (52) is introduced. One thus obtains:

$$\varrho = \left(\frac{\pi}{12}\right)^{1/3} \left(\frac{\varepsilon\lambda}{a\Delta n}\right)^{2/3} \tag{54}$$

This expression, again, refers to the top of the curve. In other parts thereof, it varies as

$$\frac{n'''(x)}{n'(x)} = \frac{x^2 - 2Dt}{(2Dt)^2} \tag{55}$$

If the correction (54) is constantly applied, the value of $F(v)$ need not be chosen in close agreement with equation (52), but can be considerably greater. For instance, if $F(v)$ is doubled, the total number of fringes is also doubled, and the relative error is four times greater. Still the result will be quite adequate if the correction (54) is applied. In this way accidental errors can be minimized when measuring small concentrations.

THE SECOND ERROR TERM

This term, containing the second derivative of the refractive index as a factor, is without influence in the top of the curve. It consists of two parts, one containing the first derivative, and one containing the angle δ. The former gives rise to a systematic error without causing blurring, as does the first error term treated above, the latter causes blurring without introducing systematical

errors, provided that the light source slit has a symmetrical position with respect to the optical axis.

The systematical error is:

$$4\ F(v)\ [F'(v) - F'(0)]\ a^2\ n'(x)\ n''(x) \tag{56}$$

and gives rise to the relative error:

$$\varrho_2 = 2\ a\ n''(x)\ [F'(v) - F'(0)] \tag{57}$$

It is at its maximum in the inflexion points, where we have:

$$n''(x_i) = \frac{\Delta n}{2Dt\sqrt{2\pi e}} \tag{58}$$

The value of $F'(v)$—$F'(0)$ is:

$$F'(v) - F'(0) = \frac{d\,v^2(m-1)\,(3+3\,m-m^2)}{2\,m^3} \tag{59}$$

We take the value of v from the first order approximation of $F(v)$:

$$F(v) = \frac{d\,v\,(m-1)}{m} \tag{60}$$

and, moreover, we choose the maximum permissible $F(v)$ value derived from the first error term, equation (52). Hence we get:

$$\varrho_2 = \frac{(3+3m-m^2)\sqrt[3]{9a\,\Delta n\,(\varepsilon\lambda)^2}}{dm\,(m-1)\sqrt{e}\,\sqrt[6]{2\pi}} \tag{61}$$

If we require that this relative error be smaller than 10^{-3}, we can formulate this condition in terms of a minimum thickness of the inclined plates:

$$d > \frac{1000\,(3+3\,m-m^2)\sqrt[3]{9\,a\,\Delta n\,(\varepsilon\lambda)^2}}{m\,(m-1)\sqrt{e}\,\sqrt[6]{2\pi}} \tag{62}$$

With the following numerical figures, which are rather unfavourable with

regard to this error, *viz.* $m = 1.5$, $a = 5$ cm, $\Delta n = 0.002$, $\varepsilon = 0.1$, and $\lambda = 5460$ Å, this becomes:

$$d > 0.43 \text{ cm} \tag{63}$$

For technical reasons already, d should be chosen at least 1 cm. It can thus be concluded that the second error term will never be able to cause any detectable systematical errors if the first error term is negligible. The conditions for a correct refractive index analysis derived in the former section are therefore sufficient conditions.

The part containing δ in the second term, which part only causes blurring and no systematical errors, is of the same order of magnitude as the part treated above since δ and $a\, n'(x)$, when they have their maximum values, are of about the same size. Hence the blurring is quite unimportant under the conditions which have to prevail for a correct analysis.

DISCUSSION

The differential interferometer described in this paper seems to be capable of giving an accuracy of about 2 parts in 1000 in the refractive index derivative under standard experimental conditions and thus compares favourably with other methods for measuring this derivative. By a simple manipulation of the optical components, the interferometer can be converted into an integral interferometer which is capable of giving the total refractive index change with a very high precision, much greater than that obtainable by integration of the Gaussian curve. Although this method cannot be expected to supersede the very precise Gouy method for measuring diffusion constants (Kegeles and Gosting [3]; Longsworth [6]; Coulson, Cox, Ogston, and Philpot [7]), it is advantageous that it makes possible a direct viewing of the Gaussian curve. This method, moreover, is useful for boundary systems, such as those appearing in electrophoresis and adsorption analysis, whereas the Gouy method is not.

The direct measurement of the maximum derivative with the aid of the scale method and the diagonal slit method is open to systematic errors which can only be corrected for by an involved wave-optical theory (Adler and Blanchard [8]; Kegeles and Gosting [3]). The differential interference method does not seem to be open to such errors.

Like the integral interferometer of Philpot and Cook [2], the differential interferometer can also be built into the optical system of the diagonal slit method (*cf.* Svensson [9]).

An entirely different method of producing interference between two vertically displaced light pencils through the cell has been reported by Vallet [10].

SUMMARY

An optical arrangement for making direct records of the refractive index derivative in stratified solutions by interferometric means has been presented. A theory for the method has been given, which shows that the phase differences between the two interfering rays occurring outside the cell neutralize each other and that the phase difference within the cell can be made very nearly proportional to the refractive index derivative in each point. The theoretical resolving power, as derived from higher terms in the analytical expression for the phase difference, compares favourably with that of other known methods for recording the refractive index derivative. The conditions for getting unblurred interferograms have been considered.

This work has been financially supported by a grant from the Swedish Technical Research Council and by *LKB-Produkter Fabriksaktiebolag*, Stockholm, which is most gratefully acknowledged.

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Received September 2, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1347–1350

Thiobenzoylation of Esters and Amides of α-Amino Acids

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In connection with an investigation in this laboratory some thiobenzoylated esters and amides of α-amino acids were required. The literature on thioacylation has recently been reviewed [1], and it appears that salts or esters of thion- or dithioacids have proved useful as thioacylating agents. Dithiobenzoic acid, however, does not react with glycine or leucine [2], nor does thiobenzoyl chloride appear to be of much value in the thiobenzoylation of amino acids [3]. Holmberg [4] prepared carboxymethyl dithiobenzoate and demonstrated the easy formation of thiobenzamide and -hydrazide upon mild treatment of the dithioacid with ammonia or hydrazine respectively. The same author [5] later extended this observation to amino acids and described in addition the successful preparation of ethyl thiobenzamidoacetate by this method, whereas thiobenzoyl-L-asparagine was not isolated, although its presence in the reaction mixture was established. Using Holmberg's procedure, Elliott [6] recently reported the preparation of DL-N-thiobenzoyl-serine methyl ester.

Holmberg investigated different methods of preparing dithiobenzoic acid, required as an intermediate in the synthesis of carboxymethyl dithiobenzoate, and concludes that the highest yield (ca. 50 %) of the latter compound is obtained when benzotrichloride is used as a starting material. The alternative route from bromobenzene and carbon disulphide was found much inferior, the yield of the final product being only 17 %. In this laboratory, however, satisfactory yields were consistently obtained by the latter method, using slightly modified conditions. It is believed that this procedure, described in some detail in the experimental section, represents the most convenient one for preparing thiobenzoylthioglycollic acid in larger quantities.

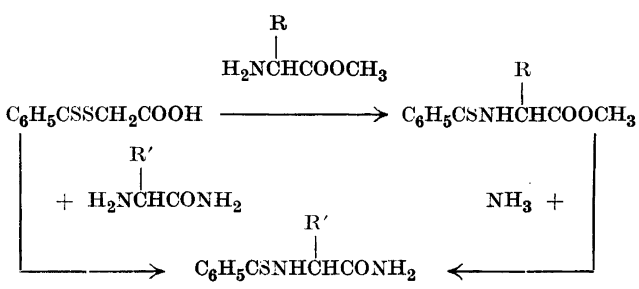
The thioacylations, as represented in the following scheme, were performed in neutral aqueous solutions at room temperature.

Tabel 1. Esters and amides

$C_6H_5CSNHCHCOR'$ (R)

R	R′	Yield, %	M. p., °C.	Formula	Car Calcd.
H	OCH_3	68[a]	73-4	$C_{10}H_{11}O_2NS$	57.38
CH_3[b]	OCH_3	78[a]	100-1	$C_{11}H_{13}O_2NS$	59.16
H	NH_2	89[c]	136-7[d]	$C_9H_{10}ON_2S$	55.63
CH_3[b]	NH_2	82[e]	204.5	$C_{10}H_{12}ON_2S$	57.65
C_6H_5[b]	NH_2	87[c]	160-1	$C_{15}H_{14}ON_2S$	66.67
p-OHC_6H_4OH[f]	NH_2	89[c,f]	185 (d.)	$C_{16}H_{16}O_2N_2S$	63.97

[a] Recrystallized from benzene-hexane. [b] DL-Configuration.
[c] Recrystallized from aqueous ethanol. [d] Form B. The dimorphous form A had m. p. 119°, but could not, on account of its ready transformation into B, be purified quite satisfactorily for analysis. Found: C, 55.31; H, 4.78; N, 14.02; S, 16.17. [e] Recrystallized from 96 % ethanol. [f] L-Configuration, $[\alpha]_D = +162.6°$ in 96 % ethanol (0.1412 g/5.00 ml, 1 dm, + 4.59°).



I	R = H	IV	R′ = CH_3
II	R = CH_3	V	R′ = C_6H_5
III	R′ = H	VI	R′ = *p*-$OHC_6H_4CH_2$

All attempts to thiobenzoylate ethyl α-amino-isobutyrate proved fruitless in spite of moderate heating and prolonged reaction time. This behaviour, which reflects a greatly diminished reactivity of the amino group, may be explained in terms of unfavorable sterical conditions.

Treating the esters (I) and (II) with methanolic ammonia yielded quantitatively the corresponding amides, (III) and (IV), identical with the products obtained upon direct thiobenzoylation of the amino amides. No difficulties were encountered in the isolation and purification of the reaction products, which are well crystallizing compounds, usually of an intensive yellow colour. Thiobenzoyl-L-tyrosineamide (VI), however, formed colourless crystals

of α-N-thiobenzoylamino acids.

bon, %	Hydrogen, %		Nitrogen, %		Sulphur, %	
Found	Calcd.	Found	Calcd.	Found	Calcd.	Found
57.92	5.30	5.12	6.69	6.51	15.32	15.48
59.36	5.87	5.62	6.27	6.15	14.35	14.29
55.34	5.19	4.92	14.42	14.19	16.50	16.44
57.75	5.81	5.93	13.45	13.43	15.39	15.32
66.67	5.22	5.22	10.36	10.55	11.86	11.93
64.04	5.37	5.04	9.33	9.49	10.67	10.27

as did one of the dimorphous forms of thiohippuramide (III) *(vide infra)*. Both colourless compounds gave intensively yellow solutions in ethanol or water. The intense absorption of an ethanolic solution of (VI) in the visible produces an abnormally high rotation at the sodium D-line.

The occurence of dimorphism in this series was noticed by Holmberg [4] in the case of thiobenzhydrazide of which two different forms were isolated and mutually converted one into the other on seeding. In the course of the present work, thiohippuramide (III) was found to exist in two forms, A and B. A crystallized in nacreous, yellow plates, m. p. 119°, and B in colourless cubes with m. p. 136-7°. Upon rapid cooling of a hot solution of B in aqueous ethanol, A separated, but could be isolated only with great difficulty, owing to the readiness with which it changed into B. Seeding a supersaturated solution of B with A, however, did not result in the crystallization of A, unless rapid cooling was applied. Although the phenomenon appears to be a case of dimorphism, it cannot on basis of the experimental facts be definitely excluded that a dynamic isomerism is implied.

Studies in ring-closure reactions of thioacylated derivatives of α-amino acids are in progress in this laboratory.

EXPERIMENTAL *

Carboxymethyl dithiobenzoate

A Grignard-solution, prepared under nitrogen in the usual way from 16.5 g (0.68 m) of magnesium, 300 ml of dry ether and 101.6 g (0.65 m) of freshly distilled bromobenzene, is transferred under nitrogen pressure to a dropping funnel, inserted in a three-necked flask with ground joints. The flask is further provided with mechanical stirring and inlet and outlet tubes for nitrogen. In the flask is placed a solution of 76.5 g (1.01 m) of dry carbon disulphide in 150 ml of dry ether, kept cold throughout the reaction by

* All melting points are uncorrected. Microanalyses were carried out in this laboratory by Mr. A. Grossmann.

means of an ice-bath. In a stream of nitrogen, the Grignard-solution is added dropwise, in the course of one hour, to the vigorously stirred and well-cooled solution. The reaction mixture is allowed to come to room temperature overnight, then cooled again in an ice-bath. To the stirred solution is added 400 g of ice, a small amount of a brown precipitate is removed by filtration, and the deep-red aqueous layer separated and extracted once with a portion of fresh ether.

To the aqueous phase is added at once a solution of 64.2 g (0.68 m) of chloroacetic acid in 250 ml of water, neutralized with 38 g (0.36 m) of sodium carbonate. After standing for 48 hours at 5°, the mixture is acidified by slowly adding a solution of 45 ml (0.83 m) of conc. sulphuric acid in 50 ml of water. The dark-red precipitate is collected by suction, and a little oily material removed by pressing the filter cake. After being washed thoroughly with water, the crystals are dried *in vacuo* over calcium chloride. Yield 76.7 g (56 %), m. p. 126-7°. An analytically pure product is obtained after one recrystallization from 350 ml of benzene, yielding 70.4 g (51.5 %) of thin, beautifully red plates, melting at 127-8°.

The thiobenzoylation of α-amino amides and -esters

In general the reactions were performed in the following way. A solution of one mole of carboxymethyl dithiobenzoate in one equivalent of 2 *N* sodium hydroxide was added in one portion to another solution of one mole of α-amino-amide or -ester in water, containing one equivalent of sodium hydroxide in case the ester or amide hydrochlorides were used. After thorough shaking, the mixture deposited within a few minutes a yellow oil, crystallizing rapidly on cooling and scratching. The crystals were collected, dried and recrystallized from suitable solvents or solvent mixtures. Yields ranging from 70—90 % were obtained. The results are summarized in Table 1.

Ammonolysis of methyl α-thiobenzamidopropionate

In a glass-stoppered flask, a solution of 1.72 g of methyl α-thiobenzamidopropionate in 5 ml of methanolic ammonia, was shaken for four hours at room temperature, when yellow plates separated from the solution. After standing for 18 hours at room temperature 1.47 g (92 %) of N-thiobenzoyl-DL-alanineamide were collected. Recrystallized once from ethanol. M. p. 205° (dec.).

SUMMARY

An improved procedure for preparing carboxymethyl dithiobenzoate from bromobenzene, carbon disulphide and chloroacetic acid is described.

A series of thiobenzoylated esters and amides of α-amino acids is prepared, and a case of dimorphism, involving differently coloured forms, is noticed.

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Received September 2, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1351–1364

Some Studies on Azo Dyes. I

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The purpose of the present investigation was to examine the effect of amino-*p*-toluic and aminoterephthalic acid structures on the properties of the related azo dyes. These two amino acids have been prepared from *p*-cymene [8,11] a by-product of the sulfite pulping industry.

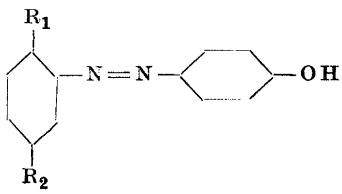
Two series of dyes were synthesized. One utilized phenol and the other β-naphthol as the phenolic component. The reason for this choice was two-fold: firstly they are the most common commercially used and secondly, they represent, in solution, the two different forms of azo compounds. That is to say, phenol dyes exist mainly in the azo form, whereas β-naphthol dyes exist mainly in the hydrazone form [1].

The following substances were used as the amine component:

aniline
o-toluidine
m-amino-benzoic acid
o-amino-benzoic acid
3-amino-*p*-toluic acid
aminoterephthalic acid

In this manner the effect of an *o*-methyl group, *o*- and *m*-carboxyl groups, and combinations of the two could be determined.

The nomenclature and formulae of the *p*-phenol dyes are shown below:



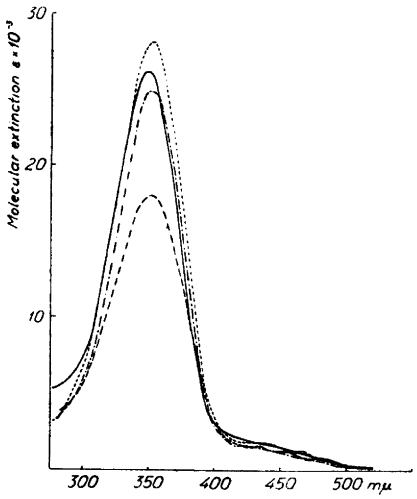


Fig. 1. Absorption curves of Phenol I, II, III, and V.

——— *Phenol I*
– – – – – *Phenol II*
—·—·—·— *Phenol III*
— — — — *Phenol V*

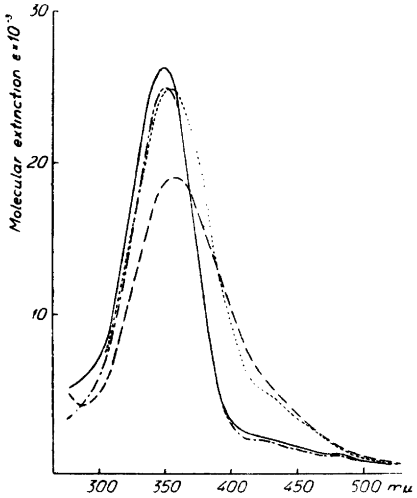


Fig. 2. Absorption curves of Phenol I, III, IV, and VI.

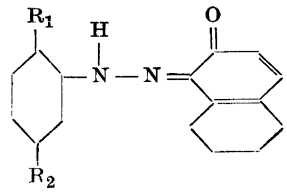
——— *Phenol I*
—·—·—·— *Phenol III*
— — — — *Phenol IV*
– – – – – *Phenol VI*

Phenol I	$R_1 = H$	$R_2 = H$
Phenol II	$R_1 = CH_3$	$R_2 = H$
Phenol III	$R_1 = H$	$R_2 = COOH$
Phenol IV	$R_1 = COOH$	$R_2 = H$
Phenol V	$R_1 = CH_3$	$R_2 = COOH$
Phenol VI	$R_1 = COOH$	$R_2 = COOH$

Fig. 1 shows the absorption curves of Phenol I, II, III, and V in 96 % alcohol. All of these dyes show a single, well-defined, almost symmetrical maximum at approximately 350 mμ, and are, in all respects, very similar.

Fig. 2 shows the absorption curves of Phenol I, III, IV, and VI. It can be seen that the both dyes containing an *o*-carboxyl group (IV and VI) show a maximum at slightly longer wave lengths than the other two, and show a strongly increased absorption in the region from 370 to 500 mμ.

The nomenclature and formulae of the dyes of the β-naphthol series are shown below:



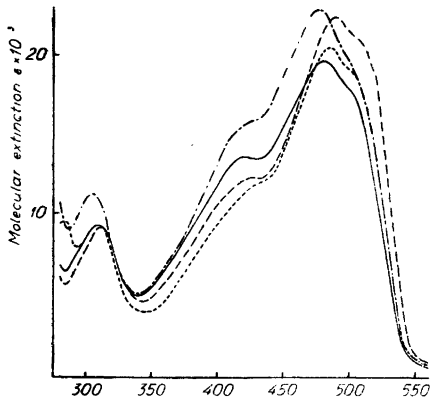


Fig. 3. Absorption curves of β-Naphthol I, II, III, and V.

——— *β-Naphthol I*
— — — — — *β-Naphthol II*
—·—·—·— *β-Naphthol III*
– – – – – – *β-Naphthol V*

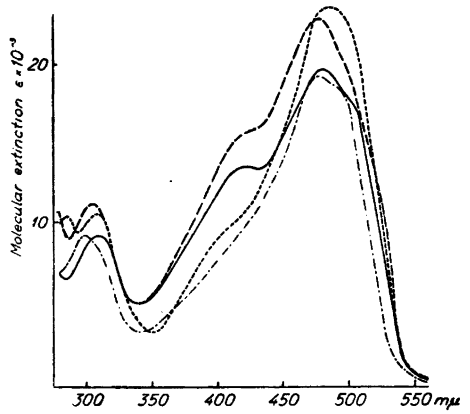


Fig. 4. Absorption curves of β-Naphthol I, III, IV, and VI.

——— *β-Naphthol I*
— — — — — *β-Naphthol III*
– – – – – – *β-Naphthol IV*
—·—·—·— *β-Naphthol VI*

β-Naphthol I	$R_1 = H$	$R_2 = H$
β-Naphthol II	$R_1 = CH_3$	$R_2 = H$
β-Naphthol III	$R_1 = H$	$R_2 = COOH$
β-Naphthol IV	$R_1 = COOH$	$R_2 = H$
β-Naphthol V	$R_1 = CH_3$	$R_2 = COOH$
β-Naphthol VI	$R_1 = COOH$	$R_2 = COOH$

Fig. 3 shows the absorption curves of β-Naphthol I, II, III, and V, in 96 % alcohol. It can be seen that all the dyes show a maximum at approximately 310 mμ, which corresponds very closely to the position of absorption of the naphthalene nucleus (311 mμ) [2]. In addition, V shows a very small maximum at 284 mμ. In the visible range, all of the dyes show either a small maximum or an inflection at approximately 425 mμ, and a large maximum at approximately 480 mμ.

Fig. 4 shows the absorption spectrum of β-Naphthol I, III, IV, and VI. The max. at approximately 310 mμ is obvious in this series also. It can be seen that the inflection points, in the region around 425 mμ, so obvious in the dyes in Fig. 3, are almost completely missing in the dyes containing an *o*-carboxyl group (IV and VI). The characteristic maximum at approximately 480 mμ is common to all the dyes.

Table 1 summarizes the positions of the absorption maxima in both series.

Table 1. The location of the absorption maxima.

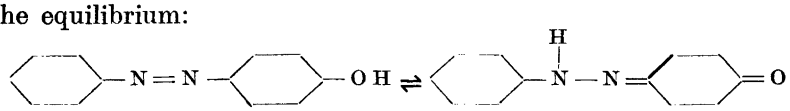
Dye		Location of max. in mμ			
β-Naphthol	I	—	312	421	480
»	II	—	312	427	488
»	III	—	305	425 *	478
»	IV	285	310	—	486
»	V	284	309	428	484
»	VI	—	298	—	478
Phenol	I	—	349	—	—
»	II	—	352	—	—
»	III	—	350	—	—
»	IV	—	358	—	—
»	V	—	351	—	—
»	VI	—	355	—	—

* Inflection point

DISCUSSION

As can be seen from Table 1, the effects of the various substituents on the location of the strongest absorption maximum are rather slight. A non-auxochromic *o*-methyl group causes a slight bathochromic effect, considerably stronger in the β-naphthol dye. An auxochromic *m*-carboxyl group has little effect, while an *o*-carboxyl group causes a relatively strong bathochromic effect in both series. The *o*-methyl and *m*-carboxyl groups together cause a deepening of the color which is intermediate between the effects of the two constituents alone. The effect of both an *o*- and *m*-carboxyl group differs in the two series. In the phenol dyes they cause a bathochromic effect, whereas in the β-naphthol dye they have little effect, if any a slight hypsochromic effect.

As mentioned previously, all of the phenol dyes are approximately symmetrical about 350 mμ except the two containing *o*-carboxyl groups. Kuhn and Bär [1] have determined that *p*-hydroxy-azo-benzene exists almost completely in the azo form, which shows a maximum at 350 mμ. The present author has shown [3] that the hydrazone form of this dye absorbs at approximately 375 mμ. The closeness of these two positions of maximum absorption would be expected to preclude the occurrence of a double maximum when the relative amount of the hydrazone form is small. The stronger absorption of Phenol IV and VI in the region above 370 mμ, however, would tend to indicate that the equilibrium:



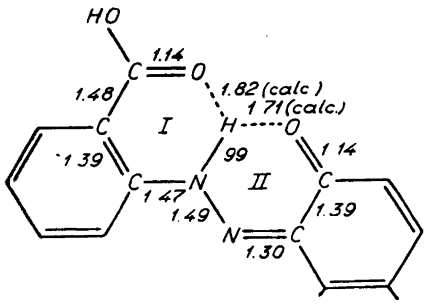


Fig. 5. Configuration of hydrazone form of o-carboxy dye

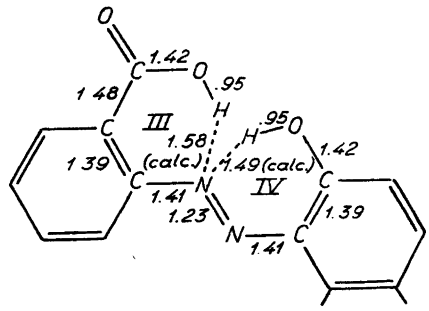


Fig. 6. Configuration of azo form of o-carboxy dye

is, in these cases, displaced to the right and that this, in turn, causes the increased absorption at the longer wave lengths. The symmetry of the other phenol dyes would tend to indicate that no such shift in the equilibrium has occured.

Figs. 5 and 6 show the interatomic distances in the hydrazone and azo forms of azo dyes, respectively. In the hydrazone form of phenol dyes containing an *o*-carboxyl group, structure I would be expected to occur. That is to say, a hydrogen bond would be expected to occur between the *o*-carboxyl group and the > N—H of the hydrazone group. The calculated O · · · · H—N distance for this bond is approximately 2.81 Å. In the azo form of the same dyes, a similiar hydrogen bond would be expected between the *o*-carboxyl group and the —N < of the azo group, as shown in structure III, Fig. 6. The interatomic O—H · · · · N distance for this bond is approximately 2.53 Å. No such hydrogen bonds are possible in any of the other phenol dyes. Each of these hydrogen bonds would be expected to exert a stabilizing influence on the corresponding form of the azo dye. The chelate ring in the hydrazone form, however, contains two conjugated double bonds. Thus it would be expected that the hydrogen bond in the hydrazone form would be more stable [4], in spite of its slightly longer O · · · · H—N distance, than that in the azo form. The resultant increased stability of the hydrazone form would thus displace the equilibrium to the right. The value of 2.8 Å for this bond corresponds well with that for the strong hydrogen bond in diketopiperazine (2.85 Å) and is considerably less than that in urea (2.98 Å) [5]. The symmetry in the curves of the other phenol dyes, including that containing a *m*-carboxyl group, would tend to indicate that only in those forms where hydrogen bonds are possible is there any shift in the equilibrium toward the hydrazone form.

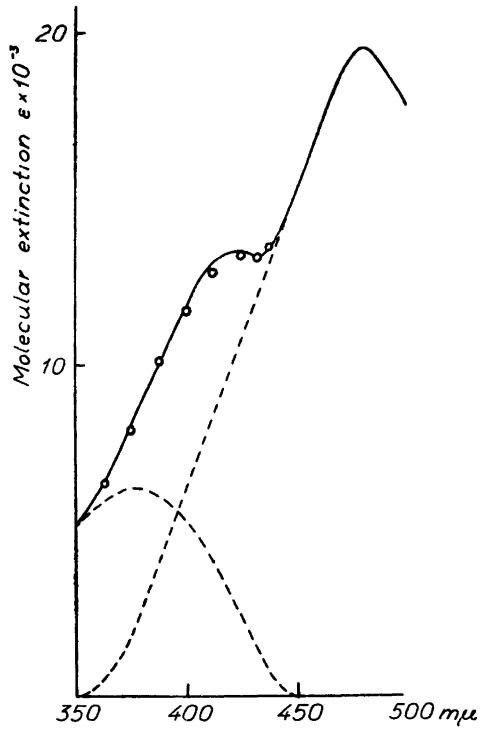
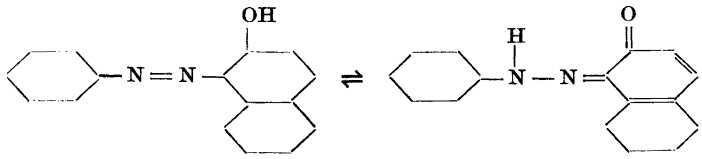


Fig. 7. Breakdown of β-Naphthol I absorption curve into Component azo and hydrazone bands.
Solid curve: Actual β-Naphthol I absorption curve.
Dotted curves: Component azo and hydrazone bands.
Circles: Addition of azo and hydrazone bands.

However, since there is no double maximum or inflection point in these curves, it is not possible to break them down into components and to calculate quantitatively the relative amounts of the two forms.

In the β-naphthol series, all of the dyes, except those containing an *o*-carboxyl group, show either a slight maximum or an inflection point at approximately 425 mμ. The results of Kuhn and Bär's studies [1] on the O acetate and O benzoate derivatives of benzene-l-azo-β-naphthol indicate that this dye exists mainly in the hydrazone form, which absorbs at 480 mμ, and that the azo form shows an absorption maximum at approximately 390 mμ. It would thus appear that the inflection at 425 mμ is due to a small amount of the dye, in the azo form, absorbing at 390 mμ. The two dyes containing an *o*-carboxyl group, however, do not show these inflection points and hence it would appear as if the equilibrium:



for these dyes has again been displaced to the right and that the amount of the azo form has been reduced to the point where it no longer manifests itself as an inflection or a maximum.

Fig. 7 shows the breakdown of benzene-1-azo-β-naphthol into its two component curves. By applying this method of breakdown to all the curves and calculating the values of $\frac{\varepsilon_H}{\varepsilon_A + \varepsilon_H}$, the figures in Table 2 were obtained.

Table 2. Relative amount of hydrazone form.

Dye		$\frac{\varepsilon_H}{\varepsilon_A + \varepsilon_H}$ *
β-Naphthol	I	.74
»	II	.75
»	III	.76
»	IV	.81
»	V	.75
»	VI	.80

* ε_H = extinction of hydrazone form
ε_A = « » azo form

The values of the molecular extinction of the pure azo and hydrazone forms are not known. If they are similar, then $\frac{\varepsilon_H}{\varepsilon_A + \varepsilon_H} \cdot 100$ approaches the percent hydrazone form present. If not, the value is nevertheless a quantitative measure of the relative amount of hydrazone form.

It can be seen from Table 2 that all the dyes, except IV and VI, show approximately the same value for $\frac{\varepsilon_H}{\varepsilon_A + \varepsilon_H}$. The values for IV and VI are higher showing that the relative amount of hydrazone form has increased.

β-Naphthol IV and VI, which contain both an *o*-carboxyl and an *o'*-hydroxyl can have either structure I or II in Fig. 5 in the hydrazone form, and either III or IV in Fig. 6 in the azo form. Both possible chelate rings in the hydrazone form contain two conjugated double bonds. The O · · · · H—N distance in structure I has been calculated to be approximately 2.81 Å, while that in structure II is 2.70 Å. Thus it would be expected that structure II would predominate, *i. e.* the hydrogen bond would exist mainly between the hydrazone H and the quinoid O of the naphthol.

In the azo form, the interatomic O—H · · · · N distance for structure III has been calculated to be approximately 2.53 Å and for IV, 2.44 Å. More-

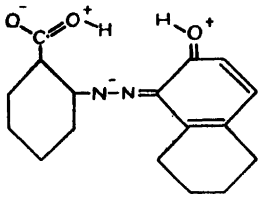


Fig. 8. Resonance structure of azo form of o-carboxy dye.

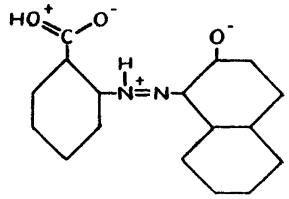


Fig. 9. Resonance structure of hydrazone form of o-carboxy dye.

over, structure IV contains two conjugated double bonds, whereas III contains only one. It would thus appear that structure IV would predominate, *i. e.* the hydrogen bond in the azo form is also between the hydroxyl group of the naphthol and the azo grouping.

Once again, these hydrogen bonds would be expected to stabilize their respective forms of the dye. The cause of the increased stabilization of the hydrazone form and the subsequent shift in the equilibrium is not as obvious, in this case, as in the phenol dyes.

Resonance in the dyes, to structures such as shown in Figs. 8 and 9, should be about equally effective in strengthening the hydrogen bonds in both forms, due to the increase in the electronegativity of one of the atoms concerned [6]. Thus one would expect no additional stabilizing effect on the hydrazone form from this cause alone.

Also, since it is assumed that the effective hydrogen bond formation is between the azo or hydrazone N and the phenolic O of the naphthol, the occurrence of only one conjugated double bond in structure III (Fig. 6) is of little importance, as this ring is not directly involved in hydrogen bond formation.

If structure IV (Fig. 6) is assumed to occur in the azo form, then the carboxyl group could not maintain the configuration shown, due to the close

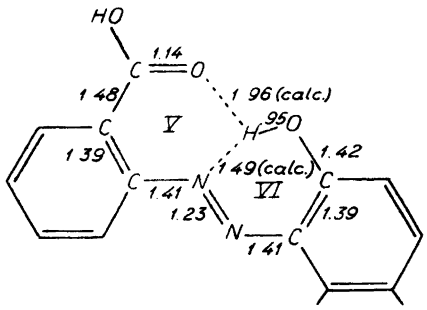


Fig. 10. Alternative configuration of azo form of o-carboxy, o'-hydroxy dye.

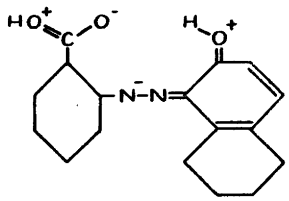


Fig. 11. Resonance structure of azo form of o-carboxy dye.

proximity of the hydrogen atoms. Resonance in the carboxyl group would probably cause it to rotate through 180° and assume the configuration shown in Fig. 10.

In this way the residual negative character of the carbonyl group would be closest to the residual positive charge of the hydrogen atom. This configuration is similar to that shown in Fig. 5 for the hydrazone form, and could resonate with structures such as shown in Fig. 11.

The calculated O—H · · · O distance, in Fig. 10, is approximately 2.91 Å.

It can be seen from Figs. 5 and 10 that in both the azo and hydrazone forms, one hydrogen atom can be attracted by two electronegative atoms. In the azo form the O—H · · · O distance (Fig. 10) is so much larger than the O—H · · · N distance that the carbonyl group would not be expected to exert much influence on the phenolic hydrogen atom, even though resonance affects the electronegativity of the two oxygen atoms (Fig. 11).

In the hydrazone form, however, the two interatomic distances are 2.70 and 2.81 Å, both of which are within the limits for strong hydrogen bonds. Resonance in this case would also tend to strengthen the bonds (Fig. 9). The difference in interatomic distances would also tend to be counteracted by the greater resonance in the carboxyl group (resonance energy 28 kcal/mole for carboxyl group and 7 kcal/mole for phenolic hydroxyl). It is thus possible that a bifurcated hydrogen bond exists between the hydrazone H and the two oxygen atoms, similar to that reported for glycine [7]. Even if an actual bifurcated bond is not formed, the carbonyl group, which is in such close proximity, undoubtedly exerts an influence on the hydrogen atom and thus stabilizes this form slightly in preference to the azo form. Thus, the equilibrium is displaced to a slight extent toward the hydrazone form.

EXPERIMENTAL

Aminoterephthalic acid

Terephthalic acid was prepared according to a previous publication by the author [8], subsequently nitrated by a modification of de la Rue and Müller's method [9], and the nitro acid reduced according to Kloeppel [10].

30 ml of pure *p*-cymene were dissolved in 100 ml of acetic acid and this solution was slowly dropped into a heated (100°) solution of 450 g CrO_3 in 600 ml H_2O, 750 ml of conc. H_2SO_4, and 2 l acetic acid. After the addition of all the cymene solution, the excess CrO_3 was destroyed with sulfurous acid and the mixture extracted with ether, in which terephthalic acid is insoluble, to remove by-products. The terephthalic acid was then removed by filtration and washed with boiling water. It was obtained as an almost white, amorphous powder. Yield approx. 70 %.

$C_8H_6O_4$ (166.13) Titration eq. (calc.) 83.0
» » (found) 83.1

9 g of terephthalic acid were added to a solution of 53 ml fuming (20 % SO_3) H_2SO_4 and 50 ml of fuming HNO_3 (d. 1.52). The mixture was heated by a direct flame to the beginning of the reaction and then on a water bath for 1 ½ hours. After this it was heated on an electric plate just to the boiling point. It was then cooled, poured on ice, and allowed to stand for 24 hours at 5°. The precipitate was removed and recrystallized from boiling water. Yield approx. 75 %.

$C_8H_5NO_6$ (211.13) Titration eq. (calc.) 105.6 M.P. (lit.) 270° (263°)
» » (found 106.2 M.P. (found) 269—270°

8 g of nitroterephthalic acid were added to a hot solution of 30 g $SnCl_2$ in 100 ml conc. HCl, and heated, with stirring, on a water bath for 1 hour. The solution was then cooled, filtered, and the precipitate washed with conc. HCl. The white crystals obtained were undoubtedly the HCl salt.

Upon recrystallization from hot water, the salt was destroyed and the free amino acid crystallized as lemon-yellow needles. Yield was approximately quantitative.

$C_8H_7NO_4$ (181.14) N (calc.) 7.73 %
N (found) 7.88 »

3-Amino-*p*-toluic acid

p-Cymene was oxidized and nitrated according to a previous publication [11] and then reduced [10].

30 ml of *p*-cymene and 17 g of MnO_2 were added to 1 500 ml of conc. HNO_3 (d. 1.40). This mixture was refluxed for one hour. The resulting clear yellow solution deposited pale yellow crystals on cooling. These were removed and washed with a little cold water. The precipitate was then dissolved in aqueous ammonia, filtered, and acidified with HCl. The resulting precipitate was removed and recrystallized from boiling water. yield 55 %.

$C_8H_7NO_4$ (181.14) N (calc.) 7.73 % M.P. (lit.) 189°
N (found) 7.70 » M.P. (found) 188—189°

7 g of 3-nitro-*p*-toluic acid were added to a hot solution of 30 g $SnCl_2$ in 100 ml conc. HCl (37 %), and treated as with nitroterephthalic acid. The HCl salt was obtained in the form of yellow crystals upon recrystallization from water.

$C_8H_9NO_2 \cdot HCl$ (187.70)	N (calc.)	7.46 %	Titration eq. (calc.)	93.8
	N (found)	7.54 »	» » (found)	93.3

The free amine melted at 166° (lit. 164—165°).

m-Aminobenzoic acid

Since no *m*-aminobenzoic acid was available, *m*-nitrobenzoic acid was reduced in the manner previously described.

$C_7H_7NO_2 \cdot HCl$ (173.7)	N (calc.)	8.06 %
	N (found)	8.13 »

The free amine melted at 173—174° (lit. 174°).

Phenol I. 4-Phenylazophenol

Since all the dyes were prepared in a similar manner [12], only the preparation of this dye will be given in detail.

1 g of aniline was placed in a beaker with 5 ml conc. HCl and a little ice. This mixture was cooled to 0 to 5° in an ice bath, and diazotized by the addition of an aqueous solution of .74 g $NaNO_2$ in 10 ml water. The temperature, during the addition of the $NaNO_2$, was not allowed to exceed 5°. When the diazotization was complete, the solution was poured into an alkaline solution of 1 g phenol. The red solution obtained was allowed to stand for several hours, after which it was acidified with HCl.

The precipitate was removed and dried at 105°. Recrystallization from aqueous alcohol yielded light brown crystals.

$C_{12}H_{10}N_2O$ (198.22)	N (calc.)	14.13 %
	N (found)	13.97 »

Phenol II. 4-(*o*-Tolylazo)-phenol

1 g of *o*-toluidine was diazotized and coupled as above. A dark oil was obtained. The solution was extracted with ether (3 × 250 ml), the ether dried over Na_2SO_4, and the solvent evaporated. The dark oil that was obtained did not crystallize even after standing at 5° for several months.

Finally, it was dissolved in the smallest possible amount of glacial acetic acid and shaken with a large excess of water. This deposited small brown needles after a week at 5°. Recrystallized from alcohol.

$C_{13}H_{12}N_2O$ (212.24)	N (calc.)	13.20 %
	N (found)	12.98 »

Phenol III. 4-(3-Carboxyphenylazo)-phenol

1 g of HCl salt of *m*-aminobenzoic acid was diazotized and coupled with phenol. Recrystallization from methanol yielded dark brown-violet crystals.

$C_{13}H_{10}N_2O_3$ (242.23) N (calc.) 11.11 %
N (found) 11.20 »

Phenol IV. 4-(2-Carboxyphenylazo)-phenol

1 g anthranilic acid was diazotized and coupled in the usual way. Recrystallization from methanol yielded light yellow-tan needles.

$C_{13}H_{10}N_2O_3$ (242.23) N (calc.) 11.11 %
N (found) 11.22 »

Phenol V. 4-(2-Methyl-5-carboxyphenylazo)-phenol

1 g of HCl salt of 3 amino-*p*-toluic acid was diazotized and coupled with phenol. Recrystallization from methanol yielded dark violet-brown crystals.

$C_{14}H_{12}N_2O_3$ (256.26) N (calc.) 10.93 %
N (found) 10.62 »

Phenol VI. 4-(2,5-Dicarboxyphenylazo)-phenol

1 g of aminoterephthalic acid was diazotized and coupled in the usual manner. Recrystallization from hot water and from ethanol yielded fine orange needles.

$C_{14}H_{10}N_2O_5$ (286.24) N (calc.) 9.80 %
N (found) 9.96 »

β-Naphthol I. 1-Phenylazo-2-hydroxynaphthalene

1 g of aniline was diazotized and coupled, as above, with β-naphthol. Recrystallization from alcohol yielded orange-red needles.

$C_{16}H_{12}N_2O$ (248.27) N (calc.) 11.31 %
N (found) 11.28 »

β-Naphthol II. 1-(*o*-Tolylazo)-2-hydroxynaphthalene

1 g *o*-toluidine was diazotized, as above, and coupled with β-naphthol. Recrystallization from ether yielded dark red crystals.

$C_{17}H_{14}N_2O$ (262.30) N (calc.) 10.68 %
N (found) 10.43 »

β-Naphthol III. 1-(3-Carboxyphenylazo)-2-hydroxynaphthalene

1 g of HCl salt of *m*-aminobenzoic acid was diazotized and coupled with β-naphthol. Recrystallization from methanol yielded dark red crystals.

$C_{17}H_{12}N_2O_3$ (292.28) N (calc.) 9.60 %
N (found) 9.50 »

β-Naphthol IV. 1-(2-Carboxyphenylazo)-2-hydroxynaphthalene

1 g anthranilic acid was diazotized and coupled as above.
Recrystallization from glacial acetic acid yielded fine red needles.

$C_{17}H_{12}N_2O_3$ (292.28) N (calc.) 9.60 %
N (found) 9.64 »

β-Naphthol V. 1-(2-Methyl-5-carboxyphenylazo)-2-hydroxynaphthalene

1 g of HCl salt of 3-amino-*p*-toluic acid was diazotized and coupled with β-naphthol.
Recrystallization from alcohol yielded dark red crystals.

$C_{18}H_{14}N_2O_3$ (306.31) N (calc.) 9.16 %
N (found) 9.24 »

This dye showed an interesting phenomena, which was later found to be common to many of the azo dyes. Different conditions of recrystallization (solvent, temp., *etc.*) seemed to yield different crystal modifications. In this particular dye, recrystallization from methanol yielded a red product, whereas recrystallization from ether yielded an orange product. The later showed strong orange fluorescence in ultra-violet light, while the red form showed no tendency to fluorescence. Heating the two forms at 200° for many hours seemed to produce an intermediate form, *i. e.* the fluorescence of the orange form decreased while that of the red form increased. The visible color also changed to an intermediate orange-red. A spectroscopic comparison of the two forms (in ethanol) gave, however, identical curves.

A chemical analysis of the two forms showed almost identical nitrogen contents. This result would eliminate the possibility of any alcohol or ether of crystallization.

The two forms were completely interchangeable by recrystallization from the corresponding solvent.

For these reasons, this phenomena, and others of similar type, was considered to be due to polymorphism.

β-Naphthol VI. 1-(2,5-Dicarboxyphenylazo)-2-hydroxynaphthalene

1 g of aminoterephthalic acid was diazotized and coupled, in usual way, with β-naphthol.
Recrystallization from glacial acetic acid yielded red-orange needles.

$C_{18}H_{12}N_2O_5$ (336.30) N (calc.) 8.33 %
N (found) 8.26 »

SUMMARY

The effect of an *o*-methyl group, *o*- and *m*-carboxyl groups, and combinations of the two on the absorption spectra of phenylazo-phenol and phenylazo-β-naphthol has been examined and the absorption curves of the dyes in 96 % alcohol are given.

The effect of these groups on the azo-hydrazone tautomerism is also discussed. The increase in the hydrazone form for dyes containing an *o*-carboxyl group is explained in terms of hydrogen bond formation. The possibility of a bifurcated hydrogen bond in the β-naphthol dye is discussed.

The author wishes to express his thanks to Prof. K. Myrbäck and Doc. E. Adler for helpful discussions, to Prof. E. Hägglund for the use of the facilities at Svenska Träforskningsinstitutet, and to Civiling. G. Gran for the analyses.

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Received September 4, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1365–1374

Veratryl Alcohol, Diveratryl Ether, and Veratryl-ethyl Ether as Lignin Models

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Holmberg [1] showed that lignin behaves as α-phenethyl alcohol in its reactions with sulphite cooking acid, thioglycollic acid, and ethanolic hydrochloric acid. For that reason he considered that these lignin reactions may be due to benzyl alcohol groups, or because of the great reactivity of these alcohol groups, to benzyl ether groups in the lignin. He also considered that the lignin molecule may be bound in the wood by benzyl ether linkages to other lignin molecules or to carbohydrate molecules.

On the assumption that phenolic groups are formed during the sulphonation of lignin, Freudenberg [2] considered that the groups responsible for the sulphonation are benzyl-*aryl* ether and benzyl alcohol groups. Later Freudenberg, Lautsch, and Piazolo [3] showed, however, that no phenolic groups are formed during the sulphonation. For that reason they considered that the ether group may be a benzyl-*alkyl* one, as, for example, in pinoresinol.

Benzyl alcohols have recently been found to be suitable lignin models in many respects [4–6]. These alcohols are easily transformed into ethers (called to the author's attention by E. Adler). Hedén and Holmberg [7] showed that α-phenethyl alcohol is transformed into α-phenyl-ethan-sulphonic acid and di-(α-phenethyl) ether if an excess of the alcohol is heated with sulphite cooking acid. If *p*-methoxy-α-phenethyl alcohol [8] and veratryl alcohol (compare experimental part) are kept at room temperature for a few months they are partly transformed into the corresponding ethers.

It has been assumed that there are two groups in lignin responsible for its sulphonation [9, 10]. On the basis of the above-mentioned results and theories, the author has examined if the reactivity of veratryl alcohol and diveratryl ether run parallell to the reactivity of the lignin groups. For that reason

the earlier studies on the reactions of veratryl alcohol [4] have been improved and extended.

Reactions of veratryl alcohol and diveratryl ether with sulphite solutions

If veratryl alcohol is heated at 135° with a normal sulphite cooking acid it readily forms 3,4-dimethoxy-toluene-ω-sulphonic acid [4]. The sulphonation rate of the alcohol decreases with increasing pH (see Table 1. All pH values were measured at room temperature). The decrease in the rate can not be due to the formation of diveratryl ether, because the unsulphonated substance which was recovered after heating for 20 hours at pH 7 was, for the main part, veratryl alcohol.

Table 1 also shows that diveratryl ether readily forms 3,4-dimethoxy-toluene-ω-sulphonic acid with sulphite solutions of pH 1.4—2.5. At pH 3.7 to 5.9 the sulphonation rate rapidly decreases. Table 2 shows the amounts (in per cent of diveratryl ether employed) of the ether which were not recovered after heating with sulphite solutions of pH 3.7—5.9.

Table 1. The yields of 3,4-dimethoxy-toluene-ω-sulphonic acid from veratryl alcohol ("A") and diveratryl ether ("E") by heating at 135° with sulphite solutions of varying pH. pH determined at room temperature.

Time, hours	pH															
	1.4		1.9	2.5	3.7		4.2		5.0		5.9		7.0	8.7	11.0	
	A	E	E	E	A	E	A	E	A	E	A	E	A	A	A	E
½	96	94														
1	96	97														
3 ½			93	91	69	24	56	3		2	27	1				
10					91	65	83	30	60	6						
20					96	81	98	91	84	10	58	2	43	40	44	0

Table 2. The amounts of diveratryl ether hydrolyzed at 135° by sulphite solutions with varying pH. pH determined at room temperature.

Time, hours	pH				
	3.7	4.2	5.0	5.9	11.0
3 ½	42	15	2	1	
10	75	42	8		
20	94	97	13	2	0

Table 3. The yields of S-veratryl-thioglycollic acid from veratryl alcohol and diveratryl ether on heating at 100° for 4 hours with thioglycollic acid solutions of varying pH, and the amounts of diveratryl ether recovered.

pH	Thioglycollic acid derivative obtained from		Diveratryl ether recovered
	the alcohol	the ether	
0.8	86	84	0
1.4	86	78	0
2.0	80	65	4
3.0	57	0	80
4.0	47	0	96

To summarize, veratryl alcohol and diveratryl ether are readily sulphonated at pH 1.4—3.7. At pH 6 and higher the ether reacts scarcely at all. The alcohol is sulphonated even at pH 11 at an appreciable rate. (Vanillyl alcohol * is sulphonated much more rapidly than veratryl alcohol at higher pH[4].)

Reactions of veratryl alcohol and diveratryl ether with thioglycollic acid

The model substances were heated at 100° for 4 hours with thioglycollic acid-sodium thioglycollate solutions of varying hydrogen ion activity. Table 3 shows the yields of S-veratryl-thioglycollic acid and the amounts of diveratryl ether recovered. Both substances readily react at low pH (0.8—1.4). At higher pH (3.0—4.0) only the alcohol reacts. The yields of veratryl-thioglycollic acid formed are up to 15 per cent (absolute) higher than those recorded in the table for some of the acid was lost during its isolation.

These two points, sulphonation and reaction with thioglycollic acid, are to be discussed in forth-coming papers concerning the sulphonation of lignin at high pH, and the reaction of lignin with thioglycollic acid.

Reactions of veratryl alcohol and diveratryl ether with sulphite solutions containing β-naphthol

If vanillyl alcohol is heated with a normal sulphite cooking acid (pH 1.5—2) containing resorcinol, the condensation with the phenol predominates over the sulphonation [4]. At pH 4.5, however, the sulphonation predominates. The

* Added in proof. A lignane with *p*-hydroxybenzyl ether groups, pinoresinol, also reacts in this way (B. O. Lindgren, forthcoming paper).

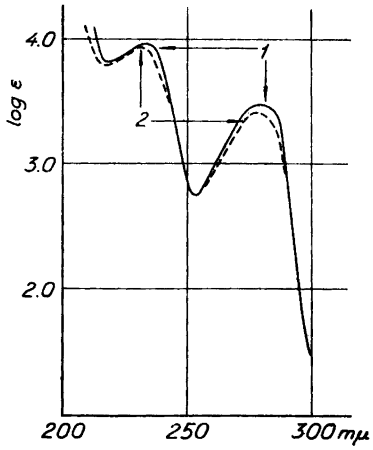

Fig. 1. Ultra-violet spectra.
1. *Diveratryl ether (in alcohol).*
2. *Sodium salt of 3,4-dimethoxy-toluene-ω-sulphonic acid (in water).*

poly-benzyl alcohols synthesized and examined by Erdtman and Leopold [5] also react in this way.

Because resorcinol is transformed into a sulphonic acid by long heating with sodium bisulphite solution [11], β-naphtol was used in the following experiments. This phenol does not react with sulphite solutions during the conditions used in these experiments (compare experimental part).

When veratryl alcohol was heated with sulphite solutions containing β-naphthol it was transformed partly into a condensation product with the phenol, and partly into the sulphonic acid. As β-naphthol has only one strongly reactive position (the α-position), it was postulated that the condensation product was formed from one molecule of the phenol and one molecule of the alcohol by loss of one molecule of water. The product was not further investigated. On this assumption Table 4 shows the yields of the condensation product from veratryl alcohol. It also shows the number of moles of naphthol (determined from the amounts of the phenol recovered) which reacted per mole of veratryl alcohol employed.

Table 4. Heating of veratryl alcohol with sulphite solutions containing β-naphtol.

	pH of the sulphite solution		
	1.4	2.8	5.4
The condensation product: weight, g	1.49	0.76	0.15
yield, %	85	43	8
Moles of β-naphtol reacted per mole of veratryl alcohol employed	0.92	0.51	0.09

Veratryl alcohol reacts similarly to the other benzyl alcohols which have been examined in this way. At low pH the condensation predominates, at higher pH the sulphonation.

At pH 2 diveratryl ether reacts as veratryl alcohol. At pH 5.3 the ether was recovered almost quantitatively (84 %) after heating for 24 hours. The rest of the ether was evidently sulphonated, as no condensation product was obtained.

Reactions of veratryl alcohol and diveratryl ether with ethanolic hydrochloric acid

Berg and Holmberg [12] have shown that α-phenethyl alcohol reacts with ethanolic hydrochloric acid with the formation of α-phenethyl-ethyl ether. They considered this as a model reaction for the introduction of ethoxyl groups into lignin when wood is treated with ethanolic hydrochloric acid.

Veratryl alcohol and diveratryl ether also yielded the corresponding ethyl ether when refluxed with ethanolic hydrochloric acid (0.1—0.55 N). During these reactions condensation products were also obtained: in the case of 1 N acid the model substances yielded 2,3,6,7-tetramethoxy-9,10-dihydro-anthracene. (This compound has been obtained by G. M. Robinson [13] by condensation of veratryl alcohol with acetic acid in the presence of hydrochloric acid.) As the complete delignification of wood by ethanolic hydrochloric acid has not yet been achieved, it is generally assumed that lignin, like the above-mentioned model substances, partly condenses during this treatment.

Treatments of veratryl alcohol and diveratryl ether with acetic anhydride and pyridine

The above-mentioned experiments show that diveratryl ether is easily hydrolyzed. To prove if dibenzyl ether linkages are split under the conditions employed in the acetylation of ligninsulphonic acids [14], diveratryl ether was treated in the same manner as these lignin preparates. The ether was recovered unchanged after the treatment. Veratryl alcohol was transformed into veratryl acetate under these conditions.

Reactions of veratryl-ethyl ether with thioglycollic acid and sulphite cooking acid

Berg and Holmberg [12] showed that ethanol lignin reacts with thioglycollic acid with a loss of ethoxyl groups. Veratryl-ethyl ether (like α-phenethyl-

ethyl ether [12]) is a good model substance for this reaction. It is easily transformed into veratryl-thioglycollic acid by thioglycollic acid and hydrochloric acid.

Erdtman and Pettersson [15] showed that ethoxylated low sulphonated ligninsulphonic acids obtained from wood according to the Kullgren process [16] can be sulphonated by normal sulphite cooking acid with loss of the ethoxyl groups. In the same way the ethoxyl group of veratryl-ethyl ether can easily be substituted for a sulphonic acid group.

EXPERIMENTAL *

The reduction of veratraldehyde by hydrogen and platinum

Veratraldehyde [17] (100 g) was reduced by hydrogen and platinum activated by iron according to the method used by Carothers and Adam [18] to synthesize several other benzyl alcohols. The oil obtained was distilled, veratryl alcohol being collected in the distillate at 157—160° (5 mm Hg) (75 g, yield 74 %).

The distillation residue crystallized on cooling. Repeated recrystallizations raised the m. p. of the product from 60—62° to 69—72° (8.3 g, yield 9 %). The product gave no m. p. depression with diveratryl ether, synthesized according to Method 1 described below.

The syntheses of diveratryl ether

1. Potassium (1.3 g) was pulverized in the usual manner in toluene (30 ml). Veratryl alcohol (5.8 g) dissolved in toluene (20 ml) was added. The mixture was refluxed for 1 hour. A further amount of veratryl alcohol (2 g) dissolved in toluene (10 ml) was added and the mixture refluxed for a further half hour. Veratryl chloride [19] (6.4 g) dissolved in toluene (25 ml) was then added and the mixture refluxed for 1 hour and then left over night at room temperature.

The potassium chloride was filtered off and the filtrate passed through a column (3 × 10.5 cm) containing aluminium oxide. The toluene was distilled off under vacuum. The residue crystallized (8.5 g). Recrystallization from methanol yielded a product with the m. p. 68—70° (7.2 g, yield 65 % calc. from the amount of veratryl chloride employed).

Repeated recrystallizations yielded a product with the m. p. 70—72.5°. The analyses agree with the compound being diveratryl ether.

$C_{18}H_{22}O_5$	Calc.	OCH_3	39.0	C	68.0	H	6.93
	Found	»	39.3	»	68.0	»	6.96

2. If veratryl alcohol was kept at room temperature a few months it was partly transformed into diveratryl ether (about 10 %).

3. See the reduction of veratraldehyde above.

* All m.p. uncorr. M.p. made on aluminium-block.

Reactions of veratryl alcohol and diveratryl ether with ethanolic hydrochloric acid

Veratryl alcohol (2 g) was heated with ethanolic hydrochloric acid (10 ml) of varying concentration (0.01, 0.1, 0.55, and 1 N) on a steam bath for 4 hours. The solution was neutralized with potassium hydroxide and evaporated in a vacuum. The residue was distilled. An oil with the boiling temperature 152—154° (20 mm Hg) and $n_D^{20} = 1.519$ was obtained. The analysis agrees with the compound being veratryl-ethyl ether.

$C_{11}H_{16}O_3$	Calc.	Alkoxyl	(calc. as OCH_3)	47.4
	Found	»	» » »	46.9

The yields of veratryl-ethyl ether were: 0.01 N acid 56 %, 0.1 N 58 %, 0.55 N 32 %, and 1 N 0 %.

During the heating with N acid a precipitate was observed. It was filtered off (m. p. 227°, yield 20 %). The product gave no m. p. depression with 2,3,6,7-tetramethoxy-9,10-dihydro-anthracene, synthesized from veratrol and formaldehyde [13].

Diveratryl ether was treated in the same way. The yields of veratryl-ethyl ether were: 0.1 N acid 49 %, 0.55 N 16 %, and 1 N 0 %.

With 1 N acid the anthracene derivative was obtained in a yield of 89 %.

Reactions of veratryl alcohol, diveratryl ether, and veratryl-ethyl ether with sulphite solutions

Diveratryl ether (0.6 g) was mixed with sulphite cooking acid (60 ml, 0.7 % NaOH and 5 % total SO_2) and heated in a rotating tube for 3 hours at 135°. After the heating all the ether was dissolved. The barium salt of the sulphonic acid was isolated by the method described before [4], its weight was 1.03 g (yield 91 %). The barium salt was transformed into the pyridinium salt [4], m. p. 144—146°. Equivalent weight; $C_{14}H_{17}NSO_5$; calc. 311, found 318.

Veratryl-ethyl ether, treated in the same way, gave the barium salt of the sulphonic acid in a yield of 91 %. The pyridinium salt melted at 144—146°. It gave no m. p. depression with the corresponding product from diveratryl ether.

Veratryl alcohol (0.1 g) was dissolved in a sulphite solution (10 ml, 5 % SO_2, varying amounts of sodium hydroxide) in a glass tube. The tube was rotated in a glycerol bath at 135°.

After the heating, the solution was extracted 3 times with ether (total 60 ml). The extract was dried over anhydrous sodium sulphate and evaporated. The residue was assumed to be veratryl alcohol. The residue obtained from the treatment at pH 7, was found to contain mainly the alcohol. This was shown by the fact that the oily residue was for the main part soluble in water, and by transforming the oil into veratryl-thioglycollic acid (see below).

The extracted water solution was diluted to a suitable degree and the absorption at 280 mμ was determined with a Beckman spectrophotometer. From the absorption (see the Figure) the amount of the sulphonic acid in the solution was calculated.

The yield of the sulphonic acid was determined by these two methods (Table 1).

Diveratryl ether was treated as veratryl alcohol above. The amount of diveratryl ether which did not react was determined by filtering off and weighing the ether. (Diveratryl ether is not soluble in water, in contrast to veratryl alcohol.) The yield of the sulphonic acid was determined according to the two methods mentioned above with the exception that the amount of recovered diveratryl ether was added to the amount of the residue from the ether extract to determine the amount of substance not transformed into the sulphonic acid.

The yield of the sulphonic acid is shown in Table 1, and the amount of diveratryl ether which had reacted, in Table 2.

Reactions of veratryl alcohol, diveratryl ether, and veratryl-ethyl ether with thioglycollic acid

Veratryl alcohol (0.5 g) was heated on a steam bath for 4 hours with a water solution (7.5 ml) of thioglycollic acid (2.5 g). The pH of the solution was adjusted to the desired value by hydrochloric acid or sodium hydroxide. After the heating the solution was extracted with ether. The extract was shaken with a solution of sodium bicarbonate. The bicarbonate solution was mixed with the extracted water solution. On acidifying the combined solutions (about 50 ml), a crystalline substance was obtained. (Some of the veratryl-thioglycollic acid obtained was lost, as the product is slightly soluble in water, 2 g/l). The m. p. was about 89—91° in the different experiments.

Recrystallizations from benzene yielded a product with m. p. 92—92.5°. The equivalent weight agrees with the compound being S-veratryl-thioglycollic acid.

$C_{11}H_{14}O_4S$	Calc.	Equivalent weight	242
	Found	» »	246.5

The yield of the thioglycollic acid derivative is shown in Table 3.

Diveratryl ether was treated in the same way. The yield of the thioglycollic acid derivative and the amount of diveratryl ether recovered are shown in Table 3.

Veratryl-ethyl ether was treated as above with a thioglycollic acid solution at pH 1.4. The yield of the thioglycollic acid derivative was 65 %.

Reactions of veratryl alcohol with sulphite solutions containing β-naphtol

Veratryl alcohol (1 g) and β-naphthol (1 g) were heated with a sulphite solution (50 ml, 5 % SO_2, pH 1.4) in a rotating tube for 20 hours at 135°.

After the heating, the solution was extracted with ether. The extract was dried over anhydrous sodium sulphate and the solvent evaporated. The weight of the residue was 1.70 g. The naphthol in the residue was removed by sublimation, its weight was 0.21 g. The residue from the sublimation (1.40 g) was considered to be a condensation product of the phenol and veratryl alcohol, but was not further investigated.

When β-naphthol was heated at 135° for 24 hours with sulphite solutions of pH 1.4 and 5.7, it was recovered almost quantitatively (87 resp. 93 %).

The Table 4 shows the yields of the condensation product at varying pH.

Treatment of veratryl alcohol and diveratryl ether with acetic anhydride and pyridine

Veratryl alcohol (2 g) was heated with a mixture of acetic anhydride (4 ml) and pyridine (36 ml) for 9 hours on a steam bath. The mixture was evaporated in a vacuum desiccator over sulphuric acid and sodium hydroxide. The residue was vacuum distilled. An oil with boiling point 170—172° (18 mm Hg) was obtained (1.8 g, yield 72 %, $n_D^{20} = 1.523$).

The analysis agrees with the compound being veratryl acetate.

$C_{11}H_{14}O_4$	Calc.	Ac	20.5
	Found	»	20.0

When diveratryl ether was treated in the same way, it was recovered quantitatively in an unchanged condition.

SUMMARY

1. The following new substances have been synthesized: diveratryl ether, veratryl-ethyl ether, veratryl acetate, and S-veratryl-thioglycollic acid.

2. The following lignin model reactions of veratryl alcohol and diveratryl ether have been examined:

a. with sulphite solutions at varying pH
b. with sulphite solutions containing β-naphtol
c. with ethanolic hydrochloric acid
d. with thioglycollic acid at varying pH.

3. The reactions of veratryl-ethyl ether with sulphite cooking acid and thioglycollic acid have been examined.

This work has been supported by a grant from Swedish Natural Science Research Council. I gratefully acknowledge the assistance of Miss Ulla Saedén.

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Received September 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1375–1385

Die Bildungsweise der Chlorhydrine

IX. Einige Additionsversuche mit Epichlorhydrin und Glyceringlycid

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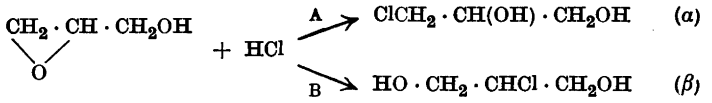
1. DER EINFLUSS DER TEMPERATUR AUF DIE ISOMERENBILDUNG BEI DER ADDITION VON CHLORWASSERSTOFF

Experimente von ÅKE SVENSSON und LARS ASSARSSON

Bei Untersuchung der Reaktion:

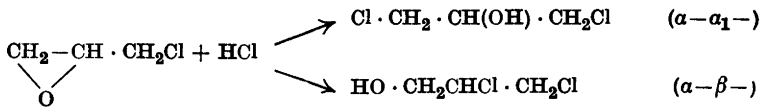


wurde gefunden[1], dass die relative Menge des β-Glycerinmonochlorhydrins mit steigender Temperatur zunimmt, z. B. in Ätherlösung bei — 75° 2,5 % β-Chlorhydrin, bei + 25° 15,5 % der genannten Verbindung. Dies muss bedeuten, dass die Additionsgeschwindigkeiten längs des Weges A (siehe oben) zu denjenigen längs des Weges B sich bei 25° wie 5,5 : 1, bei — 75° wie 40 : 1 verhalten. Solche Versuche sind neuerdings in wässeriger Lösung wiederholt worden, wobei wir bei den Additionstemperaturen 0° und 100° die Zahlen 10 %, bzw. 17 % β-Verbindung fanden *.

Der Gehalt an α-Verbindung vermindert sich mit sinkender Temperatur, doch wird niemals — bei noch so niedriger Temperatur — reine α-Verbindung entstehen können.

Man müsste annehmen, dass eine solche Gesetzmässigkeit bei allen gleichartigen Reaktionen vorhanden ist, so z. B. auch bei der Reaktion zwischen Epichlorhydrin und Chlorwasserstoff;

* Vorläufige Ziffern. Die Versuche werden wiederholt.



Hier würde man folglich auch ein Gemisch von Glycerindichlorhydrinen erwarten, das mit steigender Temperatur sich zugunsten der β-Verbindung verschieben sollte.

Aber eben für diese Reaktion ist es eine uralte Vorstellung, dass ausschliesslich α-α_1-Verbindung gebildet wird, wobei als Kriterium früher [2] vor allem die Konstanz des Siedepunktes des Reaktionsproduktes diente. Es lässt sich aber einwenden, dass dieses Kriterium eben hier keinen grösseren Wert hat, weil der Unterschied der Siedepunkte für die beiden Isomeren nur etwa 8° beträgt und eine Beimischung von einigen Prozenten der α-β-Verbindung sich durch eine Siedepunktsbestimmung mit älteren Methoden sich gar nicht nachweisen lässt.

Viel genauer ist die Bestimmung der alkalischen Zersetzungsgeschwindigkeit [3] des Reaktionsproduktes. Da diese Geschwindigkeit für die eben genannte α-α_1-Verbindung mehr als 100 mal grösser ist als für die α-β-Verbindung, würde ein Gehalt von 1—2 Prozent mit Sicherkeit beobachtet werden können, indem die Geschwindigkeitskoeffizienten des Reaktionsproduktes mit steigender Umsetzung abnehmen würden. Die ersten kinetischen Analysen dieses Reaktionsproduktes [4] wurden bei 25° ausgeführt. Das Resultat der Analyse war indessen etwas unsicher, weil die Geschwindigkeiten so gross sind, dass auch mit guter Versuchsmethodik die Versuchsfehler in der Richtung wirken, dass die ersten kinetischen Koeffizienten in jeder Serie (Zeiten 1—2 Min.) auch bei einer isomerenfreien Verbindung etwas höher als die späteren ausfallen. Infolgedessen kann man nicht entscheiden, ob bei α-α_1-Dichlorhydrin ein solcher kleiner Abfall der Konstanten Versuchsfehlern oder Einmischung von α-β-Verbindung zuzuschreiben ist.

Ich führe einige Beispiele solcher Zersetzungsgeschwindigkeiten an: die Addition von Chlorwasserstoff an Epichlorhydrin wurde »bei Zimmertemperatur» ausgeführt; die Bestimmung der Zersetzung geschah bei 25°; Konzentrationen von Chlorhydrin und Alkali etwa 0,006 mol; Probenahmen bei 30—80 % Umsetzung. Die bimolekularen Geschwindigkeitskoeffizienten einer Serie waren: 80,5; 77,9; 76,7; 77,7.

Zersetzungsversuche mit α-α_1-Dichlorhydrin unter Zuhilfenahme von *Soda-Bicarbonat*puffern sind von Laudon [5] und Bergkvist [6] ausgeführt worden. Die Reaktion geht hier sehr langsam, warum die Messungen bequem sind. Der letztgenannte hat in seinen Berechnungen auch die Einwirkung der anwesenden Ionen des Puffers auf die sek. Diss.konstante der Kohlensäure be-

rücksichtigt, und daher tadellose — also Koeffizienten ohne Gang — Geschwindigkeitskoeffizienten gefunden, die auch hinsichtlich des Absolutwertes mit den oben angeführten (mit reiner Natronlauge) gewonnenen Zahlen übereinstimmten (bei verschiedenen Pufferkonzentrationen also die Koeffizienten 74; 76 und 80). Dass hierbei die grössten Pufferkonzentrationen auch die grössten Konstanten geben — bei reiner Natronlauge ist es umgekehrt —, liegt wohl an den Berechnungen.

Indessen ist eine kinetische Analyse mit Hilfe einer Pufferlösung aus theoretischen Gründen weniger empfindlich für eine sehr kleine »Verunreinigung» der α-β-Verbindung als eine solche mit Lauge *. Es schien daher wünschenswert, die kinetischen Analysen mit reiner Lauge bei niedrigerer Temperatur und mit verbesserter Methodik auszuführen, um hinreichende experimentelle Sicherheit zu erreichen und die in dieser Weise gewonnenen Prozentzahlen mit den Resultaten der Analysen mit Pufferlösungen zu vergleichen.

Die Addition von Chlorwasserstoff an Epichlorhydrin wurde teils bei — 10°, teils bei + 100° durchgeführt, wobei besondere Vorsichtsmassregeln getroffen wurden, um die Reaktionswärme genügend schnell wegzuleiten. Die Reaktion ist bei 100° sehr heftig.

Synthese bei — 10°. Das Reaktionsgefäss war ein dreitubulierter Kolben, 150 g konc. Salzsäure enthaltend (Kühlung von aussen). 100 g neudest. Epichlorhydrin wurden durch eine kapillare Glasspirale eingeführt, derer Windungen sich in der Säure befanden. Während der Zusetzungzeit (2 Std.) wurde die Lösung intensiv turbiniert. Das Gemisch blieb noch 2 Std. bei derselben Temperatur stehen, dann wurde mit Magnesiumsulfat ausgesalzt, das Öl abgeschieden und die wässerige Lösung mit Äther extrahiert. Es schien uns nähmlich wichtig, das Reaktionsprodukt so vollständig wie möglich zur kinetischen Analyse mitzunehmen. Öl + Extrakt wurden mit Kaliumcarbonat geschüttelt und der Äther abdestilliert (Gew. des Rohproduktes 97 g). Das Produkt wurde dreimal in Vakuum destilliert, wobei solche Bedingungen eingehalten wurden, dass kein Fraktionieren der möglicherweise anwesenden beiden isomeren Dichlorhydrine stattfinden konnte. Auch bei der ersten Destillation blieb nur ein sehr unbedeutender Rückstand im Destillationskolben.

Die Synthese bei 100° wurde in derselben Weise angeführt, nur wurde 20 %-ige Salzsäure benutzt: 260 g Säure und 95 g Epichlorhydrin, Gew. des Rohproduktes 98 g. Zeit für Zusetzen der Säure 1 Stunde.
Analysen:

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* Im ersten Falle haben wir die Gleichung einer pseudomonomolekularen, im späteren einer bimolekularen Reaktion.

— 10°-Dichlorhydrin: % Cl: 55,13; gef. 54.98; $n_D^{20} = 1{,}4837$: $d_4^{20} = 1{,}3639$; $MR_D = 27{,}04$ (ber. 27,31).

+ 100°-Dichlorhydrin: % Cl: gef. 54,93; $n_D = 1{,}4838$; $d_4^{20} = 1{,}3637$; $MR_D = 27{,}04$.

Die physikalischen Konstanten sind, wie ersichtlich, innerhalb der Versuchsfehler identisch für die beiden Produkte *.

Um zu prüfen, ob bei der Reaktion bei 100° eine eventuelle isomerisierende Einwirkung der Salzsäure auf das gebildete Isomerengemisch stattfand, wurde reine α-α_1-Isomere eine Stunde mit der äquivalenten Menge Salzsäure auf 100° erhitzt. Das Chlorhydrin wurde wie früher erwähnt isoliert und danach kinetisch analysiert, wie unten beschrieben. Keine Veränderung konnte kinetisch nachgewiesen werden.

Die Synthese des α-β-Dichlorhydrins geschah nach King-Pyman[7]. Konstanten des Produkts: $n_D = 1{,}4829$; $d_4^{20} = 1{,}3546$; $MR_D = 27{,}19$.

Zersetzungsgeschwindigkeiten siehe unten.

Die Bestimmung der alkalischen Zersetzungsgeschwindigkeit — also die kinetische Analyse — wurde bei 25° und 15° unter Zuhilfenahme folgender Methodik vorgenommen. Reaktionsgefäss: weithalsige Flasche (250 ml), in welche kohlensäurefreie Luft eingeblasen wurde. Intensives Turbinieren der Lösung. Zu der alkalischen Lösung in der Flasche wurde zur Zeit t = 0 aus einem kleinen (mit Kork gewogenem) Becher die Chlorhydrinlösung in einem Zuge gegossen, wonach der Becher zurückgewogen wurde. Die Reaktionszeit wurde mit einem kontrollierten Metronom gemessen. Die Reaktion wurde durch Eingiessen von Salpetersäure unterbrochen, wonach die Chloridionen potentiometrisch bestimmt wurden. In den kinetischen Analysen bei 0° wurde die gewöhnliche[4] kinetische Methode als hinreichend genau angesehen. Die Konzentrationen (Mol/Lit.) waren in allen Versuchen etwa: Baryt = 0,01, Chlorhydrin = 0,007, Totalvolumen der Lösungen etwa 120 ml.

Originaltabellen für die alkalische Zersetzungsgeschwindigkeit werden nur für das 100°-Produkt angeführt, für die übrigen nur Mittelwerte (k_M), nebst den mittleren Fehlern (F) derselben; Δk bedeutet die (graphisch bestimmte) Abnahme des bimol. Geschwindigkeitskoeffizienten von 20 % bis 80 % Umsetzung. »Temp». gibt die Temperatur bei der *kinet. Analyse* an.

* Diese Synthesen wurden zu verschiedenen Zeiten unabhängig von einander von Svensson und Assarsson ausgeführt. Die angeführten Werte rühren von Svensson her, der mit etwas grösserer Genauigkeit gearbeitet hat. Die Ziffern Assarssons sind indessen mit denjenigen Svenssons beinahe identisch. Die Zersetzungsgeschwindigkeit mit Natronlauge wurde von Svensson bestimmt, diejenige mit Puffer von Assarsson. — Aira Halonen machte dieselben Synthesen, kinetische Bestimmungen nach einer anderen Methode. Ihre Bestimmungen waren indessen nicht genügend sicher, um kinetisch verwertet werden zu kommen. Für 100°-Synthese fand sie etwa 5 % α-β-Verbindung.

Tabelle 1.

Synthese bei	Temp.	K_M	F	F %	Δk		$\Delta k : F$
— 10°	25,00°	69,6 ± 0,3		0,4	3,1	4 %	10
— 10°	15,00°	23,14 ± 0,07					
— 10°	15,00°	23,14 ± 0,07		0,3	0,3	1 %	4
— 10°	0,02°	3,41 ± 0,002		0,06	0,01	0,3 %	5

Die »Abnahme» (Δk) ist bei »Temp» = 25° noch 10 mal grösser als der mittlere Versuchsfehler, um bei 15° und 0° auf die Hälfte dieser zu sinken. Da die letzten Serien mit verschiedener Versuchsmethodik ausgeführt sind, kann man annehmen, dass die in denselben bestehende Abnahme auf die Gegenwart der α-β-Verbindung in sehr kleiner Menge hindeutet. Über die mutmassliche Grösse derselben siehe unten. — In den Tabellen unten ist t in Min. angegeben; »%» bedeutet prozentische Umsetzung.

Tabelle 2 (Temp. = 0,02°).

(Synthese bei 100°)

t	6,5	11,0	15,0	20,0	28,5	36,0	48,0	
%	1,75	26,9	33,7	40,8	50,7	56,8	64,9	%
k	3,22	3,22	3,20	3,17	3,16	3,15	3,14	

Am einfachsten werden die Tabellen in der folgenden Weise für kinetische Analyse verwertet. Man nimmt an, dass die Synthese — 10° reine α-α_1-Isomere gibt: dass also $k_\alpha = 3{,}41$ bei 0°,02. Für die α-β-Isomere bestimmten wir (siehe unten) für 0°,02: $k_\beta = 0{,}0135$. Wird nun nach Tabelle 2 der Anfangswert extrapoliert, resultiert 3,25. Einfache Mischungsrechnung für $t = 0$ ergibt dann 4,4 % α-β-Chlorhydrin im 100°-Gemisch.

Kinetische Analyse mit *Soda-Bicarbonatpufferlösung* (25°): k_p in Tabelle 3 bedeutet der direkt gefundene Geschw.-Koeffizient (Berechnung des bimol. Koeffizienten (k) siehe Bergkvist, *l. c.*). Für das — 10°-Produkt wurde bei 25° gefunden $10^3 \cdot k_{p\alpha} = 7{,}32 \pm 0{,}07$, woraus $k = 76 \pm 0{,}7$. Ein Versuch mit dem + 100°-Produkt wird in Tabelle 3 wiedergeben.

Tabelle 3 (Temp. 25,0°).

t	15	30	60	90	120	150	180	210
%	10	19	34	46	55	62	69	73
$k_p \cdot 10^3$	7,11	7,00	7,06	7,04	6,94	6,88	6,84	6,72

Zur Zeit t_0 extrapoliert wird der Koeffizient 7,10. Zwei weitere Versuche ergaben 7,11 bzw. 7,08. Mittel also $7{,}10 \cdot 10^{-3}$. Für die α-β-Verbindung (siehe

unten) hat man (25°,0) $k_{p\beta} = 8 \cdot 10^{-5}$. Hieraus berechnet man für das Gemisch 3,0 % α-β-Verbindung. Der k-Wért für die α-β-Verbindung ist unsicher und zwar zu hoch, weil bei der sehr langsamen Reaktion in Pufferlösung gleichzeitig Wasser an das gebildete Epichlorhydrin addiert wird und das entstehende Glycerin-α-monochlorhydrin dann verhältnismässig schnell mit dem Puffer reagiert. Doch spielt in diesem Falle ein Fehler in $k_{p\beta}$ bis auf 100 % keine Rolle für die Analyse.

In Tabelle 4 wird ein Beispiel der Zersetzungsgeschwindigkeiten (Baryt) des α-β-Chlorhydrins bei 25° wiedergeben. Wegen der oben angedeuteten Komplikationen im Reaktionsmechanismus kann kein Mittel genommen werden.

Tabelle 4 (25,0°).

t	60	92	130	176	235	310	410
%	18	25	32	39	48	56	66
$k\beta$	0,359	0,353	0,351	0,344	0,351	0,361	0,384

Wie ersichtlich gehen die k-Werte durch ein Minimum, und dasselbe ist der Fall bei 15° und 0°. Das Ansteigen nach dem Minimum beruht — wie eben gesagt — auf die Bildung von Epichlorhydrin. Die Abnahme *vor* dem Minimum wird am besten erklärt durch die Anwesenheit einer Verunreinigung in kleiner Menge, aber mit grosser Zersetzungsgeschwindigkeit. Mehrere Möglichkeiten zur Bildung einer solchen sind vorhanden. Die Koeffizienten in Tabelle 4 sind alle von der Zeit $t = 0$ berechnet. Durch Rechnung mit der $(t_2 - t_1)$ — Formel unter Vernachlässigung des $(0 - t)$-Wertes wird der Einfluss der Verunreinigung eliminiert. In dieser Weise wurden folgende k_β-Werte (Baryt) (bei etwa 25 % Umsetzung) gefunden: bei 25° 0,334; bei 15° 0,0970 und bei 0,02° 0,0135 (die beiden ersten Werte sind Mittel von je 2 Serien).

2. DIE GÜLTIGKEIT VON MICHAELS REGEL BEI DER DICHLORHYDRINBILDUNG

Diese Regel lautet: »Können zwei isomere oder alloisomere Verbindungen aus einer ungesättigten * Verbindung gebildet werden, so entsteht ein direktes Verhältnis zwischen den relativen Mengen der gebildeten Additionsprodukte und den Geschwindigkeiten, mit welchen sie in die Ursubstanz zurückgeführt werden können.»

Für das anfangs erwähnte System: Glyceringlycid, Chlorwasserstoff und Glycerinmonochlorhydrin gilt, wie früher [8] gezeigt worden ist, diese Regel gut.

* »ungesättigt» im weiteren Sinne.

An der soeben zitierten Stelle habe ich geschrieben, dass »bei den Glycerindichlorhydrinen eine ausgezeichnete Übereinstimmung mit der Regel Michaels sich vorfindet». Ich habe dabei an den Umstand gedacht, dass bei Zimmertemperatur einerseits das Verhältnis der alkalischen Zersetzungsgeschwindigkeiten $k_{\alpha\alpha_1} : k_{\alpha\beta} > 100$ ist, andererseits, dass die Anwesenheit der $\alpha\beta$-Verbindung im Additionsprodukte zu dieser Zeit nicht nachgewiesen war, was auf ein Verhältnis der Additionsgeschwindigkeiten $C_{\alpha\alpha_1} : C_{\alpha\beta} > 100$ hindeutete. Eine Voraussetzung für die allgemeine Gültigkeit der genannten Regel ist jedoch, dass die bei *einer* Temperatur nachgewiesene Gleichheit der Verhältnisse der Additions- und Abspaltungsgeschwindigkeiten auch bei anderen Temperaturen erhalten bleibt. Hier wird ein Versuch gemacht, zu untersuchen, ob die Regel bei der Bildung der Dichlorhydrine bei verschiedenen Temperaturen Gültigkeit hat.

Die Temperaturkoeffizienten der Abspaltungsgeschwindigkeiten (k) werden berechnet (zwischen 25° und 0° *) nach der Formel:

$$\ln k_T = -\frac{A}{T} + B$$

Für die $\alpha\alpha_1$-Verbindung wird erhalten: $A = 9\,810$ $B = 37{,}20$
» » $\alpha\beta$- » » » : $A = 10\,440$ $B = 34{,}00$

Aus diesen Werten ergeben sich wiederum die Aktiveringsenergien $E_{\alpha\alpha_1} = 19\,500$ und $E_{\alpha\beta} = 20\,700$.

Die Geschwindigkeiten bei 100°, bzw. -10° werden hieraus berechnet und aus diesen dann die Geschwindigkeitsverhältnisse ($k_{\alpha\alpha} : k_{\alpha\beta}$). Bei 100° wird das Verhältnis = 130, bei - 10° = 270. Wäre die Regel Michaels gültig bei diesen Temperaturen, würden auch die Prozentgehalte der $\alpha\alpha_1$-Verbindung zu denjenigen der $\alpha\beta$-Verbindung im Gemisch sich wie diese Zahlen verhalten, d. h. das Reaktionsprodukt würde bei 100° 0,8 %, bei -10° 0,4 % $\alpha\beta$-Verbindung enthalten.

Bei den Synthesen wurde gefunden:
für das bei 100° dargestellte Chlorhydringemisch (siehe oben): % $\alpha\beta$-Verbindung = 4,4 bzw. 3,0, *also im Mittel 3,7* %. Für das »*-10°-Produkt*» entspricht die Abnahme Δk (Tabelle 1) bei 0,02° einem Gehalte *von 0,3* % α-β-Verbindung, allerdings eine sehr grobe Schätzung.

Die Gültigkeit der Regel bei verschiedenen Temperaturen kann durch unsere Versuche nicht als bewiesen angesehen werden. Die Nichtgültigkeit

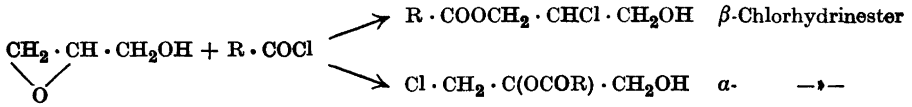
* Berechnungen unter Rücksichtsnahme auf die Werte bei 15° gibt ziemlich genau dasselbe Resultat.

vielleicht auch nicht, denn die Berechnung der Abspaltunggeschwindigkeiten bei 100° aus denjenigen bei 0—25° bedeutet eine unsichere Extrapolation, bei der α-β-Verbindung besonders unsicher aus dem Grunde, dass die Reaktion derselben mit Alkali, wie oben erwähnt wurde, einige Nebenreaktionen in sich schliesst.

3. NOTIZEN ÜBER DIE ADDITION VON SÄURECHLORIDEN AN GLYCERINGLYCID

Experimente von ANDERS KYLIN und STIG ANDERSSON

Die Reaktion zwischen Säurechloriden und Glyceringlycid ist kompliziert. Einerseits kann eine direkte Addition unter Bildung von zwei acylierten Chlorhydrinen stattfinden:



Diese werden dann nachher eventuell an der Hydroxylgruppe acyliert. Andererseits könnte das Glycid *zuerst* an dieser Gruppe angegriffen werden, wonach der gebildete Glycidester ein Molekül Säurechlorid addierte. Es ist indessen anzunehmen, dass eine Veresterung des Glycidhydroxyls keine wesentliche Veränderung der Proportionen zwischen den schliesslich gebildeten α- und β-Chlorhydrinestern verursachen wird. Substituiert wird ja der Hydroxylwasserstoff, welcher in β-Stellung zum Glycidring steht. Es ist mit andren Worten meiner Auffassung nach für die Stellung des Chloratoms im Molekül von keinem grösseren Belang, dass bei der obengenannten Reaktion der wahre Mechanismus unbekannt ist und dass als Reaktionsprodukte bisweilen Mono-, bisweilen Diester der Glycerinmonochlorhydrine entstehen.

Rider [9] hat Acetylchlorid, bzw. *p*-Nitrobenzoylchlorid an Glyceringlycid addiert und dabei durch Darstellung der krystallisierten Phenylurethanderivate nachweisen können, dass die beiden möglichen Isomeren auch gebildet werden. Quantitative Proportionen werden nicht angegeben. Er meint, dass eine Alkoholyse zu Chlorhydrin und nachfolgende Reinigung desselben Verluste durch Destillation mit sich bringen würde, welche die quantitativen Resultate einer kinetischen Analyse illusorisch machen sollten. Indessen verschieben sich durch eine Destillation ohne Dephlegmator die Proportionen zwischen den Isomeren nicht wesentlich und ein Versuch, durch kinetische Analyse den Verlauf der in Frage kommenden Addition aufzuklären, schien daher nicht aussichtslos.

Reaktion mit Acetylchlorid. Die Synthese wurde auf zwei Weisen ausgeführt. Zuerst stellten wir Monochlorhydrindiacetat dar nach der Beschreibung Riders. Das Acetylchlorid wurde unter Rückfluss *zum Sieden* erhitzt und dann unter Nachlassen der Erwärmung das Glycid tropfenweise hineingelassen. Alkoholyse mit HCl-Metanol. Die Ausbeuten waren die von Rider angegebenen; das resultierende Chlorhydringemisch war analysenrein (Cl: ber. 32,09; gef. 32,18 %) Bei der zweiten Synthese wurden 27 g Acetylchlorid in 80 ml absol. Äther zu 26 g Glycid in 80 ml *bei Zimmertemperatur* unter Rührung tropfenweise zugesetzt; Zeit etwa 1 Std. Nach weiteren 2 Stunden wurde der Äther abdestilliert. Die Reaktionskomponenten lagen in äquimolekularen Mengen vor. Es wurde nach Beendigung der Reaktion nachgewiesen, dass *keine Chloridionen gebildet worden waren:* diese Synthese stellt somit *eine reine Addition dar.* Das Produkt — das Monochlorhydrinmonoacetat (Ausbeute 67 %) — siedete (15 mm) bei 120—122°. Die Analyse zeigte, dass die Substanz bei Hydrolyse mit Alkali 2 Äquivalente Alkali verbrauchte und gleichzeitig 1 Äquivalent Chloridion produzierte.

Alkoholyse ergab Monochlorhydrin in einer Ausbeute von 80 %.

Analyse: Cl gef. 31,8 %.

Reaktion mit Benzoylchlorid. Da es gewisse Schwierigkeiten bot, das Monochlorhydrindibenzoat darzustellen, wurden äquimolekulare Mengen von Benzoylchlorid und Glycid zur Reaktion gebracht (4 Std. auf dem Wasserbade). Bei nachfolgender Destillation (etwa ½ mm) wurde eine Fraktion zwischen 125—165° genommen, eine andere zwischen 165—205° (je etwa ¼ der Gesamtmenge); jene war ziemlich reines Monobenzoat (Cl: ber. 16,83 %, gef. 17,00; 17,05 %), diese Dibenzoat (Cl: ber. 11,13; gef. 11,06; 11,05 %). Das Monobenzoat wurde mit Methanol alkoholysiert, wobei vor der abschliessenden Destillation mit Bleikarbonat geschüttelt wurde. Ausbeute an Methylbenzoat quantitativ, an Monochlorhydrin nur 66 %, wahrscheinlich wegen Destillationsverlusten bei der kleinen Substanzmenge. Analyse des Chlorhydrins: Cl: ber. 32,09, gef. 32,15. Versuche, Benzoylchlorid an Glyceringlycid in Ätherlösung zu addieren, fielen negativ aus: nach 15 stündigem Kochen unter Rückfluss war noch keine Reaktion bemerkbar.

Bei den *kinetischen Analysen* der entstehenden Chlorhydringemische wurden die Bedingungen und Rechenmethoden innegehalten, die früher angegeben sind (siehe oben). Die Zersetzungsgeschwindigkeiten wurden mit Baryt bei 25° bestimmt. Die Prozentgehalte der beiden Isomeren können entweder durch eine empirisch-mathematische Rechenmethode[10] oder durch graphische Interpolation[11], welche unten mit Prozent$_r$ (P_r), bzw. Prozent$_{gr}$ (P_{gr})bezeichnet sind, festgestellt werden.

Kinetische Doppelanalyse der Monochlorhydringemische.

1) Aus dem *Diacetat:* P_r = 22,1 und 25,0 %; Mittel = 23 ½ % β-Monochlorhydrin
   K_{gr} = 23 ½ und 23 ½ %; Mittel = 23 ½ % β-Monochlorhydrin.
2) Aus dem *Monoacetat:* 2 kinetische Serien ergaben übereinstimmend P_{gr} = 16—17 %.

Der Unterschied ist bedeutend und weit ausserhalb der Versuchsfehler. Über die Ursachen dieses sehr interessanten Unterschiedes lässt sich vorläufig keine Aussage machen: die Bedingungen der beiden Synthesen sind jedoch ziemlich verschieden: das Diacetat war z.B. bei etwa 50° ohne Lösungsmittel, das Monoacetat bei etwa 20° in Ätherlösung hergestellt worden. Eine Erhöhung der Temperatur wirkt erhöhend auf den Gehalt von β-Chlorverbindung, aber wahrscheinlich viel weniger, als es hier der Fall ist.

3) Aus dem *Monobenzoat:*

$$k_r = 17{,}3 \text{ und } 15{,}7\ \%;\ \text{Mittel} = 16{,}5\ \%$$
$$k_{gr} = 16 \quad » \quad 16\ \%;\ \text{Mittel} = 16\ \%$$

Das Diacetat und Monobenzoat sind unter gleichartigen Bedingungen, das letztere doch bei einer höheren Temperatur dargestellt. Der Temperaturunterschied würde indessen wahrscheinlich (siehe oben) eine Veränderung der Prozentgehalte in entgegensetzter Richtung bewirken. In Betracht der geringen Anzahl der Versuche und des neuen Gebietes scheint es indessen nicht angemessen über die Ursachen der ein wenig unerwarteten Resultate jetzt zu spekulieren.

Zum Vergleich soll hier daran erinnert werden, dass bei Addition von Chlorwasserstoff (bzw. Bromwasserstoff [5]) an Glyceringlycid der Prozentgehalt an β-Verbindung 10 % (bzw. 7 % [12]) ist.

ZUSAMMENFASSUNG

Bei der Addition von Chlorwasserstoff an Epichlorhydrin enthält das Produkt, sehr wahrscheinlich auch bei Reaktion bei niedriger Temperatur, ein wenig α-β-Glycerindichlorhydrin. Wir fanden von dieser Verbindung bei — 10° etwa 0,3 %, bei 100° etwa 4 %.

Die Regel von Michael über das Verhältnis zwischen Additions- und Abspaltungsgeschwindigkeiten gilt gut bei — 10°: ber. 0,4 % α-β-Verbindung, gef. 0,3 %. Inwieweit diese Regel auch bei 100° gültig ist, konnte nicht entschieden werden.

Bei Addition von Acetylchlorid an Glyceringlycid wurde gefunden: ohne Lösungsmittel (bei etwa 50°) 23 % β-Monochlorhydrin (als Diacetat), in Ätherlösung (bei Zimmertemperatur) 16—17 % β-Monochlorhydrin (als Monoacetat). Diese letzte Reaktion stellt somit eine reine *Addition* von Acetylchlorid dar.

Benzoylchlorid reagiert mit Glyceringlycid (äquiv. Mengen Wasserbad) unter Bildung von einem Gemisch von Chlormono- und Chlordibenzoaten. Das Monobenzoat enthält zu 16 % das Chloratom in β-Stellung.

Alle Prozentgehalte wurden durch kinetische Analyse ermittelt.

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Eingegangen am 6. September 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1386–1389

Action of Strong Acids on Acetylated Glycosides

VI. Transglycosidation of Galactosides *

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The previus papers in this series have dealt with the transglycosidation of acetylated glucosides. It would be of interest to know whether the results of these investigations could be applied to glycosides of other sugars. In this paper the investigation of some galactosides is reported.

β-Galactose pentaacetate, in a solution of sulfuric acid in acetic anhydride--acetic acid, was transformed into the equilibrium mixture of α- and β-acetates at a rate which was 2.5 times faster than the analogous reaction for β-glucose pentaacetate. When ethyl β-galactoside tetraacetate was dissolved in the same solvent and the optical rotation observed, it was found to change in a similar manner to that of ethyl β-glucoside tetraacetate [1] under the same conditions (Fig 1.) The maximum rotation in both cases corresponds to an accumulation of α-glycoside. This has been demonstrated by isolation of the α-galactoside from the reaction mixture when this showed maximum rotation. The rotation then decreased below the value calculated on the assumption that only α/β--galactose pentaacetate is formed by the acetolysis of the galactoside. From analogy with the glucosides one must assume that this depends upon the formation of some galactose heptaacetate, which, similar to other *al*-sugars, has a rather low optical rotation. When the α-galactoside was treated in the same way, the rotation decreased rapidly to the same value as that for the maximum mentioned above, and then the values coincided with those for the β-galactoside. *iso*-Propyl β-galactoside tetraacetate and β-chloroethyl β-galactoside tetraacetate have also been investigated. For the former the transglycosidation is faster and the percentage of heptaacetate in the final product higher than for the ethyl derivative. For the latter the transglycosidation is slower than the α/β-transformation of the acetate. Thus the analogy with the

* Part V, this journal 3 (1949) 1355.

glucose derivatives is complete. From the rotations of the pure α- and β-derivatives the percentage of α-isomer in the equilibrium mixture can be calculated. The values for galactose pentaacetate and ethyl galactoside tetraacetate are 87 % and 82 % respectively, while the values for corresponding glucose derivatives are 89 % and 89 %.

The velocity constants for the α/β-transformation and acetolysis of the galactosides, compared with those for the corresponding glucose derivatives are given in Table 1. The velocity of the transformation of β-glucose pentaacetate to the equilibrium mixture is taken as unity.

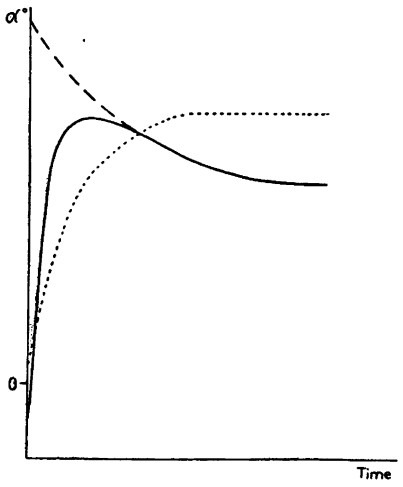

Fig. 1. Transglycosidation and acetolysis of the tetraacetates of α- and β-ethyl galactoside.

——— *Ethyl β-galactoside*
– – – – *Ethyl α-galactoside*
· · · · · · · · *β-Galactose pentaacetate*

From Table 1 it can be seen that the transglycosidation is faster for the galactose than for the glucose derivatives. The acetolysis is also faster. From this small amount of data, however, it is not possible to find any simple mathematical relationship between the different velocity constants.

Table 1. Relative velocity constants for transglycosidation and acetolysis of some acetylated galactose and glucose derivatives.*

	β-Pentaacetate	β-Ethyl		β-*iso*-Propyl		β-Chloroethyl
	$k_{\alpha/\beta\text{-transform.}}$	$k_{\text{trans-glyc.}}$	$k_{\text{aceto-lysis}}$	$k_{\text{trans-glyc.}}$	$k_{\text{aceto-lysis}}$	$k_{\text{transglyc.}}$
Galactose	2.5	31	0.35	71	0.15	0.7
Glucose	1	15	0.07	50	0.08	0.4
$\frac{k_{\text{galactose}}}{k_{\text{glucose}}}$	2.5	2.1	5	1.4	2	1.8

* The k-values are determined at different concentrations of sulfuric acid and hence not strictly comparable. See experimental part.

Ethyl α-galactoside tetraacetate was prepared by treating the β-galactoside with titanium tetrachloride. This reagent has been used chiefly for the transglycosidation of glucosides, but was, as expected, equally suitable for galactosides.

EXPERIMENTAL

Ethyl β-galactoside tetraacetate

Ethyl β-galactoside tetraacetate, which is described in the literature [2], was prepared here by the mercuric acetate method. A solution of galactose bromide tetraacetate (8.22 g) and mercuric acetate (3.03 g) in a mixture of absolute benzene (40 ml) and ethanol (16 ml) was boiled on the steam bath for 15 minutes. After cooling it was washed several times with water, dried over calcium chloride and concentrated under reduced pressure. The oily residue was dissolved in hot ethanol-water, 1 : 1, and on cooling somewhat sticky crystals were obtained, which were recrystallized from the same solvent. Yield 5.3 g (72 %) M. p. 86—87°*.

iso-Propyl β-galactoside tetraacetate

iso-Propyl β-galactoside tetraacetate was prepared by the same method as the β-ethyl galactoside and in a yield of 80 %. It was recrystallized from light petroleum. M. p. 58 $-59°$ $[\alpha]_D^{20} -9°$ (Chloroform, C = 2).

$C_9H_{14}O_6$ $(OCCH_3)_4$ (390.4). Calc. Acetyl 44.1 Found Acetyl 44.5

β-Chloroethyl β-galactoside tetraacetate

β-Chloroethyl β-galactoside tetraacetate was prepared by the same method as the ethyl β-galactoside and recrystallized from ethanol. Yield, 54 %. M. p. 115—116° $[\alpha]_D^{20} -5°$ (Chloroform, C = 2). Coles, Dodds and Bergeim [3] report the melting point as 117° but give no value for the optical rotation.

Ethyl α-galactoside tetraacetate

With titanium tetrachloride: A solution of ethyl β-galactoside tetraacetate (3 g) and titanium tetrachloride (1.5 g) in absolute chloroform (90 ml) was boiled on the steam bath for 45 minutes. When cold, the mixture was washed with ice water, dried over calcium chloride and concentrated under reduced pressure. The residue was recrystallized from light petroleum. Two recrystallizations yielded the pure substance. Yield, 2.0 g (67 %) M. p. 85—86° $[\alpha]_D^{20} + 136°$ (Chloroform, C = 2).

$C_8H_{12}O_6$ $(OCCH_3)_4$ (376.4) Calc. Acetyl 45.7 Found 45.8.

With sulfuric acid: Ethyl β-galactoside tetraacetate (2 g) was dissolved in a mixture of sulfuric acid (0.02 ml), acetic anhydride (8 ml) and acetic acid (2 ml). The optical rotation of the solution was observed, and when this showed maximum value the mixture was poured into ice water (150 ml) containing sodium acetate (1 g). A white

* All melting points uncorrected.

precipitate was slowly formed. This was filtered off and recrystallized from ethanol-water, 1 : 1. Yield, 1.1 g (55 %) M. p. 84—86°, alone or on admixture with the ethyl α-galactoside tetraacetate described above.

Typical run

The experimental conditions for the kinetic determinations were the same as in the preceding papers (Compare Part III[1]) For the transglycosidation and the acetolysis runs sulfuric acid of concentration 0.03 C and 0.5 C respectively was used. For the transglycosidation of β-chloroethyl β-galactoside however, the concentration of sulfuric acid was 0.4 C. The solvent was a mixture of acetic anhydride-acetic acid, 10 : 3.

Table 2. Transglycosidation of iso-propyl β-galactoside tetraacetate. β-Galactose pentaacetate and iso-propyl β-galactoside tetraacetate, 0.5 g of each, dissolved in 20 ml of 0.031 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min.	β-Galactose pentaacetate		*iso*-Propyl β-galactoside tetraacetate	
	α_D	k	α_D	k
0	+ 1.42		— 0.14	
4	1.46	0.00250	+ 2.42	0.0625
8	1.54	237	3.87	630
12	1.59	209	4.68	632
16	1.64	200	5.18	656
20	1.74	220	5.40	644
30	1.94	233	5.66	
40	2.06	215	5.70_{max}	
∞	5.17			
	Mean value 0.0022		Mean value 0.063	

SUMMARY

The transglycosidation of some galactosides with sulfuric acid and titanium tetrachloride has been investigated. The reaction is somewhat faster than that of the corresponding glucosides but otherwise perfectly analogous.

The authors wish to thank *Statens Naturvetenskapliga Forskningsråd* för financial support.

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Received September 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1390–1392

The Iodine-Azide Reaction

VI. The Catalytic Effect of Monoseleno- and Monotelluropentathionate Ions

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Keeping in mind that, according to Foss [1], pentathionate ions must be assumed to be monosulphur di(thiosulphate) ions, it was also to be expected that monoseleno and monotelluro di(thiosulphate) ions would show a strong catalytic effect on the reaction between azide ions and iodine.

Sodium selenopentathionate, $Na_2Se(S_2O_3)_2 \cdot 3\ H_2O$, and sodium telluropentathionate, $Na_2Te(S_2O_3)_2 \cdot 2\ H_2O$, were prepared and analyzed as outlined by Foss [2, 3].

Firstly kinetic experiments were carried out in the same way as with potassium pentathionate [4]: Into a flask containing a solution of sodium azide, iodine, and starch indicator, was pipetted 10 ml 0.0002 *M* sodium selenopentathionate. Already before this solution had completely run down into the flask, all the iodine present had been consumed with a brisk evolution of nitrogen. In case of potassium pentathionate, the corresponding time of reaction was 3.18 min (ref. 4, Expt. no. 1, Table 1). A similar experiment was then carried out with 10 ml 0.00002 *M* sodium selenopentathionate solution. In this case some of the iodine was consumed immediately, thereafter nothing more happened. The two experiments show that selenopentathionate ions, when catalyzing the iodine-azide reaction, immediately are converted into substances, which are relatively inactive towards this reaction. The substances are presumably selenious acid, tetrathionate, and sulphate; at least, these three compounds could be detected after the iodine-azide reaction had taken place.

Since solutions of potassium tetra- and pentathionate react with sodium azide with the evolution of nitrogen, it was to be expected that a similar reac-

tion would take place between selenopentathionate and azide ions. This was also the case. At the same time a considerable amount of red selenium was formed. The gas was analyzed according to Christiansen and Wulff [5]; the result was 99.8 % nitrogen and 0.2 % oxygen.

Telluropentathionate was known to undergo immediate decomposition when catalyzing the iodine-azide reaction, because it is readily oxidized by iodine, to give tellurous acid and tetrathionate [3].

However, it was possible to compare the catalytic power of seleno- and telluropentathionate with that of thiosulphate by simple titrations: Into a 500 ml Erlenmeyer flask was pipetted 10 ml 0.01 *N* iodine, which was 0.02 *M* with respect to potassium iodide, 10 ml 0.1 *M* sodium azide, 1 ml 0.5 % starch solution; and water up to a total volume of 200 ml was added. This mixture was titrated by dropwise addition of thiosulphate, and, alternatively, seleno- and telluropentathionate solutions, until all iodine was consumed. The addition was performed at a rate of approximately one drop per two seconds, and the reaction mixture was briskly swirled by hand during the addition.

Table 1. The catalytic effect of seleno- and telluropentathionate on the iodine-azide reaction compared with that of thiosulphate. Temp.: ~ 20° C.

Solutions used for the titration	Amount of solution used for the titration ml		
10^{-3} *M* thiosulphate	13.87	14.03	14.37
10^{-3} *M* selenopentathionate	3.54	3.65	3.61
5.10^{-4} »	7.58	6.70	7.16
10^{-3} *M* telluropentathionate	3.56	3.66	3.62
5.10^{-4} *M* »	6.76	7.24	6.95

The results (Table 1) in duplicate runs did not agree too well, but that was presumably due to sensitiveness to variation in experimental method (method of adding the solutions and mode of stirring). Dodd and Griffith [6] also found that same sensitiveness when carrying out similar experiments with thiosulphate as the catalyst. However, it was obvious that seleno- and telluropentathionate showed quantitatively the same catalytic effect, which

was larger than that of thiosulphate. In all three cases tetrathionate and sulphate were found after the reaction had taken place. This seemed to indicate that the catalytic effect of seleno- and telluropentathionate was caused by liberated thiosulphate ions. Dodd and Griffith [6] found that when titrating a solution of iodine and sodium azide with thiosulphate, the ratio R (moles I_2 consumed/moles $Na_2S_2O_3$ consumed) increased with decreasing concentration of thiosulphate. The reason why seleno- and telluropentathionate are found to be more catalytically active than thiosulphate, could possibly be that, in a short interval of time, they are the source of a very small thiosulphate concentration.

SUMMARY

The catalytic effect of monoseleno- and monotelluropentathionate ions toward the iodine-azide reaction was found to be larger than that of thiosulphate ions. Seleno- and telluropentathionate ions are decomposed immediately when acting as catalysts, and their effect seems actually to be a thiosulphate catalysis.

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Received September 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1393–1400

Action de l'ammoniaque et des amines aliphatiques sur les O.S-(1,2)-arylène-thiocarbonates

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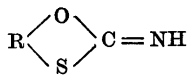
La chimie des thiocyanates appartenant à la série aromatique connait un cas d'anomalie qui était peu étudié jusqu'à maintenant.

Notamment certains phénols et naphtols, au lieu de fournir des thiocyanates normaux du type:

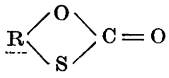
$$OH-R-SCN$$

conduisent à des 0.S-(1,2)-arylène-imino-thiocarbonates de formule générale:

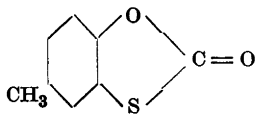


où R = arylène.

En milieu aqueux acide ces derniers se décomposent même à froid pour donner naissance à des 0.S-(1,2)-arylène-thiocarbonates exempts d'azote:

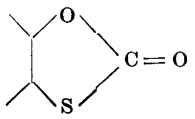


Kaufmann a décrit un ester de ce genre dérivant du para-crésol [1]:

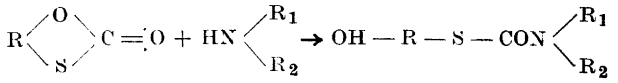


Werner en a préparé toute une série en partant des polyphénols et polynaphtols [2].

En examinant les propriétés des 0.S-(1,2)-arylène-thiocarbonates décrits par Kaufmann et Werner, nous avons pu faire la constatation suivante. — Sous l'influence de l'ammoniaque ainsi que des amines aliphatiques primaires et secondaires, ces esters subissent l'ouverture du cycle:

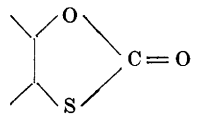


tout en se condensant avec les bases mises en présence. Les carbamates des hydroxyarylthiols, composés peu connus dans la littérature [3, 4], prennent naissance dans la réaction:



où R = arylène, R_1 et R_2 = H ou alcoyle.

Cette ouverture du cycle



se traduit naturellement par la réapparition de la réactivité du groupe hydroxyle. Ainsi, p. ex., le carbamate dérivant du para-crésol copule avec les composés diazoïques d'une façon identique à celle du para-crésol lui-même, le thiocarbonate correspondant exempt d'azote étant parfaitement inactif en ce sens, sans parler que ce dernier est insoluble dans la soude diluée.

La réaction peut être effectuée en milieux aqueux ou dans un solvant organique tel que l'acétone. Pour rendre la réaction complète on emploiera un excès d'ammoniaque ou d'amine.

Nous signalerons que parmi les facteurs régissant la vitesse de la réaction la grosseur des groupes alcoyle de l'amine ainsi que le nombre de groupes hydroxyle dans la molécule du 0.S-(1,2)-arylène-thiocarbonate, produit de départ, semblent être d'une grande importance.

L'exemple de l'empêchement stérique dû à la nature des groupes alcoyle nous est fourni par le comportement du thiocarbonate dérivant de la résorcine vis-à-vis des amines. Voici les résultats obtenus dans une série d'essais effec-

tués en milieu aqueux à 30—35° avec 2 fois la quantité théorique d'amine, la concentration de cette dernière étant 6 mol. par litre:

Nature de l'amine	Proportion de l'amine absorbée dans la réaction en espace de 10 min. par rapport à la quantité théorique
CH_3NH_2	100 %
$C_2H_5NH_2$	75 %
$(CH_3)_2NH$	100 %
$(C_2H_5)_2NH$	70 %

Quant à l'influence du nombre de groupes hydroxyle, on s'en rendra compte du tableau suivant, dont les données sont calculées de la même façon et les conditions d'expérience étant les mêmes que plus haut:

Nature du thiocarbonate	Nature de l'amine	
	$C_2H_5NH_2$	$(C_2H_5)_2NH$
Dérivé du para-crésol	100 %	85 %
Dérivé de la résorcine	75 %	70 %
Dérivé de la phloroglucine	55 %	30 %

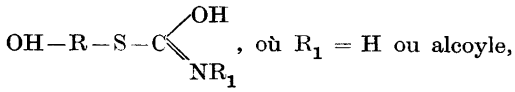
Les rendements en produits de condensation étant élevés dans plusieurs cas, la réaction peut donc constituer une méthode de synthèse de certains carbamates des mono- ou des dihydroxyarylthiols.

Notre étude du thiocarbonate dérivé d'un trihydroxybenzène (de la phloroglucine) ne nous a malheureusement pas permis d'obtenir de composés définis. Néanmoins, nous pouvons affirmer avec certitude que la condensation ait lieu dans ce cas également, la réaction en question présentant donc un caractère très général.

A côté des mono-amines du type le plus simple (telles que la méthylamine, l'éthylamine, la diméthylamine etc.) nous avons fait graviter dans notre expérimentation une diamine hétérocyclique à caractère saturé — la pipérazine. Cette diamine réagit facilement avec les thiocarbonates dérivant du para-crésol et de la résorcine, p. ex., mais ne se condense pas avec le thiocarbonate dérivant de la phloroglucine.

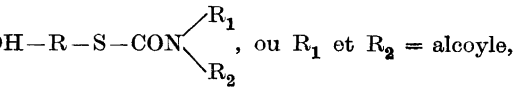
Les nouveaux produits donnent des réactions caractéristiques avec certains cations (Ag^+, Pb^{++}, Tl^+, Hg^{++} etc.), propriété signalée par Conrad et Salomon pour le carbamate d'éthane-thiol [5].

Il est à remarquer ici que les carbamates des hydroxyarylthiols, dont la constitution prévoit l'existence de la forme tautomère:



, où R_1 = H ou alcoyle,

conduisent à des composés métallifères *colorés* (cas des dérivés de l'ammoniaque et des amines primaires), tandis que les carbamates n'offrant pas de possiblité de tautomérisation

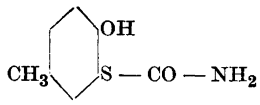


, ou R_1 et R_2 = alcoyle,

semblent inactifs ou donnent des produits *incolores*.

PARTIE EXPÉRIMENTALE

1. Carbamate du 2-hydroxy-5-méthylphénylthiol



Le produit s'obtient par action de l'ammoniaque sur le 0.S-(1,2)-[5-toluylène]-thiocarbonate. Ce dernier est préparé suivant les indications de Kaufmann (*l. c.*).

1,66 g de thiocarbonate sont agités avec un mélange de 4 cm³ d'ammoniaque 10 *N* et de 4 cm³ d'acétone jusqu'à dissolution complète. On laisse reposer la solution obtenue pendant 30 min., verse dans 8 cm³ d'eau glacée et acidifie jusqu'à réaction au Congo avec de l'acide chlorhydrique 5 *N*. Le précipité cristallin formé est filtré, lavé à l'eau froide et séché sous vide. Cristallisation dans le benzène.

Lamelles brillantes, *F* = 119°, pratiquement insolubles dans l'eau, facilement solubles dans la soude diluée, dans l'alcool, dans le chloroforme à chaud.

NO_3Ag, OHTl et $(CH_3COO)_2Pb$ en solution aqueuse provoquent, par addition à la solution hydro-acétonique du produit, la formation des précipités jaunes caractéristiques.

Le produit, dissout dans l'alcool méthylique, copule en présence d'acétate de soude anhydre avec le diazonanhydride de l'acide sulfanilique.

$C_8H_9O_2NS$ (183)	Calc.	C 52,46	H 4,92	N 7,69	S 17,48
	Trouvé	» 52,68	» 4,72	» 7,68	» 17,70

2. Méthylcarbamate du 2-hydroxy-5-méthylphénylthiol

1,66 g de 0.S-(1,2)-[5-toluylène]-thiocarbonate sont agités avec 2,8 cm³ d'une solution aqueuse de méthylamine à 7,3 mol. par litre. Après dissolution on laisse reposer pendant 15 min., ajoute 2,8 cm³ d'eau froide, acidifie avec de l'acide chlorhydrique 5N, sépare par

décantation le précipité pâteux, reprend ce dernier avec de l'eau aiguisée d'acide chlorhydrique et filtre le lendemain. Séchage sous vide. Cristallisation dans le benzène.

Propriétés analogues à celles du produit précédemment décrit. F = 130°.

$C_9H_{11}O_2NS$ (197)	Calc.	N	7,10
	Trouvé	»	7,18

3. Diméthylcarbamate du 2-hydroxy-5-méthylphénylthiol

Le produit est préparé comme son homologue obtenu avec la méthylamine. On le fait cristalliser dans un mélange à volumes égaux de chloroforme et d'éther de pétrole ($E<70°$).

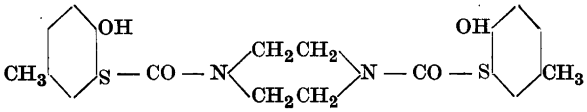
Ainsi purifié, il est facilement soluble dans l'alcool, le chloroforme et le benzène, insoluble dans l'eau, soluble dans la soude diluée. F = 116°.

Pas de réaction appréciable avec NO_3Ag, ni $(CH_3COO)_2Pb$. OHTl donne un précipité blanc.

Le produit se comporte vis-à-vis du diazoanhydride de l'acide sulfanilique d'une façon analogue à celle des carbamates décrits plus haut.

$C_{10}H_{13}O_2$ (211)	Calc.	N	6,63
	Trouvé	»	6,57

4. Pipérazine-dicarbamate du 2-hydroxy-5-méthylphénylthiol

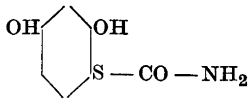


2,5 g de 0.S-(1,2)-[5-toluylène]-thiocarbonate sont dissous à chaud dans 7,5 cm³ d'acétone. Dans cette solution, refroidie à 20°, on ajoute le mélange suivant: 7,5 cm³ d'une solution aqueuse de pipérazine à 1,5 mol. par litre + 7,5 cm³ d'acétone. Après 5 min. d'agitation énergique on filtre le faible résidu insoluble et laisse reposer le filtrat pendant 30 min. Le produit de condensation précipite; il est filtré, lavé avec un mélange à volumes égaux d'acétone et d'eau, à l'eau aiguisée d'acide chlorhydrique, à l'eau pure, à l'alcool chaud et finalement à l'éther.

Le dicarbamate purifié de la sorte est insoluble dans l'eau et dans les solvants organiques usuels. Il se dissout dans la soude diluée. F = 185°.

$C_{20}H_{22}O_4N_2S_2$ (418)	Calc.	N	6,69
	Trouvé	»	6,90

5. Carbamate du 2,4-dihydroxyphénylthiol



C'est en partant du thiocarbonate dérivant de la résorcine qu'on obtient ce composé. — On dissout donc 8,4 g de 0.S-(1,2)-[4-hydroxyphénylène]-thiocarbonate de Werner dans 35 cm³ d'ammoniaque à 10 % en vol. La solution est ensuite refroidie avec de la glace

et laissée reposer à froid pendant 1 h 30 min. On la traite alors à la manière habituelle. Le produit de réaction, après dessiccation sous vide, est cristallisé dans un mélange de chloroforme et d'alcool méthylique (15 cm³ de ce dernier pour 100 cm³ de chloroforme).

Aiguilles légérement jaunâtres, solubles dans l'eau à 20° en raison de 2 % env., facilement solubles dans l'alcool, difficilement dans l'éther et le chloroforme, pratiquement insolubles dans le benzène. F = 105°. Coloration ou précipité jaune caractéristique avec NO_3Ag, OHTl, $(CH_3COO)_2Pb$, Cl_2Hg. Réactivité comparable à celle de la résorcine vis-à-vis du diazoanhydride de l'acide sulfanilique (le produit copule avec 3 molécules de diazoïque).

$C_7H_7O_3NS$ (185)	Calc.	N 7,56
	Trouvé	» 7,26

6. Méthylcarbamate du 2,4-dihydroxyphénylthiol

On opère d'une manière identique à celle de l'essai précédent, en remplaçant l'ammoniaque par une solution aqueuse de méthylamine à 3,75 mol. par litre. On fait cristalliser le produit de condensation dans un mélange de chloroforme et d'alcool méthylique renfermant 4 cm³ d'alcool pour 100 cm³ de chloroforme.

Propriétés analogues à celles du carbamate précédemment décrit. F = 139°.

$C_8H_9O_3NS$ (199)	Calc.	C 48,24	H 4,52	N 7,03	S 16,08
	Trouvé	» 48,37	» 4,73	» 6,96	» 15,49

7. Diméthylcarbamate du 2,4-dihydroxyphénylthiol

La condensation du 0.S-(1,2)-[4-hydroxyphénylène]-thiocarbonate avec la diméthylamine est effectuée en milieu aqueux suivant le mode opératoire habituel, la durée de la réaction étant de 15 min. au total. Le carbamate est cristallisé dans le chloroforme. Il est difficilement soluble dans l'eau, tandis que dans les solvants organiques il se comporte sensiblement de la même façon que les dérivés correspondants de l'ammoniaque et de la méthylamine. F = 142°.

OHTl seul provoque la formation d'un précipité qui est blanc, les sels d'argent, de plomb et de mercure ne donnant aucune réaction appréciable.

Même réactivité vis-à-vis du diazoanhydride de l'acide sulfanilique que dans le cas du carbamate correspondant non substitué en N.

$C_9H_{11}O_3NS$ (213)	Calc.	N 6,57
	Trouvé	» 6,61

8. Diéthylcarbamate du 2,4-dihydroxyphénylthiol

Nous avons préparé ce produit en opérant dans l'acétone, la concentration des corps réagissants (du 0.S-(1,2)-[4-hydroxyphénylène]-thiocarbonate et de la diéthylamine) étant la même que dans les essais précédents, effectués en milieu aqueux. Après 30 min. de repos à la température ordinaire, le mélange réactionnel, rendu acide avec de l'acide acétique en solution dans l'acétone, est versé dans l'eau froide. Le produit de la réaction est filtré le lendemain. Séché sous vide, il est purifié par cristallisation dans le chloroforme.

Propriétés similaires à celles de son homologue dérivé de la diméthylamine. $F = 150°$.

$C_{11}H_{15}O_3NS$ (241)	Calc.	N	5,90
	Trouvé	»	5,80

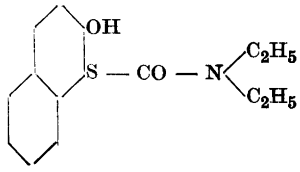
9. Pipérazine-dicarbamate du 2,4-dihydroxyphénylthiol

En utilisant à peu de choses près la même technique que dans la préparation du pipérazine-dicarbamate du 2-hydroxy-5-méthylphénylthiol, nous condensons le 0.S-(1,2)-[4-hydroxyphénylène]-thiocarbonate avec la pipérazine.

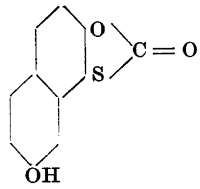
Le produit obtenu possède des propriétés très voisines de celles du dicarbamate dérivé du para-crésol. F = 215°.

$C_{18}H_{18}O_6N_2S_2$ (422)	Calc.	N	6,63
	Trouvé	»	6,82

10. Diéthylcarbamate du 2,7-dihydroxynaphtylthiol



Le produit point de départ, c. à d. le 0.S-(1,2)-[7-hydroxynaphtylène]-thiocarbonate:



n'étant que mentionné et non décrit dans la littérature (voir Werner, *l. c.*), nous donnerons ici quelques détails sur sa préparation et ses propriétés.

Pour hydrolyser l'iminothiocarbonate correspondant en thiocarbonate qui nous intéresse, on opérera en milieu hydro-alcoolique, car le chlorhydrate de l'iminothiocarbonate est beaucoup trop difficilement soluble dans l'eau pure. On utilisera le même mélange d'acide chlorhydrique, d'alcool éthylique et d'eau que préconise Kaufmann pour préparer le 0.S-(1,2)-[5-toluylène]-thiocarbonate (loc. cit.). Après 3 h d'ébullition à reflux on laissera cristalliser le thiocarbonate dans le mélange réactionnel. Le produit peut être utilisé tel quel pour la transformation ultérieure.

Recristallisé dans l'alcool dilué, il se présente sous forme de fines aiguilles incolores, fondant à 212°. Insoluble dans l'eau, il se dissout à chaud dans le benzène et chloroforme.

La condensation avec la diéthylamine est effectuée suivant le même mode opératoire que dans le cas du diéthylcarbamate du 2,4-dihydroxyphénylthiol. Le produit de réaction est purifié par cristallisation dans le benzène.

Lamelles brillantes, F = 165°, insolubles dans l'eau, facilement solubles dans l'alcool, assez difficilement dans le chloroforme. Formation d'un précipité blanc caractéristique avec OHTl. Le produit copule facilement avec le diazoanhydride de l'acide sulfanilique en présence d'acétate de soude anhydre en solution dans l'alcool méthylique.

$C_{15}H_{17}O_3NS$ (291)	Calc.	N	4,81
	Trouvé	»	4,95

RÉSUMÉ

Par action de l'ammoniaque et des amines aliphatiques primaires et secondaires, les 0.S-(1,2)-arylène-thiocarbonates sont transformés en carbamates des hydroxyarylthiols.

La réaction est susceptible d'être appliquée comme méthode de synthèse de ces derniers.

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Reçu le 25 Septembre 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1401–1407

The Probable Existence of Acid Aluminium Di-soaps

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The following paper gives a survey of the mechanisms by which fatty acid can be taken up by aluminium di-soap. The possible phase equilibria between a fatty acid solution and an aluminium soap solid are deduced thermodynamically. The relationships thus obtained are compared with experimental results of Coe, Mysels and Smith [1], and conclusions are drawn about the existence of an acid aluminium di-soap.

Precipitation of aluminium soaps from alkali soap (denoted NaL) solutions results in products of indefinite composition with an aluminium content varying from that of the di-soap $AlOHL_2$ to that of the tri-soap AlL_3 [2, 3]. By Soxhlet extraction of these products with an anhydrous organic solvent, which dissolves only the fatty acid, aluminium di-soap with the theoretical composition is obtained [4]. Consequently a true tri-soap AlL_3 had not been formed. The existence of an aluminium di-soap is shown by X-ray analysis [5].

Most organic liquids dissolve or swell aluminium di-soaps, but a few organic solvents such as acetone and some paraffin hydrocarbons do not dissolve aluminium soaps. Coe, Mysels and Smith [1] examined the equilibrium between stearic acid dissolved in acetone or isooctane and a solid consisting of aluminium stearates. They found a relationship between the stearic acid content of the solution and the mole ratio HL/Al in the solid, indicating that fatty acid was taken up by the di-soap. Thus the possible existence of an acid aluminium di-soap $AlOHL_2HL$, overlooked before [6–8], is indicated. Compare this with the established existence of acid alkali soaps aNaLbHL (a and b being integers) [9], which release their excess fatty acid on extraction [10]. It must be borne in mind that there is no principal difference between an acid di-soap $AlOHL_2HL$ and a compound represented by AlL_3H_2O, because neither of these can release water if the anhydrous tri-soap AlL_3 does not exist.

In the present paper the equilibrium between a fatty acid and its aluminium soaps is studied theoretically on the basis of possible phase compositions of the soap solid. The presence of an anhydrous solvent, capable of dissolving the fatty acid but inert to the aluminium soaps, is assumed. Relations between the fatty acid concentration in the solution and the over all composition of the soap solid are deduced, with the latter variable as the independent one. The total composition of the solid will be given as the amount of fatty acid exceeding di-soap composition and expressed by the mole ratio $HL/AlOHL_2$ ($= x$). Dissolved fatty acid is represented by its activity $a_{HL(l)}$. The pure compounds have unit activity (standard state) and ideal solutions are assumed. Differences caused by the use of different solvents are consequently eliminated. Common symbols are used, (s) and (l) denoting solid and liquid phase.

POSSIBLE PHASE COMPOSITIONS OF THE SOLID

A. The solid consists of aluminium di-soap and fatty acid, according to the following phase behaviour

1) fatty acid and di-soap form two separate phases
2) fatty acid and di-soap form a solid solution
3) fatty acid is adsorbed on the solid di-soap.

A partition equilibrium for the fatty acid exists corresponding to $HL(s) \rightleftharpoons HL(l)$.

B. The solid consists of aluminium di-soap and acid aluminium di-soap, where

1) di-soap and acid di-soap form separate phases
2) di-soap and acid di-soap form a solid solution.

The concentration of fatty acid in solution is determined by the equation $AlOHL_2HL(s) \rightleftharpoons AlOHL_2(s) + HL(l)$

Deduction of phase equilibria.

A) Solid consisting of $AlOHL_2$ and HL according to $HL(s) \rightleftharpoons HL(l)$

1) *The solid components form separate phases.* The solution will be saturated as soon as the fatty acid content of the solid is raised above that of the di-soap, giving the relation $a_{HL(l)} = 1$ as represented by curve a in Fig. 1

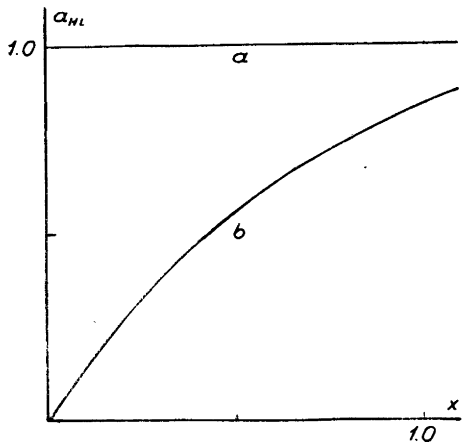

Fig. 1. Solid consisting of HL and $AlOHL_2$ (a) as separate phases, (b) in a solid solution.

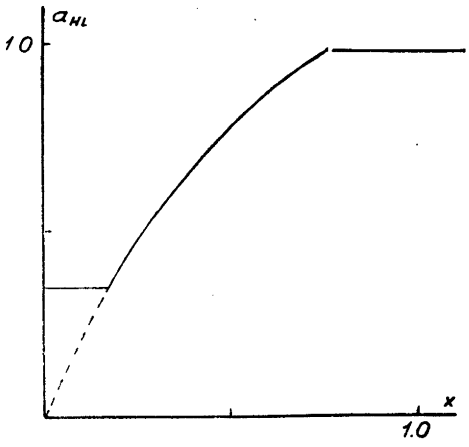

Fig. 2. Solid solution of HL and $AlOHL_2$ at lower limited mutual solubility.

2) *The solid components form a solid solution.* It can easily be shown that in this case $a_{HL(s)} = K \dfrac{n_{HL}}{n_{AlOHL_2} + n_{HL}} = a_{HL(l)}$, K being a constant. This simplifies to the relation $a_{HL(l)} = \dfrac{K \cdot x}{1 + x}$ represented by curve b in Fig. 1. In case of lower mutual solubility the relationship depicted in Fig. 2 represents the phase equilibrium.

3) *Adsorption of fatty acid on aluminium di-soap.* Applying Langmuir's concepts the number of adsorbed moles fatty acid per mole di-soap will be

$$x = \frac{p \cdot a_{HL(l)}}{1 + q \cdot a_{HL(l)}} \quad (p \text{ and } q \text{ being constants})$$

giving

$$a_{HL(l)} = \frac{k_1 \cdot x}{(1 - k_2 x)}$$

The case is shown in Fig. 3.
If Freundlich's equation is applied similar results will be obtained.

B) Solid consisting of di-soap and acid di-soap according to

$$AlOHL_2HL(s) \rightleftharpoons AlOHL_2(s) + HL(l)$$

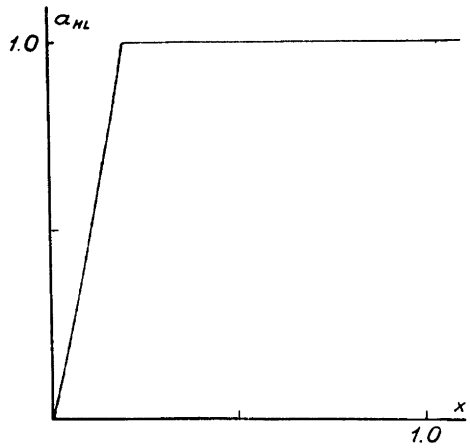

Fig. 3. Adsorption of HL on $AlOHL_2$.

1) *The solid components form separate phases.* Then the equilibrium constant is $a_{HL_2(l)} = K$ where $K < 1$. The relationship is represented by the line EFGH in Fig. 4.
2) *The components form a solid solution.* The activities of the two components are

$$a_{AlOHL_2HL_2(s)} = \frac{p \cdot n_{AlOHL_2HL_2}}{n_{AlOHL_2HL_2} + n_{AlOHL_2}}$$

$$a_{AlOHL_2(s)} = \frac{q \cdot n_{AlOHL_2}}{n_{AlOHL_2} + n_{AlOHL_2HL_2}}$$

p and q being constants. Equilibrium requires:

$$K_1 = \frac{a_{HL_2(l)} \cdot a_{AlOHL_2(s)}}{a_{AlOHL_2HL_2(s)}}$$

Thus

$$a_{HL_2(l)} = \frac{K \cdot x}{(1 - x)}$$

The equilibrium constant K determines the highest possible solubility of $AlOHL_2HL$ in $AlOHL_2$. Curve ADFGH or, in case of a limited mutual solubility curve CBDFGH, in Fig 4 represents this case. If $AlOHL_2HL$ exists only in solid solution with $AlOHL_2$ but not in a pure state, the phase equilibrium is represented by Fig 5, where fatty acid exists as a solid phase within the range AB.

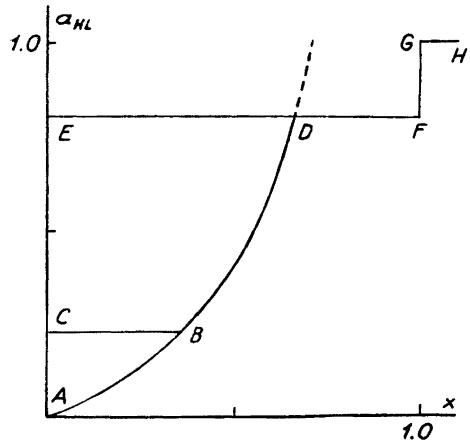

Fig. 4. Solid consisting of $AlOHL_2$ and $AlOHL_2HL$.
Line EFGH: as separate phases,
Line ADFGH or CBDFGH: in a solid solution.

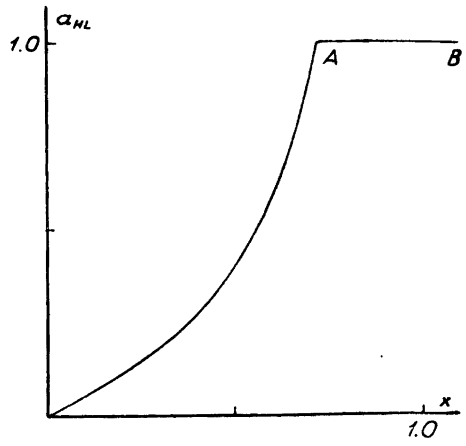

Fig. 5. Solid solution of $AlOHL_2$ and $AlOHL_2HL$, if the latter component does not exist in a pure state.

The same relation can be obtained by a statistical treatment, simultaneously giving a good picture of the structure of the single phase system di-soap—acid di-soap. Let us postulate that one molecule fatty acid can be bound as an upper limit to each di-soap molecule in the single phase system. Thus a solid solution of acid di-soap in di-soap is formed.

Then assuming fatty acid is released with the rate $v_1 = R_1 x$ and combined with the rate $v_2 = R_2\ (1 - x)\ a_{HL(l)}$, R_1 and R_2 being constants, we find at equilibrium $v_1 = v_2$ and again: $a_{HL(l)} = \dfrac{K \cdot x}{(1 - x)}$.

COMPARISION WITH EXPERIMENTAL RESULTS ON ALUMINIUM STEARATES

Coe, Mysels and Smith [1] present the experimentally obtained relationship for stearic acid and aluminium stearate at 30° C in the same way as in our figures. From a comparison it is obvious that either sorption of fatty acid on di-soaps (A : 3) or a limited formation of acid di-soap (B : 2) may account for their results. The two corresponding equations differ only by the presence of a constant k_2 in the denominator of the equation A : 3. Table 1 shows the excellent agreement with both formulae assuming in the case A : 3 that k_2 is unity. However, the high value reached for the ratio of adsorbed substance to adsorbent makes it improbable that true surface adsorption takes

Table 1. Comparison of experimental results on the system aluminium stearate — stearic acid at 30° C with the equation $a_{HL(l)} = \frac{K \cdot x}{(1-x)}$ *(Case B : 2).*

$a_{HL(l)}$	x observed *	K **	x calculated ***
1.00	0.64	0.56	0.67
0.90	0.63	0.53	0.64
0.80	0.60	0.53	0.61
0.70	0.58	0.51	0.58
0.60	0.55	0.49	0.55
0.50	0.51	0.48	0.50
0.40	0.46	0.47	0.44
0.30	0.38	0.49	0.38
0.20	0.29	0.49	0.29
0.10	0.17	0.49	0.17
		Mean 0.50	

* Values taken from Fig. 1 of Coe, Mysels and Smith [1].
** K values calculated from $a_{HL(l)}$ and x values observed.
*** Calculated using obtained mean value $K = 0.50$.

place. Consequently the best explanation for the experimental results is that the aluminium stearate solid consists of $AlOHSter_2HSter$ and $AlOHSter_2$. These components then form a one phase system up to an acid content of 0.67 mole HSter per mole $AlOHSter_2$ corresponding to $0.33(AlOHSter_2)$ 0.67 $(AlOHSter_2HSter)$, very close to $3AlOHSter_2 2HSter$.

At higher total acid content in the solid the activity of fatty acid in the solvent should be constant. This acivity must be less than unity if $AlOHL_2HL$ is present here as a pure phase (Fig. 4). Theoretically, it can reach unity, then making it difficult to draw conclusions concerning this region of the solid phase. The experiments with stearic acid may point to the existence of a pure stearic acid phase in this region together with a phase of $3AlOHSter_2 2HSter$.

Further research will be carried out on the aluminium abietate — abietic acid system.

SUMMARY

By thermodynamic treatment phase equilibria between fatty acid solutions and a solid of aluminium soaps are deduced on the basis of possible phase compositions of the solid. By comparison with earlier published experimental work evidence is obtained for the existence of an acid aluminium di-stearate

with a composition varying from $AlOHSter_2$ to a compound close to $3Al(OH)Ster_2 2HSter$, which can be interpreted as a solid solution of di-stearate $AlOHSter_2$ and acid di-stearate $AlOHSter_2HSter$.

We are indebted to Professor A. Ölander for valuable discussions and suggestions and to Professor B. Steenberg, head of our department, for encouraging interest.

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Received September 6, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1408–1412

The Dipole Moments and the Ultraviolet Absorption Spectra of Xylocaine and Two Related Compounds

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During the course of a systematic study by Löfgren and Collaborators [1] of the chemical and physical properties of local anesthetics of the type amino-acyl amide, the dipole moments of some of these anesthetics, *viz.* ω-diethyl-amino-acetanilide (*A*), ω-diethylamino-N-methylacetanilide (*M*) and ω-diethylamino-2,6-dimethylacetanilide (xylocaine) (*X*), have been measured. A qualitative investigation of the absorption spectra of these molecules has been made previously by Löfgren [1], who estimated the position of the absorption bands without measuring the extinctions quantitatively. In order to extend our knowledge of the molecules we have now made a more detailed study of the spectra. The results of the measurements of dipole moments and spectra are reported in this paper and the distribution of electrons within the molecules is discussed.

EXPERIMENTAL

Benzene. A thiophene-free product from Baker's Ltd. was stored over sodium wire for several weeks. It was distilled in an atmosphere of dry nitrogen immediately before use.

ω-Diethylamino-acetanilide, $C_{12}H_{18}N_2O$ (206.28), prepared according to Erdtman and Löfgren [2], freshly redistilled in N_2-atm., b. p. 160–161° (7 mm).

ω-Diethylamino-N-methylacetanilide, $C_{13}H_{20}N_2O$ (220.31), prepared by Löfgren [3], freshly redistilled in N_2-atm., b. p. 157° (9 mm).

ω-Diethylamino-2,6-dimethylacetanilide (*xylocaine*), $C_{14}H_{22}N_2O$ (234.33), prepared by Löfgren [3], six times recrystallized from petroleum ether, m. p. 67°.

The dipole moments were measured in dilute benzene solutions at 25° C with the apparatus recently described by one of us [4]. From the values obtained for the dielectric constants of the solutions, ε, and for the specific volumes, v, the moments were computed according to Halverstadt and Kumler [5]; for details of the calculations, see Fischer [4]. The results of the measurements are listed in Table 1, where f_2 is the mole fraction of the solute, P_2^0 the molar polarization at infinite dilution, and μ the dipole moment measured in Debye (D) = 10^{-18} e. s. u.

Table 1. Measurements on benzene solutions at 25° C. The values for the pure solvent are extrapolated.

f_2	ε	v cm³/g.
	ω-Diethylamino-acetanilide	
0.00000	2.2842	1.1435
0.01272	2.5586	1.1392
0.01971	2.6851	1.1364
0.02807	2.8635	1.1328
0.03680	3.0596	1.1297
0.05648	3.4995	1.1225
$\partial\varepsilon/\partial f_2 = 21.1 \pm 0.3$; $\partial v/\partial f_2 = -0.370 \pm 0.002$; $P_2^0 = 370 \pm 6$ cm³; $MR_D{}^1 = 62.68$ cm³; $\mu = 3.88 \pm 0.04$ D		
	ω-Diethylamino-N-methylacetanilide	
0.00000	2.2866	1.1438
0.00864	2.4202	1.1409
0.01465	2.5254	1.1386
0.01803	2.5761	1.1369
0.02050	2.6270	1.1363
0.04846	3.1049	1.1260
0.07721	3.5373	1.1156
$\partial\varepsilon/\partial f_2 = 16.4 \pm 0.3$; $\partial v/\partial f_2 = -0.367 \pm 0.002$; $P_2^0 = 306 \pm 6$ cm³; $MR_D{}^1 = 66.52$ cm³; $\mu = 3.42 \pm 0.05$ D		
	ω-Diethylamino-2,6-dimethylacetanilide	
0.00000	2.2576	1.1441
0.00188	2.3018	1.1438
0.00389	2.3538	1.1427
0.00419	2.3558	1.1426
0.00488	2.3789	1.1422
0.00971	2.4768	1.1402
0.01133	2.5276	1.1396
0.01729	2.6973	1.1370
0.01875	2.6963	1.1362
0.02686	2.8907	1.1352
0.02776	2.9285	1.1328
$\partial\varepsilon/\partial f_2 = 24.0 \pm 0.3$; $\partial v/\partial f_2 = -0.385 \pm 0.014$; $P_2^0 = 425 \pm 5$ cm³; $MR_D{}^1 = 71.25$ cm³; $\mu = 4.16 \pm 0.03$ D		

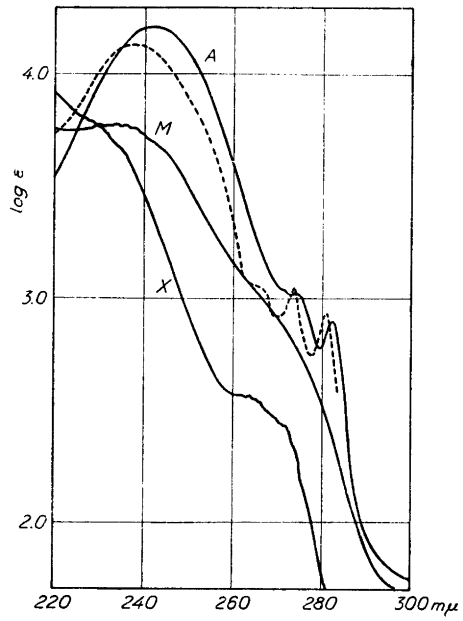


Fig. 1. Ultraviolet absorption spectra measured in hexane solutions.

——— *The present investigation of A, M and X.*

– – – – – *Acetanilide according to Klingstedt* [6].

The ultraviolet absorption spectra were measured on a Beckman quartz spectrophotometer with a hydrogen lamp as a light source. The solvent was spectroscopically pure hexane and the concentrations were about 10^{-5} mole/l. The interval between two readings was 1 mμ except in the more interesting regions where the interval was 1/4 mμ. The spectra were studied between $\lambda = 220$ mμ and $\lambda = 300$ mμ, as the earlier measurements showed that none of the molecules has any absorption band at longer wave lengths than 300 mμ. In Fig. 1 the values obtained for log ε* are plotted against the wave length λ.

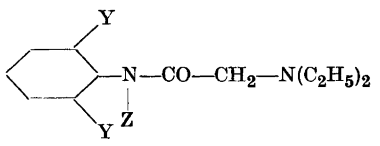
DISCUSSION

Our spectroscopic measurements confirm the earlier, qualitative results obtained by Löfgren [1]; as might be expected, the new measurements disclose more details of the bands and indicate the positions with greater accuracy. *A* has two bands. The one nearest the visible end of the spectrum has three distinct maxima which, however, overlap appreciably: $\lambda = 282.2$ mμ, log $\varepsilon = 2.90$; $\lambda = 275.2$ mμ, log $\varepsilon = 3.01$; $\lambda = 271.8$ mμ, log $\varepsilon = 3.03$. The last maximum is, moreover, strongly overlapped by the second and broader band, with the maximum at $\lambda = 242.0$ mμ, log $\varepsilon = 4.21$. Both these bands are recognizable, although they are distorted and displaced, in the spectra of

* In this connexion ε is the extinction coefficient; as confusion is unlikely to occur we have adopted the notation most commonly employed although it is the same both for the dielectric constant and the extinction coefficient.

M and *X*. In *M* the first band lies between 265 and 280 $m\mu$, but the intensity is so low and the band is so strongly overlapped by the second band that no maximum can be distinguished. The second band, $\lambda = 233$ mμ, log $\varepsilon = 3.78$, has a faint fine structure. In *X* the first band is somewhat more intense than in *M*, but it is still difficult to analyze. It contains several maxima: $\lambda = 261$ mμ, log $\varepsilon = 2.57$; $\lambda = 265$ mμ, log $\varepsilon = 2.56$; $\lambda = 272$ mμ, log $\varepsilon = 2.43$. There appear to be still further maxima in the region $\lambda = 260 - 274$ mμ, but they are too faint to be localized with precision. The broad band at $\lambda = 230$ mμ, log $\varepsilon = 3.77$, is still less sharp than in *M* and is overlapped by absorption bands at still shorter wave lengths, which cannot be measured on the available photometer. As in the case of *M*, a fine structure can be traced.

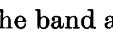
The absorption spectrum of *A*



A: Z = H, Y = H

M: Z = CH_3, Y = H

X: Z = H, Y = CH_3

is closely similar to that of acetanilide,  — NH — CO — CH_3, published by Klingstedt [6], *cf.* Fig. 1. The band at 270—290 mμ has a less distinct structure, but all the three maxima observed by Klingstedt in the band of acetanilide can also be distinguished in the spectrum of *A*. Consequently, the — $CH_2N(C_2H_5)_2$ group does not seem to influence the conjugating system φ — N — C = O to any greater extent than does a CH_3-group. In *M* and *X* the bands are displaced towards shorter wave lengths and their intensities are lowered appreciably. This change of the spectrum is analogous to the change of the spectrum of an aromatic amine when transformed into a positive ion, see *e. g.* Wohl [7], though the change is more pronounced in the latter case. This similarity suggests that the conjungation between the amide nitrogen and the phenyl group is weakened in *M* and *X*, presumably due to steric hindrance. The hindrance is less obvious in *M* than in *X*, as *M* does not contain any *o*-substituents. However, as pointed out by Klingstedt [6], the φ-N conjugation is considerably weaker in acetanilide than in aniline. Therefore, it is not unreasonable to assume that in acetanilide two substituents in the proximity of the amide nitrogen, that is, substituted either at the nitrogen or in *o*-position, will cause a steric repulsion strong enough to overcome the conjugation energy, as three substituents have this effect in aniline [4].

The values found for the dipole moments are consistent with a weak φ-N conjugation, which implies that the part of the φ-N dipole moment due to the π-electrons is small. The main part of the total dipole moment of all the

three molecules is certainly due to the $\overset{+}{N} - C = \overline{O}$ group with the indicated polarity. The consequence of the inhibited resonance in *M* and *X* will be that the moments of these molecules would be lower than the moment of *A*. Now, the moment of *M* is 0.46 *D* lower, but the moment of *X* is 0.28 *D* higher than that of *A*. This may be explained if we assume that the π-electron moment of φ-N is only a small fraction of 0.46 *D*. The rest of this difference may be due to the decrease in the positive charge on the nitrogen due to the electron-donating power of the methyl group attached to it. The high moment of *X* partly depends on the moment of the methyl groups in the *o*-positions and it may be due in part to configurational changes within the rest of the molecule. Owing to the complexity of the molecule the total dipole moment cannot be accurately accounted for. However, Löfgren's [1] suggestion, based on measurements of ionization constants, that the amino nitrogen of *A* and *X* has a resultant positive charge due to the electron-attracting power of the oxygen, is consistent with the relatively high value of the dipole moments. It is not to be expected that such an induction effect will influence the spectrum of the molecule, as the intensity of the absorption is very insensitive to such effects [8].

SUMMARY

The dipole moments and the ultraviolet absorption spectra of ω-diethylamino-acetanilide (*A*), ω-diethylamino-N-methylacetanilide (*M*) and ω-diethylamino-2,6-dimethylacetanilide, *i. e.* xylocaine (*X*) have been determined. The results suggest that the φ-N conjugation is very weak in *M* and *X*. They are consistent with Löfgren's earlier suggestion that the amino nitrogen of *A* and *X* has a resultant positive charge due to the strong induction effect of the carbonyl group.

This work has been aided by a grant from *Statens Tekniska Forskningsråd.*

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Received October 19, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1413–1436

On the Crystal Structure of Cinnabar

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PREVIOUS WORK ON MERCURY (II) HALIDES AND MERCURY (II) CHALCOGENIDES

In connection with an X ray investigation of mercury (II) oxyhalides it seemed of interest to review what is known about the type of bond between bivalent mercury and metalloids of group VII b (halogens) and VI b (chalcogens). As seen from the following retrospect much work has been done on the halogen compounds HgX_2 but relatively meagre data are available on the mercury compounds containing O, S, Se and Te.

From powder photographs of crystalline HgF_2 Ebert and Woitinek [1] concluded that this compound is isomorphous with CaF_2. The F atoms, however, contribute very sparsely to the intensities, so their positions can not be regarded as proved. With the structure of Ebert and Woitinek every Hg atom is surrounded by 8 F atoms in a cubical arrangement and every F tetrahedrically by 4 Hg atoms in the distance 2.4_0 kX and thus the structure probably is built up from Hg^{2+} and F^- ions. If this is so, HgF_2 forms an exception from the other mercury (II) halides, where mercury is coordinated with two and in one case with four X atoms.

By X ray methods crystalline $HgCl_2$ and $HgBr_2$ have been found to be molecular compounds; each molecule HgX_2 can be regarded as linear ($HgCl_2$: Braekken and Harang [2], Nieuwenkamp and Bijvoet [3], Braekken and Scholten [4]. $HgBr_2$: Verweel and Bijvoet [5]).

The distance Hg → Cl is 2.25 kX (Braekken and Scholten [4]) and Hg → Br 2.48 kX (*Strukturbericht 1928—1932*, p. 19).

Yellow HgI_2, which is stable above 127° (Smits [6]) is isomorphous with $HgBr_2$ (Verweel and Bijvoet [5], Gorskiĭ [7]). The distance Hg → I has been calculated to be 2.62 kX (Gorskiĭ).

In the vapour state the compounds HgX_2 are also found to be linear or nearly linear. The following distances are found from electron interference studies on HgX_2 (gas):

Hg → Cl 2.2_8 kX, Hg → Br 2.3_8 kX, Hg → I 2.5_5 kX (Braune and Knoke [8])
Hg → Cl 2.3_4 kX, Hg → Br 2.4_4 kX Hg → I 2.6_1 kX (Gregg *et al.*[9]).

In red HgI_2, stable at ordinary temperature, a new type of coordination occurs. Thus every Hg atom is surrounded tetrahedrically by 4 I (Havighurst [10], Bijvoet, Classen and Karssen [11], Classen [12], Huggins and Magill [13]). The distance Hg → I is 2.78 kX (*Strukturbericht 1913—1928*, p. 180).

According to Wells [14, p. 514] the colinear bonds (in $HgCl_2$ (s), $HgBr_2$ (s), yellow HgI_2 (s) and the gaseous molecules) are sp bonds, whereas the tetrahedral bonds in red HgI_2 are sp^3 bonds.

The mercury (II) oxide can be prepared with two colours, yellow and red. It has been discussed by several authors if the yellow and the red oxides are identical or not (Gay-Lussac [15], Ostwald [16, 17], Varet [18], Cohen [19], Hulett [20], Schick [21], Fuseya [22], Levi [23, 24], Goldschmidt [25], Fricke [26], Zachariasen [27], Kolkmeijer [28, 29]).

The conclusion is that the oxides are identical compounds; they give the same powder photographs and differ only in the grain size. The division of the yellow oxide is, however, finer than of the red one.

The structure of HgO is orthorhombic. Zachariasen [27] has approximately fixed the positions of the Hg atoms ($x_{Hg} \approx \frac{1}{4}$) and discussed a few possibilities for the positions of the O atoms.

Of the mercury (II) sulphide two modifications are known; the red cinnabar and the black metacinnabar.

Metacinnabar is cubic. The structure is of the B 3 type, thus isomorphous with ZnS (blende) (Kolkmeijer, Bijvoet and Karssen [30], Lehmann [31], v. Olshausen [32], Buckley and Vernon [33], Hartwig [34], Goldschmidt [25, 35]).

The Hg atoms, therefore, are tetrahedrically surrounded by S atoms; the distance Hg → S is 2.52_5 kX ($a\frac{\sqrt{3}}{4}$) since $a = 5.83_2$ kX (Goldschmidt [35]).

HgSe and HgTe are isomorphous with metacinnabar and have the cell edges $a_{HgSe} = 6.06_8$ kX and $a_{HgTe} = 6.44_0$ kX. The distance Hg → Se is 2.62_8 kX and Hg → Te 2.78_9 kX (*Strukturbericht 1913—1928*, p. 77) (Zachariasen [36], Hartwig [34], Goldschmidt [25, 35], de Jong [37]).

The four tetrahedral bonds the mercury atom forms in HgS (metacinnabar), HgSe and HgTe are probably sp^3 bonds as in red HgI_2.

In the mercaptides $Hg(SC_2H_5)_2$, $Hg(SC_4H_9)_2$ and $Hg(SC_8H_{17})_2$ the bonds S-Hg-S form straight or almost straight lines and are thus probably sp bonds. The distance Hg → S was calculated to be 2.5_0 kX from a Fourier projection, assuming the S-Hg-S bonds to be exactly colinear (Wells [38]).

The other form of HgS, cinnabar, is hexagonal. By various authors, values for a have been reported from 4.12 to 4.16 kX and for c from 9.43 to 9.54 kX. A structure has been proposed which is denoted by *Strukturbericht* as B 9 (Mauguin [39], v. Olshausen [32]).

The space groups proposed are D_3^4 or D_3^6, which give left handed or right handed spirals (Buckley and Vernon [33], de Jong and Willems [40]).

With D_3^4 the point positions are:

3 Hg in 3 (a): $x_1\ 0\ \frac{1}{3},\ 0\ x_1\ \frac{2}{3},\ \bar{x}_1\ \bar{x}_1\ 0$ and
3 S in 3 (b): $x_2\ 0\ \frac{5}{6},\ 0\ x_2\ \frac{1}{6},\ \bar{x}_2\ \bar{x}_2\ \frac{1}{2}$

For cinnabar no direct determination of the distances Hg → S has hitherto been made. The positions of the S atoms have been fixed by assuming the same distance Hg → S as found for metacinnabar. This distance is about the same as the sum of the atomic radii for covalent tetrahedral structures according to Pauling [41] (Hg = 1.48 kX, S = 1.04 kX).

From intensity calculations based on powder photographs Buckley and Vernon [33] found the value $x_1 \approx \frac{1}{3}$. With $x_2 = 0.21$ for the S parameter the same distance Hg → S was obtained as found in metacinnabar. This x_2 value also seemed to suit the intensities best.

From powder photographs de Jong and Willems [40] could conclude that x_1 is in one of the ranges 0.25—0.40 or 0.60—0.75 but could draw no conclusion as to the value of x_2. They found that with a coordination Hg → 6S or Hg → 4S, the Hg → S distances would be unbelievably long. They thus assumed that each Hg has 2 nearest S neighbours and that the distance Hg → S is 2.52 kX (the sum of the *tetrahedral* atomic radii!). They finally arrived at the values $x_1 = 0.72_5$ and $x_2 = 0.55$.

In the present work the author has tried to redetermine the positions of Hg and S in cinnabar with the aid of intensity calculations only, and without any previous assumption about distances and coordination. Work on HgO and Hg oxyhalides is in progress.

MATERIALS

For the X ray measurements of this investigation native cinnabar was used, which was kindly supplied by Professor S. Gavelin of the Mineralogical Institute, University of Stockholm.

For getting single crystals, suitable for X ray investigation, the native cinnabar crystal conglomerate was crushed. A number of fragments were picked out and examined by Laue photographs before two acceptable single crystals were found.

Reflexions, which should have the same intensities according to the Laue symmetry, showed different values because of the fact that the shape of the crystals (flat needles) did not show the same symmetry as the reciprocal lattice, and therefore the observed absorption shows a lower symmetry, too.

Of course the asymmetrical absorption effects might be diminished by grinding the crystal to cylindrical form, but it seemed that the single crystals available were too tiny for such a procedure.

Attempts to synthesize HgS crystals, suitable for X ray analysis, were not successful. Only microcrystalline cinnabar was obtained.

This synthetical cinnabar gave powder photographs identical with those of native cinnabar.

UNIT CELL AND SPACE GROUP

The dimensions of the unit cell were determined accurately from the powder photographs with CrKα radiation and focussing cameras of the Phragmén-Westgren type (Table 1).

The powder photographs could be interpreted with a hexagonal unit cell with:

$a = 4.137$ kX $= 4.146$ Å
$c = 9.477$ kX $= 9.497$ Å
$V = 140.4_6$ kX^3 $= 141.3_6$ Å^3 Accuracy about 0.05 %

The cell dimensions according to de Jong and Willems (1926) are $a = 4.12$ kX $c = 9.43$ kX.

The density according to Allen and Crenshaw[42] is 8.176, thus allowing 3 formula units per unit cell (calculated density 8.19_8).

Laue photographs, rotation and Weissenberg photographs around (001) (zero layer and first layer) and (010) (zero layer) were taken. From the Laue photographs, (incident beam parallel to the threefold axis) the Laue symmetry was found to be D_{3d}—$\bar{3}m$. The only systematic extinction found was that $00l$ was absent for $l \neq 3n$, which is characteristic of the space groups D_3^{3-6}.

Because the unit cell contains 3 formula units and because cinnabar has a definite composition the only way to get an ordered structure was to assume the Hg atoms occupy one threefold point position. For each of the space

Table 1. Powder photographs of HgS (cinnabar). CrKα radiation.

hkl	$10^4\sin^2\Theta_{calc.}$	$10^4\sin^2\Theta_{obs.}$	$I_{obs.}$	*hkl*	$10^4\sin^2\Theta_{calc.}$	$10^4\sin^2\Theta_{obs.}$	$I_{obs.}$
100	0.1018	0.1007	w	006	0.5238	0.5247	w
101	.1163	.1150	vst	203	.5381	.5386	m
003	.1310	(.1301)	(st)	106	.6256	—	—
102	.1600	.1586	vst	204	.6399	.6400	st
103	.2327	.2320	vw	115	.6691	.6688	vvw
110	.3053	.3056	st	210	.7125	.7121	w
111	.3199	.3204	m	211	.7270	.7270	m
104	.3346	.3358	st	212	.7707	.7707	m
112	.3635	(.3624)	(m)	205	.7709		
200	.4071	—	—	107	.8147	.8149	w
201	.4217	.4221	st	116	.8291	.8302	w
113	.4363	.4368	st	213	.8434	.8434	w
202	.4653	.4655	st				
105	.4655						

In the list of the powder photographs, the reflexions systematically absent and the β reflexions have been omitted. If a tabulated reflexion coincides with a β reflexion, the $\sin^2\Theta_{obs.}$ and the observed intensity of the resulting line are given in brackets. The observed intensities are indicated as follows: vst = very strong, st = strong, m = medium, w = weak, vw = very weak and vvw = very, very weak.

groups D_3^{3-6} two different sets of threefold point positions 3 (a) and 3 (b) are possible. However, on displacing the origin of the unit cell by $c/2$ the positions 3 (a) could be made identical with the positions 3 (b). It was thus arbitrarily assumed that the Hg atoms are situated in 3 (a) with the coordinates:

$$
\begin{aligned}
D_3^3&: x_1\ \bar{x}_1\ 0,\ x_1\ 2x_1\ \tfrac{1}{3},\ 2\bar{x}_1\ \bar{x}_1\ \tfrac{2}{3}\\
D_3^5&: x_1\ \bar{x}_1\ 0,\ x_1\ 2x_1\ \tfrac{2}{3},\ 2\bar{x}_1\ \bar{x}_1\ \tfrac{1}{3}\\
D_3^4&: x_1\ 0\ \tfrac{1}{3},\ 0\ \ x_1\ \tfrac{2}{3},\ \ \bar{x}_1\ \bar{x}_1\ 0\\
D_3^6&: x_1\ 0\ \tfrac{2}{3},\ 0\ \ x_1\ \tfrac{1}{3},\ \ \bar{x}_1\ \bar{x}_1\ 0
\end{aligned}
$$

The space group D_3^3 or D_3^5 being assumed, maxima for the following x values are expected, according to the interatomic distances Hg → Hg and Hg → S in the Patterson projections $P(xpz)$; $z = \frac{1}{6}$, $\frac{1}{3}$ and $\frac{1}{2}$ (Table 2).

Thus if the Hg atoms are arranged according to space groups D_3^3 or D_3^5, the Patterson projections $P(xpz)$ (Table 2) would be symmetrical curves. However, such symmetrical projections can only be expected if $I_{h0l} = I_{\bar{h}0\bar{l}}$

Table 2. Expected maxima, due to interatomic distances Hg → Hg and Hg → S in P (xpz), space groups D_3^3 and D_3^5 being assumed. x_1 is the Hg parameter and x_2 is the S parameter.

Projection	Expected x value for the maxima	
	Hg → Hg	Hg → S
$P\ (xp\ \frac{1}{6})$		$\pm\ (x_1 - x_2)$ $\pm\ (x_1 + 2x_2)$ $\pm\ (2x_1 + x_2)$
$P\ (xp\ \frac{1}{3})$	$\pm\ 3x_1$ 0	
$P\ (xp\ \frac{1}{2})$		$\pm\ (x_1 - x_2)$ $\pm\ 2(x_1 - x_2)$

and $I_{h0l} = I_{h0\bar{l}}$. Actually, $I_{h0l} \neq I_{h0\bar{l}}$, as seen from the reflexions $h0l$. Therefore D_3^3 and D_3^5 can be excluded.

The point positions in D_3^4 are mirror images of those in D_3^6 (Fig. 1). On calculating the intensities from the structure factors A and B (*International tables* [43]), D_3^4 and D_3^6 give the same result if the signs of the parameters are reverted ($x\ y\ z$ in D_3^4 correspond to $\bar{x}\ \bar{y}\ \bar{z}$ in D_3^6).

As seen from Fig. 1, with the threefold point positions of D_3^4 the atoms will be coiled into lefthanded spirals and with those of D_3^6 into right handed spirals, if the xyz axes form a right coordinate system (as in the *International tables*).

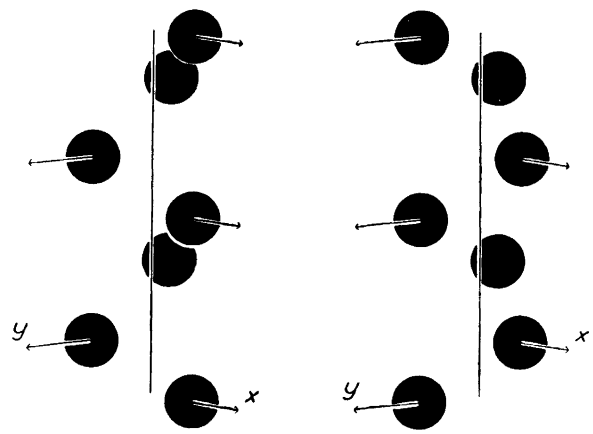


Figure 1. Spiral chains of the mercury atoms of HgS (cinnabar). The Hg atoms are arranged according to space groups D_3^4 (left) and D_3^6 (right). The arrows point in the direction of the x and y axes.

The optical rotating power of cinnabar crystals has been investigated by several authors (des Cloizeaux [44], Wyrouboff [45], Tschermak [46], Melville and Lindgren [47], Becquerel [48], Rose [49]).

According to these authors both dextrorotary and laevorotary cinnabar crystals exist. By the X ray methods it is not possible to distinguish between D_3^4 and D_3^6, nor to decide which of the dextrorotary crystal and the laevorotary crystal crystallizes with the symmetry D_3^4 and which with D_3^6.

As mentioned above the same structure factors are obtained for D_3^6 as for D_3^4. Therefore only the space group D_3^4 is considered in the following.

METHOD OF ATTACK

An attempt was first made to determine the parameters by the method of trial and error. This proved to be very laborious, due to the complicated form of the structure factors A and B. Moreover, on account of the asymmetrical absorption it was difficult to compare the calculated values of I with those observed. It seemed that the best way of attacking the structure problem was to use Patterson and Fourier analysis.

If several different projections are used for the determination of the parameters, the intensities of quite a large number of reflexions will be considered. Errors in visually estimated intensities will cause a spread of the values of the parameters obtained from these different projections. In the average value of the parameters, it may be hoped that the errors, caused by the intensities, have partially cancelled out.

The following Patterson projections were computed: $P(xpz)$ with $z = \frac{1}{6}$, $\frac{1}{3}$ and $\frac{1}{2}$, based on the reflexions $h0l$ and $P(x0p)$ and P $(2xxp)$, based on the reflexions $hk0$.

The Patterson projections were calculated using both values of I and "F^2" (see p. 1420). From the Patterson projection P $(xp\ \frac{1}{3})$ (Table 5) a preliminary value of the Hg parameter was obtained and, with the aid of this value, the signs of the amplitudes of the reflexions could be calculated.

Then Fourier analyses were computed, based on "F" ($\sqrt{\text{"}F^2\text{"}}$) values for the reflexions equivalent to $h00$, $h\bar{h}0$ *etc.* and for $h0,3L$ ($l = 3L$, $L =$ whole number) and on the obtained signs of the amplitudes. If only these reflexions are considered, the Fourier analysis will give a density function ϱ_3 which can be referred to a hypothetical unit cell with a centre of symmetry in the projection ϱ_3 (xpZ) (p. 1421—24).

The zero level is unknown in the curves obtained from the Patterson and Fourier projections, and false maxima may be introduced because of the limited number of reflexions used. However, the highest maxima in the curves

will probably give rather accurate values for the positions of the Hg atoms and it seems that the minor peaks might give information on the positions of the S atoms. If the maxima of different projections give the same values for the parameters of Hg and S, this will add to the probability of these values.

With the aid of mean values of x_1 and x_2, calculated from all these projections the value of the phase angle α_{h0l} for every reflexion $h0l$ was computed. Then it was possible to calculate a Fourier synthesis based on all reflexions $h0l$ and to obtain more accurate values for the parameters of Hg and S (p. 1426—28).

INTENSITIES

The values of I_{h0l} and I_{hk0} were estimated visually from the Weissenberg photographs.

In order to reduce the errors introduced by the absorption effects, average values of I were used for reflexions which should have the same intensities because of the Laue symmetry D_{3d}-$\bar{3}m$ (Table 3).

"F^2" for each reflexion was calculated from "F^2" $= I/f$, where I is the estimated value of the intensity, $f = 1 + \cos^2 2\Theta/\sin 2\Theta$ and Θ is the glancing angle. For each reflexion, f was taken from a curve where $1 + \cos^2 2\Theta/\sin 2\Theta$ had been plotted against $\sin^2\Theta$. $\sin^2\Theta$ was calculated from $\sin^2\Theta = k_1 (h^2 + k^2 + hk) + k_2 l^2$, where $k_1 = \lambda^2/3a^2$ and $k_2 = \lambda^2/4c^2$. a and c were obtained from the powder photographs.

Table 3. Estimated intensities and mean values of I for some reflexions h0l and hk0 from Weissenberg photographs of HgS. CuKa radiation.

	I	I		I	I
hkl	estimated	mean value	*hkl*	estimated	mean value
$20\bar{8}$	30		210	50	
$20\bar{2}$	83	45	120	40	36
204	35		$\bar{1}30$	30	
20 10	30		$\bar{2}30$	25	
$30\bar{9}$	80		310	15	
$30\bar{3}$	75	79	130	10	10
303	80		$\bar{1}40$	9	
309	80		$\bar{3}40$	4	
$40\bar{2}$	15	11	110	60	62
404	7		$\bar{1}20$	65	
			$\bar{2}10$	60	
101	15	9			
107	3		220	35	28
			$\bar{2}40$	20	

In these "F^2" values no correction was thus made for thermal movement, nor for absorption.

The mean values of I and "F^2" for $hk0$ are given in Table 4 a, for $h0l$ in Table 4 b. Values of "F" for the reflexions $h00$ are given in Table 4 c and values of "F" for the reflexions $h0,3L$ in Table 4 d.

Since the values for I, "F^2" and "F" are given in an arbitrary scale, the Patterson function and the electron density ϱ obtained in the following are also expressed on a relative scale.

ON THE FUNCTION ϱ_3

The complex structure factor of hkl can be expressed by

$$F_{hkl} = \int_0^1 \int_0^1 \int_0^1 e^{-2\pi i\,(hx + ky + lz)}\, \varrho\ (xyz)\ dxdydz$$

where ϱ is the electron density, thus $\varrho\,(xyz)\ dxdydz$ is the average number of electrons in the volume element $dxdydz$.

Table 4 a. Mean values of I and "F^2" from hk0 from Weissenberg photograph of HgS. CuKa radiation.

	I						"F^2"					
k	$0k0$	$1k0$	$2k0$	$3k0$	$4k0$	$5k0$	$0k0$	$1k0$	$2k0$	$3k0$	$4k0$	$5k0$
$\bar{5}$		34	46	46	34			7	20	20	7	
$\bar{4}$	62	10	28	10	62	34	44	10	28	10	44	7
$\bar{3}$	35	32	32	35	10	46	35	27	27	35	10	20
$\bar{2}$	4	62	4	32	28	46	2	28	2	27	28	20
$\bar{1}$	13	13	62	32	10	34	3	3	28	27	10	7
0		13	4	35	62			3	2	35	44	
1	13	62	32	10	34		3	28	27	10	7	
2	4	32	28	46			2	27	28	20		
3	35	10	46				35	10	20			
4	62	34					44	7				

Table 4 b. Mean values of I and "F^2" from h0l from Weissenberg photograph of HgS. CuKα radiation.

	I					"F^2"				
l	00l	10l	20l	30l	40l	00l	10l	20l	30l	40l
± 12										
± 6	60	10	3	47	78	41	3	2	45	28
0										
7										
1		9	116	36	12		4	72	34	5
$\bar{5}$										
$\bar{11}$										
8										
2		60	23	5	31		27	15	5	19
$\bar{4}$										
$\bar{10}$										
± 3	47	6	12	79	38	16	2	8	48	23
± 9										
10										
4		0	45	75	11		0	31	64	7
$\bar{2}$										
$\bar{8}$										
11										
5		50	27	25	28		20	17	23	14
$\bar{1}$										
$\bar{7}$										

Table 4 c. Values for "F" of the reflexions h00, calculated from the average "F^2" of h00, 0h0 and $h\bar{h}0$ in the Weissenberg photograph hk0.

hkl/	100	200	300	400
	1.7	$\overline{1.4}$	5.9	6.6

Table 4 d. Values of "F" of the reflexions h0,3L, calculated from "F^2" in the Weissenberg photograph h0l.

l/	00l	10l	20l	30l	40l
± 12					
± 6	6.4	1.6	$\overline{1.4}$	6.7	5.3
0					
± 9					
	3.9	1.4	$\overline{2.9}$	7.0	4.7
± 3					

$$F_{h0l} = \int_0^1\int_0^1 e^{-2\pi i\,(hx+lz)}\,dxdz \int_0^1 \varrho\,(xyz)dy = \int_0^1\int_0^1 e^{-2\pi i\,(hx+lz)}\,\varrho\,(xpz)\,dxdz$$

where $\varrho\,(xpz)$ is the projection of the electron density $\varrho\,(xyz)$ on the xz plane.

For the reflexions $h0,3L$

$$F_{h0,3L} = \int_0^1\int_0^1 e^{-2\pi i\,(hx+3Lz)}\,\varrho\,(xpz)\,dxdz \tag{1}$$

The electron density $\varrho\,(xpz)$ is the same for z, $z+1$, $z+2$ — — — — but the term $e^{-2\pi i\,(hx+3Lz)}$ has the same value for z, $z+\frac{1}{3}$, $z+\frac{2}{3}$ — — — — —. Thus the z period is 1 for $\varrho\,(xpz)$ and $\frac{1}{3}$ for $e^{-2\pi i\,(hx+3Lz)}$. The xz plane can therefore be divided into 3 parts, corresponding points of which give the same value for the complex term $e^{-2\pi i\,(hx+3Lz)}$.

We replace z in (1) by a parameter

$$Z = 3\,z \tag{2}$$

and find

$$F_{h0,3L} = \int_0^1\int_0^1 e^{-2\pi i\,(hx+LZ)}\,\varrho_3(xpZ)\,dxdZ \tag{3}$$

where ϱ_3 is defined by

$$\varrho\,(x,\,y,\,z) + \varrho\,(x,\,y,\,z+\tfrac{1}{3}) + \varrho\,(x,\,y,\,z+\tfrac{2}{3}) = 3\,\varrho_3\,(x,\,y,\,Z) \tag{4}$$

The Z period of $\varrho_3(xyZ)$ and its projection $\varrho_3(xpZ)$ is 1.
By reversal of (3) we find

$$\varrho_3\,(xpZ) = \sum_h\sum_L F_{h0,3L}\,e^{-2\pi i\,(hx+LZ)} = \sum_h\sum_L F_{h0,3L}\,\cos\,2\pi\,(hx+LZ) \tag{5}$$

The general point position xyz [6 (c)] of D_3^4, if transferred to the projection $\varrho_3(xpZ)$ will give points at $\pm\,(xpZ,\ \bar{y}pZ,\ (y\text{-}x)\ pZ)$. Since the projection has thus a centre of symmetry, $F_{h0,3L} = F_{\bar{h}0,3\bar{L}}$ and the sine terms cancel out in (5).

In a hypothetical cell with the electron density $\varrho_3\,(xyZ)$ each atomic position 3 (a) in the ordinary cell (p. 1415) will correspond to 3 maxima of electron density at $x00,0x0$ and $\bar{x}\bar{x}0$. The projection $\varrho_3\,(xpZ)$ from the reflexions $h0,3L$ has maxima in $xp0,0p0$ and $\bar{x}p0$ and the cut $\varrho_3\,(xp0)$ has maxima at x, 0 and $\bar{x}$.

In the same manner an atomic position 3 (b) (p. 1415) gives in the projection $\varrho_3(xpZ)$ maxima of electron density at $xp\frac{1}{2}$, $0p\frac{1}{2}$ and $\bar{x}p\frac{1}{2}$. The cut $\varrho_3(xp\frac{1}{2})$ gives maxima at x, 0 and $\bar{x}$.

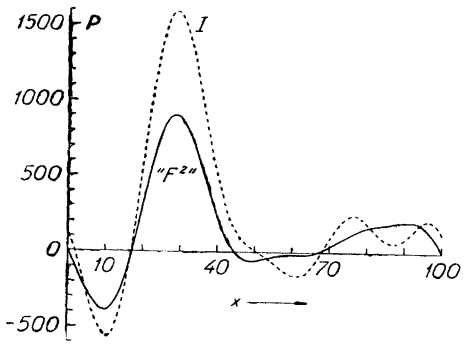


Figure 2. Patterson function $P(xp\frac{1}{3})$ of HgS (cinnabar).

Full curve: $\sum_h$"F^2"$_{h0l}\cos 2\pi(hx + \frac{1}{3})$

Dotted » $\sum_h I_{h0l}\cos 2\pi(hx + \frac{1}{3})$

For the two cuts desired, equation (5) gives:

$$\varrho_3(xp0) = \sum_h \sum_L F_{h0,3L} \cos 2\pi hx = \sum_h \cos 2\pi hx \left[\sum_{L \text{ even}} F_{h0,3L} + \sum_{L \text{ odd}} F_{h0,3L} \right] \quad (6)$$

$$\varrho_3(xp\tfrac{1}{2}) = \sum_h \sum_L F_{h0,3L} \cos 2\pi(hx + \tfrac{1}{2}) = \sum_h \cos 2\pi hx \left[\sum_{L \text{ even}} F_{h0,3L} - \sum_{L \text{ odd}} F_{h0,3L} \right] \quad (7)$$

These functions will be used in the calculations which follow.

PRELIMINARY DETERMINATION OF THE PARAMETERS

The highest maximum of the Patterson projections is found in $P(xp\frac{1}{3})$ at $x = 0.29$ (Fig. 2). This must be due to the distance Hg → Hg, which should give a maximum of weight 2 at $\bar{x}_1$ (Table 5). From this follows $x_1 = 0.71$.

In order to secure more information on the positions of the Hg atoms and especially on the positions of the S atoms the Fourier sums $\varrho_3(xp0)$ and $\varrho_3(xp\frac{1}{2})$ were calculated from (6) and (7), using solely the reflexions $h0,3L$. The signs of the amplitudes (Table 4 d) were determined using the approximate parameter $x_1 = 0.71$ and neglecting the influence of the S atoms.

Atoms in the point position 3 (a) (levels 0, $\frac{1}{3}$ and $\frac{2}{3}$) should give maxima at 0, x and $\bar{x}$ in the function $\varrho_3(xp0)$. In the same way atoms in the point position 3 (b) (levels $\frac{1}{6}$, $\frac{1}{2}$ and $\frac{5}{6}$) should give maxima at 0, x and $\bar{x}$ in $\varrho_3(xp\frac{1}{2})$. Now in each of the two cuts there is one such group of maxima (Fig. 3). The high maxima in $\varrho_3(xp0)$ are due to Hg atoms, which occupy 3 (a) according to our arbitrary assumption in calculating the signs. From the much lower maximum in $\varrho_3(xp\frac{1}{2})$ it was concluded that the S atoms occupy the point position 3 (b).

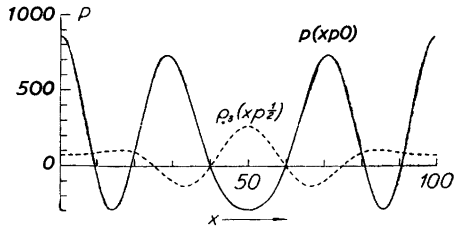


Figure 3. Fourier sums of HgS (cinnabar).
Full curve: $\varrho_3(xp0)$
Dotted » $\varrho_3(xp\frac{1}{2})$

The cut $\varrho_3(xp0)$ gives the value $x_1 = 0.71$ for the Hg parameter and $\varrho_3(xp\frac{1}{2})$ the value $x_2 \approx 0.50$ for the S parameter (Fig. 3, Table 6).

If we consider separately the sums $\sum_h \sum_{L \text{ even}} "F"_{h0,3L} \cos 2\pi hx$ and $\sum_h \sum_{L \text{ odd}} "F"_{h0,3L} \cos 2\pi hx$, which were used for calculating $\varrho_3(xp0)$ and $\varrho_3(xp\frac{1}{2})$, we find the values $x_1 = 0.72$ and $x_1 = 0.71$ (Fig. 4).

Since it seemed definite that Hg occupies 3 (a) and S 3 (b), Tables 5 and 6 were made giving the expected positions of maxima in various Patterson and Fourier cuts, the positions observed, and the derived values for x_1 and x_2.

The Fourier sum $\varrho\ (xp0)$ was computed, using the reflexions $h00$ and equivalents from the photograph $hk0$.

From this projection a value $x_1 = 0.73$ was obtained for the Hg parameter (Fig. 5, Table 6). Since the cut $\varrho\ (xp0)$ is based on only a few reflexions, less weight should be ascribed to this value $x_1 = 0.73$ than to the other values for x_1, which seem to be of greater accuracy.

From the Fourier projections (Table 6) and the Patterson projection $P(xp\frac{1}{3})$ (Fig. 2, Table 5) the average value 0.72 ± 0.01 was assumed for the Hg parameter on calculating the S parameter x_2.

As seen from Table 5 the projections based on values of I and those based on values of "F^2" gave somewhat different values for x_2.

The value $x_2 = 0.50$ from the cut $\varrho_3(xp\frac{1}{2})$ (Table 6) is not very accurate since the maximum at 0.50 (Fig. 3) may also consist of two overlapping maxima.

The average value of the S parameter was assumed to be 0.48 ± 0.02.

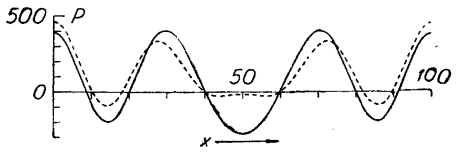


Figure 4. Fourier sums of HgS (cinnabar).
Full curve: $\sum_h \sum_{L \text{ odd}} "F"_{h0,3L} \cos 2\pi hx$
Dotted » $\sum_h \sum_{L \text{ even}} "F"_{h0,3L} \cos 2\pi hx$

Table 5. Patterson projections

x_1 is the Hg parameter and x_2 the S parameter.

Projection	x value expected distance		x value observed distance		x value calc.		rel. weight in units		rel. weight observed
	Hg → Hg	Hg → S	Hg → Hg "F^2" I	Hg → S "F^2" I	Hg	S	$(Hg)^2$	HgS	
P $(xp\frac{1}{2})$ Fig. 6		$\pm(x_1-x_2)$ 0		$\pm$ 0.23 $\pm$ 0.23		0.49		2 1	high —
P $(xp\frac{1}{3})$ Fig. 2	$\bar{x}_1$ $2x_1$		0.29 0.29		0.71		2 1		high
P $(xp\frac{1}{6})$ Fig. 7		x_1 $-(x_1+x_2)$ x_2		0.81 0.80 0.45 0.47		 0.45 0.47		2 2 2	high, broad high
P $(x0p)$ Fig. 8		$x_1 - x_2$		0.26 0.23		0.46 0.49			small
P $(2\,xxp)$ Fig. 9		$x_1 - x_2$		0.26					small

FINAL FOURIER CUTS

Finally the Fourier sums ϱ $(xp0)$ and ϱ $(xp\frac{1}{2})$ were computed using all reflexions $h0l$.

$$\varrho(xpz) = \sum_h \sum_l e^{-2\pi i(hx+lz)} F_{h0l} = \sum_h \sum_l e^{-2\pi i(hx+lz)} (A_{h0l} + iB_{h0l}) =$$

$$= \sum_h \sum_l \left[A_{h0l} \cos 2\pi(hx + lz) - B_{h0l} \sin 2\pi(hx + lz) \right]$$

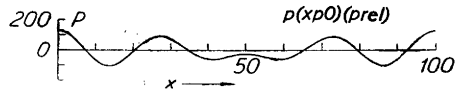
since the imaginary terms cancel out.

Table 6. Fourier sums.

x_1 is the Hg parameter and x_2 the S parameter.

cut	x value expected	x value observed	x calculated Hg	x calculated S
ϱ_3 $(xp0)$	0, $\pm x_1$	0.29, 0.71	0.71	
ϱ_3 $(xp\frac{1}{2})$	0, $\pm x_2$	0.50		$\approx$ 0.50
ϱ $(xp0)$ (prel.)	0, $\pm x_1$	0.27, 0.73	0.73	
ϱ $(xp0)$ (final)	x_1	0.720	0.720	
ϱ $(xp\frac{1}{2})$ (final)	x_2	0.48_5		0.48_5

Figure 5. Fourier sum $\sum_h$ "F"$_{h00}\cos 2\pi hx$ *of HgS (cinnabar).*



The cut $\varrho(xp0)$ was calculated to study the Hg parameter and the cut $\varrho(xp\frac{1}{2})$ to study the S parameter.

$$\varrho(xp0) = \sum_h \sum_l A_{h0l} \cos 2\pi hx - \sum_h \sum_l B_{h0l} \sin 2\pi hx$$

$$\varrho(xp\tfrac{1}{2}) = \left[\sum_h \sum_l A_{h0l} \cos 2\pi hx - \sum_h \sum_l B_{h0l} \sin 2\pi hx\right]_{l \text{ even}} -$$

$$-\left[\sum_h \sum_l A_{h0l} \cos 2\pi hx - \sum_h \sum_l B_{h0l} \sin 2\pi hx\right]_{l \text{ odd}}$$

The values of A_{h0l} and B_{h0l} that were used for these projections were calculated from $A_{h0l} = |\text{"F"}_{h0l}| \cos\alpha_{h0l}$, $B_{h0l} = |\text{"F"}_{h0l}| \sin\alpha_{h0l}$. α_{h0l} = phase angle.

For each reflexion $h0l$, A_{h0l} and B_{h0l} were also computed according to the appropriate terms of the structure factors in the *International tables* and with the aid of the values $x_1 = 0.72$, $x_2 = 0.48$ and α_{h0l} calculated from $\alpha_{h0l} = \text{arctg}\,\dfrac{B_{h0l}}{A_{h0l}}$.

"F" was calculated from the visually estimated values of I of the reflexions $h0l$ (p. 1420).

In Table 7 are given the values for $|\text{"}F\text{"}_{h0l}| \sin\alpha_{h0l}$ and in 8 the values for $|\text{"}F\text{"}_{h0l}| \cos\alpha_{h0l}$.

The projections $\varrho(xp0)$ and $\varrho(xp\frac{1}{2})$ are given in Fig. 11. The cut $\varrho(xp0)$ has a very high and well defined maximum at 0.720, from which the Hg para-

Table 7.

$|\text{"}F\text{"}_{h0l}| \sin\alpha_{h0l} = B_{h0l}$

$hl \rightarrow$ ↓	0	1	2	3	4	5
0	0			0		
1	0	1.6_{25}	4.5_{00}	0	$\overline{0}$	3.9_{00}
2	0	$\overline{7.3}_{58}$	3.3_{70}	0	$\overline{4.8}_{58}$	3.5_{51}
3	0	$\overline{5.0}_{45}$	$\overline{1.9}_{28}$	0	$\overline{6.9}_{27}$	$\overline{4.1}_{60}$
4	0	1.9_{23}	3.7_{17}	0	2.2_{88}	3.2_{65}

Table 8.

$|\text{"}F\text{"}_{h0l}| \cos\alpha_{h0l} = A_{h0l}$

$hl \rightarrow$ ↓	0	1	2	3	4	5
0	6.4_{03}			3.9_{37}		
1	1.5_{81}	$\overline{0.9}_{26}$	$\overline{2.6}_{06}$	1.4_{14}	$\overline{0}$	$\overline{2.2}_{66}$
2	$\overline{1.4}_{14}$	4.2_{55}	1.9_{82}	$\overline{2.8}_{81}$	$\overline{2.7}_{85}$	2.0_{29}
3	6.6_{86}	$\overline{2.9}_{23}$	$\overline{1.1}_{39}$	6.9_{50}	4.0_{01}	$\overline{2.3}_{94}$
4	5.3_{20}	1.1_{48}	$\overline{2.1}_{62}$	4.7_{43}	1.3_{37}	$\overline{1.8}_{42}$

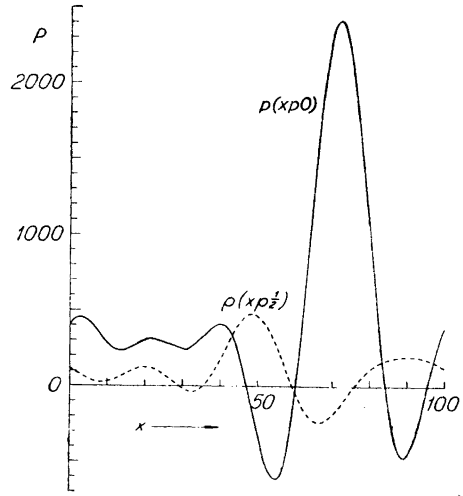


Figure 11. Fourier sums of HgS (cinnabar).
Full curve: $\varrho(xp0)$
Dotted » $\varrho(xp\frac{1}{2})$

meter was fixed at 0.720 ± 0.003. The sulphur parameter was fixed at $0.48_5 \pm 0.01$ from $\varrho(xp\frac{1}{2})$.

There seemed to be no point in trying to obtain greater accuracy with the experimental data available.

DETAILED DISCUSSION OF THE PATTERSON PROJECTIONS

$P(xp\frac{1}{3})$: (Fig. 2)

The projection has its highest maximum at $x = 0.29$ due to the distance Hg → Hg of weight 2 at $\bar{x}_1 \cdot A$ Hg → Hg maximum of weight 1 would be expected at $x \approx 0.40$. However, it seems probable that the maximum at ≈ 0.40 is concealed by the maximum at 0.29. Maxima are also expected from interatomic distances S → S in this projection. However, they are too small to stand out among the false maxima.

$P(xp\frac{1}{2})$: (Fig. 6)

The cut based on the values of I has a small maximum at $x = 0.50$ which could not be found in the cut "F^2". The projection of "F^2" sums probably gives more accurate values than that of I sums. The Hg → S maximum of weight 1 expected at 0 is not observed.

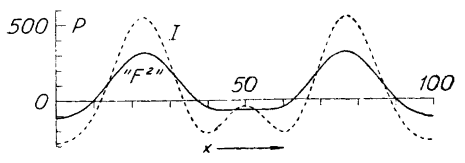


Figure 6. Patterson function $P(xp\frac{1}{2})$ of HgS (cinnabar).
Full curve: $\sum_h "F^2"_{h0l} \cos 2\pi(hx + \frac{1}{2})$
Dotted » $\sum_h I_{h0l} \cos 2\pi(hx + \frac{1}{2})$

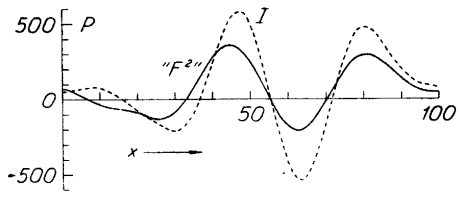


Figure 7. Patterson function $P(xp\frac{1}{6})$ of HgS (cinnabar).

Full curve: $\sum_h$"F^2"$_{h0l}\cos 2\pi(hx + \frac{1}{6})$

Dotted » $\sum_h I_{h0l}\cos 2\pi(hx + \frac{1}{6})$

$P(xp\frac{1}{6})$: (Fig. 7)

The maximum at $x = 0.80-0.81$ is not of the same height but much broader than the Hg → S maximum found at 0.45 ("F^2" sum) and 0.47 (I sum). This maximum (0.80–0.81) is probably due to overlapping of a Hg → S maximum expected at 0.72 and a Hg → S maximum, expected at 0.80.

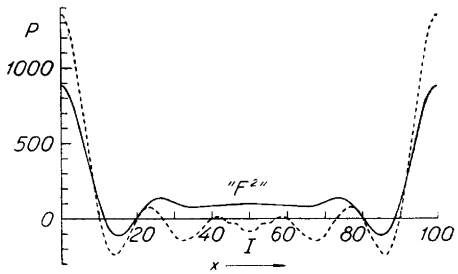


Figure 8. Patterson function $P(x0p)$ of HgS (cinnabar).

Full curve: $\sum_h$"F^2"$_{hk0}\cos 2\pi hx$

Dotted » $\sum_h I_{hk0}\cos 2\pi hx$

$P(x0p)$: (Fig. 8)

In the cut $P(x0p)$ maxima depending on the distance Hg → S are expected. The cut I gives its highest maximum at x = 0.23 and a smaller one at $x = 0.42$. In the cut "F^2" there is only one maximum at $x = 0.26$. No significance could be ascribed to the maximum at $x = 0.42$ (cut I).

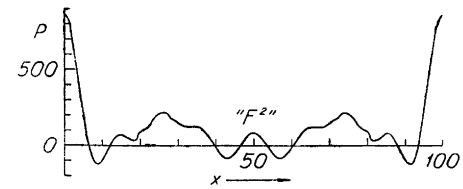


Figure 9. Patterson function $P(2xxp) = \sum_h$"F^2"$\cos 2\pi(2h + k)x$ of HgS (cinnabar).

$P(2xxp)$: (Fig. 9)

In the cut $P(2xxp)$ are expected maxima, resulting from the interaction between maxima depending on the distance Hg → Hg and maxima depending on the distance Hg → S (Fig. 10). The projection has a much too complicated appearance to give a clear understanding about the parameters of Hg and S.

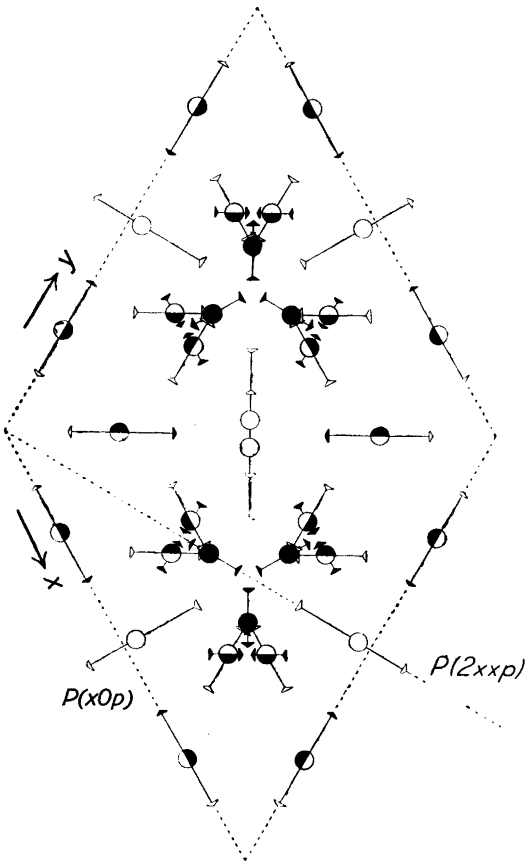


Figure 10. Expected maxima in the Patterson functions. The centres of the spheres are situated in the points calculated with the aid of the parameter values $x_{Hg} = 0.720$ *and* $x_S = 0.48_5$. *Expected Hg→Hg maxima are marked out with black spheres, S→S maxima with white spheres and Hg→S maxima with spheres, half black. The black arrows indicate the displacements of the Hg→Hg and Hg→S maxima which would follow from altering the Hg parameter. The white ones show the displacements of the S→S and Hg→S maxima, following from an alteration of the S parameter.* x_{Hg} *is assumed to be in the range 0.68—0.75 and* $x_S = 0.40—0.55$. *The arrows point in the direction of increasing parameter values.*

FINAL STRUCTURE PROPOSITION

Now that the positions of the sulphur and the mercury atoms have been determined, the intensities of the reflexions *hk*0 and *h*0*l* of the Weissenberg photographs were calculated, considering the scattering power of both the Hg and the S atoms.

In Tables 9 and 10 the calculated values of I are compared with the observed intensities of the reflexions in the Weissenberg photographs *hk*0 and *h*0*l*. They agree as closely as can be expected, bearing in mind that the "observed intensities" are mean values of the estimated intensities, which sometimes differ considerably because of asymmetrical absorption (see again Table 3).

Thus the following structure is proposed for HgS (cinnabar):
Hexagonal, $a = 4.146$ Å, $c = 9.497$ Å, 3 HgS per unit cell.

Table 9. Calculated and observed intensities of hk0 from Weissenberg photograph of HgS. CuKa radiation. The calculated intensity is given on the left of each column and the intensity estimated on the right.

$$I_{\text{calc.}} = \tfrac{2}{3}\ (F/2f_{\text{Hg}})^2\ 10^3$$

k	$\bar{5}$		$\bar{4}$		$\bar{3}$		$\bar{2}$		$\bar{1}$		0		1		2		3		4	
	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.
0k0			55	44	23	35	1	2	1	3			1	3	1	2	23	35	55	44
1k0	10	7	8	10	18	27	45	28	1	3	1	3	45	28	18	27	8	10	10	7
2k0	30	20	15	28	18	27	1	2	45	28	1	2	18	27	15	28	30	20		
3k0	30	20	8	10	23	35	18	27	18	27	23	35	8	10	30	20				
4k0	10	7	55	44	8	10	15	28	8	10	55	44	10	7						
5k0			10	7	48	20	48	20	10	7										

Table 10. Calculated and observed intensities of h0l from Weissenberg photograph of HgS. CuKa radiation. The calculated intensity is given on the left of each column and the intensity estimated on the right.

$$I_{\text{calc.}} = \tfrac{5}{9}\ (F/f_{\text{Hg}})^2\ 10^3$$

l	00l		10l		20l		30l		40l	
	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.	calc.	obs.
0 ± 12, ± 6	69	41	1	3	1	2	21	45	49	28
1, 7 $\bar{5}$, $\bar{11}$			4	4	39	72	12	34	3	5
2, 8 $\bar{4}$, $\bar{10}$			57	27	11	15	3	5	15	19
± 3 ± 9	34	16	3	2	11	8	28	48	21	23
4, 10 $\bar{2}$, $\bar{8}$			0	0	35	31	32	64	6	7
5, 11 $\bar{1}$, $\bar{7}$			37	20	8	17	8	23	9	14

Space group $D_3^4 - C3_12$

3 Hg in 3 (a): $x\ 0\ \frac{1}{3},\ 0\ x\ \frac{2}{3},\ \bar{x}\ \bar{x}\ 0$ $x = 0.720 \pm 0.003$
3 S in 3 (b): $x\ 0\ \frac{5}{6},\ 0\ x\ \frac{1}{6},\ \bar{x}\ \bar{x}\ \frac{1}{2}$ $x = 0.48_5 \pm 0.01$

DISCUSSION OF THE STRUCTURE

If S — Hg — S are to lie on a straight line, x_1 and x_2 must satisfy the equation: $x_1 = 1 - \frac{1}{2}x_2$. The Hg parameter $x_1 = 0.720 \pm 0.003$ is determined with greater accuracy than the S parameter $x_2 = 0.48_5 \pm 0.01$. If the value 0.720 for x_1 is inserted in the above equation the value $x_2 = 0.56$ is obtained and differs too greatly from the experimental value $x_2 = 0.48_5$. Thus S — Hg — S do not lie on a straight line.

The bonding angle $S_1 - Hg^* - S_1$ (Θ_1) (Fig. 12) was calculated from the formula:

$$\operatorname{tg}\frac{\Theta_1}{2} = \frac{\sqrt{0.75x_2^2 + \left(\frac{c}{a}\right)^2 \frac{1}{36}}}{1 - x_1 - 0.50x_2}$$

and the angle $Hg_1 - S_1 - Hg^*$ (Θ_2) (Fig. 12) was evaluated from:

$$\operatorname{tg}\frac{\Theta_2}{2} = \frac{\sqrt{0.75\,(1 - x_1)^2 + \left(\frac{c}{a}\right)^2 \frac{1}{36}}}{x_2 - 0.50\,(1 - x_1)}$$

With $x_1 = 0.720 \pm 0.003$ and $x_2 = 0.48_5 \pm 0.01$ the value of Θ_1 and Θ_2 are: $\Theta_1 = 172°{\cdot}4 \pm 1°{\cdot}7$, $\Theta_2 = 105°{\cdot}2 \pm 2°{\cdot}0$.

As seen Θ_1, the angle between the mercury bonds is not far from 180°, which is the angle between the mercury bonds of the halogen compounds $HgCl_2$ (s), $HgBr_2$ (s), yellow HgJ_2 (s), the gaseous molecules HgX_2 and HgSR (R = n-alkyl group) (p. 1413). Θ_2, the angle between the bonds from a sulphur atom, lies close to the values found for molecules containing 2-covalent S [SCl_2 103°, S_2Cl_2 105° ± 5° (Wells [14, p. 306])].

By assuming $x_1 = 0.720$ and $x_2 = 0.48_5$ the following distances and coordination were obtained:

The neighbourhood of one mercury atom ($\bar{x}_1\ \bar{x}_1\ 0$) Hg * in Fig. 12:

2 S at	2.3_6 Å	S_1	6 Hg at	4.1_5 Å	Hg_0
2 S	3.1_0	S_2	2 Hg	3.7_5	Hg_1
2 S	3.3_0	S_3	4 Hg	4.1_0	Hg_2, Hg_3

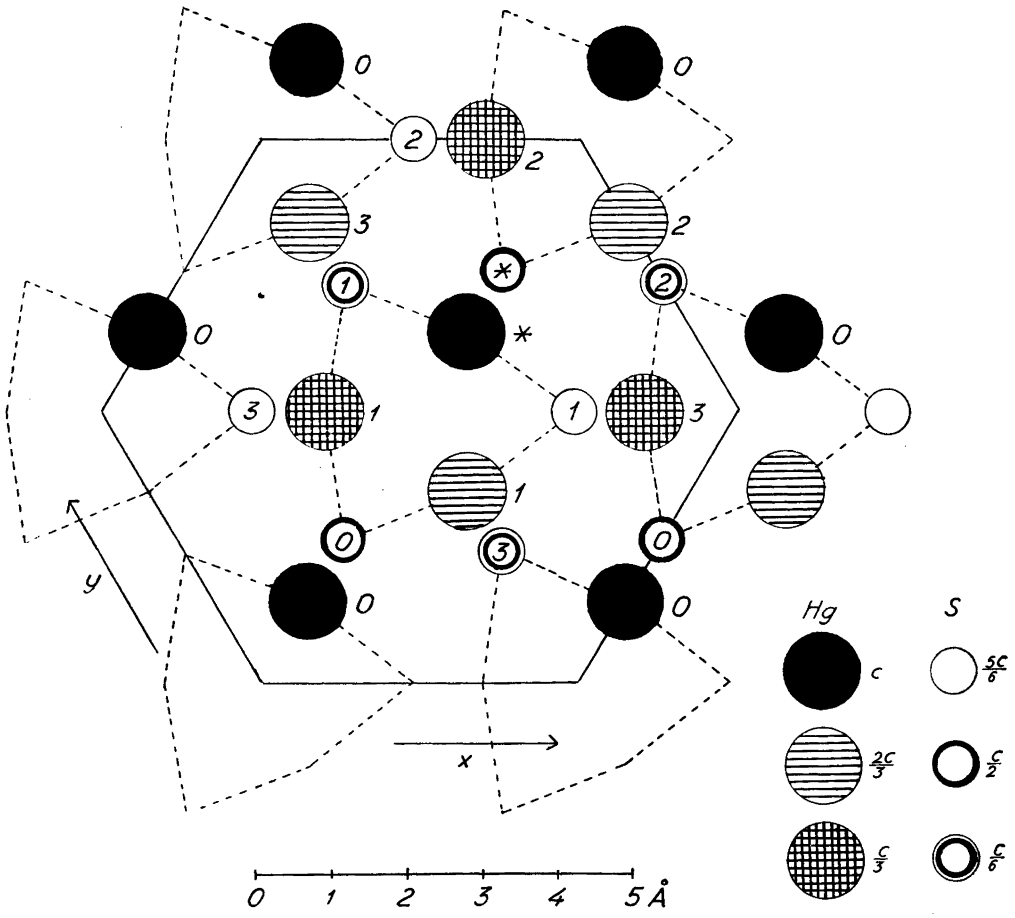


Figure 12. xy projection of the structure of cinnabar. The diagram shows the coordination of the atoms. The small circles (1), (2), (3) *show the S atoms and the larger ones* $\bigcirc_1$, $\bigcirc_2$, $\bigcirc_3$ *the Hg atoms. The distances Hg→Hg, Hg→S and S→S are listed in the text. The dotted line unites the atoms of one spiral chain.*

The neighbourhood of one sulphur atom ($\bar{x}_2$ $\bar{x}_2$ $\frac{1}{2}$) S * in figure 12:

2 Hg	at 2.3_6 Å	Hg_2	6 S	at 4.1_5 Å	S_0
2 Hg	3.1_0	Hg_3	2 S	4.8_7	S_3
2 Hg	3.3_0	Hg_1	4 S	3.7_9	S_1, S_2

(The distance Hg * → S * is 4.8_5 Å.)

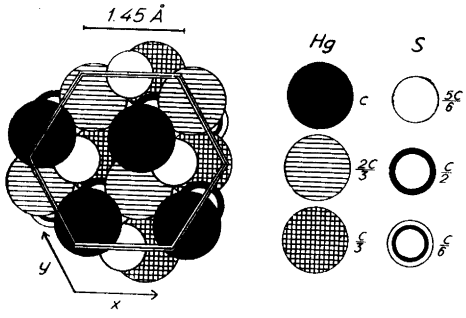


Figure 13. xy projection of the structure of cinnabar. The diagram shows the contents of one hexagon (3 hexagonal unit cells). The Hg atoms are situated at the levels $\frac{c}{3}$ $\frac{2c}{3}$ and c and the S atoms at the levels $\frac{c}{6}$ $\frac{c}{2}$ and $\frac{5c}{6}$. The distance Hg→S is 2.3_6 Å. The radii of the Hg and the S atoms are assumed to be 1.4_0 Å and 0.9_6 Å.

If HgS were built up from Hg^{2+} and S^{2-} ions, the distance Hg → S would be 2.94 kX. (Hg^{2+} : $r = 1.10$ kX, S^{2-}: $r = 1.84$ kX, according to Pauling [41]). This value is much longer than that found, 2.3_6 Å. Also, the distance calculated from covalent tetrahedral radii, 2.52 Å, seems to differ too much from the obtained value.

From the distances Hg → X in HgX_2 (gas) (Braune and Knoke [8], Gregg *et al* [9], see page 1414) and the normal covalent radii (Wells [18, p. 81]) the 2-covalent Hg radius was calculated to be $\approx 1.2_7$ Å. If the 2-covalent S radius is 1.04 Å (Wells [14, p. 81]), the distance Hg → S should be 2.3_2 Å, if the forces between the Hg and S atoms are exclusively homopolar.

The distance Hg → S actually found is 2.3_6 Å.

From this distance Hg → S 2.3_6 Å (2.3_5 kX) and the values of Θ_1 and Θ_2 the conclusion can be drawn that the bond between Hg and S is mainly homo-

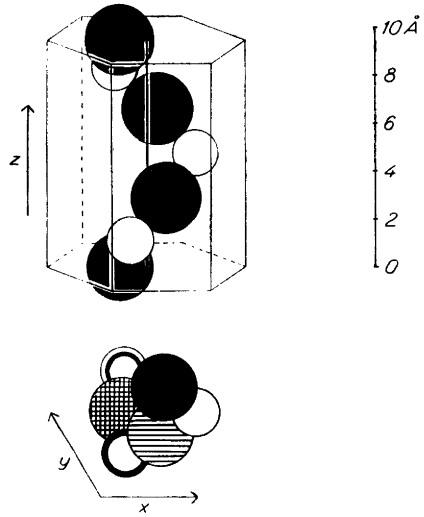


Figure 14. Diagrams from the side and above of one spiral chain —S—Hg—S— of the structure of cinnabar. The spiral chain extends indefinitely through the crystal in the vertical direction.

The atoms in the projection are seen in the centre of Fig. 13.

polar (sp type) in spite of the deviation of the angle from the normal value, 180°, for sp bonds.

Thus the structure of HgS can be described as consisting of a series of infinite spiral chains -S-Hg-S-, running parallel to the c-axis of the hexagonal unit cell (Figs. 13 and 14).

Within the chain each atom is strongly bound, chiefly by homopolar forces, to its two immediate neighbours, but between the chains the forces are far less strong, and the interatomic distances are correspondingly considerably greater than within the chain. The chains probably support each other by means of the longer Hg → S contacts. It can be noted that the Hg atoms approximate a close-packed lattice.

SUMMARY

The crystal structure of native cinnabar has been investigated. The positions of the Hg and S atoms have been determined by means of Fourier synthesis. The cell edges are: $a = 4.14_6$ Å, $c = 9.49_7$ Å (accuracy 0.05 %). The following structure is proposed:

Space group: $D_3^4 - C\ 3_1\ 2$

3 Hg in the point position 3 (a): $x\ 0\ \frac{1}{3},\ 0\ x\ \frac{2}{3},\ \bar{x}\ \bar{x}\ 0,$
$x = 0.720 \pm 0.003$

3 S in the point position 3 (b): $x\ 0\ \frac{5}{6},\ 0\ x\ \frac{1}{6},\ \bar{x}\ \bar{x}\ \frac{1}{2}$
$x = 0.48_5 \pm 0.01$

The structure is built up of infinite spiral chains, $(\text{-S-Hg-})_n$ running parallel to the c axis of the hexagonal unit cell.

The angle S-Hg-S is 172°.4 ± 1°.7 and the angle Hg-S-Hg 105°.2 ± 2°.0.

The distances Hg → S within the chain are 2.3_6 Å.

From the angles and distances the bonds seem to be mainly covalent sp bonds.

A 2-covalent Hg radius ≈ 1.2_7 Å may be probable.

I wish to thank the Head of the Department Professor A. Ölander for his kind interest. I am much indebted to Professor L. G. Sillén for suggesting the subject of this investigation and for helpful advice and continued interest during my work.

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Received June 22, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1437–1445

The Phase Diagram of Sodium Chloride and Steam Above the Critical Point

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It is well known that high pressure steam dissolves several solids, such as sodium chloride, silica, *etc.* This has caused great trouble in power plants operating with higher steam pressure than 75 kg/cm^2, as the dissolved substances will deposit on the turbine blades, heavily impairing their efficiency. Our original purpose was to ascertain the solubility of sodium chloride in steam at higher temperatures and pressures than were previously measured, but we have also studied the displacement of the critical phenomena, when salt is added to the water.

An extensive review of the work hitherto carried out in this field has recently been published by Booth and Bidwell [1], so it will be sufficient for us to direct the attention of the reader to the work of Spillner [2] and of Straub [3], both of whom investigated the salt contents of the steam phase by its electrical conductivity after cooling. Spillner also measured the dielectric constant of steam. He found that the conductivity of the solution of salt in steam was very small, so the electrolytic dissociation must be negligible, as could be expected from the low dielectric constant, the latter being only about 2.9 at the critical point. Schröer [4] measured the raising of the critical temperature when various halides were added to the water.

EXPERIMENTAL

Our measurements were carried out in a steel bomb, Fig. 1, which was lined with copper, about 5 mm thick. The steel was delivered by Wikmanshyttan. The quality used, VH 312, should, according to Swedish standards, contain 0.23—0.28 % C, 0.15—0.35 % Si, 0.50—0.80 % Mn, 0.9—1.2 % Cr

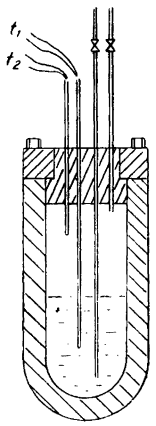

Fig. 1. High pressure vessel.

and 0.15—0.25 % Mo. Its tensile strength was ca. 72 kg/mm² at 500° C (communication from the steel work). The volume of the bomb was a little more than 5 litres. The bomb was heated by an electrical winding and was insulated with kieselguhr. The temperature was measured to 1° C with chromel-alumel-thermocouples, calibrated at the melting points of Sn, Pb and Zn. Two couples were situated in borings in the upper and lower part of the bomb and two were inside tubes running through the cover of the bomb and ending at different heights inside it. Usually the four thermocouples differed by only 1° C or less. The pressure was read to 1 kg/cm² on a Bourdon manometer calibrated by The Government Testing Institute, Stockholm, twice during the course of the investigation.

Samples were let out from the bomb through copper capillaries with 1 mm inner diameter, one ending near the top, the other near the bottom of the bomb. The capillaries passed through cooling mantles before the liquid was discharged through steel valves. Before a sample was taken for analysis an adequate quantity was let out through the capillary and discarded, in order to ensure that the sample gave the true composition of the phase in which the capillary ended. The chloride ion was titrated with silver nitrate using chromate as an indicator. The concentrations are given in weight per cent. The density of the high temperature phases was not measured, so the concentration in them cannot be given.

The bomb was filled through a steel pump. Both this and the steel valves rusted of course, but the iron hydroxide formed does not affect the measurements. The copper lining was also somewhat attacked, and especially the samples from the bottom of the bomb were usually more or less turbid from

some cuprous chloride and oxide. Presumably these compounds precipitated during the cooling of the sample and were kept in solution as complex compounds in the hot interior of the bomb. Their amount was so small that they cannot possible have interfered with the measurements. Of course there are much better materials than copper, but the vessel was available from an earlier investigation of technical nature concerning the possibility of making potable water from sea water by distillation at high pressure [5].

After the bomb was filled with a solution, it was heated, during which procedure liquid had to be let out. When the required temperature was reached and equilibrium established, samples were let out through the two capillaries, ending in the upper and lower part of the vessel. At the beginning these had the same composition, but below a certain pressure the contents parted into two phases, one at the bottom, being richer in salt, and another at the top with less salt. Near this critical pressure samples were taken at every kg/cm^2. Between the taking of every pair of samples at least 30 minutes were allowed to pass, often much more, in order to re-establish temperature equilibrium and allow the two phases to separate completely. With sinking pressure the lower phase became richer in salt, but the sampling had to be interrupted when its salt content exceeded 26 %, which is the solubility at room temperature, to avoid the capillary being obstructed by precipitated salt. The upper phase was sampled down to pressures about 150 kg/cm^2, where its salt content was of the order of magnitude 0.005 %.

MEASUREMENTS

Runs were made at intervals of about 10° from 353° C to 475° C. At temperatures higher than 445°, however, samples were taken only from the upper phase. In most cases two or three runs were carried out at every temperature. Samples were taken at every kg/cm^2 near the pressure at which the separation into two phases occurred, then at every 5 kg/cm^2, but at those pressures where only the upper phase was investigated at every 10 kg/cm^2. For every temperature the salt contents were plotted against the pressure and a smoothed curve drawn. Some of these are given in Fig. 2. At temperatures higher than 374° C, the critical temperature of pure water, the curves for the two phases will meet at a critical point, which corresponds to the highest pressure under which two phases can exist at that temperature.

In order to obtain this critical turning-point to the right of each curve one has to choose a definite critical composition of the solution, which is richer in salt the higher the temperature is. If a different composition is used, the formation of two phases will occur at a lower pressure and the middle part

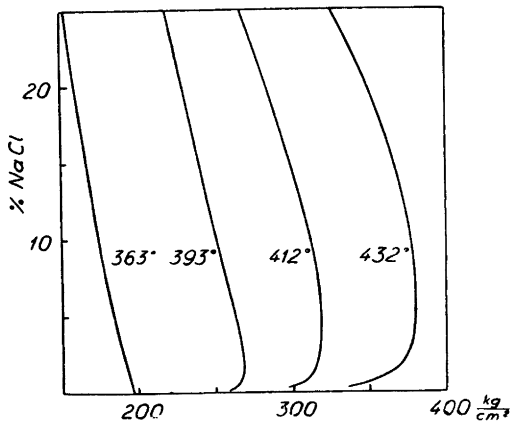

Fig. 2. Salt content of liquid, p—c-diagram.

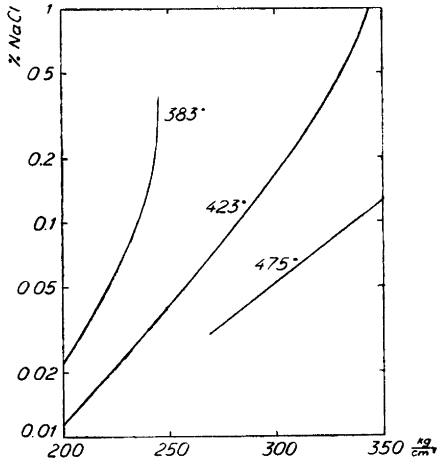

Fig. 3. Salt content of steam, p—log c-diagram.

of the curve is lost. The critical composition is obtained if the mean value of the compositions of the two phases is also plotted in the diagram. This gives a slightly bent curve which is extended to the critical pressure at that temperature. This pressure could be ascertained to within $\pm$ 1 kg/cm², as the pressure of separation was changed only a little if the original composition was not too far from the critical one. After a few preliminary experiments it was then possible to chose the correct solution for each temperature.

The logarithms of the salt contents of the upper phase, mostly less than 1 %, were also plotted against the pressure, whereby rather straight lines were obtained, Fig. 3. When all the results were plotted it was seen that the mean deviation of the measured points from the mean curves was about $\pm$ 2 kg/cm². Our measurements agree well with Spillner's few measurements at 407° C [2].

If the compositions of the phases are plotted against the temperature, we get, for each constant pressure, a curve showing the temperature *above* which two phases will occur.

At temperatures lower than 374° C the curves in Fig. 2 will start at zero salt concentration and the vapour pressure of pure water, as is shown for 363° C. This curve gives the vapour pressure of solutions up to 25 % NaCl. The steam above these solutions also contains some sodium chloride, but the amount is so small that it cannot be shown in the same figure. Fig. 4 gives the salt concentrations at two temperatures. These results are much less accurate than the other measurements. Sometimes greater concentrations were found

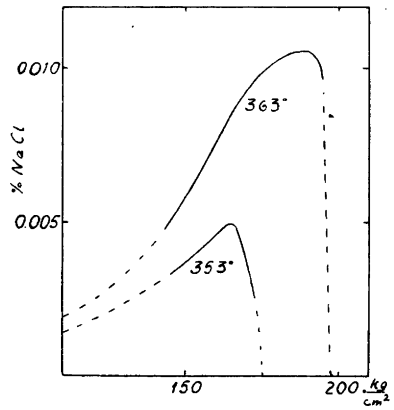

Fig. 4. Salt content of steam, p—c-diagram.

which is believed to be due to drops of the liquid solution being dragged along with the steam. The dashed parts of the curves are extrapolated.

Spillner [2] found that the steam containing salt had no electrical conductivity so there are no ions in it, but NaCl-molecules. He also measured the dielectric constant at the critical point to be 2.9, so that this water should be regarded as a "non-aqueous solvent".

RESULTS

From the smoothed curves obtained by plotting the concentration against pressure the critical compositions were found as mentioned above and these values plotted against temperature and pressure. A smoothed curve was drawn again, from which the plotted points only deviated by 0.1 %. The smoothed values are:

Table 1. Critical curve for NaCl-solutions.

% NaCl	0	0.2	0.5	1.0	1.5	2.0	3	4	5	6	7
t° C	374	379.5	383.5	388.5	394	399	408.5	416.5	424	431	437.5
p kg/cm²	224	239	248	259	271	283	309	332	354	376	397

If the values of the last two lines are plotted in a p-t-diagram a curve is obtained which forms a continuation of the vapour pressure curve of pure water, the uppermost curves in Figs. 5 and 6.

In this p-t-diagram were also plotted the smoothed pressure values corresponding to different concentrations, for all temperatures investigated. The resulting curves for the lower, concentrated phase are given in Fig. 5,

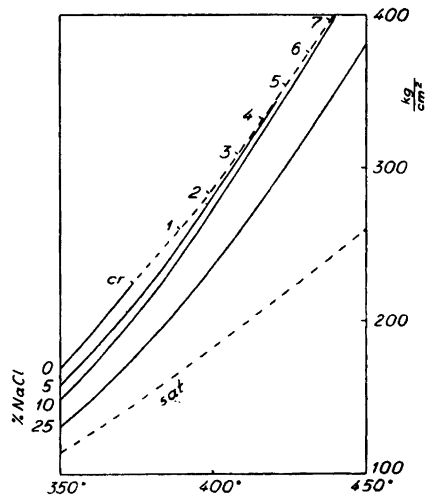

Fig. 5. Salt content of liquid, t—p-diagram.

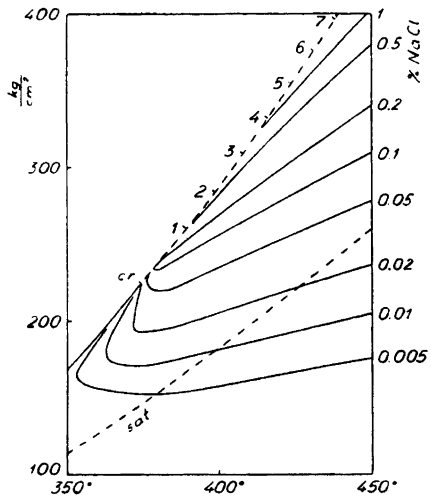

Fig. 6. Salt content of steam, t—p-diagram.

and for the upper steam phase in Fig. 6. The values, once more smoothed, are contained in Table 2. The broken line crossing the table indicates the place of the critical states, the pressures of which are given in the second line. The numbers above the broken line refer to steam hotter than 374° C, those below it to the phase at the bottom of the vessel.

In one experiment steam was let out from the vessel until the solution was saturated. This was indicated by the fact that, after letting out more steam, and raising the temperature to its original value before the letting out, the former pressure was restored. It was also found that the capillary outlet from the bottom was choked by salt, so it was difficult to pump more solution into the vessel. The pressures of the saturated solutions are given in the last line of Table 2 and the corresponding curve drawn in Figs. 5 and 6.

In the experiments in which the pressure became less than that of the saturated solution, the lower phase could not more be liquid, but was solid sodium chloride. Regarded as a phase diagram, Figs. 5 and 6 show three areas: at the left top a one-phase area, in the middle an area of two phases, liquid and steam, and at the right bottom the area of solid salt and superheated steam.

Table 3 gives approximate values of the salt contents of steam at three temperatures below the critical point of pure water in *parts per million*. Table 4 gives in *per cent* the salt contents of the solutions in equilibrium with the

Table 2. Pressure at which a phase exists containing the amount of salt shown in the first column.

t =	350	360	370	380	390	400	410	420	430	440	450	460	475° C
Crit. Pr.				240	262	286	313	343	373	405	436		
0 %	169	191	215										
0.005 %				149	154	159	163	166	169	171			
0.01				172	178	184	190	194	198	202	205	208	
0.02				193	199	206	213	220	226	231	236	241	
0.03				206	211	218	227	234	241	248	254	259	267
0.05				220	226	234	243	252	261	269	277	285	296
0.1				234	242	253	264	275	286	297	308	318	334
0.2				240	254	268	233	297	311	325	338	352	372
0.5	167	189	213	240	260	280	301	321	341	361	379	396	
1.0	166	187	211	236	262	284	309	333	358	382			
2.0	163	184	208	234	262	286	313	340	368	396			
5.0	157	178	201	226	254	283	313	343	373	403			
10	148	169	192	216	244	284	305	338	371	405			
15	140	161	184	208	233	261	291	324	369	395			
20	133	154	176	199	222	248	277	309	341	376			
25	127	147	168	189	211	235	260	289	321	256			
sat.	113	127	141	155	169	183	198	213	227	243	258	273	297

Table 3. Approximate salt content of steam below the critical point of pure water (in parts per million)

p kg/cm²	353°	363°	372°
140	30		
150	37		
160	46	77	
165	50		
170	35	93	105
175	0		
180		101	140
190		106	180
195		90	
198		0	
200			200
205			260
210			100
211			88
220			0

Table 4. Salt content of solutions in equilibrium with the steams recorded in Table 3 (in per cent)

353°	363°	372° C
21.8		
13.5	26	
7.0	18.8	
4.3	15.4	
1.9	12.3	27
0	9.2	24
	7.0	20.7
	2.9	14.3
	1.0	11.4
	0	9.7
		8.5
		5.9
		3.4
		3.0
		0

steams in Table 3 at the same temperatures and pressures. If the compositions of the two co-existing phases at some temperature above 374° are wanted, they can easily be obtained if the composition-pressure curve for that temperature is drawn, when they are found as the two values of the curve for a given pressure.

These results give an answer to the problem as to whether it is possible to free the steam from a boiler from the salt it has dissolved which is of interest in steam engineering (cf. the introduction). Spillner and others [2] are of the opinion that it is not possible to wash out the salt with feed water, but Straub [3] succeeded in purifying the steam to a high degree by running the steam through a small fractionating column with four bubble cap plates, and even with a column with only one single plate. If a phase diagram (c—t-diagram) is constructed from the measurements in this investigation, it will be seen, that the steam with salt (*cf.* Tables 3 and 4, *e. g.* at 170 kg/cm^2) will be in equilibrium with a water very much more rich in salt. Our results thus support Straub's, and it should be possible to wash the steam free from salt in a scrubber, provided the pressure is lower than 224 kg/cm^2, the critical pressure for pure water.

The results show that a salt solution at 300 kg/cm^2 and 475° C yields a steam with a little more than 0.05 % NaCl and a liquid which is almost saturated at room temperature. The advantage of this distillation procedure according to an invention by Baltzar von Platen is that the thermal efficiency is much better at this pressure and temperature than at atmospheric pressure. [5] Mr von Platen considers that water with 0.05 % NaCl, obtained from sea water, could be used for drinking and irrigation in places where potable water is scarce. A further purification by means of ion exchanger would be rather cheap. Such water could be immediately used as boiler water as the solubility of calcium and magnesium ions in the steam is very small. We have distilled sea water from Skagerrak at the west coast of Sweden with a chlorosity [6] of 14.72 g Cl^-/lit. at 300 kg/cm^2 and 450° C. The distillate contained 0.62 g/lit. Cl^-, $2.9 \cdot 10^{-4}$ mol/lit. $Ca^{2+} + Mg^{2+}$ (mean values from two experiments) and no sulphate ion detectable with barium salt.

SUMMARY

The phase diagram of the system sodium chloride — water — steam has been investigated in the region 353°—475° C and 150—400 kg/cm^2. Values are given for the vapour pressures of solutions and the solubilities of sodium chloride in steam.

A new method of making potable water from sea water is mentioned.

The apparatus was attended by Mr Fritz Wallin and Mr Gustav Eriksson. The analyses were carried out by Mr Ane Swärdh. During the last part of the investigation Baron Casper Wrede, Mr Hans Dinger and Mr Erik Lundblad also took part in the work.

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Received September 26, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1446–1449

Action of Strong Acids on Acetylated Glycosides

VII *. Transglycosidation of Xylosides

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The present paper deals with an investigation of the transglycosidation reaction with xylosides. The course of this reaction with glucosides and galactosides has been discussed in previous communications [1, 2] in which it was shown that acetylated alkyl β-glucosides and β-galactosides can be transformed into the respective α-forms by the action of strong acids, such as titanium tetrachloride, boron trifluoride or sulfuric acid. The velocity of this reaction is increased by electron repelling and decreased by electron attracting groups in the aglycone. The galactosides are transformed about twice as rapidly as the corresponding glucosides.

The xylosides have now been found to react in the same way, the only difference observed being that the reactions are much faster than for the hexosides, in fact about 50 times faster than for the corresponding glucosides. The kinetic determinations, the results of which are summarized in Table 1, were carried out in acetic anhydride-acetic acid, 10 : 3, with sulfuric acid as catalyst. From the values of the final rotations, which were much lower than if only α/β-xylose tetraacetate were formed by the acetolysis, it can be concluded that xylose hexaacetate is also formed. This is in agreement with the results found for glucosides and galactosides. All k-values are relative, the value for the transformation of β-glucose pentaacetate into the equilibrium mixture of α/β-acetates being taken as unity.

In each kinetic experiment a parallel run was made with the β-acetate, using the same catalyst solution. As the velocity constants of the individual reactions studied varied by several powers of ten, different concentrations of sulfuric acid were used. The velocities of transformation and of transglyco-

* Part VI, this journal, 4 (1950) 1386.

Table 1. Relative velocity constants for the transglycosidation and acetolysis of some acetylated xylose and glucose derivatives.

	β-Penta-acetate	β-Ethyl glycoside		β-*iso*-Propyl glycoside		β-Chloroethyl glycoside	
		$k_{transglyc.}$	$k_{acetolysis}$	$k_{transglyc.}$	$k_{acetolysis}$	$k_{transglyc.}$	$k_{acetolysis}$
Xylose	30	1 120	68	2 300	105	68	110
Glucose	1	15	0.07	50	0.08	0.4	0.03
Xylose/Glucose	30	75	970	46	1 300	170	3 600

sidation of sugar acetates vary to rather different extents with changes in the concentration of the catalyst, and therefore the values obtained are not strictly comparable.

It can be seen from Table 1 that transglycosidation occurs much more rapidly with xylosides than with glucosides. In the case of acetolysis the differences are still greater, the xylosides being acetolyzed at least 1 000 times faster than the corresponding glucosides. The ratio $k_{transglyc}/k_{acetolysis}$ for a xyloside is too small for the observed maximum in optical rotation to be used as a final value for the transglycosidation reaction. This maximum has therefore to be obtained by extrapolation, which makes the determinations rather inaccurate. In view of the errors thus introduced, together with the systematical errors discussed above, the results must be regarded as being only semi-quantitative. From the rotations of the pure α- and β-derivatives, the percentage of α-isomer in the equilibrium mixture can be calculated. The values for xylose tetraacetate and ethyl xyloside triacetate are both about 90 %.

The transglycosidation of ethyl β-xyloside triacetate by the action of titanium tetrachloride in chloroform was investigated as a preparative method; under conditions similar to those employed in the previous experiments with hexosides, a 45 % yield of ethyl α-xyloside triacetate was obtained.

EXPERIMENTAL

Ethyl β-xyloside triacetate

Ethyl β-xyloside triacetate was prepared by the mercuric acetate method. A solution of xylose bromide triacetate (6.78 g) and mercuric acetate (3.03 g) in a mixture of absolute benzene (40 ml) and ethanol (16 ml) was boiled on the steam bath for 15 minutes. After cooling, the solution was washed several times with water, dried over calcium

chloride and concentrated under reduced pressure. The crystalline residue was recrystallized from ethanol. Yield 3.1 g (51 %). M. p. 106—107° *. $[\alpha]_D^{20}$ —62° (Chloroform, C = 2)

$C_7H_{11}O_5(OCCH_3)_3$ (304.3) Calc. Acetyl 42.4
Found Acetyl 42.0

iso-Propyl β-xyloside triacetate

iso-Propyl β-xyloside triacetate was prepared by the mercuric acetate method as described above and in a yield of 62 %. It was recrystallized from ethanol. M. p. 119—120°. $[\alpha]_D^{20}$ —61° (Chloroform, C = 2)

$C_8H_{13}O_5$ $(OCCH_3)_3$ (318.3) Calc. Acetyl 40.6
Found Acetyl 41.0

β-Chloroethyl β-xyloside triacetate

β-Chloroethyl β-xyloside triacetate was also prepared by the same method as the ethyl β-xyloside and recrystallized from ethanol. Yield 49 %. M. p. 136—137°. $[\alpha]_D^{20}$ — 56° (Chloroform, C = 2). Coles, Dodds and Bergeim [3] report the melting point as 137° but give no value for the optical rotation.

Ethyl α-xyloside triacetate

Titanium tetrachloride (1.8 g) was added to a solution of ethyl β-xyloside triacetate (3 g) in absolute chloroform (90 ml). The solution became strongly yellow, but in contradistinction to the behaviour in similar reactions, no precipitate was formed. The solution was boiled for two minutes on the steam bath, then cooled, thoroughly washed with water, dried over calcium chloride and finally concentrated under reduced pressure. When the residue was crystallized from ethanol, a small amount of unchanged starting material (0.4 g) was recovered. The mother liquors were concentrated and the residue dissolved in light petroleum. From this solution crystals of the α-xyloside, m. p. 38—39°, slowly separated. Yield 1.4 g. The melting point was unchanged by further recrystallizations from the same solvent. $[\alpha]_D^{20}$ + 100°. (Chloroform, C = 2)

$C_7H_{11}O_5$ $(OCCH_3)_3$ (304.3) Calc. Acetyl 42.4
Found Acetyl 42.4

Kinetic determinations

The experimental conditions for the kinetic determinations were the same as in the preceding papers. (Compare Part III [1]). For the transglycosidation and acetolysis runs sulfuric acid of approximate concentration 0.004 C and 0.02 C respectively was used. A typical run is given in Table 2.

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* All melting points uncorrected.

Table 2. Transglycosidation of ethyl β-xyloside triacetate. β-Xylose tetraacetate and ethyl β-xyloside triacetate, 0.5 g of each, dissolved in 20 ml of 0.0043 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20°. Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min	β-Xylose tetraacetate		Ethyl β-xyloside triacetate	
	α_D	k	α_D	k
0	− 1.12°		− 3.08°	
4	− 1.00	0.0028	+ 1.82	0.116
8	− 0.91	24	+ 3.46	0.111
12	− 0.75	28	+ 3.62*	
16	− 0.63	29	+ 3.59	
20	− 0.48	31	+ 3.36	
24	− 0.34	31	+ 3.16	
30	− 0.19	30	+ 2.86	
∞	+ 3.80		+ 0.50	
	Mean value 0.0030		Mean value 0.112	

* The final value for the transglycosidation, calculated by extrapolation, is 4.40°.

SUMMARY

The transglycosidation of some xylosides with sulfuric acid and titanium tetrachloride has been investigated. The reaction is much faster than that of the corresponding hexosides investigated, but otherwise perfectly analogous.

The authors wish to thank *Statens Naturvetenskapliga Forskningsråd* for financial support.

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Received November 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1450–1463

The Number of Isomeric Aliphatic Hydrocarbons

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From time to time papers dealing with the problem of calculating the number of isomeric aliphatic hydrocarbons have been published. In the present paper recursion formulas for the calculation of isomeric hydrocarbons of the acetylene series and the ethylene series — with and without taking into consideration stereoisomerism — will be derived. These formulas presuppose the knowledge of the number of isomeric univalent radicals of the methane series, and these numbers are therefore mentioned in the sections I A and II A. The new formulas have been arrived at in I B, I C, II B and II C.

SYMBOLS USED IN THE PRESENT PAPER

n = the number of carbon atoms in a hydrocarbon molecule.
R_n = the radical C_nH_{2n+1}.
p_n = the number of isomeric primary radicals R_n
s_n = » » » » secondary » R_n
t_n = » » » » tertiary » R_n
T_n = » » » » radicals R_n
A_n = » » » » alkanes C_nH_{2n+2}
E_n = » » » » alkenes C_nH_{2n}
I_n = » » » » alkines C_nH_{2n-2}

(without taking stereoisomerism into account.)

I'_n = » » » stereoisomeric + non-stereoisomeric alkines.
$I(s)_n$ = » » » » alkines.
$I(ns)_n$ = » » » non-stereoisomeric alkines.

E'_n, $E(s)_n$, $E(ns)_n$; p'_n, $p(s)_n$, $p(ns)_n$; *etc.* are defined in the same manner.
a, b, c, d, g, h, i, j, x and y are integers > 0.

In many of the following formulas certain terms have to be left out for special values of n. It turns out that this happens for such values of n for

which the subscripts in the terms are fractions: *E. g.* in formula (2) the last term should be left out for such values of n for which the subscript $\frac{n-1}{2}$ is a fraction; that is for all even values of n. This is indicated in the formula by applying the factor λ to the last term. λ is in other words a function of n. It takes the values 1 or 0, according as the subscripts in the term to which λ is applied are integers or not.

I. STEREOISOMERISM NOT CONSIDERED

A. Hydrocarbons of the methane series (alkanes). In 1875 Cayley [1] tried to derive a formula by which the number of isomeric alkanes C_nH_{2n+2} could be calculated, and later on several attempts have been made to solve the problem (the literature until 1931 is reviewed by Henze and Blair [2, 3]). In 1931 Henze and Blair [3] succeeded in solving the problem by first deriving recursion formulas for the number of isomeric alcohols R_nOH classified in primary, secondary and tertiary alcohols [2].

From the definition of p_n, s_n, t_n and T_n it follows that

$$T_n = p_n + s_n + t_n$$

Henze and Blair found *):

$$p_n = T_{n-1} \tag{1}$$

and

$$s_n = \sum_{\substack{a < b \\ a + b = n-1}} T_a \cdot T_b + \lambda \cdot \tfrac{1}{2} \cdot T_{\frac{n-1}{2}} \cdot (T_{\frac{n-1}{2}} + 1) \tag{2}$$

and they also found a formula for t_n, which is of no interest for this paper. Using these formulas and the value $T_1 = 1$ it was possible to compute T_2, T_3, T_4, ... Henze and Blair thereafter gave direction for computing A_{2n} using the values of T_1, T_2, ... T_n and for computing A_{2n+1} using the values of T_1, T_2, ... T_{n+1}.

Using Henze and Blairs' formulas the numerical values of T_n, p_n, s_n and t_n for $n \leqq 30$ were published [2, 4], and the numerical values of A_n for $n \leqq 40$ and for $n = 60$ were also published [3, 4, 5].

* These and later formulas by Henze and Blair or others are here expressed in a more concise form than in the original papers.

B. Hydrocarbons of the acetylene series (alkines). Coffman, Blair and Henze [6] have calculated the number of isomeric alkines. They considered $R-C\equiv C-R'$, where at first they assumed R to represent a hydrogen atom and at the same time R′ an arbitrary radical R_{n-2}. Afterwards they assumed R and R′ to represent two radicals R_i and R_j, where $i < j$ and $i + j = n - 2$. The three authors found:

$$I_n = T_{n-2} + \sum_{\substack{i<j \\ i+j=n-2}} T_i \cdot T_j + \lambda \cdot \tfrac{1}{2} \cdot T_{\frac{n-2}{2}} \cdot \left(T_{\frac{n-2}{2}} + 1\right) \tag{3}$$

However, this formula can be rewritten by means of (1):

$$I_n = p_{n-1} + \sum_{\substack{1<g<h \\ g+h=n}} p_g \cdot p_h + \lambda \cdot \tfrac{1}{2} \cdot p_{\frac{n}{2}} \cdot \left(p_{\frac{n}{2}} + 1\right)$$

and using

$$p_{n-1} = p_1 \cdot p_{n-1}$$

we get:

$$I_n = \sum_{\substack{g<h \\ g+h=n}} p_g \cdot p_h + \lambda \cdot \tfrac{1}{2} \cdot p_{\frac{n}{2}} \left(p_{\frac{n}{2}} + 1\right) \tag{4}$$

The formula (4) may be derived directly from the following consideration. An alkine may be considered as consisting of two primary radicals R_g and R_h, in which two hydrogen atoms are removed from the primary carbon atom in R_g as well as in R_h, and thereafter the two resulting tervalent radicals are joined in a triple bond. This gives for n odd:

$$I_n = p_1 \cdot p_{n-1} + p_2 \cdot p_{n-2} + \ldots\ldots\ldots + p_{\frac{n-1}{2}} \cdot p_{\frac{n+1}{2}} \tag{4a}$$

and for n even:

$$I_n = p_1 \cdot p_{n-1} + p_2 \cdot p_{n-2} + \ldots\ldots\ldots + p_{\frac{n-2}{2}} \cdot p_{\frac{n+2}{2}} + \tfrac{1}{2} \cdot p_{\frac{n}{2}} \left(p_{\frac{n}{2}} + 1\right) \tag{4b}$$

The content of (4a) and (4b) is included in (4).

However, formula (4) can be further simplified. From (2) it follows that

$$s_{n-1} = \sum_{\substack{i<j \\ i+j=n-2}} T_i \cdot T_j + \lambda \cdot \tfrac{1}{2} \cdot T_{\frac{n-2}{2}} \, (T_{\frac{n-2}{2}} + 1)$$

and using (1) we get:

$$s_{n-1} = \sum_{\substack{1<g<h \\ g+h=n}} p_g \cdot p_h + \lambda \cdot \tfrac{1}{2} \cdot p_{\frac{n}{2}} \cdot (p_{\frac{n}{2}} + 1)$$

or since

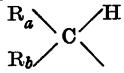
$$p_1 \cdot p_{n-1} = p_{n-1}$$

we get:

$$s_{n-1} = -p_{n-1} + \sum_{\substack{g<h \\ g+h=n}} p_g \cdot p_h + \lambda \cdot \tfrac{1}{2} \cdot p_{\frac{n}{2}} \cdot (p_{\frac{n}{2}} + 1)$$

which compared with (4) gives:

$$I_n = p_{n-1} + s_{n-1} \tag{5}$$

Formula (5) may also be derived in the following manner. In the isomeric alkines C_nH_{2n-2} α) a terminal carbon atom takes part in the triple bond, or β) no terminal carbon atom takes part in the triple bond. Alkines of type α may be derived from the primary radicals R_{n-1} by removing two hydrogen atoms from the primary carbon atom, and thereafter attaching the resulting tervalent radical to $\equiv$C—H; this procedure results in p_{n-1} different alkines. Alkines of type β may be derived from the secondary radicals R_{n-1} by removing the hydrogen atom and the radical R_a from  , and thereafter attaching the resulting tervalent radical to $\equiv$C—R_a; this procedure results in s_{n-1} different alkines.

Numerical values (found in literature [6]) of I_n for $n \leqq 30$ are checked by means of formula (5).

C. Hydrocarbons of the ethylene series (alkenes). Henze and Blair [7] have calculated the number of isomeric alkenes. They divided the problem into four parts, in accordance with the fact that all alkenes may be considered as derived from $H_2C = CH_2$ by substituting 1, 2, 3 or 4 hydrogen atoms by suitable radicals. In this manner the authors found a rather complicated method for calculating E_n, a method which may be carried through when T_x is known for $x \leqq n-2$.

However, a simple formula may be derived in a very direct manner as follows. We may think of an alkene as a hydrocarbon built up in the following way. From a primary or secondary radical R_g a hydrogen atom is removed from the primary or secondary carbon atom; and the same is done in the primary or secondary radical R_h. By joining the two resulting bivalent radicals in a double bond an alkene is produced. From this it follows that

$$E_n = \sum_{\substack{g<h \\ g+h=n}} (p_g + s_g) \cdot (p_h + s_h) + \lambda \cdot \tfrac{1}{2} \cdot (p_{\frac{n}{2}} + s_{\frac{n}{2}}) \cdot (p_{\frac{n}{2}} + s_{\frac{n}{2}} + 1) \tag{6}$$

which by means of (5) can be rewritten as:

$$E_n = \sum_{\substack{1<a<b \\ a+b=n+2}} I_a \cdot I_b + \lambda \cdot \tfrac{1}{2} \cdot I_{\frac{n+2}{2}} \cdot (I_{\frac{n+2}{2}} + 1) \tag{7}$$

Numerical values (found in literature [7, 8]) of E_n for $n \leqq 20$ are checked by means of formula (7), and the following values are calculated for $n \leqq 30$ (Table 1).

Table 1. The number, E_n, of isomeric alkenes (not considering stereoisomerism) with n carbon atoms ($20 < n \leqq 30$).

n	E_n	n	E_n
21	11 062 046	26	1 423 665 699
22	29 062 341	27	3 788 843 391
23	76 581 151	28	10 103 901 486
24	202 365 823	29	26 995 498 151
25	536 113 477	30	72 253 682 560

II. STEREOISOMERISM CONSIDERED

A. Alkanes. Blair and Henze have derived formulas for computing stereoisomeric and non-stereoisomeric primary, secondary and tertiary radicals [9] and thereafter formulas for computing stereoisomeric and non-stereoisomeric alkanes [10]. The chain of reasoning was analogous to that used by the authors in the case where stereoisomerism was not taken into account [2, 3], but the computations were for good reasons more complicated. The authors published [11] the numerical values for T'_n, $T(s)_n$, $T(ns)_n$; $p(s)_n$, $p(ns)_n$; $s(s)_n$, $s(ns)_n$; $t(s)_n$, $t(ns)_n$ for $n \leqq 20$; and also [12] for A'_n, $A(s)_n$, $A(ns)_n$ for $n \leqq 20$.

The following formulas taken from Blair and Henze are of interest for this paper:

$$p'_n = T'_{n-1} \tag{8a}$$

$$p(ns)_n = T(ns)_{n-1} \tag{8b}$$

$$s'_n = 2 \cdot \sum_{\substack{a<b \\ a+b=n-1}} T'_a \cdot T'_b + \lambda \cdot (T'_{\frac{n-1}{2}})^2 \qquad \text{for } n > 1 \tag{9}$$

B. Alkines. Coffman [11] has calculated the number of stereoisomeric and non-stereoisomeric alkines. The chain of reasoning was (apart from the fact that it was more complicated) the same as by Coffman, Blair and Henze [6], where stereoisomerism was not taken into account.

However, the considerations can be carried through in a more straightforward manner if the method which led to the formula (4) is used. No stereoisomerism is destroyed through joining two tervalent radicals, and therefore the following must be true:

$$I'_n = \sum_{\substack{g<h \\ g+h=n}} p'_g \cdot p'_h + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{n}{2}} \cdot (p'_{\frac{n}{2}} + 1) \tag{10}$$

A non-stereoisomeric alkine must necessarily be derived from two non-stereoisomeric radicals, and therefore we have:

$$I(ns)_n = \sum_{\substack{g<h \\ g+h=n}} p(ns)_g \cdot p(ns)_h + \lambda \cdot \tfrac{1}{2} \cdot p(ns)_{\frac{n}{2}} \cdot (p(ns)_{\frac{n}{2}} + 1) \tag{11}$$

Finally $I(s)_n$ is found by subtraction:

$$I(s)_n = I'_n - I(ns)_n =$$
$$\sum (p'_g \cdot p'_h - p(ns)_g \cdot p(ns)_h) + \lambda \cdot \tfrac{1}{2} \cdot p(s)_{\frac{n}{2}} \cdot (p(s)_{\frac{n}{2}} + 2 \cdot p(ns)_{\frac{n}{2}} + 1) \quad (12)$$
$$g < h$$
$$g + h = n$$

The contents of the formulas given here correspond to the results obtained by Coffman [11]. However, formula (10) can be even more simplified. From (9) it follows that

$$s'_{n-1} = 2 \cdot \sum T'_i \cdot T' + \lambda \cdot (T'_{\frac{n-2}{2}})^2 \qquad \text{for } n > 2$$
$$i < j$$
$$i + j = n-2$$

Using (8a) we get:

$$s'_{n-1} = 2 \cdot \sum p'_g \cdot p'_h + \lambda \cdot (p'_{\frac{n}{2}})^2 \qquad \text{for } n > 2$$
$$1 < g < h$$
$$g + h = n$$

or since

$$p'_1 \cdot p'_{n-1} = p'_{n-1}$$

we have

$$s'_{n-1} = -2 \cdot p'_{n-1} + 2 \cdot \sum p'_g \cdot p'_h + \lambda \cdot (p'_{\frac{n}{2}})^2 \qquad \text{for } n > 2$$
$$g < h$$
$$g + h = n$$

which compared with (10) gives:

$$I'_n = p'_{n-1} + \tfrac{1}{2} \cdot s'_{n-1} + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{n}{2}} \qquad \text{for } n > 2 \quad (13)$$

This formula is valid for $n > 2$, but it is easily found that $I'_2 = 1$.

Formula (13) may also be derived as follows. In the isomeric alkines C_nH_{2n-2} α) a terminal carbon atom takes part in the triple bond, or β) no terminal carbon atom takes part in the triple bond. Alkines of type α may be

derived from the primary radicals R_{n-1} by removing two hydrogen atoms from the primary carbon atom, and thereafter attaching the resulting tervalent radical to $\equiv C—H$. By so doing no stereoisomerism is destroyed, and this procedure results in p'_{n-1} different alkines. Alkines of type β may be derived from the secondary radicals R_{n-1} (where $n > 2$) by removing the hydrogen atom and the radical R_a from  , and thereafter attaching the resulting tervalent radical to $\equiv C—R_a$. By this procedure the number of asymmetric carbon atoms is reduced by one except in the cases where R_a is identical with R_b, and this occurs only when n is even, and in that case $a = b = \frac{n-2}{2}$. Identical groups will be attached to the secondary carbon atom of the radical R_{n-1} in $T'_{\frac{n-2}{2}} = p'_{\frac{n}{2}}$ number of cases. And therefore the number of alkines belonging to type β must be:

$$\tfrac{1}{2} \cdot (s'_{n-1} - \lambda \cdot p'_{\frac{n}{2}}) + \lambda \cdot p'_{\frac{n}{2}} = \tfrac{1}{2} \cdot s'_{n-1} + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{n}{2}}$$

Numerical values (quoted in the literature [11]) of I'_n, $I(ns)_n$ and $I(s)_n$ for $n \leqq 22$ are checked by means of (13) and (11).

C. Alkenes. This section will deal with the computation of 1) E'_n, 2) $E(ns)_n$ and 3) $E(s)_n$.

1) The total number of stereoisomeric and non-stereoisomeric alkenes, E'^n. A formula for computing E'_n can be derived by a method analogous to that which led to formula (6). We may think of an alkene as a hydrocarbon built up in the following way: From a primary or secondary radical R_g a hydrogen atom is removed from the primary or secondary carbon atom; and the same is done in the primary or secondary radical R_h. By joining the two resulting bivalent radicals in a double bond an alkene is produced. These bivalent radicals belong to one of the following two types:

α) The radicals in which two identical groups are attached to the carbon atom which takes part in the double bond,

β) the radicals in which two identical groups are not attached to the carbon atom which takes part in the double bond.

α) Let the radical of type α contain x carbon atoms.

For $x = 1$ there will be only one radical, namely $H_2C\langle$.

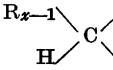
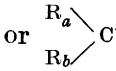
For x odd and > 1 there will be the radicals $(R_{\frac{x-1}{2}})_2C\langle$, where the two groups $R_{\frac{x-1}{2}}$ are identical. This makes a number equal to

$$T'_{\frac{x-1}{2}} = p'_{\frac{x+1}{2}}$$

For x even there will be no radicals of type α.

Finally we get that for all values of x there will be a number of radicals of type α equal to

$$\lambda \cdot p'_{\frac{x+1}{2}}$$

β) Let the radical of type β also contain x carbon atoms. The total number of bivalent radicals, containing x carbon atoms, of type α and type β is found equal to I'_{x+1}. This may be derived in the following manner. Every alkine, $C_{x+1}H_{2x}$, is of the form $R_{x-1}—C\equiv C—H$ or $R_a—C\equiv C—R_b$, where $a + b = x—1$. If the triple bond in such an alkine is broken off and the one carbon atom, contained in the triple bond, is removed, then from each such alkine one radical may be derived, namely either  or  . In this way no stereoisomerism is destroyed, and all possible bivalent radicals must appear.

For $x = 1$ there will be no radicals of type β.

For $x > 1$ there will be $(I'_{x+1} - \lambda \cdot p'_{\frac{x+1}{2}})$ radicals of type β. However, using (13) we get:

$$\begin{aligned} I'_{x+1} - \lambda \cdot p'_{\frac{x+1}{2}} &= p'_x + \tfrac{1}{2} \cdot s'_x + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{x+1}{2}} - \lambda \cdot p'_{\frac{x+1}{2}} \\ &= p'_x + \tfrac{1}{2} \cdot s'_x - \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{x+1}{2}} \end{aligned}$$

In the following considerations it is assumed that n > 2, and that each alkene may be considered as constructed of two bivalent radicals with respectively g and h carbon atoms, where $g \leqq h$, and $g + h = n$.

For $g = 1$ there will be 1 radical of type α and none of type β.

For $g > 1$ there will be $\lambda \cdot p'_{\frac{g+1}{2}}$ radicals of type α and

$(p'_g + \tfrac{1}{2} \cdot s'_g - \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{g+1}{2}})$ radicals of type β or altogether

$(p'_g + \tfrac{1}{2} \cdot s'_g + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{g+1}{2}})$ radicals.

For $h > 1$ (and this is always the case as $n > 2$) there will be $\lambda \cdot p'_{\frac{h+1}{2}}$ radicals of type α and $(p'_h + \tfrac{1}{2} \cdot s'_h - \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{h+1}{2}})$ radicals of type β or a total of $(p'_h + \tfrac{1}{2} \cdot s'_h + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{h+1}{2}})$ radicals.

When a radical of type α is joined in a double bond to a radical of type α or to a radical of type β no *cis-trans* isomerism is possible. On the other hand, if a radical of type β is joined to a radical of type β both a *cis* form and a *trans* form will occur.

Now the following can be found:

$$E'_n = p'_{n-1} + \tfrac{1}{2} \cdot s'_{n-1} + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{n}{2}} \qquad \text{(term I)}$$

$$+ \sum_{\substack{1<g<h \\ g+h=n}} (p'_g + \tfrac{1}{2} \cdot s'_g + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{g+1}{2}}) \cdot (p'_h + \tfrac{1}{2} s'_h + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{h+1}{2}}) \qquad \text{(term II)}$$

$$+ \sum_{\substack{1<g<h \\ g+h=n}} (p'_g + \tfrac{1}{2} \cdot s'_g - \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{g+1}{2}}) \cdot (p'_h + \tfrac{1}{2} \cdot s'_h - \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{h+1}{2}}) \qquad \text{(term III)}$$

$$+ \lambda \cdot \tfrac{1}{2} \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} + \tfrac{1}{2} \cdot p'_{\frac{n+2}{4}}) \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} + \tfrac{1}{2} \cdot p'_{\frac{n+2}{4}} + 1) \qquad \text{(term IV)}$$

$$+ \lambda \cdot \tfrac{1}{2} (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} - \tfrac{1}{2} p'_{\frac{n+2}{4}}) \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} - \tfrac{1}{2} \cdot p'_{\frac{n+2}{4}} + 1) \qquad \text{(term V)}$$

Term I is due to $g = 1$ and $h = n-1$.

Term II is due to $1 < g < h$, when all alkenes are counted, those appearing in both *cis* form and *trans* form are, however, counted as one.

Term III is due to $1 < g < h$, when each alkene appearing in both *cis* form and *trans* form is counted as one again.

Term IV is due to $g = h = \frac{n}{2}$, when all alkenes are counted, those appearing in both *cis* form and *trans* form are, however, counted as one.

Term V is due to $g = h = \frac{n}{2}$, when each alkene appearing in both *cis* form and *trans* form is counted as one again.

By reduction we get:

$$E'_n = p'_{n-1} + \tfrac{1}{2} \cdot s'_{n-1} + \lambda \cdot \tfrac{1}{2} p'_{\frac{n}{2}} \qquad \text{(term I)}$$

$$+ 2 \cdot \sum_{\substack{1<g<h \\ g+h=n}} (p'_g + \tfrac{1}{2} \cdot s'_g) \cdot (p'_h + \tfrac{1}{2} \cdot s'_h) + \lambda \cdot \tfrac{1}{2} \cdot \sum_{\substack{1<g<h \\ g+h=n}} p'_{\frac{g+1}{2}} \cdot p'_{\frac{h+1}{2}} \qquad \text{(term II + III)}$$

$$+ \lambda \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}}) \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} + 1) + \lambda \cdot \tfrac{1}{4} \cdot (p'_{\frac{n+2}{4}})^2 \qquad \text{(term IV + V)}$$

However, according to (9) the following must be true:

$$\lambda \cdot s'_{\frac{n}{2}} = \lambda \cdot 2 \cdot \sum_{\substack{a<b \\ a+b=\frac{n-2}{2}}} T'_a \cdot T'_b + \lambda \cdot (T'_{\frac{n-2}{4}})^2$$

and by introduction of (8a) this can be written as:

$$\lambda \cdot s'_{\frac{n}{2}} = \lambda \cdot 2 \cdot \sum_{\substack{1<c<d \\ c+d=\frac{n+2}{2}}} p'_c \cdot p'_d + \lambda \cdot (p'_{\frac{n+2}{4}})^2$$

$$= \lambda \cdot 2 \cdot \sum_{\substack{1<g<h \\ g+h=n}} p'_{\frac{g+1}{2}} \cdot p'_{\frac{h+1}{2}} + \lambda \cdot (p'_{\frac{n+2}{4}})^2$$

This expression for $s'_{\frac{n}{2}}$ is substituted in the above formula for E'_n:

$$E'_n = p'_{n-1} + \tfrac{1}{2} \cdot s'_{n-1} + \lambda \cdot \tfrac{1}{2} \cdot p'_{\frac{n}{2}} + 2 \cdot \sum_{\substack{1<g<h \\ g+h=n}} (p'_g + \tfrac{1}{2} s'_g) \cdot (p'_h + \tfrac{1}{2} \cdot s'_h)$$

$$+ \lambda \cdot \tfrac{1}{4} \cdot s'_{\frac{n}{2}} + \lambda \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}}) \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} + 1)$$

or

$$E'_n = p'_{n-1} + \tfrac{1}{2} \cdot s'_{n-1} + 2 \cdot \sum_{\substack{1<g<h \\ g+h=n}} (p'_g + \tfrac{1}{2} \cdot s'_g) \cdot (p'_h + \tfrac{1}{2} \cdot s'_h)$$

$$+ \lambda \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}}) \cdot (p'_{\frac{n}{2}} + \tfrac{1}{2} \cdot s'_{\frac{n}{2}} + \tfrac{3}{2}) \qquad (14)$$

This formula is valid for $n > 2$, but it is easily found that $E'_2 = 1$.

2) The number of non-stereoisomeric alkenes, $E(ns)_n$. The non-stereoisomeric alkenes must be derived from two non-stereoisomeric bivalent radicals, which are joined in a double bond in such a manner that no *cis-trans* isomerism is produced. These bivalent radicals belong to one of the following two types:

α) The radicals in which two identical groups are attached to the carbon atom which takes part in the double bond,

β) the radicals in which two identical groups are not attached to the carbon atom which takes part in the double bond.

α) Let the radicals of type α contain x carbon atoms.

For $x = 1$ there will be only one radical, namely $H_2C\!<$.

For x odd and > 1 there will be the radicals $(R_{\frac{x-1}{2}})_2 C\!<$, where the two non-stereoisomeric groups $R_{\frac{x-1}{2}}$ are identical. This gives altogether

$$T(ns)_{\frac{x-1}{2}} = p(ns)_{\frac{x+1}{2}}$$

For x even there will be no radicals of type α.

Finally we get that for all values of x there will be a number of radicals of type α equal to

$$\lambda \cdot p(ns)_{\frac{x+1}{2}}$$

β) Let the radical of type β also contain x carbon atoms. The total number of bivalent radicals, containing x carbon atoms, of type α as well as type β, is equal to $I(ns)_{x+1}$. This is seen in the same way as above, where the number of stereoisomeric and non-stereoisomeric bivalent radicals was found.

The number of radicals of type β is consequently for all values of x equal to

$$I(ns)_{x+1} - \lambda \cdot p(ns)_{\frac{x+1}{2}}$$

In the following considerations it is assumed that each alkene may be considered as constructed of two bivalent radicals with respectively g and h carbon atoms, where $g + h = n$. To avoid *cis-trans* isomerism one of the radicals must be of type α, while the other may be either of type α or of type β.

Now the following can be found:

$$E(ns)_n = \lambda \cdot \sum_{\substack{g<h \\ g+h=n}} p(ns)_{\frac{g+1}{2}} \cdot I(ns)_{h+1} \qquad \text{(term I)}$$

$$+ \lambda \cdot \sum_{\substack{g<h \\ g+h=n}} (I(ns)_{g+1} - p(ns)_{\frac{g+1}{2}}) \cdot p(ns)_{\frac{h+1}{2}} \qquad \text{(term II)}$$

$$+ \lambda \cdot \tfrac{1}{2} \cdot p(ns)_{\frac{n+2}{4}} \cdot (p(ns)_{\frac{n+2}{4}} + 1) \qquad \text{(term III)}$$

$$+ \lambda \cdot p(ns)_{\frac{n+2}{4}} \cdot (I(ns)_{\frac{n+2}{2}} - p(ns)_{\frac{n+2}{4}}) \qquad \text{(term IV)}$$

Term I is due to $g < h$; the radicals of type α with g carbon atoms are here combined with the total number of radicals with h carbon atoms.

Term II is due to $g < h$; the radicals of type β with g carbon atoms are here combined with the radicals of type α with h carbon atoms.

Term III is due to $g = h = \frac{n}{2}$; the radicals of type α are here combined mutually.

Term IV is due to $g = h = \frac{n}{2}$; the radicals of type α are here combined with the radicals of type β.

By reduction we get:

$$E(ns)_n = \lambda \cdot \sum_{\substack{g<h \\ g+h=n}} [p(ns)_{\frac{g+1}{2}} \cdot I(ns)_{h+1} + I(ns)_{g+1} \cdot p(ns)_{\frac{h+1}{2}}] + \lambda \cdot p(ns)_{\frac{n+2}{4}} \cdot I(ns)_{\frac{n+2}{2}}$$

$$- \lambda \cdot \sum_{\substack{g<h \\ g+h=n}} p(ns)_{\frac{g+1}{2}} \cdot p(ns)_{\frac{h+1}{2}} - \lambda \cdot \tfrac{1}{2} \cdot (p(ns)_{\frac{n+2}{4}})^2 + \lambda \cdot \tfrac{1}{2} \cdot p(ns)_{\frac{n+2}{4}}$$

However, according to (11) the following must be true:

$$\lambda \cdot I(ns)_{\frac{n+2}{2}} = \lambda \cdot \sum_{\substack{g<h \\ g+h=n}} p(ns)_{\frac{g+1}{2}} \cdot p(ns)_{\frac{h+1}{2}} + \lambda \cdot \tfrac{1}{2} \cdot (p(ns)_{\frac{n+2}{4}})^2 + \lambda \cdot \tfrac{1}{2} \cdot p(ns)_{\frac{n+2}{4}}$$

This expression is substituted in the above formula for $E(ns)_n$; we get:

$$E(ns)_n = \lambda \cdot \sum_{g+h=n} p(ns)_{\frac{g+1}{2}} \cdot I(ns)_{h+1} - \lambda \cdot I(ns)_{\frac{n+2}{2}} + \lambda \cdot p(ns)_{\frac{n+2}{4}} \qquad (15)$$

Note: The demand for $g < h$ is dropped in the first term above.

3) The number of stereoisomeric alkenes, $E(s)_n$. Finally $E(s)_n$ is found by subtraction:

$$E(s)_n = E'_n - E(ns)_n \qquad (16)$$

Numerical values of E'_n, $E(ns)_n$ and $E(s)_n$ for $n \leqq 22$ are calculated by means of the formulas (14), (15) and (16) (Table 2). The results have been checked by writing down all the formulas of the alkenes for $n \leqq 10$.

Table 2. The number, E'_n, of stereoisomeric and non-stereoisomeric alkenes, the number, $E(ns)_n$, of non-stereoisomeric alkenes and the number, $E(s)_n$, of stereoisomeric alkenes with n carbon atoms ($1 < n \leqq 22$).

n	E'_n	$E(ns)_n$	$E(s)_n$
2	1	1	0
3	1	1	0
4	4	2	2
5	6	4	2
6	18	8	10
7	42	14	28
8	118	26	92
9	314	48	266
10	895	88	807
11	2 521	159	2 362
12	7 307	287	7 020
13	21 238	516	20 722
14	62 566	924	61 642
15	185 310	1 646	183 664
16	553 288	2 925	550 363
17	1 660 490	5 184	1 655 306
18	5 011 299	9 163	5 002 136
19	15 190 665	16 155	15 174 510
20	46 244 031	28 423	46 215 608
21	141 295 042	49 912	141 245 130
22	433 200 573	87 487	433 113 086

FINAL REMARKS

The calculations in this paper follow the line which has been pointed out by Henze and Blair. Calculations in connection with isomerism can also be discussed mathematically as shown by Polya [12]. Also approximations, becoming more and more accurate the greater n becomes, can be set up [13]. Further calculations by extrapolation can be carried through [4, 5].

SUMMARY

Formulas for the number of isomeric alkines and alkenes — with and without taking stereoisomerism into account — are derived.

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Received October 12, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1464–1470

The Accurate Determination of Condensation Point Differences on Small Samples

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In search for a convenient method for the determination of boiling point (b. p.) differences on small samples, the method of Siwoloboff[1] has proved of value[2]. In this paper an improved differential Siwoloboff-method is described by which it is possible to obtain condensation point (c. p.) differences with an accuracy of 0.001 °C or less.

METHOD

In the original method of Siwoloboff a capillary tube (1 mm diameter), sealed at one end, is placed with the sealed end uppermost in a 4 to 5 mm tube containing the liquid to be tested. The tube is attached to at thermometer and immersed in a heating bath. When the temperature of the bath is gradually raised to the b. p. of the liquid, bubbles emerge slowly from the capillary and, due to the expansion of the enclosed air, are replaced by a rapid and continuous stream of gas bubbles. When the temperature falls to the condensation point, the bubbling ceases and the liquid is sucked back into the capillary. The latter procedure gives more reproducible results and has been the only one used here.

In an endeavour to increase the accuracy of the method the following changes were made:

1. The rate of cooling was carefully controlled. This was done by using an unsilvered Dewar-flask containing glycerol as the heating medium. The flask was inserted in an empty beaker, 20 mm wider then the Dewar-flask (see Fig. 1). The bath contained an electric nichrome wire heater, freely mounted around a thin-walled glass tube. The heater was fed from a variable transformer and with a constant voltage input. A constant temperature gradient of 0.0005 to 0.001 °C per second was found satisfactory. A propeller stirrer provided rapid agitation throughout the liquid.

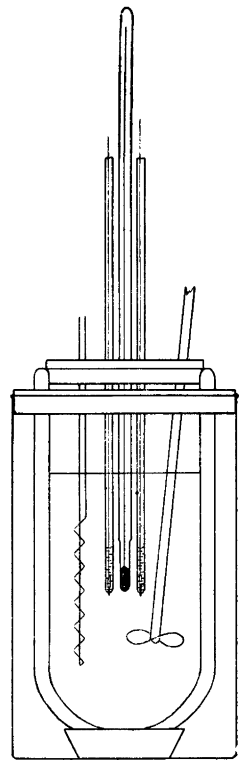

Fig. 1. Apparatus for determination of condensation point differences.

An Anschütz thermometer with graduation of 0.1 °C was placed in the bath and read under the microscope (magnification × 20). The cooling rate in seconds per 0.1 °C was measured with a stop watch by observing the times, necessary for the meniscus of the mercury stem to pass from one to another of the graduations.

2. The method of comparative measurement was strictly followed. Two boiling point tubes with an inner diameter of 3—4 mm were used. They were placed equally close to the thermometer bulb. The identical two cups made in the following way were inserted into the tubes. A thin-walled glass tube 3—4 mm in diameter was drawn out to an outer diameter of 0.8—1.0 mm. On this capillary three sharp marks, 2.00 mm apart, were ground on a lathe (see Fig. 2). The central mark was ground as deep as possible without breaking the capillary. The tube was sealed outside of the two outer marks. In this way a completely closed tube (12—14 mm in length) was obtained. At either end a long thin glass rod was fastened and the small tube was carefully broken

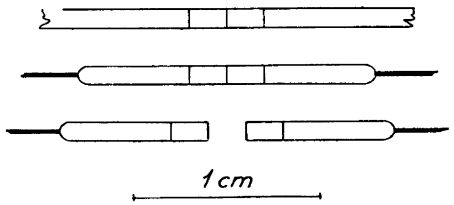

Fig. 2. Different stages in the making of cups.

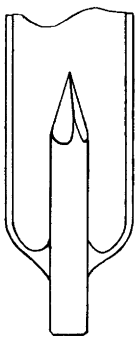

Fig. 3. Bottom part of boiling point tube.

along the central mark. Care was taken to keep the edge clean and even. The two cups were then tested for equivalence as outlined below.

3. The next step was to eliminate another disadvantage of the method. There is always a last vapor bubble (before condensation takes place) "hanging" at the opening of the cup. This bubble either leaves the orifice just before the beginning of the condensation, or the bubble tacks the opening and must be completely condensed before the liquid begins to rise into the cup. In the latter case the "sucking in" lags behind that in the first case even if all other conditions are equal. This difficulty was eliminated as is shown in Fig. 3. In the bottom of the boiling point tube a platinum wire, 1.0 mm diameter, was introduced. The end of the wire had been conically lathed (top angle 40°) and then three symmetrical planes had been chafed on the cone, the angle between the axis of the wire and the planes being 18°. The cup was placed on the triangular point thus obtained. The point extends about 0.5 mm into the cup. The exit area is thus reduced and the small bubbles will leave the orifice instantaneously.

EXPERIMENTAL PROCEDURE

The b. p. tubes and the cups were carefully cleaned and dried. They had been stored over phosphorous pentoxide. First of all the equivalence of the two cups was checked. The b. p. tubes were filled with the aid of a micro-pipette up to a height of about 20 mm with the same pure liquid (ether, acetone, ethanol, water or toluene). The marked cups (a and b) were inserted into the tubes (l and r) and the tubes were placed in the heating bath.

The heating bath was pre-heated to the boiling point of the liquid 30 minutes before the tubes were inserted, in order to establish equilibrium

between the apparatus and the surroundings. The b. p. tubes were then slowly inserted in the bath at a temperature just below the b. p. The bath was gradually heated to about 0.5 °C above the b. p. of the liquid. A rapid stream of bubbles emerged from the cups and the air inside the cups was slowly replaced by the vapor of the liquid. It is most important, that the gas phase is totally free from air, a step requiring 5 to 10 minutes. Now, the current through the heater was changed to a previously determined value in order to assure a rate of cooling of about 0.001 °C per second. The cooling rate was determined starting from 0.4 °C above the b. p.

In the vicinity of the condensation point the bubbling slowed down and then ceased; during a short time the meniscus was seen at the opening between the platinum pin and the cup wall. The meniscus ascended into the cup and when it passed the mark 2 mm above the orifice, another stop watch was started. When the liquid in the second b. p. tube passed the mark of the cup, the watch was stopped, thus giving the time difference between the passage of the menisci. When the determination was repeated immediately, the heating current was switched on before the cups were completely filled with liquid, which occured, if the temperature was allowed to decrease to much. Within a few seconds the b. p. was reached and then the measurement was

Table 1.

Time for meniscus passage in capillary in sec.		Time diff. reduced to 0.001 °C/sec.	Condensation point (uncorr.)	Actual cooling rate sec./0.1°
a	*b*			
(in l)	(in r)			
0	1.2	1.1	77.94	110
0	0.8	0.7	77.94	120
0	1.0	0.9	77.95	115
0	3.5	2.2	77.95	160
(in r)	(in l)			
0	0	0	77.95	150
0	1.1	0.6	77.95	185
0	2.7	1.4	77.95	190
0	3.4	1.8	77.95	190
(in l)	(in r)			
0	2.2	2.0	77.92	110
0	2.1	1.3	77.92	160
0	1.1	0.6	—	180

Mean value = 1.3 sec.
Standard deviation = 0.7 sec.
Standard deviation of the mean = 0.2 sec.

carried out again. A new series of determinations was done after cleaning the tubes and cups, but this time with the cups interchanged (in the first series *a* in r and *b* in l, in the second *b* in r and *a* in l).

Table 1 gives the values for one set of determinations.

In the first and second column the times observed for the menisci to pass the respective mark are given at the actual cooling rate shown in the last column. The time differences are reduced to a constant cooling rate of 0.001 °C per sec. (column 3). The actual temperature of the c. p., as read on the Anschütz-thermometer, is given in column 4.

If the reduced time difference exceeds 3 sec. it is advisable to correct the volumes of the cups with the aid of a small micro flame and repeat the measurement.

The liquid levels must be controlled after each determination. Different heights of liquid in the two tubes give different pressures inside the cups and thus the c. p. difference found will be incorrect. It is useful to engrave a scale on the b. p. tubes, beginning at the Pt-point. A difference in height is read directly on these scales and the pressure dependence of the b. p. is easily found from an additional experiment. Low boiling liquid will evaporate more or less even if the b. p.tubes are as long as shown in Fig. 2. Furthermore a slight fractionation is inevitable even at total reflux, due to the formation of vapor inside the tubes. This phenomenon, however, seems to be of less importance.

Comparing different fractions from a fractional distillation, one fraction has to be taken as a reference standard. First, the equivalence of the cups is checked with the reference fraction in both tubes and then the b. p. differences between the reference standard and the fractions are determined. It is necessary to replace the reference liquid for each determination. Extreme cleanliness is very important as even the smallest contamination may cause misleading results.

The maximum c. p. difference for which the method outlined above is useful, is less than 1 °C and it is somewhat dependent on the boiling point of the sample. At a cooling rate of 0.001 °C per sec. a c. p. difference of 1 °C corresponds to a time difference of about 17 minutes. The lower boiling sample will be more superheated than the higher boiling sample and, furthermore, it has to boil for a longer time. For low boiling substances the loss of substance due to evaporation is appreciable and consequently the results will be unreliable. By inceasing the rate of cooling the time of boiling is cut down and the evaporation diminished.

The method has been used for temperatures between 35 and 140 °C. Above this temperature the glycerol soon becomes dark and good observations are

thereby prevented. With silicone oil as a heating medium it will probably be possible to use the method at higher temperatures.

Tables 2 and 3 give the results from two successive distillations of *n*-amyl chloride *. In the first series the cooling rates were varied within wide limits, in the second they were kept as constant as possible. The last column in the tables gives the difference between the read (mean) temperatures of the fractions during the distillation. Fraction F4 from the first distillation, which was believed to be rather pure, gave on second distillation a first fraction boiling 0.04 °C below the main fraction. This was probably due to the presence of a small quantity of water, driven off as an azeotrop (*cf.* Swietoslawski [3, p. 84]).

Table 2.

Fractions	Rate of cooling sec. per 0.1 °C	C.p.diff. °C	Temperature diff. (mean) from distillation
F4-A10	84	0.640	0.64
	333	0.635	
F4-C8	108	0.255	0.26
	80	0.252	
F4-C9	273	0.160	0.16
	221	0.158	
F4-F1	167	0.097	0.10
	292	0.097	
	94	0.096	
F4-F2	227	0.017	0.01
	460	0.017	
	166	0.018	
	88	0.019	
F4-F3	260	0.003	—
	172	0.003	
	160	0.003	
	207	0.003	
	162	0.003	

The order of fractions: Al-C3 (5 fractions) together 80 g, A 10 6.0 g; C8 7.1 g; C9 6.5 g; Fl 9.4 g; F2 15.2 g; F3 14.8 g; F4 29.9 g.

* We are indebted to Prof. L. Smith for permission to study the fractions in question. The distillations were done by Mr. L. Bjellerup.

Table 3.

Fractions	Rate of cooling sec. per 0.1 °C.	C.p.diff. °C.	Temperature diff. from distillation
B9-C6	159	0.040	0.04
	160	0.040	
	161	0.040	
B9-B6	160	0.002_5	
	161	0.002_5	
	152	0.003_0	
	154	0.003_0	
B9-B8	162	0.001_0	
	164	0.001_5	
	165	0.001_0	
	165	0.001_0	
B9-E2	164	$-\ 0.004_5$	
	164	$-\ 0.006_0$	
	167	$-\ 0.005_0$	

Fraction F4 from Table 2 distilled. Order of fractions: C6 5.6 g.; B6 5.7 g.; B8 5.3 g.; B9 4.7 g. and E2 residue 5.1 g.

The method outlined above was worked out mainly as an aid in estimating the purity of substances for combustion calorimetry where the available amounts are often rather limited and the requirements on the purity of the substances burned are very high.

SUMMARY

A description is given of a differential Siwoloboff-method allowing the determination of condensation point differences with an accuracy of 0.001 °C on samples as small as 0.1 ml. The maximum c. p. difference hitherto determinable with this accuracy is about 0.5 °C. The method is useful for the estimation of the purity of fractions in fractional distillation of small samples.

The investigation was supported by a grant from Swedish Natural Science Research Council for which we feel greatly indebted.

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Received October 29, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1471–1489

On Ion Exchange Equilibria

I. The Silver-Hydrogen Equilibrium on Wofatit KS and Dowex 50

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Way [1] in 1850 observed the phenomenon of ion exchange and Eichhorn [2] showed that the ion exchange process is reversible. Since then many quantitative investigations have been performed on ion exchange equilibria, and a number of experimental methods have been developed.

In earlier investigations empirical equations were often used to represent the experimental data. One equation that has frequently been applied is that of Rothmund and Kornfeld [3]. Bacon [4] and Møller [5] indicated that the mass action law was applicable to ion exchange equilibria. Recent investigations show that it is sometimes approximately obeyed within small concentration ranges: cf Samuelson [6], Boyd, Schubert and Adamson [7], Marinsky [8], Kressman and Kitchener [9], and Duncan and Lister [10, 11]. Some of these authors, however, point out that the mass action "constant" is really a variable [6, 8, 10, 11].

Samuelson [6] measured the equilibria: $Na^+ - H^+$, $NH_4^+ - H^+$ and $Li^+ - H^+$ on a commercial sulfonic acid exchanger. He found that the equilibrium quotient (which would be $\varkappa_{Me,H}$ with the notation in eq. (3) below) increased with decreasing mole fraction (β_{MeR}) of Na^+ and NH_4^+ in the exchanger (Me = Na^+, NH_4^+, R^- = negative group of the exchanger) within the concentration interval $0.2 \leqq \beta_{MeR} \leqq 0.8$. For the exchange $Li^+ - H^+$, $\varkappa_{Li,H}$ increased in the same manner but to a lesser degree.

Boyd, Schubert and Adamson[7] studied a number of exchange equilibria on Amberlite IR-1. Data were given for the exchange $Na^+ - H^+$ within the interval $0.26 \leqq \beta_{NaR} \leqq 0.93$. Just as in Samuelson's work, $\varkappa_{Na,H}$ showed a small but marked tendency to increase with decreasing β_{NaR}. However, these authors preferred to regard it as a constant.

Marinsky [8] studied the exchange reactions $Na^+ - H^+$, $Ca^{2+} - H^+$, $Ba^{2+} - H^+$ and $Ce^{3+} - H^+$ on especially treated Dowex 50. The exchange $Na^+ - H^+$ was studied within the range $6.10^{-4} \leqq \beta_{NaR} \leqq 0.95$, and $\varkappa_{Na,H}$ was found to increase with increasing β_{NaR}. The other exchange reactions also gave equilibrium quotients that varied considerably within the concentration ranges studied. Marinsky demonstrated by careful analyses that the assumption of equivalent exchange was valid within the limits of experimental error.

Kressman and Kitchener [9] studied a number of exchange reactions on a resin synthesized by themselves. For the exchange between NH_4^+ on one side, and Li^+, H^+, Na^+, K^+, Rb^+, Cs^+, Ag^+ or Tl^+ on the other, they obtained nearly straight lines when plotting $\left(\frac{\beta_{NH_4R}}{\beta_{MeR}}\right)_{resin}$ against $\left(\frac{[NH_4^+]}{[Me^+]}\right)_{aq}$ within the interval $0.3 \leqq \beta_{MeR} \leqq 0.7$ This means that $\varkappa$ is almost constant.

Actually, although this was not stressed by these authors, there is generally a steady increase of $\varkappa_{Me,NH_4}$ with decreasing β_{MeR}. The approximate constancy probably depends on the small β range covered. Kressman and Kitchener also studied the reaction at different T and found that $\varkappa$ is little affected by temperature changes.

Duncan and Lister [10] have studied the exchange $Na^+ - H^+$ on Amberlite IR — 100 H and Dowex 50. For Amberlite IR — 100 H, $\varkappa_{Na,H}$ varied from 1.4 to 1.2 when β_{NaR} varied from 0.03 to 0.8. For Dowex 50, a maximum in $\varkappa$ was obtained for $\beta_{NaR} \approx 0.1$. The equilibrium on Dowex 50 was also studied at 87° C. The maximum was found to be flatter at this temperature. The differences between their results and Marinsky's are probably due to the pretreatment of Marinsky's resin with 6 C NaOH for 48 h at 95° C.

The aim of the present work was to make a thorough investigation of the exchange equilibrium between Ag^+ and H^+ on two ion exchangers at various ratios $Ag^+ : H^+$ and at different ionic strengths.

The combination $Ag^+ - H^+$ was chosen because in this case a very wide concentration range could be covered by use of chemical and emf methods.

Two different ion exchangers were used: Wofatit KS, the same as used in the filtration experiments,[12, 13] and Dowex 50. Both exchangers contain nuclear sulfonic acid groups. Dowex 50 is considered to be more homogeneous; it has a higher capacity and reacts more slowly.

The temperature was kept constant at 25° C, although variations of a few degrees seem to be of little influence on ion exchange processes [9]. The experiments were carried out at two different ionic strengths: 100 mC and 20 mC. Since the equilibrium quotient (3) proved to be independent of ionic strength

within the limits of experimental error, experiments with lower ionic strengths did not seem necessary.

The experiments were carried out by shaking weighed-out resin samples and 100 ml of a solution of $AgNO_3$ and HNO_3 in a thermostat and analysing them as described below.

Symbols: The reaction studied can be written as (R^- = anion framework of resin):

	Ag^+	+	HR	$\rightleftharpoons$	H^+	+	AgR (1)
concentration	αa_0		$s_0\ (1-\beta)$		$(1-\alpha)a_0$		βs_0
number of moles in batch	n_{Ag^+}		n_{HR}		n_{H^+}		n_{AgR}

a_0 is the ionic strength, s_0 is the sorption capacity of the exchanger and α and β are the mole fractions of silver in the solution and the resin.

$$\alpha = \frac{n_{Ag^+}}{n_{Ag^+} + n_{H^+}} \text{ and } \beta = \frac{n_{AgR}}{n_{AgR} + n_{HR}} \tag{2}$$

We shall define the equilibrium quotient $\varkappa$, or more exactly $\varkappa_{Ag,H}$ by:

$$\varkappa_{Ag,H} = \varkappa = \frac{(1-\alpha)\ \beta}{\alpha\ (1-\beta)} = \frac{n_{H^+} \cdot n_{AgR}}{n_{Ag^+} \cdot n_{HR}} = \frac{[H^+]\ n_{AgR}}{[Ag^+]\ n_{HR}} \tag{3}$$

The last equality is approximately valid, since the activity factors of H^+ and Ag^+ almost cancel out.

EXPERIMENTS

Ion exchangers. 1. Wofatit KS with a grain size of 0.5 — 1.0 mm diameter was purified and saturated with H^+ as described in ref. [12]. After purification, part of it was dried in air and kept in a glass-stoppered bottle. 2. Dowex 50 of the same grain size was saturated with H^+ in the same manner as Wofatit KS. Then it was washed with dist. water until methyl red gave neutral reaction. The sorbent was placed in a glass filter funnel and dried by sucking a filtered air stream through it for 10—20 hours.

Silver saturated sorbent (AgR) was prepared from the H^+ form (HR) by running an excess of 1 C $AgNO_3$ through it (*cf.* Fig. 2 c in ref. [12]). It was washed with dist. water until the wash liquid was free from Ag^+, and then dried in a filtered air stream for about 5 hours.

Chemicals and standard solutions. 100.0 mC (millimoles per liter) HNO_3 was prepared from conc. nitric acid (Baker's analysed, sp. gr. 1.42) and dist. water. It was standardized against weighed HgO and KI [14]. 100.0 mC $AgNO_3$

was prepared from high grade pure $AgNO_3$ and dist. water. It was standardized according to Volhard against 0.1 C *KSCN* which was prepared from pure (Baker's analysed) KSCN and dist. water and standardized against weighed silver wire. 0.1 C *NaOH* was prepared from a solution of about 50 % NaOH and CO_2-free dist. water, and standardized against the 100.0 mC HNO_3 with methyl red as indicator.

Batches. Solutions of accurately known concentrations of Ag^+ and H^+, and of the ionic strength desired, were prepared from the standard solutions of $AgNO_3$, and HNO_3. With $a_0 = 100.0$ mC about 30, and with $a_0 = 20.0$ mC about 10 different solutions were prepared.

A batch was made by weighing out 1.000 g of HR or 1.500 g of AgR in 150 ml glass bottles, pipetting 100.00 ± 0.05 ml $Ag^+ - H^+$ solution to the sample, and then sealing the bottles. The bottles were shaken to equilibrium in the thermostat at $25.0 \pm 0.1°$ C. The period of shaking was never less than 24 hours. It was found to be important that the batches with AgR were mixed immediately after the AgR had been dried, because when left standing, silver ions are easily reduced in the dry sorbent. In our first experiments a number of batches were prepared in 150 ml bottles with rubber stoppers. Since the rubber might reduce the Ag^+ and hence give rise to errors, the batches were later on prepared in 150 ml pyrex glass bottles with constricted necks, which were then sealed.

Analysis. After shaking, the equilibrium solution was sucked off through a glass filter funnel and the sorbent was washed with about 100 ml dist. water.

For small silver concentrations in the *equilibrium solution* the wash water was discarded, the Ag^+ content determined by measuring the emf of a concentration cell, and the H^+ content determined by pipetting 20.00 ml of the solution and titrating with 0.1 C NaOH and methyl red as indicator.

For higher silver concentrations in the solution, the wash liquid was mixed with the equilibrium solution, and the total amounts of H^+ and Ag^+ (n_{H^+} and n_{Ag^+}) were determined by titration with NaOH and KSCN. These titrations were performed in tall 2 l beakers. After the titration with NaOH, 25—50 ml nitrobenzene and a few ml of conc. HNO_3 were added. The beaker was shaken until all methyl red had been transferred to the nitrobenzene phase. Iron (III) alum was added, and the silver titrated with 0.1 C KSCN.

After the *sorbent* had been separated from the solution, 0.5—1 l 2 C KNO_3 was run through it to expel Ag^+ and H^+. The arrangement was the same as shown in Fig. 2 c ref. [12]. Then the total amounts of AgR and HR (n_{AgR} and n_{HR}) were determined as described above. For small AgR contents, the most accurate way of obtaining n_{AgR} was to subtract n_{Ag^+} (which was determined by emf measurements) from the total amount of Ag in the batch.

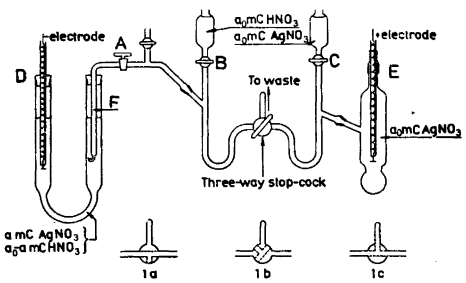


Fig. 1. Side view of emf apparatus for measuring $[Ag^+]$. *The stop-cock A is opened only when measuring emf. The stop-cocks B and C are opened when rinsing the three-way stop-cock. D and E are the electrode vessels. The narrow part of the U-tube D prevents* a_0 *mC* HNO_3 *from F to diffuse to the negative electrode. The tube F is filled with* a_0 *mC* HNO_3. *The three-way stop-cock 1 a) set for measuring emf, 1 b) closed and 1 c) set for rinsing.*

In order to test whether all Ag^+ was expelled by the KNO_3 solution, the AgR forms of Wofatit KS and Dowex 50 were treated with KNO_3 in the same manner as described above. The resins were then ignited in porcelain crucibles. After ignition HNO_3 was added to dissolve the silver. The presence of silver was tested with HCl. With Wofatit KS a very faint turbidity was obtained, and with Dowex 50 no turbidity at all. This test also shows that the reduction of silver during the exchange process is negligible.

EMF MEASUREMENTS

The apparatus used for the emf measurements was of the type designed by Forsling and Sillén (to be published). It was immersed in a paraffin oil thermostat with the temperature 25.0 $\pm$ 0.1° C in a thermostat room of 23.5° C.

The emf was measured by means of a Jensen potentiometer, with a Multiflex galvanometer MG2 as zero instrument.

The electrodes were Ag, AgI electrodes of the same kind as used in refs. 12 and 13. The cell can be described schematically by:

$$-\text{Ag/unknown } [Ag^+]/a_0 \text{ mC } HNO_3/a_0 \text{ mC } AgNO_3/\text{Ag} + \qquad (4)$$

Fig. 1 gives a picture of the apparatus.

In the following way a fresh diffusion layer was always obtained. Previous to each measurement the three-way stop-cock was rinsed with a_0 mC HNO_3 and a_0 mC $AgNO_3$. Stop-cocks B and C were opened simultaneously for a moment, and the three-way stop-cock was kept in the position shown in Fig. 1 c. Then the three-way stop-cock was turned to the position shown in Fig. 1 a. The stop-cock A was opened and the emf of the cell measured.

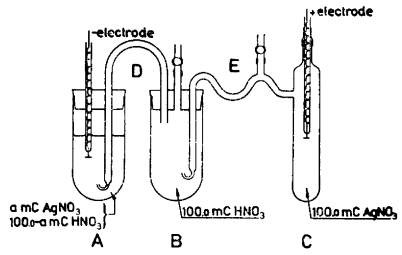


Fig. 2. Side view of first emf apparatus for measuring $[Ag^+]$. *A and C are the electrode vessels. Tube B with 100.0 mC* HNO_3 *prevents silver ions from C from diffusing to the electrode vessel A. D and E are bridges. D containing 100.0 mC* HNO_3 *and E 100.0 mC* $AgNO_3$.

THE DIFFUSION POTENTIAL

Before the apparatus in Fig 1 was used, measurements were made in a simpler apparatus (Fig. 2). The minus electrode at A is connected to the tube B containing 100.0 mC HNO_3 with the bridge D, also containing 100.0 mC HNO_3. Tube B is connected to the positive electrode at C with the bridge E containing 100.0 mC $AgNO_3$. The solutions in A and C are a mC $AgNO_3$ + + (100.0-a) mC HNO_3 and 100.0 mC $AgNO_3$. The cell was thus of the same type (4) as described above, though the diffusion layer could not be so easily renewed. The diffusion layer was developed in tube B between bridge E and the solution in B. The diffusion potential between the test solution in A and the HNO_3 solution in D could be expected to vary but slightly in the concentration interval studied. Moreover it is small in comparison to the diffusion potential in B. The variation should depend only upon the diffusion layer between B and E. The various electrodes used were compared with each other in the same silver solution; no potential difference was detected between them.

Some attempts were made to get a defined diffusion layer. When the layer was developed in sand (Bjerrum [15]), the resistance of the cell became too high for accurate measurements with our potentiometer. When the diffusion layer was placed in a cotton plug, the diffusion potential was constant for a few hours, but then began to decrease; simultaneously the cotton was coloured black by reduction of silver ions to silver. When finally the diffusion layer was placed in the three-way stop-cock, the diffusion potential decreased by 1 mV in 24 hours.

When the Forsling-Sillén apparatus was used as described above, the emf was reproducible within $\pm$ 0.1 mV. The emf changed by less than 0.1 mV during the first minutes after the three-way stop-cock was rinsed and opened for measurement. Then it rose fast to a maximum value, and finally decreased slowly to values even lower than the initial one. The maximum value was not reproducible.

If Henderson's equation is applicable, the emf of our cell should be the same as that of a cell without the insertion of pure HNO_3. This latter cell can be described by:

$$-Ag/[Ag^+]=\alpha a_0;\ [H^+]=a_0\,(1-\alpha);\ [NO_3^-]=a_0/[Ag^+]=a_0;\ [NO_3^-]=a_0/Ag+ \quad (5)$$

$$E = E_{corr} + E_d \quad (6)$$

where E = the measured emf, E_d = the diffusion potential, and E_{corr} = the corrected emf:

$$E_{corr} = \frac{RT}{F} \ln \frac{1}{\alpha} = 59.16^1 \log \frac{1}{\alpha} \text{ mV at } 25.0^\circ \text{ C} \quad (7)$$

At both ionic strengths, E was measured for a number of solutions with known silver concentration (αa_0) and ionic strength (a_0). E_{corr} was calculated from (7), after which E_d could be calculated from (6). It was found that equation (13b) in [12] satisfied the measurements if the values $d = 2.26$ for $a_0 = 100.0$ mC and $d = 2.15$ for $a_0 = 20.00$ mC were used:

$$(13b)\ [12] \qquad E_d = 59.16 \log (1 + d\ (1 - \alpha)) \quad (8)$$

Table 1 gives the values of E_d from (6) and (7) for $a_0 = 100.0$ mC and the value of d calculated from these E_d and (8). The last column gives the value for E_d calculated from (8) with the average value $d = 2.26$.

Table 2 gives the values of E_d for $a_0 = 20.00$ mC and the d values calculated from these E_d values and (8). The last column gives the values of E_d from (8) with the average value $d = 2.15$.

Table 1. Emf and diffusion potential in cells (5) with $a_0 = 100.0$ mC.

$\alpha \cdot a_0$ mC	E mV	E_{corr} from (7) mV	E_d from (6) mV	d from (8)	E_d from (8) mV $d = 2.26$
0.0100	266.9	236.6	30.3	2.25	30.4
0.1000	207.8	177.5	30.3	2.25	30.3
1.000	148.6	118.3	30.3	2.27	30.2
5.000	106.6	77.0	29.6	2.27	29.5
10.00	87.8	59.2	28.6	2.27	28.5

Table 2. The diffusion potential at a_0 = 20.00 mC.

$a \cdot a_0$ mC	E mV	E_{corr} from (7) mV	E_d from (6) mV	d from (8)	E_d from (8) mV d = 2.15
0.0500	183.2	153.9	29.3	2.13	29.4
0.2500	142.1	112.6	29.5	2.18	29.3
0.5000	123.8	94.8	29.0	2.15	29.0
1.000	105.6	77.0	28.6	2.15	28.6
5.000	60.2	35.6	24.6	2.14	24.7
10.00	36.4	17.8	18.6	2.13	18.8

The agreement is seen to be good. It can be noted that the average d value 2.15 in Table 2 is in close agreement with the value d = 2.16 obtained by Sillén and Ekedahl [12] for a_0 = 20.2 mC.

THE TIME FOR ATTAINMENT OF EQUILIBRIUM

A few experiments were made to study whether equilibrium was reached within one day. Dowex 50 was chosen because it is known to react more slowly than most other organic ion exchangers.

Four batches containing the HR form of the sorbent and one common standard solution were prepared. They were taken from the thermostat and analysed after 1, 2, 3, or 7 days. n_{Ag+} and thus a was determined by emf measurements. n_{AgR} and thus β was calculated from $n_{Ag+\,tot} - n_{Ag+}$ and n_{H+} and n_{HR} were determined by titration with 0.1 C NaOH as described above. Table 3 gives the values of the equilibrium quotient ($\varkappa$) and the time of shaking.

Table 3. The influence of the time of shaking a_0 = 100.0 mC. Sealed pyrex bottles.

Time of shaking Days	$a \cdot 10^4$	$\beta \cdot 10^3$	$\varkappa$ from (3)
1	21.3	20.6	9.9
2	19.8	20.7	10.7
3	21.0	20.6	10.0
7	20.3	20.9	10.5

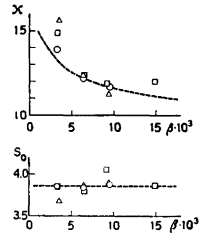


Fig. 3. Comparison of a) the equilibrium quotient $\varkappa$ and b) the exchange capacity s_0 for Dowex 50 of different particle sizes.
β = silver fraction in ion exchanger.
○ particle size 0.5 — 1.0 mm
△ » » 0.13 »
□ » » 0.04 »
The dotted line in Fig. 3 a is a smooth curve based on all determinations in Tables 8 a and c.
The dotted line in Fig. 3 b is the mean of the s_0 values in Table 4.

From Table 3 is seen that within the limits of experimental error the equilibrium is attained within one or two days; probably it is attained within even shorter time than one day. Nachod and Wood [16] have shown that for a number of different ion exchangers equilibrium is practically attained within 30 minutes. These results have been corroborated by the later findings of Holm and Westermark.[21] Marinsky [8] found that for Dowex 50 3.3 hours was enough for attaining equilibrium.

THE INFLUENCE OF THE PARTICLE SIZE

To study whether $\varkappa$ and s_0 depend upon the particle size, dry Dowex 50 in the HR form was ground in a mortar. In that process particles of all sizes between colloidal and unbroken grains were obtained. Two different times of grinding, and thus different mean particle sizes were used. The diameters of 100 particles from each sample were measured in a microscope with

Table 4. The influence of the particle size.
a_0 = 100.0 mC, sealed pyrex bottles. HR form of Dowex 50.

Mean particle size	$n_{Ag^+ tot}$ mmol	n_{Ag^+} 10^3 mmol	n_{H^+} mmol	n_{HR} mmol	s_0 mmol	$\alpha \cdot 10^4$	$\beta \cdot 10^3$	$\varkappa$ from (3)
0.5—1.0	0.01500	2.367	10.04	3.856	3.868	2.367	3.265	13.9
— » —	0.03000	5.278	9.90	3.815	3.840	5.278	6.438	12.2
— » —	0.04500	8.193	10.01	3.837	3.874	8.193	9.502	11.7
0.13	0.01500	2.227	—	3.671	3.684	2.227	3.466	15.6
— » —	0.03000	5.237	—	3.852	3.877	5.237	6.386	12.3
— » —	0.04500	8.387	—	3.859	3.896	8.387	9.397	11.3
0.04	0.01500	2.227	—	3.849	3.862	2.227	3.367	14.9
— » —	0.03000	5.278	—	3.764	3.789	5.278	6.524	12.4
— » —	0.04500	7.789	—	4.023	4.060	7.789	9.165	11.9
— » —	0.0700	12.57	—	3.790	3.847	12.57	14.93	12.0

a calibrated ocular micrometer. The mean diameters were found to be 0.13 and 0.04 mm. Batches with both samples were prepared, and after shaking in thermostat they were analysed as described above with the exception that the equilibrium solution was centrifuged from the sorbent at 1 500 r. p. m. for 0.5 — 1 h instead of filtrated. Table 4 and Fig. 3 give the results of the analyses compared with those for unground particles.

In Table 4 $n_{Ag^+\ tot}$ = the silver amount in mmol per 100 ml of the Ag^+ — H^+ solution. Only directly determined n-values are listed; n_{AgR} was determined indirectly from $(n_{Ag^+\ tot} - n_{Ag^+})$. The titrated n_{H^+}-values for the ground samples were too high and are not listed. This is due to the fact that even after centrifuging a certain quantity of resin particles containing H^+ remained in the equilibrium solution. Thus it was preferred to calculate n_{H^+} from $(0.1\ a_0 - n_{Ag^+})$. The total capacity s_0 is defined by:

$$s_0 = n_{HR} + n_{AgR} \tag{9}$$

Fig. 3 indicates that both $\varkappa$ and s_0 seem to be independent of the particle size within the limits of experimental error.

VARIOUS WAYS OF CALCULATING $\varkappa$

Since both solution and sorbent were analysed, the equilibrium quotient $\varkappa$ could be calculated in a number of different ways by excluding one or two determinations and calculating $\varkappa$ from the rest of the data. Table 5 gives the different methods possible.

Table 5. Possible combinations of experimental data to calculate the equilibrium quotient $\varkappa$.

Method of evaluatn.	n_{H^+}	n_{Ag^+}	n_{HR}	n_{AgR}	$0.1\ a_0$	$n_{H^+\ tot}$	$n_{Ag^+\ tot}$	$n_{H^+\ tot}$ known	$n_{Ag^+\ tot}$ known
1	+	+	+	+	—	—	—	x	x
2	+	—	+	+	+	—	—	x	(x)
3	—	+	+	+	+	—	—	(x)	x
4	+	+	+	—	—	—	+		x
5	+	—	+	+	—	—	+		(x)
6	+	—	+	—	+	—	+		(x)
7	—	+	+	—	+	—	+		x
8	—	—	+	+	+	—	+		(x)
9	+	+	—	+	—	+	—	x	
10	—	+	+	+	—	+	—	(x)	
11	+	—	—	+	+	+	—	x	
12	—	+	—	+	+	+	—	(x)	
13	—	—	+	+	+	+	—	(x)	

Between the quantities in Table 5 the following relations are valid:

$$0.1\ a_0 = n_{H^+} + n_{Ag^+} \quad (10); \qquad n_{Ag^+\,tot} = n_{Ag^+} + n_{AgR} \quad (11)$$

$$n_{H^+\,tot} = n_{HR} + n_{H^+} \quad (12)$$

Of the relations (11) and (12), only one can be applied to a certain experiment since only $n_{Ag^+\,tot}$ is known when starting from the HR form of the resin and only $n_{H^+\,tot}$ when starting from the AgR form. + means the data used and — the data excluded.

The last two columns show which method can be used when n_{H^+tot} ($n_{Ag^+\,tot}$) is known. "x" means that the method is applicable and "(x)" that it is inaccurate if the known quantity $n_{H^+\,tot}$ (or $n_{Ag^+\,tot}$) is small.

Tables 6 a and b give the values of $\varkappa$ calculated by different methods for four batches of Wofatit KS, two starting from the HR form and two from the AgR form.

Table 6 a. Comparison of $\varkappa$ values calculated by methods 1—8 (Table 5). a_0 = 100.0 mC. Bottles with rubber stoppers. HR form of Wofatit KS.

Method of evaluatn.	$n_{Ag^+\,tot}$ mmol	n_{Ag^+} mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	0.1 a_0 mmol	$\beta \cdot 10^3$	$\varkappa$ from (3)
1	3.500	2.439	7.533	1.038	0.638	1.676	10.00	619.3	5.02
2	— » —	(2.467)	7.533	1.038	0.638	1.676	—»—	619.3	4.97
3	— » —	2.439	(7.561)	1.038	0.638	1.676	—»—	619.3	5.04
4	— » —	2.439	7.533	(1.061)	0.638	1.699	—»—	624.5	5.14
5	— » —	(2.462)	7.533	1.038	0.638	1.676	—»—	619.3	4.98
6	— » —	(2.467)	7.533	(1.033)	0.638	1.671	—»—	618.2	4.94
7	— » —	2.439	(7.561)	(1.061)	0.638	1.699	—»—	624.5	5.16
8	— » —	(2.462)	(7.538)	1.038	0.638	1.676	—»—	619.3	4.98
								Mean:	5.03 ± 0.13
1	2.700	1.776	8.214	0.898	0.782	1.680	—»—	534.5	5.31
2	— » —	(1.786)	8.214	0.898	0.782	1.680	—»—	534.5	5.28
3	— » —	1.776	(8.224)	0.898	0.782	1.680	—»—	534.5	5.32
4	— » —	1.776	8.214	(0.924)	0.782	1.706	—»—	541.6	5.46
5	— » —	(1.802)	8.214	0.898	0.782	1.680	—»—	534.5	5.23
6	— » —	(1.786)	8.214	(0.914)	0.782	1.696	—»—	538.9	5.38
7	— » —	1.776	(8.224)	(0.924)	0.782	1.706	—»—	541.6	5.47
8	— » —	(1.802)	(8.198)	0.898	0.782	1.680	—»—	534.5	5.22
								Mean:	5.33 ± 0.14

The values in parenthesis are those calculated from the other data.

Table 6 b. Comparison of $\varkappa$ values calculated by methods 1—3 and 9—13 (Table 5). a_0 = 100.0 mC. Pyrex bottles. AgR form of Wofatit KS.

Method of evaluatn.	n_{H+tot} mmol	n_{Ag+} mmol	n_{H+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	0.1 a_0 mmol	$\beta \cdot 10^3$	$\varkappa$ from (3)
1	9.000	2.092	7.939	1.314	1.051	2.365	10.00	555.6	4.74
2	— » —	(2.061)	7.939	1.314	1.051	2.365	—»—	555.6	4.82
3	— » —	2.092	(7.908)	1.314	1.051	2.365	—»—	555.6	4.73
9	— » —	2.092	7.939	1.314	(1.061)	2.375	—»—	553.2	4.70
10	— » —	2.092	(7.949)	1.314	1.051	2.365	—»—	555.6	4.75
11	— » —	(2.061)	7.939	1.314	(1.061)	2.375	—»—	553.2	4.77
12	— » —	2.092	(7.908)	1.314	(1.092)	2.406	—»—	546.1	[4.55]
13	— » —	(2.051)	(7.949)	1.314	1.051	2.365	—»—	555.6	4.85
								Mean:	4.77 ± 0.08
1	8.000	2.899	7.160	1.522	0.827	2.349	—»—	647.9	4.55
2	— » —	(2.840)	7.160	1.522	0.827	2.349	—»—	647.9	4.64
3	— » —	2.899	(7.101)	1.522	0.827	2.349	—»—	647.9	4.51
9	— » —	2.899	7.160	1.522	(0.840)	2.362	—»—	644.4	4.48
10	— » —	2.899	(7.173)	1.522	0.827	2.349	—»—	647.9	4.55
11	— » —	(2.840)	7.160	1.522	(0.840)	2.362	—»—	644.4	4.57
12	— » —	2.899	(7.101)	1.522	(0.899)	2.421	—»—	628.7	[4.15]
13	— » —	(2.827)	(7.173)	1.522	0.827	2.349	—»—	647.9	4.67
								Mean:	4·57 ± 0.10

The n values in parenthesis are those calculated from the other data. From Table 6 is seen that the method of evaluation does not affect the $\varkappa$ values in any significant manner. The $\varkappa$ values calculated by the especially inaccurate method 12 are given in brackets in the last column and have been omitted in the calculation of the mean $\varkappa$. All other values are within the limits ± 3 %.

In the Tables 7 and 8, $\varkappa$ has as far as possible been calculated with method 1. Only directly determined n values are listed. If it was not possible to use 1, one of the methods 4 and 7 were used.

The authors have preferred to plot $\varkappa$ as a function of β instead of β as a function of α, which is commonly used, since the former mode of representation is more sensitive to small variations in $\varkappa$ with β. In Figs. 4 and 5, $\varkappa$ is plotted against β for Wofatit KS and Dowex 50. Obviously $\varkappa$ is far from being constant and the points fall on a smooth curve. If it had been constant, the isotherm would have been of the Langmuir type. From Figs. 4 b and 5 b it is seen that the error in $\varkappa$ is about ± 5 %. It is also seen that, within the limits of experimental error, $\varkappa$ is independent of ionic strength. As is seen in the

Table 7 a. $a_0 = 100.0$ *mC. Bottles with rubber stopper. * denotes pyrex bottles. HR form of Wofatit KS.*

$n_{Ag^+ tot}$ mmol	$n_{Ag^+} \cdot 10^3$ mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
0.00500	0.8023	10.00	—	1.724	1.728	0.8023	2.429	4	30.4
*0.00500	0.9980	10.06	—	1.810	1.814	0.9980	2.206	4	22.3
0.01000	1.788	10.01	—	1.729	1.737	1.788	4.728	4	26.6
0.01500	3.097	—	—	1.720	1.732	3.097	6.871	7	22.3
*0.01500	2.831	10.00	—	1.764	1.776	2.831	6.852	4	24.4
0.02000	3.942	10.01	—	1.721	1.737	3.942	9.246	4	23.7
0.02500	5.116	10.00	—	1.720	1.740	5.116	11.43	4	22.6
0.03000	6.718	10.00	—	1.720	1.743	6.718	13.36	4	20.1
*0.03000	6.931	10.00	—	1.754	1.777	6.931	12.98	4	19.0
0.03500	8.098	—	—	1.716	1.743	8.098	15.43	7	19.3
0.04500	11.06	—	—	1.710	1.744	11.06	19.46	7	17.9
0.05000	12.24	9.95	—	1.708	1.746	12.24	21.63	4	18.0
*0.07000	18.63	10.04	—	1.777	1.828	18.63	28.10	4	15.6
0.1000	28.36	9.94	—	1.663	1.735	28.36	41.29	4	15.1
*0.1500	46.49	10.00	—	1.712	1.816	46.49	56.99	4	13.0
0.2000	68.88	9.91	—	1.598	1.729	68.88	75.82	4	11.8
0.3000	116.5	9.89	—	1.553	1.737	116.5	105.6	4	10.0
0.4000	165.0	9.82	0.218	1.435	1.653	165.0	131.9	1	9.0
0.4150	168.6	9.81	—	1.491	1.737	168.6	141.9	4	9.6
0.5600	236.5	9.74	—	1.426	1.750	236.5	184.9	4	9.3
0.6800	319.2	9.67	—	1.378	1.739	319.2	207.5	4	7.9
0.8000	374.4	9.60	—	1.333	1.759	374.4	242.0	4	8.2
0.9000	468.0	9.53	0.412	1.226	1.638	468.0	251.5	1	6.8
0.9200	451.3	9.55	—	1.304	1.773	451.3	264.4	4	7.6
1.040	537.7	9.45	—	1.236	1.738	537.7	289.0	4	7.1
1.180	616.2	9.36	—	1.192	1.756	616.2	321.1	4	7.2
1.400	800.0	9.208	0.587	1.072	1.659	800.0	353.8	1	6.3
*1.750	1020	9.057	0.730	1.080	1.810	1020	403.3	1	6.0
2.000	1228	8.678	0.731	0.919	1.650	1228	443.0	1	5.6
*2.350	1354	8.691	0.996	0.956	1.952	1354	510.2	1	6.7
2.700	1776	8.214	0.898	0.782	1.680	1776	534.5	1	5.3
3.500	2439	7.533	1.038	0.638	1.676	2439	619.3	1	5.0
*4.350	3119	6.935	1.231	0.564	1.795	3119	685.8	1	4.9
4.600	3389	6.539	1.178	0.484	1.662	3389	708.8	1	4.7
6.000	4638	5.358	1.357	0.354	1.711	4638	793.1	1	4.4
8.300	6766	3.212	1.509	0.201	1.710	6766	882.5	1	3.6
*10.00	8379	1.682	1.621	0.150	1.771	8379	915.3	1	2.2

Table 7 b. $a_0 = 100.0$ *mC. Pyrex bottles. AgR form of Wofatit KS.*

n_{H^+tot} mmol	n_{Ag^+} mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
10.00	1.260	8.740	0.943	1.236	2.179	1260	432.8	1	5.3
10.00	1.238	8.762	0.921	1.227	2.148	1238	428.8	1	5.3
10.00	1.239	8.761	0.916	1.234	2.150	1239	426.0	1	5.2
9.000	2.092	7.939	1.314	1.051	2.365	2092	555.6	1	4.7
9.000	2.068	7.933	1.317	1.045	2.362	2068	557.6	1	4.8
8.000	2.899	7.160	1.522	0.827	2.349	2899	647.9	1	4.5
8.000	2.873	7.178	1.485	0.815	2.300	2873	645.7	1	4.6
7.000	3.731	6.329	1.666	0.639	2.323	3731	717.2	1	4.4
7.000	3.736	6.320	1.679	0.657	2.336	3736	718.8	1	4.3
6.000	4.549	5.449	1.659	0.531	2.190	4549	757.5	1	3.7
6.000	4.548	5.449	1.650	0.521	2.171	4548	760.0	1	3.8
4.000	6.347	3.641	1.864	0.337	2.201	6347	846.9	1	3.2
4.000	6.407	3.602	2.121	0.372	2.493	6407	850.8	1	3.2
2.000	8.360	1.766	2.347	0.216	2.563	8360	915.7	1	2.3
2.000	8.353	1.770	2.363	0.213	2.576	8353	917.3	1	2.4
1.000	9.264	0.789	2.642	0.128	2.770	9264	953.8	1	1.8
1.000	9.404	0.831	2.467	0.109	2.576	9404	957.7	1	2.0

Table 7 c. $a_0 = 20.00$ *mC. Pyrex bottles. HR form of Wofatit KS.*

n_{Ag^+tot} mmol	$n_{Ag^+} \cdot 10^3$ mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
0.00500	0.2313	2.01	—	1.813	1.818	0.1157	2.623	4	22.9
0.00500	0.2239	2.01	—	1.761	1.766	0.1119	2.704	4	24.3
0.02500	1.318	2.01	—	1.774	1.798	0.6590	13.17	4	20.4
0.02500	1.328	2.01	—	1.850	1.874	0.6640	12.63	4	19.4
0.05000	3.030	2.00	—	1.816	1.863	1.515	25.21	4	17.1
0.05000	3.238	2.01	—	1.816	1.863	1.619	25.10	4	16.0
0.1000	7.594	2.00	—	1.760	1.852	3.797	49.90	4	13.8
0.1000	7.684	1.99	—	1.745	1.837	3.842	50.26	4	13.7
0.5000	7.344	1.93	0.4081	1.433	1.841	3.672	221.7	1	7.5
0.5000	7.316	1.93	0.4133	1.457	1.870	3.658	221.0	1	7.5
1.000	21.1	1.788	0.7755	1.115	1.891	10.6	410.1	1	5.9
1.000	22.0	1.789	0.7755	1.058	1.834	11.0	422.8	1	6.0
1.500	41.0	1.607	1.081	0.765	1.846	20.5	585.6	1	5.5
1.500	40.5	1.595	1.090	0.780	1.870	20.3	582.9	1	5.5
2.000	65.3	1.331	1.322	0.546	1.868	32.7	707.7	1	4.9
2.000	64.5	1.337	1.323	0.557	1.880	32.3	703.7	1	4.9

Table 8 a. $a_0 = 100.0$ *mC. Pyrex bottles. HR form of Dowex 50*

$n_{Ag^+ tot}$ mmol	$n_{Ag^+} \cdot 10^3$ mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$\alpha \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
0.00500	0.7422	10.01	—	3.886	3.890	0.7422	1.095	4	14.8
0.00500	0.9521	10.11	—	3.848	3.852	0.9521	1.051	4	11.2
0.01000	1.537	10.02	—	3.863	3.871	1.537	2.186	4	14.3
0.01000	1.592	10.11	—	3.868	3.876	1.592	2.169	4	13.8
0.01500	2.367	10.04	—	3.856	3.868	2.367	3.265	4	13.9
0.01500	2.433	10.14	—	3.865	3.878	2.433	3.241	4	13.6
0.02000	3.426	10.01	—	3.856	3.873	3.426	4.278	4	12.6
0.02500	4.344	10.02	—	3.842	3.863	4.344	5.348	4	12.4
0.03000	5.278	9.90	—	3.815	3.840	5.278	6.438	4	12.2
0.03000	5.176	10.10	—	3.856	3.881	5.176	6.395	4	12.6
0.03500	6.264	9.99	—	3.836	3.865	6.264	7.436	4	11.9
0.03500	6.024	9.99	—	3.845	3.874	6.024	7.481	4	12.5
0.04000	7.206	10.01	—	3.845	3.878	7.206	8.455	4	11.8
0.04500	8.193	10.01	—	3.837	3.874	8.193	9.502	4	11.7
0.04500	7.911	10.09	—	3.856	3.893	7.911	9.527	4	12.3
0.05000	9.462	10.00	—	3.808	3.849	9.462	10.53	4	11.3
0.06000	11.32	9.99	—	3.805	3.854	11.32	12.63	4	11.3
0.06000	10.97	10.11	—	3.715	3.764	10.97	13.03	4	12.2
0.08000	15.82	10.01	—	3.827	3.891	15.82	16.49	4	10.6
0.09000	18.20	9.99	—	3.825	3.897	18.20	18.42	4	10.3
0.09000	17.57	10.10	—	3.812	3.884	17.57	18.65	4	10.9
0.1000	20.29	10.01	—	3.792	3.872	20.29	20.59	4	10.4
0.2000	44.20	9.96	—	3.732	3.888	44.20	40.07	4	9.4
0.3000	69.41	9.92	—	3.648	3.879	69.41	59.45	4	9.0
0.4000	95.14	9.83	—	3.528	3.833	95.14	79.55	4	8.9
0.5000	123.5	9.87	—	3.475	3.852	123.5	97.74	4	8.7
0.6000	150.3	9.85	—	3.392	3.842	150.3	117.0	4	8.7
0.7000	180.1	9.82	—	3.323	3.843	180.1	135.3	4	8.5
0.8000	205.6	9.81	—	3.260	3.854	205.6	154.2	4	8.7
0.9000	216.9	9.78	—	3.201	3.884	216.9	175.9	4	9.6
0.9000	228.4	9.69	—	3.135	3.807	228.4	176.4	4	9.1
0.9500	240.3	9.84	—	3.195	3.905	240.3	181.7	4	9.1
0.9500	242.1	9.84	—	3.155	3.863	242.1	183.3	4	9.1
1.000	264.8	9.80	—	3.125	3.860	264.8	190.5	4	8.7
1.500	405.3	9.601	1.065	2.789	3.854	405.3	276.3	1	9.0
1.500	430.1	9.584	1.036	2.528	3.564	430.1	290.7	1	9.1
1.500	437.3	9.582	1.030	2.520	3.550	437.3	290.1	1	9.0
2.000	589.6	9.437	1.412	2.464	3.876	589.6	364.3	1	9.2
2.500	761.0	9.240	1.691	2.150	3.841	761.0	440.2	1	9.5
2.500	756.9	9.248	1.702	2.183	3.885	756.9	438.1	1	9.5
3.000	992.3	9.001	2.015	1.895	3.910	992.3	515.3	1	9.6

Table 8 a (continued).
a_0 = 100.0 mC. Pyrex bottles. HR form of Dowex 50.

n_{Ag^+tot} mmol	$n_{Ag^+} \cdot 10^3$ mmol	n_{H^+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
4.000	1599	8.356	2.365	1.218	3.583	1599	660.1	1	10.1
4.000	1607	8.354	2.370	1.209	3.579	1607	662.2	1	10.2
4.000	1471	8.479	2.433	1.367	3.800	1471	640.3	1	10.3
4.000	1471	8.541	2.473	1.400	3.873	1471	638.5	1	10.3
5.000	2130	7.873	2.875	1.013	3.888	2130	739.5	1	10.5
6.000	2804	7.170	3.083	0.653	3.736	2804	825.2	1	12.1
6.000	2774	7.202	3.150	0.726	3.876	2774	812.7	1	11.3
6.000	2977	6.969	2.928	0.616	3.544	2977	826.2	1	11.1
7.000	3885	6.153	3.147	0.445	3.592	3885	876.1	1	11.2
7.000	3888	6.151	3.151	0.445	3.596	3888	876.3	1	11.2
7.000	3665	6.359	3.357	0.536	3.893	3665	862.3	1	10.9
7.000	3660	6.399	3.353	0.505	3.858	3660	869.1	1	11.6
8.000	4496	5.493	3.493	0.372	3.865	4496	903.8	1	11.5
10.00	6378	3.708	3.719	0.173	3.892	6378	955.5	1	12.5

tables, s_0 shows a good constancy with a few exceptions. These are probably due to differences in water content of the dry resin.

The $\varkappa$ (β) curve for Dowex 50 differs much from that of Wofatit KS but both increase for small β. The curve for Dowex 50 has a minimum at $\beta \approx 0.15$.

Table 8 b. a_0 = 100.0 mC. Pyrex bottles. AgR form of Dowex 50.

n_{H^+tot} mmol	n_{Ag^+} mmol	n_{H^+} mmol	n_{AgR} mmol	n_{RH} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
10.00	1.469	8.531	2.589	1.481	4.070	1469	636.1	1	10.2
10.00	1.471	8.529	2.596	1.493	4.089	1471	634.9	1	10.1
10.00	1.476	8.524	2.612	1.469	4.081	1476	640.0	1	10.3
10.00	1.480	8.520	2.595	1.471	4.066	1480	638.2	1	10.2
6.000	4.380	5.611	3.682	0.383	4.065	4380	905.8	1	12.3
6.000	4.384	5.603	3.648	0.390	4.038	4384	903.4	1	12.0
4.000	6.133	3.810	3.899	0.214	4.113	6133	948.0	1	11.3
4.000	6.159	3.795	4.087	0.224	4.311	6159	948.0	1	11.2
2.000	8.111	1.929	4.194	0.075	4.269	8111	982.4	1	13.3
2.000	8.127	1.926	4.105	0.086	4.191	8127	979.5	1	11.3

Table 8 c. $a_0 = 20.00$ *mC. Pyrex bottles. HR form of Dowex 50.*

$n_{Ag+\ tot}$ mmol	$n_{Ag+} \cdot 10^3$ mmol	n_{H+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
0.00500	0.2482	2.00	—	3.563	3.568	1.241	1.332	4	10.7
0.00500	0.1875	1.99	—	3.539	3.544	0.938	1.358	4	14.4
0.02500	1.028	2.00	—	3.537	3.561	5.140	6.731	4	13.2
0,02500	1.043	1.99	—	3.534	3.558	5.215	6.734	4	12.9
0.05000	2.344	1.99	—	3.500	3.548	11.72	13.43	4	11.6
0.05000	2.326	1.99	—	3.552	3.600	11.63	13.24	4	11.5
0.1000	4.930	2.00	—	3.407	3.502	24.65	27.14	4	11.3
0.1000	4.969	1.99	—	3.463	3.558	24.85	26.71	4	11.0
0.5000	31.30	1.97	0.4565	3.084	3.541	156.5	128.9	1	9.3
0.5000	31.93	1.97	0.4498	3.081	3.531	159.7	127.4	1	9.0
1.000	70.91	1.93	0.9130	2.636	3.549	354.6	257.3	1	9.4
1.000	71.75	1.93	0.9082	2.630	3.538	358.8	256.7	1	9.3
1.500	120	1.868	1.362	2.218	3.580	599.0	380.4	1	9.6
1.500	117	1.863	1.360	2.210	3.570	585.0	381.0	1	9.8
2.000	185	1.795	1.781	1.792	3.573	922.5	498.5	1	9.6
2.000	184	1.792	1.774	1.778	3.552	917.5	499.4	1	9.7

Table 8 d. $a_0 = 20.00$ *mC. Pyrex bottles. AgR form of Dowex 50.*

$n_{H+\ tot}$ mmol	n_{Ag+} mmol	n_{H+} mmol	n_{AgR} mmol	n_{HR} mmol	s_0 mmol	$a \cdot 10^4$	$\beta \cdot 10^3$	Method of evaluatn.	$\varkappa$ from (3)
1.500	0.877	1.111	3.699	0.383	4.082	438	906.2	1	12.2
1.500	0.881	1.105	3.699	0.374	4.073	440	908.2	1	12.4
0.5000	1.576	0.413	4.010	0.091	4.101	788	977.8	1	11.5
0.5000	1.580	0.410	4.017	0.087	4.104	790	978.8	1	12.0

It is interesting to note that our results are in qualitative agreement with those of Samuelson [6] and Duncan and Lister [10], who have also shown that $\varkappa_{Me,H}$, increases with decreasing β_{MeR}.

In papers to follow, the results in this paper will be compared with those obtained by Ekedahl from leaking curves [17], and it will be shown how data on ion exchange equilibria can be used for calculating the activities of the components in the resin. Some of these latter results have already been published in preliminary communications [18–20].

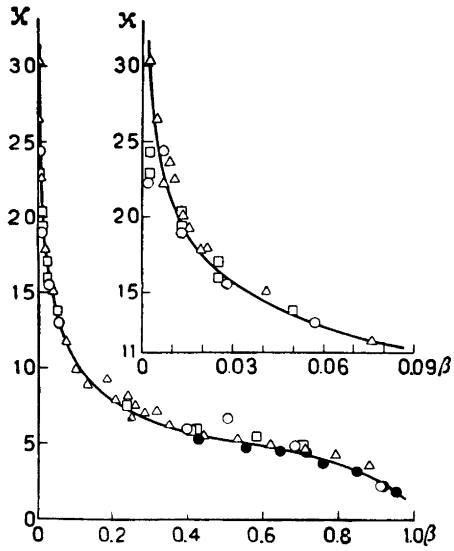


Fig. 4. The equilibrium quotient $\varkappa$ as function of the silver fraction for Wofatit KS. The same data as in Tables 7 a—c. The range $\beta < 0.09$ is also given on a larger scale.

△ *Starting with HR form, $a_0 = 100.0$ mC. Bottles with rubber stoppers.*
○ *Starting with HR form, $a_0 = 100.0$ mC. Pyrex bottles.*
● *Starting with AgR form, $a_0 = 100.0$ mC. Pyrex bottles.*
□ *Starting with HR form, $a_0 = 20.00$ mC. Pyrex bottles.*

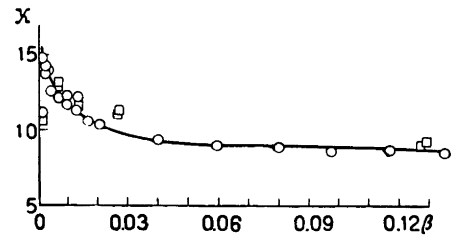
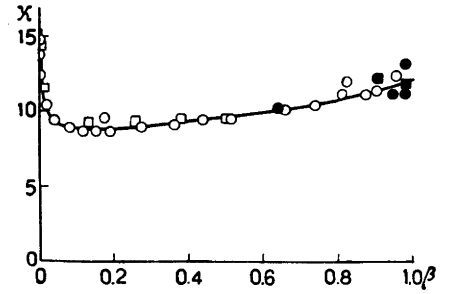


Fig. 5. The equilibrium quotient $\varkappa$ as function of the silver fraction β for Dowex 50. The same data as in Table 8 a-d. The range $\beta < 0.13$ is also given on a larger scale.

○ *Starting with HR form, $a_0 = 100.0$ mC. Pyrex bottles.*
● *Starting with AgR form, $a_0 = 100.0$ mC. Pyrex bottles.*
□ *Starting with HR form, $a_0 = 20.00$ mC. Pyrex bottles.*
■ *Starting with AgR form, $a_0 = 20.00$ mC. Pyrex bottles.*



SUMMARY

The exchange equilibrium $Ag^+ — H^+$ has been studied at two ionic strengths ($I = 0.100$ and 0.020) on Wofatit KS and Dowex 50. A broad concentration range was covered $0.001 \leqq \beta \leqq 0.98$, using emf and chemical methods. The equilibrium was attained from both sides. No corrections for activities were tried.

The authors have preferred to represent their measurements with $\varkappa$ (β) curves Figs. 4 and 5, where $\varkappa$ is the equilibrium quotient, and β the molar fraction of AgR. The $\varkappa$ (β) curves are very different for the two exchangers but both increase for small β. The $\varkappa$ (β) curve for Dowex 50 has a minimum at $\beta \approx 0.15$. The error in $\varkappa$ is about $\pm$ 5 %.

$\varkappa$ is found to be independent of the ionic strength for both exchangers. For Dowex 50, $\varkappa$ and s_0 did not change when the particle size was decreased by grinding. It was also checked that the equilibrium is attained within the shortest time of shaking (24 hours).

The authors wish to thank Professor Gustaf Ljunggren for his continuous and encouraging interest in our work and Professor Arne Ölander for his kindness in placing rooms at our disposal. We also wish to thank Miss Gunilla Bergström for her valuable aid in our work.

This work was supported by a grant from *Statens Tekniska Forskningsråd.*

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Received September 15, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1490–1494

Studies in the Tropolone Series

III. Infra-red Spectra

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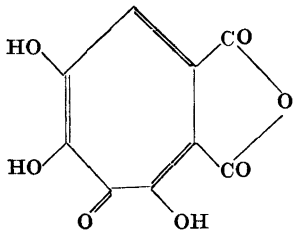
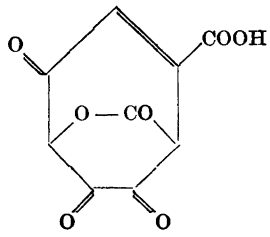
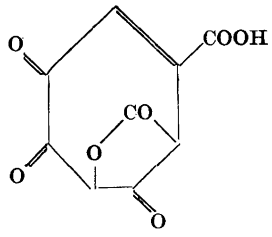
It was recently stated in a short note in this journal [1] that ultra-violet absorption studies on puberulonic acid [2–4] and related compounds at various hydrogen ion concentrations had led one of the present authors to propose a new formula (I) for the acid instead of the lactone structures (II) and (III) put



(I)



(II)



(III)

forward by Todd *et al.*[4]. In the same note it was briefly mentioned that the anhydride formula was also supported by infra-red absorption data. We understand that similar work in the infra-red field is now being done in other European and American laboratories and, hence, consider it justifiable to publish the results of our infra-red studies prior to those of the ultra-violet investigations although the chronological order is thereby reversed.

The main purpose of the present investigation was to confirm the structure (I) for puberulonic acid. This structure contains a tropolone skeleton and an acid anhydride ring, but no carboxyl group. The vibrational frequencies of these structural elements are known from earlier investigations on a variety of organic substances (*cf.* below). They are well-characterized although naturally somewhat influenced by neighbouring parts of the molecules. Hence, the

infra-red spectrum of a compound possessing structure (I) must be easily distinguishable from those of substances like (II) and (III), especially if compared with the spectra of closely related compounds. Stipitatic [5–7] and puberulic [2–4] acids as well as β- and γ-thujaplicins [8, 9] were used as reference compounds in this investigation. In connection with this work, the infra-red spectrum of nootkatin [10] was also determined.

Some work on the infra-red absorption properties of tropolones has been reported during the current year. Scott and Tarbell [11] examined solutions of colchicine and some of its derivatives as well as γ-thujaplicin and β-methyltropolone. They found a series of three or four absorption bands in the region 1 255—1 625 cm^{-1} which occurred in all the tropolone derivatives and were therefore considered typical for the tropolone ring system. The approximate wave-numbers of the bands were: 1 280, (1 350), 1 550 and 1 615 cm^{-1}. In a later communication from the same laboratories [12] bands of similar wave-numbers were reported for 4,5-benztropolone and derivatives thereof. A synthetic carbethoxytropolone was found by Bartels-Keith and Johnson [13] to exhibit absorption maxima at 1 250, 1 440, 1 478, 1 560 and 1 620 cm^{-1} which were ascribed to the tropolone nucleus.

A comparison of the bands reported for tropolone derivatives shows that they all absorb in the following regions: 1 250—1 280, 1 538—1 567 and 1 605—1 624 cm^{-1}. For β-methyltropolone and γ-thujaplicin, in the 1 450—1 500 cm^{-1} region (*cf.* the 1 478 band of carbethoxytropolone), Scott and Tarbell observed general absorption but no well-defined bands. Not only structural differences between the substances but also the solvents employed cause minor variations in the wave-numbers of the maxima.

EXPERIMENTAL

The samples * were investigated in the form of paraffin smears (1 part of the sample in approximately 8 parts of paraffin except for puberulonic acid, where the ratio was 1 : 2.5). Transmission of 0.025 mm layers was determined with a Perkin-Elmer Model 12 B infra-red spectrometer using a rock salt prism. The slit-width was varied with the wave-numbers according to the following scheme (wave-numbers in cm^{-1}, slit-widths in mm in parentheses): 662—738 (0.520), —904 (0.230), —1 052 (0.177), —1 309 (0.097), —1 552 (0.069), —1 987 (0.033), —2 000 (0.015).

* The provision of pure specimens of puberulonic, stipitatic and puberulic acids by Professor H. Raistrick, and of the thujaplicins and nootkatin by Professor H. Erdtman is gratefully acknowledged.

RESULTS AND DISCUSSION

Fig. 1 shows the percentage absorption curves and Table 1 gives the wave-numbers for the bands of main interest. It is seen that all of the substances investigated exhibit absorption bands within, or very close to, the three regions mentioned above as being characteristic for the tropolone nucleus. This, of course, furnishes strong evidence in favour of the tropolone structure for

Table 1.

Substance	Wave-numbers of bands (cm^{-1})						
β-Thujaplicin	1 270	(1 480)	1 545	1 615			
γ-Thujaplicin	1 265	(1 490)	1 560	1 605, 1 620			
Stipitatic acid	1 285	(1 480)	1 570	1 615	1 700		
Puberulic acid	1 285	1 505	1 535	1 595	1 680		
Puberulonic acid	1 285	1 505	1 545	1 620		1 770	1 830
Nootkatin	1 270	(1 480)	1 550	1 595			

puberulonic acid and the tropolone structure of nootkatin is also confirmed.

The 1 480—1 505 cm^{-1} maxima are most probably analogous to the 1 478 cm^{-1} band for carbethoxytropolone and also ascribable to the tropolone ring system.

The strong absorption maxima at 1 680 and 1 700 cm^{-1} for puberulic and stipitatic acids respectively are due to the carboxyl groups [14]. The absence of a corresponding maximum in the puberulonic acid absorption curve is inconsistent with the two formulae (II) and (III).

The intense bands at 1 770 and 1 830 cm^{-1}, exhibited by puberulonic acid alone, constitute the pair typical for acid anhydrides. The exact position of this pair varies with the structure of the anhydride concerned. The wave-numbers found for puberulonic acid, however, agree well with *e. g.* the approximate values 1 775 and 1 845 cm^{-1} for phthalic anhydride [14] and 1 795 and 1 850 for maleic anhydride [14].

The results reported above, together with chemical and ultra-violet spectral evidence, conclusively establish that puberulonic acid is (I) or a tautomeride thereof, differing in the arrangement of the nuclear CO and —OH groups. This mould metabolite, puberulonic acid, discovered by Birkinshaw and Raistrick in 1932, is thus not only a tropolone but also one of the rare naturally-occurring acid anhydrides.

For nootkatin, the infra-red data together with ultra-violet evidence [10] shows that this substance also is a tropolone. Chemical investigation [15] (isolation of acetone as a degradation product) shows that it contains an isopropylidene group, but the infra-red absorption curve offers no certain evidence

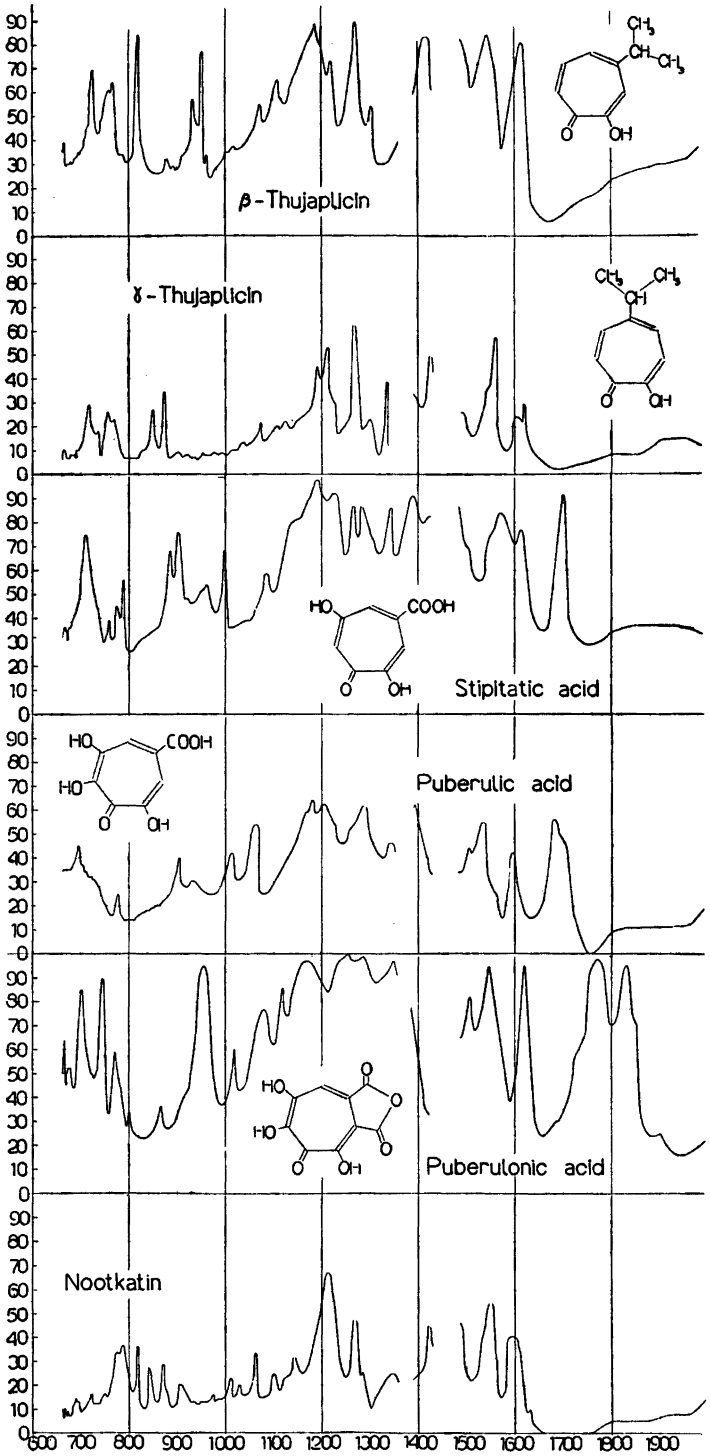


Fig. 1. Percentage absorption. Wave-numbers in cm^{-1}.

regarding the existence of an extra-nuclear double bond in the molecule. Its stretching band may, however, be obscured by the tropolone band at 1 595 cm^{-1}.

SUMMARY

Infra-red spectra have been determined for paraffin smears of puberulonic, puberulic and stipitatic acids, β- and γ-thujaplicins and nootkatin. Four maxima, characteristic for the tropolone ring system, were found in all cases. No carboxyl band but anhydride bands at 1 770 and 1 830 cm^{-1} were found in the puberulonic acid curve. This shows that puberulonic acid possesses structure (I) or a tautomeric formula, not the structure (II) or (III). The formula (I) has been suggested earlier on the basis of ultra-violet spectroscopic evidence.

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Received December 19, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1495–1500

Short Communications

Microrefractometry with Abbe-Type Refractometer

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For evaluating the refractive and dispersive constants of a liquid several types of refractometers are available, of which the so-called Abbe-type refractometer [1–3] is the most common.

This type is both rapid and simple by the measurements and has a precision ($\pm$ 0.0001 units in n_D) that is sufficient for most purposes. Because it is provided with compensator [4], the dispersive constants ν (n_D-1/n_F-n_C) may be obtained with close approximation and also n_F and n_C separately [2, 5]. By this the refractometer is also useful for investigations of structure [6] and for quantitative analysis of complicated liquid mixtures [7].

To render a measurement possible it is necessary with a continuous liquid layer between the lower, illuminating prism (Fig. 1 a) and the upper polished prism (Fig. 1 b), where the light is reflected. The required quantities of liquid depend consequently on the dimensions of the free space that exists between the prisms in closed position. The surface of the base is 13 × 28 mm (Abbe-type refractometer model 1 [8]). The height *i.e.* the distance between the prisms, which varies slightly among the instruments, is about 0.15 mm. This makes a volume of about 50 mm^3.

If the test volume is less than the above-mentioned the field of view will be irregularly illuminated and rapidly completely dark according to the fact that air has replaced the liquid film between the prisms. Such factors as the viscosity and volatility of the sample, and the technique used by the supply will also influence the amount that is necessary.

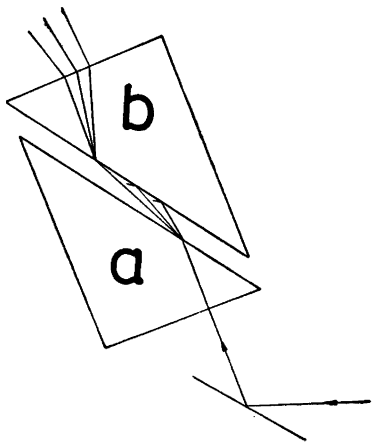

Fig. 1. Schematic diagram of the prism optic of the Abbe-refractometer.

For works with microquantities, *i.e.* a few mm^3, microrefractometers have been designed. Comprehensive reviews of these instruments and works have been made among others by Alber and Bryant [9] and Wilson [10]. Most of these specially designed refractometers are however comparatively tedious and expensive and none unites both the versatility and rapidness of the Abbe-type refractometer. The difference in the last-mentioned factor will be very noticeable by measurements of a large number of test samples *e.g.* by identification of fractions from separation of liquids [11].

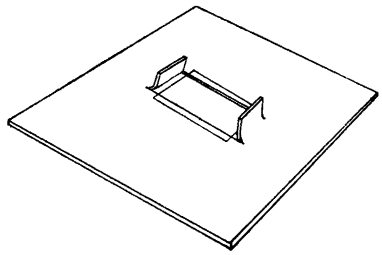

Fig. 2. Inserting of the paper in the measuring plate.

The amount of sample that the Abbe-type refractometer requires can however be reduced if the free space between the prisms is partly filled by a transparent material in which the liquid is adsorbed. Alber and Bryant (*l. c.*) point out that the volume in certain cases may be reduced to about 10 mm^3, if the sample is taken up in a "japanese lens paper" of the size 10 × 15 mm.

This technique has been further developed and here will be described a technique according to which samples of less than 1 mm^3 may be measured. If the free space is limited by a non-adsorbing, inert material (metal) so that only an opening of a few mm^2 is left and a thin paper is fixed in this opening, a very small amount of sample will be sufficient for establishing liquid contact between the prisms. The flow of light will be reduced by this but is still sufficient for fine adjustment of the cross hairs and achromatizing prisms.

The prism surfaces, especially the upper, polished one, are the most sensitive parts of the refractometer and by careless handling the instrument will be damaged [12]. As by this technique a solid body is placed between the prisms great care should be taken. Properly performed no damage of the prisms can happen, partly because the metal only rests between the prisms due to forces of adhesion (not mechanical pressing) and partly because it is made from very soft material.

Making the measuring plate:
Material: 0.08 mm sheet of soft metal i.e. Sn or Al foil, and 0.03 mm paper (resinfree, bleached and hardpressed, of tissue-type).

In the metal foil, 10 × 10 mm, two parallel slits of 5 mm:s length are cut 2 mm apart. A rectangle of 3 mm:s length is cut between the slits and the two laps are unfolded. The paper, 3 × 4 mm, is fitted into the opening and is fixed by folding down the laps (Fig. 2). The plate is then pressed and controlled (micrometer screw) for absolute smoothness and evenness.

Measurement:

The plate is placed with the opening in the middle of the lower prism and the sample is fed from a capillary tube (Fig. 3). By closing the prisms it should be noted that the lower prism is held in as horisontal position as possible until they are locked together, in order to prevent sliding of the plate.

After the measurement the plate is carefully removed and washed in ether. Before measuring an unknown sample, t e technique should be tested at a liquid with

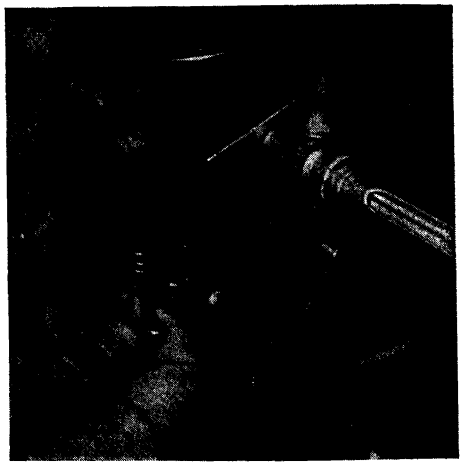

Fig. 3. Photograph of the measuring technique. The sample is fed from a capillary tube to the measuring plate which is resting on the lower prism.

known refractive index in order to control the accuracy and the proper inserting of the paper. The procedure will not take much longer time than a measurement with normal quantities and may be used with all liquids which do not react with the paper. The minimum quantity for a measurement is ¼ – ½ mm³.

1. Abbe, E. *Neue Apparate zur Bestimmung des Brechungs- und Zerstreuungsvermögens fester und flüssiger Körper.* Jena (1874).
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3. Gibb, T. R. T. *Optical methods of chemical analysis.* (1942).
4. Abbe, E. *Gesammelte Abhandlungen.* Jena. **2** (1906) 130.
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Received November 15, 1950.

Inability of two Hydantoins to act as Precursors of Pyrimidines in Ribonucleic Acid

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In earlier work we have found that the rat can utilize orotic acid as a precursor for the pyrimidines of polynucleotides [1]. During the course of work on further possible intermediates in the synthesis of polynucleotides in the rat we have investigated the utilization of two N^{15} marked intermediates in the synthesis of orotic from aspartic acid according to Mitchell and Nyc [2] *i. e.* 5-acetylhydantoin and 5(carboxymethylidine)-hydantoin. The latter compound is transformed into orotic acid by alkali under mild conditions. A similar transformation might conceivably be caused by the hydantoinsplitting enzyme recently found in rat tissues by Bernheim and Bernheim [3].

Starting from N^{15} aspartic acid the two hydantoins were synthesized according to the method of Nyc and Mitchell [2]. They contained an over-all excess of 16.2 atom % N^{15}. Each hydantoin was injected subcutanously into two different groups of two rats at a level of 125 mg/kilo of body weight per day. The injections were carried out twice daily with approximately 12 hourly intervals over a period of 3 days. The animals were killed 12 hours after the last injection. In each group the polynucleotides from the pooled livers were prepared and separated into desoxyribo- and ribonucleic acids according to Hammarsten [4]. The pyrimidine nucleosides were prepared from ribonucleic acids according to Reichard [5]. Both the mixed polynucleotides and the pyrimidine ribosides were analyzed for N^{15}.

In no case could a significant incorporation of the isotope be demonstrated thus indicating that none of the two hydantoins had been used for the synthesis of polynucleotide components.

1. Bergström, S., Arvidsson, H., Eliasson, N. A., Hammarsten, E., Reichard, P., and von Ubisch, H. *J. Biol. Chem.* **177** (1949) 495, **179** (1929) 169.
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Received December 8, 1950.

Paper Chromatographic Analysis of Urine from a Few Cases of Porphyrinuria

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Totter *et al.* have reported a connexion between the amount of glycine in the diet and the porphyrin excretion [1]. Dent and Rimington recently discussed the possibility that a peptide containing methionine sulphone may be the porphyrinurogenic agent [2]. During investigations of the amino acid and peptide excretion in some diseases [3], urines from three cases of porphyrinuria were analyzed by two-dimensional paper partition chromatography. For technical details see references 3, 4. In two cases an orange-yellow-coloured spot was seen on the papers directly under the alaninethreonine spot (see Fig. 1).

It was thought that the spot corresponded to porphobilinogen, a substance earlier described by Waldenström and Wahlquist [5] as present in such urines. To verify this hypothesis three parallel runs were made for each sample of urine. One paper was developed with ninhydrin, the other two papers were treated with Ehrlich reagent [6], or with diazotized sulfanilic acid [4]. On positions corresponding to the yellow ninhydrin spot, positive reactions were obtained in both cases. Therefore, it seems likely, that the yellow spot corresponds to porphobilinogen. This would then appear to be another example of the large variety of substances which react with ninhydrin. The orange-yellow colour resembles the proline and hydroxyproline colours, and supports the conclusion of Waldenström and Wahlquist that porphobilinogen also is a pyrrole derivate.

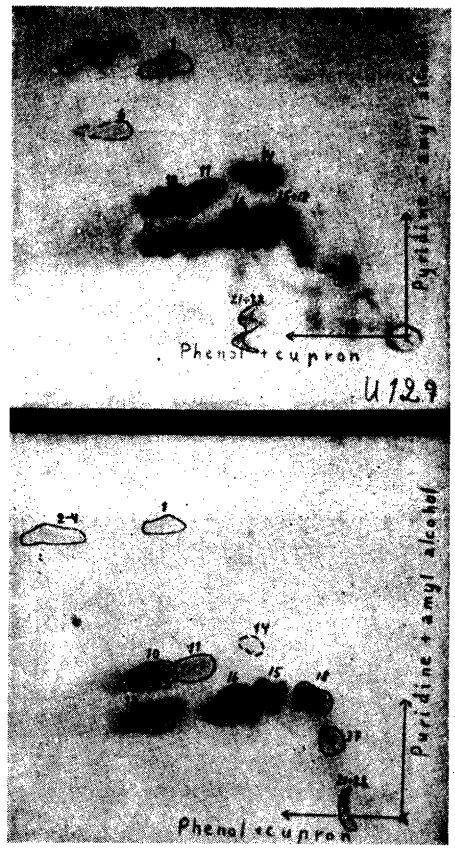

Fig. 1. Photograph of a two-dimensional chromatogram of urine from a case of porphyrinuria, above, and normal urine, below. In both cases an amount of urine containing 1.2 mg creatinine was used.

1. = *Tyrosine*
2 = *Phenylalanine*
3 = *Isoleucine*
4 = *Leucine*
6 = *Valine*
10 = *Alanine*
11 = *Threonine*
12 = *Glutamine*
14 = *Taurine*
15 = *Serine*
16 = *Glycine*
18 = *Histidine*
21 = *Arginine*
22 = *Lysine*
37 = *An orange spot, which did not give any ninhydrin - reacting substances after hydrolysis*

The yellow spot is not numbered, but is encircled directly under the threonine spot.

There was no pronounced difference in the amino acid pattern of urines from normals and cases of porphyrinuria.

1. Totter, J. R., Amos, E. S., and Keith, C. K. *J. Biol. Chem.* **178** (1949) 847.
2. Dent, C. E., and Rimington, C. *Biochem. J.* **41** (1947) 253.
3. de Verdier, C.-H. *Acta Med. Scand.* **138** (1950) 344.
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6. Synge, R. L. M. *Biochem. J.* **38** (1944) 285.

Received December 1, 1950.

Reactions of Diselenium Dichloride with Sodium Sulphinates

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This note is a preliminary report on the synthesis of two new types of selenium sulphur compounds, *viz.*, diselenium and triselenium disulphinates.

The diselenium disulphinates, $Se_2(SO_2R)_2$, are formed when diselenium dichloride reacts, under cooling in ice, with an excess of finely powdered, anhydrous sodium benzene- or *p*-toluenesulphinate, suspended in benzene:

$$Se_2Cl_2 + 2\ RSO_2Na = Se_2(SO_2R)_2 + 2\ NaCl$$

The following proportions of reagents have been used: 10 g of sodium benzenesulphinate in 20 ml of benzene, or 12 g of sodium *p*-toluenesulphinate in 40 ml of benzene; 2 ml of diselenium dichloride. The contents of the flask are stirred with a glass rod for a few minutes, until the diselenium dichloride colour disappears. The mixture is subsequently heated rapidly, the sodium chloride and the excess of sodium sulphinate are filtered off, and ligroin is added to the filtrate.

The benzene compound melts at 79—80° C, the *p*-toluene compound at 104—106° C (dec.).

0.1801 g substance: 33.34 ml of 0.09754 *N* iodine (Norris & Fay).
$Se_2(SO_2C_6H_5)_2$ (440.3)
Calc. Se 35.86 Found Se 35.63
0.1408 g substance: 23.61 ml of 0.1022 *N* iodine.
$Se_2(SO_2C_7H_7\text{-}p)_2$ (468.3)
Calc. Se 33.72 Found Se 33.83

The triselenium disulphinates, $Se_3(SO_2R)_2$, result if, for the above process, the sodium sulphinates are suspended in dry ether instead of in benzene. Probably a rearrangement of primarily formed diselenium disulphinate takes place:

$$2\ Se_2(SO_2R)_2 = Se(SO_2R)_2 + Se_3(SO_2R)_2$$

The last change is in accordance with the behaviour of the corresponding sulphur compounds, $S_2(SO_2R)_2$, which undergo rearrangement to give trithionic and pentathionic compounds [1, 2].

Triselenium di(benzenesulphinate) melts and decomposes rapidly at 101—102° C, while the triselenium di(*p*-toluenesulphinate) crystals liberate selenium at 130—140° C (preheated bath).

0.1069 g substance: 25.33 ml of 0.09754 *N* iodine.
$Se_3(SO_2C_6H_5)_2$ (519.2)
Calc. Se 45.61 Found Se 45.62
0.1073 g substance: 24.21 ml of 0.09754 *N* iodine.
$Se_3(SO_2C_7H_7\text{-}p)_2$ (547.3)
Calc. Se 43.28 Found Se 43.44

The diselenium and triselenium disulphinates form greenish yellow crystals, which appear to be relatively stable at room temperature; thus, some samples have so far been kept unchanged for several weeks.

There was no pronounced difference in the amino acid pattern of urines from normals and cases of porphyrinuria.

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The diselenium and triselenium disulphinates form greenish yellow crystals, which appear to be relatively stable at room temperature; thus, some samples have so far been kept unchanged for several weeks.

Apart from the alkali triselenides, the only compounds known hitherto, with three selenium atoms, mutually bonded, in the molecule, appear to be the selenium selenocyanate, $Se(SeCN)_2$, of Verneuil [3], and the diethyl triselenide, $Se_3(C_2H_5)_2$, of Baroni [4].

Further work on reactions of diselenium dichloride with sodium sulphinates, and also with sodium or potassium thiosulphonates, is in progress.

The author is indebted to *Norges Almenvitenskapelige Forskningsråd* for a grant.

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Received December 14, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1501–1502

New Books

W. A. Sexton, *Chemical Constitution and Biological Activity*. (Industrial Chemistry Series). E. and F. N. Spon, Ltd., London, 1949. 55s., pp. XXI + 412.

This work is divided into two sections, of which the first deals with the chemical compounds and processes that play a part in biological activity in general; the second part deals with selected subjects chosen, as far as possible, with a view of providing illustrations for the principles outlined in the earlier chapters. The latter part includes various growth factors, choline and its derivatives, cancer problems, antigens and antibodies.

In one of the first chapters the author declares that biological activity "must be considered as a function of the molecule as a whole". One feels grateful for this clear formulation of an extremely important principle, which is often being sinned against by other writers in this field. One further notes with satisfaction that the author presents in detail the results of Ferguson's investigations on general narcotics; as shown by Ferguson, the thermodynamic activity corresponding to the minimal effective concentrations of the narcotics is approximately constant. — The author is exceptionally well-read and gives a great number of references. It is a surprise, however, that so few general views are given concerning the main theme, which is the connection of chemical constitution with physiological effect. Many pharmacodynamically related groups of substances have by now been arranged in well-defined structure schemes, *e. g.* analgetics, histaminolytics, antispasmodics, local anesthetics and curare-active substances. The author treats these fields very superficially. — One looks in vain for a mention of the great importance of the amino group for drugs; the part of the book which deals with basic groups consists of only 20 lines. — As an example for the isosteric principle the author mentions, *inter alia*, morphine and pethidine, but as the author does not treat analgetics in greater detail, the reader may easily get the impression that these drugs are dependent on a morphine-like steric configuration; This is of course not the case. — Further the author seems rather uncritical when he completely accepts some questionable concepts concerning the role of acetylcholine in the activity of the nervous system. — The parts of the book dealing with vitamins, hormones, enzymes and cancerogenic compounds are treated very thoroughly and in great detail.

Despite the inadequacies mentioned above this book has so many definite merits that it may be warmly recommended. The material treated is very extensive and the author has made a consistent effort to cite the sources. The author's good stylistic ability should also contribute to its success.

Nils Löfgren

R. H. F. Manske and H. L. Holmes. *The Alkaloids. Chemistry and Physiology*. Volume I. Academic Press Inc., New York, 1950. viii + 525 pp. $10.00.

This is the first volume of a series of five (according to the preface by the editors) which will gather together "all the

pertinent knowledge of the chemistry and pharmacology of the alkaloids". The pharmacological aspects of the alkaloids, the chemistry of which are so thoroughly described in this volume, is missing. It is therefore to be supposed that one of the following volumes will deal with these aspects of the subject. The physiology of the alkaloids, that is alkaloid formation and metabolism in plants, is dealt with in one of the chapters in the present volume. According to the statement given in the preface the sub-title should include "Pharmacology" instead of "Physiology".

One of the editors, R. H. F. Manske, is the author of the first Chapter: Sources of alkaloids and their isolation (11 pp.). It gives a short introduction to the natural occurrence of alkaloids and their distribution in the plant. It also contains general remarks on isolation, separation, and purification. The second editor, H. L. Holmes, writes about the tropane alkaloids (97 pp.) and the strychnos alkaloids (140 pp.). Both chapters represent excellent reviews of the pure chemistry of these groups of alkaloids. They contain several clearly arranged charts illustrating the relationship of transformation products of the better known substances. The accompanying tables of physical constants for the alkaloids and their transformation products are most valuable. The table for the tropane alkaloids occupies 54 pp., and for the strychnos alkaloids 57 pp. Especially noteworthy is the section on the elucidation of the structure of strychnine and brucine, although the recent contribution of Robinson (1947-1948) to the constitution of strychnine, brucine, and vomicine has not been considered. Generally, the authors of the various chapters have considered the literature up to 1946-1947. The editors, however, have planned to issue supplements at periodic intervals.

W. O. James has contributed with a fine chapter (75 pp.) on the biological aspects of the alkaloids in the plant. He gives an account of the biosynthesis of alkaloids, considering the site of alkaloid formation and the factors affecting it, in addition to the distribution of alkaloids in various physiological parts of the plant and alkaloids in grafts.

L. Marion writes two chapters, one on the comparatively few naturally occurring pyrrolidine alkaloids (17 pp.), and the other on the pyridine alkaloids (105 pp.). The latter compounds are described in the following order: the alkaloids of pepper, the areca nut, trigonelline, pomegranate root bark, *Lobelia*, *Ricinus*, *Leucaena*, poison hemlock, tobacco, and of *Ammodendron*. The chapter constitutes a fine survey on the chemistry of all these compounds and is accompanied by a comprehensive list of references (518 ref.).

Another chapter (59 pp.) by N. J. Leonard deals with the *Senecio* alkaloids. Similar alkaloids in the *Heliotropium*, *Trachelanthus*, and *Trichodesma* genera and in *Crotalaria* plants are also described here. All these compounds, the chemistry of which has been elucidated during the last 20 years, are closely related alkaloids, most of them alkamine esters with only one nitrogen atom.

An author index and a comprehensive subject index make the book easy to use as a "Handbuch".

Altogether this first volume is a valuable contribution to the ever growing literature in the chemical field of natural products. It will be exceedingly helpful to all working with alkaloids, chemists as well as pharmacologists.

Klas-Bertil Augustinsson

ACTA CHEMICA SCANDINAVICA 4 (1950) 1503–1522

On the Complex Formation Between Thorium and Acetylacetone

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It was once believed that complex compounds are formed directly in one step like $Me + mX \rightarrow MeX_m$, where Me is a metal ion and X is an uncharged molecule or a negative ion (ligand). Accordingly no other complexes would be been formed in appreciable amounts.

During the last few decades a number of complex systems have been thoroughly studied; in all cases not only the complex with maximum number m of ligands appears to be formed, but also, to some extent, all intermediate steps MeX_n, where $n = 1, 2, \ldots, m-1$. This is true both for systems with inorganic ions like $Cr^{3+} - SCN^-$ [1] and $Hg^{2+} - Cl^-$ [2], for systems where the ligand is uncharged, like metal-ammine systems [3], and for systems involving organic ions like $Cu^{2+} - CH_3COO^-$ [4].

Organic molecules with two coordinating groups (*e. g.* salicylaldehyde, oxine, cupferron, acetylacetone) combine with metals to form so-called chelates, or inner complexes. One might suspect that these chelates will be formed in the same stepwise manner. Recently Calvin and Wilson [5] have calculated equilibrium constants for the formation of a great number of copper(II) chelates, CuX_2 and the intermediate step CuX^+, in a mixture of dioxane and water; in most cases the accuracy was rather low. However there is still a widespread opinion that in the formation of chelates only the uncharged complex is obtained. Hückel [6], when discussing the formation of acetylacetone chelates, states that "Die Zahl der innerkomplex gebundenen Acetylacetonreste entspricht fast durchweg der Wertigkeit der als Zentralatom fungierenden Metalles, so dass sich die allgemeine Formel für die Acetylacetonate $Me(CH_3COCH : COCH_3)_m$ in der m die Wertigkeit des Metalles bedeutet, aufstellen lässt."

In this paper the results are given of an investigation of the complex formation between Th^{4+} and acetylacetone (HAa). The data could be explained well *only* by assuming that *all* complexes $ThAa^{3+}$, $ThAa_2^{2+}$, $ThAa_3^{+}$ and $ThAa_4$ are formed; with this assumption the mass action constants for the reaction steps could be calculated.

METHOD OF INVESTIGATION

To be able to calculate the constants for a complex equilibrium one has to know the total amount of central atom and ligand and the free concentration of at least one definite species. Sometimes emf measurements have been used to find the concentration of central ion or ligand. This was not possible in the present case, as no reproducible thorium or acetylacetonate electrode had been constructed. Nor could optical methods be used, at least not with visible light, since the thorium-acetylacetone complexes are uncoloured.

In his classical investigation on the complex formation between Hg^{2+} and X^- ($X = Cl^-$, Br^-, I^-) Morse[7] made use of the fact that HgX_2 molecules, but not ions, are soluble in toluene. From the distribution of mercury between toluene and water as a function of $[X^-]$ he could calculate the concentration of HgX^+ and HgX_2.

An analogous method has been used here; the distribution of thorium between benzene and water has been measured as a function of the concentration of free Aa^- in the aqueous phase. The radioactive isotope Th^{234} (UX_1) was used in tracer amounts, never exceeding 10^{-10} mol/l.

By the use of radioactive tracers instead of macro amounts several advantages are gained: a) the ligand concentration can be increased without exceeding the solubility product of the uncharged complex, b) the concentrations of other substances in solution are not affected by the addition of tracer amounts of the central atom; this will simplify the mathematical treatment, and c) in general the radiometric method of analysis is much simpler than other analytical methods. Grahame and Seaborg[8] have shown that the distribution ratio of tracer isotopes between two phases is equal to that for macro amounts of the same element.

Zirconium gives a complex with thenoyltrifluoracetone (TTA) soluble in benzene. Connick and McVey[20] have investigated the distribution of radioactive Zr^{95} between benzene and water, in which some TTA had been dissolved, as a function of the concentration of different organic and inorganic anions. From these measurements they could calculate some complex formation constants. This method is an extension of that used by Morse, and

may in the future be of great value for determining complexes where emf and optical methods fail. However the constants obtained by these authors should not be regarded as definite until some of the assumptions made concerning the system zirconium-TTA-water have proved to be true.

DEPENDENCE OF THE DISTRIBUTION COEFFICIENT ON THE HA*a* CONCENTRATION

Many authors have given general mathematical treatments of the formation of mononuclear [e.g. 3, 9] and polynuclear [e.g. 4, 9, 10] complexes. The discussion will treat only the special case of thorium complexes in aqueous solution.

It is presumed that no ions exist in the benzene phase. In the following the index aq and the charges of ions are omitted for the sake of simplicity (*E. g.* $[ThAa_2]$ means the concentration of the ion $ThAa_2^{2+}$ in the aqueous phase). Since all solutions have an approximately constant ionic strength of 0.01, all activity coefficients are constant, and by using concentrations in the law of mass action, stoichiometric constants are obtained.

In an aqueous solution of thorium and acetylacetone mononuclear complexes $ThAa_n$ and polynuclear complexes Th_mAa_n might occur. Moreover we have products of hydrolysis, which can be written as $Th(OH)_n$, ... $Th_m(OH)_n$, and mixed complexes $Th_mAa_n(OH)_p$. If hydrolysis can be neglected, mixed complexes can probably also be neglected. Indeed, no evidence for their existence was obtained, and no mathematical treatment for mixed complexes will be made here. The following section I will deal with the simplest case, when only mononuclear complexes are formed. In II the general case is treated.

I. Mononuclear complexes are formed

We define the complex product $\varkappa_n$ by

$$\varkappa_n = [ThAa_n]\ [Th]^{-1}\ [Aa]^{-n} \tag{1}$$

If the hydrolysis product, β_n, is defined analogous to the complex product, we have

$$\beta_n = [Th(OH)_n]\ [Th]^{-1}\ [OH]^{-n} \tag{2}$$

The total concentration of thorium in the aqueous phase $[Th]_{aq,\,tot}$ must then be

$$[\mathrm{Th}]_{\mathrm{aq,\,tot}} = [\mathrm{Th}] + \sum_{n=1}^{n=N} [\mathrm{ThAa}_n] + \sum_{n=1}^{n=P} [\mathrm{Th(OH)}_n] \tag{3}$$

ThAa_N and $\mathrm{Th(OH)}_P$ are the complexes with maximum number ligands respectively. Now the measurements are made on the radioactive Th^{234} in solution. The radioactivity in the aqueous phase, I_{aq} disintegrations/minute, under defined conditions must be proportional to $[\mathrm{Th}]_{\mathrm{aq,\,tot}}$:

$$[\mathrm{Th}]_{\mathrm{aq,\,tot}} = \Lambda_{\mathrm{aq}} \cdot I_{\mathrm{aq}} \tag{4a}$$

The only existing form of thorium in the benzene phase at these low concentrations is ThAa_4. At macro concentrations some association between ThAa_4 molecules takes place; the evidence for this is apparent from the studies of Biltz and Clinch [11]. Using the subscript be for the benzene phase, we have

$$[\mathrm{Th}]_{\mathrm{be,\,tot}} = [\mathrm{ThAa}_4]_{\mathrm{be}} \tag{5}$$

If the distribution factor of ThAa_4 is λ_4,

$$\lambda_4 = [\mathrm{ThAa}_4]_{\mathrm{be}}/[\mathrm{ThAa}_4]_{\mathrm{aq}} \tag{6}$$

we get

$$[\mathrm{Th}]_{\mathrm{be,\,tot}} = \lambda_4\ [\mathrm{ThAa}_4]_{\mathrm{aq}} \tag{7}$$

If the measurements on the benzene phase are also made under defined conditions we obtain

$$[\mathrm{Th}]_{\mathrm{be,\,tot}} = \Lambda_{\mathrm{be}} \cdot I_{\mathrm{be}} \tag{4b}$$

Now we may define the apparent distribution coefficient q:

$$q = I_{\mathrm{be}}/I_{\mathrm{aq}} = [\mathrm{Th}]_{\mathrm{be,\,tot}} \cdot \Lambda_{\mathrm{aq}}/[\mathrm{Th}]_{\mathrm{aq,\,tot}} \cdot \Lambda_{\mathrm{be}} \tag{8}$$

We define λ_4' by

$$\lambda_4' = \lambda_4 \cdot \Lambda_{\mathrm{aq}}/\Lambda_{\mathrm{be}} \tag{9}$$

It will be shown later that $\Lambda_{\mathrm{be}}/\Lambda_{\mathrm{aq}}$ is approximately 1 in the present case. With Equations (1) — (9) we get

$$q = \frac{\lambda_4'\ \varkappa_4\ [\mathrm{Aa}]^4}{1 + \sum \varkappa_n\ [\mathrm{Aa}]^n + \sum \beta_n\ [\mathrm{OH}]^n} \tag{10a}$$

This equation shows that different curves $q = f$ [Aa] will be obtained for different pH, if $\sum \beta_n [OH]^n$ cannot be neglected when compared with $1 + \sum \varkappa_n [Aa]^n$. If the hydrolysis is negligible, Equation (10a) will take the form (note that $\varkappa_0 = 1$)

$$q = \frac{\lambda_4' \cdot \varkappa_4 [Aa]^4}{\sum_{n=0}^{n=N} \varkappa_n [Aa]^n} \tag{10b}$$

If we introduce a set of new constants φ_n, where

$$\varphi_{4-n} = [ThAa_n] [ThAa_4]^{-1} [Aa]^{4-n} = \varkappa_n \cdot \varkappa_4^{-1} \tag{11}$$

Equation (10b) can be simplified to

$$q = \lambda_4' (\sum \varphi_{4-n} [Aa]^{n-4})^{-1} \tag{12a}$$

We have $\varphi_0 = \varkappa_0 = 1$. If no negatively charged complexes are formed, then

$$q = \frac{\lambda_4'}{\varphi_4 [Aa]^{-4} + \varphi_3 [Aa]^{-3} + \varphi_2 [Aa]^{-2} + \varphi_1 [Aa]^{-1} + 1} \tag{12b}$$

In case negative complexes $ThAa_5^-$, $ThAa_6^{2-}$, etc., are formed additional terms φ_{-1} [Aa], φ_{-2} $[Aa]^2$, *etc.*, occur in the denominator of Equation (12b).

Acetylacetone is a weak acid with the dissociation constant $K_a = 1.17 \cdot 10^{-9}$ at 25° C[12]. If V_{be}° ml benzene with an acetylacetone concentration of $[HAa]_{be}^{\circ}$ is shaken with V_{aq}° ml water containing no acetylacetone, we find after equilibrium that

$$[HAa]_{aq} = [HAa]_{be}^{\circ} (1 + K_a [H^+]^{-1} + D V_{be}/V_{aq}) \cdot V_{aq}/V_{be} \tag{13}$$

where D is the distribution coefficient of acetylacetone between the two liquids. D is practically constant for pH between 1 and 7, and an ionic strength less than 0.02 mol/l; D was found to be 5.95 [13]. V_{aq} and V_{be} are the equilibrium volumes of the two phases; in this case we have assumed $V_{aq}^{\circ} = V_{aq}$ and $V_{be}^{\circ} = V_{be}$, which is true for low acetylacetone concentrations. If pAa = $-\log[Aa^-]$ and $V_{aq} = V_{be}$ we have

$$pAa = pK_a - pH - \log [HAa]_{be}^{\circ}/(D + 1 + [H^+]^{-1} K_a) \tag{14}$$

For $1 < pH < 7$ we can in the case of acetylacetone neglect $[H^+].K_a$ when compared to $D + 1$. Introducing the values of the constants we get

$$pAa = 9.77 - pH - \log[HAa]^{\circ}_{be} \tag{15}$$

The addition of tracer amounts of thorium to the acetylacetone solution will bind some Aa^-, but this amount is negligible when compared to the amount of acetylacetone in the two phases.

II. Polynuclear complexes are formed

If complexes with two or more central atoms are formed the complex product must be defined by

$$\varkappa_{m,n} = [Th_mAa_n]\ [Th]^{-m}[Aa]^{-n} \tag{16}$$

in which $\varkappa_{1,n} = \varkappa_n$ from Equation (1). For the analogous hydrolysis constants $\beta_{m,n}$ can be defined. Since Equations (4) — (9) are still valid, we get

$$q = \frac{\lambda'_4 \varkappa_4 [Aa]^4}{1 + \sum\sum m\beta_{m,n} [Th]^{m-1} [OH]^n + \sum\sum m \varkappa_{m,n} [Th]^{m-1} [Aa]^n} \tag{17}$$

In this equation the apparent distribution coefficient q is a function of not only pH and [Aa] but also of the thorium concentration [Th]. It would be a difficult task to determine the constants $\varkappa_{m,n}$ and $\beta_{m,n}$ in this general case; however under the experimental conditions of the present work, case I seems to be realised, as might have been expected because of the very low thorium concentration.

EXPERIMENTAL

Chemicals used

Except for thorium and acetylacetone all chemicals employed were of analytical grade and were used without further purification or analysis.

Pure acetylacetone was prepared according to a method described elsewhere [13]. It was kept in benzene solution.

The thorium isotope Th^{234} (UX_1) is a natural disintegration product of U^{238} (UI), the most common uranium isotope. The uranium now produced for use in reactors contains no other beta active thorium isotope than Th^{234} and Th^{231} (UY), which is a disintegration product of U^{235}, and disintegrates by soft β-emission. When radioactive equilibrium exists between UI and UX_1, 1 mg of uranium will give 738 beta particles per minute from UX_1. The energy of these beta particles is too low (0.12 MeV) to be mea-

sured by common glass-wall GM-counters. However the next member in the disintegration series UX_2 (Pa^{234}) has a beta energy of 2.32 MeV, and this high energy makes it easily detectable. Radioactive equilibrium between UX_1 and UX_2 will be practically reached within 10 minutes; after this time the radioactivity of UX_2 indicates where UX_1 is to be found. The half-life of UX_1 is 24.1 days [21].

Dyrssen [14] has given a fairly simple method for preparing carrier-free UX_1. A few grams of uranyl nitrate are shaken with some 10 ml of ether, previously saturated with water. In the small aqueous layer formed, practically all UX_1 is to be found. This aquous phase is diluted with a few ml of 0.1 *M* HCl and run through ani on exchanger column (Wofatit KS): Th^{4+} (UX_1) and UO_2^{2+} are adsorbed. The uranyl ions are washed out with 2 *M* HCl. UX_1 is desorbed by 0.5 *M* oxalic acid, and the eluate is evaporated. The oxalic acid then sublimes, leaving carrier free UX_1, which is dissolved in a small amount of strong $HClO_4$.

Distribution experiments

The two phases were prepared in a 60 ml separatory funnel in the following way: To 0.03 ml of UX_1 solution, calculated amounts of water, $HClO_4$ and NaOH were added so as to give 15 ml solution of ionic strength 0.01 and the acidity desired. Finally 15 ml of benzene of a known acetylacetone concentration, $[HAa]^\circ_{be}$, was added.

The separatory funnels were sealed with glass stoppers and shaken for about 20 hours at 25.0 ± 0.2° C. The loss of solution due to possible leakage was less than 0.1 %. The phases were allowed to separate in the thermostat, definite volumes of both phases were taken out, and their radioactivity I_{aq} and I_{be} was measured in a reproducible manner (see next section).

The pH of the aqueous phase was measured with a glass electrode and a standard pH-meter; the accuracy was ± 0.02 pH-units. From the amount of $HClO_4$ and NaOH added, and $[HAa]^\circ_{be}$, the pH could be roughly predicted for pH $\leq$ 4, but not for higher pH. No buffer solutions were added since these might give complexes with Th^{4+} and thus compete with the acetylacetone; no complexes are formed between sodium and acetylacetone [15, 16].

The radioactive measurements

The radioactivity of UX_1 (or actually UX_2) could be measured either in the solution directly or after evaporation to dryness. The first method was found to be the most convenient.

The radioactive liquid to be measured is contained in a special "immersion cup". The arrangement with the GM-tube (Type B6, 20th Century Electronics, *London;* wall thickness about 30 mg/cm^2) is shown in Fig. 1. When inserting the GM-tube in the immersion cup, liquid is pressed up and surrounds the sensitive part of the counter tube. The GM-tube is well centered in the cup, but separate experiments have shown that this is not too important. This is also to be expected since the mean range of beta particles from UX_2 is 3 mm in water, while the thickness of the liquid layer is about 1 mm.

On each cup a line has been etched. When liquid is filled to this line and the GM-tube is inserted, the liquid surface will reach the same height for different cups. As these cups are not exactly of the same internal diameter (± 0.2 mm) different cups will contain different volumes of liquid. In the experiment recorded in Fig. 2, different volumes of a solution of constant specific radioactivity were brought into a certain immersion cup, and the number of counts per minute, *I*, measured. On this cup the line was etched

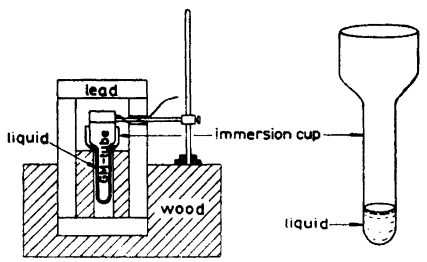

Fig. 1. The experimental arrangement with the GM-tube inserted in the immersion cup.

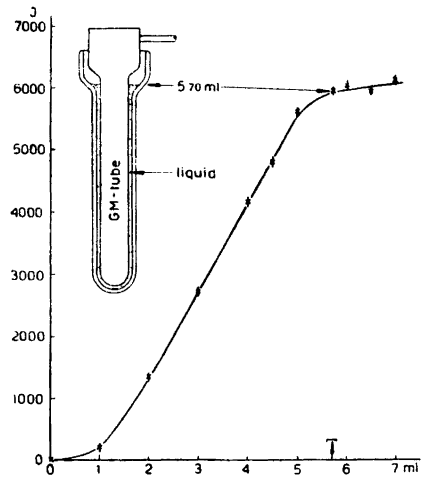

Fig. 2. The variation of radioactivity measured (I counts/minute) as a function of the volume in ml of an UX_1-solution of constant specific activity.

at 5.7 ml, where a variation of $\pm$ 0.1 ml will change I by less than 1 %. Due to the different volumes of liquid in the cups these have been calibrated against each other with a standard UX_1-solution; this introduces the calibration factor, ψ. The ψ-scale was arbitrarily chosen by making $\psi = 1$ for one cup, the "standard immersion cup".

Before the measurements on UX_1 the cup is filled with pure water and the zero effect I_0 is determined. If the following measurement on the aqueous UX_1-solution gives I_m, we have the corrected activity I_{aq}:

$$I_{aq} = \psi \ (I_m - I_0) \tag{18}$$

For the activity in the benzene phase an analogous equation is obtained; in this case the zero effect is taken on pure benzene. The radioactivity adsorbed on the glass wall of the GM-tube is desorbed by washing with H_2SO_4-$K_2Cr_2O_7$.

The deviations given in Table 1 are standard errors (the square root ot the total number of counts divided by the time of measurement). Only in one case the activity was so high, that corrections for the resolving time of the counter had to be made.

To determine the efficiency of the counting arrangement an immersion cup was filled with a known amount of uranyl nitrate solution, and the radioactivity was measured; this gave 131 $\pm$ 1 counts/min. mg U. It is known that 1 mg U in equilibrium with UX_1 and UX_2 gives 738 beta UX_2/min. From these two values we calculate that 131/738 = 17.8 % of all betas from UX_2 in the solution are counted by the electronic circuit.

From 1 g uranium, which was completely freed from thorium one year ago, there will now be about $7.2 \cdot 10^{-13}$ mol Th^{230} (Io) + Th^{234}. This amount of Th will give approximately $7.4 \cdot 10^5$ β disintegrations/minute. We can now find for the standard immersion

cup, which has a $\psi = 1$ and a volume of 5.20 ml, that 1 count/min corresponds to $7.2 \cdot 10^{-13}/7.4 \cdot 10^{5} \cdot 5.20 \cdot 10^{-3} \cdot 0.178 = 1.05 \cdot 10^{-15}$ mol Th/l. According to Equation (4a) this value must be A_{aq}; thus we have

$$[\mathrm{Th}]_{\mathrm{aq,\ tot}} = 1.05 \cdot 10^{-15} \cdot I_{aq} \qquad \mathrm{mol/l} \tag{19}$$

The absorption of beta particles varies with the density of the absorber *e.g.* [17]. The exponential relationship $I = I_0 \cdot e^{-ux}$, where x is the thickness in cm of the absorbing layer and u is the absorption coefficient, is roughly valid for beta particles. The mass absorption coefficient u/d, where d is the density, is nearly independent of the atomic number of the absorber. If E_m is the maximum energy of the betas, one can use the empirical relationship $E_m^{1.33} \cdot u = 22 \cdot d$ to find u. For $E_m = 2.32$ MeV and $d_{be} = 0.897$ we can calculate $I/I_0 = 0.697$ for a water layer of 0.5 mm, and 0.729 for a benzene layer of the same thickness. This means that the absorption of beta particles from UX_2 in 0.5 mm of water is 0.729/0.697 times that in benzene. If the experimental conditions are the same for the measurements on the benzene solution to those for the aqueous solution, we have $A_{be} = A_{aq} \cdot 0.697/0.729$, or $A_{be} = 1.00 \cdot 10^{-15}$ mol. min/l · counts. Thus we have

$$[\mathrm{Th}]_{\mathrm{be,\ tot}} = 1.00 \cdot 10^{-15} \cdot I_{be} \qquad \mathrm{mol/l} \tag{20}$$

ANALYSIS OF THE DISTRIBUTION CURVE

In Table 1 the experimental data ($[\mathrm{HAa}]^{\circ}_{be}$, pH, I_{be}, I_{aq}) are tabulated. From pH and $[\mathrm{HAa}]^{\circ}_{be}$ we can calculate $[\mathrm{HAa}]_{aq}$ and pAa by means of Equation (15). With Equation (8) I_{be} and I_{aq} gives q. The symbols in the last column of Table 1 are the same as shown in Fig. 3, where log q is given as a function of pAa (this curve will be referred to as the "distribution curve"). Table 1 is divided in five sections, each representing a definite pH-range.

In Fig. 3 almost all points are on one continuous curve. For the five symbols ♯ and ▲ the concentration of acetylacetone in the benzene phase is 5-molar; *i. e.* the benzene solution consists of 50 % acetylacetone. The q measured for these points is not the distribution of UX_1 between benzene and water, but the distribution between 50 % benzene + 50 % acetylacetone and water. Thus the low values of q obtained here are either due to that ThAa_4 is more soluble in benzene than in acetylacetone, which seems improbable, or that ThAa_4 is less soluble in pure water than in water containing much acetylacetone. These five q values have not been used in the calculation of the complex constants.

In the deduction of Equation (10a) it was pointed out that if the thorium hydrolysis influences the equilibrium between thorium and acetylacetone, different curves $q = f[\mathrm{Aa}]$ should be obtained for different pH. At pH > 3 the thorium hydrolysis is considerable[18]. As the pH was widely different

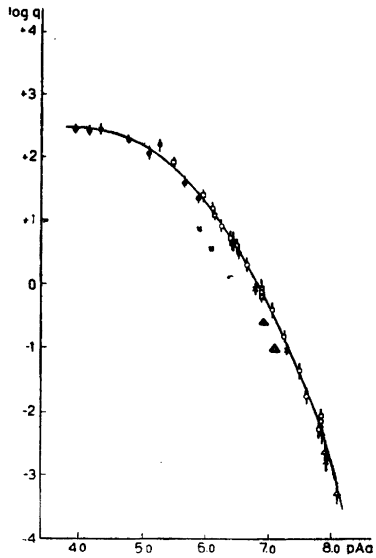

Fig. 3. The distribution of UX_1 between benzene and water (q) as a function of the concentration of acetylacetonate ions ($pAa = -\log[Aa^-]$). The different signs relate to pH-values according to Table 1 : △ $pH \approx 2$; × $pH \approx 3$; ○ $pH \approx 4$; □ $pH \approx 5$; ● $pH \approx 6$. The curve was drawn to fit the experimental points; it was used for calculating $\varkappa_n$ by method A.

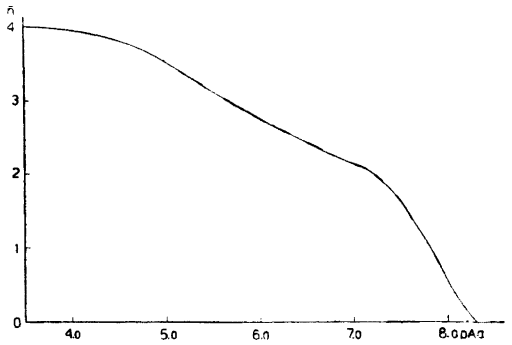

Fig. 4. The average number of ligands per central atom ($\bar{n}$) as a function of the concentration of free ligand ($pAa = -\log[Aa^-]$), calculated from the smooth curve in Figure 3.

in the five series of measurement (see Table 1) the curvature in Fig. 3 can not be due to the thorium hydrolysis, but solely to the complex formation between thorium and acetylacetone.

It is shown in Equation (17) that at constant pH and pAa, q will be a function of [Th] if polynuclear complexes are formed. A separate investigation at pH 3 and pAa 6.9 showed that a variation of I_{aq} from 10 to over 1,000 counts/min had no influence on q, which was about 1. It is thus concluded that no polynuclear complexes are formed at these low concentrations.

THE CALCULATION OF COMPLEX CONSTANTS FROM THE DISTRIBUTION CURVE

It is clear from the considerations in the preceeding section that hydrolysis and polynuclear complexes can be neglected; then Equation (10b) is valid. There are many ways to evaluate the constants in this equation; here two independent methods will be used.

A. The ligand number $\bar{n}$ is generally defined as the mean number of ligands per central atom. In our case we have

$$\bar{n} = \sum n[\mathrm{ThAa}_n]_{\mathrm{aq}}/[\mathrm{Th}]_{\mathrm{aq,\ tot}} \tag{21}$$

The fraction of Th present in form of the n^{th} complex is

$$\alpha_n = [\mathrm{ThAa}_n]_{\mathrm{aq}}/[\mathrm{Th}]_{\mathrm{aq,\ tot}} \tag{22}$$

Comparing this with Equations (6) — (9) we find that

$$q = \alpha_4 \cdot \lambda_4' \tag{23}$$

From Equations (21) and (22) we can deduce

$$\bar{n} = n + d(\log\ \alpha_n)/d(\mathrm{pAa}) \tag{24}$$

which gives together with (23)

$$\bar{n} = 4 + d(\log\ q)/(d\mathrm{pAa}) \tag{25}$$

Fig. 3 shows log q as a function of pAa. By graphical derivation of this curve using Equation (25) we can obtain $\bar{n} = f$ (pAa), the complex formation curve, which is shown in Fig. 4. We transform Equation (21) to a form more suitable for mathematical treatment [3]:

$$\begin{aligned}\bar{n} + (\bar{n} - 1)\,\varkappa_1\,[\mathrm{Aa}] + (\bar{n} - 2)\,\varkappa_2\,[\mathrm{Aa}]^2 + (\bar{n} - 3)\,\varkappa_3\,[\mathrm{Aa}]^3 + \\ + (\bar{n} - 4)\,\varkappa_4\,[\mathrm{Aa}]^4 = 0\end{aligned} \tag{26}$$

Each point on the curve in Fig. 4 will give an equation of this kind. Four points on the curve will suffice for the determination of all the constants; we take $\bar{n} = 0.5$, 1.5, 2.5 and 3.5. The solution of the set of equations thus obtained is achieved by using determinants. From these values of $\varkappa_n$ we use Equation (11) to calculate φ_n, and thus obtain the following values:

$$\varphi_1 = 1.13 \cdot 10^{-5},\quad \varphi_2 = 4.38 \cdot 10^{-12},\quad \varphi_3 = 1.63 \cdot 10^{-19},\quad \varphi_4 = 1{\cdot}15 \cdot 10^{-27}.$$

The values $\bar{n} = n - 0.5$ are taken because at each of these points two complexes dominate, and the errors from the presence of the other complexes are negligible. Consequently we find

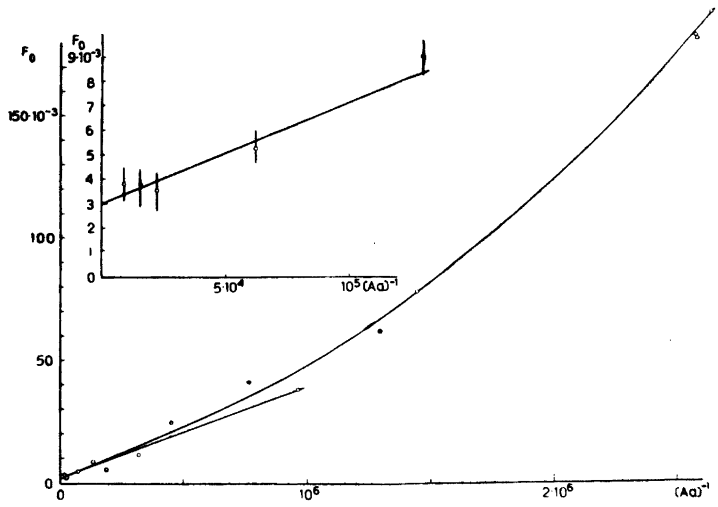

Fig. 5. The variation of $F_0 = 1/q$ with $[Aa^-]^{-1}$ calculated from the experimental points in Figure 3. The intercept on the F_0-axis gives $1/\lambda'_4$ and the slope of the curve at this point is φ_1/γ'_4.

$$[\mathrm{Aa}]^{-1}_{\bar{n}\,=\,n\,-\,0.5} \approx \varkappa_n \varkappa^-_{n-1} = k_n \tag{27}$$

where k_n is called the complex formation constant:

$$k_n = [\mathrm{ThAa}_n]\ [\mathrm{ThAa}_{n-1}]^{-1}\ [\mathrm{Aa}]^{-1} \tag{28}$$

B. Before proceeding to calculate the complex formation constants, we will see what values of φ_n will be obtained by the second method, which is analogous to that used by Leden [9]; with this method the influence of the experimental errors is more clearly seen, and the uncertain procedure of taking derivatives on a smoothed curve is avoided.

Equation (12b) is transformed to

$$F_0 = 1/q = (1 + \varphi_1\,[\mathrm{Aa}]^{-1} + \varphi_2\,[\mathrm{Aa}]^{-2} + \varphi_3\,[\mathrm{Aa}]^{-3} + \varphi_4\,[\mathrm{Aa}]^{-4})\,\lambda'^{-1}_4 \tag{29}$$

In Table 2 F_0 and $[\mathrm{Aa}]^{-1}$ are calculated from the data in Table 1 (not from the smooth curve in Fig. 3). In Fig. 5 F_0 is given as a function of $[\mathrm{Aa}]^{-1}$. The intercept on the F_0-axis gives $1/\lambda'_4$ and the slope of the curve at this point gives φ_1/λ'_4 according to Equation (29). We then get $\lambda'_4 = 330 \pm 30$ and $\varphi_1 = (1.2 \pm 0.2)10^{-5}$.

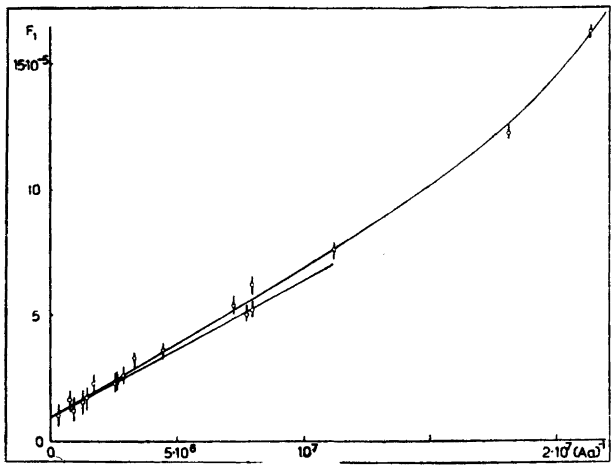

Fig. 6. F_1 calculated according to Equation (30) as a function of $[Aa^-]^{-1}$. The intercept on the F_1-axis gives φ_1 and the slope of the curve at this point φ_2.

In an analogous manner we derive functions F_1, F_2, *etc.*,

$$F_1 = \left(\frac{\lambda_4'}{q} - 1\right)[\mathrm{Aa}] = \varphi_1 + \varphi_2\,[\mathrm{Aa}]^{-1} + \varphi_3\,[\mathrm{Aa}]^{-2} + \varphi_4\,[\mathrm{Aa}]^{-3} \qquad (30)$$

$$F_2 = (F_1 - \varphi_1)\,[\mathrm{Aa}] = \varphi_2 + \varphi_3\,[\mathrm{Aa}]^{-1} + \varphi_4\,[\mathrm{Aa}]^{-2} \qquad (31)$$

The values of F_1 and F_2 are found in Table 2. In Figs. 6 and 7 they are given as functions of $[\mathrm{Aa}]^{-1}$. From Fig. 6 we obtain $\varphi_1 = (1.0 \pm 0.1)\,10^{-5}$ and $\varphi_2 = (5.4 \pm 0.3)\,10^{-12}$. This φ_1 is somewhat lower than the φ_1 obtained from Fig. 5, but as it is more accurate it has been used for the calculation of F_2. From Fig. 7 we have $\varphi_2 = (5 \pm 1)\,10^{-12}$; no calculation of F_3 has been done because the errors will be to great. As the value of φ_2 from Fig. 6 is more exact than that from Fig. 7, the value $\varphi_2 = (5.4 \pm 0.3)\,10^{-12}$ has been accepted.

As the errors in the calculation of F_2 are considerable, no attempt to find F_3 and F_4 has been done. To obtain φ_3 and φ_4 we deduce

$$F_5 = \left(\frac{\lambda_4'}{q} - 1\right)[\mathrm{Aa}]^4 = \varphi_4 + \varphi_3\,[\mathrm{Aa}] + \varphi_2\,[\mathrm{Aa}]^2 + \varphi_1\,[\mathrm{Aa}]^3 \qquad (32)$$

F_5 is calculated in Table 2 and given as a function of [Aa] in Fig. 8. From this curve we find $\varphi_4 = (1.5 \pm 0.5)\,10^{-27}$ and $\varphi_3 = (1.0 \pm 0.2)\,10^{-19}$.

Table 1. Calculation of the distribution curve from experimental data.

pH	$[HAa]^0_{be}$	$[HAa]_{aq}$	pAa	I_{be}	I_{aq}	q	log q	
1.90	0.605	0.087	8.10	10 ± 1	18200	0.00055	− 3.26	△
1.98	6.73	0.967	6.97	282	1179	0.240	− 0.62	▲
1.98	5.38	0.775	7.07	81	826	0.098	− 1.01	▲
2.00	0.865	0.124	7.85	6.7 ± 3	1510	0.0044	− 2.30	△
1.94	0.865	0.124	7.89	2.7 ± 1	1106	0.0024	− 2.70	△
3.06	6.73	0.967	5.89	1030	143	7.20	+ 0.86	⧓
2.88	6.73	0.967	6.07	1290	359	3.59	+ 0.56	⧓
2.66	5.38	0.775	6.39	750	595	1.26	+ 0.10	⧓
2.99	0.865	0.124	6.86	429	511	0.84	− 0.08	×
2.98	0.288	0.0415	7.33	81 ± 5	856	0.095	− 1.02	×
3.20	0.0450	0.0065	7.93	1.6 ± 1.2	974	0.0016	− 2.70	×
3.74	0.865	0.124	6.11	1238	78 ± 1	16.0	+ 1.20	○
3.72	0.806	0.116	6.16	1253	99 ± 8	12.7	+ 1.10	○
3.90	0.450	0.0645	6.23	474	59 ± 3	8.03	+ 0.90	○
4.02	0.225	0.0323	6.41	468	86	5.45	+ 0.74	○
3.85	0.323	0.0465	6.42	1047	203	5.16	+ 0.71	○
3.86	0.288	0.0465	6.46	996	223	4.33	+ 0.64	○
4.00	0.134	0.0193	6.65	1120	560	2.00	+ 0.30	○
4.05	0.0673	0.0097	6.90	339	430	0.79	− 0.10	○
3.90	0.0673	0.0097	7.05	290	752	0.386	− 0.41	○
3.75	0.0578	0.0083	7.26	92 ± 5	617	0.149	− 0.82	○
3.93	0.0225	0.00323	7.50	35 ± 2	814	0.043	− 1.37	○
3.70	0.0278	0.00397	7.63	11 ± 3	635	0.0173	− 1.76	○
4.00	0.0090	0.00129	7.83	4.3 ± 2.0	867	0.0050	− 2.30	○
3.88	0.0092	0.00133	7.85	4.0 ± 2.0	471	0.0084	− 2.10	○
3.98	0.0090	0.00129	7.85	5.7 ± 2.0	791	0.0072	− 2.15	○
4.93	0.225	0.0323	5.50	1100	14 ± 2	79.0	+ 1.90	□
4.97	0.0673	0.0097	5.98	790	31 ± 2	25.7	+ 1.41	□
4.54	0.0673	0.0097	6.41	668	120	5.57	+ 0.75	□
4.54	0.0225	0.00323	6.89	282	333	0.848	− 0.07	□
4.75	0.0134	0.00194	6.90	228	342	0.667	− 0.18	□
5.92	0.865	0.124	3.93	2210	8.5 ± 0.9	260	+ 2.42	●
5.69	0.806	0.116	4.18	1432	5.4 ± 2.0	265	+ 2.42	●
6.62	0.0673	0.0097	4.33	915	3.2 ± 1.8	285	+ 2.45	●
6.64	0.0225	0.00323	4.79	689	3.6 ± 1.5	190	+ 2.28	●
5.84	0.0673	0.0097	5.11	720	6.7 ± 3.0	110	+ 2.04	●
5.68	0.0673	0.0097	5.27	1190	7.2 ± 2	165	+ 2.18	●
6.00	0.0134	0.00193	5.65	247	6.4 ± 2	38.6	+ 1.59	●
5.55	0.0225	0.00323	5.88	562	23 ± 2	24.4	+ 1.38	●
5.61	0.0045	0.00065	6.52	720	241	3.0	+ 0.48	●

Table 2. Calculation of F_n from the data in Table 1 according to Equations (29) – (32). In Figures 5 to 8 these F_n are used for the determination of φ_n.

[Aa]	1/[Aa]	F_0	F_1	F_2	F_5
$11.8 \cdot 10^{-5}$	$8.5 \cdot 10^{3}$	0.00385			
6.62	15.1	0.00377			
4.67	21.4	0.00351			
1.62	61.6	0.00526			
$78.0 \cdot 10^{-7}$	$1.29 \cdot 10^{5}$	0.00909			
53.8	1.86	0.00606			
31.6	3.16	0.01266	$10.04 \cdot 10^{-6}$		
22.4	4.47	0.02564	16.71		
13.2	7.59	0.04167	16.83		
10.46	9.56	0.03846	12.23	$2.33 \cdot 10^{-12}$	
7.75	12.9	0.06250	15.21	4.04	
6.94	14.4	0.07874	17.34	5.09	
5.88	17.0	0.1245	23.6	8.00	
3.89	25.7	0.1828	23.1	5.10	
3.89	25.7	0.1835	23.2	5.13	
3.80	26.3	0.1938	23.9	5.28	
3.47	28.8	0.2309	26.1	5.59	
3.02	33.1	0.333	32.9	6.92	
2.24	44.6	0.500	36.7	5.98	$41.2 \cdot 10^{-26}$
1.38	72.4	1.190	54.1	6.09	14.3
$12.89 \cdot 10^{-8}$	77.5	1.179	50.0	5.16	10.7
12.59	79.4	1.250	51.9	5.28	10.34
12.59	79.4	1.50	62.2	6.57	12.4
8.93	$1.12 \cdot 10^{7}$	2.59	76.3	5.92	5.39
5.49	1.82	6.71	$1.215 \cdot 10^{-4}$	6.12	2.02
4.67	2.14	10.5	1.616	6.61	1.65
3.16	3.16	23.3	2.43	7.36	0.77 ± 0.05
2.34	4.27	57.8	4.46	10.20	0.58 ± 0.14
1.48	6.76	200	9.77	14.31	0.32 ± 0.15
1.41	7.07	227	10.56	14.75	0.30 ± 0.10
1.41	7.07	118	5.48	7.59	0.16 ± 0.06
1.41	7.07	140	6.51	9.04	0.18 ± 0.07
1.29	7.75	417	17.75	22.77	0.38 ± 0.12
1.27	7.85	625	26.20	33.1	0.54 ± 0.40
0.79	12.6	1820	47.4	37.4	0.24 ± 0.02

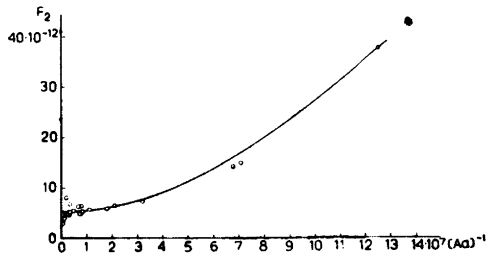

Fig. 7. F_2 calculated according to Equation (310 as a function of $[Aa^-]^{1-}$. The intercept on the F_2-axis gives an approximate value of φ_2; the scattering of the points is to great to make any calculation of φ_3 possible from the slope of the line at $F_2 = O$.

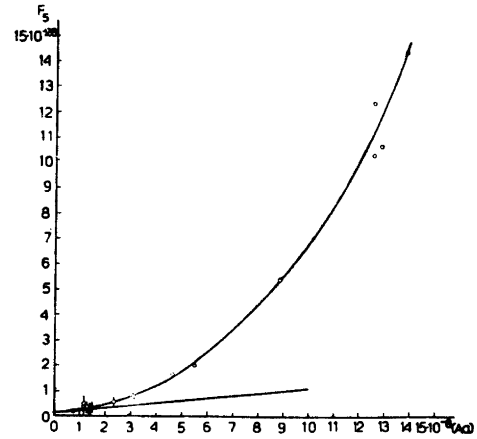

Fig. 8. F_5 calculated with Equation (32) as a function of $[Aa^-]$. The intercept on the F_5-axis gives φ_4 and the slope of the line φ_3. The values of F_5 are given in Table 2.

Table 3. Comparision of φ_n obtained by method A and B.

	Method A	Method B
φ_1	$1.13 \cdot 10^{-5}$	$(1.0 \pm 0.1)\ 10^{-5}$
φ_2	$4.38 \cdot 10^{-12}$	$(5.4 \pm 0.3)\ 10^{-12}$
φ_3	$1.63 \cdot 10^{-19}$	$(1.0 \pm 0.2)\ 10^{-19}$
φ_4	$1.15 \cdot 10^{-27}$	$(1.5 \pm 0.5)\ 10^{-27}$

In Table 3 the values of φ obtained by the two methods are compared. These values have been used in Equation (12b) to calculate q as a function of [Aa]. The two resultant curves, q_A and q_B, are shown in Fig. 9, where the symbols are taken from Table 1. The difference between the two curves is very small; the closest agreement to the measured points is given by q_B. With the values from Method B k_n is calculated:

$$k_1 = (0.7 \pm 0.3)\ 10^{8} \quad \text{1/mol}$$
$$k_2 = (5.4 \pm 1.7)\ 10^{7} \quad \text{»}$$
$$k_3 = (1.9 \pm 0.3)\ 10^{6} \quad \text{»}$$
$$k_4 = (1.0 \pm 0.1)\ 10^{5} \quad \text{»}$$

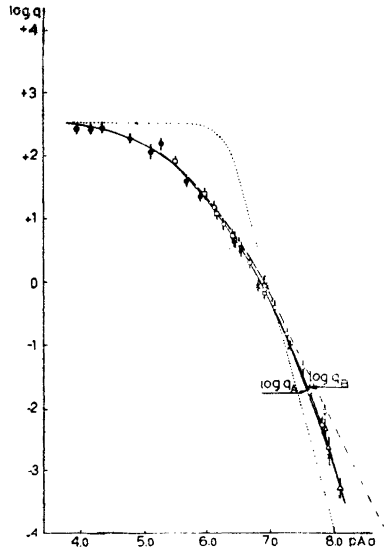

Fig. 9. Log q is calculated as a function of pAa under the following assumptions: The only thorium species existing in the aqueous phase are a/ Th^{4+} and $ThAa_4$ (. . . . .); b/ $ThAa_4$, $ThAa_3^+$, $ThAa_2^{2+}$ (— — — —); c/ all species $ThAa_n^{4-n}$ for $0 \leq n \leq 4$ (————). The experimental points are the same as in Figure 3.

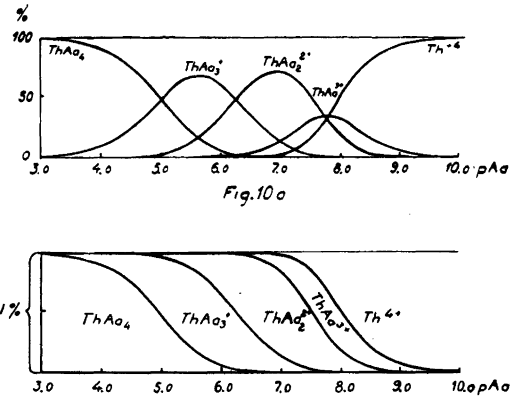

Fig. 10 a and Fig. 10 b. The percentage of the different complexes as a function of the concentration of acetylacetonate ions ($pAa = -\log [Aa^-]$) in two different graphical representations: in a/ the percentage of a complex for a certain pAa is the height of the curve over the zero line; in b/ the percentage is the length of the vertical line at this pAa which falls in the area of that complex.

Equation (22) is transformed to

$$\alpha_n = \frac{\varphi_{4-n}\,[\mathrm{Aa}]^{n-4}}{\varphi_4\,[\mathrm{Aa}]^{-4} + \varphi_3\,[\mathrm{Aa}]^{-3} + \varphi_2\,[\mathrm{Aa}]^{-2} + \varphi_1\,[\mathrm{Aa}]^{-1} + 1} \tag{33}$$

α_n is calculated with φ from method B. Fig. 10 a gives directly the percentage of the n^{th} complex ($= 100\alpha_n$) at a certain pAa. The same result is given in an other graphical representation in Fig. 10 b; the percentage of a complex is here given by the height of the vertical line which falls in the region of that complex for a certain pAa. Thus the areas in Fig. 10 b gives the ranges of existence of the individual complexes.

The true distribution factor of $ThAa_4$ between benzene and water is given by Equation (9), where $\Lambda_{be}/\Lambda_{aq} = 0.96$ and $\lambda'_\zeta = 330 \pm 30$. We then have $\lambda_4 = 315 \pm 30$. This may be compared with the value of 80 for the distri-

bution of $PuAa_4$ between benzene and water, found by Harvey *et al.*[19] However they report no data on the concentration of acetylacetone.

DISCUSSION

In the calculations made here it has been presumed from the beginning that all complexes $ThAa_n^{4-n}$ should exist. Attempts have been made to find if any complexes could be eliminated.

If we assume that the equilibrium between thorium and acetylacetone in water is completely described by

$$Th^{4+} + 4\,Aa^- \rightleftharpoons ThAa_4$$

we can deduce the equation

$$\left(\frac{\lambda_4'}{q} - 1\right)[Aa]^4 = \varkappa_4^{-1} \tag{34}$$

Comparing Equations (32) and (34) it is seen that $F_5 = \varkappa_4^{-1}$, if Equation (34) is valid. In Table 2 the values for F_5 are given; as no constant value is obtained, it may be concluded that the assumption for the deduction of Equation (34) is certainly wrong. The dotted line in Fig. 9 gives q as a function of [Aa] according to Equation (34); $\varkappa_4^{-1}$ was chosen $3.3 \cdot 10^{-26}$. If the $\varkappa_4^{-1}$ value is varied the curve will be displaced along the pAa-axis retaining its form constant. It is easily seen that a good agreement with the measured q cannot be obtained.

If we assume that [Th] = [ThAa] = 0, we can obtain a curve, which fits the upper part of the experimental distribution curve very well; this gives the dashed line in Fig. 9. However the agreement with the experimental points is bad for $\log q < 0$. Thus it may be concluded that all species Th^{4+}, $ThAa^{3+}$, $ThAa_2^{2+}$, $ThAa_3^+$ and $ThAa_4$ exist in the aqueous solution.

If negatively charged complexes like $ThAa_5^-$, $ThAa_6^{2-}$, *etc.*, were formed, q should reach a maximum at a certain pAa, and then decrease for higher concentrations of Aa^-. From Fig. 3 it is seen that q approaches to a maximum. It has not been possible to make measurements on higher [Aa] than $\approx 10^{-4}$, and negative complexes may exist at still higher [Aa]. It is unlikely on steric grounds, however, that negative complexes are formed. The present results give no evidence of higher complexes than $ThAa_4$.

SUMMARY

The complexes of Th^{4+} and acetylacetone (HAa) in aqueous solution have been investigated by measuring the distribution of tracer amounts of Th^{234} (UX_1) between benzene and aqueous solutions of various [Aa^-]. The measurements have been performed at 25° C and at an approximately constant ionic strength of 0.01 mol/l, which was brought about by adding $HClO_4$, and NaOH. The hydrolysis of Th^{4+} was found to be negligible, and no polynuclear complexes seemed to be formed at these low concentrations. The results could be explained only by assuming that all complexes $ThAa^{3+}$, $ThAa_2^{2+}$, $ThAa_3^+$ and $ThAa_4$ exist. The constants for the stepwise addition of Aa^- to Th^{4+} (k_1, k_2, ...) and those for the over all addition ($\bar{\varkappa}_1$, $\varkappa_2$, . . .) were calculated by two independent methods, and consistent results were obtained;

$$k_1 = (0.7 \pm 0.3)\ 10^8 \qquad \varkappa_1 = 0.7 \cdot 10^8$$
$$k_2 = (5.4 \pm 1.7)\ 10^7 \qquad \varkappa_2 = 3.8 \cdot 10^{15}$$
$$k_3 = (1.9 \pm 0.3)\ 10^6 \qquad \varkappa_3 = 7.2 \cdot 10^{21}$$
$$k_4 = (1.0 \pm 0.1)\ 10^5 \qquad \varkappa_4 = 7.2 \cdot 10^{26}$$

The distribution constant of $ThAa_4$ between benzene and water was found to be $\lambda_4 = 315 \pm 30$.

I wish to thank the head of FOA 1, Professor Gustav Ljunggren, for helping me in many ways, and Professor Arne Ölander for the interest he has shown in this work. I am greatly indebted to Professor Lars Gunnar Sillén for the many valuable suggestions, which have proved so helpful to me. With my friends Laborator Roland Rynninger and Fil.mag. David Dyrssen I have had many fruitful discussions.

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Received September 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1523–1537

Aromatic Keto- and Hydroxy-polyethers as Lignin Models. III *

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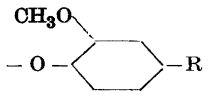
A large number of methods have been employed for the direct oxidation of lignin, most of which, however, give poor yields of identifiable degradation products of any importance in relation to the structure of lignin.

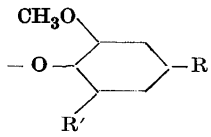
About ten years ago Freudenberg *et al.* [1, 2] very successfully applied the method of oxidation with nitrobenzene and alkali to spruce lignin. With this lignin, *in situ* or in the form of "isolated lignin", for example lignin sulphonic acids, fairly high yields of vanillin and related compounds were obtained. In the following years this method was extensively used by v. Wacek and Kratzl [3–7] in their studies on the oxidation of various synthetic lignin models, mainly of the phenyl propane type. They investigated the influence of substituents on the course of the reaction and found that most phenyl propanes of type I furnished relatively large yields of identifiable oxidation products, especially if the side chain (R) carries several oxygen atoms or a double bond.

Much evidence can be brought forward in favour of the view that spruce lignin contains elements of types I—III [8, 9] **.

Consequently it was of interest to study the oxidative degradation under comparable conditions of synthetic mononuclear compounds possessing these



I



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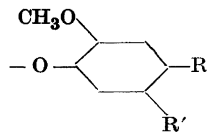


III

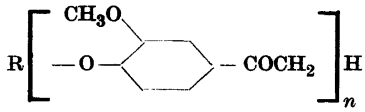
* Part II. *Acta Chem. Scand.* 3 (1949) 1358.

** Note added in proof: Richtzenhain [30] has recently shown that, in all probability, structures of type III do not exist as such in spruce lignin, but are formed during the isolation of the material.

general structures. (Tables 2 and 3.) Many of these compounds have been oxidized by v. Wacek and Kratzl and other workers. They, however, employed conditions not comparable to those chosen in this work.

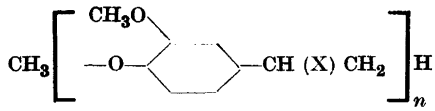
Special interest is attached to the degradation of synthetic chain molecules containing only "open elements" (I) or a combination of "open" and "condensed" (II or III) elements. This makes it possible to investigate whether an appreciable change in the yields of oxidation products is caused by the combination of the different elements into larger molecules. Furthermore, by comparing the yield of, for example, vanillin from lignin and from such model compounds one might be able to obtain valuable information regarding the relative frequency of open and condensed elements in lignin.

In Part II two series of compounds (V and VI), containing only elements of type I, were described.



V

R = H or CH_3
n = 2, 3 or 4

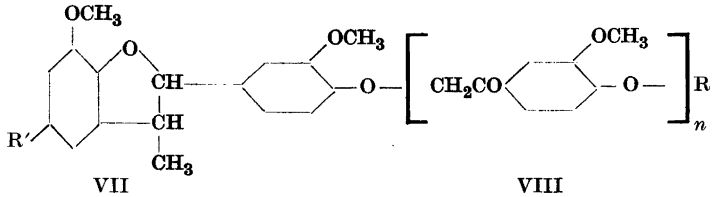


VI

X = OH or $SO_3\frac{Ba}{2}$
n = 2, 3 or 4

As reported in Part II, the compounds V ($R = COC_6H_5$) could not be properly reduced to the corresponding benzyl alcohols owing to deacylation and other complications. A new attempt to synthesize such alcohols containing a free phenolic hydroxyl group has now been made, starting from the benzyl ethers ($R = CH_2C_6H_5$) instead of the corresponding benzoates. The method, however, failed as the subsequent cleavage of the benzyl ethers with sodium and alcohol (*cf.* Richtzenhain [10]) only resulted in unidentifiable amorphous products.

Another series of compounds (VII and VIII), containing elements of both type I and II, have also been synthesized.



VII

$R' = -CH=CH\cdot CH_3$
R = H n = 0 or 1
$R = CH_3$ n = 0 or 1

VIII

$R' = -CH_2CH_2CH_3$
R = H n = 0 or 1
$R = CH_3$ n = 1 or 2

The syntheses were accomplished by reacting dehydrodi-*iso*eugenol [11] (VII, R = H, $n = 0$) or dihydrodehydrodi-*iso*eugenol [12] (VIII, R = H, $n = 0$) with an appropriate bromo derivative in a manner closely analogous to the syntheses of the members of series V. (*Cf.* experimental part.)

The members of the series V, VI, VII, and VIII were subjected to oxidation with nitrobenzene and alkali. (Tables 4—7.) Some of the compounds were also heated with alkali alone. (Table 1.) The results of the latter experiments possess considerable interest in relation to the probable mechanism of the nitrobenzene oxidation process.

RESULTS

Heating with alkali

Table 1 summarizes the results obtained. All experiments were carried out by heating 2 g of the substance at 180° C for two hours with 60 ml of 2 *N* sodium hydroxide solution. The yields are calculated on the basis of the theoretically possible figures.

Table 1.

Compound	Veratric acid %	Other compounds %	Undissolved material %
V, R = CH_3, $n = 2$	14	Acetoguaiacone (IX) * 75	0
V, R = CH_3, $n = 3$	18	Acetoguaiacone 75 Vanillic acid 12	2
VI, X = OH, $n = 2$	3	Vinyl guaiacol (XI) * 18	7
VI, X = OH, $n = 3$	trace	Vinyl guaiacol 23	0

* See Table 2, p. 1527.

Oxidation with nitrobenzene and alkali

Very little has been reported in the literature on the conditions required for obtaining optimal vanillin yields [2, 13]. Therefore, the vanillin yields from a number of compounds were first studied as functions of temperature and time. All substances investigated behaved closely alike in this respect as is shown in Figs. 1 and 2.

A temperature of 180° and a reaction time of two hours is evidently sufficiently near the optimal experimental conditions.

The influence of the alkali concentration and the amounts of nitrobenzene and alkali was also studied.

Fig. 3 shows that in general 2 *N* sodium hydroxide gives the best results, but in the case of series VI (chain molecules containing benzyl alcoholic groupings) the use of stronger alkali greatly increases the vanillin yield. As seen from Figs. 4 and 5, the amounts of nitrobenzene and alkali seem to have little influence, provided they exceed a minimum value.

Even though only some of the compounds recorded in Tables 2—7 have been studied under varying conditions, it may safely be assumed that in general the optimum conditions are those described on p. 1527.

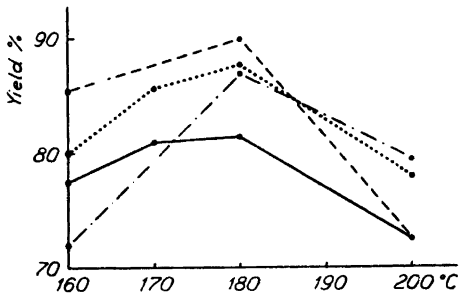


Fig. 1. Vanillin yield as function of reaction temperature (at optimal time). 60 ml/g of NaOH and 8 ml/g of nitrobenzene.

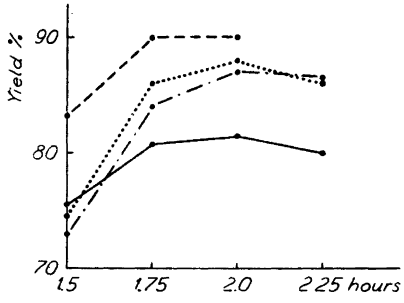


Fig. 2. Vanillin yield as function of reaction time at 180°. 60 ml/g of NaOH and 8 ml/g of nitrobenzene.

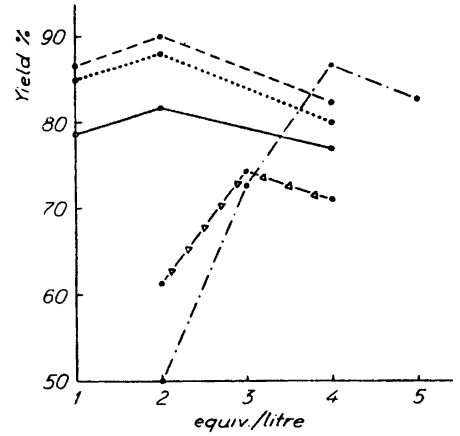


Fig. 3. Vanillin yield as function of alkali concentration. Reaction temp. 180°; time 2 hours. 60 ml/g of NaOH and 8 ml/g of nitrobenzene.

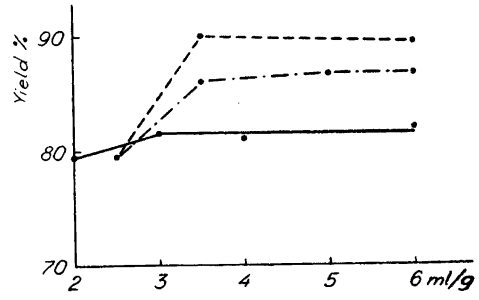


Fig. 4. Vanillin yield as function of the amount of nitrobenzene. Reaction temp. 180°; time 2 hours. 60 ml/g of NaOH.

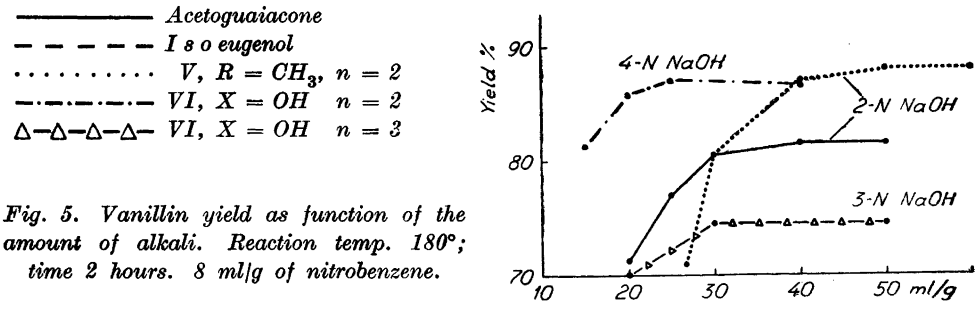


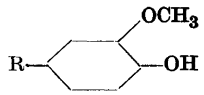
Fig. 5. Vanillin yield as function of the amount of alkali. Reaction temp. 180°; time 2 hours. 8 ml/g of nitrobenzene.

The following tables give the results of the oxidation experiments. The yields are calculated on the basis of the theoretically possible figures.

"Total yield" is the sum of the yields of vanillin and vanillic acid.

In all experiments 0.5—2 g of substance were employed. The reaction was carried out at 180° with a reaction time of two hours. 2 *N* sodium hydroxide solution (60 ml per g of substance) was used unless otherwise stated. The amount of nitrobenzene was 8 ml per g of substance.

Table 2. Oxidation of compounds of the general structure:



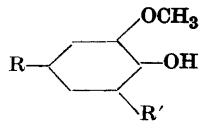
No.	R	Vanillin %	Vanillic acid %	Total yield %	Yields reported by other authors %
IX*	$-CH_2OH$	82+	3	85	"very high" [17]
X*	$-CH_2SO_3\frac{Ba}{2}$	80	—	—	—
XI[14]	$-CH=CH_2$	80	6	86	—
XII	$-COCH_3$	81	4	85	93 [4]; 83 [18]
XIII[15]	$-COCH_2OH$	66	10	76	—
XIV	$-CH_2CH_2CH_3$	17	—	—	6 [5]
XV	$-COCH_2CH_3$	30	—	—	11 [4]
XVI[16]	$-COCH(OH)CH_3$	33	20	53	45 [4]
XVII	$-CH_2-CH=CH_2$	88	—	—	—
XVIII	$-CH=CH-CH_3$	90	trace	90	89 [19]
XIX**	$-CH=CHCHO$	86	4	90	—

* Kindly supplied by Dr. B. Lindgren.

** Kindly supplied by Dr. E. Adler.

\+ Alkali concentration 4 equiv./litre.

Table 3. Oxidation of compounds of the general structure:



No.	R	R′	Vanillin %	XXIII % (R = R′ = CHO)	XXIV % (R = CHO, R′ = COOH)
XX[20]	$-CH_2OH$	$-CH_2OH$	0.8	13	6
XXI[21]	$-CH_2-CH=CH_2$	$-CH_2OH$	2.2	9	11
XXII[22]	$-CHO$	$-CH_2CH=CH_2$	1.6	20	6
XXIII[23]	$-CHO$	$-CHO$	1.5	12	26
XXIV *	$-CHO$	$-COOH$	2	—	—

* This experiment is quoted from Freudenberg [24].

In Tables 4—7 the yields are calculated on the following assumptions:

a) Elements with the para hydroxyl groups methylated yield no vanillin. This seems to be justified by the fact that neither acetoveratrone nor dehydrodi-*iso*eugenol methyl ether (VII, R = CH_3, $n = 0$) gives any vanillin.

b) Elements of type II yield no vanillin. This is justified by the results summarized in Table 3.

Table 4. Oxidation of the members of series V (see p. 1524).

Compound	Vanillin %	Vanillic acid %	Total yield %
R = H, $n = 2$	63	11	74
R = H, $n = 3$	60	12	72
R = H, $n = 4$	60	—	—
R = CH_3, $n = 2$ *	88	0	88
R = CH_3, $n = 3$	71	4	75
R = CH_3, $n = 4$	73	—	—

* A similar compound containing bromine has been oxidized by Kratzl [6].

Table 5. Oxidation of the members of series VI (see p. 1524).

Compound	Vanillin %	Vanillic acid %	Total yield %	Alkali concentration equiv./litre
X=OH, $n = 2$	87	0	87	4
X=OH, $n = 3$	74	4	78	3
X=OH, $n = 4$	72	—	—	3
$X=SO_3\frac{Ba}{2}$, $n = 2$	83	0	83	4
$X=SO_3\frac{Ba}{2}$, $n = 3$	70	—	—	3
$X=SO_3\frac{Ba}{2}$, $n = 4$	71	—	—	3

Table 6. Oxidation of the members of series VII (see p. 1524).

Compound	Vanillin %	Vanillic acid %	Total yield %	XXIII %	XXIV %
R = H, $n = 0$	69	6	75	1	11
R = H, $n = 1$	66	8	74	trace	9
$R = CH_3$, $n = 0$ [11]	0	0	—	—	—
$R = CH_3$, $n = 1$	70	3	73	2	12

Table 7. Oxidation of the members of series VIII (see p. 1524).

Compound	Vanillin %	Vanillic acid %	Total yield %
R = H, $n = 0$	60	6	66
R = H, $n = 1$	67	—	—
$R = CH_3$, $n = 1$	67	5	72
$R = CH_3$, $n = 2$	62	—	—

DISCUSSION

The results from the heating with alkali alone (Table 1) show that under the conditions employed the ether linkages are split by alkali, since the compounds were completely dissolved and degradation products corresponding to the "elements" were isolated. The yields in the last two experiments were low, probably due to polymerizations and condensations.

These results strongly support the view, first expressed by Lautsch [25], that the oxidation is preceded by a fission of ether linkages and subsequent dehydration. The vinyl guaiacol formed from the members of series VI is evidently a dehydration product.

The oxidation of "condensed" compounds of type II (Table 3), containing side chains (R') of varying structure, clearly shows that no appreciable amounts of vanillin are formed from such elements. Yields amounting to 20—40 % of 5-formyl vanillin (XXIII) and 5-vanillin carboxylic acid (XXIV) were however obtained. The rest was amorphous and could not be identified.

Finally, Tables 4—7 show that with a few exceptions (the first members of series V, R = CH_3, VI, and VIII) the total yields from the "open" elements vary only slightly, ranging from 72 to 78 %. The yields conform approximately with those obtained from the corresponding mononuclear compounds.

The sulphonic acids listed in Table 5 give approximately the same yields as the corresponding alcohols.

It is interesting to note that the main product obtained from the condensed elements in Table 6, 5-vanillin carboxylic acid (XXIV), has actually been isolated by Freudenberg [1] from the products obtained by oxidizing wood.

From the results discussed above it is evident that the vanillin yield obtained from simple mononuclear elements of type I is substantially the same as that obtained from chain molecules containing these elements connected by ether linkages (series V and VI). On the other hand, little or no vanillin is formed from elements of type II. Chains containing both types of elements connected by ether linkages will furnish vanillin in a yield corresponding to the amount of elements of type I present in the starting material. Consequently it appears to be possible to estimate the relative amounts of elements of the types I and II (or III?) in lignin preparations. This question will be discussed in a forthcoming paper on the oxidation of various lignin preparations and derivatives.

EXPERIMENTAL

Dehydrodi-*iso*eugenol. (VII, R = H, $n = 0$)

This compound was prepared by a modification of the method described by Erdtman [11]: 50 g of *iso*eugenol (freshly distilled) were dissolved in 450 ml of alcohol (95 %) and 200 ml of water. Then 70 g of ferric chloride (crystallized) in 200 ml of water were added. The solution turned dark green and cloudy. A few crystals of dehydrodi-*iso*eugenol were added. After 24 hours in the refrigerator, a reddish white crystalline mass had separated and the solution was reddish yellow. The crystals were collected by filtration and washed with 45 % alcohol. Recrystallization from a small amount of alcohol yielded 15 g (30 %) of a colourless product. M. p. 132—133°.

Acetoguaiacyl-dehydrodi-*iso*eugenol methyl ether

(VII, R = CH_3, $n = 1$)

5 g of ω-bromoacetoveratrone (see Part II), 6.3 g of dehydrodi-*iso*eugenol and 10 g of anhydrous potassium carbonate in 100 ml of absolute methyl ethyl ketone were refluxed under anhydrous conditions for 30 minutes. The mixture was washed with water, the methyl ethyl ketone layer dried over anhydrous sodium sulphate and concentrated *in vacuo*. An oil was obtained, which crystallized on cooling and trituration with alcohol. Yield 7.95 g (82 %). M. p. 112—120°. Repeated recrystallizations from alcohol yielded flat crystals of m. p. 123—124°.

$C_{26}H_{20}O_3$ $(OCH_3)_4$	Calc.	C 71.4	H 6.40	OCH_3 24.6
	Found	» 71.8	» 6.46	» 24.7

Acetoguaiacyl-dehydrodi-*iso*eugenol benzoate

(VII, R = C_6H_5CO, $n = 1$)

4.2 g of ω-bromobenzoylacetoguaiacone (see Part II), 4 g of dehydrodi-*iso*eugenol and 10 g of potassium carbonate in 100 ml of methyl ethyl ketone were reacted as described above. Yield 3.7 g (51 %). Recrystallization from alcohol yielded needles of m. p. 124—125°.

$C_{33}H_{25}O_5(OCH_3)_3$ Calc. OCH_3 15.7 Found OCH_3 15.9

Acetoguaiacyl-dehydrodi-*iso*eugenol

(VII, R = H, $n = 1$)

2.7 g of the above compound were refluxed with 2 ml of piperidine in 75 ml of alcohol for 30 minutes. The mixture was poured into water and acidified. An amorphous powder separated, which crystallized on treatment with alcohol. Yield 1.6 g (73 %). The crystals were dissolved in chloroform, the solution filtered through aluminium oxide and the

chloroform evaporated. The residue was recrystallized from dilute alcohol. Repeated recrystallizations yielded almost colourless crystals of m. p. 129—130°.

$C_{26}H_{21}O_4(OCH_3)_3$ Calc. OCH_3 19.0 Found OCH_3 18.8

Methylation with diazomethane in ether furnished a methyl ether identical with VII, R = CH_3, n = 1 (m. p. 123—124°).

Acetoguaiacyl-dihydrodehydrodi-*iso*eugenol methyl ether

(VIII, R = CH_3, n = 1)

4 g of ω-bromoacetoveratrone, 5 g of dihydrodehydrodi-*iso*eugenol[12] and 10 g of potassium carbonate in 100 ml of methyl ethyl ketone were reacted as described above. Yield 6 g (78 %). Three recrystallizations from alcohol yielded flat crystals of m. p. 99—100°.

$C_{26}H_{22}O_3(OCH_3)_4$	Calc.	C 71.2	H 6.77	OCH_3 24.5
	Found	» 71.4	» 6.45	» 24.3

Di-(acetoguaiacyl)-dihydrodehydrodi-*iso*eugenol methyl ether

(VIII, R = CH_3, n = 2)

5 g of ω-bromoacetoguaiacyl-acetoguaiacone methyl ether (see Part II), 3.88 g of dihydrodehydrodi-*iso*eugenol and 7.5 g of potassium carbonate in 75 ml of methyl ethyl ketone were reacted as described above. Yield 6.25 g (79 %). On recrystallization from methanol and then from acetone almost colourless crystals of m. p. 114—115° were obtained.

$C_{34}H_{27}O_5(OCH_3)_5$	Calc.	C 69.9	H 6.32	OCH_3 23.1
	Found	» 69.7	» 6.29	» 23.0

Acetoguaiacyl-dihydrodehydrodi-*iso*eugenol benzoate

(VIII, R = C_6H_5CO, n = 1)

5.3 g of ω-bromobenzoylacetoguaiacone, 5 g of dihydrodehydrodi-*iso*eugenol and 10 g of potassium carbonate in 100 ml of methyl ethyl ketone were reacted as described above. Yield 4.9 g (54 %). Two recrystallizations from methanol yielded needles of m. p. 98—99°.

$C_{33}H_{27}O_5(OCH_3)_3$ Calc. OCH_3 15.6 Found OCH_3 15.2

Acetoguaiacyl-dihydrodehydrodi-*iso*eugenol

(VIII, R = H, n = 1)

The above compound was debenzoylated with piperidine as described for VII, R = H, n = 1 (p. 1531). Yield 73 %. Repeated recrystallizations from methanol yielded crystals of m. p. 82—83°.

$C_{26}H_{23}O_4(OCH_3)_3$ Calc. OCH_3 18.9 Found OCH_3 18.7

Methylation with diazomethane in ether furnished a methyl ether identical with VIII, R = CH_3, $n = 1$ (m. p. 100—101°).

Acetoguaiacone benzyl ether

10 g of acetoguaiacone and 15 ml of benzyl chloride were dissolved in 150 ml of alcohol and 3.7 g of sodium hydroxide were added. After refluxing for 1.5 hours, the mixture was poured into water and extracted with ether. The ether solution was washed with water, dried over anhydrous sodium sulphate and evaporated. An oil was obtained which crystallized on cooling. Yield 13.3 g (86 %). M. p. 81—84°. Two recrystallizations from petroleum (b. p. 40—60°) yielded needles of m. p. 86—86.5°.

$C_{15}H_{13}O_2(OCH_3)$ Calc. OCH_3 12.1 Found OCH_3 12.1

ω-Bromoacetoguaiacone benzyl ether

10 g of acetoguaiacone benzyl ether were dissolved in 100 ml of chloroform. A solution of 6.25 g of bromine in 50 ml of chloroform was added in small portions. When all the bromine had reacted, the mixture was washed with bicarbonate solution and then with water. The chloroform solution was dried over anhydrous sodium sulphate and evaporated. An oil was obtained which crystallized on cooling. Yield 9.7 g (75 %). Three recrystallizations from benzene-petroleum (b. p. 120—140°) yielded needles of m. p. 102.5—103°.

$C_{15}H_{12}O_2Br(OCH_3)$	Calc.	OCH_3	9.3	Br	23.9
	Found	»	9.2	»	23.8

3-Methoxy-4-benzyloxyphenacyl coerulignol ether

5 g of ω-bromoacetoguaiacone benzyl ether, 2.55 g of coerulignol and 5 g of anhydrous potassium carbonate in 50 ml of absolute methyl ethyl ketone were reacted as described on p. 1531. The resulting oil solidified to a yellowish mass. Yield 5.5 g (90 %). Recrystallization from a small amount of ether yielded colourless needles of m.p.86.5—87.5°.

$C_{24}H_{22}O_3(OCH_3)_2$	Calc.	C	74.3	H	6.72	OCH_3	14.8
	Found	»	74.0	»	6.50	»	14.9

Dihydro-3-methoxy-4-benzyloxyphenacyl coerulignol ether

4 g of the above compound were reduced with 4 g of aluminium *iso*propoxide in 30 ml of absolute *iso*propyl alcohol following the procedure described in Part II. Yield 3.5 g (88 %). Recrystallization from ether yielded colourless plates of m. p. 55—57°.

$C_{24}H_{24}O_3(OCH_3)_2 + H_2O$	Calc.	OCH_3	14.1	H_2O	4.1
	Found	»	14.2	»	4.0

On drying the compound *in vacuo* at 70° a decrease in weight corresponding to one mole of water of crystallization was observed. The anhydrous substance had a m. p. of 62—63°.

$C_{24}H_{24}O_3(OCH_3)_2$ Calc. OCH_3 14.7 Found OCH_3 14.8

Oxidation experiments

Apparatus

The oxidations were carried out in 100 ml autoclaves of stainless steel, rotating in an electrically heated oil bath. The temperature could be controlled with an accuracy of ± 1°.

A preliminary determination was made of the time taken for the contents of the autoclaves to attain the temperature of the oil-bath after immersion in it. This was done by placing in the autoclaves, each containing 50 ml of water, capillary tubes charged with substances of melting points ranging from 80 to 180° and immersing the autoclaves in the oil bath, which was maintained at 180°, for different lengths of time. From subsequent examination of the materials in the capillary tubes it was concluded that 8—9 minutes were required for the contents of the autoclaves to reach oil-bath temperature and this time is included in the reaction times reported in this paper.

Procedure

The autoclaves were charged, heated in the oil bath and then cooled with running water. The contents were subjected to steam distillation to remove nitrogenous compounds. After acidification, the residues were continuously extracted with benzene for about 24 hours.

Examination of the products

The benzene extracts were shaken with bisulphite, bicarbonate and sodium hydroxide in the usual manner. The various fractions were then investigated in the following way:

a) Bisulphite fraction.

(The procedure for the separation of all three of the aldehydic compounds isolated is given. In most cases, however, this fraction contained only vanillin.)

After acidification, the solution was freed from sulphur dioxide by passing a stream of carbon dioxide through the mixture at about 90° for 15—30 minutes. On cooling, a precipitate was obtained in some cases. This was removed by filtration, the filtrate extracted with ether and the ether solution dried over anhydrous sodium sulphate and evaporated. The residue was washed with small amounts of ether (A). The undissolved material together with the precipitate referred to above were triturated with a little acetone to remove coloured impurities. On recrystallization from water almost colourless needles of m. p. 252—254° were obtained. (Freudenberg and Klink[26] reported 255° for *5-vanillin carboxylic acid*, XXIV.)

$C_8H_5O_4(OCH_3)$	Calc.	OCH_3	15.8	eq.wt.	196
	Found	»	15.8	» »	195

The ether solution (A) was evaporated and the residue stirred with hot petroleum (b. p. 120—140°) (B). The undissolved residue was recrystallized from water, yielding yellowish crystals of m. p. 119—120°. The compound gave no depression with an authentic sample of *5-formyl vanillin* (XXIII).

From the petroleum solution (B) yellowish *vanillin* crystals separated. The final purification was effected by filtering an ether solution of this material through a column of aluminium oxide. On evaporation almost colourless vanillin, m. p. 79—81°, was obtained.

The analytical determination of vanillin was carried out using a modification of the method recommended by Mr. J. R. Salvesen of Marathon Corporation, Wisconsin, U. S. A., and kindly put at our disposal by him.

An aliquot of the original benzene extract (equivalent to about 50 mg of vanillin) is filtered through a column of aluminium oxide, previously treated with hydrochloric acid. The column is then thoroughly washed with ether and in this way the aldehydes XXIII and XXIV are adsorbed and only vanillin passes through. (If, as in most cases, vanillin is the only aldehyde present, this step is of course omitted.) The solution is then placed in a distilling flask together with 50 ml of water containing 0.4 g of sodium bisulphite and the solvent removed by distillation. The residual aqueous solution is filtered into a stoppered 300 ml Erlenmeyer flask. When the solution has attained room temperature, another 0.4 g of sodium bisulphite, a few drops of starch solution, and 2 ml of 10 % potassium iodide solution are added. After standing for 20 minutes the mixture is cooled to 0°. At this temperature 5 % potassium iodate solution is added from a burette until the equivalence point is almost reached. The mixture is then titrated with 0.05 *N* iodine solution to a faintly blue colour. Then 20 ml of 0.05 *N* iodine solution are added with a pipette. The mixture is heated on the water bath to 20°, 10 ml of concentrated hydrochloric acid added, and the solution heated to 35°. Then the flask is stoppered and allowed to stand at room temperature for five minutes, and the excess of iodine estimated by titration with 0.05 *N* sodium thiosulphate solution. One ml of 0.05 *N* iodine solution corresponds to 3.8 mg of vanillin.

The results obtained by this method were occasionally checked by gravimetric analyses employing 2,4-dinitrophenyl hydrazine (according to Pearl [27]) or 3-nitrobenzoyl hydrazine (according to Tomlinson and Hibbert [28]). These control experiments showed that the titration method gives results which are 2—3 % low. This was confirmed by model experiments with pure vanillin. All vanillin yields reported, therefore, have been corrected.

b) *Bicarbonate fraction.*

From this fraction only *vanillic acid* (m. p. 207—208°) and *veratric acid* (m. p. 175—176°) could be isolated. These compounds could easily be purified by vacuum sublimation and recrystallization from water or benzene-petroleum (b. p. 40—60°). When the two acids occurred together, however, it was very difficult to accomplish a quantitative separation. Several attempts to effect a separation by chromatographic adsorption on different media were unsuccessful. However, by fractional vacuum sublimation essentially as described by Hibbert *et al.*[29] for the separation of vanillin and syringic aldehyde, a fairly good separation could be achieved. The first fraction was collected at 120° (1 mm) and had a melting point ranging between 160 and 190° (veratric acid containing vanillic acid). The second fraction was collected at 150°. M. p. 204—207°. (Almost pure

vanillic acid.) On resublimation, the first fraction yielded further amounts of vanillic acid. This method is of course somewhat inaccurate and the reported yields of vanillic acid are probably not quantitative.

c) Sodium hydroxide fraction.

After filtering the ether extract through a column of aluminium oxide and evaporating the ether, these fractions generally yielded brown, sticky residues. In most cases orange red crystals of p-*hydroxy azobenzene* [1] (m. p. 150—151°) soon appeared. As a rule, no other crystalline compounds could be isolated, but a distinct smell of *guaiacol* was generally observed.

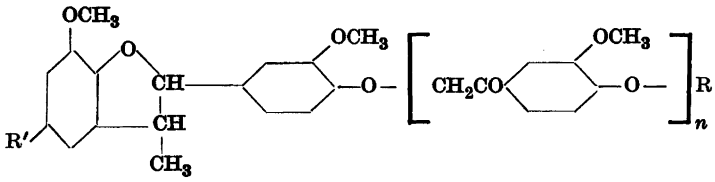
Only in the case of products of alkali fission experiments (see Table 1) could appreciable amounts of phenols be isolated from this fraction.

Vinyl guaiacol (XI) was isolated from the acidified solutions by steam distillation. The distillate was extracted with ether, the ether evaporated and the residue distilled *in vacuo*. A colourless oil, boiling at about 100° (5 mm), was obtained. The oil was benzoylated with benzoyl chloride in pyridine, yielding colourless crystals (from alcohol) of m. p. 109—111°. No depression was observed with an authentic sample of vinyl guaiacol benzoate [14].

Acetoguaiacone (IX) was purified by vacuum sublimation and recrystallization from water. M. p. 114—115°.

SUMMARY

A series of lignin models of the general formula



have been synthesized. The members of this series and of two series described in Part II, containing only ether linked elements, have been subjected to oxidation with nitrobenzene and alkali. In addition some of the compounds have been heated with alkali alone.

The results show that the oxidation is preceded by alkaline fission of the ether linkages. Elements connected only by ether linkages give high yields of vanillin and vanillic acid; elements connected by carbon-carbon linkages give neither of these products.

The author wishes to acknowledge his indebtedness to *Statens Tekniska Forskningsråd* for financial support, and to Miss I. Malmström for skilful assistance.

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Received September 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1538–1540

A Revision of the Mechanism of the Ferric Salt Catalyzed Hydrogen Peroxide Decomposition

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By means of recently published kinetic experiments at different temperatures it is shown that the mechanism of the ferric salt catalyzed hydrogen peroxide decomposition must be represented by a sequence which contains an active form of hydrogen peroxide anion instead of oxygen atoms as formerly assumed.

One of us has in a previous paper [1] given a reaction scheme for the ferric salt catalyzed decomposition of hydrogen peroxide.

The investigations are later [2] extended to include the dependence of the velocity of reaction on the temperature for the interval between 10° and 35° C.

The result of these investigations was, that the sequence proposed:

$Fe^{+++} + HO_2^- \rightleftharpoons FeOOH^{++}$	12		
$FeOOH^{++} \rightleftharpoons FeOH^{++} + O$	25	II	
$O + HO_2^- \rightarrow O_2 + OH^-$	50		

led to an unexpectedly high value for the ratio between the velocity constants for the two processes 50 and 52. The ratio was found to be equal to about 10^9 for the temperature interval mentioned above. As it is practically independent of the temperature one would have expected a value of the order of magnitude 1.

To avoid the above result we have now been compelled to modify the sequence as follows:

$Fe^{+++} + HO_2^- \rightleftharpoons FeOOH^{++}$	12		
$FeOOH^{++} \rightleftharpoons Fe^{+++} + \overset{*}{HO}_2^-$	25	III	
$\overset{*}{HO}_2^- + H_2O_2 \rightarrow O_2 + H_2O + OH^-$	50		

$\overset{*}{HO}_2^-$ symbolizes an active HO_2^--ion, possibly HO^-: O.

Table 1. Figures used for calculation of the ratio between the velocity constants for the two processes 50 and 52 ind the sequence III. k_{50}/k_{52} in column (6) are calculated by means of (35) and k_{50}/k_{52} in column (7) are calculated by means of (36).

1	2	3	4	5	6	7
T	c_{Ferric}	$A \cdot 10^3$	c_{H^+}	$K_{Fe} \cdot 10^3$	k_{50}/k_{52} exp. Eqn (35)	k_{50}/k_{52} calcd. Eqn (36)
283.15	0.004	1.50	0.0102	0.66	1.08	1.15
288.15	0.004	1.50	0.0103	0.98	1.06	1.07
293.15	0.004	1.50	0.0105	1.41	1.02	1.00
298.15	0.004	1.50	0.0106	2.00	0.97	0.91
298.15	0.002	0.80	0.0103	2.00	0.91	0.91
303.15	0.002	0.80	0.0104	2.82	0.86	0.85
308.15	0.002	0.80	0.0106	3.98	0.79	0.79

The modified sequence leads to the same value of k_{25} as originally calculated. But now we get for the calculation of the ratio k_{50}/k_{52}:

$$A = 0.4343 \frac{k_{52} \cdot c_{H^+} \cdot c_{\text{Ferric}}}{k_{50} (K_{Fe} + c_{H^+})} \tag{35}$$

which gives a value for k_{50}/k_{52} of the order of magnitude one.

The values necessary for the calculation of k_{50}/k_{52} are given in Table 1; the values for A are taken from the above cited paper [2], the values for K_{Fe} are calculated from equation (27) (*l. c.*); the hydrogen ion concentration, which in the cited paper was presumed equal to the concentration of nitric acid is corrected by means of the equation:

$$c_{H^+} = c_{HNO_3} + \alpha \cdot c_{\text{Ferric}}$$

where α is the degree of hydrolysis of the ferric ion in the solutions in question. The values for k_{50}/k_{52} which are calculated from equation (35) are given in column (6) in the table.

The table shows that the ratio varies a little with the temperature. By means of the usual method of calculation it can be shown that:

$$k_{50}/k_{52} = 10^{-1,88} \cdot e^{\frac{2514}{RT}} \tag{36}$$

for the temperature interval in question.

The values for k_{50}/k_{52} which are given in Table 1 column (7) are calculated by means of (36).

The order of magnitude which has been found for the frequency factor in (36) is in good agreement with the fact that 52 is supposed to be a reaction between two oppositely charged ions and that 50 is supposed to be a reaction between an ion and an uncharged molecule.

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Received November 8, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1541–1553

Retene Investigations

XVIII. The Mannich Reaction with Monohydroxy Derivatives of Phenanthrene and Retene

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The condensation of ammonia or a primary or secondary amine with formaldehyde and an organic compound containing at least one hydrogen atom of pronounced reactivity is known as the Mannich reaction. At this reaction the active hydrogen atom is replaced by an aminomethyl or substituted aminomethyl group according to the formula:

$$AH + CH_2O + R_1R_2NH \rightarrow ACH_2NR_1R_2 + H_2O$$

This type of reaction has found during recent years wide application in organic preparations in several different classes of organic compounds, above all on ketones. The Mannich reaction has been less investigated with phenols, where, however, the *o*- and *p*-hydrogen atoms are sufficiently active to enter into this reaction. Thus, *p*-cresol gives a mono- and a disubstituted product [1] and phenol mono-di-and tri-substituted derivatives [1, 2].

In the naphthalene series 1-naphthol gives substitution in the 2-position (not, however, in the 4-position as far as I have found in the literature), while 2-naphthol is substituted in the 1-position [3].

As for monohydroxy derivatives in the phenanthrene series no information exists in the literature concerning their ability to enter into the Mannich reaction. Due to this the present investigation was carried out. It comprises the following phenols: 2- and 3-hydroxyphenanthrene, 2- and 3-hydroxyretene and 4-methyl-3-hydroxyretene. Piperidine and morpholine, with, to a lesser degree, dimethylamine have been the amines mainly used.

The standard preparation has been the following. The phenol in question was dissolved in glacial acetic acid and an excess of formalin and amine was

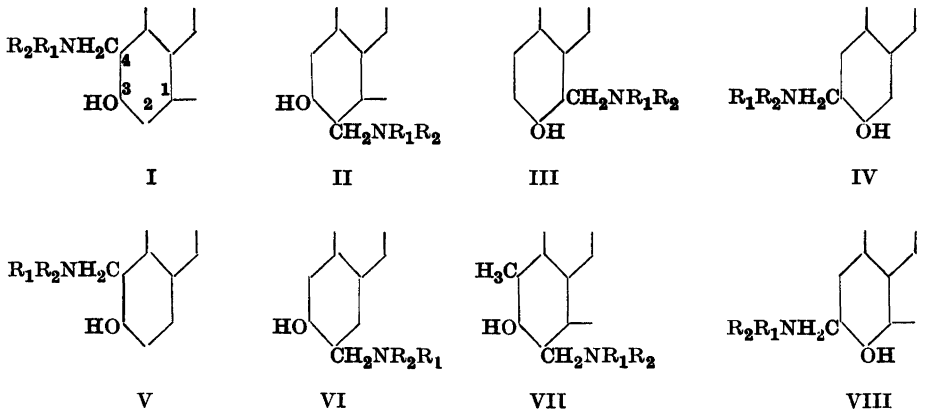

Fig. 1. Possible isomeres.

added at room temperature, after which the reaction mixture was allowed to stand 15—20 hours. Then the reaction product was precipitated with water and purified in various ways. This resulted in 2- and 3-hydroxyphenanthrene and 3-hydroxyretene reacting easily under formation of monoderivatives, whereas 2-hydroxyretene and 4-methyl-3-hydroxyretene did not react under the standard conditions. Reaction, however, could be obtained with 2-hydroxyretene, if the reaction mixture was boiled for several hours. A monosubstituted derivative was then formed.

It must be considered highly probable that substitution occurs in that benzene ring, to which the hydroxyl group is attached and as there are no free *para* positions in relation to this group, only *ortho* positions are available for substitution. The phenols in question can then give rise to eight different compounds (Fig. 1), namely 3-hydroxyretene I and II, 2-hydroxyphenanthrene III and IV, 3-hydroxy-phenanthrene V and VI, 4-methyl-3-hydroxyretene VII and 2-hydroxyretene VIII. The three phenols first named, which easily react, only give one reaction product, even if theoretically two isomeres could be formed from each phenol. As 4-methyl-3-hydroxyretene does not react, while 3-hydroxyretene gives a monoderivative, there is reason to suspect, that the last-mentioned compound is substituted in the 4-position with the group $-CH_2NR_1R_2$. If this position, however, is occupied (as in case the of 4-methyl-3-hydroxyretene) no reaction takes place. This assumption has been shown to be correct (see further). As, furthermore, 2-hydroxyphenanthrene gives a monoderivative, while 2-hydroxyretene, having occupied 1-position, does not react under the standard conditions, there is reason to suppose, that 2-hydroxyphenanthrene is substituted in the 1-position.

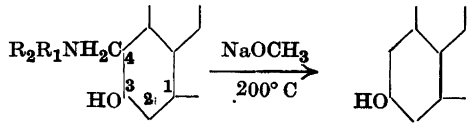

Fig. 2. Reaction with sodium methoxide.

This has also been found to be the case. Both these examples constitute yet another contribution to the well-known fact, that the two *ortho*-positions in a hydroxyl-substituted phenanthrene nucleus are not equivalent.

In order to clear up the structure of the Mannich bases obtained we have considered it suitable to try to transform these to the corresponding methyl-derivatives. This involves a cleavage of the carbon-nitrogen bond according to the formula:

$$HOArCH_2NR_1R_2 \rightarrow HOArCH_3 + R_1R_2NH$$

This metod has been launched by Caldwell and Thompson [4] for the preparation of phenols, methyl substituted in the nucleus. They performed the cleavage by hydrogenation over copper-chromit catalyst in dioxane solution. We have tried to use their method but obtained an viscous oil and well crystallizing hydrocarbons, which were not subjected to a closer examination, as they were of no interest in this connection.

Instead, attempts were made to cleave the carbon-nitrogen bond by heating the compound in question with sodium methoxide in methanol solution in an autoclave at 130—200 °C. Cornforth, Cornforth and Robinson [5] have, namely, in this manner treated 2,7-dihydroxy-1,8-bispiperidinomethylphenanthrene and obtained the corresponding 1,8-dimethyl derivative. In our case, however, this method resulted in a cleavage of the carbon-carbon bond, thus giving back the original phenol (see Fig. 2). This process is therefore of no value for the determination of the structure. Morpholinomethyl-2-hydroxyretene is, however, an exception. It remains unchanged when heated with sodium methoxid for 14 hours at 200 °C. In this connection it is of interest to point out, that 2-hydroxyretene only reacts with formaldehyde and morpholine under rigorous conditions in contrast to the other phenols.

Mc Cleary and Roberts [6] have recently found that Mannich bases of phenols in the benzene series react with thiols under formation of thioethers according to the formula:

$$HOC_6H_4CH_2N(CH_3)_2 + HSR \rightarrow HOC_6H_4CH_2SR + (CH_3)_2NH$$

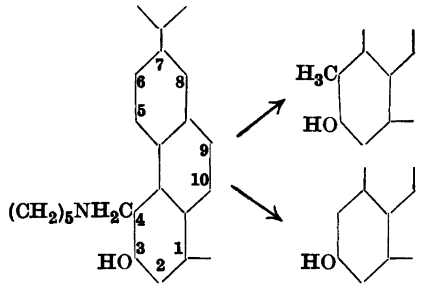

Fig. 3. Hydrogenation over Raney nickel.

By hydrogenation of the thioethers thus obtained the desired methyl derivatives should be obtained. Preliminary attempts have shown, however, that our Mannich bases react with thiols under cleavage of the carbon-carbon bond. The process is thus of no interest for the determination of the structure. It should be noted, however, that morpholinomethyl-2-hydroxyretene is in this case also extremely stable and remains unchanged.

The result of attempts to cleave the carbon-nitrogen bond with cyanogen bromide was that under normal reaction conditions no reaction was obtained. Harder conditions resulted in oily, indefinite products.

By hydrogenation in dioxane solution over Raney-nickel at 150—180°C and 140—160 at the undesirable, original phenol (cleavage of the carbon-carbon bond) was obtained together with much resinous products. Reduction of the hydrogen pressure to 80—100 at (with the same temperature as before) resulted as previously in oily products and original phenol, but in this case also the desired methylphenol could be isolated. By systematic and tedious methods of purification it was possible to isolate in each special case from the complicated reaction mixture so much of the methylphenol, that its identity could with certainty be established. By the last-mentioned hydrogenation the following two reactions (illustrated in Fig. 3 for piperidinomethyl-3-hydroxyretene) occur.

The hydrogenations gave the following results. Morpholinomethyl-3-hydroxyretene gave a methyl-3-hydroxyretene, which proved to be identical with 4-methyl-3-hydroxyretene. The latter has been quite recently prepared [7]. By hydrogenation of the corresponding piperidino derivative the same methylphenol is obtained. Morpholinomethyl-3-hydroxyphenanthrene gave a methylphenol, which is not described in the literature. With regard to the preceding results it could be expected that it was 4-methyl-3-hydroxyphenanthrene. This compound has been prepared by us from 3-hydroxyphenanthrene-

4-aldehyde by reduction according to Clemmensen. This compound is identical with that obtained by hydrogenation of morpholinomethyl-3-hydroxyphenanthrene. The Mannich reaction with both 3-hydroxy-retene and -phenanthrene thus results in substitution in the 4-position.

Morpholino- (and piperidino-) methyl-2-hydroxyphenanthrene gave one and the same methylphenol, identical with 1-methyl-2-hydroxy-phenanthrene, already described in the literature [8]. This compound has been prepared by us through reduction according to Clemmensen of 2-hydroxyphenanthrene-1-aldehyde.

2-Hydroxyretene has only one free ortho position and it must therefore be considered highly probable that substitution occurs in the 3-position at the Mannich reaction. As, however, we have hitherto not succeeded in preparing 3-methyl-2-hydroxy-retene, we have not performed any hydrogenations with this Mannich base.

As we have found, that 3-hydroxyretene with occupied 4-position does not enter into the Mannich reaction it was of a certain interest to investigate, whether 3-hydroxyretene, substituted in another position, would react. For this purpose use was made of 9-(or 10) bromo-3-hydroxyretene and 3-hydroxyretene-9-(or 10) sulfonic acid, which both gave the expected products.

Whereas morpholine and piperidine respectively easily react with 3-hydroxyretene and formaldehyde we, in spite of varying reaction conditions, failed to obtain reaction with aliphatic amines, such as dimethylamine.

In the Mannich bases here described the hydroxylgroup and the substituted aminomethyl group are placed in ortho position and chelation between them is probable [9]. Thus the reactivity of the hydroxylgroup is diminished. It does not react, *i. g.*, with acetic anhydride, not even at boiling. If, on the other hand, sodium hydroxide is added acetylation of the hydroxyl group with acetic anhydride easily occurs at room temperature; benzoylation is also easy to perform in alkaline medium. It is remarkable, that these Mannich bases are not attacked by boiling acetic anhydride under formation of the corresponding hydroxymethyl derivatives. Attempts to methylate showed that both dimethyl sulphate and diazomethane do not react with the hydroxylgroup. Snyder and Brewster [10] have quite recently found that phenolic Mannich bases with the substituents in *ortho* position cannot be methylated. *m*-Nitrobenzazid in boiling xylene for several hours does not react with the hydroxyl group. We have made attempts to replace the hydroxyl group with an amino group by heating morpholino- (and piperidino-) methyl-3-hydroxyretene with ammonium chloride, glacial acetic acid and anhydrous sodium acetate in an autoclave at 260 °C. The result was that 3-acetaminoretene was formed in a high

yield, which indicates that the substituted aminomethyl group has been split off and the hydroxyl group aminated.

As these Mannich bases are tertiary amines we have tried to prepare their methiodides but always without result. Snyder and Brewster [10] have not succeeded in preparing the quaternary salts of, *i. g.*, 1-dimethylaminomethyl-2-hydroxynaphthalene.

EXPERIMENTAL

Preparation of the Mannichs bases. Derivatives

4-Morpholinomethyl-3-hydroxyretene, $HOC_{18}H_{16}CH_2N(CH_2)_4O$. 3-Hydroxyretene (10 g, 0.04 mole) with the melting point 161—163 °C was dissolved in 250 ml of glacial acetic acid by gentle warming. The solution was then cooled to 20 °C whereupon a mixture of 10 ml (0.12 mole) of morpholine and 10 ml (0.1 mole) of 30 % formalin was added in portions. The solution thus obtained was stirred for a short while and then allowed to stand for 15—20 hours at room temperature. Then it was poured into two liters of cold water whereby a milky precipitate was formed. On addition of 200 ml 5 % sodium hydroxide the precipitate coagulates and is then easy to filter. After washing with diluted sodium hydroxide and water, and drying, 12 g (85 %) of a yellow-white powder was obtained. Recrystallized from a mixture of 96 % ethanol and acetone (5 : 1) 4.6 g of beautiful rectangular plates are obtained which melt at 176—177 °C.

Calc.	C	79.0	H	7.79	N	4.01
Found	»	78.7	»	7.66	»	4.09

The picrate, $HOC_{18}H_{16}CH_2N(CH_2)_4O.HOC_6H_2(NO_2)_3$ was obtained from the components in ethanol solution in the form of yellow needles with the melting point 159—160 °C.

Calcd.	C	60.2	H	5.22
Found	»	59.5	»	5.23

4-Morpholinomethyl-3-benzoxyretene, $C_6H_5COOC_{18}H_{16}CH_2N(CH_2)_4O$, was prepared by benzoylation according to Schotten-Baumann. The crude product was recrystallized from ethanol and obtained as white, transparent, prismatic crystals, melting at 182—183 °C.

Calcd.	C	79.4	H	6.89
Found	»	79.0	»	6.84

4-Morpholinomethyl-3-acetoxyretene, $CH_3COOC_{18}H_{16}CH_2N(CH_2)_4O$, was obtained through shaking a suspension of the Mannichs base in diluted sodium hydroxide with acetic anhydride at room temperature. Recrystallization from ethanol gave thick plates. M. p. 172.5—173.5 °C.

Calcd.	C	76.7	H	7.47
Found	»	76.8	»	7.51

Morpholinomethyl-3-hydroxy-9-(or 10)bromoretene, $HO(Br)C_{18}H_{15}CH_2N(CH_2)_4O$. 3-Hydroxy-9-(or 10) bromoretene (1.0 g) was dissolved in 40 ml of glacial acetic acid. A mixture of 1.5 ml of morpholine and 1.5 ml of 30 % formalin was added under stirring at room temperature. After 24 hours 200 ml of 1 % sodium hydroxide was added and the white precipitate formed was filtered off an washed with water. The crude product (1.0 g) was recrystallized from 96 % ethanol, and the pure compound obtained as thin plates with the melting point of 161—162 °C. Yield 0.3 g.

Calcd.	C	63.8	H	5.84	Br	19.3
Found	»	63.7	»	5.83	»	20.2

4-Piperidinomethyl-3-hydroxyretene, $HOC_{18}H_{16}CH_2(CH_2)_5$. To 3-hydroxyretene (10 g., 0.04 mole, m. p. 161—163 °C) dissolved in 250 ml of glacial acetic acid was added in portions while stirring a mixture of 10 ml (0.12 mole) of piperidine and 10 ml (0.1 mole) of 30 % formalin at room temperature. After 15—20 hours two liters of 1 % sodium hydroxide was added whereby a yellowish-white precipitate and a sticky oil is obtained, the later solidifying gradually. After washing with water and drying, the product (11 g) was recrystallized from 96 % ethanol. Yield 4.8 g of thin, rectangular plates melting at 155—156 °C.

Calcd.	C	82.9	H	8.42	N	4.03
Found	»	83.5	»	8.33	»	4.13

The picrate, $HOC_{18}H_{16}CH_2N(CH_2)_5 \cdot 2\ HOC_6H_2(NO_2)_3$ was obtained from the components in ethanol solution as garnet red needles, which melt at 148.5—149.5 °C. It is of interest that this picrate contains two molecules of picric acid while all the other picrates described in this paper only contain one. The colour of the picrate (garnet red) is also quite different from that of all the other picrates (yellow).

Calcd.	C	53.7	H	4.38
Found	»	53.4	»	4.36

4-Piperidinomethyl-3-benzoxyretene, $C_6H_5COOC_{18}H_{16}CH_2N(CH_2)_5$. The benzoylation was performed according to Schotten-Baumann. Recrystallization from ethanol gave hexagonal plates melting at 139—140 °C.

Calcd.	C	82.4	H	7.36
Found	»	81.2	»	7.20

Piperidinomethyl-3-hydroxyretene-9-(or 10) sulfonic acid, $HO_3S(HO)C_{18}H_{15}CH_2N(CH_2)_5$. The sulfonic acid (0.5 g), 0.5 ml of piperidine and 0.5 ml of 30 % formalin were dissolved in 10 ml of glacial acetic acid at room temperature. The reaction mixture was treated as before and a white powder was obtained.

Calcd.	N	3.28	S	7.49
Found	»	3.15	»	7.31

Morpholinomethyl-2-hydroxyretene, $HOC_{18}H_{16}CH_2N(CH_2)_4O$. 2-Hydroxyretene (5.0 g, 0.02 mole, m. p. 199—201 °C), 2.0 g (0.025 mole of morpholine and 5 ml (0.05 mole) of

30 % formalin were dissolved in 200 ml of glacial acetic acid and refluxed for 5 hours. After cooling, the solution was poured into 1 liter of 1 % sodium hydroxide, the precipitate formed filtered off and thoroughly washed with water. After drying, 6 g of a yellowish-white powder was obtained, which on recrystallization from a mixture of ethanol and acetone (5 : 1) gave thin, transparent plates, m. p. 176—177 °C. Yield 3.0 g.

Calcd.	C	79.0	H	7.79
Found	»	78.9	»	7.62

The picrate, $HOC_{13}H_{16}CH_2N(CH_2)_4O \cdot HOC_6H_2(NO_2)_3$ was obtained from the components in ethanol solution in the form of yellow needles, which melt at 230 °C (decomposition).

Calcd.	C	60.2	H	5.22
Found	»	59.2	»	5.13

4-Morpholinomethyl-3-hydroxyphenanthrene, $HOC_{14}H_8CH_2N(CH_2)_4O$. To 3-hydroxyphenanthrene (5.0 g, 0.026 mole, m. p. 114—116 °C) dissolved in 100 ml of glacial acetic acid was added a mixture of 5 ml (0.06 mole) of morpholine and 5 ml (0.05 mole) of 30 % formalin under stirring. The reaction mixture was left one day at room temperature and then poured into 1 % sodium hydroxide. The precipitate thus formed was washed with much water and dried. The crude product is a white powder and amounts to 6 g. Recrystallization from 96 % ethanol gave 3 g of long, needle-shaped crystals melting at 146—147 °C.

Calcd.	C	77.8	H	6.53	N	4.77
Found	»	76.9	»	6.27	»	4.82

The picrate, $HOC_{14}H_8CH_2N(CH_2)_4O \cdot HOC_6H_2(NO_2)_3$ was prepared in the usual manner in ethanol solution. Yellow needles, decomposition at 181—183 °C.

Calcd.	C	57.6	H	4.25
Found	»	57.1	»	4.32

1-Morpholinomethyl-2-hydroxyphenanthrene, $HOC_{14}H_8CH_2N(CH_2)_4O$. 2-Hydroxyphenanthrene (0.20 g, m. p. 168 °C), 0.2 ml of morpholine and 0.2 ml of 30 % formalin were dissolved in 8 ml of glacial acetic acid and left for one day at room temperature. Precipitation with 1 % sodium hydroxide in the usual manner gave 0.25 g of a white powder. By recrystallization from 96 % ethanol the pure compound was obtained as lustrous scales. Melting point 194—195 °C. Yield 0.22 g.

Calcd.	C	77.8	H	6.53
Found	»	77.0	»	6.50

The picrate $HOC_{14}H_8CH_2N(CH_2)_4O \cdot HOC_6H_2(NO_2)_3$ was prepared in ethanol solution. Yellow needles melting at 205 °C (decomp.).

Calcd.	C	57.6	H	4.25
Found	»	56.7	»	4.21

1-Piperidinomethyl-2-hydroxyphenanthrene, $HOC_{14}H_8CH_2N(CH_2)_5$. 2-Hydroxyphenanthrene (0.50 g, m. p. 168 °C), 0.5 ml of piperidine and 0.5 ml of 30 % formalin were dissolved in 25 ml of glacial acetic acid. After one day at room temperature 200 ml of 1 % sodium hydroxide were added and the precipitate formed drawn off, washed and dried. Yield 0.70 g. Recrystallization from 96 % ethanol gave 0.57 g of long needles with the melting point 153—154 °C.

Calcd.	C	82.5	H	7.29	N	4.81
Found	»	81.4	»	7.11	»	4.76

The picrate, $HOC_{14}H_8CH_2N(CH_2)_5 \cdot HOC_6H_2(NO_2)_3$ from ethanol. Yellow needles. M. p. 198 °C (decomp.).

Calcd.	C	60.0	H	4.66
Found	»	58.5	»	4.60

Amination. 4-Piperidinomethyl-3-hydroxyretene (1.00 g), 1.0 g of ammonium chloride, 1.7 g of anhydrous sodium acetate and 1.0 g of glacial acetic acid were heated together in a sealed tube at 250 °C for 14 hours. The reaction product was filtered off, washed with water and dried, giving 0.78 g of a brown powder. On recrystallization from toluene 0.43 g of a white substance was obtained, melting at 239—241 °C.

$CH_3CONHC_{18}H_{17}$	Calcd.	C	82.4	H	7.28	N	4.81
	Found	»	83.1	»	7.32	»	4.87

Mixing the product with an authentic sample of 3-acetaminoretene did not result in any depression of the melting point. By boiling for some hours with acetic anhydride the diacetate was formed, m. p. 222—223 °C (*cf.* Karrman and Sihlbom [11] 221.5—222 °C).

4-Morpholinomethyl-3-hydroxyretene treated in the same manner also gave 3-acetaminoretene.

Attempts to determine the structure of the Mannichs bases

1. *With cyanogen bromide.* 4-Piperidinomethyl-3-hydroxyretene (0.5 g) and cyanogen bromide (0.2 g) were dissolved in ether. No noticeable reaction was observed. The solution was boiled for a few minutes; after evaporation of the solvent a yellow resinous oil remained. 4-Morpholinomethyl-3-benzoxyretene (0.2 g) and cyanogen bromide (0.05 g) dissolved in 5 ml of ether and 10 ml of 96 % ethanol were boiled for twenty minutes. After evaporation of the solvent the unchanged substance was recovered. This was again treated with cyanogen bromide in chloroform in a sealed tube at 100 °C for six hours. Unchanged substance together with a resinous oil was obtained.

2. *With sodium methoxide.* To a solution of 0.65 g of 4-piperidinomethyl-3-hydroxyretene in 10 ml of methanol in a tube 1.0 g of sodium was added. When the later has dissolved the tube was sealed and heated to 170—180 °C for two and a half hours. The viscous reaction product was treated with diluted hydrochloric acid and extracted twice with 20 ml of ether. The ether solution was dried with anhydrous sodium sulphate; after evaporation of the solvent 0.54 g of a substance, m. p. 162—163 was left behind.

On mixing with an authentic sample of 3-hydroxyretene no depression of the melting point was observed.

Calcd.	C	85.0	H	7.25
Found	»	85.5	»	7.03

The above experiment was repeated at 190—200 °C and a heating time of 43 hours. Only resinous products were obtained. Under milder conditions (130 °C, 20 hours) 3-hydroxyretene was formed in a high yield (80 per cent).

4-Morpholinomethyl-3-hydroxyretene (0.5 g), 10 ml of methanol and 1.0 g of sodium in a sealed tube at 130 °C for 14 hours gave 3-hydroxyretene in a yield of 75 per cent.

A mixture of 4-morpholinomethyl-3-hydroxyphenanthrene (0.5 g), 14 ml of methanol and 1 g of sodium was heated to 200 °C for 14 hours. The reaction product was treated with diluted hydrochloric acid and after drying the precipitate was recrystallized twice from a mixture of bensene and ligroin. M. p. 119—120 °C, picrate, m. p. 159 °C. The compound was identical with 3-hydroxyphenanthrene. The same result was obtained with 4-piperidinomethyl-3-hydroxyphenanthrene.

4-Morpholinomethyl-2-hydroxyphenanthrene was treated exactly as the 3-isomer above. The reaction resulted in a complicated mixture, from which 2-hydroxyphenanthrene could be isolated as the picrate, m. p. 156—158 °C. The picrate was dissolved in benzene and filtered through aluminium oxide giving pure 2-hydroxyphenanthrene. M. p. 168 °C.

3. *With thiols.* 4-Piperidinomethyl-3-hydroxyretene (0.20 g) and 0.3 g of isoamylthiol were heated together in a sealed tube for 6 hours at 130 °C. The reaction product was poured into water and the precipitate formed recrystallized from ligroin. Yield 0.1 g, m. p. 161—163 °C. The compound was identical with 3-hydroxyretene.

Hydrogenations

1. *Over copper chromite*[12]. 4-Piperidinomethyl-3-hydroxyretene (0.7 g), 15 ml of dioxane and 0.5 g of catalyst were heated to 180 °C for 3 hours at a hydrogen pressure of 200 at. After removal of catalyst, solvent and basic compounds, 0.6 g of a brown crystalline mass was obtained. Purification by repeated recrystallizations from ethanol gives 0.1 g of coulerless plates, m. p. 85—87 °C.

Found C 91.8 H 7.45 $\sum$ 99.3

The product is thus a hydrocarbon, probably a mixture of chiefly retene and dihydroretene.

2. *Over Raney-nickel*[13]. 1-Morpholinomethyl-2-hydroxyphenanthrene (2.0 g), 125 ml of dioxane and about 1 g of catalyst are hydrogenated at 185 °C for 3 hours under 100 at. Then the catalyst is filtered off and the solvent removed giving 2.3 g of a brown oil. This was dissolved in ether, gaseous hydrochloric acid was passed into the solution, the precipitate formed drawn of, the solution dried with anhydrous sodium sulphate and carbonate and evaporated to dryness. 1.3 g of a half-solid mass was obtained which on attempts at recrystallization from benzene-ligroin only gave an oil. The solution was then diluted with more solvent and filtered through aluminium oxide (Brockmann) and then partly evaporated, whereby a mixture of oil and crystals were obtained. The crystals were separated and recrystallized from benzene-ligroin; yield 0.11 g m. p. 195.5—197 °C.

(According to the literature [8] 1-methyl-2-hydroxyphenanthrene melts at 196—197 °C.) Mixed with an authentic sample of 1-methyl-2-hydroxyphenanthrene no depression of the melting point could be observed.

The picrate, $HOC_{14}H_8CH_3 \cdot HOC_6H_2(NO_2)_3$, had a m. p. of 162—164 °C.

Calcd.	C	57.7	H	3.45
Found	»	58.4	»	3.44

4-Morpholinomethyl-3-hydroxyphenanthrene (2.6 g), 75 ml of dioxane and about 1 g of catalyst were hydrogenated at 150 °C for 3 ½ hours. Hydrogen pressure 80 at. From the reaction mixture the catalyst and the solvent were removed leaving behind 2.8 g of a brown oil. This oil was dissolved in ether and gaseous hydrochloric acid passed into the solution. The precipitate was filtered off and the solution dried and evaporated to dryness, giving 1.4 g of a brown oil. This oil was dissolved in a mixture of benzene and ligroin and filtered through aluminium oxide; after removing of the solvent 1.2 g of a brown oil remained. The oil was dissolved in benzene and picric acid added, giving a solid picrate, which melted at 140—145 °C. After one recrystallization from benzene the m. p. was 144—146 °C. This product, dissolved in benzene, was filtered through aluminium oxide (Brockmann), the solvent removed, and a white product was obtained, 0.2 g, m. p. 90.5—92 °C. Crystallization from a mixture of benzene and hexane gave 0.1 g with the melting point 92—93 °C. Further recrystallizations did not change the melting point. The product was a methyl-3-hydroxyphenanthrene according to the analysis.

$C_{15}H_{12}O$	Calcd.	C	86.6	H	5.81
	Found	»	86.5	»	6.05

For comparision 4-methyl-3-hydroxyphenanthrene was prepared from 3-hydroxyphenanthrene-4-aldehyde by reduction according to Clemmensen. The aldehyde (0.40 g m. p. 92—93.4 °C) was dissolved in 20 ml of absolute ethanol whereupon 5 ml of concentrated hydrochloric acid and about two grams of amalgamated zinc were added. The originally yellow reaction mixture, gradually grows ligther during reflux and was after two hours colourless. After cooling it was poured into water and the precipitate crystallized from 96 % ethanol. Yield 0.25 g, m. p. 91.5—92.5 °C.

$C_{15}H_{12}O$	Calcd.	C	86.6	H	5.81
	Found	»	85.7	»	5.67

The methyl-3-hydroxyphenanthrene obtained by hydrogenation did not depress the melting point of 4-methyl-3-hydroxyphenanthrene prepared as above.

Also the picrates and the benzoylderivatives have been prepared and the melting points compared as is seen in table 1.

4-Morpholinomethyl-3-hydroxyretene (7.5 g), 100 ml of dioxane and about 2 g of catalyst at 160 °C for two hours. Hydrogen pressure 100 at. The further treatment of the reaction mixture showed that it was much more difficult here to isolate definite chemical compounds than in the preceeding cases. However, through systematic recrystallizations, purification over picrates and chromatographic separation with aluminium oxide among others 0.3 g of 3-hydroxyreten and 0.3 g of 4-methyl-3-hydroxyretene m. p.

Table 1. Comparision of the methylphenols.

Substance	M. p.	Picrate m. p.	Benzoylderiv. m. p.
4-Methyl-3-hydroxyphenanthrene	91.5—92.5	154—156	142—143
Methyl-3-hydroxyphenanthrene from hydrogenation	92—93	153—155	141—142

127 °C were obtained. The last mentioned compound gave a *picrate* m. p. 139—140 °C (Karrman[7] 139—140 °C) and a benzoylderivative m. p. 150—151° Karrman[7] 151—152°). The corresponding piperidino-derivative gave the same result by hydrogenation.

SUMMARY

Mannich bases have been prepared from 2- and 3-hydroxyphenanthrene and 2- and 3-hydroxyretene. These phenols with the exception of 2-hydroxyretene condense easily with formaldehyde and piperidine or morpholine in glacial acetic acid solution at room temperature under the formation of monoderivatives. 2-Hydroxyretene reacts only under more rigorous conditions.

By hydrogenation at a pressure of 80—100 at over Raney-nickel among other things a cleavage of the carbon-nitrogen bond occurs under the formation of the corresponding methylphenols. In this way 4-methyl-3-hydroxyretene was obtained from morpholino- (and piperidino-) methyl-3-hydroxyretene and 4-methyl-3-hydroxyphenanthrene from morpholino-methyl-3-hydroxyphenanthrene. 1-Methyl-2-hydroxyphenanthrene was obtained by the hydrogenation of morpholino- (and piperidino-) methyl-2-hydroxyphenanthrene. Thus the structure of these Mannich bases has been fully established. It is very probable that the Mannich base of 2-hydroxyretene is substituted in the 3-position.

The reactivity of the hydroxyl group and substituted aminomethyl group was shown to be diminished, which probably is due to chelation.

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Received October 13, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1554–1559

Investigations in the Retene Field

III. 4,9-Dinitroretene

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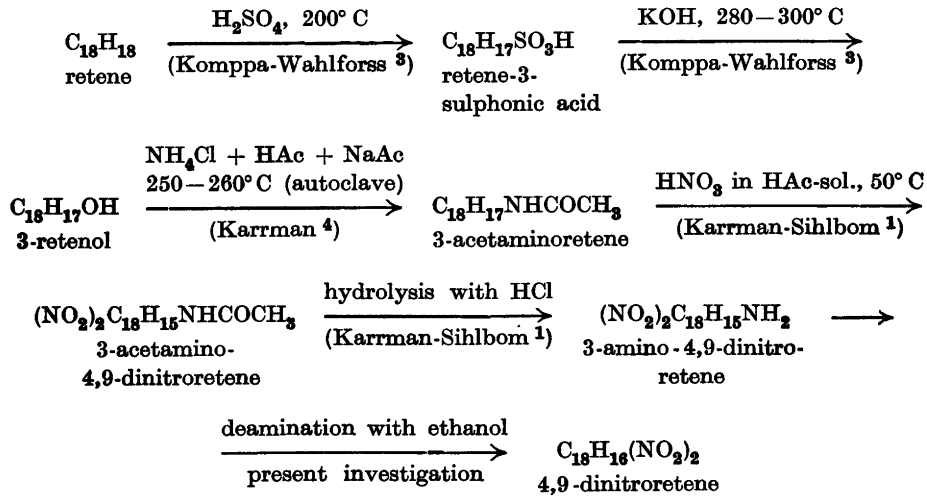
The nitration of 3-acetaminoretene, under mild conditions, yields both 3-acetamino-4-nitroretene and 3-acetamino-9-nitroretene [1-2]. From these derivatives, 4-nitroretene (Sihlbom [2]) and 9-nitroretene (Karrman-Sihlbom [1]) have been prepared. These were the first nitro-derivatives of retene to be synthesized. By utilizing stronger nitrating conditions, a 55 % yield of 3-acetamino-4,9-dinitroretene can be obtained from 3-acetaminoretene [1-2]. In the present paper the preparation of 4,9-dinitroretene from 3-acetamino-4,9-dinitroretene is described.

The following reaction scheme shows the synthesis of 4,9-dinitroretene from retene (the first part of this scheme is also presented in another retene investigation by Karrman-Sihlbom [1]):



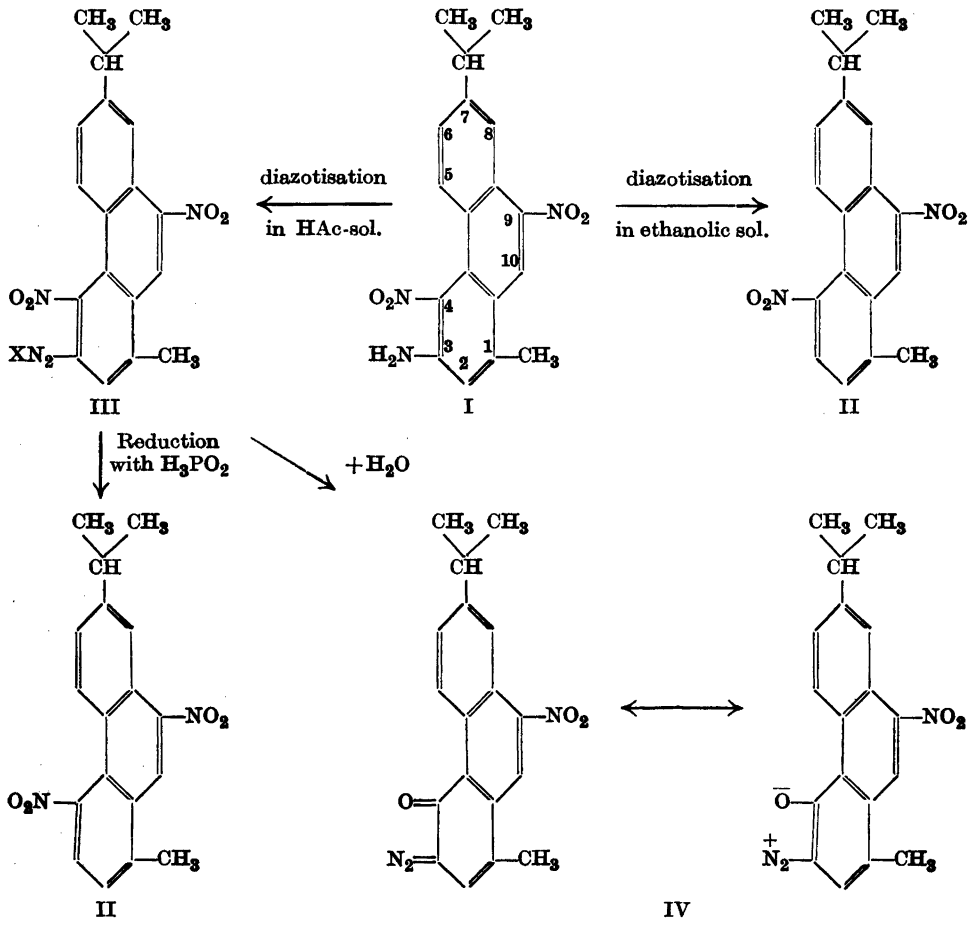
The yield is approximately 10 % for the total synthesis.

The hydrolysis of 3-acetamino-4,9-dinitroretene in ethanol, containing conc. hydrochloric acid, yielded the free amine and not its hydrochloride. This result indicates that the two nitro-groups have considerably reduced the basic characteristics of the amine. Various attempts have been made to obtain the salt of this nitro-amine. If an excess of conc. sulphuric acid is added to a warm solution of the amine in either ethanol or glacial acetic acid, the amine sulphate crystallizes on cooling. The acetic acid solution requires a relatively small excess of sulphuric acid.

If an excess of conc. hydrochloric acid is added to the warm glacial acetic acid solution, the nitro-amine decomposes. In this solution, it appears as if the hydrochloric acid causes a nitro-group to split off. At the same time, a diazonium salt is formed which then gradually decomposes. This reaction will be discussed further in a later paper. — If conc. hydrochloric acid is added to a cool solution of the nitro-amine in glacial acetic acid, the amine hydrochloride can be obtained.

As can be seen from the reaction scheme below, 4,9-dinitroretene (II) has been prepared by the deamination of 3-amino-4,9-dinitroretene (I). The diazonium salt (III) was reduced with hypophosphorous acid or ethanol. The latter reducing agent is preferable, as the reaction solution should contain as little water as possible. The presence of water causes the diazonium salt to decompose into a brick-red product, probably 9-nitroretene-3-diazo-4-oxide (IV). This substance is apparently decomposed by light, since exposure to daylight for some hours causes the colour to change from brick-red to grey. In the dark, the colour does not change even after a considerable time. The diazo-oxide is slightly soluble in the usual organic solvents and decomposes violently on heating. An ethanolic solution of the diazo-oxide gives a green colour with an alkaline resorcinol solution. These proporties are in agreement with those expected for a diazo-oxide [5]. This substance will be further discussed in a later publication. — Other examples of the formation of diazo-oxides from aqueous solutions of diazonium salts of nitro-amines, are given by Kornblum in *Organic reactions* [6].

In view of the abovementioned characteristics of the nitro-amine and its diazonium salt, the deamination was accomplished in ethanol in the presence of a large amount of sulphuric acid. The nitrous acid was added as nitrosyl sulphuric acid. The diazonium salt is reduced as fast as it is formed, to 4,9-dinitroretene (II) which precipitates and can easily be removed by filtration. The crude product obtained was purified by chromatography. Pure dinitroretene was obtained in a yield of 72 %, calculated on the nitro-amine consu-



med. By the chromatographic purification, 15 % of unreacted nitro-amine was also isolated.

The reduction of 4,9-dinitroretene with stannous chloride yielded the corresponding diamine. This was obtained in the form of a difficultly soluble tin complex which, contrary to the corresponding complex of 3,4-diaminoretene [2], precipitated in a very impure form and was therefore not further examined. In order to obtain the free amine, the tin complex was suspended in ether and washed with an aqueous alkali solution. These reactions must be accomplished in a nitrogen atmosphere, since the amine solution otherwise becomes a deep red colour. The 4,9-diaminoretene has been characterized by the following simple derivatives: the hydrochloride, the trinitrobenzene double compound, the picrate, and the N,N'-diacetyl compound.

EXPERIMENTAL

4,9-Dinitroretene

5.0 g of 3-amino-4,9-dinitroretene was dissolved in 700 ml of boiling ethanol. The solution was cooled to 50° C when 200 ml of conc. sulphuric acid was added with cooling and stirring. When the solution was cooled to 5—10° C, rather large quantities of amine sulphate separated as crystals. At this temperature, a solution of 2.5 g of sodium nitrite in 35 ml of conc. sulphuric acid was added, with stirring, in the course of one hour. The diazonium salt formed was rapidly reduced by the ethanol to 4,9-dinitroretene, which was obtained as a yellow flocculent precipitate. The reduction was complete after the addition of the nitrite solution (no coupling reaction with β-naphtol). In order to assure complete precipitation, approx. 1 000 ml of water was added. The yield of crude product was 4.4 g. This was dissolved in a mixture of 75 ml of benzene and 75 ml of ligroin (b. p. 65—90° C). A little residue remained undissolved. From the dark solution, 4,9-dinitroretene and unchanged nitro-amine were isolated chromatographically. The solution was taken up in a column of alumina, 30 cm high and 3.5 cm diameter. On passing a mixture of 2 vol. of benzene and 3 vol. of petroleum ether (b. p. 40—60° C), 4,9-dinitroretene moves through the column as a faintly yellow band. From the percolate 2.9 g of completely pure dinitroretene (m. p. 170—171° C) was isolated. Recrystallization did not raise the melting point. *n*-Propanol is a suitable recrystallization solvent; in 100 ml of *n*-propanol 6 g of the compound can be dissolved at the boiling point and 0.5 g at 10° C. The compound is also readily soluble in acetone, benzene, and chloroform, slightly soluble in ligroin and ethanol. It crystallizes from propanol as pale-yellow needles.

$C_{18}H_{16}N_2O_4$ (324.3)	Calc.	C 66.6	H 4.97	N 8.64
	Found	» 66.8	» 5.04	» 8.72

In the chromatographic purification there was also formed a sharp reddish-brown band which moved very slowly through the column. After the addition of pure benzene, the chromatogram was better developed, and from the reddish-brown band 0.8 g of 3-amino-4,9-dinitroretene was isolated. Therefore, the yield of 4,9-dinitroretene, based on the amount of nitro-amine actually consumed, is 72 %.

9-Nitroretene-3-diazo-4-oxide

0.5 g of 3-amino-4,9-dinitroretene was dissolved in 80 ml of warm glacial acetic acid. 1 ml of conc. sulphuric acid was added and the solution was cooled to about 20° C. Most of the amine sulphate separated as nearly colourless crystals. A small excess of 20 % sodium nitrite solution was added. The clear diazonium salt solution was diluted with 80 ml of water. Almost immediately a brick-red precipitate began to separate. After 30 minutes it was filtered off, and washed with dilute acetic acid, ethanol, and ether. On heating it decomposes explosively and shows no definite melting point.

$C_{18}H_{15}N_3O_3$ (321.3)	Calc.	C 67.3	H 4.71	N 13.08
	Found	« 66.6	» 4.71	» 12.50

The colour of the diazo-oxide changes from brick-red to grey when exposed to daylight for about 6 hours. An ethanolic solution of the diazo-oxide gives a green colour with an alkaline resorcinol solution.

4,9-Diaminoretene

16 g of $SnCl_2 . 2H_2O$ was dissolved in 45 ml of glacial acetic acid containing hydrogen chloride. With cooling and stirring, 2.0 g of finely powdered 4,9-dinitroretene was added in small portions. The temperature was kept at about 20° C. As the reduction proceeds, 4,9-dinitroretene gradually dissolves, but a clear solution is not obtained because a white fine-grained product begins to separate before all the dinitroretene is reduced. After one hour, when the reduction was complete, the reaction mixture was saturated with hydrogen chloride and the precipitate was filtered off. This precipitate consists of tin salts and a tin salt complex of 4,9-diaminoretene (a pure tin salt complex like that of 3,4-diaminoretene [2] is not easily obtained in this case). Yield about 2.7 g. — As a solution of the diamine in ether turns red in the presence of air, the following operations were performed in a nitrogen atmosphere. — A suspension of the crude product in ether was shaken with a 10 % sodium hydroxide solution containing a few tenths of a gram of sodium hydrosulfite. After washing and drying the ether solution, the solvent was evaporated. The residue, a faintly reddish-brown viscous oil, soon crystallized forming a pale-yellow crystalline mass. Yield 1.1 g, corresponding to 67.5 % of the theoretical. One recrystallization from a mixture of 4 ml of benzene and 10 ml of ligroin (b. p. 65—90° C) yielded 0.9 g of the pure product, m. p. 114—115° C. It is readily soluble in ethanol, acetic acid, and benzene, slightly soluble in ligroin. 4,9-diaminoretene crystallizes as nearly colourless plates.

$C_{18}H_{20}N_2$ (264.4)	Calc.	C 81.8	H 7.63	N 10.60
	Found	» 82.1	» 7.60	» 10.45

The *hydrochloride* was obtained as white needle-shaped crystals when conc. hydrochloric acid was added to a solution of 4,9-diaminoretene in ethanol. The compound has no definite melting point. It gradually decomposes and turns dark at about 150° C. Titration showed that a dihydrochloride had been formed.

$C_{18}H_{22}N_2Cl_2$ (337.3) Calc. Cl 21.0 Found Cl 21.3

The *picrate.* 0.1 g of 4,9-diaminoretene was dissolved in 3 ml of hot ethanol containing 0.15 g of picric acid. On cooling, the picrate crystallized as flat yellow prisms. At 135° C it begins to decompose rather rapidly, but there is no definite melting point.

$C_{24}H_{23}N_5O_7$ (493.5)	Calc.	C 58.4	H 4.70
	Found	» 57.6	» 4.86

The *trinitrobenzene double compound* is very rapidly formed when two etanolic solutions containing sym. trinitrobenzene and 4,9-diaminoretene, respectively, are mixed. The double compound is only slightly soluble in ethanol, but can be recrystallized from this solvent. It crystallizes as long thin needles with dark greyish-brown colour. M. p. 224—225° C (decomp.).

$C_{24}H_{23}N_5O_6$ (477.5)	Calc.	C 60.4	H 4.85
	Found	» 60.3	» 4.81

N, N'-Diacetyl-4,9-diaminoretene. 1.0 g of 4,9-diaminoretene was dissolved in 25 ml of 80 % acetic acid. Two hours after the addition of 2 ml of acetic anhydride, the diacetyl compound formed was filtered off. Yield 0.9 g. M. p. 299—300° C. Recrystallization did not raise the melting point. Thus the compound is obtained directly in a pure state. It is slightly soluble in ethanol and benzene, and somewhat more soluble in glacial acetic acid. The substance crystallizes as short white needles.

$C_{22}H_{24}N_2O_2$ (348.4)	Calc.	C 75.8	H 6.94
	Found	» 75.8	» 6.98

SUMMARY

4,9-Dinitroretene and the corresponding 4,9-diaminoretene have been synthesized from 3-amino-4,9-dinitroretene. Some simple derivatives of the diamine have been prepared.

In the course of these syntheses it was found that the diazonium salt of 3-amino-4,9-dinitroretene is hydrolyzed by water to a brick-red substance, probably 9-nitroretene-3-diazo-4-oxide.

It has also been found that 3-amino-4,9-dinitroretene, dissolved in hot glacial acetic acid, reacts with conc. hydrochloric acid forming a diazonium salt. It may be assumed that the first step of this intramolecular diazotization is the replacement of one of the two nitro-groups by chlorine.

I wish to express my gratitude to *Stiftelsen Bokförlaget Natur och Kultur* for financial support.

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Received October 19, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1560–1566

Isomorphous Thiosulphates of Divalent Sulphur, Selenium and Tellurium

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The purpose of this article is to report the preliminary results of a crystallographic study of some salts of pentathionic, selenopentathionic and telluropentathionic acids. The anions of these acids, *viz.* :

$$S(S_2O_3)_2^{--}; \qquad Se(S_2O_3)_2^{--}; \qquad Te(S_2O_3)_2^{--}$$

are, as indicated in the title, thiosulphates of divalent sulphur, selenium and tellurium [1-3]. The salts investigated include potassium and rubidium pentathionate, and ammonium, potassium, rubidium and cesium selenopentathionate and telluropentathionate.

SYNTHESIS OF NEW SALTS

Potassium pentathionate hemitrihydrate, the first salt of pentathionic acid isolated in a pure state, has been known since 1888 [4]. Rubidium pentathionate, described for the first time in this article, was prepared from the potassium salt by metathesis with sodium perchlorate and, subsequently, with rubidium chloride. The rubidium salt, like the potassium analogue, crystallizes with one and a half mole of water.

The only salts of selenopentathionic and telluropentathionic acids prepared hitherto are the sodium and potassium salts, which were isolated by one of us recently [2,3]. Thus, the ammonium, rubidium and cesium salts, described below, are new.

Ammonium selenopentathionate and telluropentathionate were obtained from ammonium thiosulphate and selenious or tellurous acid in the same way as the sodium salts [2,3]. The salts are less soluble than ammonium tetrathionate, which is formed in these reactions in equimolar proportions, and

crystallize from the mixtures contaminated with only a few per cent of the tetrathionate, as do the sodium salts [2,3]. In most cases a single recrystallization suffices to give the pure salts.

Rubidium and cesium selenopentathionate and telluropentathionate were prepared from the sodium salts by metathesis with rubidium and cesium chlorides.

Of the potassium, ammonium, rubidium and cesium selenopentathionates and telluropentathionates, only three salts are anhydrous, *viz.*, potassium and ammonium telluropentathionate, and cesium selenopentathionate. The remaining ones crystallize with one and a half mole of crystal water, like potassium and rubidium pentathionate, as appears from the following table:

$K_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	$K_2Te(S_2O_3)_2$
$(NH_4)_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	$(NH_4)_2Te(S_2O_3)_2$
$Rb_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	$Rb_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$
$Cs_2Se(S_2O_3)_2$	$Cs_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$

The hydrates in most cases crystallize more readily and show better developed forms than the anhydrous salts. Also, the hydrates have a brighter colour; thus, among the telluropentathionates the rubidium and cesium salts are distinctly orange red while the potassium and ammonium salts are yellow. The crystal water of the hydrates is kept very firmly, and is not liberated *in vacuo* over sulphuric acid.

The salts, when pure, may be kept for weeks without any change; however, gradually selenium or tellurium appears within some of the crystals of a sample.

The solubilities in water of the selenopentathionates and telluropentathionates decrease in the order sodium, ammonium, potassium, rubidium and cesium salts. The telluropentathionates are a little less soluble than the corresponding selenopentathionates. The sodium and ammonium salts are appreciably soluble in methanol, and also to a slight extent in 96 % ethanol, while the potassium, rubidium and cesium salts are insoluble in alcohols.

EXPERIMENTAL

Rubidium pentathionate. To 1.8 g (5 millimole) of potassium pentathionate dissolved, by gentle heating, in 3 ml of 0.5 *N* hydrochloric acid, was added 1.2 g (10 millimole) of sodium perchlorate dissolved in 2 ml of water. The mixture was cooled for a few minutes in ice water, the potassium perchlorate then filtered off, and 1.2 g (10 millimole) of rubidium chloride was added to the filtrate. On cooling in ice water, rubidium pentathionate crystallized as flat needles or oblong plates. Yield, 1.2 g (53 % of the theoretical amount). 1 g of the product was dissolved in 2 ml of 0.5 *N* hydrochloric acid at 40° C. On slow

cooling, rhombic bipyramidal crystals were obtained, of the shape described by Groth [5] for potassium pentathionate.

For analysis, about 0.25 millimole was dissolved to 50 ml in a volumetric flask, and 20 ml samples were pipetted out and analyzed by means of the sulphite [6] and the cyanide [1,6] method, using 0.01 *N* iodine.

0.1283 g substance: 20.56 ml (sulphite) and 10.32 ml (cyanide) of 0.01094 *N* iodine (for pentathionates, the sulphite value is, theoretically, twice the cyanide value).

$Rb_2S(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$ (454.3)	Calc.	S	35.29
	Found	»	35.18

Ammonium selenopentathionate and telluropentathionate. The reactions were carried out as described earlier for the sodium salts [2,3]. Suitable proportions of reagents are: 20 g of selenious acid (H_2SeO_3) dissolved in 20 ml of water and 100 ml of glacial acetic acid; 80 g of ammonium thiosulphate in 40 ml of water. 18.8 g of tellurium dioxide (from 15 g of tellurium) dissolved in 45 ml of concentrated hydrochloric acid and 75 ml of glacial acetic acid; 66 g of ammonium thiosulphate in 40 ml of water. The thiosulphate solutions were added drop-wise, under mechanical stirring, to the solutions of selenious and tellurous acid which were kept at a temperature of about 0° C by means of an ice-salt freezing mixture. The stirring and cooling were afterwards continued for about 10 minutes. The products, which contained 30—35 g of the desired salts, with 3—8 mole % of ammonium tetrathionate, were subsequently filtered off, drained well, washed with a little ethanol and with ether, and dried *in vacuo* over sulphuric acid. The selenium salt was recrystallized by dissolving in about an equal amount of 0.5 *N* hydrochloric acid at about 40° C, filtering with suction through a sintered glass filter, and cooling in ice-water. The tellurium salt was dissolved in three halfs of its weight (or a little less) of the same solvent at about 35° C, and after filtering, an equal amount of ethanol was added. Yields, about 20 g of pure products.

0.2071 g substance: 21.56 ml of 0.1074 *N* sodium thiosulphate (Norris & Fay, after oxidation by means of bromate as described earlier [2]).

$(NH_4)_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$ (366.3)	Calc.	Se	21.55
	Found	»	21.46

0.2050 g substance: 20.92 ml of 0.1007 *N* iodine (titrated directly, as described earlier[3]).

$(NH_4)_2Te(S_2O_3)_2$ (388.0)	Calc.	Te	32.89
	Found	»	32.76

Rubidium and cesium selenopentathionate and telluropentathionate. To 0.5 millimole of the sodium salts (2.1 g and 2.2 g, respectively) dissolved in 5 ml of 0.5 *N* hydrochloric acid (traces of selenium or tellurium were filtered off, when necessary) were added, at room temperature, 10 millimole of rubidium or cesium chloride (1.2 g and 1.7 g, respectively) dissolved in 5 ml of water. The salts rapidly crystallized in a pure state.

Samples, which on standing had liberated selenium or tellurium, were purified by dissolving in 0.2 *N* hydrochloric acid at 40—50° C, filtering with suction through a sintered glass filter, and cooling in ice water. The same procedure was used for recrystallizations.

The analyses were performed volumetrically in the same way as for the ammonium salts.

0.3881 g substance: 29.89 ml of 0.1027 *N* sodium thiosulphate.

$Rb_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$ (501.2)	Calc.	Se	15.75
	Found	»	15.62

0.07594 g substance: 5.19 ml of 0.1027 *N* sodium thiosulphate.

$Cs_2Se(S_2O_3)_2$ (569.0)	Calc.	Se	13.88
	Found	»	13.86

0.3090 g substance: 23.11 ml of 0.09754 *N* iodine.

$Rb_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$ (549.9)	Calc.	Te	23.20
	Found	»	23.27

0.2487 g substance: 15.80 ml of 0.09754 *N* iodine.

$Cs_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$ (644.7)	Calc.	Te	19.79
	Found	»	19.76

X-RAY DATA

The unit cells and space groups were determined from rotation, oscillation and Weissenberg photograhs on small single-crystal specimens, using FeK_α radiation, $\lambda = 1.934$ Å. The values recorded below for axial lengths are accurate to within 0.5 %. Density determinations were made by flotation in suitable solvents.

Six of the salts have the same orthorhombic bipyramidal space group and unit cells of nearly the same size, and are thus isomorphous. These salts include potassium pentathionate, ammonium selenopentathionate, cesium telluropentathionate, and the three rubidium salts.

Table 1. Isomorphous hemitrihydrates. Axial lengths in Å.

Salt	*a*	*b*	*c*	Density	
				calc.	found
$K_2S(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	20.09	9.17	12.30	2.12	2.11 [5]
$Rb_2S(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	20.81	9.26	12.50	2.51	2.50
$(NH_4)_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	20.58	9.24	12.57	2.03	2.01
$Rb_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	20.84	9.23	12.48	2.76	2.71
$Rb_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	21.14	9.35	12.46	2.96	2.91
$Cs_2Te(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$	21.76	9.55	12.91	3.19	3.16

There are eight molecules per unit cell. Absent spectra, for all six salts: $(hk0)$ when $h+k$ is odd, $(h0l)$ when l is odd, and $(0kl)$ when k is odd. The space group is therefore D_{2h}^{14}—*Pbcn*.

Of the above salts, potassium pentathionate, the only one which is not new, is described by Groth [5], as orthorhombic bipyramidal with axial ratios $a:b:c = 0.4564:1:0.3051$. The X-ray data give the ratios $0.4565:1:0.6124$ which show that the c axis of Groth must be doubled. In the X-ray unit, the a and b axes of Groth have been interchanged in order to comply with the International Space Group Nomenclature; the c axis of Groth (needle axis) has been retained.

The crystals of the five other salts of Table 1 show the same morphology as potassium pentathionate as described by Groth. Occasionally, in some preparations, other shapes were observed; *e. g.*, rubidium pentathionate was first obtained as flat needles elongated along the c axis and flattened along the b axis of the X-ray unit, the most prominent form thus being $\{100\}$.

Potassium selenopentathionate, $K_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$, has the same axial lengths as the hemitrihydrates of Table 1: $a = 20.18$ Å, $b = 9.21$ Å, $c = 12.24$ Å. There are, likewise, eight molecules per unit cell; density, calc. 2.38, found 2.36. However, the only reflexions which are entirely absent are $(0kl)$ when k is odd, which is characteristic for the space group D_{2h}^{5}—*Pbmm* and the space groups of lower symmetry, C_{2v}^{2} or C_{2v}^{4}. The morphology of the crystals is the same as that of the other hemitrihydrates, *viz.*, orthorhombic bipyramidal, indicating that D_{2h}^{5}—*Pbmm* may be the correct space group. The reflections distinguishing from D_{2h}^{14}—*Pbcn*, *viz.*, $(hk0)$ when $h + k$ is odd and $(h0l)$ when l is odd, are present only in a limited number. The elongation of the crystals along the c axis is frequently quite pronounced, thus giving the crystals the appearance of prismatic needles.

The only selenopentathionate, of the four ones examined, which crystallizes without crystal water, is the cesium salt, $Cs_2Se(S_2O_3)_2$. It appears as prisms having a great tendency to branch off at the ends and which in most cases are twins. From oscillation and Weissenberg photographs around the a axis (needle axis) the crystals definitely appear to be orthorhombic, with the axial lengths, $a = 6.40$ Å, $b = 18.16$ Å, $c = 9.74$ Å. Density, calc. on the basis of four molecules per unit cell, 3.32, found, 3.30. Absent reflections, $(hk0)$ when k is odd, and $(00l)$ when l is odd. These extinctions do not, however, fit with any orthorhombic space group, and further work is required.

Potassium and ammonium telluropentathionate, both anhydrous, are monoclinic prismatic, but not isomorphous. They appear as prisms, the former elongated along the b axis, the latter along the a axis. In both cases, as for cesium selenopentathionate, the needle axis is the shortest axis. The salts frequently crystallize in rosette-shaped aggregates of crystals too tiny for single-crystal X-ray work. The cell dimensions are as follows.

Potassium telluropentathionate, $K_2Te(S_2O_3)_2$. $a = 11.20$ Å, $b = 5.54$ Å, $c = 16.64$ Å, $\beta = 91.2°$. Four molecules per unit cell; density, calc. 2.75,

found 2.74. Absent spectra, ($h0l$) when $h + l$ is odd, and ($0k0$) when k is odd. Space group, C_{2h}^5—$P2_1/n$.

Ammonium telluropentathionate, $(NH_4)_2Te(S_2O_3)_2 \cdot a = 5.03$ Å, $b = 18.90$ Å, $c = 11.48$ Å, $\beta = 91.0°$. Four molecules per unit cell; density, calc. 2.35, found 2.34. Absent spectra, ($h0l$) when l is odd, and ($0k0$) when k is odd. Space group, C_{2h}^5—$P2_1/c$.

STRUCTURAL RELATIONSHIP OF THE ANIONS

The fact that pentathionic, selenopentathionic and telluropentathionic acids give salts which are isomorphous, strongly indicates that the anions have analogous structures. This finding is in accordance with the chemical reactivity of the anions [1–3].

The possibilities for the salts to crystallize as hemitrihydrates appear to depend upon the relative sizes of the cations and of the divalent atoms, sulphur, selenium and tellurium. The ionic radii of the cations and the covalent radii of the atoms (in Å) are [7]:

K^+	NH_4^+	Rb^+	Cs^+	S	Se	Te
1.33	1.48	1.48	1.69	1.04	1.17	1.37

The cation should apparently be larger than the divalent atom but not too much larger, as evidenced by the fact that potassium telluropentathionate, on one side, and cesium selenopentathionate, on the other side, do not fit into the isomorphous series. In other words, the cation and the divalent atom should not have too different atomic numbers. Rubidium, which is of medium size, is the only cation which gives isomorphous salts with all three acids. Although the cesium sulphur salt, cesium pentathionate, has not been investigated, it may be predicted, on this basis, that it is not a hemitrihydrate.

SUMMARY

Some new salts of pentathionic, selenopentathionic and telluropentathionic acids are described, *viz.*, rubidium pentathionate, and ammonium, rubidium and cesium selenopentathionate and telluropentathionate.

Unit cells and space groups are reported for these and for the potassium salts. Six of the salts, two of each acid, are isomorphous, which shows that the anions have analogous structures.

The authors are indebted to Prof. H. Haraldsen for the use of his X-ray apparatus.

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Received October 25, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1567–1574

Studies Related to Naturally-Occuring Acetylene Compounds

V. * Dehydro Matricaria Ester (Methyl *n*-decene-triynoate) from the Essential Oil of *Artemisia vulgaris* L.

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The two plants, *Lachnophyllum gossypinum* Bge. and *Matricaria inodora* L., which belong to the family *Compositeae*, have as components of their essential oils highly unsaturated derivatives of methyl caprate: *viz.* the lachnophyllum ester (I) [1], the matricaria ester (II)[2], and the cumulene hexahydro

$$H_3C-CH_2-CH_2-C\equiv C-C\equiv C-CH = CH-COOCH_3 \quad (I)$$

$$H_3C-CH = CH-C\equiv C-C\equiv C-CH = CH-COOCH_3 \quad (II)$$

$$H_3C-CH_2-CH_2-CH_2-CH_2-CH = C = C = CH-COOCH_3 \quad (III)$$

matricaria ester (III)[3]. As mentioned in a previous communication of this series [4], a crystalline compound m. p. 32.5—33° belonging to this enyne class was isolated in a pure state, as early as 1907, from the essential oil of a plant from Java, which the collector Dr. Carthaus named *Artemisia lavandulaefolia* [5]. Chemical investigations on this crystalline compound were reported in 1910 [6]. The composition was given as $C_{12}H_{14}O_2$, and with our knowledge of the properties and reactions of the compounds I—III it became obvious that the compound $C_{12}H_{14}O_2$ described by Dr. Carthaus must be closely related. Consequently, we were anxious to re-investigate the constituents of Artemisia oil. The paper published in 1910 states, however, that the plant must have been erroneously identified, as *A. lavandulaefolia* does not occur in Java, and in

* It is considered convenient to classify our papers on natural enynes under a common heading. The first communication of this series is reference 2 of this paper, the second, R. Holman and N. A. Sørensen, *Acta Chem. Scand.* **4** (1950) 416, the third reference 4, and the fourth reference 3 of this paper.

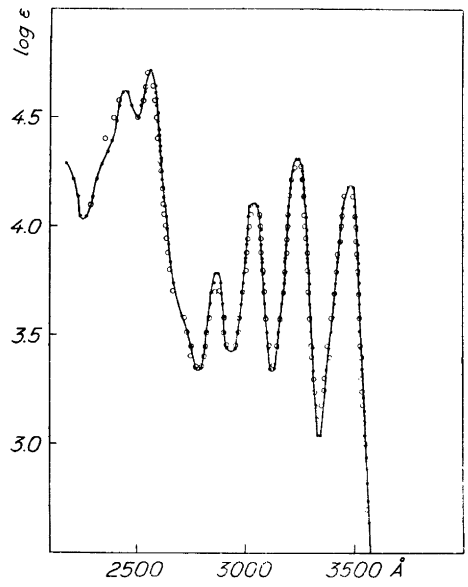

Fig. 1. Ultraviolet absorption of dehydro matricaria ester in hexane.

▬·▬·▬·▬ *from the essential oil of Artemisia vulgaris L.*

○ ○ ○ ○ *from cold extracts of Artemisia vulgaris L.*

$(\varepsilon = \frac{\log I_0/I}{c \cdot d}$, *c in mol/l, d in cm)*

fact Dr. H. M. van Raalte of Treub Laboratorium, Bogor-Djawa (formerly Buitenzorg) states in a private communication that *A. lavandulaefolia* does not exist at all. This name has arisen through a misunderstanding of a Latin term used by N. L. Burman in his *Flora Indica*. According to Dr. van Raalte only *Artemisia vulgaris* L. does occur in Java.

The essential oil of *Artemisia vulgaris* L. and some of its subspecies and variants, which have had a widespread use in medicine, have been investigated a number of times and the older investigations have been summarized by Gildemeister and Hoffmann [7]. The only definitely identified components of the essential oil are cineol and thujyl alcohol.

In anticipation of a forthcoming investigation of *A. vulgaris* L. from Java, we have carried out a reinvestigation of the essential oil of European mugwort (*Artemisia vulgaris* L. s. s.). The oil from the leaves and flowering parts of the plant does not show any longwave U. V.-absorption and consequently must be devoid of highly unsaturated compounds such as I—III. The essential oil from the root, however, contains at least three different compounds of this class, two of which crystallize well, but are quite different from the crystalline compound $C_{12}H_{14}O_2$ described by Dr. Carthaus. Steam distillation of roots yields an oil, which is remarkably dark in colour and which deposits crystals immediately on cooling, a behaviour observed as long ago as 1826 [8].

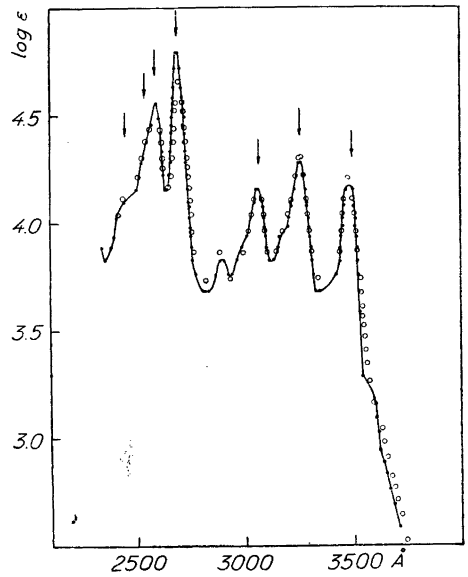

Fig. 2. Ultraviolet absorption of "Centaur X" fractions.

▬·▬·▬·▬ *best chromatographic fraction from mugwort root oil*

○ ○ ○ ○ *same fraction distilled 65°/10^{-4} mm Hg*

arrows: "Centaur X" maxima after Löfgren [9]

Crystallisation from petroleum ether gave slightly yellow crystalline material of m. p. 112° C which had the composition $C_{11}H_8O_2$, thus containing two hydrogen atoms less than the matricaria ester. On hydrogenation it consumed 7 moles of H_2, the perhydro compound obtained being methyl caprate as is the case with matricaria ester. The only remaining question then is the positions of the ethylenic and the three acetylenic bonds in the carbon chain. It seems probable on account of the high m. p. and the pronounced acetylenic fine structure of the long wave U. V.-absorption band — compare Fig. 1 — that the three acetylenic links lie in conjugation, and so there are the two possible formulae, IV a and IV b.

$$H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-COOCH_3 \qquad \text{IV a}$$

$$H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-COOCH_3 \qquad \text{IV b}$$

The choice between these two possibilities will be treated in a following communication.

By extraction with acetone at room temperature and chromatographic analysis of the extract we have established that this dehydro matricaria ester is a genuine component of the mugwort root oil.

Simultaneously, spectrographic investigation of the products from the chromatogram revealed that the mugwort contains at least two more compounds of the same class. Whereas petroleum ether elutes only a sesquiterpene,

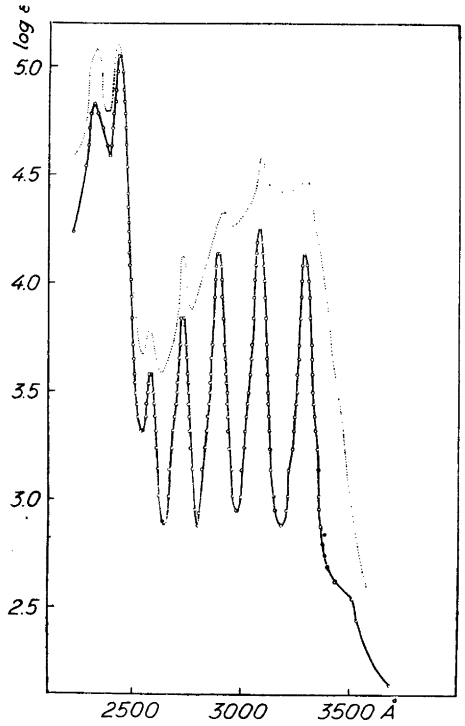

Fig. 3. Ultraviolet absorption of the compound $C_{12}H_{10}O$ from mugwort root oil in hexane (—○—○—○—) and of its hydrazone with phenylhydrazine p-carbonic acid in abs. ethanol (·····)

petroleum ether containing 20 % of benzene takes out a liquid component with a U. V.-spectrum as sharply structured as that of dehydromatricaria ester (compare Fig. 2).

In Fig. 2 the arrows show the positions given by Löfgren [9] for the U. V.-maxima of "Centaur X", a substance of unknown structure discovered by Löfgren in many *Centaurea* species. As will be seen, five of the maxima given by Löfgren coincide with the maxima from this mugwort fraction, and, as far as we can judge from his photographs, the relative intensities of these five bands, which are the main bands, are also in complete agreement. It may be mentioned that a very similar spectrum has been obtained from products prepared by chromatography of extracts of *Matricaria inodora* flowers and from the root of *Erigeron canadense* L., so that we are inclined to suppose that Löfgrens "Centaur X" has a rather widespread occurrence in compositae plants.

Whereas the "Centaur X" extracts from *Matricaria inodora* flowers were very dilute, the mugwort gave more concentrated solutions, with high extinction coefficients. Since "Centaur X" is predominantly epiphasic between petroleum ether b. p. $< 40°$ C and 92 % methanol, whereas matricaria ester and dehydro matricaria ester are predominantly hypophasic, "Centaur X"

should be a highly unsaturated hydrocarbon. A preliminary analysis carried out on an insufficient amount of material supports this assumption. "Centaur X" may be distilled without decomposition at 65° C 10^{-4} mm Hg.

The third enyne component of the mugwort oil is eluted along with the dehydro matricaria ester and this mixture is rather difficult to separate because mixed crystals form readily. Only very small amounts of the new compound, which crystallises in colourless needles m. p. 52—52.5° C, have been isolated free from dehydro matricaria ester. The U. V.-spectrum of this third component has the most outstanding fine structure of them all (compare Fig. 3). The fine structure is one of the most beautiful so far observed in solution spectra. The spectrum of benzene, for example, which is well known for the fine structure of its weak long wave U. V.-bands has a half width of only 22 Å, and a ratio (at room temperature) between a maximum and the following minimum of about 4. The corresponding figures for the third component of the mugwort oil are 45 Å and not less than 19.

The elementary composition seems to be $C_{12}H_{10}O$ and the oxygen atom is present as a carbonyl group as the compound gives a beautifully crystalline hydrazone with phenylhydrazine *p*-carbonic acid. The pronounced acetylenic spacing in the fine structure of the long wave U. V.-spectrum indicates a poly-yne structure. In a carbonyl compound $C_{12}H_{10}O$ this implies an aliphatic structure containing 14 hydrogen atoms less that the corresponding saturated carbonyl compound. We are inclined to believe that the compound $C_{12}H_{10}O$, like dehydro matricaria ester, contains 3 /≡ and 1 /=. We are returning to the structure of this interesting compound as soon as more starting material is available.

EXPERIMENTAL

40 kg of the flowering tops of common mugwort was distilled with steam. The essential oil could be separated directly and amounted to 20 g. This flower oil does not solidify at − 15° C and is devoid of selective absorption above 2 900 Å although the spectrum has a weak inflection, at ca. 3 400 Å.

5.5 kg of the root of mugwort was distilled in the same manner and yielded 1.1 g of yellow-brown oil which was taken up in petroleum ether. The pale yellow needles which separated readily from the petrol solution had m. p. 104—5°, raised by recrystallization to the constant value 113°.

U. V.-spectrum in hexane solution, Fig. 1.

λ_{max}	3 486	3 240	3 037	2 867	2 560	2 445
ν_{max} x10^{-12}	860.6	925.9	987.8	1 046.4	1 171.9	1 227.0
$\Delta\nu$ »		65.3	61.9	58.6		

$C_{11}H_8O_2$ (172.1) Calc. C = 76.73 H = 4.68
Found » = 76.8 » = 4.9

71.4 mg was hydrogenated at room temperature with 110 mgs of PdO_2-CaCO_3 catalyst. The activity of the catalyst was somewhat too great for the analytical measurement of the hydrogen consumption, as the initial rate of absorption (12 ml/min) made the zero reading somewhat uncertain.

$C_{11}H_8O_2$, 3 /=, 1/=	Calc.	71.7 ml H_2 (758, 20.5°)
	Found	67.0 ml H_2 (758, 20.5°)

This corresponds to 6.53/=.

The perhydro compound which had the characteristic fine strawberry odour of methyl caprate was saponified. The crude acid obtained melted at 27—28° C, and was at once converted via the acid chloride into the amide, m. p. 96° corr., undepressed by an authentic specimen of capric amide.

1.84 kgs of mugwort root was extracted with acetone at room temperature for 2 days. The acetone extract was diluted with distilled water and extracted with petroleum ether in a atmosphere of carbon dioxide. The combined petroleum ether extracts were cautiously shaken with distilled water until free of acetone, dried over a little anhydrous sodium sulphate, and concentrated below 25° C.

All long-wave absorbing substances were adsorbed when this solution was passed through a column of slightly deactivated alumina. The petroleum ether filtrate gave a colourless liquid residue, distilling at about $50°/10^{-3}$ mm Hg.

Dispersion of sesquiterpene from *Artemisia vulgaris* L.

$d_4^{20} = 0.9270$ $\lambda_0 = 962.4$ Å $R_{\lambda=\infty} = 62.9625$

λ	n_λ^{20}	$R_{\lambda, \text{obs.}}$	$R_{\lambda, \text{calc.}}$
6678.1	1.49540	64.29	64.30
5895.9	1.49905	64.69	64.69
5875.7	1.49918	64.70	64.70
5015.6	1.50526	65.37	65.37
4921.9	1.50613	65.46	65.46
4713.1	1.50830	65.70	65.70
4471.5	1.51122	66.02	66.02

R_D tricyclic sesquiterpenes = 64.40

"Centaur X" fraction.

A 20 % benzene/petroleum ether mixture eluted a liquid compound with a remarkable fine structure spectrum, the position of the main bands being in excellent agreement with those recorded for the "Centaur X" of Löfgren[9]. This liquid compound may be distilled unchanged at 10^{-4} mm/65° (air bath temperature), but the distillation path, must be short, and the time of heating not too prolonged, to avoid serious decomposition. As the extinction coefficient of the distillate is identical with that of the entire 20 % benzene/petroleum ether eluate, "Centaur X" appears to be the only substance eluted by this solvent mixture.

A semi-micro carbon and hydrogen analysis using an inadequate amount of substance gave C + H > 100 %.

U. V.-spectrum in hexane solution, Fig. 2.

λ_{max} "Centaur X" Löfgren[9]:	3 500,	3 260,	3 070,		2 690,	2 590,	2 540,	2 450 Å
λ_{max} Mugwort hydrocarbon:	3 485,	3 260,	3 064,	2 895,	2 694,	2 592,	—	— »
ν_{max} x10^{-12} » » :	860.8	920.2	979.1	1 036.3	1 113.6	1 157.4		cm^{-1}
$\Delta\nu_{max}$ » » :	59.4	58.9	57.2					

Spectrographical investigation of the epiphase and hypophase of the crude mugwort extract in petrol, revealed that "Centaur X" was predominantly epiphasic (to about 3/4) whereas the rest of the chromophoric substances were predominantly hypophasic (to about 4/5) between petroleum ether and 90 % methanol.

Benzene eluate: Benzene eluted a yellow fraction which crystallized after removal of the solvent *in vacuo*. This material exhibited not less than 8 sharp maxima between 2 320 and 3 490 Å. As different eluates and crude crystals gave identical spectral curves we were at first inclined to suppose that the fraction was homogeneous. However, the m. p. of the first crystals was not sharp (52—103°) and differed appreciably from preparation to preparation.

When a dilute solution of this material in petroleum ether was kept for some days at — 15° pale yellow needles of dehydro matricaria ester, m. p. 113°, were deposited (spectrum: Fig. 1). The mother liquor gave crystals whose spectrum again showed 8 maxima with somewhat distorted extinction coefficients. It was found that the mixture could be separated by crystallization from ca. 50 % ethanol at — 10° C; the third component then crystallized first in colourless needles, m. p. 52—52.5° C.

U. V.-spectrum of its hexane solution Fig. 3.

λ_{max}	3 286,	3 081,	2 890,	2 725,	2 577,	2 425,	2 310 Å
$\nu_{max} \times 10^{-12}$	913.3	973.7	1 038.1	1 100.9	1 164.1	1 237.1	1 298.7 cm
$\Delta\nu_{max} \times 10^{-12}$	60.4	64.4	62.8	63.2		61.6	»

19.6 mg gave 60.6 mg CO_2 11.15 mg H_2O

$C_{12}H_{10}O$	Calc.	C	84.66	H	5.92
	Found	»	84.3	»	6.32

2.9 mg gave no methoxyl in the semimicro apparatus. (Test with 0.73 mg matricaria ester: Found: OCH_3 15.6 %, calc. 17.8 %.)

With phenylhydrazine *p*-carbonic acid in diluted ethanol at room temperature the compound $C_{12}H_{10}O$ slowly yielded beautiful yellow crystals insoluble in petroleum ether. At 180° the hydrazone turns dark and decomposition takes place rapidly, but the substance does not liquify at 230° C.

The U. V.-spectrum in abs. ethanol does not show the beautiful fine structure of the compound $C_{12}H_{10}O$ (compare Fig. 3) but the maxima do occur at the same wavelengths.

SUMMARY

Re-investigation of the essential oil from the root of common mugwort (*Artemisia vulgaris* L.) has shown that the readily crystallizing substance in this oil — noticed as early as 1826 — is a methyl *n*-decene-triynoate (IV) or dehydro matricaria ester. Chromatographic analysis of acetone extracts from mugwort root demonstrated that this extremely unsaturated compound occurs as such in the plant.

Together with dehydro matricaria ester, the mugwort root contains a highly unsaturated hydrocarbon with a U. V.-spectrum very similar to that of "Centaur X" of Löfgren and a crystalline acetylenic carbonyl compound, $C_{12}H_{10}O$, m. p. 52—52.5° C.

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Received September 27, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1575–1583

Studies Related to Naturally-Occuring Acetylene Compounds

VI. The Essential Oils of Some Species of *Erigeron*

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In connection with our investigations on the highly unsaturated derivatives of methyl caprate, so far only found in some *Compositae* plants, our attention was directed to some *Erigeron* oils. The only commercially available *Erigeron*- or fleabane-oil from *Erigeron canadense* L. = marestail, horseweed, or butterweed is officinal in U. S. A. The only known components of *Erigeron canadense* oil are *d*-limonene which accounts for the major part of the oil, and *rac.* α-terpineol [1].

The crude marestail oils are remarkable in that their refractive indicas $n_D^{20} = 1.499$—1. 506 are appreciably higher, than the corresponding constants for the two main known constituents (Limonene; $n_D^{20} = 1.4750$, α-Terpineol: $n_D^{20} = 1.4826$). Sometimes these oils were noticed to deposit crystals in the cold [2].

Through Magnus, Mabee & Reynard Inc. we obtained a sample of "genuine" marestail oil distilled in the 1947 season. The sample showed $n_D^{20} = 1.4893$, a value that is amongst the lowest recorded in literature. No crystallisation took place at — 16°. The U. V.-absorption spectrum of the oil, however, was in close agreement with that of matricaria ester (I), the spectrographically estimated concentration in the crude oil being about 0.2 %.

$$H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3 \qquad (I)$$

The matricaria ester is predominantly hypophasic (73 %) between petroleum ether and 90 % methanol. By means of four consecutive partitions between these solvents we obtained an epiphasic portion (85 % of the oil) practically devoid of longwave U. V.-absorption, and a hypophasic portion consisting of terpineol, a volatile carbonyl compound of unknown structure, and the compound with the matricaria ester spectrum. On destillation at

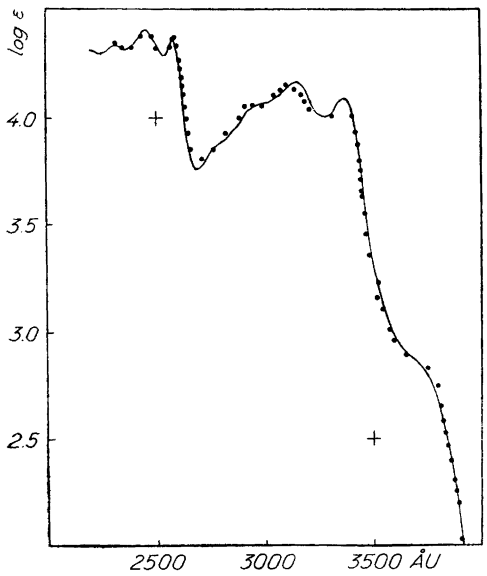

Fig. 1. Ultraviolet absorption of matricaria ester in hexane.

——— *R. T. Holman and N. A. Sørensen*[4].
● ● ● *Matricaria ester from Erigeron canadense L.*
($\varepsilon = \frac{\log I_0/I}{c \cdot d}$, *c in mol/l, d in cm*)

0.5—1 mm Hg the carbonyl compound passed over at 57—58°, then at 75—90° a fraction which solidified on cooling. Recrystallization from petroleum ether gave colourless needles m. p. 32.2—32.5°, raised on admixture with matricaria ester from *Matricaria inodora* L. to 33°. Since the crystals from *Erigeron canadense* were colourless, whereas matricaria ester from scentless mayweed is slightly straw yellow, even after repeated recrystallization, Debye-Scherrer diagrams were taken and confirmed the identity. The reason for this difference in colour is a small contamination of matricaria ester from scentless mayweed by its hexahydro compound ("Composit-cumulen I") which is yellow[3]. After chromatographic purification on deactivated alumina the matricaria ester from scentless mayweed also crystallized in colourless needles. When colourless, matricaria ester is remarkably more stable to oxidation in air than the original substance. Preliminary measurements by Dr. Ralph T. Holman (Department of Biochemistry and Nutrition, College Station, Texas) showed a rate of oxidation of only 1/10 of that of the original substance. The amount of impurity, "Composit cumulen I", is very small. In Fig. 1 the drawn curve shows the spectrum of matricaria ester from scentless mayweed according to Holman and Sörensen[4], and the dotted curve our measurements on the pure ester from fleabane oil.

The occurrence of matricaria ester in the 1947 sample of marestail oil was thus definitely established. The small yield, and the description in American periodicals of the fleabane oil industry *Cf.*[5]:

"The plant is never cultivated, but when it grows very abundantly in a field in the mint or wormwood growing section the "crop" is likely to be bought for a few dollars by somebody who owns a still and the oil destilled from it. The buyer harvests it with a binder or mower —."

gave rise to the postulate that the isolated matricaria ester might originate by contamination of the raw material of marestail with the widely distributed weed, scentless mayweed. In fact about 0.4 % of scentless mayweed in the starting weed material would be sufficient to give the observed quantity of matricaria ester in the marestail oil.

Through the courtesy of Dr. Ralph T. Holman, we obtained a sample of marestail seeds from Texas, and, in the summer of 1949 tried to cultivate some marestail in Trondheim. The plants did not flower, possibly because of the extremely bad summer, or because *E. canadense* L. is a short day plant. The leaves and stems were distilled and gave a good yield of essential oil, which, however, was spectroscopically devoid of matricaria ester.

Because of the lack of marestail flowers we decided to try flowers of some other *Erigeron* species. *Erigeron boreale* (Vierh.) Simm. is a small calciferous plant of scattered occurrence in Norwegian mountains. In Fagerlidalen, Trollheimen, there are some good localities which gave us 160 g of air dry flowers. These yielded 0.25 g of essential oil, which crystallized in the cold. The U. V.-spectrogram of the crude oil was identical with that of matricaria ester and corresponded to a concentration of 51 % of the latter in the crude essential oil. Recrystallization from petroleum ether gave colourless crystals, m. p. 32.2—32.5°.

The ability of the genus *Erigeron* to synthesise matricaria ester was thus definitely established and the high content of matricaria ester in the *E. boreale* oil suggested an investigation of the other available Norwegian *Erigeron* species. In southern Norway these are *E. acre* L., *E. politum* Fr. and *E. uniflorum* L. and although these 3 *Erigeron* species are practically odourless, the flowers as well as the non floral parts gave good yields of essential oils.

The essential oil from *E. politum* Fr. solidified at room temperature. U. V.-spectrograms of the crude flower oil gave a perfect curve for matricaria ester and indicated a concentration of 83 % of matricaria ester in the crude oil The essential oil from the non floral parts showed the same behaviour and, in addition, the somewhat astonishing concentration of 100 % of matricaria ester as determined spectrographically. A single crystallization from petroleum gave colourless crystals m. p., and mixed m. p. with matricaria ester, 33°.

Since the crude essential oil from *E. acre* L. flowers — and also the oil from the non floral parts — partly solidified at room temperature, we expected matricaria ester to be a general component of *Erigeron* oils. The U. V.-spectrograms,

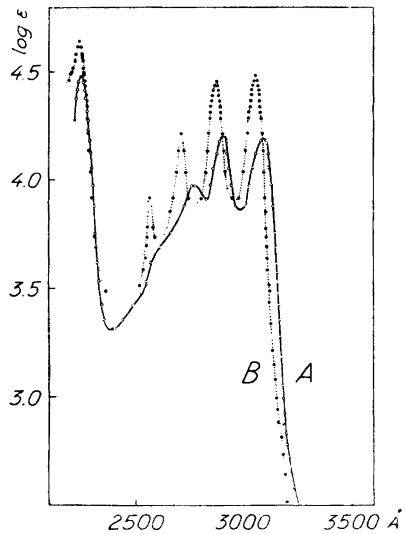

Fig. 2. Ultraviolet absorption of lachnophyllum ester in hexane.

A: —○—○—○— *cis lachnophyllum ester from Erigeron acre L.*

B: ···●···●···●··· *trans lachnophyllum ester; Bruun, Haug and Sørensen*[7].

however, revealed the occurrence of another polyene chromophore with one less double bond. Crystallization from petroleum ether or diluted ethanol gave quite colourless needles m. p. 32°. The elementary composition was $C_{11}H_{12}O_2$, that is dihydro matricaria ester. A dihydro matricaria ester with m. p. 32.6—32.8° was isolated in 1935 from the compositae plant *Lachnophyllum gossypinum*, Bge, by the Russian chemists Wiljams, Smirnow and Goljmow [6]. They proved the constitution to be the *cis* form of II, — for which the name *lachnophyllum* ester is proposed

$$CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH = CH-COOCH_3 \qquad (II)$$

The U. V. spectrum of the pure *cis*-lachnophyllum ester from *Erigeron acre* L. is given in Fig. 2, together with the spectrum of *trans*-lachnophyllum ester synthesized a short time ago by Bruun, Haug and Sörensen [7]. It will be seen that the two spectra are very similar. The difference is in agreement with experience in the polyene series [8], in that the synthetic *trans* compound shows the most pronounced fine structure. The maxima of the natural *cis* compound, however, occur at about 40 Å longer wave lengths, whereas *cis* compounds in general absorb at shorter wavelengths than the corresponding *trans* isomers [8].

We have not been able to find any report on *cis*-lachnophyllum ester since the interesting paper of the discoverers [6]. The concentration of lachnophyllum ester in the flower oil of *E. acre* L. is 81%, in the essential oil from the non floral parts close to 100%.

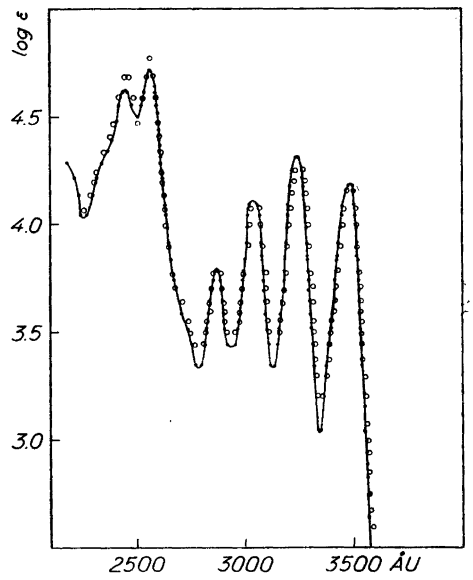

Fig. 3. Ultraviolet absorption of dehydro matricaria ester in hexane.

●—●—● *K. Stavholt and N. A. Sørensen*[9].
○○○○○ *dehydro matricaria ester from Erigeron canadense L.*

The difference in composition of the essential oils of *E. acre* L. and *E. politum* Fr. may be of some botanical interest. *E. politum* Fr. has for a long time been treated as only a subspecies of *E. acre*; *E. acre* subsp. *elongatus* Ledeb. being its botanical synonym.

Erigeron uniflorum L. is a close botanical relative to *E. boreale* (Vierh.) Simm. The whole plant of *E. uniflorum* yielded 1.5 % of essential oil. The U. V.-spectrum of this oil was rather more complex than the previously investigated *Erigeron* oils. Strong absorption started at longer wave lengths than with matricaria ester, the first definite maximum at 3 360 Å corresponded with the first maximum of matricaria ester; then followed with appreciably higher intensities, the maxima at 3 080, 2 915 and 2 240 Å characteristic of *cis*-lachnophyllum ester. Chromatography led to the ready isolation of a yellow compound with the properties of the hexahydro-matricaria ester, III, ("Composit cumulen I"), isolated from scentless mayweed [3].

$$CH_3-CH_2-CH_2-CH_2-CH_2-\overset{H}{C}=C=C=\overset{H}{C}-COOCH_3 \qquad \text{III}$$

We have not yet found convenient chromatographic procedures for separating *cis*-lachnophyllum ester and matricaria ester. Both are absorbed on slightly activated alumina, 30 % benzene-petroleum ether does not elute either of them and 70 % benzene-petroleum ether elutes both. The matricaria ester was concentrated in the last fractions, but we have only succeeded in a perfect

separation, after recrystallization, of the main component, the *cis*-lachnophyllum ester. The matricaria ester from the mother liquors still remained impure.

Some investigations on other plants, belonging to the family *Compositae*, this summer have revealed that enynes of this class may occur preferentially in the root. Accordingly, a preliminary study of the essential oil of marestail root was carried out using the rest of our Texas culture which had hibernated in a window culture. The U. V.-spectrum of the marestail root oil exhibited not less than 6 prominent maxima between 3 470 and 2 560 Å. Of these 6 maxima, 5 belonged to dehydro matricaria ester, to date the most highly unsaturated member of this series, isolated a short time ago from the root oil of common mugwort *(Artemisia vulgaris* L.). By crystallization from petroleum ether the dehydro matricaria ester was isolated in a pure condition, m. p. 112.5—112.7°. The spectral measurements are given in Fig. 3, together with the curve for the original dehydro matricaria ester [9].

The remaining peak in the U. V.-spectrum of marestail root oil lies at 2 690 Å and stands out with a remarkable sharpness. This peak has so far been observed only in one compound, the "Centaur X" which Löfgren [10] has demonstrated to be a component of some species of the composite genus *Centaurea*. As shown in the fifth communication of this series [9] "Centaur X" is also a component of the mugwort root oil. Its structure is still obscure.

All the 5 species of *Erigeron* so far investigated contain enyne-derivatives of methyl caprate. The results are summarized in Table 1.

It is of course to be expected that the known enynes will prove to be widely spread amongst *Erigeron* species and many foreign *Erigeron* species are at present under cultivation to give some general indication of the occurrence of enynes in this genus which contains more than 150 good species. Botanically the genus *Erigeron* is closely related to *Lachnophyllum*, which provided the first compound of elucidated structure in this series. The rela-

Table 1. Enyne components of the investigated Erigeron oils.

Compound isolated	Fleabane species				
	E. acre L	*E. boreale* Simm.	*E. canadense* L.	*E. politum* Fr.	*E. uniflorum* L.
Lachnophyllum ester	+				+
Matricaria ester		+	+	+	+
Dehydro matricaria ester			+		
"Composite cumulene I"					+
"Centaur X"			+		

tionship to *Matricaria* and *Artemisia* is, however, very remote. An account of our investigations on essential oils from different compositae genera will appear later.

EXPERIMENTAL

Erigeron canadense L. 100 mls of essential oil of *Erigeron canadense* L. (Magnus, Mabee & Reynard Inc.) in 300 mls of petroleum ether was given 4 consecutive extractions with 90 % methanol, the methanol phase being re-extracted with one third of its volume of petroleum ether. The combined epiphasic portion from 505 mls of marestail oil amounted to 440 mls, i. e. 87 %. This epiphasic fraction was devoid of long wave U. V.-absorption.

The hypophasic fraction (68 g) was distilled at 0.5—1 mm. At 57—58° there distilled a fraction with very penetrating mint-like odour. By condensation with phenylhydrazine *p*-sulphonic acid and regeneration with steam in acid solution this forerun fraction gave a liquid carbonyl compound showing an inflexion by 3250 Å (log ε = ca. 1.5) followed, at 2300 Å, by a maximum with log ε = ca. 3.6.

The fraction distilling between 75° and 90° (ca. 1 g.) solidified on cooling. The crude material melted at 18° and after several crystallisations from petroleum ether, formed colourless needles, m. p. 31.5°, raised on admixture with matricaria ester to 33°. U. V.-absorption spectrum: Fig. 1, dotted curve.

Essential oil from marestail leafs and stems. 4 kg of fresh material gave about 2 ‰ of essential oil on steam distillation. This oil was devoid of long wave U. V.-absorption.

Essential oil from marestail roots. 121 gs of 1 ½ year old marestail root gave 0.2 g of essential oil on steam distillation. The oil partially crystallized in the cooler. The crude oil showed the following sharp maxima in the ultra violet.

λ	3 473	3 235	3 040	2 865	2 690	2 560	Å
log ε	3.41	3.55	3.35	3.16	3.54	3.98	

(The extinction coefficients are calculated on a molecular weight of 172 for dehydro matricaria ester.) Recrystallisation from petroleum ether gave slightly yellow needles m. p. 112.5—112.7 corr. U. V.-absorption: Fig. 3, dotted curve.

Erigeron boreale (Vierh.) Simm. The material was collected on August 28th, 1949 in Fagerlidalen, Storlidalen, Trollheimen. The flowers were dried at room temperature in shadow and then weighed 160 g. On steam distillation they yielded 0.25 g of essential oil which crystallized immediately on cooling. The U. V.-absorption spectrum in hexane solution showed main maxima at 3 375 Å (log ε = 3.75), 3 110 Å (log ε = 3.9) and 2 580 Å (log ε = 4.15). The position of these main maxima and the shape of the curve are in excellent agreement with matricaria ester at a calculated concentration of 51 %. Crystallization from petroleum ether gave colourless crystals of m. p. 32.2—32.5°, having U. V.-absorption curve identical with that of matricaria ester.

Erigeron politum Fr. The material was collected on August 17th, 1950 in Vinstradalen, Oppdal. The flowers were separated from the non-flowering parts, and both were dried in shadow at room temperature; dry flowers 45 g; plant residue 137 g. Steam distillation of the flowers: yield 0.13 g of essential oil; from the plant residue 0.55 g. Both oils solidified at room temperature. The U. V.-absorption curves of both oils were in complete agree-

ment with that of matricaria ester; flower oil concentration, 83 %; concentration in the oil from the non floral parts 100 % of matricaria ester.

Erigeron acre L. Through the kind assistance of Mrs. Helene Sörensen we obtained 85 gs of air dry flowers and 200 g of air dry material of the non-flowering parts of *Erigeron acre* L collected in mid-July, 1950, at Lykkja, Hemsedal. Steam distillation gave 0.20 g of flower oil and 0.30 g of oil from the non floral parts. Both oils solidified at 0°. Although the oils were transparent at higher wawe-lengths, intense absorption commenced abruptly at about 3 200 Å and continued to shorter wawe-lengths with sharp maxima as follows:

	λ	3 100	2 916	2 750	2 250
flowers:	log ε	4.11	4.03	3.89	4.37
non floral parts:	λ	3 088	2 911	2 770	2 240
	log ε	4.20	4.16	3.98	4.55

Both oils gave colourless needles, m. p. 32° C, after one crystallization from petroleum ether.

$C_{11}H_{12}O_2$ (176.1)	Calc.	C = 74.98	H = 6.88
	Found	» = 74.8	» = 7.20

U. V.-spectrum of the pure substance Fig. 2, curve A.

Erigeron uniflorum L. The material was collected below Ryphuskollen, Oppdal, on August 19th, 1950, and brought fresh to the laboratory for steam distillation. The whole plant material (= 214 gs) was distilled in one batch, yielding 0.32 g of essential oil. The crude oil was slightly yellow, and its U. V.-absorption started even in the violet region with appreciable absorption in the range 3 800—4 000 Å, where matricaria ester is transparent. The long wave maximum of matricaria ester at 3 375 Å occurred (log ε = 3.53), then followed the three main maxima of the lachnophyllum ester:

λ	3 080	2 915	2 240
log ε	4.21	4.20	4.57

Chromatography of the crude oil in petroleum ether solution on slightly deactivated alumina gave a yellow zone at the top of the column and the petroleum ether filtrate was devoid of long wave U. V.-absorption. Elution with a 30 % benzene-petroleum ether mixture removed only small amounts of compounds, also devoid of polyenyne spectra. On elution with a 70 % benzene-petroleum ether mixture, both *cis*-lachnophyllum and matricaria ester were removed. The ratio of the two esters was 10 in the first fractions of 100 mls of the 70 % benzene-petrol eluate, but decreased to 4, 4 and 3.2 in the following fractions. The weights of unsaturated methyl caprate derivatives were 71, 85, 32.6, 4.7 and 0.5 mg. The first two fractions were united, the solvent removed, and the residue recrystallized once from petroleum ether, then from dilute ethanol until the U. V.-spectrum showed it to be free of matricaria ester. The m. p. of the resulting colourless needles was 32° C, and the U. V.-absorption curve was identical with that of *cis*-lachnophyllum ester from *Erigeron acre* L.

The chromatogram was then eluted with 96 % ethanol, the lipoids transferred to ether, and distilled at 10^{+4} mm Hg. At 52—54° C (air bath temperature) there distilled 15 mgs of yellow oil with a penetrating odour. The U. V.-spectrum of this oil agreed with that

recorded for the hexahydro-matricaria ester [3] ("Composite cumulene I"). As the value of log ε was about 0.08 units low throughout the entire spectrum, the fraction should contain about 17 % of a transparent impurity. The small amount did not allow any further purification.

SUMMARY

The essential oils of the following species of the genus *Erigeron* have been investigated for their content of enyne derivatives of methyl caprate: *E. acre* L, *E. boreale* (Vierh.) Simm., *E. canadense* L., *E. politum* Fr. and *E. uniflorum* L. All these fleabanes contain enynes. Lachnophyllum ester, matricaria ester and dehydro matricaria ester were isolated in pure condition: Hexahydro matricaria ester ("Composit cumulen I") and "Centaur X" was detected spectrographically. The distribution of the enynes among the investigated fleabanes is summarized in Table 1.

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Received September 27, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1584–1596

Crystal Structure of "*Cis*-Naphthodioxane" — Bi-1,3-Dioxacyclopentyl(2)

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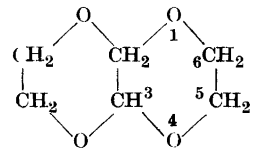
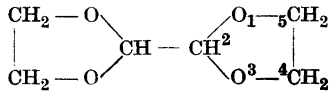
If ethylene glycol reacts either with 2,3-dichlorodioxane or with glyoxal two solid substances are obtained, one melting at 111° the other at 136° C (Böeseken, Tellegen and Cohen Henriquez [1]). Both substances have been assumed to be "naphthodioxanes" (I) and to correspond to the two stereoisomeric decalins.



(I)

Böeseken and co-workers have also, however, considered the possibility that one of the two substances might have the formula



(II)

but subsequently rejected this possibility on the basis of chemical arguments. The dipole moment of the higher melting isomer (*"trans"*) was found to be very small (0.7 D), that of the lower melting substance (*"cis"*), however, was found equal to 1.7 D. In order to account for a moment of this magnitude "movable" forms of the dioxane rings had to be assumed.

We have considered this problem important in connection with earlier work on six-membered rings carried out in our laboratory, and have therefore undertaken crystallographic investigations of the two isomers in question.

PREPARATION OF THE SUBSTANCES

The substances were prepared from 2,3-dichlorodioxane and ethylene glycol using benzene as a solvent. In most cases a mixture of the two isomeric 2,3-dichlorodioxanes was used, but in one case the pure solid isomer of m. p. 32° C was employed. The proportion of higher and lower melting isomers in the reaction product seemed, however, to be the same in both cases, in conformity with the result obtained by Baker and Shannon [2]. By recrystallisation it was easy to isolate considerable quantities of the 136° isomer in a pure state. In addition a mixture of both isomers, melting at about 90°, was obtained, from which the lower melting substance could be isolated, although with some difficulty. The isolation could be performed either by partially melting the mixture and separating the solid part from the liquid, or by dissolving the mixture in water and slowly evaporating the solvent. In the latter case good single crystals of both isomers were obtained which might be separated by hand under the microscope.

In one case a solution from which the greater part of the higher melting isomer and a mixture of both isomers had already been removed, on further concentration yielded large needles apparently different from crystals of the higher melting substance. X-ray diagrams revealed an identity period of 4.46 Å along the needle axis. Weissenberg photographs recording reflexions on the first and second layer lines proved, however, that the needles were twins and not true single crystals. Later diagrams of well developed single crystals of the lower melting isomer showed that the needle-shaped crystals had the lattice of this isomer.

The formation of mixed crystals containing both isomers was not observed, neither by crystallisation from solutions nor by sublimation.

As pointed out in a preliminary communication [3], a complete crystal structure determination of the higher melting isomer does not appear promising. The crystal structure of the lower melting isomer, on the other hand, could more easily be carried out, and a detailed report of this investigation is given below.

MORPHOLOGY, UNIT CELL AND SPACE GROUP

The crystals are monoclinic, and may occur in different habits, either as plates (100) with b and c along the diagonals of a rhombus, or as plates (010) elongated along the a axis. Needle-shaped twins elongated along the a axis were also observed (see above).

From X-ray oscillation photographs the following unit cell dimensions were derived: $a = 4.46$ Å, $b = 7.76$ Å and $c = 11.64$ Å (all $\pm$ 1 %). Copper ($\lambda = 1.54$ Å) and iron ($\lambda = 1.934$ Å) radiation were used. The monoclinic angle β was obtained from a Weissenberg photograph about the b axis, and found equal to 121.5°. By flotation of the crystals in a mixture of benzene and carbon tetrachloride a density of about 1.40 g/cm³ was measured, corresponding to *two* (calc. 1.97) molecules $C_6H_{10}O_4$ in the unit cell.

Systematic absences occur in the $h0l$ reflexions for odd values of l, and in the $0k0$ reflexions for odd values of k. The space group is therefore $P2_1/c$, from which follows that the molecule must be centrosymmetrical, with its centre of symmetry in special positions in the unit cell.

DETERMINATION OF THE STRUCTURE FACTORS $F_{obs.}$

A needle-shaped crystal elongated along a and with a cross-section of 0.4×0.4 mm was used to take a series of Weissenberg photographs recording the $0kl$ reflexions. The compound is volatile and was therefore kept in a thin-walled glass capillary tube. Exposure times varied between 2 h and 60 h. 75 of the 97 reflexions attainable with Cu $K\alpha$ radiation were recorded on the film with measureable intensity. The intensities were estimated visually and corrected for the Lorentz polarization factor, using the method of Goldschmidt and Pitt [14]. No correction was applied for absorption or extinction. Later on, the values were converted to an approximately absolute scale by comparison of observed and calculated structure factors.

For the b axis photographs a tabular crystal with a cross-section of roughly 0.1×0.6 mm was used. The shape of the reflected beams as recorded on the film showed great variations, and a correction for this "spot shape" effect had to be applied, following the procedure described by Broomhead [4]. It was found that the observed intensities had to be multiplied by factors varying between 1.0 and 0.5, taking the factor 1.0 for beams leaving the crystal perpendicularly to the dominating face (100). 43 of the 55 $h0l$ reflexions theoretically available were recorded.

ATTEMPTS TO DETERMINE THE STRUCTURE USING THE NAPHTHODIOXANE FORMULA

A crystal structure determination was first attempted using the naphthodioxane formula (I). As mentioned in the preliminary report [3] it was found impossible to work out a satisfactory structure along these lines, but we would nevertheless like to include a brief account of the trials, as we think they elucidate certain fundamental aspects of crystal structure determination.

Because of the shortness of the a axis, the projection in the direction of this axis was first considered. An approximate structure was soon found, which gave a fairly good agreement between observed and calculated structure factors for the low order reflexions. A Fourier synthesis was carried out, giving a promising electron density map, which improved during the following four refinements. In the course of this process the value of $R = \Sigma||F_{obs.}| - |F_{calc.}|| \,/\, \Sigma|F_{obs.}|$ decreased gradually from about 0.55 to 0.38, when no more sign changes occurred. The final electron density map is shown in Fig. 1. The two condensed six-membered rings appear clearly on the map, with all the atoms well resolved and with peak heights corresponding about to 5e.Å^{-2} for the three carbon atoms

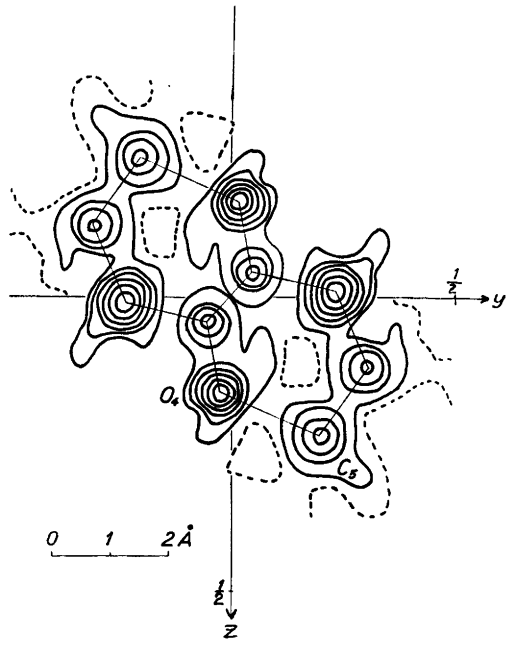


Fig. 1. Electron density map obtained by using the naphthodioxane formula. Contours at intervals of 1 e. Å^{-2}, the unit contour line being dotted.

and 7 e. Å^{-2} for the two oxygen atoms. However, the structure cannot be the correct one for the following reasons:

1) The agreement between $F_{obs.}$ and $F_{calc.}$ is not satisfactory. The value 0.38 for the reliability factor R is too high for a structure of this type, although it is low enough to indicate that the structure might be *essentially* correct. (As will be shown later, an R-value of 0.13 was obtained for the correct structure.)

2) The distance between the peaks corresponding to the atoms O_4 and C_5 is 1.80 Å, a value which is not compatible with our present knowledge of bond lengths in organic compounds.

3) Small spurious peaks and distortions occur in the electron density map shown in Fig. 1.

Many more trials were carried out with naphthodioxane models, but we never succeeded in getting values of R below 0.38. However, it is not safe to conclude from these negative results that the substance cannot be an isomer of naphthodioxane. The definite proof of this was obtained by the subsequent finding of a structure, against which the objections in 1), 2) and 3) cannot be raised.

THE STRUCTURE DETERMINATION BASED ON THE BI-DIOXA*CYCLO*-PENTYL (2) FORMULA

Our failure of establishing the crystal structure on the basis of the naphthodioxane formula made us consider the possibility, that the substance might be bi-1,3-dioxa*cyclo*pentyl(2), although the chemical evidence, as interpreted by Böeseken *et al.* [1], has not substantiated this view.

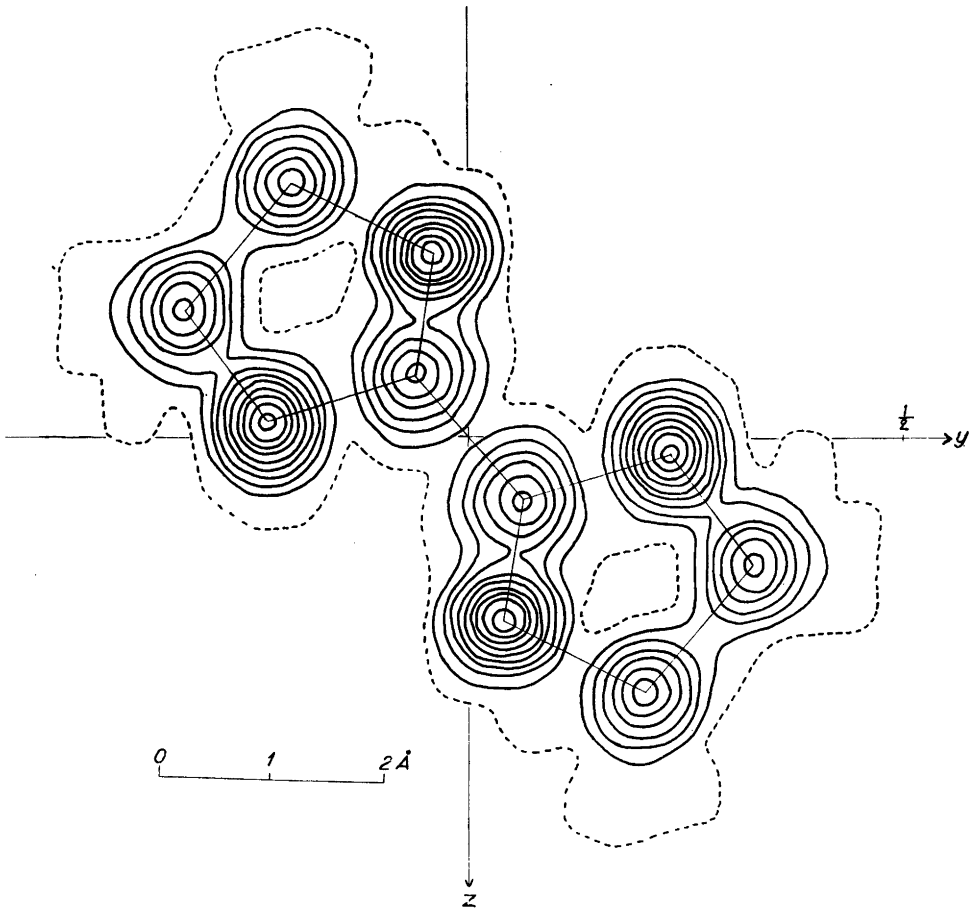


Fig. 2. Fourier projection in direction of a axis. Contours at intervals of 1 e. Å^{-2}, the unit contour line being dotted. The electron density is calculated at 1/60th of b and c axes.

a axis projection. It will be seen from models that molecules corresponding to the formulae (I) and (II) may be very similar in projection, except for the position of the atom C_2. Indeed, the first postulated co-ordinates based on the bi-dioxa*cyclo*pentyl formula (II) differed from the original structure postulated for (I) only in the y co-ordinate of the atom C_2, which was changed from $-y_2$ to $+y_2$. The factor R was about the same in both cases (0.55), but whereas the refinement could not be improved beyond $R = 0.38$ if (I) was used, the value 0.13 was reached if the calculations were based on (II).

The resulting electron density map (Fig. 2) has reasonable inter-peak distances and no unexplainable peaks or distortions. The three carbon atoms

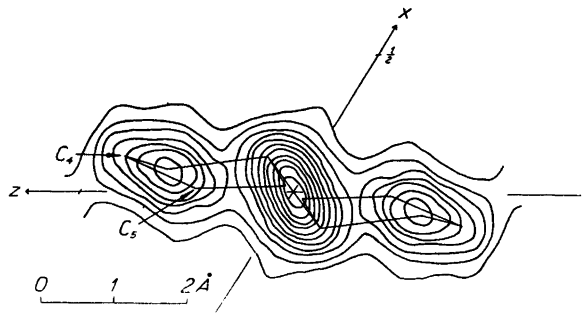


Fig. 3. Fourier projection in direction of b axis. Contours at intervals of 2 e. Å^{-2}.

have almost identical peak heights (7.1 ± 0.1 e. Å^{-2}) and the same holds for the two oxygen atoms (10.4 ± 0.1 e. Å^{-2}). All the atoms are well resolved, and their co-ordinates may be derived directly from the map. The presence of hydrogen atoms at C_4 and C_5 is also indicated.

In the first stages of the analysis the experimental values of the atomic scattering factor *f* given by Robertson [5] for hydrocarbons were used, weighting oxygen and carbon as 8 to 6. No separate account was taken for the hydrogen atoms. In this way an *R*-value of 0.17 was obtained. When inserting the hydrogen atoms in the positions which appeared reasonable, the agreement became no better. In view of the relatively great importance of the oxygen atoms in the structure, the theoretical Hartree *f*-curves (see *International Tables*) should be expected to be more satisfactory. The structure factors thus obtained gave an *R*-value of 0.16, and now the influence of the hydrogen atoms was very noticeable, as their inclusion made *R* drop to 0.13. It is interesting to notice that the introduction of more accurate *f*-curves made one reflexion (034) change sign, which again caused displacements of about 0.01 Å for the atoms O_1 and C_4. Inaccurate *f*-values *may* thus lead to errors in the co-ordinates.

When using the theoretical *f*-curves a temperature factor $e^{-B(\sin\Theta/\lambda)^2}$ must of course be introduced. The value of *B* was found to be 3.2 Å^2 by comparison of observed and calculated structure factors.

By the calculation of the reliability factor *R* the reflexions which are too weak to be observed, were treated in the following way: If $F_{calc.}$ is less than the lowest observable value of $F_{obs.}$, the error is taken equal to zero. If $F_{calc.}$ is greater, the error is taken as the difference between $F_{calc.}$ and the smallest value of $F_{obs.}$. In $\Sigma|F_{obs.}|$ the non-observed reflexions are not included.

In Table 3 $F_{obs.}$ and $F_{calc.}$ are compared for all $0kl$ and $h0l$ reflexions available with Cu *Kα* radiation.

b axis projection. The knowledge of the z co-ordinates and approximate bond lengths made it easy to work out this projection, and after only two approximations the final electron density map shown in Fig. 3 was obtained. As the peaks overlap extensively, it is not possible to derive very accurate co-ordinates from the map. The x co-ordinates were determined by finding the maxima of electron density along lines corresponding to the known z co-ordinates, the influence of the neighbouring peaks being roughly corrected for by subtracting them from the map, using the standard shape of the Fourier peak as given in a paper by Booth [6]. Obviously the procedure is not very satisfactory, and the x co-ordinates can only be given to two-figure accuracy (see Table 1).

The structure factors were first calculated using the Hartree f-curves with the same temperature factor as in the a projection ($B = 3.2$). This gave good agreement between $F_{obs.}$ and $F_{calc.}$ for planes roughly parallel to (001). However, for all other planes $F_{obs.}$ was decreasing much more rapidly with increas-

Table 1. Atomic co-ordinates as fractions of the corresponding cell edge.

Atom	x	y	z
O_1	0.04	0.232	0.015
C_2	0.13	0.061	0.055
O_3	0.08	0.040	0.164
C_4	0.13	0.201	0.228
C_5	0.015	0.328	0.114

ing Θ than $F_{calc.}$, and for planes approximately perpendicular to (001) the value of $B = 5.2$ was found. This is believed to be due to oriented thermal vibrations of the molecule; it lies roughly in the (100) plane and would therefore be expected to vibrate chiefly in a direction normal to this plane. In accordance with this view (100) gives a strongly diffuse reflexion on Laue photographs. The temperature factor depends consequently not only on $\sin \Theta/\lambda$ but also on the orientation of the plane in question. In order to account for this, the reciprocal lattice was divided in sectors, and B-values varying between 3.2 and 5.2 were assigned to the sectors by comparison of observed and calculated structure factors for high values of Θ.

The reliability factor for the b axis projection is 0.17, being reduced to 0.16 when inserting the hydrogen atoms.

Discussion of errors. The co-ordinates derived from electron density maps are liable to errors from different sources, especially when only two-dimensional methods are used, as in the present case.

Errors in $F_{obs.}$ are shown by Booth [6,7] and Robertson [8] to be of minor importance, in general leading to displacements in the peak positions of less than 0.01—0.02 Å. This

was substantiated in the present case by an independent determination of $F_{obs.}$ for the $0kl$ reflexions, using Fe $K\alpha$ radiation (Cu radiation was not available when the investigation was started), another crystal specimen (cross-section 0.05 × 0.13 mm), the intensity estimations being carried out by an independent observer. The agreement between the two sets of $F_{obs.}$ was satisfactory, the mean difference between the two observations being 0.9, or in general 10—15 %. This was found to cause deviations of 0.01—0.04 Å in the peak positions on the Fourier map (reflexions not available with Fe radiation were taken from diagrams obtained with Cu radiation). These deviations are somewhat greater than those quoted by Booth and Robertson, which was also to be expected because different radiations were used.

The finite summation errors were investigated following the procedure described by Booth [6]. It was found that these errors cause atomic displacements varying from 0.01 to 0.035 Å, but application of these corrections did not give better agreement between $F_{obs.}$ and $F_{calc.}$. It is thus hardly possible to decide whether the corrected co-ordinates are better than the original ones or not.

Overlapping of peaks and thermal vibrations give rise to very considerable errors in the x co-ordinates, which, it is believed, may possibly be as great as 0.06 Å. However, the molecule lies roughly in the (100) plane, and errors in the x co-ordinates do not influence the bond lengths appreciably. Considering the different sources of errors, we think that the maximum possible error in the bond lengths may be about 0.06 Å, but that it is likely to be smaller, say 0.03 Å.

DESCRIPTION AND DISCUSSION OF THE STRUCTURE

The molecular structure. The bond lengths calculated from the co-ordinates in Table 1 are given in Table 2, column I. After correction for finite summation errors the values in column II were obtained. It will be seen that the four independent determinations of the C—O bond agree very closely with the generally accepted value (1.42 Å) for a normal single bond, their mean value being 1.41 Å. The deviations of the two C—C bond lengths from the standard

Table 2. Bond lengths (in Ångström units) and bond angles.

Bond	I	II	Angle	
C_2-O_3	1.407	1.414	$\angle C_2'C_2O_3$	106°
O_3-C_4	1.408	1.413	$\angle C_2'C_2O_1$	111°
C_5-O_1	1.420	1.416	$\angle O_1C_2O_3$	105°
O_1-C_2	1.394	1.402	$\angle C_2O_3C_4$	109°
C_4-C_5	1.507	1.534	$\angle O_3C_4C_5$	102°
C_2-C_2'	1.523	1.523	$\angle C_4C_5O_1$	104°
			$\angle C_5O_1C_2$	111°

value 1.54 Å are also well within the limits of error. As for the bond angles, their deviations from the value 108—109° are not believed to be significant, except for the angles at C_4 and C_5 which are as small as 102° and 104° respectively.

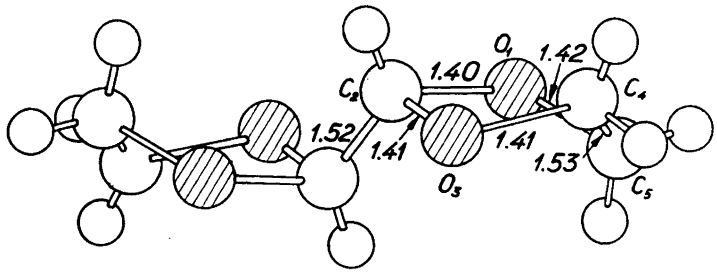


Fig. 4. Å model of the molecule, with bond lengths (in Å units).

A plane defined by the three atoms O_1, C_2 and O_3 do not pass through either C_5 or C_4, the distances from these atoms to the plane being about 0.25 Å and 0.6 Å respectively. C_4 and C_5 lie on the same side of the plane. Because of the inaccuracy of the x co-ordinates, the displacement of C_5 from the plane may not be significant, whereas C_4 definitely appears to be out of the rough plane through the four other atoms. Thus the five-membered ring would seem to deviate from a planar ring in roughly the same way as described earlier for fructofuranose [9] and *d*-ribose [10].

The deviation from planarity is probably due mainly to repulsive forces between the hydrogen atoms. With a planar ring the hydrogen atoms at C_4 and C_5 are in *cis* position and only 2.25 Å apart. It will be seen by means of models (compare Fig. 4) that by moving the atom C_4 out of the plane of the ring by about 0.5 Å, the hydrogen atoms will approximately be brought into *trans* position, with a separation of 2.4—2.5 Å, a distance corresponding to the sum of the van der Waals radii. It appears therefore that favourable H—H distances are most easily obtained by a deformation of the five-membered ring from the planar model. The great importance of forces acting between non-bonded atoms has earlier been demonstrated in the case of six-membered rings (cyclohexane and related substances) [13].

The packing of the molecules. The shortest intermolecular atomic distances are those between the atom O_3 and the atoms C_4 and C_5, the separation being 3.25 Å in both cases (see Fig. 5). This is somewhat shorter than the distances found between oxygen atoms and methylene groups in glycine [11] (3.38 Å, 3.52 Å) and diketopiperazine [12] (3.32 Å, 3.33 Å). The distances from the oxygen atom O_1 to C_4 and C_5 in different neighbouring molecules are 3.57 Å and 3.68 Å. Unfortunately only very approximate positions of the hydrogen atoms can be given, but calculations seem to indicate (compare Fig. 5) that neighbouring atoms belonging to different molecules are separated by distances corresponding roughly to the normal values of the van der Waals radii (1.2 Å for hydrogen atoms and 1.4 Å for oxygen atoms).

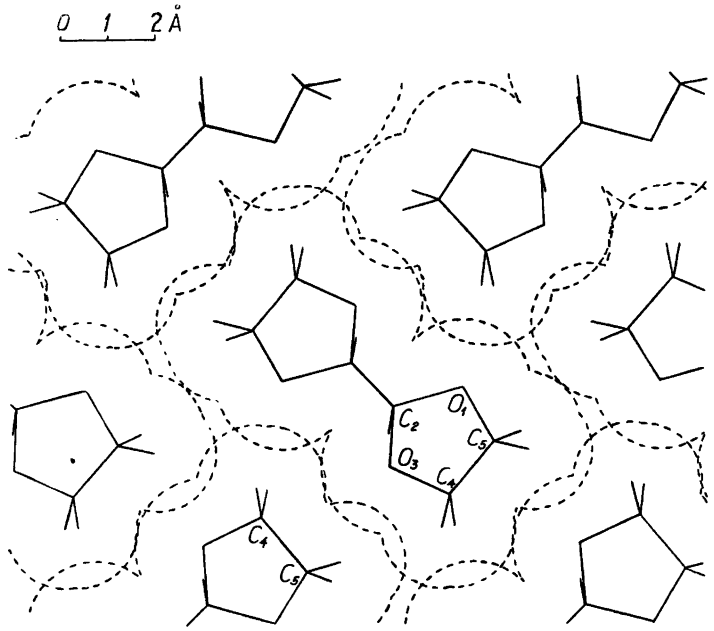
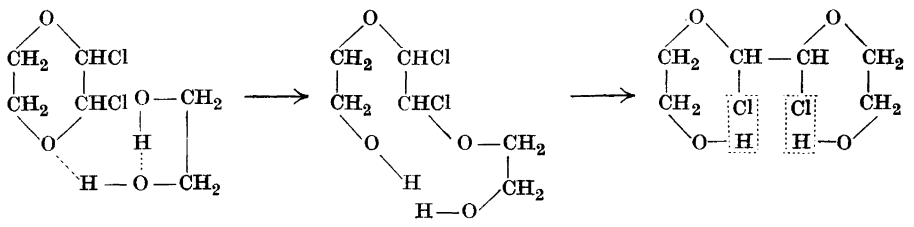


Fig. 5. The crystal structure projected in direction of a axis. Van der Waals contacts are shown dotted.

CHEMICAL ASPECTS

Since the structure analysis has established beyond doubt that the lower melting isomer is bi-1,3-dioxa*cyclo*pentyl(2), a few remarks on the possible reactions leading to this product would perhaps be of interest.

It is easy to understand that both isomers ((I) and (II)) may be formed by the reaction between glyoxal and ethylene glycol. The formation of five-membered rings from 2,3-dichlorodioxane and ethylene glycol seems, however, more difficult to explain. At least two possibilities would appear to deserve consideration. In the first place the reaction may start for example with a complex containing two hydrogen bonds according to the scheme:



Secondly, if not both the solvent and the ethylene glycol are completely free from traces of water, the primary process may be a hydrolysis of 2,3-dichlorodioxane giving (besides glycol) a quantity of glyoxal equivalent to the amount of water originally present. Bi-1,3-dioxa*cyclo*pentyl(2) would then be formed by the reaction between *glyoxal* and ethylene glycol, a process which again yields water, thus enabling the reaction to continue.

Table 3. Comparison of observed and calculated structure factors.

hkl	$F_{obs.}$	$F_{calc.}$	*hkl*	$F_{obs.}$	$F_{calc.}$
002	9.7	+ 10.4	033	17.9	− 17.6
004	8.0	+ 6.1	034	2.7	+ 3.2
006	10.6	+ 10.0	035	8.9	− 8.2
008	3.5	+ 4.3	036	3.0	+ 2.5
0 0 10	1.6	− 1.5	037	3.5	+ 3.3
0 0 12	1.9	+ 0.7	038	4.1	− 3.2
			039	4.8	− 4.1
011	22.3	+ 25.7	0 3 10	5.1	+ 6.3
012	28.5	− 33.4	0 3 11	< 1.2	+ 1.1
013	6.7	− 8.7			
014	5.0	− 7.3	040	22.5	+ 20.4
015	11.7	+ 11.1	041	8.6	− 7.8
016	6.0	− 4.9	042	4.8	+ 4.8
017	< 1.4	+ 0.1	043	17.5	− 16.3
018	< 1.4	− 0.9	044	10.6	+ 10.0
019	7.1	− 8.6	045	9.4	+ 8.8
0 1 10	4.8	− 6.6	046	9.4	+ 8.9
0 1 11	< 1.3	− 1.3	047	4.2	− 3.8
0 1 12	< 1.0	− 0.7	048	< 1.5	+ 1.4
			049	< 1.4	+ 1.1
			0 4 11	2.1	+ 1.6
02υ	13.8	− 12.5	0 4 10	< 1.2	− 0.3
021	12.6	− 15.1			
022	7.3	− 9.8	051	3.9	+ 3.5
023	8.1	+ 8.0	052	10.2	− 9.7
024	20.8	− 24.7	053	< 1.4	+ 0.7
025	7.1	− 7.0	054	< 1.4	+ 0.5
026	3.2	+ 2.9	055	9.0	+ 8.6
027	8.5	− 8.9	056	7.7	− 7.7
028	10.1	− 12.5	057	< 1.5	+ 0.4
029	2.1	− 1.4	058	6.9	− 7.1
0 2 10	5.7	− 6.2	059	1.8	+ 1.7
0 2 11	2.7	+ 3.5	0 5 10	1.7	+ 1.7
0 2 12	1.4	+ 0.9			
			060	4.1	− 3.6
031	14.9	+ 12.9	061	11.5	− 12.4
032	13.6	− 15.2	062	8.4	− 8.5

hkl	$F_{obs.}$	$F_{calc.}$	*hkl*	$F_{obs.}$	$F_{calc.}$
063	< 1.4	+ 0.6	300	1.9	+ 1.8
064	8.0	− 7.3	302	2.6	− 3.0
065	2.5	− 2.5	304	< 1.8	− 0.9
066	4.2	− 3.3	306	< 1.2	+ 0.3
067	4.6	− 4.4			
068	< 1.4	+ 2.2	400	2.1	− 1.7
069	2.1	− 2.1	402	< 1.4	+ 0.0
0 6 10	< 0.9	+ 1.2			
			$\bar{1}02$	30.3	+ 36.9
071	8.5	− 8.4	$\bar{1}04$	3.7	+ 3.5
072	8.5	− 8.5	$\bar{1}06$	24.0	+ 22.9
073	3.7	− 2.5	$\bar{1}08$	9.7	+ 9.7
074	3.0	+ 3.1	$\bar{1}$ 0 10	< 2.0	+ 1.1
075	4.1	− 3.0	$\bar{1}$ 0 12	< 1.7	− 2.2
076	2.3	+ 1.7			
077	< 1.3	+ 1.1	$\bar{2}02$	28.5	+ 28.8
078	< 1.1	+ 0.5	$\bar{2}04$	5.9	− 4.6
079	0.9	− 0.7	$\bar{2}06$	24.2	+ 26.9
			$\bar{2}08$	11.6	+ 13.9
			$\bar{2}$ 0 10	4.1	+ 5.0
080	5.8	− 6.5	$\bar{2}$ 0 12	< 1.2	− 1.1
081	< 1.4	+ 0.5	$\bar{2}$ 0 14	1.2	+ 0.9
082	2.5	+ 2.4			
083	< 1.4	+ 0.4	$\bar{3}02$	12.7	+ 15.1
084	2.8	+ 1.9	$\bar{3}04$	4.4	− 4.7
085	3.5	+ 3.3	$\bar{3}06$	11.3	+ 11.2
086	2.5	+ 2.8	$\bar{3}08$	11.9	+ 14.5
087	2.1	− 2.5	$\bar{3}$ 0 10	4.3	+ 6.6
			$\bar{3}$ 0 12	2.6	+ 0.7
091	1.9	+ 1.0	$\bar{3}$ 0 14	1.5	+ 1.1
092	< 1.2	− 0.2			
093	< 1.1	+ 0.9	$\bar{4}02$	2.3	+ 4.9
094	< 1.0	+ 0.9	$\bar{4}04$	3.3	− 4.0
			$\bar{4}06$	2.6	+ 1.1
100	69.9	+ 79.0	$\bar{4}08$	7.3	+ 9.0
102	6.1	− 7.1	$\bar{4}$ 0 10	3.2	+ 5.5
104	5.2	+ 4.8	$\bar{4}$ 0 12	2.5	+ 2.4
106	3.5	+ 2.1	$\bar{4}$ 0 14	1.3	+ 1.0
108	< 2.0	+ 2.0			
1 0 10	< 1.5	− 0.7	$\bar{5}02$	< 1.5	+ 1.2
			$\bar{5}04$	2.0	− 1.3
200	21.6	+ 24.2	$\bar{5}06$	2.2	− 2.0
202	6.2	− 7.5	$\bar{5}08$	3.4	+ 4.0
204	< 2.0	+ 1.4	$\bar{5}$ 0 10	1.7	+ 2.0
206	< 1.8	− 0.8	$\bar{5}$ 0 12	1.8	+ 1.7

SUMMARY

The crystal structure of "*cis*-naphthodioxane" m. p. 111° has been determined by two-dimensional Fourier syntheses. It is shown that the chemical formula assigned to this substance by Böeseken *et al.* is wrong; the compound is not an isomer of naphthodioxane, but bi-1,3-dioxa*cyclo*pentyl(2).

In the crystal the molecule has a centre of symmetry. Bond lengths in close agreement with the normal values (1.42 Å and 1.54 Å) for C—O and C—C single bonds are found. The five-membered ring appears to be non-planar, which is thought to be due to repulsion between hydrogen atoms belonging to neighbouring methylene groups.

We are very much indebted to Cand. real. E. Amble who has carried out most of the preparative work, but had to interrupt his investigation when he left this country for the United States. We also want to express our gratitude to Cand.polyt. M. Danö for her very valuable assistance during the first stages of the structure analysis. One of us (S. F.) wishes to thank *Norges Teknisk-Naturvitenskapelige Forskningsråd* for a grant.

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Received November 17, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1950) 1597–1606

Some Halogen Derivatives of *Cyclo*hexane

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The general knowledge about the chemistry of even the simplest derivatives of *cyclo*hexane is still rather incomplete and the number of substances accessible for molecular structure analysis comparatively small. Before this part of chemistry has been more thoroughly investigated, workers interested in the stereochemistry of six-membered rings will have to carry out a great deal of preparative work in order to supply compounds suited for molecular structure determinations.

We have considered halogen derivatives of *cyclo*hexane especially well suited for such determinations, and in the course of our structural investigations some preparative work dealing with those substances has therefore been carried out which has so far not been published. In most cases we had only comparatively small quantities of substance at our disposal and we therefore limited our work to crystalline substances the purity of which is more easily stated than in the case of liquids.

DI- AND TRIHALOGEN DERIVATIVES

We first bring some observations related to disubstituted *cyclo*hexanes: The lower melting dibromo- and diiodo-*cyclo*hexanes obtained besides the *trans* 1,4-compounds when resorcitol or chinitol is treated with strong hydrobromic resp. hydroiodic acid should be expected to be corresponding compounds and perhaps isomorphous. The bromo-compound does not appear to be easily obtained in a pure state, and the melting points recorded by different authors differ appreciably. The highest figure earlier reported is 48° C [1], but we observed a melting point of 51° C using single crystals. The crystals are monoclinic and the unit cell indicates a close crystallographic relationship between this compound and the 1,4*(trans)* compound, a rather surprising result which is being further investigated in our laboratory. The diiodo-compound

turned out to be triclinic and not isomorphous with the dibromo-compound. At a temperature not far from the melting point earlier reported [2] (67.5° C), however, it undergoes a transition into a new modification which may perhaps be monoclinic and correspond to that of the dibromocompound. The melting point of this modification is 84° C. It may be cooled and kept below the transition temperature for some time without suffering transition into the modification stable at room temperature.

Some experiments were performed in order to prepare 1,3,5-trihalogen-*cyclo*hexanes treating α or β phloroglucitol with strong hydrochloric and hydrobromic acid or in some cases with other reactants usually employed in order to replace hydroxy groups by halogens. These attempts were unsuccessful; when hydrochloric or hydrobromic acid was used the formation of benzene could be observed. It would seem that the 1,3,5-trihalogen compounds are unstable at higher temperatures.

1,2,3,4,-TETRAHALOGEN DERIVATIVES

Of the six possible substances containing four bromine atoms (Fig. 1) *two* have been known with certainty for a couple of years, having the melting points 156° and 90° respectively [3]. The existence of a third substance melting at 141° [4] has been questioned [3]. Our investigation clearly proves that this substance really does exist. According to the crystal structure determination carried out in our laboratory by Wang Lund [5], this substance has the configuration $\varkappa\varkappa\varkappa\varkappa$ (Fig. 1 a). The unit cells and space groups of the higher and the lower melting substances have also been reported in Wang Lunds paper. Unfortunately the crystal structure determination is more involved in these cases. With the aim of facilitating the structure determination we have prepared a dichlorodibromocompound from 1,3-*cyclo*hexadiene by first adding two atoms of chlorine and subsequently two atoms of bromine. This compound melting at 128° C would be expected to be a 1,4-dichloro-2,3-dibromo-*cyclo*hexane and as a matter of fact it turned out to be isomorphous with the 1,2,3,4-tetrabromocompound of m. p. 156° C:

	a (Å)	b (Å)	c (Å)	β	Space group
1,2,3,4-tetrabromo*cyclo*hexane	9.91	8.52	8.06	133°	$P\ 2_1$
1,4-dichloro-2,3-dibromo*cyclo*hexane	9.58	8.31	7.32	129°	$P\ 2_1$

An attempt to prepare 1,4-dibromo-2,3-dichloro*cyclo*hexane by adding two atoms of chlorine to 1,4-dibromo*cyclo*hexene(2) was unsuccessful. Bromine was liberated and chlorine substitution products formed, among them β benzene hexachloride.

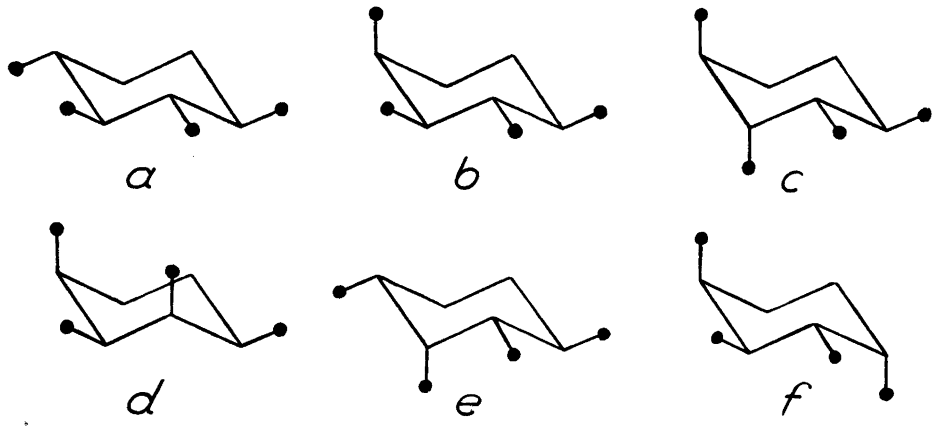

*Fig. 1. Possible configurations of 1,2,3,4-tetrabromo*cyclo*hexanes. a, b, e, f may exist in independent mirror image forms; the enantiomorphic forms of c and d are transformed into each other by a "conversion" of the carbon ring.*

If a stream of chlorine is led into 1,3-*cyclo*hexadiene both addition to the double bonds and substitution takes place, and considerable quantities of *α* benzene hexachloride are formed. From the reaction product obtained by adding the theoretical amount of chlorine to the diene, however, two different tetrachloro*cyclo*hexanes could be isolated, one melting at 112° C, the other at 116°. Both compounds would be expected to be 1,2,3,4-tetrachloro*cyclo*hexanes. We have not, however, been able to find any indication of isomorphism between at least the lower melting isomer and any of the 1,2,3,4-tetrahalogen*cyclo*hexanes mentioned above.

The fact that we have been able to prepare *three* different 1,2,3,4-tetrabromo*cyclo*hexanes by adding two atoms of bromine to the pure 1,4-dibromo-*cyclo*hexene(2) of m. p. 108° clearly demonstrates that caution is needed when drawing conclusions about the steric course of addition processes. If we assume that only *trans* addition to the double bond takes place, it would not be possible to obtain more than *two* different 1,2,3,4-tetrabromo*cyclo*hexanes starting with one of the two possible forms of 1,4-dibromo*cyclo*hexene(2). It has to be borne in mind, however, that the actual reaction scheme may include either an isomeric change from 1,4-dibromo*cyclo*hexene(2) into 1,2-dibromo-*cyclo*hexene(3) or a partial *cis-trans* transition of the 1,4-dibromo*cyclo*-hexene(2).

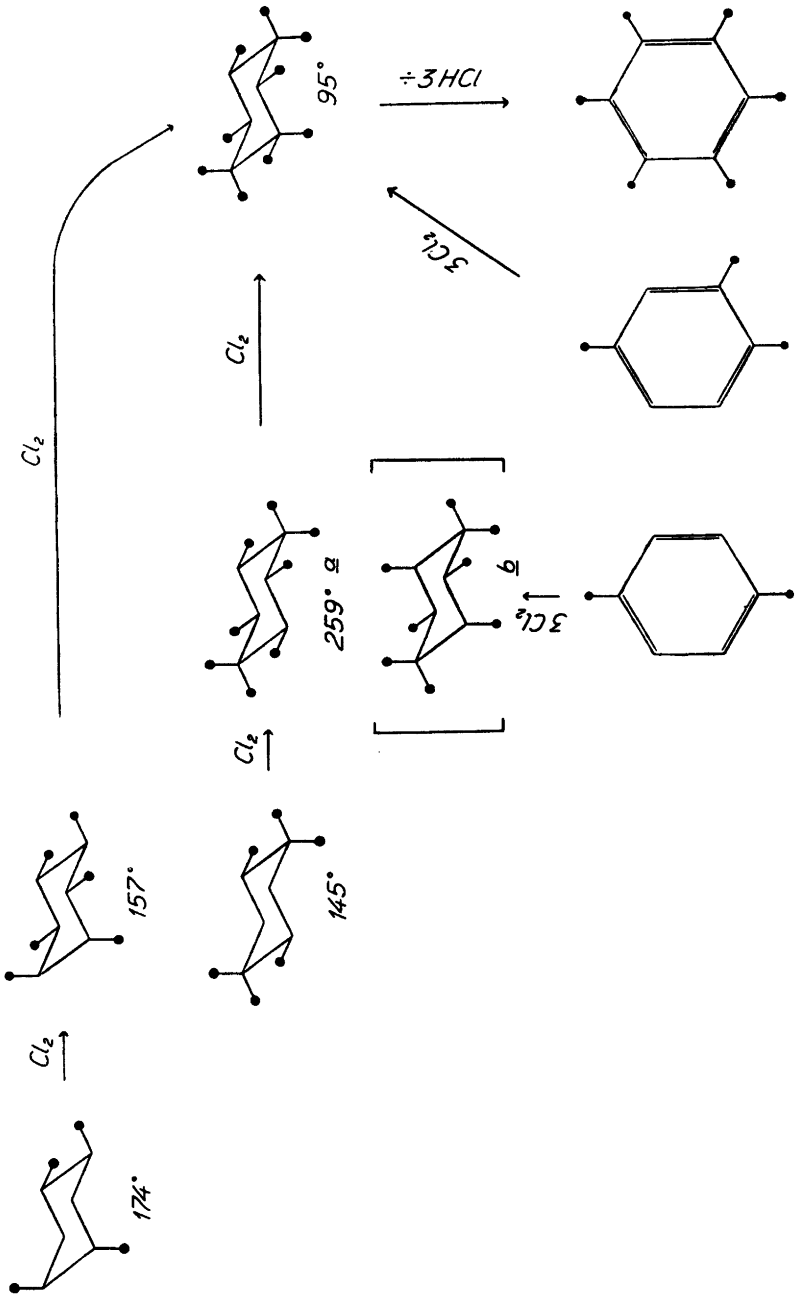

Fig. 2. The mutual relationship between some chlorinated cyclohexanes and the connection between some of these substances and chloroderivatives of benzene.

CHLORINATION PRODUCTS OF *CYCLO*HEXANE

Many experiments have already been carried out on the chlorination of *cyclo*hexane, and a number of new substances has thus been obtained. In most cases, however, the positions of the chlorine atoms have not been stated and very little is also known about the mutual relationship between the compounds successively formed during the chlorination. Systematic work dealing with the continued chlorination of intermediate products isolated in a pure state would no doubt be of great value in determining the real structure of higher chlorinated compounds.

The simplest assumption to be made when discussing the structural relationship of the chlorination products successively formed, is that bonds between carbon and chlorine already existing are not broken during the chlorination processes.

In the course of a chlorination continued until further absorbtion of chlorine could hardly be detected the following substances were isolated in a pure state: tetrachloro*cyclo*hexane (m. p. 174°), *α* benzene hexachloride (157°), hexachloro*cyclo*hexane (145°), hexachlorobenzene (227°), octachloro*cyclo*hexane (259°) and nonachloro*cyclo*hexane (95°).

Here the *α* benzene hexachloride is probably formed from the tetrachloro*cyclo*hexane of m. p. 174°, the former having the configuration $1\varepsilon\ 2\varepsilon\ 3\varkappa\ 4\varkappa\ 5\varkappa\ 6\varkappa$ (Fig. 2, (6)—157°) and the latter the configuration $1\varepsilon\ 2\varepsilon\ 4\varkappa\ 5\varkappa$ [6] (Fig. 2, (4)—174°). As a matter of fact we have detected *α* benzene hexachloride in the chlorination product of pure tetrachloro*cyclo*hexane (174°).

It has already been pointed out that the hexachloro*cyclo*hexane of m. p. 145° cannot be a benzene hexachloride [7]. Recently results of X-ray analysis and electron diffraction work has shown this substance to have the configuration $1(\varkappa\varepsilon)\ 2\varkappa\ 4(\varkappa\varepsilon)\ 5\varkappa$ [8] (Fig. 2, (6)—145°). The octachlorocompound of m. p. 259°, according to (unpublished) results obtained by E. Wang Lund, has zero dipole moment, and the X-ray analysis requires centrosymmetric molecules to be present in the crystals. By the kindness of this investigator we are able to report the crystallographical data of the substance:

$a = 8.05$ Å, $b = 11.01$ Å, $c = 6.74$ Å, $\beta = 108.5°$ Space group $P2_1/a$

The density is 2.06, and the unit cell therefore contains *two* molecules. This octachloro-compound is identical with the substance obtained by addition of chlorine to *p*-dichlorobenzene. Therefore, only *two* possible configurations of this substance should have to be considered a) $1(\varkappa\varepsilon)\ 2\varkappa\ 3\varkappa\ 4(\varkappa\varepsilon)\ 5\varkappa\ 6\varkappa$ and

b) 1($\varkappa\varepsilon$) 2ε 3$\varkappa$ 4($\varkappa\varepsilon$) 5ε 6$\varkappa$ (Fig. 2 (8)—259°). The complete structure analysis has not yet been finished, but configuration *a* must no doubt be regarded more probable than *b*. We have indeed found that the octachloro*cyclo*hexane (259°) may be obtained if the hexachloro-compound (145°) is chlorinated using the amount of chlorine theoretically necessary to form an octachloro-*cyclo*hexane. The nonachloro*cyclo*hexane (95°), however, is the main product even under these circumstances.

This nonachloro-compound (95°) is probably identical with the compound earlier obtained [9] by treating 1,2,4-trichlorobenzene with chlorine. We have obtained this substance both from *p*-dichlorobenzene and chlorine and by chlorination of octachloro*cyclo*hexane (259°). It is somewhat surprising, however, that the chlorination of the octachloro*cyclo*hexane into nonachloro-*cyclo*hexane appears to proceed very slowly, as during the chlorination of the hexachloro*cyclo*hexane (145°) into the nonachloro-compounds we would expect the octachloro*cyclo*hexane (259°) to represent an intermediate stage. If the octachloro*cyclo*hexane (259°) is assumed to have the configuration *a* (see above) the nonachloro*cyclo*hexane must have the configuration 1($\varkappa\varepsilon$) 2($\varkappa\varepsilon$) 3$\varkappa$ 4($\varkappa\varepsilon$) 5$\varkappa$ 6$\varkappa$ (Fig. 2 (9)—95°).

The presence of hexachlorobenzene in the distillation product of chlorinated *cyclo*hexane mentioned above is no doubt due to thermal decomposition of the nona-compound present in the chlorination mixture. We have indeed observed that the distillation *in vacuo* of pure nona-compound results in the partial formation of hexachlorobenzene, another part of the nona-compound distilling without decomposition.

The structure (Fig. 2 (9)—95°) attributed to the nona-compound is in good agreement with the observation that this compound is obtained by chlorination of tetrachloro*cyclo*hexane (174°), of α benzene hexachloride, of hexachloro-*cyclo*hexane (145°) and of octachloro*cyclo*hexane (259°). This view is supported by the observation made by Oiwa and coworkers [10] who obtained the nona-compound also by chlorinating β, δ and ε benzene hexachlorides and finally by chlorination of octachloro*cyclo*hexane of m. p. 149°. This octa-compound is obtained by addition of chlorine to o-dichlorobenzene and the authors assume it to have the configuration 1($\varkappa\varepsilon$) 2($\varkappa\varepsilon$) 3$\varkappa$ 4$\varkappa$ 5$\varkappa$ 6$\varkappa$.

As the nona-compound of m. p. 95° is the end product of chlorination of a number of different chlorinated *cyclo*hexanes it seemed of interest to establish its crystallographic data in order to make its identification quite unambigous. E. Wang Lund has kindly placed his results at our disposal. The crystals are monoclinic with 4 molecules in the unit cell. The monoclinic angle is 109.7° and the lattice constants are $a = 8.54$ Å, $b = 26.3$ Å, $c = 5.87$ Å (density calc. 2.12).

EXPERIMENTAL

Reaction of phloroglucitol with hydrochloric (hydrobromic) acid

5 g *α* or *β* phloroglucitol were heated to 100—120 ° in sealed tubes with an excess of concentrated acids (saturated at 0 ° C). The upper layer formed during the reaction was isolated, washed with a solution of sodium bicarbonate and with pure water, dried and distilled. The liquid boiling at 80—81 ° C had the odour of benzene and was identified as such by nitration into dinitrobenzene. Neither *α* nor *β* phloroglucitol seem to react with PCl_5 in benzene solution, with PCl_3 or $POCl_3$.

1,2,3,4-Tetrabromo*cyclo*hexanes

The *cyclo*hexadiene used in preparing 1,2,3,4-tetrahalogen-compounds was prepared according to Crossley[11]. 0.2 moles of the diene were dissolved in 100 ml chloroform and 0.4 moles of bromine added drop by drop under stirring. The first 0.2 moles of bromine are immediately absorbed. The remaining part within two or three days when the reaction mixture is kept in a dark and cool place. The solvent is evaporated *in vacuo*, a possible small excess of bromine being simultaneously removed during this process. After being kept at 0 ° C for a few days the remaining oily substance crystallizes partially. After two recrystallizations from small amounts of ethyl ether the solid consists chiefly of large compact crystals, but partially also of thin needles. A preliminary separation of the two sorts of crystals could easily be performed by hand. After recrystallization of the former crystals from ethyl ether, well developed polyhedrons of m. p. 89—90 °C were obtained:

($C_6H_8Br_4$)	Calc.	Br	80.00
	Found	»	80.35

The thin needles were recrystallized from methanol until the m. p. was constant — 156° C. Having only a limited quantity of substance at our disposal the analysis was performed on a sample containing a mixture of the two isomers and melting from 135—142°:

($C_6H_8Br_4$)	Calc.	Br	80.00
	Found	»	80.01

We have also prepared the two tetrabromo*cyclo*hexanes just mentioned by adding one mole of bromine to 1,4-dibromo*cyclo*hexene(2) in CCl_4 solution. In this case the raw product was first recrystallized from methanol. Beside the needle-shaped crystals of the higher melting isomer the first fraction also contained very thin crystal plates of a substance melting at 140°. This third isomer could be prepared in a pure state by adding less than 0.2 moles of bromine to 0.1 moles of diene dissolved in 100 ml of carbon tetrachloride. After being kept for a couple of days in the dark at room temperature the solution was colourless and a white, crystalline precipitate was formed. By recrystallization from ligroin fibre-like crystals melting at 142° were obtained. The crystals obtained from ethyl ether were well developed pyramids:

($C_6H_8Br_4$)	Calc.	Br	80.00
	Found	»	80.37

Dichloro-dibromo-*cyclo*hexane

A solution of 0.1 moles of chlorine in carbon tetrachloride is added to a cooled solution of 0.1 moles of diene in the same solvent. Subsequently a solution of 0.1 moles of bromine in carbon tetrachloride is added and the solution is kept in the dark for two or three days. After evaporation of the solvent from the (colourless) solution an oily substance is obtained which crystallizes partially after being kept at 0° C for some time. After recrystallization from ligroin needle-shaped crystals are obtained melting at 128° C:

($C_6H_8Br_2Cl_2$)	Calc.	Mol.wt.	311	Cl	22.81	Br	51.40
	Found	» »	305	»	22.68	»	51.14

0.1 moles of chlorine were added to 0.1 moles 1,4-dibromo*cyclo*hexene(2) in carbon tetrachloride solution. When the solution is kept in the dark for a couple of days bromine is liberated. After evaporation *in vacuo* the oily substance left is cooled and then deposits crystals which after recrystallization from benzene have a melting point of 308° C and which could be identified as β benzene hexachloride.

Tetrachloro*cyclo*hexanes

When a stream of chlorine is led into a solution of the diene without taking special precautions, both addition of chlorine and substitution takes place even in the dark, and higher chlorinated products, among them α benzene hexachloride, are formed. If, however, working in the dark and at $-10°$ C, a solution of 0.2 moles of chlorine in carbon tetrachloride is added to a solution of 0.1 moles of diene, and the mixture kept at 0° C for some days, the oily product obtained after evaporation of the solvent deposits crystals of a new kind. If the remaining oil is destilled *in vacuo* additional crystals are obtained from the fraction boiling between 120 and 125° (12 mm). After recrystallization from ethyl ether needle-shaped crystals of m. p. 112° are obtained:

($C_6H_8Cl_4$)	Calc.	Cl	63.91
	Found	»	63.51

Once, however, under similar conditions, a different tetrachlorocompound crystallizing in extremely thin needles was obtained having a melting point of 116° C:

($C_6H_8Cl_4$)	Calc.	Cl	63.91
	Found	»	63.68

Chlorination products of *cyclo*hexane

On chlorinating *cyclo*hexane or monochloro-*cyclo*hexane in artificial light a deposit of tetrachloro*cyclo*hexane (m. p. 174° C) is formed. Additional quantities may be obtained from the first fraction of the remaining liquid boiling between 110° and 130° C (10 mm). When a solution of this tetrachlorocompound, recrystallized from carbon tetrachloride and again dissolved in the same solvent, is treated with chlorine using artificial light, the end product of the chlorination is nonachloro*cyclo*hexane of m. p. 95° C. If the chlorination is interrupted after 15 minutes and the solvent, the excess of chlorine and the hydrogen chloride removed *in vacuo*, an oily substance remains, a fraction of which, boiling at

150—160° (10 mm) and kept for some time at 0° C, deposits crystals which could be identified as *α* benzene hexachloride.

α benzene hexachloride may also be isolated from the fraction boiling at 150—160° (10 mm) after removing the solid tetrachlorocompound from the product obtained by direct chlorination of *cyclo*hexane. From the same fraction hexachloro*cyclo*hexane of m. p. 145° is also deposited if it is kept at a low temperature for some time.

The direct chlorination of a solution of the 145° substance in carbon tetrachloride leads to nonachloro*cyclo*hexane of m. p. 95° C, which is identical with the substance obtained from tetrachloro*cyclo*hexane of m. p. 174° (see above) and the nonachloro-*cyclo*hexane which we obtained on leading a stream of chlorine into a solution of *p*-dichlorobenzene in CCl_4 using artificial light. The chief product in the last mentioned experiment is octachloro*cyclo*hexane of m. p. 259° C ("*β*-*p*-dichlorobenzene hexachloride"). If, however, the chlorination of the 145° substance is performed by adding a solution of 1.2 g chlorine in carbon tetrachloride to a solution containing 5 g of the substance and this mixture exposed to artificial light for 24 hours, octachloro*cyclo*hexane of m. p. 259° is obtained beside the nonachloro*cyclo*hexane.

Returning to the direct chlorination of *cyclo*hexane it may be stated that the fraction of the chlorinated product distilling at 160—175° deposits very thin needles melting at 226° which are identified as hexachlorobenzene. After some time the mother liquid yielded new crystals which could be identified as nonachloro*cyclo*hexane of m. p. 95°. If heated, this compound begins to split off hydrogen chloride at about 160° C. If the temperature is maintained at 200° C for some time this reaction is nearly complete and the nonachloro*cyclo*hexane is transformed into hexachlorobenzene. A distillation carried out at a pressure of about 10—12 mm transforms about 30 % of the nonacompound into hexachlorobenzene, the rest distilling unchanged.

The fraction boiling between 175° and 185° deposits crystals of the octachloro*cyclo*hexane of m. p. 259°. This substance, after being dissolved in carbon tetrachloride, was chlorinated for 24 hours in artificial light. After the solvent had been evaporated the greater part of the octachloro-compound is deposited unchanged, but an oily rest is left which subsequently deposits crystals of nonachloro*cyclo*hexane of m. p. 95°.

SUMMARY

Some observations regarding the lower melting dibromo- and diiodo*cyclo*hexanes obtained from 1,4- and 1,3-*cyclo*hexandiols and concentrated hydrogen-halogen acids are reported. Attempts to prepare 1,3,5-trihalogen-*cyclo*hexanes from phloroglucitol and hydrochloric or hydrobromic acids were unsuccessful and led to the formation of benzene.

It could be proved that the tetrabromo*cyclo*hexane of m. p. 145° obtained from 1,3-*cyclo*hexadiene and bromine is a well-defined chemical individual. A complete crystal structure determination by E. Wang Lund has proved that the bromine atoms occupy the positions: 1*α* 2*α* 3*α* 4*α*. A dichlorodibromo-*cyclo*hexane of m. p. 128° has been prepared which is isomorphous with the tetrabromocompound of m. p. 156°. Two new tetrachloro*cyclo*hexanes, melting at 112° and 116° respectively, have been prepared from 1,3-diene and chlorine.

Experiments in which *cyclo*hexane was chlorinated in artificial light are described. In one fraction of the distillation product hexachlorobenzene was observed. This substance is formed from nonachloro-*cyclo*hexane (m. p. 95°) by thermal decomposition. It is found that the configurations now attributed to the different chlorination products successively formed by substituting hydrogen by chlorine agree with those to be expected if we assume that carbon-chlorine bonds already established are not broken in the course of further substitution reactions.

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Received November 9, 1950.

ACTA CHEMICA SCANDINAVICA 4 (1951) 1607–1610

Short Communications

Cytochrome c in Sea-Urchin Eggs

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It is a matter of dispute whether the ordinary cytochrome system occurs in sea-urchin eggs. Runnström [1] found hemochromogen bands (512 and 548 mμ) in alcohol extracts, while a pyridine hemochromogen band at 550–560 mμ can be readily obtained [2, 3]. Although several workers [4–7] have unsuccessfully sought for cytochrome c, the bands of a and b have recently [8] been observed in eggs at liquid-air temperature. Studies of the effect of inhibitors on egg respiration [8–10] and experiments with cell extracts [7, 11, 12] have proved the presence of an oxidase closely allied to, or identical with, cytochrome oxidase. The ability of the extracts to oxidise cytochrome c strongly suggests the presence of cytochrome oxidase.

Cytochrome bands have been detected in sea-urchin spermatozoa [2, 3, 11] and Rothschild [13] has identified bands of the components a, a_3, b and c and also observed the CO-complex of a_3 (cytochrome oxidase). Recent studies of spectra at liquid-air temperature disclosed the presence of a component e [14].

In the present investigation suspensions of sea-urchin egg powder (*Echinus esculentus;* eggs extracted with acetone and ether, disintegrated in the Waring blendor, dried at room temperature) showed, after $Na_2S_2O_4$ addition, absorption bands at 605–600 (cytochrome a?) and 535–530 mμ, but no trace of cytochrome c bands. A very strong pyridine hemochromogen band (560 mμ) could be produced. It is not likely that the extraction and drying procedure has destroyed cytochrome c, since apparently the more sensitive cytochrome a is left unaffected, and, furthermore, acetone washing and drying have been used without ill-effects in ordinary cytochrome c preparation.

Krahl and co-workers [7] found that egg-extracts (*Arbacia*) had no specific absorption at 550 mμ, and could not replace cytochrome c in biochemical test systems. The authors concluded that the cytochrome c content, if any, was less than 10^{-2} μg per mg dry eggs. In the present investigation *Echinus* egg powder was analyzed for cytochrome c, using a new micromethod [15]. No cytochrome c was found, thus placing the upper limit of its concentration in the eggs at 5×10^{-4} μg per mg dry matter. Since the extractability of cytochrome c from dried egg material is not known this figure must be considered as approximate.

Baker's yeast has a Q_{0_2} round 200, and contains about 2.6 μg cytochrome c per mg dry matter [16]. Calculated from Borei's [17] figures (egg volume = 5.84×10^{-4} μl; dry matter round 20 %; oxygen consumption = 1.84×10^{-4} μl per egg and hour) the fertilized *Psammechinus* egg has a Q_{0_2} of 1.5. Accordingly, if the sea-urchin egg has a cytochrome system similar to that of baker's yeast, it would contain 2×10^{-2} μg cytochrome c per mg dry matter. Actually, however, the upper concentration limit probably lies much lower, as found above. This comparison is, however, only valid on assumption that there is a general proportionality between cytochrome c content and Q_{0_2} in different organisms.

Nevertheless, it must be born in mind that the oxidases of the echinoderm egg and of yeast or mammalian heart were found [12] to resemble each other very much. It is probable that the oxidase will have a different protein for each species (*cf.* hemoglobins), and that the proteins of the oxidases from heart and yeast may differ from each other just as much as either differs from that of echinoderm eggs. Furthermore, cytochrome c has been demonstrated in sea-urchin spermatozoa [13,14], and has been found to catalyze oxidations through the echinoderm egg oxidase [12]. A small amount of cytochrome c (apparently less than 5×10^{-4} μg per mg dry matter) is thus quite possible in the sea-urchin egg. In such a case the mammalian and the echinoderm cytochrome systems differ not in fundamental composition, but merely in concentration of their components.

My gratitude is due to the Director and Staff of the Scottish Marine Biological Station, Millport, where this investigation was carried out.

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Received January 11, 1951.

An Improved Method for Selfcondensations of Esters by means of Alkali Ethoxides

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A general method for the preparation of β-ketoesters of the formula $RCH_2COCH(R)CO_2C_5H_2$ is the forced self-condensation of esters with sodium ethoxide, as described by Mc Elvain [1–3]. The yields of the β-ketoesters obtained by this method are usually very good, but the practical procedure can be somewhat simplified.

The preparation of dry, powdered sodium or potassium ethoxide is a rather cumbersome process, but the preparation of a suspension of the same reagent is easily performed in an inert solvent whose boiling point is higher than the melting point of the metal, which can then be very finely divided and hence is very reactive.

The choice of the solvent is dependent upon the boiling point of the ester used. In order not to remove some of the ester by azetropic distillation with the alcohol formed in the reaction, the boiling point of the ester should be some degrees higher than that of the solvent. For esters with high boiling points, over 140°, xylene b. p. 135–140° is a useful solvent; for those in the region 115–140° toluene b. p. 111° can be used; and for those with a low boiling point, below 115°, the best solvent seems to be benzene, b. p. 81°, but in this case sodium m. p. 97.8° is preferrably replaced by potassium m. p. 63.5°. Of course sodium may be used if it is first powdered

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under xylene, and then the solvent replaced by benzene, but the time for the preparation of the ethoxide suspension is then increased to about 12 hours.

The alcohol formed is continuously removed by careful distillation through an efficient column, and the end of the reaction, which is reached when no more alcohol is formed, is indicated by a rise in temperature until near the boiling point of the solvent. In this way, the end-point is much more readily determined than by the method of Mc Elvain [1], who for the low boiling esters, followed the progress of the reaction by measuring the refractive index of the distillate. For the high boiling esters [2], he removed the alcohol under reduced pressure and the end of the reaction was taken to be the point when the boiling of the mixture ceased.

By this modification the same yields are obtained as by the old method, but the reactions are more easily controlled and the operations need very little attention. For the low-boiling esters, only the theoretical amount of ester is used, whereas Mc Elvain had to use a considerable excess much of which was lost in the isolation process [3].

EXPERIMENTAL

Ethyl propionyl-propionate. In a 1-l three-necked flask fitted with a mercury seal stirrer, a dropping funnel and a 30 cm Widmer column with a total reflux variable take-off still head, were placed 500 ml of dry benzene and 19.5 g (0.5 atom) of potassium. The flask was heated in an oil bath and refluxed for some minutes in order to expel the air, and *then* the stirrer was started. To the boiling suspension, 23 g (0.5 mole) of absolute alcohol were added carefully from the dropping funnel. After the addition of about 1 ml, the oil bath was removed and the addition continued at such a rate that rapid refluxing was secured. When all the alcohol had been added, the oil bath was replaced and the mixture refluxed for half an hour to complete the reaction.

If the procedure above is not followed and air is allowed to enter the flask with the finely powdered potassium, an explosion may occur when the alcohol is added.

102 g of dry ethyl propionate (1 mole) were added and the alcohol formed removed by careful fractionating. The take-off was so regulated, that the temperature at the top of the column did not exceed 70°. When no more alcohol could be obtained, about 100 ml of benzene were distilled off, and the mixture allowed to cool.

These operations take about 15 hours, and as the reaction needs very little attention, the distillation of the alcohol can be made overnight after careful adjustement of the take-off.

The clear, deeply coloured solution thus obtained was poured over 20 ml of conc. sulphuric acid and an excess of ice. The organic layer was separated, and the water extracted with ether. The combined organic layers were washed with water and then with a solution of sodium bicarbonate. After drying over a little anhydrous sodium sulphate, the ether and benzene were removed under reduced pressure, and the residue fractionated *in vacuo*. Yield 59 g (75 %) of ethyl propionyl-propionate boiling at 88–90°/12 mm.

Ethyl n-*butyryl*-n-*butyrate.* In the same apparatus as above were placed 400 ml of dry toluene and 11.5 g (0.5 atom) of sodium. The flask was heated in a wax bath until the sodium melted. The stirrer was started, the wax bath removed, and 23 g of absolute alcohol were carefully added through the dropping funnel.

The procedure after this was the same as that above except that 116 g of dry ethyl *n*-butyrate (1 mole) were added instead of the ethyl propionate, and the alcohol removed below 90°. Yield 70 g (75 %) of ethyl *n*-butyryl-*n*-butyrate boiling at 103°/11 mm.

Ethyl n-*valeryl*-n-*valerate*. The same procedure as above was used, except that 400 ml of xylene was used instead of the toluene and 130 g (1 mole) of dry ethyl *n*-valerate instead of the ethyl *n*-butyrate. Yield 80 g (75 %) of pure ethyl *n*-valeryl-*n*-valerate boiling at 124–126°/10 mm.

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2. Briese, R. R., and Mc Elvain, S. M. *J. Am. Chem. Soc.* **55** (1933) 1697.
3. *Organic Reactions* **I** (1942) 281.

Received January 19. 1951.

Quaternary Derivatives of 2-Benzylphenoxyethylamines

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In the search for new adrenergic blocking agents related to the basic ethers of phenol [1] several quaternary ammonium compounds derived from *o*- and *p*-benzylphenol were prepared. The physical and chemical data for four such compounds are presented below (see table).

Because of the incidental relation to 2-benzylphenoxydimethylaminoethane HCl which was found by Cheney *et al.*[2] to be a potent histamine antagonist it was of interest to investigate the above mentioned quaternary compounds in this respect.

The pharmacological results, the details of which will be dealt with by others elsewhere, indicate that all these compounds are considerably less active than the hydrobromide of 2-benzylphenoxydimethylaminoethane which is as active as the corresponding hydrochloride described by Cheney *et al.*[2], and approximately twice as active as Benadryl.

The quaternaries are somewhat less active than Benadryl.

The ammonium compounds were prepared by refluxing for three hours two moles of the appropriate tertiary amine with one mole of crude 2-benzylphenoxyethylhalide and removing excess volatile amine *in vacuo*, or, more conveniently, by refluxing the free tertiary benzylphenoxyethylamine prepared according to ref. 2 with an excess of the appropriate halide in ethanol. The yields were from 85 to 90 % of the theoretical.

1. Kahane, E., and Lévy, J. *Bull. soc. chim. biol.* **27** (1945) 562.
2. Cheney, L. C., Smith, R. R., and Binkley, S. B. *J. Am. Chem. Soc.* **71** (1949) 60.

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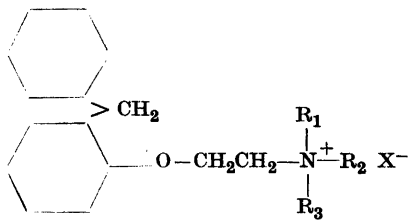
Table 1. 2-Benzylphenoxyethylammonium halides.



	m. p. °C	% X calc.	% X found	cryst. from	solubility in water
$R_1=CH_3$, $R_2=CH_3$, $R_3=CH_3$, X=I	159	32.17	32.04	abs. ethanol	sl. soluble
$R_1=C_2H_5$, $R_2=C_2H_5$, $R_3=C_2H_5$, X=Br	151	20.38	20.26	abs. ethanol-ether (1 : 1)	»
$R_1=CH_3$, $R_2=CH_3$, $R_3=C_2H_5$, X=Br	110	21.94	21.46	» »	very »
$R_1=CH_3$, R_2CH_3, $R_3=CH_2COOC_2H_5$, X=Cl	154	9.40	9.72	ethylacetate: alcohol (1 : 1)	» »

Ethyl n-*valeryl*-n-*valerate*. The same procedure as above was used, except that 400 ml of xylene was used instead of the toluene and 130 g (1 mole) of dry ethyl *n*-valerate instead of the ethyl *n*-butyrate. Yield 80 g (75 %) of pure ethyl *n*-valeryl-*n*-valerate boiling at 124–126°/10 mm.

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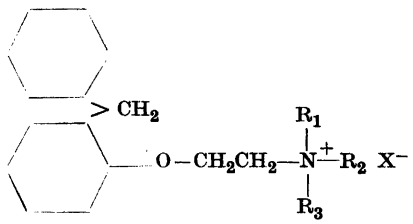

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$R_1=CH_3$, $R_2=CH_3$, $R_3=CH_3$, X=I	159	32.17	32.04	abs. ethanol	sl. soluble
$R_1=C_2H_5$, $R_2=C_2H_5$, $R_3=C_2H_5$, X=Br	151	20.38	20.26	abs. ethanol-ether (1 : 1)	»
$R_1=CH_3$, $R_2=CH_3$, $R_3=C_2H_5$, X=Br	110	21.94	21.46	» »	very »
$R_1=CH_3$, R_2CH_3, $R_3=CH_2COOC_2H_5$, X=Cl	154	9.40	9.72	ethylacetate: alcohol (1 : 1)	» »