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REAKTIONEN ENOLISCHER ZUCKERDERIVATE

TEIL I. HYDROBORIERUNG ENOLISCHER ZUCKERDERIVATE, EIN WEG ZUR DARSTELLUNG SCHWER ZUGÄNGLICHER HEXOSEN UND ZUR SPEZIFISCHEN MARKIERUNG MIT TRITIUM*

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EINFÜHRUNG

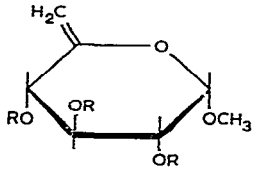
In zahlreichen Arbeiten wurde von H. C. Brown *et al.*[1] die Anlagerung von Diboran an Olefine beschrieben, die unter der Bezeichnung Hydroborierung vielfache Anwendung in der präparativen organischen Chemie gefunden hat. Die *cis*-Addition von Boran, die, nach Verseifung, zur Bildung von Alkoholen führt, verläuft mit wenigen Ausnahmen anti-Markownikoff. Erst vor etwa zwei Jahren konnten Wolfrom *et al.*[2] die Brauchbarkeit der Methode für die Kohlenhydratchemie zeigen, indem sie 5,6-Didesoxy-1,2-*O*-isopropyliden-α-D-*xylo*-hexofuranose-5-en über eine Hydroborierung der nicht enolischen Doppelbindung zu 5-Desoxy-1,2-*O*-isopropyliden-α-D-*xylo*-hexofuranose umsetzten. Ein anderes Beispiel für die Hydroborierung eines ungesättigten Zuckers wurde inzwischen auch von Arzoumanian *et al.*[3] veröffentlicht. Im Folgenden soll an einigen Beispielen die Reaktion von Diboran mit enolischen Zuckerderivaten beschrieben werden. Je nach Lage der Doppelbindung entstehen dabei die entsprechenden Hexose-Isomeren. Damit wird ein neues Verfahren beschrieben, um Epimerisierungen an asymmetrischen C-Atomen von Zuckern vorzunehmen. Bei Verwendung von Diboran-^{3}H eignet sich die Methode zur Darstellung von Zuckern, die spezifisch mit Tritium markiert sind.

DISKUSSION

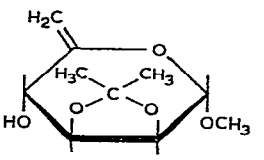
Im Gegensatz zu stereospezifisch verlaufenden radikalischen Additionen die nur zu einem Reaktionsprodukt führen[4], erfolgt die Hydroborierung eines enolischen Zuckers, wenn es extreme sterische Verhältnisse nicht ausschließen, unter Bildung von mindestens zwei Isomeren. Dieses meist unerwünschte Reaktionsergebnis hat im Falle der Hydroborierung von enolischen Zuckerderivaten Vorteile, lassen sich doch aus leicht zugänglichen seltener vorkommende Zucker darstellen. Es gelang, durch Hydroborierung einer endständigen enolischen Doppelbindung aus Derivaten der D-Glucose solche der L-Idose und aus D-Mannose-Derivaten L-Gulose-Derivate herzustellen. Die Hydroborierung von Galaktosid-5-en, die neben D-Galaktose

*Vorläufige Mitteilung: J. LEHMANN, *Angew. Chem.*, 77 (1965) 863.

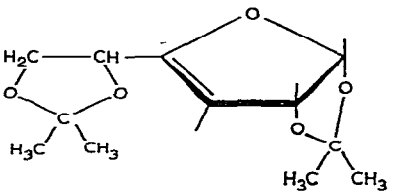
auch L-Altrose ergeben sollte, wurde nicht durchgeführt, dürfte aber analog verlaufen. Das Mengenverhältnis der entstehenden Isomeren richtet sich offenbar nach den sterischen Verhältnissen in der Muttersubstanz und kann durch den Einfluß geeigneter Substituenten mehr in die eine oder andere Richtung gelenkt werden (Tabelle I). Neben dieser präparativ interessanten Anwendung eignet sich die Hydroborierung enolischer Zuckerderivate zur spezifischen Markierung von Zuckern mit Tritium oder Deuterium. Zucker mit wohldefinierter radioaktiver Markierung sind für die Aufklärung von Mechanismen chemischer oder biochemischer



I R = -H
Ia R = -H
Ib R = -Si(CH$_3$)$_3$



II



III

TABELLE I

BILDUNG VON HEXOSE-DERIVATEN DURCH HYDROBORIERUNG VON ENOLISCHEN ZUCKERDERIVATEN

Enol	*Additionsprodukte nach Aufarbeitung*[a]	*Mengenverhältnis der Reaktionsprodukte*[b]
Methyl-6-desoxy-glucopyranosid-5-en (I)	Methyl-α-D-glucopyranosid Methyl-β-L-idopyranosid	1:2.5
Methyl-2,3,4-tri-*O*-methyl-6-desoxy-α-glucopyranosid-5-en (Ia)	Methyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosid Methyl-2,3,4-tri-*O*-methyl-β-L-idopyranosid	1:2
Methyl-2,3,4-tris-*O*-trimethylsilyl-6-desoxy-α-glucopyranosid-5-en (Ib)	Methyl-α-D-glucopyranosid Methyl-β-L-idopyranosid	1:0.6
Methyl-2,3-*O*-isopropyliden-6-desoxy-α-mannopyranosid-5-en (II)	Methyl-α-D-mannopyranosid Methyl-β-L-gulopyranosid	1:2
1,2:5,6-Di-*O*-isopropyliden-3-desoxy-D-glucose-3-en (III)	D-Galaktose	—

[a]Bei diesen Verbindungen handelt es sich um Anti-Markownikoff-Additionsprodukte. Markownikoff-Additionsprodukte wurden in keinem Fall beobachtet.
[b]Die Mengenverhältnisse wurden durch quantitative Auswertung von Papierchromatogrammen der tritierten Gemische mit Hilfe des fensterlosen Isotopenzählgerätes bestimmt. Das Verhältnis der Produkte aus Ia wurde aus Gaschromatogrammen ermittelt.

Umwandlungen (Epimerisierung, Synthese oder Abbau) der Kohlehydrate von Bedeutung.

Markierungen mit Tritium oder Deuterium an sekundären C-Atomen der Zucker gelangen bisher nur durch Reduktion verhältnismäßig schwer zugänglicher Ketoderivate mit markierten komplexen Hydriden. Die ausschließliche Markierung am C-Atom, welches zuvor die Ketogruppe trug, nach diesem Verfahren wurde wenigstens in einem Falle in Zweifel gestellt. Bevill *et al.*[5] fanden nämlich, daß bei der Reduktion von Methyl-2,3,6-tri-*O*-methyl-4-keto-α-D-glucosid mit $NaBH_4$-^3H Tritium nicht nur in 4-Stellung, sondern auch in 6-Stellung gefunden werden konnte und zwar in dem bemerkenswerten Verhältnis von 40% ^3H in 6- und 60% in 4-Stellung.

Die Reaktion von enolischen Zuckerderivaten mit Diboran soll am Beispiel von Derivaten des Methyl-6-desoxy-α-glucopyranosid-5-en (I)[6], von 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-glucose-3-en (III)[7], und von Methyl-2,3-*O*-isopropyliden-6-desoxy-α-mannopyranosid-5-en (II)[4] ausführlich beschrieben werden.

Hydroborierungen werden im allgemeinen erreicht, indem man im Reaktionsgemisch Diboran aus Natriumborhydrid mit Hilfe einer Säure freisetzt und mit dem Olefin reagieren läßt. Für Hydroborierungen von säureempfindlichen enolischen Zuckern ist diese Methode jedoch kaum geeignet, da einerseits durch teilweise Hydrolyse Verluste eintreten können und andererseits die Aufarbeitung in manchen Fällen Schwierigkeiten bereitet. Das Diboran wurde deshalb separat durch Zutropfen einer Natriumborhydridlösung in Diglym zu Bortrifluoridätherat gebildet[8] und mit einem Inertgas in den Reaktionskolben übergespült. Wie durch dünnschichtchromatographische Analyse des Reaktionsgemisches festgestellt werden konnte, ist die Umsetzung im allgemeinen nach 30 bis 40 Minuten beendet.

Auch unvollständig substituierte Zuckerderivate mit enolischer Doppelbindung lassen sich in Tetrahydrofuran hydroborieren, eignen sich jedoch weniger als Ausgangsmaterial, weil die freien Hydroxylgruppen mit Diboran unter Bildung von vernetzten Borsäureestern reagieren. Deshalb müssen grössere Mengen Diboran verwendet werden, was bei der Anwendung von Diboran-^3H sehr kostspielig werden kann. Außerdem fallen die Ester teilweise aus und verhindern damit den glatten Ablauf der Reaktion. Trotzdem wurde Methyl-6-desoxy-α-glucopyranosid-5-en (I) mit Diboran umgesetzt. Nach Aufarbeitung konnten Methyl-α-D-glucosid und Methyl-β-L-idosid papierchromatographisch im Verhältnis 1:2,5 nachgewiesen werden. Eine glattere Reaktion erfolgt bei Verwendung des Methyl-2,3,4-tri-*O*-methyl-6-desoxy-α-glucopyranosid-5-en (Ia) als Ausgangsprodukt. Die Reaktionsprodukte können in Form ihrer 6-Phenylazobenzoylester an einer Silicagelsäule mit Essigester als Elutionsmittel voneinander getrennt und kristallin erhalten werden. Methyl-2,3,4-tri-*O*-methyl-6-*O*-phenylazobenzoyl-α-D-glucopyranosid (IV) konnte mit Hilfe der auf anderem Wege dargestellten gleichen Verbindung identifiziert werden.

Verseifung der 6-Phenylazobenzoylester und Abspaltung der Methylgruppen mit Bortrichlorid in Methylenchlorid führt nur im Fall des Glucosid-Derivates zum freien Zucker. Das Idosid-Derivat zersetzt sich bei der Behandlung mit Bortrichlorid

unter Schwarzfärbung. Noch besser eignet sich der Tris-(trimethylsilyl)-äther des Methyl-6-desoxy-α-glucopyranosid-5-ens (Ib) als Ausgangsprodukt. Die Entfernung der Trimethylsilyl-Schutzgruppen gelingt ohne Verluste mit verdünnter Essigsäure in Methanol. Methyl-α-D-glucosid kann nach Verseifung des Borangemisches und Aufarbeitung direkt aus der Reaktionsmischung mit Aethanol kristallisiert werden. Auf diese Weise gelingt die Trennung von Methyl-β-L-idosid, das in Aethanol leicht löslich ist. Die weitere Reinigung des Methyl-β-L-idosids kann durch präparative Papierchromatographie erfolgen. Nach Säurebehandlung geht es in L-Idosan über, das als Triacetyl-Derivat identifiziert wurde. Es ist bemerkenswert, daß bei der Hydroborierung von Methyl-2,3,4-tri-*O*-methyl-6-desoxy-α-glucopyranosid-5-en (Ia) das Mengenverhältnis der gebildeten D-Glucosid- zu L-Idosid-Derivaten wie 1:2 ist, während bei Verwendung von Methyl-2,3,4-tris-*O*-trimethylsilyl-6-desoxy-α-glucopyranosid-5-en (Ib) das Verhältnis auf 1:0,6 zugunsten des Glucosid-Derivates verschoben wird (Tabelle I).

Wie schon erwähnt eignet sich die Hydroborierung enolischer Zuckerderivate zur Tritium- bzw. Deuterium-Markierung von Zuckern, wenn markiertes Diboran verwendet wird. Mit Diboran-^{3}H entsteht aus Methyl-6-desoxy-α-glucopyranosid-5-en (I) Methyl-α-D-glucosid-5-^{3}H und Methyl-β-L-idosid-5-^{3}H. Die ausschließliche Markierung in 5-Stellung wurde durch den Abbau der Glucose-5-^{3}H nach einer Methode von Simon *et al.*[9] gesichert (Tabelle II).

TABELLE II

ABBAU VON D-GLUCOSE-^{3}H[a]

Gemessene Verbindung	*dpm/mMol* × 10^3	*erfaßte C-Atome*
D-Glucose	1150	1 2 3 4 5 6
"D-Glucose"-phenylosotriazol	1160	1 3 4 5 6
4-Formyl-2-phenyl-1,2,3-triazol	0	1 3
Formaldehyd	0	6
2 Mol Ameisensäure	1130	4 5
"6-*O*-Benzoyl-D-glucose"-phenylosotriazol	1145	1 3 4 5 6
Ameisensäure aus "6-*O*-Benzoyl-D-glucose"-phenylosotriazol	26[b]	4

[a]Spezifische Aktivität wurde mit Hilfe eines Flüssigkeitsscintillationszählers ermittelt.
[b]Aktivität ist auf eine geringe Verunreinigung von 6-*O*-Benzoyl-D-glucose-phenylosotriazol mit D-Glucose-phenylosotriazol zurückzuführen, die durch Umkristallisieren nicht vollständig entfernt werden kann.

Ähnlich wie die Hydroborierung von Methyl-2,3,4-tri-*O*-methyl-6-desoxy-α-glucopyranosid-5-en (Ia) verläuft die Reaktion von Methyl-2,3-*O*-isopropyliden-6-desoxy-α-mannopyranosid-5-en (II) mit Diboran. Die Reaktion wurde mit Diboran-^{3}H durchgeführt und ergibt nach Aufarbeitung und papierchromatographischer Trennung Methyl-α-D-mannosid-5-^{3}H und Methyl-β-L-gulosid-5-^{3}H im Verhältnis 1:2. Ersteres wurde durch Cokristallisation mit Methyl-α-D-mannosid identifiziert, letzteres nach saurer Hydrolyse als 2-Benzyl-2-phenyl-hydrazon durch Cokristallisation mit authentischem L-Gulose-2-benzyl-2-phenyl-hydrazon[10].

Nicht nur exocyclische, sondern auch endocyclische Doppelbindungen enolischer Zuckerderivate können, wie das Beispiel des 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-glucose-3-en (III) zeigte, in glatter Reaktion hydroboriert werden*. Eine Probe vom Schmelzpunkt 51°, die nach den Literaturangaben[11] rein sein sollte, ergibt nach Reaktion mit Diboran-^{3}H und Aufarbeitung neben Galaktose als Hauptkomponente zwei Nebenprodukte. Diese Nebenprodukte sind auf Verunreinigungen zurückzuführen, die im 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-glucose-3-en (III) vom Schmelzpunkt 51° vorhanden sind. Die Verunreinigungen können durch analytische Gaschromatographie nachgewiesen werden, sie wurden jedoch nicht eindeutig identifiziert. Wahrscheinlich handelt es sich um Anhydrozucker mit mindestens einer Doppelbindung. Nach mehrmaliger Sublimation erhält man das Ausgangsprodukt (III) mit einem Schmelzpunkt von 52.5–53.5°. Geringe Mengen der Nebenprodukte lassen sich jedoch weder durch wiederholte Sublimation noch Rekristallisation vollständig entfernen. Die Nebenprodukte der Hydroborierung verringern sich mit zunehmender Reinheit des Ausgangsproduktes (III). Es konnte jedenfalls sichergestellt werden, daß es sich bei den Nebenprodukten der Hydroborierung nicht um die möglicherweise aus III entstandene D-Allose oder das Markownikoff-Additionsprodukt handeln kann. Ersteres wurde durch Cokristallisation des tritierten Reaktionsgemisches mit D-Allose ausgeschlossen, letzteres nach Reduktion der Mischung mit Natriumborhydrid und Copapierchromatographie mit 3-Desoxy-dulcit.

Aus den beschriebenen Untersuchungen geht hervor, daß die sterischen Verhältnisse in enolischen Zuckerderivaten einen starken Einfluß auf die Anlagerung von Diboran ausüben. Am Modell läßt sich dies veranschaulichen. Während das freie oder mit Methylgruppen substituierte Methyl-6-desoxy-α-glucopyranosid-5-en (I) einen Angriff des Diborans bevorzugt an der verhältnismäßig offen liegenden Seite der Doppelbindung zuläßt, was zu einem L-Idosid-Derivat mit axialer Hydroxymethyl-Gruppe führt, schirmt die raumerfüllende Silyl-Gruppe in 4-Stellung, da sie von den übrigen Silyl-Substituenten aus ihrer Lage gedrängt wird, die "Idosid-Lage" der Doppelbindung ab. Sie erlaubt dadurch einen relativ bevorzugten Angriff des Diborans in der "Glucosid-Lage", wobei eine äquatoriale Stellung der Hydroxymethyl-Gruppe resultiert. Eine etwas langsamere Reaktionsgeschwindigkeit der Silylglucosid-5-ene gegenüber den methylsubstituierten Glucosid-5-enen deutet auf eine allgemeine Behinderung der Additionsreaktion bei enolischen Zuckerderivaten mit raumerfüllenden Substituenten hin. Extreme Verhältnisse liegen bei einem starren Molekül wie dem 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-glucose-3-en (III) vor. Hier ist die "Allose-Lage" durch die Isopropyliden-Gruppe in 1,2-Stellung vollständig blockiert, während die "Galaktose-Lage" eine ungehinderte Anlagerung des Diborans erlaubt. Additionsprodukte gemäß der Markownikoff-Regel konnten in keinem der untersuchten Fälle beobachtet werden.

*Mit Hilfe der gleichen Reaktion wurde von Dr. H. Paulsen 1,2:5,6-Di-*O*-isopropyliden-D-galaktose als stark bevorzugtes Umsetzungsprodukt erhalten (persönl. Mittlg. vom 20.10.1965).

EXPERIMENTELLER TEIL

Allgemeine Methoden

Papierchromatographie. Papierchromatogramme wurden absteigend auf Whatman Nr. 4 – Chromatographiepapier in *n*-Butanol/Propionsäure/Wasser (142:71:100, v/v/v) angefertigt. Den zu trennenden Gemischen wurden jeweils die entsprechenden mit Tritium markierten Gemische zugesetzt und die Zonen bzw. Flecke mit Hilfe eines fensterlosen Isotopenzählgerätes (Modell 380 der Packard Instrument Company) lokalisiert. Bei unverdünnten tritierten Gemischen wurden die Substanzen durch Autoradiographie mit blauempfindlichen Röntgenfilmen sichtbar gemacht. Es ist dabei zu beachten, daß tritiummarkierte Substanzen bei etwa gleicher Zerfallsrate, in Desintegrationen pro Minute gemessen, eine etwa zwanzigfache Belichtungszeit wie entsprechende ^{14}C-markierte Verbindungen benötigen. Elution der Flecken erfolgt in allen Fällen mit Wasser.

Gaschromatographie. Es wurde das Gerät Modell 1609 der F und M Scientific Corporation mit Flammenionisationsdetektor verwendet. Als Trägermaterial wurde mit Säure gewaschenes und siliconiertes Gaschrom P (Applied Science Laboratories Inc.) verwendet. Die flüssige Phase besteht aus SE 52 Silicongummi (F u. M Scientific Corporation) in einer Menge von 3% des Trägermaterials.

Hydroxylgruppen enthaltende Reaktionsgemische werden vor der gaschromatographischen Analyse trimethylsilyliert[12].

Hydroborierungen. Diboran wird nach H. C. Brown[8] extern durch Zutropfen einer 1 M $NaBH_4$-Lösung in abs. Diglym zu Bortrifluoridätherat im Verhältnis 1:2 Mol gebildet. Für die Hydroborierung von kleinen Mengen wurde die von H.C. Brown angegebene Versuchsanordnung wie folgt modifiziert. Es handelt sich im Prinzip um drei gleichsinnig hintereinandergeschaltete, nach unten spitz zulaufende 70 ml-Waschflaschen, deren Einleitungsrohre dicht über dem Boden enden und sich nach unten verengen. Der Austrittsstutzen des Systems ist mit einem Quecksilberventil verschlossen. Jede der drei, am Kolbenhals offenen Einheiten wird mit einer Gummikappe abgedichtet, durch die Lösung eingespritzt werden kann.

Beim Arbeiten mit nicht markiertem Diboran werden nur zwei der drei Einheiten benutzt, die erste Einheit bleibt leer. In der zweiten befindet sich das Bortrifluoridätherat und in der dritten eine 0.25 M Lösung des Enols in abs. Tetrahydrofuran. Vor Beginn der Reaktion wird die Luft in der Apparatur durch Reinst-Stickstoff verdrängt. Dann wird mit Hilfe einer Injektionsspritze durch Durchstechen der Gummikappe die $NaBH_4$-Diglym-Lösung in das vorgelegte Bortrifluoridätherat eingetropft, wobei das gebildete Diboran mit dem Stickstoffstrom in die eisgekühlte Enol-Lösung gespült wird. Es werden pro Mol Enol 1,5 Mole $NaBH_4$ verwendet; für jede freie Hydroxyl-Gruppe im Enol muß zusätzlich 1 Mol $NaBH_4$ eingesetzt werden. Nach Zugabe des $NaBH_4$ läßt man die Reaktionslösung etwa 2 Std. stehen und arbeitet dann wie bei den einzelnen Versuchen beschrieben auf.

Bei der Umsetzung mit Diboran-^3H wird ein Unterschuß an $NaBH_4$-^3H (0.3 Mol pro Mol Olefin) in Diglym verwendet und sonst wie beschrieben verfahren.

Eine Standard-Lösung von $NaBH_4$-^3H wird durch Auflösen von 100 mc $NaBH_4$-^3H hoher spezifischer Aktivität (100 mc/mmol) in 10 ml M $NaBH_4$-Diglym-Lösung hergestellt. Nach Reaktionsende wird in der ersten Einheit noch 1 Mol nicht markiertes $NaBH_4$ mit der entsprechenden Menge Bortrifluoridätherat zu Diboran umgesetzt, um damit markiertes Diboran in der zweiten Einheit quantitativ in die Enol-Lösung zu überführen und eine vollständige Umsetzung zu erreichen. Die Ausbeuten an fixiertem ^3H betragen zwischen 2 und 10% bezogen auf die Aktivitätsangaben für käufliches $NaBH_4$-^3H.

Die Ausbeuten an Reaktionsprodukten liegen zwischen 80 und 90% d. Th., bezogen auf eingesetztes Enol.

Methyl-2,3,4-tri-O-methyl-6-desoxy-α-glucopyranosid-5-en (Ia)

Methyl-6-desoxy-α-glucopyranosid-5-en (I, 4.6 g) wird nach der Methode von Glen *et al.*[13] in Aceton (25 ml) mit Dimethylsulfat (12.6 ml) in Gegenwart von pulverisiertem NaOH (9.6 g) methyliert. Nach Zugabe von Wasser wird mit Chloroform extrahiert, die organische Phase mit Wasser gewaschen und über Na_2SO_4 getrocknet. Den Rückstand nach Abziehen des Lösungsmittels destilliert man im Vakuum. 3.4 g = 60% d. Th., $Kp_{0.3}$ 72–74°; $[\alpha]_{5780}^{22}$ +69° (*c* 2, $CHCl_3$) (Ber. für $C_{10}H_{18}O_5$: C, 55.01; H, 8.31; gef.: C, 55.25; H, 8.51%).

Methyl-2,3,4-tris-O-trimethylsilyl-6-desoxy-α-glucopyranosid-5-en (Ib)

I (15 g) werden nach einer Methode von Bentley *et al.*[12] in abs. Pyridin (120 ml) durch Zugabe von Hexamethyldisilazan (47 g) und Trimethylchlorsilan (47 g) umgesetzt. Die Reaktionsmischung bleibt 4 Std. bei Zimmertemperatur stehen, das Pyridin wird unter vermindertem Druck abgezogen, der Rückstand in Tetrachlorkohlenstoff aufgenommen, mit Wasser gewaschen und über Na_2SO_4 getrocknet. Nach Abziehen des Lösungsmittels wird der Rückstand destilliert. 24 g = 72% d. Th., $Kp_{0.3}$ 110–115°. Das farblose Destillat kristallisiert nach einigen Stunden bei 0°. Fp 40–41°; $[\alpha]_{5780}^{22}$ +46° (*c* 2, $CHCl_3$) (Ber. für $C_{16}H_{36}O_5Si_3$: C, 48.93; H, 9.24; gef.: C, 48.75; H, 9.11%).

Hydroborierung von Methyl-6-desoxy-α-glucopyranosid-5-en (I)

Die Hydroborierung wurde mit 1 mmol I (0.18 g) unter den oben beschriebenen Bedingungen mit Diboran-^3H durchgeführt. Nach Reaktionsende wurde überschüssiges Diboran mit einigen Tropfen Methanol zerstört, das Lösungsmittel im Vakuum abgezogen. Den Rückstand nimmt man in 0.4 g NaOH in 5 ml Wasser auf und versetzt mit 30%igem Wasserstoffperoxid (0.5 ml). Nach 3 Stunden bei Zimmertemperatur werden Natriumionen durch Rühren mit einer ausreichenden Menge Dowex 50 (400 mesh) entfernt. Danach zentrifugiert man den Austauscher ab, wäscht ihn mit 10 ml Wasser nach und zerstört überschüssiges Wasserstoffperoxid im schwach sauren Überstand durch Zugabe einer Spur PtO_2. Die filtrierte Lösung wird im Vakuum eingedampft und der Rückstand durch mehrmaliges Eindampfen mit Methanol von Borsäure befreit. Den Rückstand löst man in wenig Wasser und

chromatographiert auf Papier. Es können zwei Substanzen lokalisiert werden, von denen die eine Methyl-α-D-glucopyranosid-5-^{3}H (R_F 0.50 ± 0.05), nach Elution der Zone mit Wasser Cokristallisation* mit authentischem Methyl-α-D-glucopyranosid, die andere Methyl-β-L-idopyranosid-5-^{3}H (R_F 0.65) ist. Das Methyl-β-L-idopyranosid-5-^{3}H wurde zu Triacetyl-L-idosan umgesetzt und durch Cokristallisation mit Triacetyl-L-idosan identifiziert.

Hydroborierung von Methyl-2,3,4-tris-O-trimethylsilyl-6-desoxy-α-glucopyranosid-5-en (Ib)

10 mMol Ib (4 g) werden mit der entsprechenden Menge nicht markiertem Diboran hydroboriert. Nach Vernichtung überschüssigen Diborans mit Methanol wird im Vakuum eingedampft und der Rückstand in Methanol aufgenommen. Zu der Lösung (40 ml) gibt man 30%ige Essigsäure (40 ml) und schüttelt 4 Std. bei Zimmertemperatur. Die Mischung wird mit Wasser (50 ml) verdünnt und das Trimethylsilanol mit Chloroform extrahiert. Die wässrige Lösung wird im Vakuum eingedampft, der sirupöse Rückstand in 8%iger Natronlauge (25 ml) aufgenommen und mit 30%igem Wasserstoffperoxid (2,5 ml) versetzt. Nach 3 Std. wird der Ansatz analog dem zuvor beschriebenen aufgearbeitet. Nach der Abtrennung der Borsäure wird der Rückstand in wenig warmen Acthanol aufgenommen. Dei bei 0° abgeschiedenen Kristalle haben einen Fp von 164–165°. Der Mischschmelzpunkt mit Methyl-α-D-glucopyranosid zeigt keine Depression. Durch Einengen und Abkühlen der Mutterlauge wird das Methyl-α-D-glucopyranosid weitgehend abgeschieden, wie die gaschromatographische Analyse dieser Mutterlauge zeigt.

Triacetyl-L-idosan. Die Mutterlauge wird durch Zugabe von wenig tritiertem Material, das aus der Umsetzung von Ib mit Diboran-^{3}H nach der gegebenen Vorschrift erhalten wurde, radioaktiv markiert und in präparativem Maßstab papierchromatographisch aufgetrennt. Es werden etwa 100 mg Substanz in 10%iger wässriger Lösung auf einen Bogen von 60 cm Breite aufgetragen. Die schneller wandernde Zone des Methyl-β-L-idosids wird ausgeschnitten und eluiert. Nach Eindampfen der Eluate im Vakuum löst man den sirupösen Rückstand (insgesamt 0.4 g) in N Schwefelsäure (10 ml) und erhitzt 6 Std. auf dem Dampfbad. Das resultierende Reaktionsgemisch wird nach den Angaben von Wiggins[14] für D-Idosan aufgearbeitet und acetyliert. Ausbeute: 0.29 g = 49%; Fp 85–85.5°; $[\alpha]_{5780}^{25}$ +76.5° (*c* 1, $CHCl_3$); $[\alpha]_D^{25}$ +73° (*c* 1, $CHCl_3$); Schmelzpunkt und Drehwert stimmen mit den Werten für D-Idosan[14] überein (Ber. für $C_{12}H_{16}O_8$: C, 50.00; H, 5.60; gef.: C, 50.01; H, 5.65%).

Verbindung (Ib) wird auch mit Diboran-^{3}H umgesetzt. Man verfährt dabei wie bereits unter "Hydroborierungen" beschrieben. Die Aufarbeitung erfolgt wie

*Nach Vermischen einer Spur der radioaktiv markierten Verbindung mit einer authentischen Probe wird 2–3mal bis zur Konstanz der spezifischen Aktivität aus einem geeigneten Lösungsmittel umkristallisiert. Die Messung der spezifischen Aktivität erfolgt nach Auflösen einer gewogenen Probe in Methanol (5 ml) und Vermischen der Lösung mit Scintillationsflüssigkeit (10 ml) in einem Scintillationszählgerät (720 Series, Liquid Scintillation System der Nuclear Chicago Company).

im vorhergehenden Abschnitt für Hydroborierung von Ib mit nicht markiertem Diboran beschrieben, unter Verwendung entsprechend kleinerer Mengen. Die Trennung des Reaktionsgemisches wird papierchromatographisch ohne vorhergehende Kristallisation von Methyl-α-D-glucopyranosid-5-^{3}H durchgeführt.

Hydroborierung von Methyl-2,3,4-tri-O-methyl-α-glucopyranosid-5-en (Ia)

0.01 Mol Ia (2.2 g) werden mit nicht markiertem Diboran umgesetzt. Nach Zerstörung überschüssigen Diborans mit Methanol und Entfernung von Tetrahydrofuran und Methanol wird der Rückstand in abs. Aethanol (10 ml) aufgenommen. Man versetzt unter Eiskühlung mit einer Lösung von pulverisiertem NaOH (1.5 g) in abs. Aethanol (15 ml) und tropft dann unter kräftigem Rühren 30%iges Wasserstoffperoxid (2 ml) zu. Nach zweistündigem Rühren bei Zimmertemperatur wird der anorganische Niederschlag abzentrifugiert, mit wenig abs. Aethanol gewaschen und erneut zentrifugiert. Die vereinten Überstände werden mit Dowex-50 (400 mesh) von Natriumionen und durch wiederholte Destillation mit Methanol von Borsäure befreit. Das Lösungsmittel wird abgezogen und der sirupöse Rückstand im Vakuum destilliert. 1.9 g = 80% d. Th.; $Kp_{0.2}$ 90–98°. Die gaschromatographische Analyse zeigt, daß das Gemisch aus zwei Komponenten besteht.

Methyl-2,3,4-tri-O-methyl-6-O-phenylazobenzoyl-α-D-glucopyranosid (IV) und Methyl-2,3,4-tri-O-methyl-6-O-phenylazobenzoyl-β-L-idosid (V)

Das Destillat vom $Kp_{0.2}$ 90–98° (0.25 g) wird in abs. Pyridin (2.5 ml) mit Phenylazobenzoylchlorid (0.3 g) 3 Std. auf 100° erhitzt. Aufarbeitung erfolgt wie bei Foster *et al.*[15] beschrieben. Das gewonnene Azoylester-Gemisch kristallisiert teilweise aus. Aus Aethanol kristallisiert ein dünnschichtchromatographisch reines Produkt. Es handelt sich hierbei um V, das allerdings nicht eindeutig durch Vergleich mit authentischem Material identifiziert werden konnte. Die Analogie der Reaktion von Ia mit Diboran zu der von I und Ib läßt jedoch auf V schließen. Durch Säulenchromatographie (siehe unten) läßt sich noch eine kleine Menge des Produktes gewinnen: 0.31 g. Fp 119–120° (Ber. für $C_{23}H_{28}O_7N_2$: C, 62.16; H, 6.35; N, 6.31; gef.: C, 61.91; H, 6.37; N, 6.52%).

Die Mutterlauge wird eingeengt und auf einer Silicagel-Säule mit Essigester chromatographiert. Die schneller laufende Zone wird aufgefangen und das Eluat eingedampft. Der Rückstand kristallisiert spontan und wird aus Petroläther (60–80°) umkristallisiert. Es handelt sich um VI. Ausbeute: 0.16 g; Fp 80° (Ber. für $C_{23}H_{28}O_7N_2$: C, 62.16; H, 6.35; N, 6.31; gef.: C, 62.10; H, 6.47; N, 6.44%).

Daneben kann durch weitere Elution noch eine kleine Menge V erhalten werden.

Verseifung von V zu Methyl-2,3,4-tri-O-methyl-β-L-idopyranosid (VI)

V (0.8 g) wird in einer Lösung von KOH (1.2 g) in Aethanol (30 ml) aufgenommen und eine Stunde am Rückfluß gekocht. Man läßt abkühlen und dann eine

Stunde bei 0° stehen. Das vom ausgefallenen Niederschlag abgetrennte fast farblose Filtrat wird im Vakuum bis fast zur Trockene eingedampft, in Wasser aufgenommen und mit Chloroform extrahiert. Nach Waschen und Trocknen der organischen Phase über Na_2SO_4 wird das Lösungsmittel im Vakuum abgezogen und der ölige Rückstand destilliert. Ausbeute: 0.39 g = 87% d. Th.; $Kp_{0.2}$ 84–85°; $[\alpha]_{5780}^{22}$ +102° (*c* 2.2, $CHCl_3$) (Ber. für $C_{10}H_{20}O_6$: C, 50.82; H, 8.53; gef.: C, 50.95; H, 8.76%).

*Verseifung von IV zu Methyl-2,3,4-tri-*O*-methyl-α-*D*-glucopyranosid (VII)*

IV wird wie für V beschrieben verseift und aufgearbeitet. Ausbeute: 85% d. Th.; $Kp_{0.2}$ 89–92°; $[\alpha]_{5780}^{22}$ +154° (*c* 1.5, $CHCl_3$) (Ber. für $C_{10}H_{20}O_6$: C, 50.82; H, 8.53; gef.: C, 51.02; H, 8.76%).

*Darstellung von Methyl-2,3,4-tri-*O*-methyl-α-*D*-glucopyranosid aus Methyl-6-*O*-triphenylmethyl-α-*D*-glucopyranosid (VIII)*

Zur eindeutigen Identifizierung von VII wird VIII[16] auf herkömmlichen Weg durch Permethylierung nach der Methode von Glenn[13] umgesetzt. Der Schmelzpuntk des gewonnenen Produktes, Methyl-2,3,4-tri-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranosid (IX), stimmt nicht mit dem Literaturwert überein[16] (109° gegenüber 166–167°). Es wurde daher die von Robertson *et al.*[16] angegebene Permethylierung mit Silberoxid und Methyljodid nachgearbeitet, wobei ebenfalls ein Produkt vom Fp 167–168° resultierte. Es kann sich jedoch nicht um die gewünschte Verbindung IX handeln, da das i.r.-Spektrum eine starke Hydroxylabsorption zeigt, die bei dem Produkt vom Fp 109° nicht auftritt (Ber. für IX, $C_{29}H_{24}O_6$: C, 72.77; H, 7.17; gef.: C, 73.07; H, 6.82%).

Enttritylierung[16] von IX ergibt ein Öl, das nach der Destillation beim Abkühlen kristallin erstarrt. Fp 23°; $Kp_{0.2}$ 90–94°; $[\alpha]_{5780}^{22}$ +162° (*c* 3, $CHCl_3$) (Ber. für $C_{10}H_{20}O_6$: C, 50.82; H, 8.53; gef.: C, 50.80; H, 8.56%). Es handelt sich hierbei um bisher nicht beschriebenes Methyl-2,3,4-tri-*O*-methyl-α-D-glucopyranosid. Es ist gaschromatographisch nicht von dem durch Hydroborierung erhaltenen Produkt zu unterscheiden. Die i.r.-Spektren sind ebenfalls identisch. Die Umsetzung zum 6-Phenylazobenzoylester IV ergibt ein Produkt, das bei 82° schmilzt. Der Mischschmelzpunkt mit dem über die Hydroborierung gewonnenen Produkt zeigt keine Depression. Ebensowenig zeigen die i.r.-Spektren irgendwelche Unterschiede.

*Entmethylierung von Methyl-2,3,4-tri-*O*-methyl-α-*D*-glucopyranosid (VII)*

Die Entmethylierung wurde nach einer Methode von Bonner *et al.*[17] mit Bortrichlorid durchgeführt. VII (0.1 g) wird in Dichlormethan (1 ml) gelöst, mit Aceton/Trockeneis gekühlt und unter Ausschluß von Feuchtigkeit mit Bortrichlorid (5 ml) versetzt. Man läßt etwa 30 Minuten im Kältebad stehen und dampft dann überschüssiges Bortrichlorid und das Dichlormethan bei 30–40° ab. Der Rückstand wird 30 Minuten auf 50–60° erwärmt und nach Kühlung vorsichtig mit Methanol versetzt. Man dampft die Lösung zur Trockene ein und wiederholt den gesamten Arbeitsgang dreimal. Beim letzten Arbeitsgang wird anstelle reinen Methanols

50%iges wässriges Methanol zugesetzt. Nach Eindampfen erweist sich der Rückstand (0.055 g) als papierchromatographisch reine D-Glucose.

Die Entmethylierung von VI gelang unter den angegebenen Bedingungen nicht, da sich die Reaktionslösung bei der Zugabe von Bortrichlorid dunkel färbt und sich beim Aufarbeiten zersetzt.

Hydroborierung von Methyl-2,3-O-isopropyliden-6-desoxy-α-mannopyranosid-5-en(II)

Die Hydroborierung von II (1 mmol) wurde mit Diboran-^{3}H durchgeführt. Aufarbeitung erfolgt wie für Ia beschrieben. Nach der Entfernung von Natriumionen und Borsäure wird die Isopropyliden-Gruppe durch einstündiges Erhitzen auf dem Dampfbad in 50%iger Essigsäure (10 ml) abgespalten. Die Essigsäure wird mit Wasser azeotrop abdestilliert und schließlich die Lösung im Vakuum zur Trockene eingedampft. Das tritierte Gemisch kann papierchromatographisch in zwei Komponenten zerlegt werden. Die schneller laufende Zone (R_F 0.45) erwies sich nach Elution und Cokristallisation mit authentischem Methyl-α-D-mannopyranosid als Methyl-α-D-mannopyranosid-5-^{3}H. Die langsamer laufende Komponente (R_F 0.36) wird eluiert, das Eluat nach dem Eindampfen mit N HCl (3 ml) bei 100° in 3 Std. hydrolysiert und nach Umsetzung mit 1-Benzyl-phenylhydrazin[10] durch Cokristallisation mit authentischem L-Gulose-benzyl-phenylhydrazon[10] als L-Gulose-5-^{3}H-benzyl-phenylhydrazon identifiziert.

Hydroborierung von 1,2:5,6-Di-O-isopropyliden-3-desoxy-glucose-3-en (III)

III vom Fp 52.5–53° (1 mmol) wird mit Diboran-^{3}H hydroboriert. Aufarbeitung erfolgt wie für II beschrieben. Nach papierchromatographischer Analyse kann nur ein Produkt aufgefunden werden. Elution der Zone und Acetylierung eines Teils des Verdampfungsrückstandes (0.01 g) mit Acetanhydrid (0,1 ml) und wasserfreiem Natriumacetat ergibt nach der üblichen Aufarbeitung einen Rückstand (0.009 g), der ohne Aktivitätsverlust mit Penta-*O*-acetyl-β-D-galaktopyranose cokristallisiert werden kann.

Damit ist bewiesen, daß das einzige bei der Reaktion entstandene Produkt D-Galaktose-4-^{3}H ist.

DANK

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ZUSAMMENFASSUNG

Durch Hydroborierung von enolischen Hexose-Derivaten mit Diboran-^{3}H kann eine spezifische Markierung von Hexosen mit Tritium erreicht werden. Es wird die Synthese von Methyl-α-D-glucopyranosid-5-^{3}H, Methyl-ß-L-idopyranosid-5-^{3}H, Methyl-α-D-mannopyranosid-5-^{3}H, Methyl-β-L-gulopyranosid-5-^{3}H, und D-Galaktose-4-^{3}H beschrieben.

Die Methode eignet sich auch zur teilweisen Umwandlung leicht zugänglicher Zucker, wie D-Glucose, D-Mannose, und D-Galaktose in die entsprechenden seltenen Zucker L-Idose, L-Gulose, und L-Altrose über enolische Derivate der Ausgangsprodukte. Im Falle des 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-glucose-3-ens konnte eine vollständige Umwandlung von D-Glucose in D-Galaktose erreicht werden.

In allen Fällen konnte nur anti-Markownikoff-Addition von Diboran an enolische Zuckerderivate beobachtet werden.

Die sterischen Effekte von Substituenten auf die Addition von Diboran an enolische Zuckerderivate werden diskutiert.

SUMMARY

By hydroboration of enolic hexose derivatives with diborane-^{3}H, specific labelling with tritium can be achieved in hexoses. Syntheses of methyl α-D-glucopyranoside-5-^{3}H, methyl β-L-idopyranoside-5-^{3}H, methyl α-D-mannopyranoside-5-^{3}H, methyl β-L-gulopyranoside-5-^{3}H, and D-galactose-4-^{3}H are described.

This method can also be used to change partially easily accessible sugars such as D-glucose, D-mannose, and D-galactose into rare sugars such as L-idose, L-gulose, and L-altrose, respectively, via enolic derivatives of the starting materials. In the case of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hexofuran-3-enose a complete conversion of D-glucose into D-galactose has been achieved.

In all cases, anti-Markownikoff addition of diborane to enolic sugar derivatives was observed.

The steric effects of substituents on the addition of diborane to enolic sugar derivatives are discussed.

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DERIVATIVES OF 2-HYDROXY-1,4-OXATHIANE AND 2-HYDROXYMORPHOLINE. A NEW CLASS OF SUGAR*

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INTRODUCTION

An approach[1] to the stereospecific synthesis of naturally-occurring, optically-active sulphoxides[2] and of asymmetric quaternary derivatives of nitrogen (and other hetero atoms) having known absolute configuration requires, initially, the incorporation of the hetero atom into an asymmetric molecular framework of known absolute stereochemistry, so that the stereochemistry of the conversion of the hetero atom into the higher valency state may be ascertained. This initial stage may be effected by the conversion of suitably blocked glycopyranosides into derivatives of 2-hydroxy-1,4-oxathiane and 2-hydroxymorpholine. We now report on some of these compounds which, in effect, constitute a new class of sugar containing two hetero atoms in a six-membered, cyclic hemi-acetal. There are numerous examples of sugar derivatives in which the ring oxygen atom has been replaced by sulphur[3] or nitrogen[4].

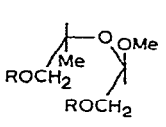
RESULTS AND DISCUSSION

Oxidation of methyl α-L-rhamnopyranoside with sodium metaperiodate, and reduction of the resulting dialdehyde with sodium borohydride afforded (2*S*,1′*R*)-2-(2′-hydroxy-1′-methoxyethyloxy)propan-1-ol[5] (I), which was characterised as the di-*p*-phenylazobenzoate. When the ditosylate (II) was treated with sodium sulphide in boiling methanol, smooth conversion into (2*R*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane (III) occurred; in the reaction sequence leading to the oxathiane (III), the absolute configuration at positions 2 and 6 is unaffected. Similarly, the ditosylate (IV) (derived[6] from 1,6-anhydro-β-D-glucopyranose by application in sequence of periodate oxidation, borohydride reduction, and tosylation) was converted into (1*R*,4*R*)-2,8-dioxa-6-thiabicyclo[3,2,1]octane (V).

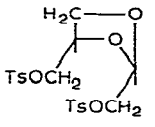
The conversion of methyl α-D-glucopyranoside into a 1,4-oxathiane derivative could not be effected directly. Treatment of methyl 6-*O*-trityl-α-D-glucopyranoside with lead tetra-acetate in pyridine (or chloroform) followed by reduction of the product with sodium borohydride gave a mixture of diol and triol arising from

*A preliminary report of some of these results has been published: K. W. BUCK, A. B. FOSTER, A. R. PERRY, AND J. M. WEBBER, *Chem. Commun.*, (1965) 433.

incomplete oxidation of the glucoside derivative, and a second treatment with the reagents was necessary to effect complete conversion into (2*R*,1′*S*)-2-*O*-(2′-hydroxy-1′-methoxyethyl)-1-*O*-tritylglycerol (VI). The crystalline ditosylate of the syrupy diol (VI) readily afforded (2*S*,6*R*)-2-methoxy-6-trityloxymethyl-1,4-oxathiane (VII) on treatment with sodium sulphide in boiling methanol. Detritylation of the 1,4-oxathiane derivative (VII) was accomplished by hydrogenolysis over palladised charcoal to give (2*S*,6*R*)-6-hydroxymethyl-2-methoxy-1,4-oxathiane (VIII).



I R=H
II R=Ts



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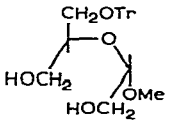
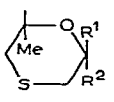
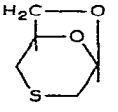


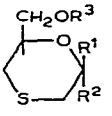
VI



III R^1 = OMe, R^2 = H
X R^1, R^2 = H, OH
XI R^1 = H, R^2 = OAc
XIII R^1 = H, R^2 = OMe
XIX R^1 = OAc, R^2 = H



V



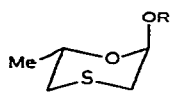
VII R^1 = H, R^2 = OMe, R^3 = Tr
VIII R^1 = R^3 = H, R^2 = OMe
IX R^1, R^2 = H, OH, R^3 = H

The 1,4-oxathiane derivatives (III), (V), and (VIII) show a sensitivity towards acid comparable to that of methyl 2-deoxyglycopyranosides[7]. Thus, compound (VIII) was hydrolysed to crystalline (6*R*)-2-hydroxy-6-hydroxymethyl-1,4-oxathiane (IX) by N sulphuric acid during 12–15 h at room temperature. The 1,4-oxathiane derivative (IX) was also obtained on acid hydrolysis of compound (V). Acid hydrolysis of compound (III) afforded (6*S*)-2-hydroxy-6-methyl-1,4-oxathiane (X) which, with acetic anhydride in pyridine, gave (2*R*,6*S*)-2-acetoxy-6-methyl-1,4-oxathiane* (XI) (the configuration at position 2 was ascertained by n.m.r. spectroscopy, as discussed below).

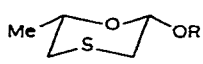
Construction of a molecular model of (2*R*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane (III), using bond angles[8] of 105° and 111° for C–S–C and C–O–C, respectively, and bond lengths[8] of 1.82 and 1.42 Å for C–S and C–O, respectively, indicated that the molecule can adopt a somewhat distorted chair conformation in which the axial substituents on C-2 and C-6 are noticeably inward-pointing, and those on C-3 and C-5 are noticeably outward-pointing. Compound (III) probably adopts conformation (XII), since the n.m.r. spectrum (acetonitrile) showed, *inter alia*, a

*Although the configuration at position 2 in the transformation (III) → (XI) is inverted, the absolute configuration[5] remains *R*.

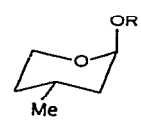
triplet at τ 5.25 ($J_{ae} + J_{ee}$ *ca.* 5 c.p.s.) for the glycosidic proton (H-2), typical[9] of an equatorial proton equally coupled to the axial and equatorial protons of a vicinal methylene group. In acetonitrile containing toluene-*p*-sulphonic acid at 20°, the *trans*-glycoside (III) equilibrated to a mixture containing 75% of the *cis*-isomer, (2*S*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane (XIII), which probably adopts the chair conformation (XIV), since the glycosidic proton signal was a quartet (τ 5.48, $J_{ae} + J_{aa}$ *ca.* 11 c.p.s.) typical[9] of an axial proton coupled to vicinal axial and equatorial protons. A contrast is provided with the behaviour of the apparently closely-related compounds *cis*- and *trans*-2-methoxy-4-methyltetrahydropyran[10] which, under similar equilibrating conditions, gave a mixture containing 65% of the *trans*-isomer. The chair conformations (XV) and (XVI) were established[10] for the *trans*- and *cis*-components of this equilibrium mixture, and the preponderance of the *trans*-isomer was held to reflect the operation of the anomeric effect[11]. The cause of the destabilisation of the *trans*-oxathiane derivative (XII) relative to the *cis*-isomer (XIV), under the equilibrating conditions, is not clear, but a contribution may be made by the non-bonded interaction between the axial methoxyl group and the axial C-6 proton. Because of the geometry of the 1,4-oxathiane ring (as noted above), this interaction would be significantly greater than the corresponding interaction in the *trans*-tetrahydropyran derivative (XV).



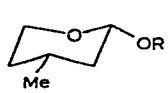
XII R = Me
XVIII R = Ac
XXIII R = H



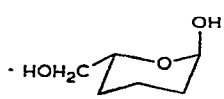
XIV R = Me
XVII R = Ac
XXII R = H



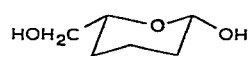
XV R = Me
XXI R = Ac



XVI R = Me
XX R = Ac



XXIV

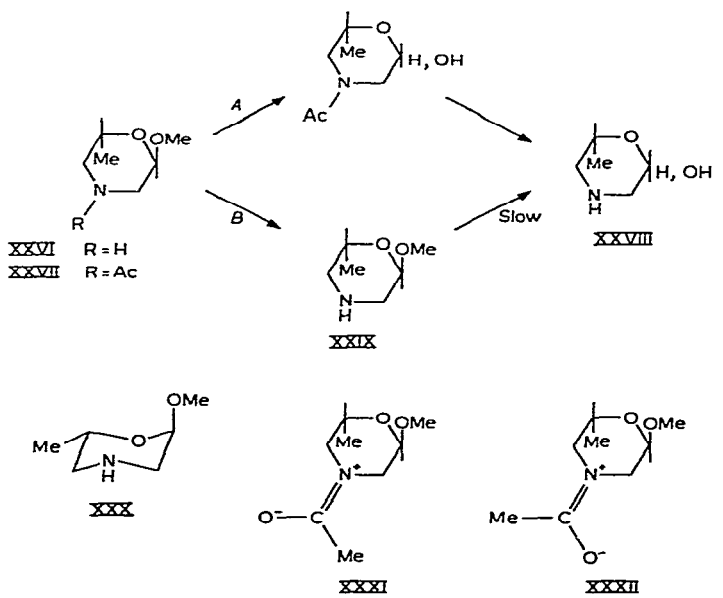


XXV

The n.m.r. spectrum (carbon tetrachloride) of the acetate (XI) showed, *inter alia*, a quartet at τ' (see Experimental) 4.25 ($J_{aa} + J_{ae}$ 12 c.p.s.) for the glycosidic proton (H-2), typical[9] of an axial proton unequally coupled to the axial and equatorial protons of a vicinal methylene group, and consistent with conformation (XVII). Equilibration of the *cis*-acetate (XI) at 20° in an equimolar acetic acid–acetic anhydride mixture containing toluene-*p*-sulphonic acid gave a *cis*,*trans*-mixture containing 67% of the *cis*-isomer. The *trans*-isomer, (2*S*,6*S*)-2-acetoxy-6-methyl-1,4-oxathiane, probably assumes the chair conformation (XVIII), since, in the reaction mixture, the signal for the glycosidic proton was a triplet at 3.87 ($J_{ee} + J_{ea}$

ca. 5.5 c.p.s.) typical[9] of an equatorial proton equally coupled to vicinal axial and equatorial protons [the corresponding signal for the *cis*-isomer (XVII) was a quartet at 4.24 ($J_{ae}+J_{aa}$ 11.8 c.p.s.)]. Thus, as for the methoxy compounds (III) and (XIII), acid-catalysed equilibration of the acetates (XI) and (XIX) favours the *cis*-isomer (XI), and a contrast is again provided with the tetrahydropyran series, where similar equilibration[10] of the *cis*- (XX) and *trans*-2-acetoxy-4-methyl derivatives (XXI) gives a mixture containing 72% of the *trans*-isomer.

An aqueous solution of the crystalline (6*R*)-2-hydroxy-6-hydroxymethyl-1,4-oxathiane (IX) showed no detectable mutarotation. The n.m.r. spectrum of a solution of (6*S*)-2-hydroxy-6-methyl-1,4-oxathiane in pyridine showed, *inter alia*, a quartet for the anomeric proton at τ 4.79 ($J_{aa}+J_{ae}$ 11.5 c.p.s.) indicative of conformation (XXII), but in deuterochloroform, the ratio of conformations (XXII) and (XXIII) was *ca.* 2:1. A comparable situation exists for 2-hydroxy-6-hydroxymethyltetrahydropyran, the *trans*- and *cis*-isomers of which can adopt the conformations (XXIV) and (XXV). The n.m.r. spectrum of the equilibrium mixture in pyridine at room temperature showed, *inter alia*, unresolved multiplets at τ 4.98 ($J_{aa}+J_{ae}$ *ca.* 11.5 c.p.s.) and 4.38 ($J_{ae}+J_{ee}$ *ca.* 5.5 c.p.s.) for the anomeric protons of conformations (XXV) and (XXIV), respectively. Integration of the H-2 signals indicated the ratio of conformations (XXIV):(XXV) to be *ca.* 1:2.



Treatment of the ditosylate (II) with methanolic ammonia at 120° afforded (2*R*,6*S*)-2-methoxy-6-methylmorpholine (XXVI), which readily gave the *N*-acetyl derivative (XXVII). The acid hydrolyses of compounds (XXVI) and (XXVII) were conveniently followed by using n.m.r. spectroscopy to observe the change in intensities of the signals for the protons in the *N*-acetyl group and in the released acetic

acid, and the change in the ratio of signal intensities for the protons in the glycosidic methoxyl group, both during the reaction and in the product. Hydrolysis of the *N*-acetyl derivative (XXVII) with 2N hydrochloric acid at 75° was rapid (80% in 15 min, *ca.* 99% in 30 min), and conversion into the amino alcohol (XXVIII) was almost complete. Hydrolysis must therefore have followed pathway *A*, with cleavage of the glycosidic methoxyl group preceding hydrolysis of the *N*-acetyl residue, since the amino glycoside (XXIX), which would be formed in pathway *B*, was relatively resistant to acid (35% hydrolysis in 210 min), presumably because of electrostatic shielding by the protonated amino group. The glycosidic substituent and the nitrogen atom in the *N*-acetyl derivative (XXVII) are separated by the same number of bonds as are the corresponding groups in the methyl 2-acetamido-2-deoxy-D-glucopyranosides, but a significant percentage of each of the latter compounds is hydrolysed in acid[12] by a pathway analogous to *B*, possibly because of the greater steric accessibility of the acetamido group.

The n.m.r. spectrum (carbon tetrachloride) of (2*R*,6*S*)-2-methoxy-6-methylmorpholine (XXVI) was consistent[9] with the chair conformation (XXX), in that the signal for the anomeric proton (H-2) was an unresolved multiplet at τ 5.60 (half band width, 4 c.p.s.). The *N*-acetyl derivative (XXVII) gave a similar signal.

At 35° in carbon tetrachloride solution, the signals for the methoxyl and acetyl protons in the n.m.r. spectrum of the *N*-acetyl derivative (XXVII) were sharp singlets, but at *ca.* −70°, the methoxyl proton signal became a doublet and the acetyl proton signal became asymmetric. The latter data are consistent with the existence of geometrical isomers of the types (XXXI) and (XXXII), analogous to those observed for *N*-alkyl amides[13] and for sugar derivatives in which the ring oxygen atom has been replaced by an acetamido group[14]. The energy barrier between the geometrical isomers (XXXI) and (XXXII) for the *N*-acetyl derivative (XXVII), where the *N*-acetyl group is flanked by methylene groups, is significantly lower than in the analogous sugar derivatives[14], where the flanking groups are bulkier.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on Kieselgel, and detection was effected with vanillin–sulphuric acid[15] and/or iodine vapour. Organic solvents were dried with $MgSO_4$. N.m.r. spectra were determined on *ca.* 20% solutions, with 5% tetramethylsilane as internal reference (τ) or 6% tetramethylsilane in chloroform as external reference (τ'), by using a Varian A60 spectrometer under normal working conditions. Optical rotations were measured at *ca.* 30°.

(2S,1'R)-2-(2'-Hydroxy-1'-methoxyethyloxy)propan-1-ol (I)

A solution of sodium metaperiodate (44.2 g) in water (200 ml) was added to a solution of methyl α-L-rhamnopyranoside[16] (16 g, m.p. 104–106°, $[\alpha]_D$ −68° in water) in phosphate buffer[17] (150 ml, pH 7), and the mixture was stored overnight at room temperature. The excess of periodate was precipitated with 10% aqueous

barium chloride. Sodium borohydride (8 g) was then added to the filtered solution and, after storage overnight at room temperature, the solution was extracted continuously with chloroform during 48 h. Concentration of the dried extract gave the title compound (10.5 g, 78%), b.p. 142–144°/16 mm, which was characterised[18] as the di-*p*-phenylazobenzoate, m.p. 84–85°, $[\alpha]_{5461}$ +98° (*c* 0.2, ethanol) (Found: C, 67.8; H, 5.5; N, 9.9. $C_{32}H_{30}N_4O_6$ calc.: C, 67.8; H, 5.3; N, 9.9%), and the ditosylate (II), m.p. 54–55°, $[\alpha]_D$ +6.5° (*c* 1.0, ethanol) (Found: C, 52.1; H, 5.7; S, 14.25. $C_{20}H_{26}O_8S_2$ calc.: C, 52.4; H, 5.7; S, 14.0%).

*(2R,1'S)-2-O-(1'-Methoxy-2'-toluene-*p*-sulphonyloxyethyl)-1-O-triphenylmethyl-3-O-toluene-*p*-sulphonylglycerol*

Lead tetra-acetate (93.2 g) was added to a solution of methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside[19] (51.5 g) in pyridine (200 ml) whilst the temperature was kept below 40°. After the mixture had been stirred for 2 h, oxalic acid was added to remove the excess of lead ions. Insoluble material was collected and washed well with chloroform, and the combined and dried filtrate and washings were concentrated to *ca.* 100 ml. Chloroform (400 ml) was added, and the solution was washed with water (2 × 300 ml), 10% aqueous sodium hydrogen carbonate (2 × 300 ml), and water, dried, and concentrated. To a solution of the residue (52.4 g) in ethanol (250 ml) at 0°, sodium borohydride (11 g) was added. After storage overnight, the solution was neutralised with acetic acid and concentrated in the presence of potassium carbonate. The resulting sludge was extracted with chloroform (500 ml), and the extract was concentrated to yield a syrup (47.1 g) which contained two main components (R_F *ca.* 0.20 and *ca.* 0.35; t.l.c., benzene–methanol, 9:1) indicative of incomplete oxidation. The product was therefore re-treated with lead tetra-acetate (58 g) in pyridine (200 ml) as described above, and the resultant syrup was treated with sodium borohydride (11 g) in ethanol (250 ml) to give slightly impure (2*R*,1'*S*)-2-*O*-(2'-hydroxy-1'-methoxyethyl)-1-*O*-triphenylmethylglycerol (VI, 30.7 g), R_F *ca.* 0.35.

The crude diol (0.976 g) was esterified with tosyl chloride (1.04 g) and pyridine (4 ml) in the usual manner, and the crude product (1.75 g) was recrystallised from ethanol to give the title compound (1.16 g, 67%), m.p. 129.5–130°, $[\alpha]_D$ −9° (*c* 1.15, chloroform) (Found: C, 65.1; H, 5.7; S, 8.8. $C_{39}H_{40}O_9S_2$ calc.: C, 65.3; H, 5.6; S, 8.95%).

In subsequent experiments, it was found more convenient to use chloroform as solvent in the lead tetra-acetate oxidation. Thus, lead tetra-acetate (265 g) was added in portions with cooling to a solution of methyl 6-*O*-triphenylmethyl-α-D-glucopyranoside (145 g) in dry chloroform (1.2 l). After the mixture had been stirred for 1.5 h, excess of lead ions were removed with oxalic acid, and the solution was washed with water, 10% aqueous sodium hydrogen carbonate, and water. The dried solution was concentrated to give the crude dialdehyde which was reduced as described above.

Preparation of 1,4-oxathiane derivatives

(*a*) A solution of the ditosylate (II) (18 g) and sodium sulphide (10.5 g) in methanol (150 ml) was boiled under reflux overnight. The mixture was diluted with water and extracted with chloroform (3 × 75 ml). Concentration of the combined and dried extracts and distillation of the residue gave (2*R*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane (III, 3.6 g, 66%), b.p. 60–62°/2 mm, $[\alpha]_D$ −55° (*c* 1.9, ethanol) (Found: C, 48.3; H, 8.3; S, 21.6. $C_6H_{12}O_2S$ calc.: C, 48.6; H, 8.2; S, 21.6%). The n.m.r. spectrum (acetonitrile) showed, *inter alia*, signals at τ 6.67 (OMe protons) and 8.83 (doublet, *J ca.*7 c.p.s., CMe protons).

(*b*) By essentially the above method, the ditosylate[6] (IV) was converted into (1*R*,4*R*)-2,8-dioxa-6-thiabicyclo[3,2,1]octane (V, 62%), b.p. 47–49°/0.1 mm, $[\alpha]_D$ −40° (*c* 6.4, chloroform) (Found: C, 45.6; H, 6.3; S, 24.4. $C_5H_8O_2S$ calc.: C, 45.5; H, 6.1; S, 24.2%).

(*c*) Likewise, the ditosylate of diol (VI) was converted into (2*S*,6*R*)-2-methoxy-6-triphenylmethoxymethyl-1,4-oxathiane (VII, 95%), m.p. 107.5–108.5° (from ethanol), $[\alpha]_D$ +18° (*c* 1.2, chloroform) (Found: C, 74.2; H, 6.5; S, 7.8. $C_{25}H_{26}O_3S$ calc.: C, 73.9; H, 6.45; S, 7.8%).

(2S,6R)-6-Hydroxymethyl-2-methoxy-1,4-oxathiane (VIII)

A solution of (2*S*,6*R*)-2-methoxy-6-triphenylmethoxymethyl-1,4-oxathiane (VII, 4.58 g) in methanol (250 ml) was shaken with hydrogen, at a pressure slightly greater than 1 atmosphere in the presence of 5% palladised charcoal, until the reaction was complete. It was necessary to add several portions of the catalyst, and the reaction times were not reproducible (up to 5 days). After removal of the catalyst and evaporation of the solvent, the product was partitioned between light petroleum (100 ml, b.p. 60–80°) and water (100 ml). The aqueous layer was then extracted with ether continuously overnight. Evaporation of the dried extract and distillation of the residue gave the title compound (1.02 g, 55%), b.p. 140° (bath)/0.2 mm, $[\alpha]_D$ +67° (*c* 1.2, chloroform) (Found: C, 43.95; H, 7.5; S, 19.3. $C_6H_{12}O_3S$ calc.: C, 43.9; H, 7.4; S, 19.5%).

(6R)-2-Hydroxy-6-hydroxymethyl-1,4-oxathiane (IX)

(*a*) A solution of (1*R*,4*R*)-2,8-dioxa-6-thiabicyclo[3,2,1]octane (V, 0.5 g) in N sulphuric acid (25 ml) was stored at 67.5°; α_D changed from −0.75° (0 min) to −0.14° (430 min, final constant value). The cooled solution was neutralised with barium carbonate, filtered, and extracted with chloroform continuously overnight. The dried extract was concentrated, and the residue was recrystallised from chloroform–light petroleum (b.p. 60–80°) to give the title compound (IX, 0.3 g, 53%), m.p. 92–94.5°, $[\alpha]_{5461}$ +7° (*c* 0.6, water, no detectable mutarotation) (Found: C, 39.9; H, 6.6; S, 21.0. $C_5H_{10}O_3S$ calc.: C, 40.0; H, 6.7; S, 21.3%).

(*b*) A solution of (2*S*,6*R*)-6-hydroxymethyl-2-methoxy-1,4-oxathiane (VIII, 140 mg) in N sulphuric acid (15 ml) was stored at room temperature overnight and then neutralised with barium carbonate. The filtered solution was extracted with

chloroform continuously for 18 h, and the dried extract was concentrated. Recrystallisation of the residue, as in (*a*), gave the title compound (IX, 46 mg), m.p. 91–94°, which was identical with the authentic product described in (*a*), $[\alpha]_{5461}$ +7° (*c* 0.6, water, no detectable mutarotation).

(2R,6S)-2-Acetoxy-6-methyl-1,4-oxathiane (XI)

(2*R*,6*S*)-2-Methoxy-6-methyl-1,4-oxathiane (III, 1.4 g) was stirred with 4 N hydrochloric acid (4 ml) at 65° until the mixture became homogeneous (4 h). The hydrolysate was neutralised with 10% aqueous sodium hydrogen carbonate and then extracted with chloroform. The dried extract was concentrated, and the residue was recrystallised from light petroleum (b.p. 60–80°) to give (6*S*)-2-hydroxy-6-methyl-1,4-oxathiane (X, 0.825 g, 66%), m.p. 74–76°, $[\alpha]_{5461}$ +25° (*c* 0.4, water) (Found: C, 44.8; H, 7.6; S, 23.7. $C_5H_{10}O_2S$ calc.: C, 44.8; H, 7.5; S, 23.8%).

A solution of the foregoing alcohol (X, 0.57 g) in pyridine (3 ml) and acetic anhydride (0.85 g) was stored at 0° overnight and then diluted with water (25 ml). The mixture was extracted with chloroform, the dried extract was concentrated, and the residue was distilled to give the title compound (XI, 95%), b.p. 67–69°/0.15 mm, $[\alpha]_D$ +36.5° (*c* 1.1, ethanol) (Found: C, 47.7; H, 6.6. $C_7H_{12}O_3S$ calc.: C, 47.7; H, 6.6%). The n.m.r. spectrum (carbon tetrachloride) had signals, *inter alia*, at τ' 7.90 (acetyl protons) and 8.67 (doublet, *J* 6 c.p.s., *C*-methyl protons).

Equilibration experiments

(*a*) A solution of (2*R*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane (III, 100 mg) in acetonitrile (0.5 ml) containing toluene-*p*-sulphonic acid (1%) was stored at 20° until the n.m.r. spectrum showed no further change (*ca.* 1 day). The proportion of 2*S*:2*R* isomers in the mixture was determined by integration of the areas of the signals for the anomeric protons (see discussion) and the methoxyl protons (τ 6.64 and 6.60, respectively).

(*b*) A solution of (2*R*,6*S*)-2-acetoxy-6-methyl-1,4-oxathiane (XI, 200 mg) in an equimolar mixture of acetic acid and acetic anhydride (1 ml) containing toluene-*p*-sulphonic acid (0.001 M) was stored at 20° until the n.m.r. spectrum showed no further change (*ca.* 8 days). The ratio of 2*S*:2*R* isomers in the mixture was determined by integration of the areas of the signals for the anomeric protons (see discussion).

(2R,6S)-2-Methoxy-6-methylmorpholine (XXVI)

A solution of the ditosylate (II, 26 g) in methanol (650 ml) saturated with ammonia at room temperature was stored in an autoclave at 120° for 20 h and then evaporated. A solution of the residue in 2 N sodium hydroxide (180 ml) was extracted with chloroform (3 × 180 ml), and the combined and dried extracts were concentrated. Distillation of the residue gave the title compound (3.7 g, 50%), b.p. 40–50°/16 mm, $[\alpha]_D$ −92° (*c* 0.3, chloroform) (Found: C, 54.4; H, 10.2; N, 10.7. $C_6H_{13}NO_2$ calc.: C, 54.9; H, 10.0; N, 10.45%).

Treatment of the foregoing amine with acetic anhydride and pyridine, in the usual manner, gave (2*R*,6*S*)-*N*-acetyl-2-methoxy-6-methylmorpholine (XXVII), b.p. 114–115°/0.25 mm, $[\alpha]_D$ −146° (*c* 0.3, chloroform) (Found: C, 55.7; H, 9.0; N, 8.5. $C_8H_{15}NO_3$ calc.: C, 55.5; H, 8.7; N, 8.1%).

Hydrolysis experiments

(*a*) A solution of (2*R*,6*S*)-2-methoxy-6-methylmorpholine (XXVI, 100 mg) in 2N hydrochloric acid (1 ml) containing *tert*-butyl alcohol (internal quantitative standard) was stored at 75°. The n.m.r. spectrum was recorded at 0.0 and 3.5 h, and the extent of hydrolysis was calculated from the integrated areas of the signals for the glycoside methoxyl and methanol protons. The hydrolysate was then concentrated *in vacuo* at 25°, and the extent of hydrolysis was calculated from the n.m.r. spectrum of an aqueous solution of the residue by using the ratio of the integrated signals for *O*- and *C*-methyl protons.

On concentration of a solution of the starting material (XXVI) in 2N hydrochloric acid *in vacuo* at 25° negligible hydrolysis occurred.

(*b*) A solution of (2*R*,6*S*)-*N*-acetyl-2-methoxy-6-methylmorpholine (XXVII, 100 mg) in 2N hydrochloric acid (1 ml) containing *tert*-butyl alcohol (1%) was stored at 75°, and the n.m.r. spectrum was recorded after 15, 30, and 210 min. The solution was then concentrated *in vacuo* at 25°. Since the n.m.r. spectrum of an aqueous solution of the residue had no signals for *O*-methyl or *N*-acetyl protons, complete hydrolysis was indicated. The extent of hydrolysis after 15 and 30 min was estimated from the ratio of the integrated signals for the *N*-acetyl and acetic acid protons (τ *ca.* 7.71 and 7.77, respectively).

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SUMMARY

Application in sequence of periodate oxidation, borohydride reduction, tosylation, and treatment with sodium sulphide to methyl α-L-rhamnopyranoside affords (2*R*,6*S*)-2-methoxy-6-methyl-1,4-oxathiane. Likewise, methyl 6-*O*-trityl-α-D-glucopyranoside and 1,6-anhydro-β-D-glucopyranose were converted into 1,4-oxathiane derivatives. Acid-catalysed equilibration of (2*R*,6*S*)-2-methoxy- and (2*R*,6*S*)-2-acetoxy-6-methyl-1,4-oxathiane affords, in each case and in contrast to structurally related tetrahydropyran derivatives, a mixture containing *ca.* 70% of the *cis* (diequatorial) isomer.

Replacement of sodium sulphide by methanolic ammonia in the above reaction sequence results in the conversion of the rhamnoside into (2*R*,6*S*)-2-methoxy-6-methylmorpholine. The behaviour of the free amine and the *N*-acetyl derivative on

acid hydrolysis is described. At low temperature, the n.m.r. spectrum of the *N*-acetyl derivative is consistent with the existence of geometrical isomers.

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THE HEMICELLULOSES OF BRACKEN

PART I. AN ACIDIC XYLAN

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INTRODUCTION

The cell walls of many higher land-plants have been shown to contain polysaccharides known as the xylans. These are polymers of β-D-xylopyranose residues; xylans from different plants differ in fine structure and in average degree of polymerisation. Most work has been done on xylans from the *Angiosperms*, the *Gymnosperms*[1], and the *Gramineae*[1,2]. Generally, the xylans from the *Angiosperms* have terminal 4-*O*-methyl-D-glucuronic acid residues on some of the C-2 positions of the D-xylose residues of the main chain; those from the *Gymnosperms* have, in addition, terminal L-arabinofuranose residues attached to C-3 positions. The xylans from the *Gramineae* have a lower proportion of L-arabinose residues than is found in the wood xylans, and they contain not only residues of 4-*O*-methyl-D-glucuronic acid, but also of D-glucuronic acid. Some of these xylans contain L-arabinose residues. There is evidence that certain of the xylans have branches of D-xylopyranose residues and, in view of recent work, even where the xylans were believed to be unbranched, it may be that the evidence requires to be reconsidered[3]. Our studies relate to work on the hemicelluloses from a fern, bracken (*Pteridium aquilinum*), and, in the present paper, to an acidic xylan from that source. The hemicelluloses from cinnamon fern have been studied by Timell[10], and a brief study made of polysaccharides from other ferns.

RESULTS AND DISCUSSION

The dried stems of bracken were milled, delignified, and treated with aqueous sodium hydroxide under nitrogen. The polysaccharides extracted by 4% alkali contained 16% of uronic acid residues. The main sugar in a hydrolysate was xylose, and there were smaller amounts of acidic components, mannose, glucose, and galactose, and a trace of arabinose. By contrast, polysaccharides then extracted by 10% alkali, on hydrolysis, yielded acidic components, equal amounts of xylose and mannose, a smaller amount of glucose, and traces of arabinose and galactose. The former material was the source of an acidic xylan now reported on, and the latter the source of a glucomannan to be dealt with in a later paper.

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By addition of dilute acetic acid to an alkaline solution of the material extracted by 4% alkali, a xylan-enriched precipitate was obtained, and several repetitions of this procedure led to the isolation of the water-soluble xylan subsequently studied. A hydrolysate of this material contained xylose, acidic components, and a trace of arabinose. In addition, there was a small proportion of glucose, which arose from a contaminating polysaccharide later removed during the methylation procedures. The xylan was homogeneous when examined by glass-paper electrophoresis in borate buffer.

By quantitative decarboxylation studies[4] and by methylation analysis, the xylan was found to contain approximately one uronic acid residue to every seven xylose residues. On hydrolysis, a sample of the xylan gave three acidic products, which were separated from neutral sugars by use of an anion-exchange resin and then fractionated by paper chromatography. An aldobiouronic acid was identified as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose by its chromatographic behaviour and by the formation of the crystalline acetate of its methyl ester methyl glycoside. Hydrolysis of the reduced methyl ester methyl glycoside gave xylose and 4-*O*-methyl-D-glucose, in equimolar amounts. Other acidic components were concluded to be a related aldotriouronic acid and 4-*O*-methyl-D-glucuronic acid.

After an extended methylation procedure, the product was fractionated (Table II) by dissolution in mixtures of chloroform and light petroleum, and a fraction having a methoxyl content of 38.3% (theoretical, 39.2%) was methanolysed and hydrolysed. It yielded the components shown in Table I, and these were separated chromatographically and identified as detailed later; together, they accounted for 94% of the material hydrolysed.

TABLE I

FRACTIONATION OF METHYLATED SUGARS

Fraction	*Component*	*Weight (mg)*	*Molar percent*
1	2,3,4-Tri-*O*-methyl-D-xylose[a]	37	1.8
2	2,3-Di-*O*-methyl-D-xylose	1495	77.7
3	3-*O*-Methyl-D-xylose[b]	75	4.2
4	2-*O*-(2,3,4-Tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranose	656	15.9

[a]Plus a trace of 2,3,5-tri-*O*-methyl-L-arabinose. [b]Plus a trace of 2-*O*-methyl-D-xylose.

The isolation of 2,3-di-*O*-methyl-D-xylose indicates that, in common with other land-plant xylans hitherto investigated, the polysaccharide consists of a chain of (1→4)-linked D-xylopyranose residues[1,2]; the negative specific rotations of the parent xylan and of the methylated derivative indicate that the principal mode of linkage is by β-D-xylosidic bonds. The 2,3,4-tri-*O*-methyl-D-xylose must be derived from terminal, non-reducing residues of xylose. An unbranched xylan would have

only one such residue and, on the basis of the methylation evidence, would have a degree of polymerisation (*DP*) of 64. That the molecule was unbranched was also indicated by the molecular-weight determination by ebulliometry[5] on the methylated xylan; this indicated the *DP* to be *ca.* 42. Foaming of the benzene solution diminished the accuracy of the determination, but the value obtained indicates the molecule to be unbranched. Periodate-oxidation studies on the xylan gave a *DP* of 67 (assuming the molecule to be unbranched), a value in excellent agreement with that obtained by the methylation analysis.

A common problem in methylation analysis of xylans is that the proportion of mono-*O*-methyl-D-xyloses is in excess of that which can be accounted for by a fully methylated structure. In this work, 3-*O*-methyl-D-xylose equivalent to about two residues per xylan molecule was isolated, but only a trace of 2-*O*-methyl-D-xylose. Aspinall and Carter[6] obtained similar results when studying the acidic xylan from Norway Spruce. It is usual to attribute an excess of mono-*O*-methyl-D-xyloses either to undermethylation of the xylan, or to demethylation[7,8] during the methanolysis and hydrolysis of the methylated xylan, but neither explanation can satisfactorily account for preponderance of 3-*O*-methyl-D-xylose over the 2-*O*-methyl analogue. Demethylation would not be expected to lead to such selectivity. It is even less likely that undermethylation is the cause of such disparity, as much evidence[9] shows that, during methylation of xylans or of cellulose, the C-2 hydroxyl group is much more readily attacked than that at C-3. Another possible explanation is that 2,3,4-tri-*O*-methyl-D-glucuronic acid is liberated, together with an equimolar amount of 3-*O*-methyl-D-xylose, but this explanation can be discounted, as no significant amount of such a uronic acid was detected. Should the hydrolysis of the methylated xylan be accompanied by simple decarboxylation of the acid residues, these would yield 2,3,4-tri-*O*-methyl-D-xylose, accompanied by an equimolar amount of 3-*O*-methyl-D-xylose from the adjacent residue on the main chain. There is insufficient tri-*O*-methyl-D-xylose for this to be a principal cause. It is suggested that, under the conditions pertaining during the later, non-aqueous stages of methylation, the acidic groups of the xylan, or possibly the acidity of the solvent, may have catalysed an esterification of certain of the C-2 hydroxyl groups by uronic acid residues. If such an esterification does take place, it could lead to the blocking of C-2 hydroxyl groups and permit the preferential methylation at C-3. Recent work by Timell[3] indicates that there is a preferential loss of 2,3,4-tri-*O*-methyl-D-xylose during hydrolysis procedures. If such a loss occurred in the present case, it would indicate that, on average, the xylan molecules have a single side-chain attached to a C-2 position of a xylose residue on the main chain, the chain being terminated by a xylopyranosyl residue. Neither molecular-weight nor periodate-oxidation evidence supports such a structure in the present instance.

The trace of 2,3,5-tri-*O*-methyl-L-arabinose found in the hydrolysate indicates that such residues occur as terminal arabinofuranosyl residues. In view of the structures reported for many other xylans[1,2], and particularly the structure of the cinnamon-fern xylan[10], it is probable that the attachment is directly to the main chain

by a (1→3)-glycosidic linkage. This would accord with the finding of a small proportion of 2-*O*-methyl-D-xylose, although other explanations are possible.

The isolation of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose and of 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranose shows that the 4-*O*-methyl-D-glucuronic acid residues in the xylan occur as single-unit side-chains linked to D-xylose residues in the main chain by α-(1→2)-glycosidic bonds.

In summary, the xylan has a chain of β-(1→4)-linked D-xylopyranose residues, one in every seven of which, on average, has a terminal 4-*O*-methyl-D-glucuronic acid residue at C-2. A few of the xylan molecules carry terminal L-arabinofuranosyl residues, probably linked to the main chain by (1→3)-glycosidic linkages. The results of periodate-oxidation studies are in good agreement with those from methylation analysis. The reduction of periodate was 0.87 mol. per sugar residue; the structure proposed for the xylan would lead to the reduction of 0.88 mol. A *DP* of 67 was found from methylation and periodate-oxidation studies; the value, obtained by ebulliometry, for the methylated xylan was lower.

The structure of the bracken xylan is similar to that of the xylan from cinnamon fern[10], but the latter has a higher proportion both of residues of arabinose (3%) and of uronic acid (*ca.* 17%), and also a higher *DP* (*ca.* 130). Timell reported that the L-arabinose content of bracken xylan was lower than that of cinnamon fern, and the greatest care was taken throughout the present work to exclude conditions that would promote the loss of L-arabinose residues. The occurrence of 4-*O*-methyl-D-glucuronic acid and the absence of D-glucuronic acid point to the similarity of the xylan from bracken to those from woods. Bracken xylan, with its low proportion of L-arabinose residues, is intermediate between the arabinose-richer softwoods and the normally arabinose-deficient, hardwood xylans. The *DP* of the bracken xylan is lower than those of wood xylans, which range from 90 upwards; cereals and grasses, on the other hand, contain xylans having similar degrees of polymerisation.

EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 and 3 MM papers, and thin-layer chromatography (t.l.c.) on glass plates (20×5 cm) coated with Kieselgel G (Merck, Darmstadt). The irrigants (v/v) used were (1) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (2) butan-1-ol–ethanol–water (4:1:5); (3) butan-1-ol–benzene–pyridine–water (5:1:3:3); (4) butan-2-one–water (2:1); (5) ethyl acetate–pyridine–water (3.6:1.15:1). Chromatographic spray reagents were *p*-anisidine hydrochloride or alkaline silver nitrate. Gas chromatography[11] was effected on a Pye Argon Gas Chromatograph. The columns (120×0.5 cm) contained as liquid phase: *A*, 20% Apiezon M; *B*, 20% Carbowax 20M; *C*, butane-1,4-diol succinate[11]; the support was either alkali-washed (*A*) or acid-washed (*B* and *C*) Celite (mesh 80–120). The argon flow-rate was 30–60 ml/min. Electrophoretic examination of monosaccharides and of oligosaccharides was carried out in either 0.1 M borax or in 0.05 M

sodium germanate buffer[12] on sheets of Whatman No. 1 or 3 MM paper (30 cm long) at 100–200 volts. Polysaccharides were examined similarly on Whatman GF/A glass-paper (120 cm long), at 1,000–1,500 volts, and were detected by spraying with alkaline potassium permanganate. Quantitative estimations of sugars in hydrolysates were made by the phenol–sulphuric acid method[13]. The uronic-acid determinations were made by the method of Anderson *et al.*[4]. Optical rotations were observed at 18±3°. Polysaccharide samples were hydrolysed in sealed tubes with N sulphuric acid for 12–16 h at 100°; the hydrolysates were neutralised with barium carbonate.

Isolation of hemicelluloses

Bracken was collected at the Hill of Fare (Aberdeenshire) in late August 1960. The stems, cut in short lengths, were immediately immersed in acetone at −40°. Enzymes were inactivated by immersing the cold, crushed stems in boiling alcohol. The plantstuff was dried at 40° and reduced to a fibrous state by milling. Batches of this plantstuff (4×200 g) were delignified by treatment with sodium chlorite–acetic acid in the usual way[14]. The temperature rose briefly to 60–65° and was thereafter maintained at 30° and at pH 4.0 for 5 h. The residual material was again treated in the same way, and the resultant holocellulose was washed successively with cold water and alcohol and dried at 40°. Yield, *ca.* 500 g.

A portion (447 g) of the holocellulose was treated successively for 14-h periods with 4% (3×5 l) and 10% (4×5 l) sodium hydroxide under nitrogen. The extracts were carefully made nearly neutral with dilute hydrochloric acid and then made faintly acid with acetic acid. Acetone (2 vol.) was added and the resulting precipitate was collected, washed with ethanol and ether, and dried. Samples of the various precipitates were hydrolysed, and the neutralised hydrolysates were examined by chromatography. The 4% alkaline extracts were similar and were combined to give Fraction I (83.3 g, 18.5% of the holocellulose); $[\alpha]_D$ −29.5° (*c* 0.81, N sodium hydroxide and uronic-acid content, 16.0%. On hydrolysis, a sample gave xylose, glucose, mannose, galactose, and arabinose, in the molar ratios of 6:1:2:1:trace, and three other components, one of which was chromatographically indistinguishable from 4-*O*-methyl-D-glucuronic acid. The 10% alkaline extracts were similar to one another and were combined to give Fraction II. Fraction I was the richer in xylose residues, and was the source of the xylan studied.

Isolation of the xylan

Preliminary studies ruled out the use of Fehling's solution and of barium hydroxide for the sub-fractionation of Fraction I. A sample (52 g) of Fraction I was dissolved in water (2 l) and ethanol (2.4 l) was added. A gel formed and was reduced in volume by the addition of an excess of acetone to give Gel *A*. Great difficulty was experienced in obtaining a non-gelatinous precipitate. Gel *A* was dispersed in water (2 l), and glacial acetic acid (400 ml) was added. The precipitate (*AP*) that formed was separated by centrifugation. The supernatant solution, on treatment with acetone (2 vol.), yielded a precipitate (*AS*) which was washed and dried as earlier. The

proportion of hexose residues in Fraction I was much higher than in the derived fraction (*AP1*). The latter material was dispersed in water (800 ml), and 2 N sodium hydroxide (160 ml) was added, followed by glacial acetic acid (400 ml). The resulting precipitate (*AP2*) was washed and dried. Similar, further fractionations yielded, successively, Fractions *AP3* and *AP4*, hydrolysates of which were chromatographically indistinguishable. Fraction *AP4* (16.3 g, 5.7% of the holocellulose) had $[\alpha]_D$ −53.6° (*c* 0.91, N sodium hydroxide). Whereas Fraction I contained two electrophoretically separable components, Fraction *AP4* contained only one component. The electrophoretic homogeneity of the material in Fraction *AP4* was further indicated by excision of the areas of paper corresponding to the leading and trailing edges of the migrated material. The polysaccharide was eluted from both areas, hydrolysed, and examined chromatographically; the hydrolysates were indistinguishable. Fraction *AP4* was selected for further studies, and is hereafter called 'the xylan'.

Examination of the xylan hydrolysate

A sample (1.35 g) of the xylan was treated with N sulphuric acid (60 ml, 12 h at 100°). After neutralisation with barium carbonate, the hydrolysate was passed through a column Amberlite IR-120(H^+ form). The concentrated eluate was applied to a column of Amberlite IR-4B (acetate form) which was then washed with water to remove neutral sugars. Acidic components were eluted by irrigation with 30% acetic acid. Paper chromatography (Irrigant 1) revealed three acidic components having R_{Xyl} values: *A*, 1.30; *B*, 0.67; *C*, 0.18. They were fractionated on paper chromatograms (Irrigant 1). Yields: *A*, 35 mg; *B*, 56 mg; *C*, 5 mg.

Acid A. The acid was indistinguishable on chromatograms (Irrigants 1 and 3) from 4-*O*-methyl-D-glucuronic acid and had $[\alpha]_D$ +40° (*c* 0.9, water). The acid (35 mg) was treated (6 h) with boiling 3% methanolic hydrogen chloride (92.5 ml), and the solution was neutralised with silver carbonate. After concentration, the solution was treated with sodium borohydride (10 mg) for 18 h. The excess of reductant was destroyed by Amberlite IR-120(H^+ form), the resin and solvent were removed, and the borate was volatilised as methyl borate. The product on hydrolysis was chromatographically indistinguishable (Irrigants 1 and 3) from 4-*O*-methyl-D-glucose.

Acid B. The acid (56 mg) had $[\alpha]_D$ +101° (*c* 1.5, water). Its chromatographic behaviour indicated it to be an aldobiouronic acid. A sample (5 mg) was converted into the methyl ester methyl glycoside and then reduced with sodium borohydride. After deionisation, the product was hydrolysed with N sulphuric acid at 100°. Chromatographic examination (Irrigants 1 and 3) of the hydrolysate indicated equimolar amounts of 4-*O*-methyl-D-glucose and of D-xylose. Another sample (51 mg) of the acid was converted into its methyl ester methyl glycoside and treated in freshly distilled pyridine (0.75 ml) with acetic anhydride (0.25 ml). The products were partitioned between chloroform and water, and, from the chloroform, a crystalline product was obtained, which on recrystallisation from ethanol–ether, had m.p. 200–202° and $[\alpha]_D$ +106° (*c* 1.0, chloroform); these values correspond with those

for methyl 2-*O*-(2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronic acid)-3,4-di-*O*-acetyl-D-xylopyranoside[15]. The infrared spectrum was similar to that reported for an authentic sample of the above compound[15].

Acid C. The acid (5 mg) was reduced through its methyl ester methyl glycoside as above. The product, on hydrolysis, yielded components which were chromatographically indistinguishable (Irrigants 1 and 3) from xylose and 4-*O*-methyl-D-glucose (molar ratio, 2:1).

Periodate oxidation of the xylan[16]

A sample (115 mg) of the xylan, dissolved in water (50 ml), was treated in the dark at 5° with 0.2 M sodium metaperiodate (50 ml). Samples (5 ml) were withdrawn at intervals, and the amount of periodate consumed (mol. per residue, assuming the ratio of uronic acid to pentose residues to be 1:7) was as follows: 0.69 (27 h); 0.90 (70 h); 0.91 (99 h); 0.94 (147 h); 1.03 (243 h); 1.06 (336 h).

A sample (226 mg) of the xylan, dissolved in water (250 ml), was treated with potassium metaperiodate (1.44 g) in the dark at 5°. Samples (20 ml) were withdrawn at intervals, and the liberated formic acid was determined. The results are expressed as the number of sugar residues per mol. of formic acid liberated (assuming the ratio of uronic acid to pentose residues to be 1:7): 48.2 (20 h); 31.7 (38 h); 33.5 (86 h); 33.5 (158 h); 31.7 (188 h). A value of 33.5 was obtained on extrapolation to zero time.

Methylation of the xylan

A sample (10 g) of the xylan was methylated with 40% sodium hydroxide (10×150 ml) and dimethyl sulphate (10×100 ml). The product had OMe, 28.0%. Methylation was continued by using methyl iodide (100 ml), methanol (5 ml), and silver oxide (25 g), and boiling under reflux. Further additions were made daily of methyl iodide (14×25 ml) and of oxide (14×25 g). The methylated product (7.24 g) had OMe, 38.8%[15]. It was fractionated by dissolution in mixtures of chloroform and light petroleum (b.p. 60–80°), and the soluble material was recovered[15].

TABLE II

FRACTIONATION OF METHYLATED XYLAN

Fraction	*Chloroform/light petroleum (v/v)*	*Weight, mg*	*OMe, %*	$[\alpha]_D$ (chloroform)
1	1/5	50.2	—	—
2	1/4	37.3	—	—
3	2/7	1198	38.3	−36.4 (*c* 1.15)
4	1/3	3644	38.1	−33.9 (*c* 2.66)

Samples (10 mg) of all fractions were dissolved in 72% sulphuric acid (0.2 ml), and, after 30 min, the solution was diluted to 12% and heated at 100°. Chromato-

graphic examination (Irrigants 1 and 2) showed that Fractions 3 and 4 were similar, but different from Fractions 1 and 2. The hydrolysates of the former fractions had components which were chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-, and/or 3-, *O*-methyl-D-xylose(s), and 2,3,5-tri-*O*-methyl-L-arabinose (trace). A methylated, acidic component was present. The hydrolysate of Fractions 1 and 2 appeared to contain in addition a trace of tri-*O*-methylhexose. Fractions 3 and 4 were combined and used in all subsequent work.

Hydrolysis of the methylated xylan

A sample of methylated xylan was boiled under reflux with 1.5% anhydrous methanolic hydrogen chloride. The methanolysate (2.72 g) was saponified with saturated barium hydroxide (50 ml) at 60° for 2 h, neutralised with solid carbon dioxide, and deionised with Amberlite IR-120 (H^+ form). An unsuccessful attempt was made to separate the acidic and neutral glycosides on a column of Permutit De-Acidite E (acetate form). The recovered eluate was hydrolysed with N sulphuric acid (50 ml) for 16 h at 100° and neutralised with barium carbonate. On concentration, it gave a pale brown syrup.

Fractionation of the methylated sugars

The mixture of methylated sugars was fractionated chromatographically on a cellulose column (70×5 cm) by using light petroleum (b.p. 100–120°)–butan-1-ol (7:3, v/v), saturated with water, as irrigant. The fractions that contained more than one component were refractionated on Whatman No. 3MM paper (Irrigant 2). Recovery, 94% (Table I).

Examination of the fractions from the hydrolysed methylated xylan

Fraction 1. The syrup had $[\alpha]_D$ +18° (*c* 0.5, water). Paper chromatography (Irrigants 1, 2, and 4), and t.l.c. on silica-gel (Irrigant 4), showed components indistinguishable from 2,3,4-tri-*O*-methyl-D-xylose and 2,3,5-tri-*O*-methyl-L-arabinose (trace). The syrup crystallised on seeding with 2,3,4-tri-*O*-methyl-D-xylose and, on recrystallisation from ether, had m.p. and mixed m.p. 89–90°. A sample (5 mg) of the fraction was boiled under reflux for 6 h with 3% methanolic hydrogen chloride (1 ml). The product was neutralised and the solvent removed. The retention times of the methyl glycosides on gas chromatograms (liquid phases *A* and *B*, at 150°) were identical with those of methyl 2,3,4-tri-*O*-methyl-D-xyloside. A sample (2 mg) of Fraction 1 was demethylated with boron trichloride; only xylose was detectable on a chromatogram (Irrigant 5) and on an electrophoretogram (borate buffer).

Fraction 2. The syrup had $[\alpha]_D$ +23.7° (*c* 1.8, water) and, on seeding with 2,3-di-*O*-methyl-D-xylose, gave crystals having m.p. and mixed m.p. 80–82°. Chromatographic examination of the syrup on paper (Irrigants 1, 2, and 4) and on silica-gel plates (Irrigants 2 and 4) indicated the same compound. The methyl glycosides were

formed and had retention-times on gas chromatograms (liquid phases *A* and *B*) identical with those of methyl 2,3-di-*O*-methyl-D-xyloside. The crystalline derivative, 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, was formed and had m.p. 123° (ethyl acetate).

Fraction 3. The syrup had $[\alpha]_D$ +15° (*c* 0.5, water). A sample was chromatographically indistinguishable from 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose. Electrophoretic examination (borate and germanate buffers; 200 volts for 12 h) showed components indistinguishable from 3-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose (trace). After demethylation of a sample of the syrup, only xylose could be chromatographically or electrophoretically detected. The major component was characterised by conversion into crystalline 3-*O*-methyl-*N*-phenyl-D-xylosylamine, m.p. 134° (from acetone).

Fraction 4. A sample (384 mg) was converted into the methyl ester methyl glycoside (312 mg). A solution of the product in dry ether was reduced with lithium aluminium hydride and, after the addition of water to destroy the excess of hydride, was neutralised with dilute sulphuric acid. Insoluble salts were removed and, on treatment with chloroform, the solution gave an aqueous and a chloroform extract. The chloroform extract was dried and concentrated. The residue (136 mg), on recrystallisation from ethyl acetate, gave colourless crystals of methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-D-xylopyranoside[15], m.p. and mixed m.p. 164–166°; the infrared spectrum exhibited absorption maxima corresponding to values quoted for an authentic sample. A slightly impure sample of the reduced product was treated with sulphuric acid (12 h at 100°), and the neutralised hydrolysate was examined by paper chromatography (Irrigants 1 and 2) and electrophoresis (borate and germanate buffers). The principal products were indistinguishable from 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose; a minor product was indistinguishable from 2,3-di-*O*-methyl-D-xylose. A sample (16 mg) of the reduced methyl ester of the aldobiouronic acid was treated in the dark at room temperature with methyl iodide (3×3 ml) and silver oxide (3×3 g). The product was recovered by extraction with chloroform and was hydrolysed with sulphuric acid. Chromatographic (Irrigants 1 and 2) and electrophoretic examination (borate and germanate buffers) of the hydrolysate indicated the presence of 2,3,4,6-tetra-*O*-methyl-D-glucose and of 3,4-di-*O*-methyl-D-xylose; traces of 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose were also noted and were assumed to have arisen from incomplete methylation.

The aqueous extract, referred to earlier in this section, was concentrated to 2 ml and treated with N sulphuric acid (2 ml) in a sealed tube (2 h at 100°). The hydrolysate was neutralised and, on concentration, gave a syrup (108 mg) which, on chromatographic fractionation (Irrigant 2), yielded four components.

Component a (26 mg) was chromatographically indistinguishable from 2,3,4-tri-*O*-methyl-D-glucose (Irrigants 1, 2, and 4) and, on demethylation with boron trichloride, gave only glucose. The methyl glycoside of component *a* gave two peaks

on gas chromatograms (liquid phases *A* and *B*) which were indistinguishable from those given by methyl 2,3,4-tri-*O*-methyl-D-glucopyranosides.

Component b (8 mg) was chromatographically indistinguishable from 2,3-di-*O*-methyl-D-xylose.

Component c (23 mg), $[\alpha]_D$ +20° (*c* 0.5, water), was indistinguishable on paper chromatograms (Irrigants 1 and 2) and on electrophoretograms (germanate and borate buffers) from 3-*O*-methyl-D-xylose.

Component d (36 mg) appeared on chromatograms to be unreduced, methylated uronic acid.

Molecular weight of the methylated xylan

The molecular weight of the methylated xylan, determined by ebulliometry, was 6750±500. Due to foaming of the benzene solution of the methylated xylan during this determination, there was an appreciable drift in the readings.

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SUMMARY

The acidic xylan from the stem of the fern *Pteridium aquilinum* is similar to xylans from woods, but has a lower degree of polymerisation. It has a chain of β-(1→4)-linked D-xylopyranose residues, one in every seven of which carries at its C-2 position a 4-*O*-methyl-D-glucuronic acid residue. A few of the molecules carry L-arabinofuranose residues, probably linked to the main chain by (1→3)-glycosidic links. The degree of polymerisation, based on periodate-oxidation and methylation-analysis studies, is about 67; a lower value was obtained by ebulliometric studies on the methylated xylan.

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REACTION OF AMMONIA WITH SOME ACETYLATED AND BENZOYLATED MONOSACCHARIDES

PART X*. ASSIGNMENT OF THE α-D CONFIGURATION TO THE *N*-ACETYL-D-GLYCOFURANOSYLAMINES OBTAINED

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INTRODUCTION

When acetylated or benzoylated monosaccharides, and some of their derivatives, are submitted to ammonolysis in methanol, the reaction products usually isolated are the 1,1-bis(acylamido)-1-deoxyalditols. In a few cases, *N*-acylglycosylamines having a furanoid or pyranoid structure have also been obtained. Examples of the production of *N*-acylglycopyranosylamines are the formation of *N*-benzoyl-β-D-mannopyranosylamine from 1,2,3,4,6-penta-*O*-benzoyl-α- or -β-D-mannose[2] or from hexa-*O*-benzoyl-D-*glycero*-D-*galacto*-heptononitrile[3], and of *N*-benzoyl-α-L-rhamnopyranosylamine from 1,2,3,4-tetra-*O*-benzoyl-L-rhamnose[4]. To the best of our knowledge, the only example in which a *N*-acyl-D-glycofuranosylamine has been obtained in a similar reaction is the formation of *N*-acetyl-D-glucofuranosylamine (Ia) by the ammonolysis of acetylated D-glucoses or derivatives, namely, 2,3,4,5,6-penta-*O*-acetyl-D-glucose[5], 1,2,3,4,6-penta-*O*-acetyl-β-D-glucose[6], hexa-*O*-acetyl-D-*glycero*-D-*gluco*-heptononitrile [5], and hexa-*O*-acetyl-D-*glycero*-D-*ido*-heptononitrile[7].

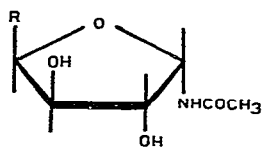
RESULTS AND DISCUSSION

We have now found that, by ammonolysis of 1,2,3,4-tetra-*O*-acetyl-β-D-xylose, which has a pyranoid ring having the same group configuration as that of D-glucose, two cyclic *N*-acetyl-D-xylosylamines are formed; these were obtained in crystalline condition by applying partition chromatography to the products of the reaction. This has a certain interest, because, when the classical methods of separation were applied, ammonolysis of the acetylated derivatives of D-xylose has never yielded crystalline products [5,8].

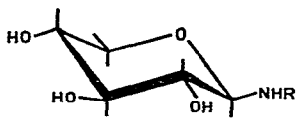
One of the glycosylamines now isolated proved to be the known *N*-acetyl-β-D-xylopyranosylamine (IIb), and the other was found to be a *N*-acetyl-D-xylofuranosylamine (Ib). The two structures were readily differentiated by periodate oxidation (Fleury–Lange method[9]); whereas the pyranosylamine consumed two moles of

*Part IX, see ref. 1.

periodic acid per mole and produced one mole of formic acid, the furanoid form consumed only one mole per mole without formation of formic acid. Formaldehyde was not detected in either case (chromotropic acid method[10]).



(Ia, R=CHOH-CH_2OH)
(Ib, R=CH_2OH)



(IIa, R=H)
(IIb, R=$COCH_3$)

It was found that the furanoid form was the α-D anomer and that the (formerly known) *N*-acetyl-D-glucofuranosylamine also has the α-D configuration at C-1.

The assignments were reached: (*a*) by determination of the optical rotation of the polyaldehydes obtained on periodate oxidation of the *N*-acetyl-D-glycosylamines and, especially, of the rotation of the polyhydric alcohols produced by reduction of the polyaldehydes with sodium borohydride; and (*b*) by application of Hudson's isorotation rules.

TABLE I

SPECIFIC ROTATIONS OF THE PRODUCTS OF OXIDATION AND FURTHER REDUCTION OF SOME *N*-ACETYL-D-GLYCOSYLAMINES

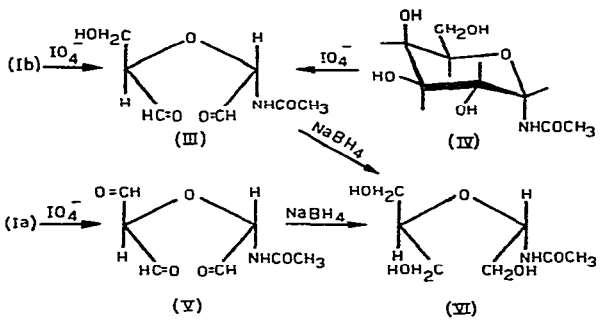
N-Acetyl derivative of	$[\alpha]_D$		
	Original products	*Polyaldehydes*[a]	*Reduced polyaldehydes*[a]
β-D-Galacto-pyranosylamine	− 9.8	−95.4[b] −97.3	− −10.8
β-D-Gluco-pyranosylamine	− 22.0	−95.6[c] −95.4	− − 9.4
β-D-Manno-pyranosylamine	− 47.8	−98.9[c] −99.9	− −10.6
β-D-Xylo-pyranosylamine (IIb)	− 1.0	−39.3[c] −40.5	− − 8.6
β-D-Lyxo-pyranosylamine[d]	− 47.0	−43.3	−10.9
α-D-Galacto-pyranosylamine (IV)	+194.9	+56.7[b] +59.2	− + 9.3
α-D-Gluco-furanosylamine (Ia)	+ 87.9	+10.7	+ 9.5
α-D-Xylo-furanosylamine (Ib)	+100.0	+57.4	+ 7.1

[a]Calculated on the basis of the weight of the original substance. [b]See Reference 11. [c]See Reference 12. [d]Compound prepared by S. Delpy (Ph. D. Thesis, University of Buenos Aires, 1962); m.p. 164–165°, $[\alpha]_D^{20}$ −47.0° (water).

Frush and Isbell[11] were the first to apply periodate oxidation to an investigation of the anomers of *N*-acetyl-D-galactopyranosylamine. Later[12], they extended their method to other *N*-acetyl-β-D-hexopyranosylamines. Some of the rotation values obtained by them for the dialdehydes are indicated in Table I, together with some determinations that we have performed which confirm their results.

When periodate oxidation was applied by Isbell and Frush[12] to the *N*-acetyl-β-D-xylopyranosylamine (IIb), a value of $[\alpha]_D$ −39.3° was obtained for the dialdehyde; this is much lower than the average of $[\alpha]_D$ −97.0° found for the dialdehydes obtained from the *N*-acetyl-D-hexosylamines (see Table I). A similar rotation, $[\alpha]_D$ −43.3°, has been observed by us for the dialdehyde produced by periodate oxidation of another pentose derivative, *viz.*, *N*-acetyl-β-D-lyxosylamine.

We have elaborated this method slightly by reducing (with sodium borohydride) the reactive aldehyde groups to the more stable alcohol groups, as was done by Smith and van Cleve[13] in their studies on the periodate oxidation of the methyl glycopyranosides. In the alcohols, the only asymmetric carbon atom is the formerly anomeric carbon atom, because, for the hexoses, the original asymmetry of C-5 is eliminated by the reduction.



When this procedure was applied to *N*-acetyl-α-D-galactopyranosylamine (IV), the anomeric configuration of which is known[11], and to *N*-acetyl-D-xylofuranosylamine (Ib) and *N*-acetyl-D-glucofuranosylamine (Ia), the resulting alcohol (VI), common to all three, had a positive rotation of $[\alpha]_D$ +7.1° to +9.5°; in our opinion, this result permits assigning of the α-D configuration to the anomeric carbon atom of both of these furanosylamines.

Compounds (Ib) and (IV) must afford the same dialdehyde (III) by periodate oxidation and, in fact, the same rotation was recorded at this stage for both substances (see Table I).

The application of Hudson's rules to the determination of the configurations of the furanose compounds has not given an agreement such as that described by Isbell and Frush[12] for a series of glycosylamines, but the results obtained confirm, in our opinion, that both of the *N*-acetyl-D-glycofuranosylamines are the α-D anomers.

For the contribution (to the rotation) of the anomeric carbon atom, we have used the A values obtained by them[11] for the anomeric pair of *N*-acetyl-D-gal-

actopyranosylamines, namely, +204°. To calculate the rotation of the *N*-acetyl-α-D-glucofuranosylamine, we employed the B value derived from the anomeric methyl D-glucofuranosides[14], namely, +39° and also from the two ethyl D-glucofuranosides[15], namely, +16°. When the first B value was used, a calculated value of $[\phi]_D$ +243° was obtained for the *N*-acetyl-α-D-glucofuranosylamine; the second B value gave $[\phi]_D$ +220°. Both results are in good agreement, in sign and order of magnitude, with the experimental value of $[\phi]_D$ +194°. For the β-D anomer, the $[\phi]_D$ calculated is −165° and −198°, respectively.

For *N*-acetyl-α-D-xylofuranosylamine, a B value of +76° was employed, derived from the anomers of methyl D-xylofuranoside[16]. This gave a calculated value of $[\phi]_D$ +280°, as against the experimental value of $[\phi]_D$ +191°. (The value calculated for the β-D anomer is $[\phi]_D$ −128°.)

The two *N*-acetyl-D-xylosylamines isolated from the ammonolysis of 1,2,3,4-tetra-*O*-acetyl-β-D-xylose are the stable products of a complex series of reactions, and one of the peculiar aspects of their formation is that, while the furanoid form has the α-D configuration, the pyranoid form is obtained as the β-D anomer. This is a result of the different geometry of the intermediates which produce them, a geometry which may be influenced by the number and position of the acetyl groups present.

It is worth noting, in connection with the β-D configuration of the pyranoid form, that, for D-xylopyranosylamine, the equilibrium is displaced so much in favor of the β anomer (IIa) that it is essentially the only one present in solution[12].

That the α-D anomer is exclusively produced when the furanoid ring is formed is also interesting, because, if we suppose, for argument, that the ring is planar, then the compound will have a rather strained structure in which there is one pair of eclipsed substituents on each side of the ring, *viz.*, the hydroxymethyl group and the hydroxyl group at C-3 on one side, and the hydroxyl group at C-2 and the acetamido group at C-1 on the other side.

The β-D anomer of the furanoid form will have three bulky substituents on one side, although the acetamido group at C-1 will not be very close to the other two (the hydroxymethyl group and the hydroxyl group at C-3).

A twist conformation will relieve part of the strain in both anomers, and a Stuart–Briegleb model revealed that, in this conformation, the β-D anomer is less strained, although the difference in energy content between the anomers must be small.

The favored formation of the α-D anomer has some similarity to the results obtained by Bishop and Cooper[17] on methyl glycosidation of D-xylose. They found that, at the beginning of the reaction, methyl α-D-xylofuranoside is formed faster than the β-D anomer, although the variation of free energy, in the interconversion of both anomers, reflects a small difference of stability in favor of the latter. In the course of time, this leads to a slight preponderance of the β-D anomer.

If the formation of *N*-acetyl-α-D-xylofuranosylamine is much faster than that of the β-D anomer, it will, because of its stability to the conditions of ammonolysis, remain as the only furanose anomer found among the products of the reaction (even if it is thermodynamically less stable).

EXPERIMENTAL

Melting points are uncorrected. Paper chromatography was performed on Whatman No. 1 paper, using ethyl acetate–pyridine–water (10:4:3) as the solvent. A 5:1:2 mixture[18] of 0.3% methanolic silver nitrate, 16% (w/v) methanolic ammonia, and 7% of sodium in methanol was used as the spray reagent.

*Ammonolysis of 1,2,3,4-tetra-*O*-acetyl-β-*D*-xylose*

1,2,3,4-Tetra-*O*-acetyl-β-D-xylose (7.3 g) was slowly added, with shaking, to 250 ml of methanolic ammonia (16% w/v). It dissolved very fast and, after being kept for 5 h at room temperature, the solution was evaporated to dryness under diminished pressure. The residue was well dried in a desiccator, and extracted with ethyl acetate to remove acetamide. The insoluble fraction (4.73 g) was found by paper chromatography to contain three different products (R_F 0.57, 0.41, and 0.25) and a large proportion of substances having a lower R_F. The residue was chromatographed on a small column of cellulose (1.5 × 30 cm) and two eluates were collected, the first containing the compounds of R_F 0.57 and 0.41 and the second, the products with R_F 0.25 (D-xylosylamine) or lower.

The first eluate, on evaporation, gave a residue showing an infrared spectrum with strong amido bands; the second eluate was discarded, as these absorption bands were absent from its spectrum. The dried residue from the first eluate (1.18 g) was rechromatographed on a larger column of cellulose (3.5 × 65 cm), and the fractions that contained the substances with R_F 0.57 and 0.41 were collected separately.

N-*Acetyl-α-D-xylofuranosylamine*

The fractions containing the products with R_F 0.57 were evaporated to dryness. The sirupy residue (which gave a negative spot-test with the aniline phthalate reagent) crystallized on treatment with a small amount of absolute ethanol; prisms (0.4 g; 9.2%), m.p. 141–143°. After several recrystallizations from the same solvent, they had m.p. 147–148° (cap.), 152–154° (Kofler); $[\alpha]_D^{20}$ + 100.0° (*c* 0.5, water).

Anal. Calc. for $C_7H_{13}NO_5$: C, 43.98; H, 6.81; N, 7.85. Found: C, 44.16; H, 7.20; N, 7.33.

N-*Acetyl*-2,3,5-*tri*-O-*benzoyl-α-D-xylosylamine* was obtained by benzoylation in the cold with the usual mixture of benzoyl chloride and pyridine. After three recrystallizations from 1:1 methanol–water, the product had m.p. 139–141° (cap.), 141–143° (Kofler); $[\alpha]_D^{20}$ +31.1° (c 0.5, chloroform).

Anal. Calc. for $C_{28}H_{25}NO_8$: C, 66.80; H, 5.00; N, 2.78. Found: C, 66.58; H, 5.09; N, 2.94.

Fractions that, on paper chromatograms, showed a spot with R_F 0.41 yielded, on evaporation, a sirup (0.51 g) which gave a positive reaction with the aniline phthalate reagent. On treatment with sodium borohydride in the usual way, xylitol (R_F 0.29) was produced; and this was readily separated, by chromotography on cellulose, from the product with R_F 0.41. Evaporation of the fractions which contained the

latter substance yielded a sirup, that, on treatment with absolute ethanol, gave 0.14 g (3.1%) of crystals of m.p. 206–208°. Recrystallization from the same solvent produced plates of m.p. 212–213° (cap.), 218–220° (Kofler); $[\alpha]_D^{20}$ −1.2° (c 0.5, water). It was identified as *N*-acetyl-β-D-xylopyranosylamine by mixed m.p. and i.r. spectrum. Isbell and Frush[12] gave m.p. 213°, $[\alpha]_D$ −0.7° (water).

Oxidation of the N-*acetyl*-D-*glycosylamines to aldehydes, and subsequent reduction to polyhydric alcohols*

To 0.1 mmole of the *N*-acetyl-D-glycosylamine, 3.5 ml (0.35 mmole) of 0.1 N sodium periodate was added, and the solution was kept at 10° in the dark; the optical rotation was measured at fixed intervals of time for 30 h. A constant rotation was observed for the *N*-acetyl-D-glycopyranosylamines after 2 h; but, for the furanoid compounds, the consumption of periodate was slower, and the final values of the rotations were obtained only after 24 h (see Table I). The solution was then made alkaline by adding 10 mg of sodium hydrogen carbonate, and treated with 10 mg of sodium borohydride. After the mixture had been kept for 2 h, the rotation was recorded; it remained constant for at least 24 h. Simultaneously with recording of the polarimeter readings, the oxidations were controlled by the determination of the consumption of periodate (using Aspinall and Ferrier's spectrophotometric method[19]).

SUMMARY

The *N*-acetyl-D-glycofuranosylamines produced in the ammonolysis of acetylated D-xylopyranose and acetylated D-glucopyranose have been found to be the α-D anomers. These assignments were obtained by destroying the asymmetry of all of the carbon atoms but the anomeric one (by periodate oxidation) followed by reduction of the resulting aldehydes with sodium borohydride, and comparing the optical rotations of the resulting products with those of known configuration. Application of Hudson's isorotation rules led to the same result. In the ammonolysis of tetra-*O*-acetyl-β-D-xylopyranose, the known *N*-acetyl-β-D-xylopyranosylamine is also produced.

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SYNTHESIS OF α AND β ANOMERS OF L-SERINE D-XYLOPYRANOSIDE

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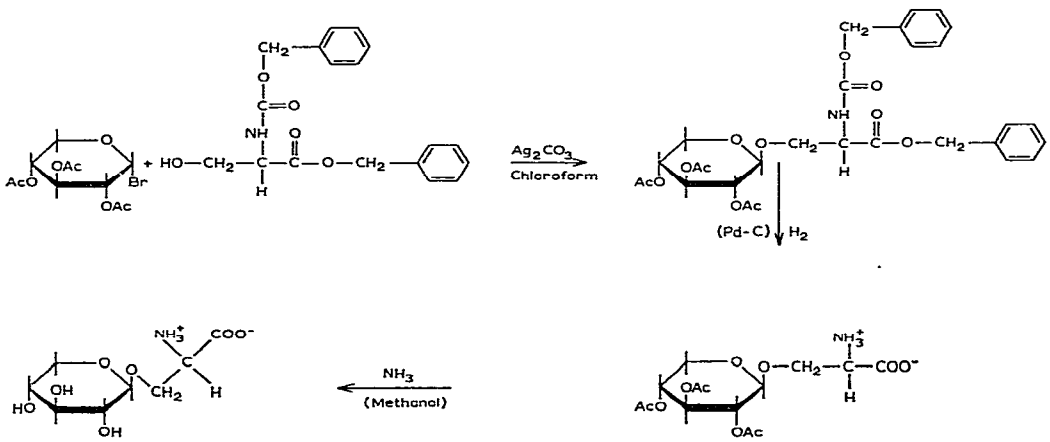
INTRODUCTION

A "xylosylserine" of unknown linkage was isolated by Rodén *et al.*[1] from partial hydrolysates of heparin and cartilage fractions. It was assumed that the *O*-xylosyl serine glycosidic bond constitutes one of the covalent linkages between peptide and polysaccharide chains in the above mentioned and similar protein polysaccharide complexes. The hydrolysis products of the isolated material showed a molar ratio of serine: xylose of 1:1, and the compound was readily separated from serine on Dowex-50 resin, by paper chromatography, or paper electrophoresis. The o.r.d. behavior of the "xylosylserine" was compared with that of methyl β-D-xyloside and L-serine and strongly indicated that the configuration of the D-xylosyl linkage was β and that the natural product had the L-serine β-D-xylopyranoside structure. In order to prove the structure of the isolated compound, we undertook the synthesis of both the α and β anomers of L-serine D-xylopyranoside. At the same time, the L-serine β-D-xylopyranoside was desired as starting material for experiments involving the alkaline cleavage (β-elimination) of polysaccharide–protein complexes from cartilage.

The projected synthetic route is shown in the accompanying diagram. Formation of the β-D-glycosidic bond is favored by the Koenigs–Knorr reaction[2–5], but we hoped to isolate the α-D-anomer as well, since this is usually formed in the reaction.



N-Benzyloxycarbonyl-L-serine-benzyl ester was used so that both the amino and the carboxyl protecting groups could be removed in one step to yield the *O*-acetylated "xylosylserines". Under mild alkaline conditions, these should yield the free "xylosylserines". The expected anomeric mixture may be resolved by crystallization or by chromatographic separation.

EXPERIMENTAL

Solutions were evaporated *in vacuo* using a rotary evaporator. Melting points were determined on a Fisher–Johns melting point apparatus. Amino acid analyses were carried out on a Technicon amino acid analyzer (separation on Dowex 50 X-12 with buffer gradient) as well as on a Beckman Spinco amino acid analyzer model 120 B (separation on Dowex 50 X-8 with a fixed buffer). Descending paper chromatography was performed using Whatman No. 1 and 3 MM paper in the following systems: butan-1-ol–pyridine–water, 6:4:3 (Solvent A), butan-1-ol–acetic acid–water, 5:2:1 (Solvent B), and butan-1-ol–ethanol–water, 10:3:5 (Solvent C). Electrophoresis was carried out on Whatman No. 3 MM paper in 1.6 M acetic acid adjusted to pH 2 with formic acid, at 20–30 V $\times$ cm^{-1} for 2–3 h. Xylose assays were performed according to a modified Mejbaum[6] procedure (orcinol reaction) using xylose standards. The values given are averages of 10 measurements. Serine was determined on the Technicon amino acid analyser after acid hydrolysis, using serine standards which were treated with acid in the same way as the "xylosylserine" samples. Optical rotations and o.r.d. were measured in a Cary 60 spectropolarimeter at 25°.

2,3,4-Tri-O-acetyl-α-D-xylosyl bromide[7,8]

A suspension of D-xylose (5 g) in acetic anhydride (30 ml) and acetic acid (20 ml) was cooled in an ice bath, stirred, and a stream of dry hydrogen bromide gas blown onto the surface of the suspension. After 30–60 min all the xylose was dissolved and the hydrogen bromide content of the solution was then increased to saturation at 0°. The hydrogen bromide stream should be regulated in such a way that the whole operation is over after 2 h, or 1 h after the xylose has gone into solution. The yellow solution was allowed to warm up to room temperature and kept at that temperature for another 2 h. The solvents were removed *in vacuo* at 50°, the oily residue was further dried by codistillation three times each with toluene (30 ml) (distilled over sodium) and finally dissolved in absolute ether (10 ml). After adding a boiling chip to promote crystallization, the resulting ether solution was cooled to −70°. The white crystalline precipitate which formed was filtered off under dry nitrogen. Yield 9.2 g (70%) m.p. 99° $[\alpha]_D^{25}$ +211° (*c* 1, chloroform).

L-Serine 2,3,4-tri-O-acetyl-β-D-xylopyranoside[9,10]

N-Benzyloxycarbonyl-L-serine-benzyl ester (Cyclo Chemical Corp.) (2 g) was dissolved in dry, alcohol-free chloroform (100 ml). Silver carbonate (10 g) and Drierite (20 g) were added, and the slurry stirred for 24 h at room temperature under protec-

tion from moisture. Twice recrystallized 2,3,4-tri-*O*-acetyl-α-D-xylopyranosyl bromide (5 g) was then added, the vessel was tightly closed (carefully greased ground joints), and the reaction mixture was magnetically stirred for 16 days in the dark at room temperature. Preliminary experiments had shown that the reaction had to be carried out at a moderate temperature for a rather long time. In order to remove the excess of 2,3,4-tri-*O*-acetyl-α-D-xylosyl bromide, absolute ethanol (100 ml) was added and the mixture stirred for an additional 24 h. The slurry was centrifuged, the supernatant was decanted, and the pellet was resuspended in alcohol and the suspension centrifuged. The combined supernatants were evaporated *in vacuo* to a sirup which was extracted thoroughly with hot water (300 ml). The latter treatment removed most of the ethyl 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside as well as other compounds formed as by-products. These compounds could also be separated by a rather involved chromatographic process. The remaining sirup was dissolved in a mixture of ethanol and water 1:1 and hydrogenated for 24 h in the presence of freshly prepared 10% palladium on charcoal catalyst. Prior to use, the catalyst was washed to neutrality with distilled water saturated with nitrogen. The solution was filtered, washed with ethanol, and the filtrate and washings evaporated *in vacuo* to a sirup. The sirup was extracted with ether in order to remove the last traces of ethyl 2,3,4-tri-*O*-acetyl-β-D-xyloside and other contaminants whereupon the sirup crystallized. Paper chromatography using solvent (A) showed three major compounds, which could not be separated completely by fractional crystallization from various solvents. The crude mixture (2 g) was therefore dissolved in butan-1-ol saturated with water, mixed with microcrystalline cellulose (5 g), and dried. The dry powder was placed on top of a cellulose powder column (2.5 cm diameter), prepared from 50 g of cellulose, which had been equilibrated for 24 h with butan-1-ol saturated with water. The column was developed with butan-1-ol saturated with water, and fractions of 5 ml were collected. Fractions 15–30 contained most of the acetylated "xylosylserines", but there was considerable tailing. After collection of 80 fractions the column was washed with water, and a second ninhydrin positive peak (serine) was obtained in fractions 90–100. Fractions 15–30 were pooled, evaporated and dissolved in a minimum amount of boiling absolute ethanol. The solution was seeded with a few crystals obtained from tube 21. After a few days, a white crystalline compound precipitated; it was filtered, washed and dried with alcohol and ether, yield 250 mg (11.5%) m.p. 194° (dec.), $[\alpha]_D^{25}$ −51° (*c* 0.33, water).

Fig. 1 describes the o.r.d. Xylose and serine assays showed a molar ratio of xylose to serine of 1:1 (± 3%).

Anal. Calc. for $C_{14}H_{21}O_{10}N$: C, 46.28; H, 5.83; N, 3.86. Found: C, 46.23; H, 5.84; N, 3.98.

The mother liquor was kept for the isolation of the α-D-anomer.

L-*Serine β-D-xylopyranoside* (I)

A small amount of the above triacetate was dissolved in absolute methanol and ammonia gas was introduced at a fairly rapid rate while the vessel was cooled to −10°.

The vessel was sealed and kept at +5° for 2 days, after which time the ammonia-saturated methanol was evaporated *in vacuo* (bath temperature below 25°). The sirupy residue crystallized after a few days and exhibited a variable melting point 220–230° (dec. at about 180°) which was dependent on the rate of heating. The compound had the same mobility as the natural material "xylosylserine" on paper chromatograms with R_{serine} 0.58 in Solvent A, 0.71 in Solvent B, and 0.73 in Solvent C. The corresponding α-D-anomer (II) was clearly resolved from both the natural and synthetic

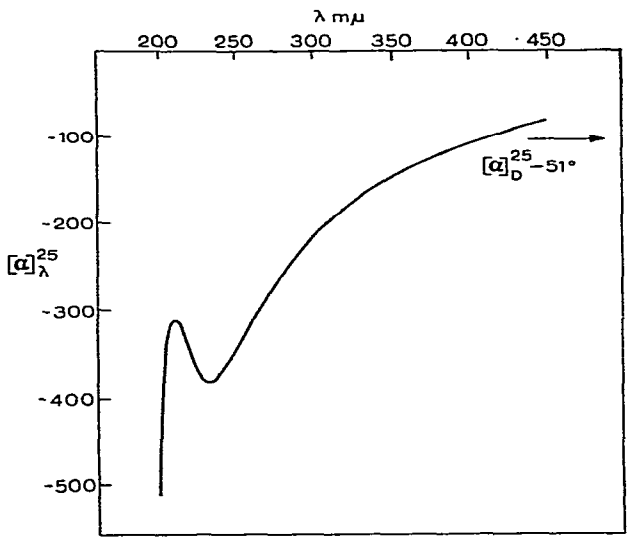


Fig. 1. O.r.d. of L-serine 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside.

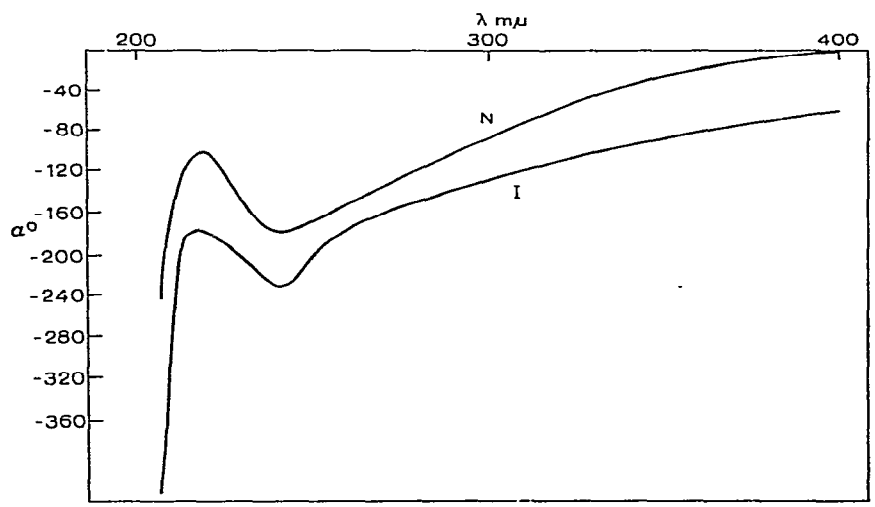


Fig. 2. O.r.d. of natural (N) "xylosylserine" and L-serine β-D-xylopyranoside (I).

products, as well as from serine. The β-D-anomer had the same mobility as the natural material on electrophoresis in 1.6 M acetic acid and showed the same retention volume on the Technicon amino acid analyzer (main peak at 2.5 h, two secondary peaks at 2.3 and 3.4 h). Finally its o.r.d. gave the same general pattern as the natural compound (Fig. 2) as well as being similar to that obtained by superimposing the o.r.d. curve of methyl α-D-xylopyranoside on that of L-serine (Fig. 3).

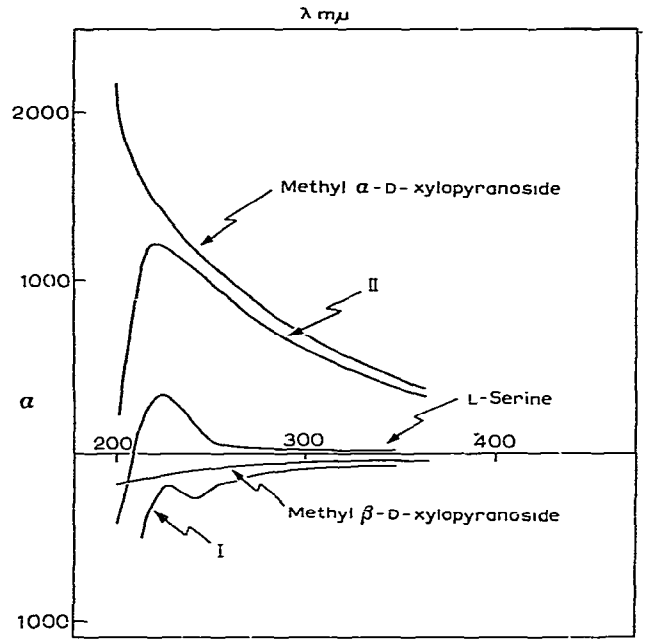


Fig. 3. O.r.d. of synthetic L-serine α- and β-D-xylopyranoside (I and II), L-serine, and methyl α- and β-D-xylopyranoside.

L-Serine α-D-xylopyranoside (II)

The mother liquors from the crystallization of L-serine 2,3,4-tri-*O*-acetyl-β-D-xylopyranoside were evaporated and the residue was dissolved in absolute methanol and deacetylated with ammonia as described above. The sirupy residue did not crystallize and on chromatography proved to be a mixture of two ninhydrin positive substances which could be separated by preparative paper chromatography, on Whatman No. 3 MM sheets using Solvent A with a developing time of 40 h. A second chromatography using solvent B for 70 h completely resolved the two fractions. The major component (I) migrated with the same R_F as that of the natural material. A minor component (II) had a slightly higher R_F value, R_{serine} 0.71 in Solvent A, 0.82 in Solvent B and 0.83 in Solvent C. A third fraction was identified as L-serine. The latter fraction could also be separated from "xylosylserines" using Dowex 50 X-12 (pyridinium form) and developing with a non-linear buffer gradient of 2M pyridine acetate between pH 2.65 and 2.85. The separation of I and II could not be

achieved by ion-exchange chromatography. However, such chromatography did reveal a small peak moving just in front of either I or II. This peak showed the presence of "xylosylserine" and was present in varying amounts depending on the conditions of hydrolysis of the L-serine 2,3,4-tri-*O*-acetyl-D-xylopyranosides. II was separated by preparative paper chromatography and finally purified by chromatography on Dowex 50 X-12. This material exhibited an o.r.d. very similar to that obtained by superimposing the o.r.d. curves of methyl D-xylopyranoside and L-serine. Therefore the structure of L-serine α-D-xylopyranoside is assigned II, it has not yet been obtained in crystalline form.

DISCUSSION

Jones *et al.*[9] synthesized L-serine 2-acetamido-2-deoxy-β-D-glucopyranoside starting from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride and *N*-benzyloxycarbonyl-L-serine-methyl ester. During alkaline hydrolysis of the acetylated glycoside intermediate, partial β-elimination of 2-acetamido-2-deoxy-D-glucopyranose occurred. This is in agreement with the findings of Riley *et al.*[11] who observed β-elimination as a side reaction during hydrolysis of *N*-benzyloxycarbonyl-*O*-(diphenylphosphoryl) L-serine-methyl ester. This elimination could be prevented by prior removal of the benzyloxycarbonyl group by catalytic hydrogenation.

A methyl ester group attached to the serine residue might enhance the tendency to β-elimination while the carboxylate ion would protect the molecule by decreasing the possibility of proton removal from the adjacent carbon atom.

N-Benzyloxycarbonyl-L-serine-benzyl ester was used as starting material so that both protecting groups could be removed by hydrogenation. This sequence led to the exclusion of any β-elimination but the bulky starting material was somewhat hindered. The sluggish reaction gave a moderately low yield of the desired product and a relatively high amount of the α-D-anomer was also obtained. I was the major product and its o.r.d. behavior could be explained as a superposition of the o.r.d. curves of methyl β-D-xylopyranoside and L-serine. In view of this, and since it derived from a crystalline acetate, $[\alpha]_D^{25}$ −51°, it was assigned the structure L-serine β-D-xylopyranoside. II, which did not separate from I on Dowex 50 resin, but did separate on paper chromatography had an o.r.d. curve which could be easily explained in terms of a superposition of the curves of methyl α-D-xylopyranoside and L-serine, and was therefore assigned the structure L-serine α-D-xylopyranoside (Fig. 3). In preliminary experiments, a small amount of contamination of both I and II (identical analysis) was detected on the Dowex-50 separations, but not on paper chromatography. The fact that these contaminants disappeared as soon as milder deacylation conditions were used, makes it most likely that they are the respective D-serine compounds. Interestingly, the α-D- and β-D- anomers separate on paper but not on Dowex 50, while the apparent diastereoisomeric pairs L-serine β-D-xylopyranoside and D-serine β-D-xylopyranoside, separate on Dowex 50 but not on paper.

ACKNOWLEDGMENT

We are indebted to Dr Lennart Rodén for a sample of natural "xylosylserine". This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, United States Public Health Service (Grants A-2903-C6 and A-4315-C4).

SUMMARY

The synthesis of L-serine β-D-xylopyranoside as well as of its α-anomer is described. Both compounds were compared with the natural material isolated from heparin and from cartilage chondroitin sulfate. The natural material is identical with L-serine β-D-xylopyranoside.

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ISOLATION OF HYALURONIC ACID FROM HUMAN SYNOVIAL FLUID BY PRONASE DIGESTION AND GEL FILTRATION

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INTRODUCTION

In previous studies[1], we isolated hyaluronic acid, largely freed from proteins, by repeated deproteinisation of human synovial fluids with 1,1,2-trichloro-1,2,2-trifluoroethane. This method has the advantages that the presence of the organic phase would be expected to denature any enzyme capable of degrading hyaluronic acid and to prevent degradation by bacteria introduced after removal of the fluid from the joint. Also, the end product is a representative sample of the whole range of hyaluronic acid molecules originally present. One drawback to this procedure is that normal and pathological fluids require different numbers of deproteinisations, and the actual act of deproteinisation (by shaking in the presence of oxygen) might of itself induce some degradation. In the present study, we sought to remove these obstacles by using a fixed time of incubation with a proteolytic enzyme, pronase, under sterile conditions and at a neutral pH. Our choice of pronase, in preference to another wide-spectrum protease (for example, ficin), was guided by the knowledge that pronase does not require a reducing agent, such as L-cysteine, for activation; L-cysteine may participate in oxidative–reductive degradation[2] of hyaluronic acid.

MATERIALS AND METHODS

Buffers of standard composition[3] were used in this work.

Behaviour of synovial fluid on Sephadex G-200

Synovial fluid (0.3 ml, from a patient having rheumatoid arthritis) was diluted with M sodium chloride (buffered to pH 7 with 0.01M phosphate; 2.7 ml). The mixture was centrifuged to remove tissue debris and then passed, under gravity flow at 4°, through a column (2 × 50 cm) of Sephadex G-200 in the presence of the same buffer. Fractions (4.5 ml) were collected and assayed for uronic acid by the Gregory[4] modification of the Dische[5] carbazole reaction. Ultraviolet absorbance at 280 mμ was measured in 1-cm cuvettes on a Unicam SP 500 spectrophotometer. The results are shown in Fig. 1a.

Time required for pronase digestion

Mixtures of pronase (Calbiochem, B grade; 7 mg), centrifuged synovial-fluid (4 ml, from a patient having rheumatoid arthritis), and 7 ml of phosphate buffer

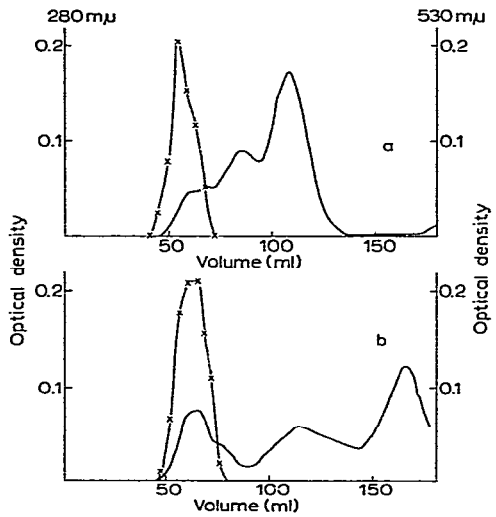

Fig. 1. Separation on Sephadex G-200 of untreated synovial fluid (*a*) and synovial fluid after pronase digestion in Tris buffer (*b*). —— Optical density (o.d.) at 280 mμ (protein or peptide debris); x-x-x, o.d. at 530 mμ (uronic acid).

(0.1 I, pH 7 or pH 8, saturated with calcium chloride) were incubated for 50 h (Table I); the digests had pH 7 and 7.6, respectively. Samples (0.1 ml) were taken during the digestion and diluted (to 5 ml), and the ninhydrin reactivity was assayed on 1-ml portions by the method of Yem and Cocking[6].

TABLE I

OPTICAL DENSITY AT 570 mμ

	1.5 h	*22 h*	*50 h*
pH 7	0.23	0.58	0.64
pH 8	0.26	0.59	0.73

Pronase digestion of synovial fluid using phosphate buffer

Synovial fluid (6 ml, combined samples obtained from rheumatoid-arthritic patients by aseptic aspiration) was mixed with a pronase (B grade) solution (4 ml; 2 mg/ml of phosphate buffer, 0.1 I, pH 7), previously sterilised by passage through a Hemming filter. The mixture was centrifuged (10 min at 4° and 17 000 r.p.m. in an MSE 17 000 centrifuge), under sterile conditions, to remove tissue debris, aseptically transferred to a sterile-dialysis apparatus[7], and incubated at 37°. The outer compart-

ment contained phosphate buffer (pH 7) saturated with calcium chloride. The buffer was changed three times during the 48-h digestion. The product was freeze-dried and reconstituted in water (6 ml). Half of this sample was then passed through a column (50 × 2.4 cm) of Sephadex G-200 (Fig. 2a) with phosphate buffer (0.1 I, pH 7).

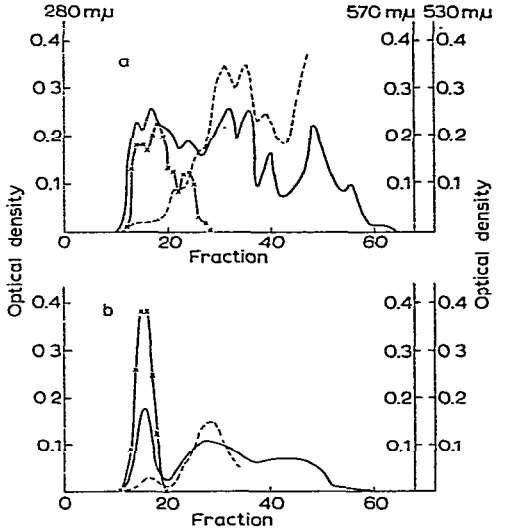

Fig. 2. Separation on Sephadex G-200 of synovial fluid after pronase digestion in phosphate buffer (*a*), and redigestion of uronic acid peak (*b*). ——, o.d. at 280 mμ (u.v.-absorbance); x–x–x–, o.d. at 350 mμ (uronic acid); ·····, o.d. at 570 mμ (ninhydrin).

Assays were performed, as described above, by measuring optical density at 280 mμ, ninhydrin reactivity[6], and uronic-acid content[4]. The fractions containing uronic acid were combined and freeze-dried. The reconstituted material was then redigested using purified pronase (Fractions 24–52, Fig. 3) and again passed through Sephadex G-200 (Fig. 2b).

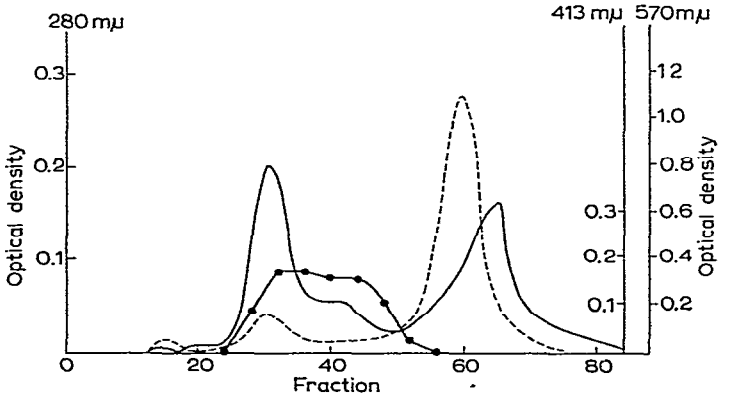

Fig. 3. Separation on Sephadex G-75 of pronase.—— o.d. at 280 mμ (u.v.-absorbance); ·····, o.d. at 570 mμ (ninhydrin); –•–•–•–, o.d. at 413 mμ reduction (haemoglobin).

Purification of pronase on Sephadex G-75

The supernatant liquid from pronase (B grade, 12 mg) dissolved in phosphate buffer (0.1 I, pH 7, 2 ml) was passed through a column (2.4 × 50 cm) of Sephadex G-75 (bead form) under gravity flow with the same buffer. Fractions (50 drops; *ca.* 3.5 ml) were collected, and the optical density at 280 mμ and the ninhydrin reactivity[6] were measured as above. Haemoglobin was used as a control for proteolytic activity; a portion (1 ml) of every third fraction was added to 0.007% haemoglobin (5 ml) in phosphate buffer (0.05 I, pH 8), and the mixture was incubated at 37° for 4 h. The decrease in optical density at 413 mμ was measured using 1-cm cells; the optical density of a 1% solution of haemoglobin was taken as 83.3. The results are shown in Fig. 3.

Isolation of [^{14}C]-hyaluronic acid from tissue culture.

Selected slices of tissue (0.5 g, wet wt.) from the synovial membrane of a healthy subject were incubated for 7 h at 37° in a protein-free medium (5 ml) containing [^{14}C]-D-glucose (50 μc), mineral salts, amino acids, insulin, and antibiotics. The culture was transferred to sterile, Mickle-disintegrator tubes, sterile beads were added, and the cells were disintegrated by 30 operations of 1-min duration, at intervals of 1 minute; the disintegrator was cooled[8] throughout by solid carbon dioxide. Using sterile, phosphate buffer (pH 7, 5 ml), the suspension was washed into a digestion apparatus, incubated with purified pronase overnight, and then passed through Sephadex G-200, as described above. The results are shown in Fig. 4. Radioactivity was determined by drying a 0.05-ml sample on a planchet and counting for 15 min in an end-window counter.

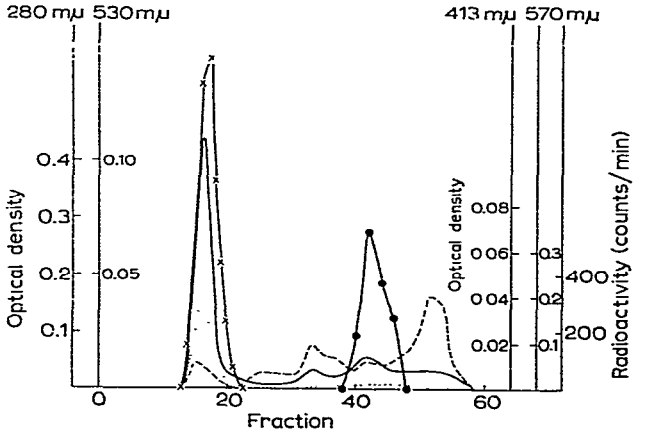

Fig. 4. Separation on Sephadex G-200 of tissue-culture digestion. ——, o.d. at 280mμ (u.v.-absorbance); x-x-x, o.d. at 530 mμ (uronic acid); ·····, o.d. at 570 mμ (ninhydrin); –•–•–•–, o.d. at 413 mμ reduction (haemoglobin); ....., radio-activity.

Pronase activity in Tris and phosphate buffers

Solutions of pronase (B grade) in Tris [tris(hydroxymethyl)aminomethane] –HCl buffer (pH 7, 0.05 I) and in phosphate buffer (pH 7, 0.1 I) containing 1.4 mg/ml

and 2.0 mg/ml, respectively, were prepared. Aliquot portions (0.1 ml) were added to 0.006% haemoglobin solutions in Tris–HCl buffer (pH 8, 0.05 I) and in phosphate buffer (pH 8, 0.1 I). The mixtures were incubated at 36° for 3 h, and the absorbances at 413 mμ were compared with those of the haemoglobin solutions after similar incubation (Table II).

TABLE II

DECREASE IN OPTICAL DENSITY AT 413 mμ

Buffer for enzyme	*Buffer for haemoglobin*	*1.4 mg/ml*	*2 mg/ml*
Tris–HCl	Tris–HCl	0.115	0.154
Tris–HCl	Phosphate	0.083	0.118
Phosphate	Phosphate	0.073	0.094

Pronase digestion of synovial fluid in the presence of Tris buffer

Synovial fluid (5 ml, combined samples) mixed with sterile pronase solution [7.5 ml; 1.5 mg of pronase-B/ml, tris–HCl buffer (0.05 I, pH 7.9)], which was 0.005M in calcium chloride, was centrifuged at 4°, and equal aliquots were dialysed under sterile conditions, overnight at 4°, against more of the same Tris buffer. The aim was to remove any small molecules that could act as reducing agents (*e.g.*, ascorbic acid) and might participate in oxidative–reductive degradation of hyaluronic acid. All digests were incubated at 37° for different lengths of time; the 24-h digest was given a further change of buffer after 8 h. Viscosities were then measured on a 1-ml sample, diluted to 10 ml with the Tris buffer, by using a suspended-level viscometer (water flow-time, 183.50 sec) maintained at 20 ± 0.002° in a water bath.

Sample	0 h	4 h	8 h	16 h	24 h
Specific viscosity	0.251	0.260	0.257	0.170	0.186

The remainder (1.5 ml ≡ 0.6 ml of synovial fluid) of the 8-h sample, with sucrose added to increase its density, was passed through a column (2 × 50 cm) of Sephadex G-200 at 4°, with M sodium chloride. Fractions (4 ml) were collected, and the uronic acid content[4] and optical density at 280 mμ were measured as above (Fig. 1b).

The fractions containing uronic acid were combined, dialysed, and freeze-dried. The protein content of the preparation was determined by submitting a hydrolysed sample to amino-acid analysis using the Technicon Autoanalyser, and corresponded to 16.5%.

RESULTS AND DISCUSSION

It was found that a partial separation of the hyaluronic acid component from the numerous proteins present in human synovial fluid could be accomplished by the application of diluted aliquots to a Sephadex G-200 column (Fig. 1a). Hyaluronic

acid is largely excluded from the gel and hence is eluted just before the synovial fluid proteins whose molecular weight lies below 200,000. However, the possibility of enzymic degradation[9] of the hyaluronic acid, particularly for pathological fluids, during the process of separation, makes it advisable for digestions to be performed under completely sterile conditions. Assays showed that such digestion with pronase was largely complete after 48 h in phosphate buffer at pH 7, and the subsequent separation of a typical pronase–phosphate-digested synovial fluid is shown in Fig. 2a. A ninhydrin scan showed that the hyaluronic acid was, in the main, separated from the protein debris, but the polysaccharide fractions were turbid, and a u.v.-scan of optical density at 280 mμ for proteins was therefore misleading. Absorption curves of isolated fractions showed that the early fractions, which contained polysaccharide, showed only a very broad absorption having a maximum in the 225-235 mμ region, with only a suggestion of an inflexion due to proteins at 280 mμ, whereas subsequent fractions, which contained protein debris, showed definite maxima at 280 mμ.

The source of this turbidity was not impurities of high molecular weight in the pronase (B grade), since passage of the enzyme through Sephadex G-75 (Fig. 3) showed that such impurities were absent. Enzyme assay with haemoglobin indicated that pronase-B contained at least two proteolytic components, together with larger amounts of inactive peptides having lower molecular-weights. Redigestion of the synovial-fluid hyaluronic acid, isolated after one treatment with pronase, still showed the turbidity (Fig. 2a) associated with the hyaluronic acid, although more protein contaminant had been removed. The hyaluronic acid component isolated after one, two, and three digestions with pronase reacted with the carbazole[4] (for uronic acid) and ninhydrin[6] reagents (for amino groups) to give optical density ratios of 1:1, 4:1, and 10:1, respectively. Pronase is excluded on Sephadex G-50 and hence was found to be eluted with the hyaluronic acid component of a pronase-digested synovial fluid. Sephadex G-75 therefore represents the lower limit for a feasible separation of hyaluronic acid and pronase, but Sephadex G-200 is much to be preferred for removal of the maximal amount of protein debris.

Whilst hyaluronic acid normally absorbs in the region 225–235 mμ, due mainly to contributions from the carbonyl groups of the 2-acetamido-2-deoxy-D-glucose (λ_{max} 220–225 mμ) and D-glucuronic acid (lactone, λ_{max} 215 mμ) residues, the absorption associated with the hyaluronic acid component, after digestion in pronase-phosphate, is much greater than could be attributed to this source. It accompanied the [^{14}C]-hyaluronic acid isolated from a culture of normal, human, synovial tissue (Fig. 4). The turbidity appeared to arise from the presence of calcium phosphate in the pronase buffer.

Tris buffer was therefore investigated as a substitute for phosphate buffer. Pronase then exhibited greater activity, and this feature, together with the higher pH (7.9), enabled the incubation time for digestion to be shortened considerably. As an additional precaution, the digests were dialysed overnight at 4° to remove natural reducing-agents that might cause free-radical degradation of the hyaluronic acid. Viscosity determinations showed that no degradation was evident after subse-

quent incubation with pronase for 8 h at 37°. This incubation time was sufficient to give an excellent separation thereafter on Sephadex G-200 (Fig. 1b). Furthermore, the turbidity previously encountered with the hyaluronic acid had disappeared.

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SUMMARY

The isolation of hyaluronic acid from human synovial fluid by passage down Sephadex G-200, following sterile digestion with pronase in Tris buffer (pH 7.9) for 8 h at 37°, affords a product without the occurrence of the enzymic or chemical degradation that may accompany other methods of isolation. Use of phosphate buffer, under similar conditions, gives turbid solutions of hyaluronic acid.

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SYNTHESIS OF 6-DEOXY-4-*O*-METHYL-D-GALACTOSE (D-CURACOSE)

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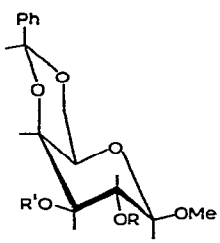
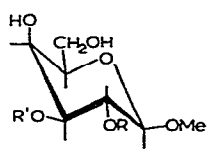
Curamycin is the name given to an antibiotic substance produced by *Streptomyces curacoi*[1]. Hydrolysis of curamycin with dilute hydrochloric acid yielded a crystalline compound (curacin) and a water-soluble fraction from which three sugars were isolated. Only one of the sugars was identified (as L-lyxose); the others were designated D-curacose and sugar I. The latter is still under study, but D-curacose has been identified[1] as the hitherto unknown 6-deoxy-4-*O*-methyl-D-galactose on the basis of chemical evidence.

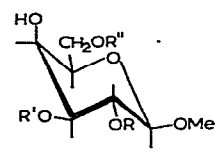
The following stereospecific synthesis of 6-deoxy-4-*O*-methyl-D-galactose confirm this assignment of structure. The synthesis was achieved in two related ways. Benzylidenation of methyl β-D-galactopyranoside (I) gave the known 4,6-benzylidene acetal



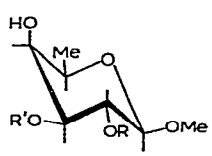
II: R = R' = H
III: R = R' = *p*-Tolylsulfonyl
IV: R = R' = Benzyl



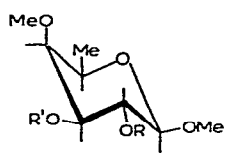
V: R = R' = *p*-Tolylsulfonyl
VI: R = R' = Benzyl



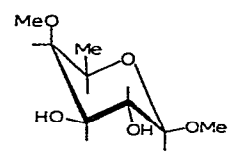
VII R = R' = R'' = *p*-Tolylsulfonyl
VIII R = R' = Benzyl
R'' = *p*-Tolylsulfonyl



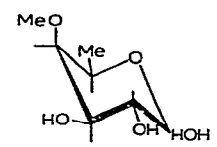
IX: R = R' = Benzyl



X: R = R' = Benzyl



XI



XII

(II) which, in the first method, was converted into methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside[3] (IV) on treatment with benzyl chloride and sodium hydride in *N*,*N*-dimethylformamide. The n.m.r. spectrum showed that one of

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the benzyl groups (probably that at C-2) has a restricted rotation, because the signal of one of the methylene groups appears as an AB system (τ 5.24 and 5.04; J 11 c.p.s.), whereas the other produces a singlet at τ 5.23.

Debenzylidenation of IVyielded methyl 2,3-di-*O*-benzyl-β-D-galactopyranoside[3] (VI) which, on mono-*p*-toluenesulfonation afforded syrupy methyl 2,3-di-*O*-benzyl-6-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (VIII). This, on desulfonyloxylation with lithium aluminum hydride was converted into methyl 2,3-di-*O*-benzyl-6-deoxy-β-D-galactopyranoside (IX), and thence, by methylation with methyl iodide and silver oxide, into methyl 2,3-di-*O*-benzyl-6-deoxy-4-*O*-methyl-β-D-galactopyranoside (X). Catalytic debenzylation[4] of the glycoside (X) gave methyl 6-deoxy-4-*O*-methyl-β-D-galactopyranoside (XI) which, on hydrolysis with N hydrochloric acid, was converted into 6-deoxy-4-*O*-methyl-D-galactose (XII), m.p. 132–133°, $[\alpha]_D^{24}$ +102.6 → +80.6° (final; *c* 0.92, water). The melting point of the synthetic sugar was not depressed on admixture with D-curacose. Galmarini and Deulofeu[1] reported m.p. 131-132°, $[\alpha]_D^{24}$ +82.0° (*c* 1.0, water) for D-curacose.

The n.m.r. spectrum of the synthetic sugar in deuterium oxide showed that both anomers were present; H-1 exhibited two resonance patterns corresponding to H-1 equatorial (α-D form) and H-1 axial (β-D form), respectively. The signal of the former gave a doublet at τ 4.84 ($J_{1,2}$ 3 c.p.s.) and that of the latter appeared as a doublet at τ 5.50 ($J_{1,2}$ 8 c.p.s.). Measurement of the spectrum of the deuterium oxide solution after 4 h revealed an increase in the relative intensity of the β-D form doublet as compared to the α-D form doublet, hence a shift of the equilibrium in favor of the equatorial orientation of the hydroxyl group at C-1 had occurred; this shift accompanies the mutarotation.

In the second approach, compound II was converted into methyl 4,6-*O*-benzylidene-2,3-di-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (III) which, on debenzylidenation yielded methyl 2,3-di-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside[3] (V). Mono-*p*-toluenesulfonation afforded crystalline methyl 2,3,6-tri-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (VII), which was exhaustively methylated with methyl iodide and silver oxide to give a syrupy product presumed to be methyl 4-*O*-methyl-2,3,6-tri-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside. The total methylation of compound VII was not easy to accomplish, owing to the axial orientation of the hydroxyl group at C-4, and was further hindered by the presence of the bulky *p*-tolylsulfonyloxy group at C-6; this difficulty was also encountered for compound IX, although it was not so marked as in the preceding case. On desulfonyloxylation with lithium aluminum hydride and subsequent hydrolysis with N hydrochloric acid, methylated VII was converted into syrupy 6-deoxy-4-*O*-methyl-D-galactose (XII). Partition chromatography on cellulose yielded chromatographically pure XII, which crystallized on nucleation.

The first method is the better, since the intermediates could be purified after each step, leading to a better overall yield. The infrared spectrum and chromatographic properties of the synthetic sugar were indistinguishable from those of D-curacose.

EXPERIMENTAL

Melting points are uncorrected. Infrared spectra were recorded with a Perkin–Elmer Infracord spectrophotometer. Nuclear magnetic resonance spectra were determined in deuteriochloroform or deuterium oxide, with tetramethylsilane as the external reference, using a Varian A-60 spectrometer. Thin-layer chromatography was performed on silica gel, using benzene–methanol as the mobile phase; the materials were detected with iodine vapor. Paper chromatography was performed on Whatman paper No. 1 with 4:1:5 1-butanol–ethanol–water (upper phase), and the reducing sugars were detected with aniline hydrogen phthalate. Solvents were usually removed under diminished pressure below 50°.

Methyl β-D-galactopyranoside (I)

D-Galactose (100 g) was acetylated with acetic anhydride (540 ml) and sodium acetate (44 g). The penta-*O*-acetyl-β-D-galactopyranose (120 g) had m.p.[5] 137–138°. This compound was treated with 30–32% hydrobromic acid in acetic acid (500 g), and processed as already described[6]; the tetra-*O*-acetyl-α-D-galactopyranosyl bromide had m.p. 80–81°. This compound (122 g) was dissolved in dry methanol (1.9 l), and silver carbonate (116 g) and Drierite (10 g) were added. The mixture was stirred overnight; the silver salts were filtered off and the filtrate was evaporated to a residue (109 g) presumed to be methyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside. A solution of this product in dry methanol (1.0 l) was saturated with ammonia, while being kept at 0°. The solution was maintained for 20 h at room temperature. The crystalline residue obtained on evaporation of the methanol was extracted with ether (3 × 100 ml) and the extracts were decanted and discarded; the product was then recrystallized from ethanol to give pure I (52 g), m.p. 178-179°, $[\alpha]_D^{24}$ +1.2° (*c* 0.98, water)[7].

Methyl 4,6-O-benzylidene-β-D-galactopyranoside (II)

Methyl β-D-galactopyranoside (I, 47 g) was mixed with freshly distilled benzaldehyde (125 ml) and anhydrous zinc chloride (40 g), and the mixture was shaken for 20 h. A saturated solution of sodium carbonate was added, and the insoluble salts were filtered off and washed with warm methanol. The residue obtained on evaporation of the filtrate was extracted twice with petroleum ether (b.p. 60–80°) and the extracts were discarded; the solid residue was extracted with warm chloroform (4 × 300 ml), the solid being filtered after each extraction. The chloroform solution was dried (magnesium sulfate) and evaporated to a residue which crystallized from ethanol, yielding pure II (57 g), m.p. 203–205°, $[\alpha]_D^{24}$ −30.1° (*c* 1.02, chloroform)[2]. The n.m.r. spectrum showed the benzylidene tertiary proton at τ 4.28.

Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranoside (IV)

Compound II (30 g) was dissolved in *N*,*N*-dimethylformamide (1.0 l), and 50% sodium hydride in oil (50 g) was added in small portions. After 1 h, benzyl

chloride (80 ml) was added dropwise, and the mixture was kept for 20 h at room temperature. The excess of sodium hydride was decomposed by addition of methanol, and the solution was evaporated. The residue was extracted with chloroform, and the extract was washed with water, dried (magnesium sulfate) and evaporated. The syrupy residue crystallized from petroleum ether (b.p. 60–80°) and was recrystallized (twice) from ethanol, yielding pure IV (32 g), m.p. 135–136°, $[\alpha]_D^{24}$ +48.5° (*c* 0.92, chloroform)[3] The n.m.r. spectrum showed an AB quartet (τ 5.24, 5.04; *J* 11 c.p.s.) corresponding to one of the benzylic methylene groups, the other appearing as a singlet at τ 5.23; the tertiary proton (benzylidene) appeared as a sharp singlet at τ 4.48.

*Methyl 2,3-di-*O*-benzyl-β-*D*-galactopyranoside (VI)*

A solution of IV (30 g) in methanol (360 ml) and N hydrochloric acid (30 ml) was heated for 5 h under reflux. Water (50 ml) and sodium hydrogen carbonate (1 g) were added, and the mixture was concentrated to remove the methanol. Water (200 ml) was added, and the mixture was again evaporated; this procedure was repeated three times. The aqueous suspension was extracted with chloroform, and the extract was washed with water and dried. Evaporation of the chloroform gave a syrupy residue which crystallized from petroleum ether (b.p. 60–80°). The compound (21 g), m.p. 68–70°, $[\alpha]_D^{24}$ + 12.4° (*c* 1.12, chloroform)[3] showed in its n.m.r. spectrum the absence of the one-proton signal at τ 4.48, indicating the removal of the benzylidene group.

*Methyl 2,3-di-*O*-benzyl-6-*O*-p-tolylsulfonyl-β-*D*-galactopyranoside (VIII)*

Compound VI (20 g) was dissolved in dry pyridine (40 ml) and maintained at 0° while a solution of *p*-toluenesulfonyl chloride (10.18 g) in dry pyridine (20 ml) was slowly added. The mixture was kept for 5 days at room temperature, poured onto ice-water and extracted with chloroform; the combined extracts were washed with 2 N hydrochloric acid and with water, and dried (magnesium sulfate). The residue (21.9 g) obtained by evaporation of the chloroform was shown by t.l.c. to be a mixture of the desired product with unreacted VI. The oily substance was chromatographed on a silica gel (100–200 mesh) column with benzene–methanol mixtures. The chromatographically pure compound (13.1 g), $[\alpha]_D^{24}$ +8.9° (*c* 0.99, chloroform) showed, in the i.r. spectrum, bands at 3450 (hydroxyl), 1360, and 1175 cm^{-1} (sulfonyl).

Anal. Calc. for $C_{28}H_{32}O_8S$: C, 63.69; H, 6.10; S, 6.07. Found: C, 63.97; H, 6.30; S, 5.90.

*Methyl 6-deoxy-2,3-di-*O*-benzyl-β-*D*-galactopyranoside (IX)*

Compound VIII (12 g) in dry ether–benzene (400:200 ml) was mixed with lithium aluminum hydride (6 g) and the mixture was heated for 15 h under reflux. The excess of lithium aluminum hydride was decomposed with ethyl acetate and ice. The salts were filtered off washed with ether, and the filtrate was washed with water and dried (magnesium sulfate). The syrupy residue (6.9 g) obtained on removal of the solvent was distilled at 125°/10^{-3} mm to give pure IX, $[\alpha]_D^{24}$ +31.5° (*c* 1.08, chloroform). Spectroscopy (i.r. and n.m.r.) showed the loss of the *p*-tolylsulfonyloxy group and

the presence of a secondary methyl group (doublet at τ 8.64, J 6.2 c.p.s.).

Anal. Calc.for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.63; H, 7.26.

Methyl 6-deoxy-2,3-di-O-benzyl-4-O-methyl-β-D-galactopyranoside (X)

Compound IX (3.76 g) was heated with methyl iodide (160 ml) and silver oxide (7 g) for 2 days under reflux, additional amounts of silver oxide (2 × 1.5 g) being added during the reaction. The cooled solution was filtered, the solid was washed with ether, and the combined filtrates were evaporated to a syrup (3.8 g). Distillation (twice) of the syrup at 110–113°/10^{-3} mm afforded pure X, $[\alpha]_D^{24}$ +8.5° (*c* 1.21, chloroform). The i.r. and n.m.r. spectra agreed with the assigned structure.

Anal. Calc. for $C_{22}H_{28}O_5$: C, 70.90; H, 7.58; Found: C, 70.96; H, 7.27.

Methyl 6-deoxy-4-O-methyl-β-D-galactopyranoside (XI)

A solution of X (2.88 g) in ethanol (220 ml) containing 5% palladium on charcoal (1.5 g) was stirred for 2 days at room temperature with hydrogen under slight pressure. The catalyst was filtered off, and the filtrate was evaporated to a crystalline residue (1.8 g). Recrystallization (twice) from acetone–petroleum ether (b.p. 60–80°) afforded XI (805 mg), m.p. 144–145°, $[\alpha]_D^{24}$ −14.6° (*c* 0.95, methanol). The n.m.r. spectrum agreed with that expected for the proposed structure.

Anal. Calc. for $C_8H_{16}O_5$: C, 49.99; H, 8.39. Found: C, 50.00; H, 8.53.

Methyl 4,6-O-benzylidene-2,3-di-O-p-tolylsulfonyl-β-D-galactopyranoside (III)

A solution of II (15 g) in dry pyridine (45 ml) was treated with *p*-toluenesulfonyl chloride (30 g) and the mixture was kept for 2 days at 36°. It was poured onto ice–water and the crystalline precipitate was filtered off and washed with water. The product (45 g) was recrystallized (twice) from ethanol, yielding pure III (22.5 g), m.p. 165–165°, $[\alpha]_D^{24}$ +27.0° (*c* 1.18, chloroform)[2,3].

Methyl 2,3-di-O-p-tolylsulfonyl-β-D-galactopyranoside (V)

Compound III (16 g) in ethanol–water (180:300 ml) was treated with N hydrochloric acid (40 ml) and the suspension was heated for 4 h under reflux (after 2 h it gave a clear solution). The ethanol was removed, and the aqueous solution remaining deposited a crystalline precipitate on cooling; the solid was filtered off and washed with water. The product was recrystallized (twice) from acetone–petroleum ether (b.p. 60–80°) to give pure V (8.6 g), m.p. 140–142°, $[\alpha]_D^{24}$ +19.4° (*c* 1.43, chloroform)[3].

Methyl 2,3,6-tri-O-p-tolylsulfonyl-β-D-galactopyranoside (VII)

In a typical experiment, compound V (1 g) in dry pyridine (2 ml) was cooled to 0° and *p*-toluenesulfonyl chloride (400 mg) was added in small portions. The mixture was kept for 4 days at room temperature and poured onto ice-water; the crystalline precipitate was filtered off, washed with water, and dried (1.34 g). Recrystallization (twice) from ethanol afforded pure VII (450 mg), m.p. 166–168°, $[\alpha]_D^{24}$ +10.7° (*c* 1.08, chloroform). The spectra agreed with those to be expected for the proposed structure.

Anal. Calc. for $C_{28}H_{32}O_{12}S_3$: C, 51.20; H, 4.92; S, 14.65. Found: C, 50.91; H, 5.12; S, 14.33.

When *p*-toluenesulfonyl chloride was used in excess[8], the product obtained was methyl 2,3,4,6-tetra-*O*-*p*-tolylsulfonyl-*β*-D-galactopyranoside, m.p. 167–168°(depressed by 10–15° on admixture with methyl 2,3,6-tri-*O*-*p*-tolylsulfonyl-*β*-D-galactopyranoside), $[\alpha]_D^{24}$ +14.3° (*c* 1.01, chloroform), showing no hydroxyl bands in its i.r. spectrum.

Anal. Calc. for $C_{35}H_{38}O_{14}S_4$: C, 51.84; H, 4.72; S, 15.82. Found: C, 51.96; H, 4.71; S, 15.43.

6-Deoxy-4-O-methyl-D-galactose (D-curacose) (XII)

(a) From methyl 6-deoxy-4-O-methyl-β-D-galactopyranoside (XI). A solution of XI (200 mg) in N hydrochloric acid (10 ml) was heated in a water bath for 2 h at 90–100°. After the solution had been cooled, it was neutralized with Dowex-3 (OH^-) resin, and the mixture was immediately filtered. The residue obtained on lyophilization of the filtrate was taken up in boiling ethyl acetate; the hot solution was filtered through Filtercel and the filtrate was kept at room temperature. The crystalline precipitate (73 mg) was recrystallized from ethyl acetate. The pure XII (33 mg) had m.p. 132–133° (undepressed on admixture with D-curacose), $[\alpha]_D^{24}$ +102.6 (10 min) → +80.6° (final; *c* 0.92, water). The spectra and chromatographic properties of the synthetic sugar and D-curacose were indistinguishable.

(b) From methyl 2,3,6-tri-O-p-tolylsulfonyl-β-D-galactopyranoside (VII). A solution of VII (4 g) in methyl iodide (25 ml) and acetone (15 ml) was heated for 8 h under reflux in the presence of silver oxide (2 g). The procedure was repeated four times until the hydroxyl band in the i.r. spectrum disappeared. The solid was filtered off and washed with acetone, and the filtrate was evaporated to a syrup (4.7 g). This syrup was dissolved in dry ether–benzene (30:18 ml), and the solution was heated for 2 days under reflux in the presence of lithium aluminum hydride (3.5 g). After the excess of lithium aluminum hydride had been destroyed with ethyl acetate and ice, the salts were filtered off and washed with ether. The syrup (1.1 g) obtained on removal of the solvent had no *p*-tolylsulfonyloxy bands in its i.r. spectrum.

This compound was hydrolyzed by refluxing it with N hydrochloric acid (15 ml) for 2 h. The cooled solution was neutralized with Dowex-3 (OH^-) resin and filtered, and the filtrate was evaporated to an oily residue (350 mg). The product was chromatographed on a cellulose (Whatman, Standard grade) column, being developed with 3:1:1 1-butanol–ethanol–water. The eluted fractions were monitored by paper chromatography. The fractions having the same R_F were combined and lyophilized. The residue (60 mg) crystallized from ethyl acetate on nucleation; the pure XII (23 mg) had m.p. 129–131° and its i.r. spectrum was identical with that of D-curacose.

6-Deoxy-4-O-methyl-D-galactose (p-tolylsulfonyl)hydrazone

Compound XII (20 mg) and (*p*-tolylsulfonyl)hydrazine (20 mg) in methanol (1.2 ml) was heated for 30 min under reflux and cooled. The crystalline precipitate

was filtered off and recrystallized from acetonitrile. The product had m.p. 133–134°, $[\alpha]_D^{24}$ −11.0 → −4.0° (final; *c* 0.87, pyridine), in good agreement with the values reported for D-curacose (*p*-tolylsulfonyl)hydrazone[1].

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SUMMARY

The stereospecific synthesis of 6-deoxy-4-*O*-methyl-D-galactose has been accomplished by using somewhat different intermediates in two related ways. The synthetic sugar is shown to be identical with D-curacose, a constituent of the antibiotic curamycin. Some of the known compounds used in the syntheses have been prepared by improved methods.

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STUDIES ON URONIC ACID MATERIALS

PART XIII[1]. THE COMPOSITION OF GUM EXUDATES FROM *Albizia sericocephala* AND *Albizia glaberrima* WITH AN APPENDIX ON BOTANICAL NOMENCLATURE IN THE GENUS *Albizia*

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INTRODUCTION

The genus *Albizia* (family *Leguminosae*, sub-family *Mimosoideae*) contains 100–150 species[2]; although it is distinguishable botanically from the closely related genera *Inga* and *Acacia*, considerable confusion existed between these three genera in the past. Consequently, complex series of synonyms exist for many *Albizia* species (see APPENDIX). It is unfortunate that "*A.*" is used to abbreviate both "*Albizia*" and "*Acacia*", and it is important that confusion between these genera be avoided. Although the spelling "*Albizzia*" has been used consistently by chemical authors to date, the correct botanical form[2] is *Albizia*.

Although Adriaens[3] reported the exudation of gum by *Albizia fastigiata* and *Albizia gummifera*, a structural study of *Albizia zygia*[4] and a note on the composition of *Albizia glaberrima*[5], published simultaneously in September 1961, appear to have been the first significant studies of *Albizia* exudates. These contributions were followed by notes on the composition of the gums from *Albizia lebbeck*[6] and *Albizia procera*[7], and on the aldobiouronic acids in *A. procera*[8]. It is therefore clear that Drummond and Percival were incorrect in stating[4] "the genus *Albizia* contains some twenty-six species, of which only two, *A. zygia* and *A. sassa*, produce gum".

This paper primarily presents results from an analytical study of the composition of the exudate from *Albizia sericocephala* Benth., now known[2] to be an African subspecies of *Albizia amara* (Roxb.) Boiv., which occurs almost exclusively in Asia (see APPENDIX).

Comparison of our results with those reported previously for *A. zygia*[4], *A. glaberrima*[5], *A. lebbeck*[6], and *A. procera*[7] (see Tables I and II) indicated that some of the analytical data for *A. glaberrima*[5] were inconsistent with the broad characteristic features of the other species studied to date. Thus, Torto reported[5] *A. glaberrima* gum:

(*a*) to be insoluble;

(*b*) to have a methoxyl content of 3.5%: this value is unusually high–more than would be present, indeed, if *all* of its glucuronic acid (*ca.* 16%, by calculation from the neutralisation equivalent reported[5]) were present in the 4-*O*-methyl form;

(*c*) to contain galactose, arabinose, and rhamnose, in the ratio 3:1:2–allowing

for 16% of uronic acid, it follows that the gum contained *ca.* 28% of rhamnose, an unusually high value for a plant gum.

Re-investigation of the composition of *A. glaberrima* gum was therefore desirable, and, in Tables I and II, we compare our results for an authenticated specimen with those reported[5] by Torto.

EXPERIMENTAL AND RESULTS

Analytical methods

The methods used have been described[1], except that paper chromatography was carried out with the following solvent systems(v/v): (*a*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*b*) ethyl acetate–pyridine–water (10:4:3); (*c*) butan-1-ol–pyridine–water–benzene (5:3:3:1, top layer); (*d*) butanone–acetic acid–water, saturated with boric acid (9:1:1).

Origin of specimens

Gum from *Albizia sericocephala* (clean, pale-yellow nodules, closely similar to commercial grades of gum arabic (*Acacia senegal*) in appearance) was collected at El Obeid in February 1965 by the Gum Research Officer, Republic of the Sudan. *Albizia glaberrima* gum (a brown, semi-plastic mass) was collected, near Entebbe in June 1965, by the Conservator (Research) of Forests, Uganda.

Examination of the crude gums

Both specimens dissolved when shaken in cold water for 36 h. *A. sericocephala* gave a very viscous solution ($[\eta] = 92.6$; *cf. Acacia senegal*, $[\eta] = 15$–25). In contrast, *A. glaberrima* gave a very low viscosity, $[\eta] = 4.7$.

Table I presents the results of other analyses and gives comparisons with the corresponding data available for *A. zygia*[4], *A. lebbeck*[6], and *A. procera*[7].

TABLE I

ANALYTICAL DATA FOR CRUDE *Albizia* GUMS

	A. sericocephala	*A. glaberrima*	*A. zygia*[4]	*A. lebbeck*[6]	*A. procera*[7]
Moisture, %[a]	11	27	17	11	12
Ash, %	4.4	5.7	5.8	4.6	5.7
Limiting flow-time number[b]	92.6	4.7	n.d[c]	n.d	n.d
Nitrogen, %	0.74	0.50	0[d]	0.26	0.2
Methoxyl, %	0.69	0.83	ca. 1	n.d	n.d
Uronic anhydride, %	17.4	20.6	n.d	n.d	n.d
Solubility	Complete in water	Complete in water	Partial in N NaOH	Complete in 2% NaOH	Complete in water

[a]Other analyses are corrected for these values. [b]In 4% saline at 25.0°. [c]n.d, not determined. [d]Our reference specimen of *A. zygia* gives N, 0.89%.

TABLE II

THE COMPOSITION OF PURIFIED *Albizia* GUMS

	A. sericocephala	*A. glaberrima*	*A. glaberrima*[5]	*A. zygia*[4]		*A. lebbeck*[6]	*A. procera*[7]
Method of Purification[a]	e	e	p		p	p	p
Yield, %	70	67	60	58[b]	16[c]	55	65
Ash, %	nil	nil	nil	0.25	0.22	n.d[d]	trace
Nitrogen, %	0.57	0.39	nil	n.d	n.d	n.d	n.d
Methoxyl, %	0.65	0.58	3.5	1.3	n.d	n.d	1.5
Neutralisation equivalent,	840	896	1100	723	n.d	1500	n.d
Hence, uronic anhydride, %[e]	21	20	16	24	n.d	12	n.d
Uronic anhydride, % (decarboxylation)	18	22	n.d	23.5	32.6	n.d	11.1
Specific rotation (water)	$[\alpha]_D^{17}$ −12° (*c* 0.7)	$[\alpha]_D^{17}$ −11° (*c* 1.0)	$[\alpha]_D^{17}$ −12° (*c* 0.3, NaOH)	+21° (*c* 0.49)	+38.7° (*c* 0.32, 0.1N NaOH)	n.d	$[\alpha]_D^{30}$ +15° (*c* 0.2)
Limiting flow-time No.[f]	57.5	4.7	n.d	n.d	n.d	n.d	n.d
Sugar composition[g]			calc.	calc.	calc.	i	calc. Ref. 7 calc. Ref. 8
Galactose, %	30	34	42	27	40	+	47 48
Mannose, %	10	6	trace	10	13		trace trace
Arabinose, %	30	30	14	40	16	+	31 32
Rhamnose, %	12	8	28	trace	—	+	11 8
Glucuronic acid, %	14	19	16	15	21		11 11
4-*O*-Methylglucuronic acid, %	4	3		8	11		

[a] e, electrodialysis; p, precipitation by alcohol.
[b] Material extracted by cold, dilute alkali.
[c] Material extracted by hot water.
[d] n.d, not determined.
[e] Assuming all titratable acidity arises from uronic acid groups.
[f] In 4% saline at 25°.
[g] Calc., calculated from sugar ratios reported; i, insufficient information available for calculation.

Purification

The crude gums were each shaken for 36 h with sufficient cold water to give 5% (w/v) solutions. After filtration through several layers of fine muslin, followed by filtrations through acid-hardened paper, the gum solutions were electrodialysed at 330 volts, in a grease-free, perspex cell fitted with cooling coils, until current ceased to flow.

During the electrodialysis of *A. glaberrima*, a fine, brown precipitate was deposited; this material (yield, 4%) was proteinaceous (N, 2.06%) and has not yet been investigated.

The free gum-acids were isolated by freeze-drying. *A. sericocephala* gave a white product (yield, 70%), and *A. glaberrima* a pale-brown product (yield, 67%).

Analysis of the purified gums

Table II presents the results obtained for *A. sericocephala* and *A. glaberrima*, and compares these with the data reported for *A. glaberrima*[5], *A. zygia*[4], *A. lebbeck*[6], and *A. procera*[7].

Examination of aldobiouronic acids in A. sericocephala *and* A. glaberrima

Samples were partially hydrolysed (N sulphuric acid, 100°, 8 h), neutralised (barium carbonate), filtered, de-ionised [Amberlite IR-120 (H^+ form) resin)], and concentrated at 35°. Comparison of the partial hydrolysates on the same chromatogram [Whatman No. 1 paper, solvent (*a*)] revealed that each gum contained three aldobiouronic acids, having the mobilities R_{Gal} = 0.21, 0.42, and 0.58. A mixed-indicator spray[9], specific for the detection of acidic saccharides, was used to locate the separated components after the chromatogram had been dried in a current of air to remove all traces of the acidic solvent. The acid having R_{Gal} = 0.42 was present in trace amount only in each gum, and its identity has not been investigated.

For each gum, the aldobiouronic acids having R_{Gal} = 0.21 and 0.58 were separated [Whatman 3MM paper, solvent (*a*)], located (by spraying side strips), and isolated by elution from the paper. The aldobiouronic acids from each gum were hydrolysed (2N sulphuric acid, 100°, 6 h), neutralised (barium carbonate), filtered, de-ionised [IR-120 (H^+ form) resin], concentrated at 35°, and examined in solvents (*a*), (*b*), and (*c*). The aldobiouronic acid having R_{Gal} = 0.21 gave glucuronic acid, glucurone, and galactose; the acid having R_{Gal} = 0.58 gave 4-*O*-methylglucuronic acid and galactose. The faster-moving acid was then shown to be identical chromatographically in solvent (*a*) with 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, and distinct from 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose. The slower-moving aldobiouronic acid was chromatographically identical with 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose.

DISCUSSION

The exudates from *A. lebbeck*[6] and *A. procera*[7] are used as substitutes for,

and adulterants of, gum arabic (*Acacia senegal*). *A. sericocephala* is found in those parts of the Sudan at which gum cultivation is concentrated; its exudate is so similar in appearance to commercial grades of *A. senegal* that admixture of the two gums would be extremely difficult to detect. Studies on any commercial sample of gum must always be treated with reserve.

A. sericocephala is not normally tapped; our specimen originated from natural exudation. Its exceptional viscosity, in comparison with the *Acacia* gums, makes it of possible commercial interest. We hope to secure, for study, a specimen of the gum exuded by *A. sericocephala* in response to tapping.

Although both specimens of *A. glaberrima* have the same rotation, there are considerable differences between some of the other results found by Torto[5] and by ourselves. Despite a careful check on our results, the differences remain. It is difficult to find an explanation for this, although *A. glaberrima* is now known (see APPENDIX) to exist in different varieties, and the two specimens involved originated from widely separated parts of Africa. Torto's specimen was obtained from trees heavily infested with moth larvae, and this may be significant; in view of the complete solubility of other *Albizia* species, it is interesting that both Torto's specimen and the other species originating from Ghana, *A. zygia*, were virtually insoluble in water.

The rhamnose content reported by Torto[5] is three times our value, and does not align with the trend set by the other species studied to date. The same comment applies to Torto's methoxyl content of 3.5%, which is so high, in relation to the uronic acid content of *A. glaberrima*, as to suggest that this species has some structural feature which is not typical of the other *Albizia* species studied so far. We found no support for this in our analysis, and we suggest that Torto's high value may be due to solvent retention[10] of the methanol used to effect purification by precipitation. In order to detect such possible artifacts, we have always considered it essential[11,12] to report a limited number of exploratory analyses on any gum prior to attempting its purification.

A. procera has now been reported[8] to contain the same two aldobiouronic acids identified in *A. zygia*[4] gum. Although *A. zygia* gum was fractionated, *A. procera* gum is claimed to be a homogeneous polysaccharide. We have confirmed Torto's report[5] that *A. glaberrima* contains residues of three aldobiouronic acids; *A. sericocephala* appears to contain the same three acids, and chromatographic evidence indicates that the two major aldobiouronic acids in *A. sericocephala* and *A. glaberrima* are different from those in *A. zygia* and *A. procera*.

The *Albizia* exudates therefore have a number of interesting features, although two of the distinctions made by Drummond and Percival[4] are no longer correct; rhamnose is not a major constituent[13] in all *Acacia* gums, and the presence of two uronic acids is not an unusual feature[14] in the *Mimosoideae* family. Structural investigations on *A. sericocephala* will be carried out, and analytical studies of further *Albizia* species are required to give a broader view of the characteristics of this genus.

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SUMMARY

The compositions of the gum exudates from *Albizia sericocephala* and *Albizia glaberrima* have been investigated. The results of analyses are compared with data available for the other *Albizia* species studied previously. The main aldobiouronic acids present in *A. sericocephala* and *A. glaberrima* differ from those found in *A. zygia* and *A. procera*. The analytical results obtained for *A. glaberrima* differ in a number of respects from those reported previously for this species by Torto.

An APPENDIX lists several *Albizia* species for which a number of botanical synonyms exist, often reflecting earlier confusion with the genus *Acacia*.

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ACID-CATALYZED HYDROLYSIS OF MALTOSE AND SELECTED MALTOSE DERIVATIVES*

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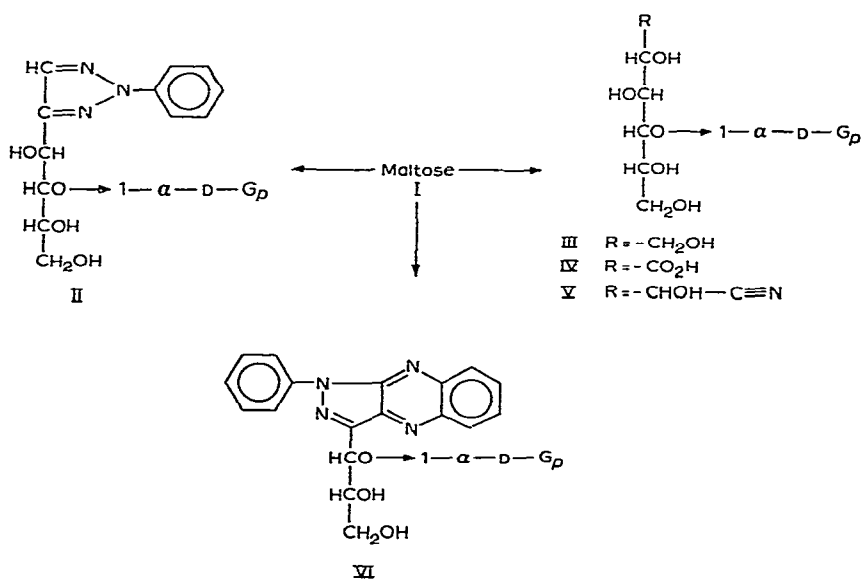
INTRODUCTION

Many investigations have been reported of the acid-catalyzed hydrolysis of glycosides, oligosaccharides, and polysaccharides, and various inductive and steric explanations of the results have been given[1]. The literature is, however, almost devoid of examples of the hydrolysis of modified oligosaccharides, that is, of glycosides in which the aglycon is a sugar derivative.

Rogovin and co-workers[2] reported that cellobionic acid is hydrolyzed slightly faster than cellobiose at 45° but slightly slower at 60° and 75°. Jones and co-workers[3] reported that the rate constants for acid hydrolysis were in the order maltose < maltobionic acid < maltitol, and isomaltitol < isomaltose. Again the individual differences in rate were very small.



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We have examined the rates of hydrolysis of maltose and selected maltose derivatives. The hydrolysis of each of the following compounds was effected at 80°, 60°, and 40°, in 0.998 N sulfuric acid: maltose (I), maltose phenylosotriazole (II) [4-(2-*O*-α-D-glucopyranosyl-D-*arabino*-tetrahydroxybutyl)-2-phenyl-2*H*-1,2,3-triazole; 4-*O*-α-D-glucopyranosyl-D-*arabino*-hexulose phenylosotriazole], maltitol (III) (4-*O*-α-D-glucopyranosyl-D-glucitol), maltobionic acid (IV) (4-*O*-α-D-glucopyranosyl-D-gluconic acid), maltose cyanohydrin (V) [5-*O*-α-D-glucopyranosyl-D-*glycero*-D-*gulo*(and D-*ido*)-heptononitrile], and maltose 1-phenylflavazole (VI) [3-(1-*O*-α-D-glucopyranosyl-D-*erythro*-trihydroxypropyl)-1-phenylpyrazolo[3,4-*b*]quinoxaline].

The objective of preparing these derivatives was to introduce electron-withdrawing groups. The latter have, in general, been found to facilitate hydrolysis, probably by facilitating the formation of the glucosyl carbonium ion which is a necessary step in the hydrolysis mechanism. In the 1-phenylflavazole derivative (VI) the functional group is adjacent to the glucosidic bond; in the phenylosotriazole derivative (II) the functional group is removed by one carbon atom from the glucosidic bond; in maltitol (III) and maltobionic acid (IV) the groups in question are separated from the glucosidic bond by two carbon atoms, and in the cyanohydrin (V) the nitrile group is separated by three carbon atoms.

EXPERIMENTAL

Maltose (I)

Maltose was purified by peracetylation, recrystallization of the octaacetate, and deacetylation[4]. Homogeneity of the preparation was established by paper chromatography.

Maltitol (III)

Pure maltose was reduced in aqueous solution with sodium borohydride[5]. Crude maltitol was purified by peracetylation, recrystallization of the nonaacetate, and deacetylation; m.p. of nonaacetate 83–85°.

Maltose phenylosotriazole (II)

Maltose phenylosazone was prepared by a standard procedure[6]; yield 42%, m.p. 201–203°. A suspension of maltose phenylosazone (10 g) and copper(II) sulfate pentahydrate (6 g) in a solution of water (730 ml) and isopropyl alcohol (470 ml) was heated for 30 min at reflux. The solution was allowed to cool to room temperature, and a saturated solution of barium hydroxide was added until barium sulfate and copper hydroxide were no longer precipitated. Carbon dioxide was added until formation of a precipitate of barium carbonate was no longer evidenced. The small proportion of copper ion remaining in solution was then removed by the addition of hydrogen sulfide. The solution was evaporated under reduced pressure to a thick sirup. The product was purified by descending paper chromatography on Whatman No. 3MM paper using an irrigant of 10:1:1 v/v isopropyl alcohol–conc. ammonium hydroxide–

water. The maltose phenylosotriazole band was located by its fluorescence, and the eluted product was obtained after drying as a thick sirup.

Maltose cyanohydrin (V)

The procedure used to prepare cyanohydrin derivatives of monosaccharides was applied to maltose[7,8]. Pure maltose (4 g) was dissolved in water (50 ml), and the solution was cooled in an ice bath. To the cold solution was added sodium cyanide (4 g) in 30 ml of 0° water. The reaction flask was stoppered and kept for 10 days at a temperature of 0–5° to ensure complete reaction. (Fehling test was negative). The solution was diluted and stirred several hours at 0° in a hood with 86 ml of Amberlite IR-120 (H^+) ion-exchange resin. The solution was filtered, transferred to a flash evaporator, and evaporated at room temperature to a thick sirup. The sirup was covered with methanol and allowed to stand several days before the methanol solution was decanted. Addition of acetone to the methanol solution precipitated the cyanohydrin as an amorphous, hygroscopic powder. Purity of the product was established by the absence of maltose when examined by paper chromatography. The preparation was used as obtained, without separation of the two epimers.

Lithium maltobionate

Lithium maltobionate was prepared by previously described procedures[9,10].

Maltose 1-phenylflavazole (VI)

Maltose 1-phenylflavazole was prepared by the standard procedure[11]; yield 33%, m.p. 263–265°.

Investigation of hydrolysis rate

The following were prepared: 0.8% solutions of I, III, V, and lithium maltobionate in water, 0.8% solutions of II and maltose phenylosazone in ethylene glycol, and a 0.4% solution of VI in ethylene glycol. Rates of hydrolysis were determined at 80°, 60°, and 40° after each of the above solutions had been mixed with an equal volume of 1.966 N sulfuric acid.

Hydrolyses were followed by observing the change in optical rotation as the reaction progressed, by means of a Bendix ETL-NPL Automatic Polarimeter equipped with a 546-mμ (mercury green line) interference filter. All optical rotations were determined at 80°.

Hydrolyses at 80° were effected in a water-jacketed polarimeter cell, and were allowed to proceed to completion. Hydrolyses at 60° and 40° were accomplished in constant-temperature baths. Aliquots were removed, and injected into the polarimeter cell jacketed at 80°. Optical rotations at the time of sampling were determined by extrapolation to the time of injection, but, since the time of temperature equilibration in the 1-cm cell was less than 1 min, and since very little or no change in rotation was observed during this period, only very small corrections, if any, were needed in most cases.

Reaction rate-constants were determined by least-squares analysis of the data by an IBM-1620 computer according to the first-order rate equation $k = [1/t \ln (R_0 - R_\infty)/(R_t - R_\infty)]$ in which R_0, R_t, and R_∞ are the specific optical rotations at the beginning, during, and after completion of hydrolysis, respectively.

The Arrhenius energy-of-activation constant, E_a, was calculated from the equation:

$$E_a = \log_{10} \frac{k_2}{k_1} \cdot \frac{2.303RT_1T_2}{T_2 - T_1}$$

Methanolysis of maltose 1-phenylflavazole (VI)

Substance VI was refluxed for 100 h in methanol made N with respect to sulfuric acid. The reaction mixture was cooled to room temperature, diluted with methanol, neutralized with barium carbonate, and filtered. The residue was washed with methanol and the combined filtrate and washings were evaporated to dryness at 50° under reduced pressure. The residue was extracted with warm water to remove methyl D-glucosides, and the water-insoluble residue was crystallized and recrystallized from methanol–propyl alcohol; m.p. 215°. D-Glucose 1-phenylflavazole (m.p. 218°) was subjected to the same procedure; m.p. 215° after treatment with acidic methanol. The two products each consumed 2.2 ± 0.3 mole of periodate per mole (triplicate) after 24 h of oxidation in pH 4.0 acetate buffer, with a further slow consumption.

Unbuffered periodate oxidation[13] of D-glucose 1-phenylflavazole (both before and after refluxing with acidic methanol) and of the flavazole product from the methanolysis of maltose 1-phenylflavazole, yielded in each case 3-formyl-1-phenylpyrazolo-[3,4-*b*]quinoxaline (1-phenylflavazolaldehyde); yield 88–95%; m.p. 144–147°, reported 144°. (Ref. 14).

RESULTS AND DISCUSSION

Maltitol (III), maltobionic acid (IV), maltose cyanohydrin (V), and maltose 1-phenylflavazole (VI) were prepared by standard procedures. Maltose phenylosotriazole (II) was isolated by a new procedure. The standard method[16–18] involves heating the phenylosazone with copper(II) sulfate followed by removal of excess copper(II) ion with hydrogen sulfide, a procedure which generates sulfuric acid. Although the solution was kept cold and the acid was neutralized as soon as possible with barium carbonate, some hydrolysis of the disaccharide phenylosotriazole occurred. The new procedure employed barium hydroxide to precipitate sulfate and copper(II) ions and carbon dioxide to precipitate excess barium ions, and the solution was thus kept neutral or basic during the isolation.

Rate constants, as provided by least-squares analyses, are plotted in Figures 1, 2, and 3. Figure 1 gives the first-order rates for hydrolysis at 80°. Here the 1-phenylflavazole (VI) undergoes hydrolysis more slowly than the other compounds at 80°. Figure 2 gives the hydrolysis rates at 60°, and indicates that the order has changed somewhat. Figure 3 gives the hydrolysis rates at 40°. Here it is evident that the

1-phenylflavazole (VI) undergoes hydrolysis much faster than the other compounds [illegible].

Nordin and French[12] previously reported that the α-D-(1→4) bond adjacent to the flavazole unit of 1-phenylflavazoles of maltose, maltotriose, and starch dextrins is apparently more resistant to acid hydrolysis at 100° than other α-D-(1→4) bonds

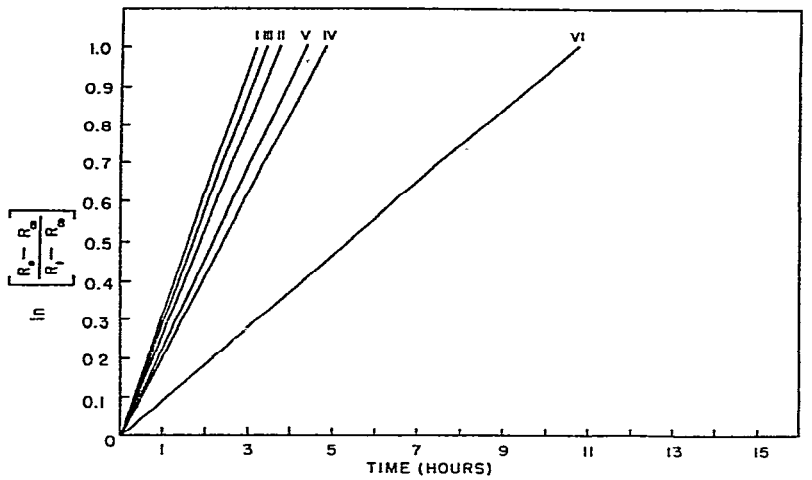

Fig. 1. Hydrolysis rate curves at 80°.

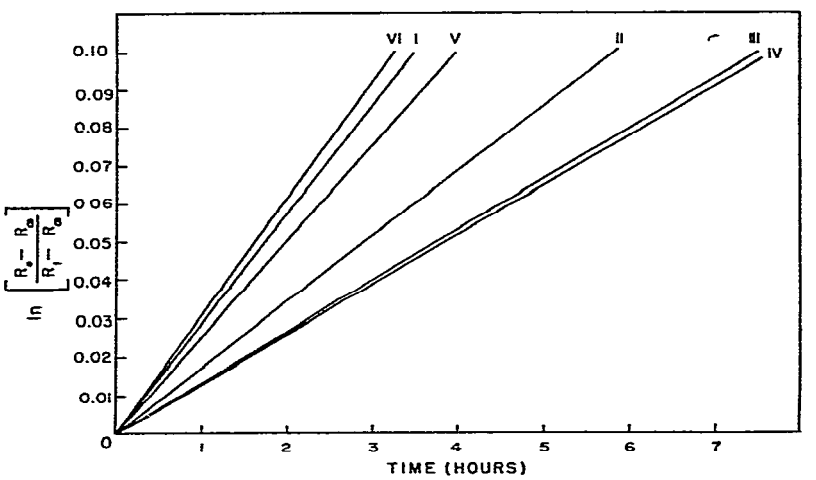

Fig. 2. Hydrolysis rate curves at 60°.

and that acid hydrolytic conditions do not destroy 1-phenylflavazoles[12]. Here also, the hydrolysis products from maltose 1-phenylflavazole were shown to be D-glucose 1-phenylflavazole and D-glucose. This proves that the rotational change is not "anomalous".

The rate of hydrolysis is plotted against temperature in Fig. 4. The data have

again been fitted to a straight line, on the apparently valid assumption that the activation energies are independent of temperature over the range investigated. The lines given by maltose (I), maltose phenylosotriazole (II), maltitol (III), maltobionic acid (IV), and maltose cyanohydrin (V) all give molar activation-energies in the range 30.0–33.0 kcal.mole^{-1}, typical for glycosides (Table I). Maltose 1-phenylflavazole (VI) has a quite different molar activation-energy, in this case 13.2 kcal.mole^{-1}.

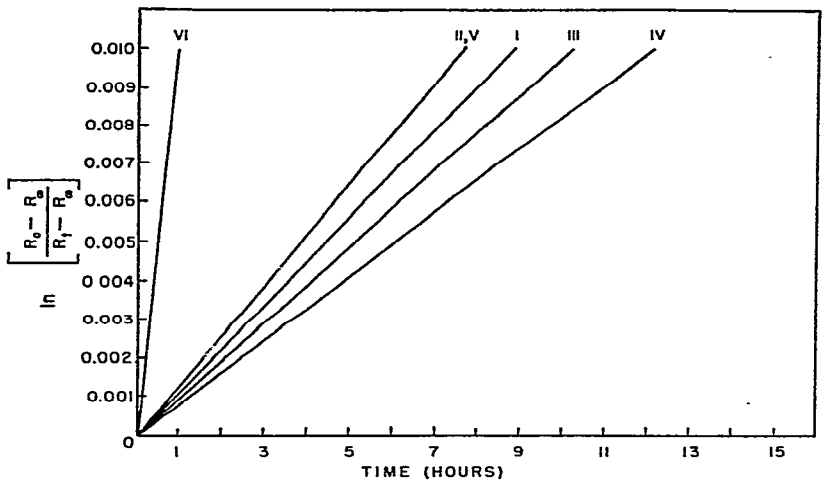

Fig. 3. Hydrolysis rate curves at 40°.

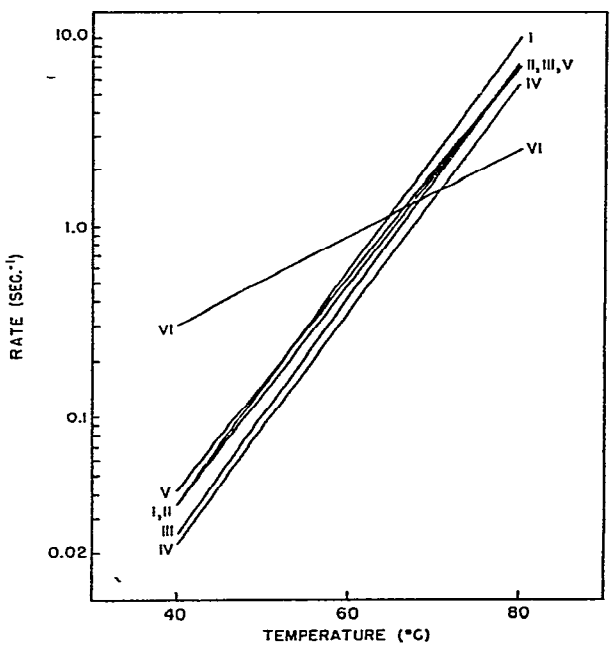

Fig. 4. Rate *versus* temperature curves.

The 1-phenylflavazole also has a strikingly different entropy of activation. Large negative entropies of activation are normally found for A-2 reactions involving attack by solvent on the protonated intermediate[19]. Negative $\Delta S^{\neq}$ values in A-1 reactions have been attributed either to high solvation of the conjugate acids or transition states, or restriction of rotation about the breaking bond in the transition state[20], but these have been found to be small negative values in the cases examined[21]. It is also possible that VI would undergo hydrolysis as does *tert*-butyl β-D-glucopyranoside, with alkyl carbon–glycosidic oxygen fission[22], but this has been disproved by methanolysis of VI. Under methanolysis conditions, the 1-phenylflavazole VI would give D-glucose 1-phenylflavazole if the usual glycosyl carbonium ion is formed, and 4-*O*-methyl-D-glucose 1-phenylflavazole if the aglycon carbonium ion is formed. The methanolysis product has been identified as D-glucose 1-phenylflavazole, since periodate oxidation data eliminated 4-*O*-methyl-D-glucose 1-phenylflavazole as a product. Furthermore, the acid hydrolysis of *tert*-butyl and 1,1-diethylpropyl β-D-glucopyranosides gives $\Delta S^{\neq}$ values of +11 to 17 (Ref. 23).

An A-2 mechanism for the hydrolysis of certain D-glucopyranosides was proposed by Bunnett[24] on the basis of an empirical mechanistic criterion, rather than on entropy differences, which did not differ from the usual values. His conclusion has been criticized on the basis of the apparent generality of the A-1 mechanism for hydrolysis[19] of acetals. An A-2 mechanism has also been suggested for the hydrolysis of ethyl β-D-galactofuranoside by Overend and co-workers[25] on the basis of its lower $\Delta S^{\neq}$ (−7.1 cal.mole^{-1}degree^{-1}). Its molar energy of activation was also lower (22.7 kcal. mole^{-1}), but neither of these values approaches those found for the 1-phenylflavazole VI. The markedly different entropy of activation found for the acid-catalyzed hydrolysis of VI is certainly indicative of a process other than the A-1 mechanism, but we have no evidence what that mechanism might be or why this compound should undergo hydrolysis by a different mechanism.

A possible mechanism for the hydrolysis of VI would involve intramolecular catalysis after protonation of the heterocyclic ring. That protonated forms might be important is suggested by the fact that the initial rotation in water is quite different from that in 0.998 N sulfuric acid at 80°. If this is true, the calculated thermodynamic values would be the sum of those for protonation and hydrolysis. This possibility is being further investigated.

The fact that conversion into the flavazole derivative does appreciably affect the hydrolysis rate of the glycosidic bond confirms many of the results already reported, which indicate that the electronic character of the aglycon can influence the hydrolysis rate by affecting the electron density around the glycosidic oxygen atom[1]. The fact that the flavazole showed the most significant change in hydrolysis rate also confirms the results of Timell[26], who reported that substituent groups at a distance of more than one carbon atom from the glycosidic bond had little effect on the hydrolysis rate. However, as pointed out above, it remains to be established whether the observed effect is one of induction or intramolecular catalysis.

Least-squares analysis, as obtained by the computer, provided the equation

for the corrected plot of ln $(R_0-R_\infty)/(R_t-R_\infty)$ *versus* time. By using the slope obtained, k (Table I), the corrected rate-curves were constructed for each of the three temperatures used and appear in the Figures 1–3. The curves are constructed to pass through the origin, although the actual equations obtained from the computer

TABLE I

HYDROLYTIC RATE DATA[a]

Compound	Rate, $k \times 10^5$ ($sec.^{-1}$)			E_a ($kcal.mole^{-1}$)	$\Delta H^{\neq}$ ($kcal.mole^{-1}$)	$\Delta S^{\neq}$ ($cal.mole^{-1}degree^{-1}$)
	40°	60°	80°			
I	0.0361	0.600	10.2	33.1	32.4	+ 14.7
II	0.0361	0.506	7.28	31.2	30.5	+ 8.6
III	0.0250	0.439	7.55	33.2	32.5	+ 14.5
IV	0.0222	0.369	6.00	32.6	31.9	+ 12.1
V	0.0417	0.550	7.34	30.3	29.6	+ 6.2
VI	0.308	0.892	2.58	12.4	11.7	− 46.7

[a]In 0.998 N sulfuric acid.

contained a y intercept. This deviation is attributed to errors in the determination of initial specific optical rotation, which was determined by extrapolation of a plot of rotation *versus* time, to zero time for each compound. Since this method involved extrapolation of the most rapidly changing portion of the curve, it was deemed necessary to assess the accuracy of the values used. One method used involved mixing the stock solutions of the derivatives with equal volumes of water instead of 1.996 N sulfuric acid, so that rotations of the neutral solutions could be determined at 80° without hydrolysis. The resultant values, R_{0,H_2O}, are recorded in Table II along

TABLE II

INITIAL ROTATION VALUES

Substance	$[\alpha]_{546}^{80}$			
	$R_{0,\,extrap.}$	$R_{0,\,calc.}$	Difference	R_{0,H_2O}
I	156°	154.5°	1.5°	161°
II	44.5°	45°	0.5°	50°
III	122°	128°	6°	127°
IV	92°	91°	1°	102° (Li-salt)
V	108°	92°	16°	116°
VI	152°	151°	1°	77.5°

with the values obtained by extrapolation, $R_{0,extrap}$. Since the equations for the rate curve obtained by the computer contained a y intercept term, indicating an error in the R_0 value, a corrected R_0 was calculated from the least-square equation by using a y intercept of zero. These values also appear in Table II as $R_{0,calc.}$. In most cases

there seems to be good correlation between the initial rotations as determined by the three methods. In addition, rate constants determined by the Guggenheim method, which is independent of R_9, agree in those cases which could be checked with the constants determined by the computer.

The computer also provided the correlation coefficients for each curve (Table III). They indicate that a high degree of agreement exists between the points as determined experimentally and the resultant least-square plot of the data.

TABLE III

CORRELATION COEFFICIENTS

Compound	*Temperature*		
	80°	*60°*	*40°*
I	0.9973	0.9618	0.8756
II	0.9981	0.9948	0.8869
III	0.9950	0.9533	0.9331
IV	0.9980	0.9119	0.8514
V	0.9944	0.9851	0.9861
VI	0.9936	0.9990	0.9685

Rates at temperatures below 40° were investigated but were found to be too low to be measured successfully by this method. Hydrolysis of the phenylosazone was also investigated. Very poor results were obtained, probably because of side reactions giving the monoanhydro derivative, cyclic forms, the hexosulose, and the phenylhydrazone.

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The authors acknowledge the help of Mr. Dwight Fitzgerald who conducted the methanolysis experiment, and the Corn Industries Research Foundation for a grant supporting this work.

SUMMARY

Acid-catalyzed hydrolysis of maltitol, maltose phenylosotriazole, maltose cyanohydrin, and maltobionic acid differ very little from that of the parent compound, maltose, as determined by energies (30–33 kcal.mole^{-1}) and entropies (+6 to 15 cal.mole^{-1}degree^{-1}) of activation. The hydrolysis of maltose 1-phenylflavazole, however, had a much lower molar activation energy (13.2 kcal.mole^{-1}) and a much more negative entropy of activation (−46.7 cal.mole^{-1}degree^{-1}). It is suggested that these differences are indicative of a different reaction mechanism for the hydrolysis of the latter compound.

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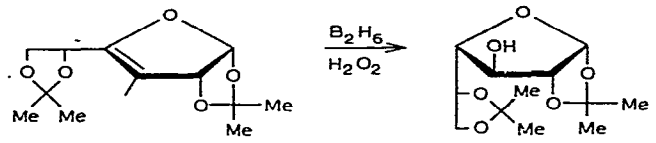
Notes

Umwandlung von 1,2:5,6-Di-*O*-isopropyliden-3-desoxy-α-D-glucose-3-en in 1,2:5,6-Di-*O*-isopropyliden-α-D-galactofuranose durch selektive Hydroborierung

Die Hydroborierung von Olefinen nach H. C. Brown[1] erfolgt stets unter *cis*-Addition entgegen der Markownikoff-Regel. Die oxydative Spaltung der dabei gebildeten Diboran-Addukte verläuft immer unter Retention zu den entsprechenden Alkoholen. Das Verfahren stellt somit eine Methode dar, in Olefinzucker selektiv Hydroxylgruppen einzuführen. Die leicht zugängliche 1,2:5,6-Di-*O*-isopropyliden-3 desoxy-α-D-glucose-3-en(I)[2] reagiert glatt mit Diboran unter *cis*-Addition an die Enol-Doppelbindung, wobei der Boranrest am C-3 eintritt.



I II

Von den beiden möglichen Reaktionsprodukten ensteht nur das, bei dem die Addition von oben von der ungehinderten Seite des Furanoseringes erfolgt, wobei Derivate der *Galacto*-Konfiguration entstehen. Die Reaktion entspricht der katalytischen Hydrierung von I, die 3-Desoxy-D-*Galacto*-Verbindungen liefert[2]. Der Angriff von unten ist durch den anellierten Fünfring der Isopropyliden-Acetal-Gruppe in I stark sterisch gehindert. 1,2:5,6-Di-*O*-isopropyliden-α-D-glucofuranose mit gleichem Ringsystem zeigt eine entsprechende sterische Hinderung, denn am C-3 ist keine katalytische Oxydation[3] und keine nucleophile Substitution mit Azid durchführbar[4]. Die oxydative Spaltung des Diboran-Adduktes von I liefert kristallisierte 1,2:5,6-Di-*O*-isopropyliden-α-D-galactofuranose (II). Die Reaktion erlaubt somit eine Umwandlung von Glucofuranose-Derivaten in Galactofuranose-Derivate, welche auf anderem Wege nur schwierig zugänglich sind. Lehmann[5] hat gefunden, dass die gleiche Umsetzung zur Darstellung von am C-4 mit Tritium markierter Galactose benutzt werden kann.

EXPERIMENTELLER TEIL

1,2:5,6-Di-O-isopropyliden-α-D-galactofuranose (II). Das nach Zinner[2] dargestellte Zuckerolefin (I) musste zur Reinigung in Petroläther gelöst und die Lösung dreimal mit Wasser ausgeschüttelt werden. 3 g gereinigtes I wurde in 9 ml abs. Tetrahy-

drofuran gelöst und in die Lösung 2–3 Stdn. aus einem Diboran-Generator Diboran (Trägergas: getrockneter Stickstoff) eingeleitet. Diboran wurde aus 5.1 g BF_3-Ätherat in 36 ml Diglyme durch Eintropfen von 25 mMol $NaBH_4$ in 20 ml Diglyme entwickelt. Anschliessend wurde überschüssiges Diboran mit Wasser-Tetrahydrofuran zersetzt und unter Kühlung 9 ml 2N NaOH und 4 ml H_2O_2 (30%) zugefügt. Die Lösung wurde i. Vak. zur Trockne eingeengt, der Rückstand in 25 ml Wasser aufgenommen und viermal mit 25 ml Äther extrahiert. Nach Einengen des Äthers verbleibt 1.8 g chromatographisch reiner Sirup, welcher beim Stehen kristallisierte. Umkristallisation aus Cyclohexan gibt 0.8 g (25%) II, Smp.[6] 97.5°–98°, $[\alpha]_D^{20}$ −35.3° (*c* 0.8, Methanol). Dünnschichtchromatographie im Laufmittel Benzol: Äthanol (3:1) + 3% Wasser.

Anal. Ber. für $C_{12}H_{20}O_6$: C 55.39 H 7.69. Gef.: C 55.19 H 7.80.

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(Eingegangen den 15. November, 1965)

Carbohydrate Res., 2 (1966) 80-81

On the association–dissociation of submaxillary mucin

Weight-average molecular weights between 4×10^6 and 8×10^6 have been reported for submaxillary mucins[1,2]. The prevalent concept of the mucin structure is that the protein occupies the central core of the molecule to which are attached short, carbohydrate side-chains[3,4]. Since the protein content of mucins is in the range from 37% [for bovine submaxillary mucin[5] (BSM)] to 48% (for porcine submaxillary mucin[2]), this would give a molecular weight of 1.5×10^6 to 4×10^6 for the protein core.

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Proteins of very high molecular weight have been found to be aggregates of subunits, and most of the evidence supports the belief that single polypeptide chains having a molecular weight of greater than 6.6×10^4 do not exist[6]. Urinary muco-proteins (mol. wt., 7×10^6) dissociate in urea to small subunits having molecular weights of 1.8×10^5 or less[7,8].

Although BSM is polydisperse and contains[1] a fraction having a molecular weight of 2×10^5, there remains the question of whether the fraction having a molecular weight of millions can be dissociated into smaller fragments by urea. To answer this, a BSM preparation[1,2,5] was chromatographed on Sephadex G-200 (Pharmacia Lot No To-6471; particle size, 40–120 μ; water regain, 20 ± 2 g) in aqueous 0.2M sodium chloride and in 7M urea containing 0.2M sodium chloride. The length of the column was 55.5 cm and its volume was 201 ml.

A 3.2-ml sample of an approximately 1% solution of BSM was placed on the column equilibrated with 0.2M sodium chloride. The mucin was eluted with 0.2M sodium chloride at a flow rate of approximately 4 ml/h, and 2-ml aliquots of the eluted samples were treated with 2 ml of Ehrlich's reagent[9]; sialic acid was thereby used to indicate the presence of the mucin. A mixture of a 0.1-ml sample of the original BSM solution, 1.9 ml of water, and 2 ml of Ehrlich's reagent gave an optical density of 0.165 at 565 mμ. The chromatogram of BSM in 0.2M aqueous sodium chloride (Fig. 1a) clearly shows the polydispersity of the BSM, as reported previously[1]. In order to obtain the void volume of the column, 1 ml of a 1% solution of dextran having a molecular weight of 2×10^6 (Pharmacia, FDR 922) was run on the same column under identical conditions. The eluted dextran was reacted with anthrone[10], and the result is presented in Fig. 1a.

Similarly, 3 ml of BSM and 1 ml of dextran (1% solutions) were chromatographed on Sephadex G-200 in 7M urea and 0.2M sodium chloride. The results are given in Fig. 1b. The dextran gel swelled in urea and, therefore, less material was needed to fill the column. The flow rate could only be kept at approximately 0.5 ml/h. The detection, with Ehrlich's reagent, of the sialic acid-containing material in the eluate was done at 625 mμ (rather than at 565 mμ as in the aqueous solution) since the absorption maximum in urea solution occurs at this wavelength. Aliquots (2 ml) of the eluate were reacted with 2 ml of Ehrlich's reagent. A mixture of a 0.1-ml sample of the original BSM solution, 1.9 ml of urea solution, and 2 ml of Ehrlich's reagent gave an optical density of 0.102 at 625 mμ.

In order to determine the swelling of the gel in urea, the column (packed in urea) was washed with 0.2M sodium chloride, and the void volume was redetermined with dextran. It had increased from 78 to 91 ml, indicating a swelling of the gel grains in urea to 1.14 times their original volume in 0.2M sodium chloride.

The results of chromatography in the two media are relatively similar; the differences are a slight increase in elution volume for the mucin in urea compared to that in the aqueous medium and a more pronounced shoulder at an elution volume of 125 ml. The average distribution coefficient, K_{av}, between the gel phase and liquid phase of the BSM in aqueous medium was 0.19 and in 7M urea was 0.23. These figures

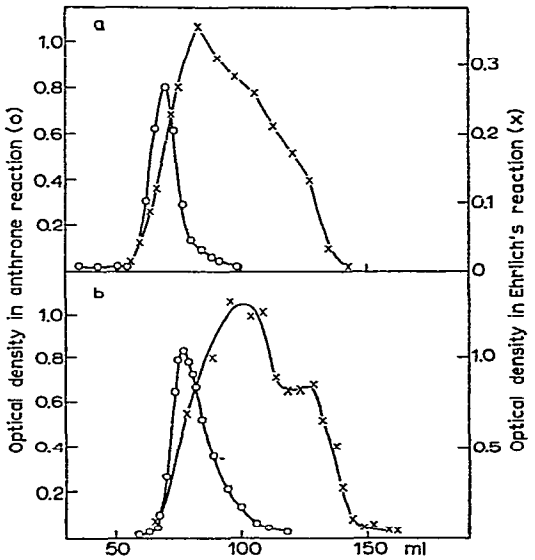

Fig. 1. Chromatography of BSM (×) and dextran (○) on Sephadex G-200 in 0.2M NaCl (a) and in 7M urea and 0.2M NaCl (b).

were calculated from the elution volumes (see ref. 11) when half of the material had been eluted. This slight increase in the capacity of the gel in urea for BSM is of the order expected from the degree of swelling of the gel and is thus not a sign of a change in the molecular parameters of BSM. Also, the more pronounced shoulder in Fig. 1b is explained by the change to a higher K_{av}-value when the chromatographic resolution increases.

It was thus not possible to show a dissociation of BSM into smaller subunits by urea, as can be done for urinary mucoproteins[7,8].

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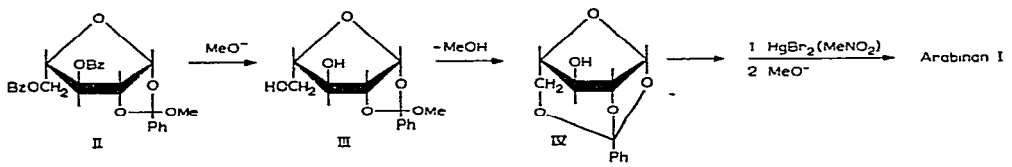
Preliminary communications

A new route to the synthesis of polysaccharides

Stereospecific synthesis of polysaccharides can serve as an important tool in chemical and biochemical investigations of these polymers. Syntheses already reported either do not give polymers of predicted structure[1,2], or are not general methods[3–5], or give rise only to oligomers [5,6]. We now report a new route to the synthesis of polysaccharides having predictable types of glycosidic linkage.

Sugar orthoesters, a new type of glycosylating reagent[7], are used as starting materials. The new route is illustrated by the synthesis of an arabinan (I) containing predominantly α-(1→5)-L-arabinofuranosidic linkages, obtained by the polymerisation of β-L-arabinofuranose 1,2,5-orthobenzoate (IV).



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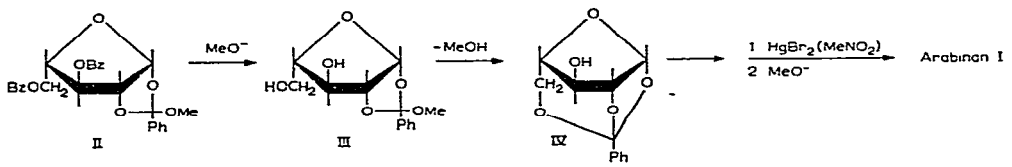

The orthoester (IV) was synthesized as follows. Syrupy β-L-arabinofuranose 1,2-(methyl orthobenzoate) 3,5-dibenzoate (II), $[\alpha]_D$ + 19° (chloroform), n_D^{37} 1.5610, was saponified to give β-L-arabinofuranose 1,2-(methyl orthobenzoate) (III), which reacted spontaneously to afford compound (IV), m.p. 148–149°, $[\alpha]_D$ + 30° (chloroform) (Found: C, 61.3; H, 5.2; active H, 0.42. $C_{12}H_{12}O_5$ calc.: C, 61.0; H, 5.1; active H, 0.42%). Other tricyclic monosaccharide-orthoesters of type (IV) are known[8,9].

The polymerisation of the orthoester (IV) was performed analogously to the glycosylation of alcohols using sugar orthoesters[7], *i.e.*, in nitromethane with catalytic amounts of mercuric bromide. Any residual orthoester-linkages present in the polymer were hydrolysed with 0.01N sulphuric acid in aqueous acetone (20°, 1 h). The resulting, partially benzoylated arabinan was saponified with sodium methoxide, and the crude polysaccharide was purified by gel-filtration on Sephadex G-25. Elution with water gave, in the first fractions, the arabinan (I, 50%) as a colourless solid, $[\alpha]_D$ −91° (water). Natural arabinan[10] with α-L-arabinofuranosidic linkages has $[\alpha]_D$ −114°, so that the optical rotation of the arabinan (I) indicates α-L-glycosidic bonds. The behaviour of the arabinan (I) on Sephadex G-25 and G-50 was consistent with polydispersity and indicated the average molecular weight to be *ca.* 4000-10000, in agreement with the value obtained by hypoiodite oxidation.

The arabinan (I) was completely hydrolysed by 0.1N sulphuric acid (100°, 3 h), arabinose being the only product. On periodate oxidation, the polymer consumed *ca.* 0.7 mol. of oxidant per anhydroarabinose unit. When the periodate-oxidised polymer was reduced with sodium borohydride and then hydrolysed by acid, *ca.* 30% of the arabinose units originally present survived. Thus, *ca.* 70% of the arabinose residues in the arabinan (I) are involved in 1→5 linkages.

The polymerisation of compound (IV) seems to proceed with splitting of one of the orthoester bonds, followed by glycosylation of the hydroxyl group thereby formed. The periodate-resistant units in the polymer may arise by glycosylation of the C-3 hydroxyl group resulting in the formation of 1→3 linkages. Obviously, the polymerisation of analogues of the orthoester (IV) having position 3 blocked should afford uniformly linked polysaccharides. In this connection, we are studying the polymerisation of the 3-*O*-benzoyl derivative of compound (IV).

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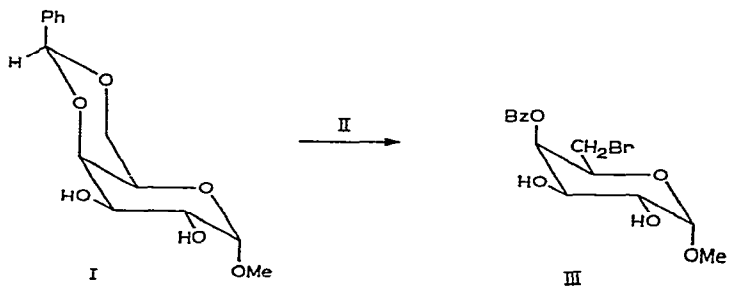
The reaction of *O*-benzylidene sugars with *N*-bromosuccinimide

I. Methyl 4,6-*O*-benzylidenehexopyranosides

This communication reports a novel ring-opening of benzylidene acetals of sugars under the influence of *N*-bromosuccinimide (II), which affords, in the case of the 4,6-*O*-benzylidene derivatives, the corresponding 6-bromo 4-benzoates in high yield.



I III

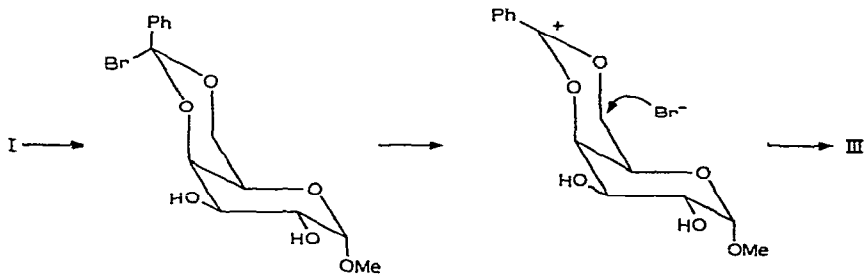
The reaction products are versatile intermediates for further synthetic work in the carbohydrate series since they possess the combined advantages of a good leaving-group at C-6 and a selectively blocked hydroxyl function at C-4. This facile introduction of a benzoate group at C-4, without any change of stereochemistry, could be advantageous in cases where this function is desired as a neighboring participant in conversions at C-3.

The reaction is performed by stirring for 2 hours at reflux temperature a solution of the *O*-benzylidene derivative (1 mole) and II (1.1 mole) in dry carbon tetrachloride containing excess barium carbonate. The products are isolated by extraction of the evaporated residues into ether or other suitable solvent, followed by conventional processing*. Thus, methyl 4,6-*O*-benzylidene-α-D-galactopyranoside[1] (I) afforded methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-galactopyranoside (III) as a colorless solid, $[\alpha]_D^{25}$ + 156° (*c* 0.56, methanol), in over 90% yield. The latter was converted by catalytic debenzoylation into the crystalline methyl 6-bromo-6-deoxy-α-D-

*The reaction products were investigated by t.l.c. (silica gel) by using the solvent system 20:1 chloroform–methanol, and were obtained in pure form by chromatography on silicic acid or by direct crystallization. All compounds reported herein gave correct analyses and had n.m.r. and i.r. spectra which were compatible with their structures. Melting points are uncorrected.

galactopyranoside, m.p. 174–175°; $[\alpha]_D^{26}$ +157° (*c* 0.5, water) in 82% yield, and ultimately into methyl 6-deoxy-α-D-galactopyranoside. The versatility of such intermediates as III was also demonstrated by conversion of III into crystalline methyl 6-azido-6-deoxy-α-D-galactopyranoside[2] by debenzoylation followed by treatment with sodium azide in *N,N*-dimethylformamide.

A probable mechanism* for the formation of III would involve initial attack of a free radical at the benzylic hydrogen atom to give the unstable bromoacetal (IV), which could collapse to the cyclic ion (V) and bromide ion. The reaction would then assume ionic character, and the more-susceptible, less-hindered C-6 would be attacked preferentially by bromide ion, to give the observed product**.



In order to investigate the influence of the stereochemistry at C-4, and hence the ring junction, on product distribution, the reaction was attempted with the D-*gluco* analog[5] of I. The major product was crystalline methyl 4-*O*-benzoyl-6-bromo-6-deoxy-α-D-glucopyranoside, m.p. 115–116°; $[\alpha]_D^{25}$ + 89° (*c* 0,52, methanol) which was converted into crystalline methyl 6-bromo-6-deoxy-α-D-glucopyranoside, m.p. 126–127°; $[\alpha]_D^{25}$ + 137° (*c* 0.54, methanol), and ultimately into methyl 6-deoxy-α-D-glucopyranoside, in good yield.

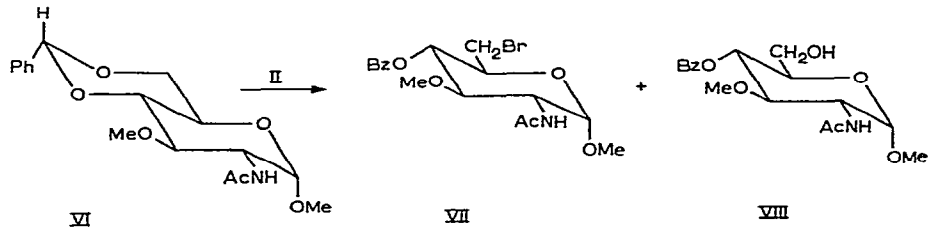
The applicability of this reaction to amino sugar derivatives was also investigated. When methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-methyl-α-D-glucopyranoside hydrate[6] (VI) (Calc. H_2O, 5.32; found, 4.97) was treated with II in tetrachloroethane at 85°, the only products formed, in approximately equal amounts, were methyl 2-acetamido-4-*O*-benzoyl-6-bromo-2,6-dideoxy-3-*O*-methyl-α-D-glucopyranoside (VII), $[\alpha]_D^{25}$ + 38° (*c* 0.523, chloroform)***, and crystalline methyl 2-acetamido-4-*O*-benzoyl-2-deoxy-3-*O*-methyl-α-D-glucopyranoside[7] (VIII), m.p. 155–157°; $[\alpha]_D^{25}$ + 23° (*c* 0.516, chloroform). The structure of VIII was proved by spectral

*The reaction of II with benzaldehyde diethyl acetal was first studied by Marvel and Joncich[3] who demonstrated the formation of ethyl benzoate. More recently, the reaction has been reported with *O*-benzylidene-1,2-cyclohexanediol[4].

**An overall free-radical mechanism, in which bromine radical is the attacking species, is also possible. The predominant attack on C-6, however, can best be rationalized in terms of an ionic termination process.

***This material crystallizes slowly from a mixture of acetone, ether, and pentane, but has a tendency to form a gel. The product, although chromatographically homogeneous and analytically pure, does not have a characteristic melting point, m.p. *ca.* 95°. It is soluble in ether.

data and by its conversion into the known debenzoylated product[6], m.p. 208–210°, and into the known 4,6-dibenzoate[7], m.p. 120–122°. The hydroxyl ion resulting from the water of hydration apparently competes with bromide ion in the attack on the intermediate cyclic ion, thus forming compound VIII*.



VI VII VIII

Many of the commonly used blocking groups in the carbohydrate series, such as azido, methoxyl, methylsulfonyl, and various ester functions, were found to be unaffected by the reaction conditions.

The reaction with II has been applied to other benzylidene acetals, such as those formed from secondary diols of furanosides, pyranosides, and acyclic systems, and those involving both cyclic and acyclic secondary hydroxyl groups (furanoses). In contrast to the 4,6-*O*-benzylidene derivatives, isomeric bromo benzoates are formed in most of these cases–a feature which broadens the scope of this reaction considerably. Results pertaining to these are to be reported.

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*When a sample of VI was dried for 2 h at 150° under vacuum, and subsequently treated with II, the preponderant product was VII. Compound VIII was still formed, presumably due to traces of moisture or incomplete drying.

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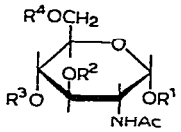
Mass spectrometry of hexosamines

A new approach to the identification of partially methylated monosaccharides[1], developed in this laboratory, was applied recently to different types of neutral monosaccharide[2,3]. The advantages of this method for investigation of polysaccharide structure prompted an examination of *N*-acetylhexosamines, which are components of mucopolysaccharides, glycopeptides, and other polymers of biological importance. Hence, we have studied the mass spectra of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-glucopyranoside (I) and its deuterated analogues, having CD_3 groups in the 3- (II), 4,6- (III), and 1,3,4,6-positions.



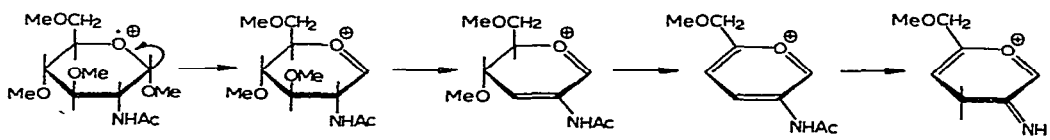
I $R^1 = R^2 = R^3 = R^4 = CH_3$

II $R^1 = R^3 = R^4 = CH_3$; $R^2 = CD_3$

III $R^1 = R^2 = CH_3$; $R^3 = R^4 = CD_3$

IV $R^1 = R^2 = R^3 = R^4 = CD_3$

Compounds II–IV were prepared from the corresponding, partially methylated *N*-acetyl-D-glucosamine derivatives by methylation with CD_3I, according to Kuhn's procedure[4]. The physical constants were in agreement with those reported[5] for compound (I). The mass spectra were measured by means of an MX-1303 mass spectrometer (temperature of inlet system, 200°; ionizing potential, 70 eV). The data* on compounds (I)–(IV), treated as described[2,3] previously, permitted establishment of the structures of the fragments and the main features of the fragmentation pattern, which must be the same for other *N*-acetylhexosamines, since stereochemical differences in the monosaccharide molecules do not change[2] the fragmentation pattern.



A_1 m/e 246 A_2 m/e 214 A_3 m/e 182 A'_3 m/e 140

*Full details of this investigation will be published elsewhere.

The fragmentation of compound (I) is, in principle, analogous to that of methyl tetra-*O*-methyl-α-D-glucopyranoside[3], resulting in appearance of most of the series of peaks[2] observed in the mass spectra of the neutral monosaccharides.

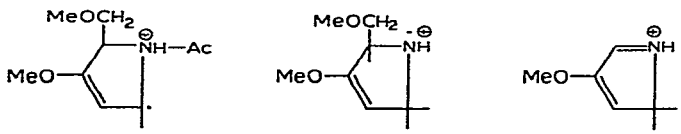
A-series. The ions having m/e 246 (A_1), 214 (A_2), 182 (A_3), and 140 (A_3') belong to this series. The last ion arises by loss of ketene from the A_3-ion. Such a process is characteristic for *N*- and *O*-acetylated compounds[6].

B-series. This consists of two ions, B_1(m/e 203) and B_2(m/e 172); B_2 arises by loss of $CH_3O^{\bullet}$ from B_1:

B_1, m/e 203 B_2, m/e 172

The B_1 peak (m/e 203) is more intensive than A_2 (m/e 214), so that the rule established for permethylated methyl hexopyranosides[7] is invalid in the case of methyl hexosaminides.

C-series. The formation of the parent ion [C_2 (m/e 185)] of this series from amino sugars differs from that of neutral monosaccharides. In the former case, methanol is lost instead of a $CH_3O^{\bullet}$-radical. This process is followed by the expulsion of ketene and then of a $^{\bullet}CH_2OCH_3$-radical. Such processes were not observed for neutral methyl hexopyranosides. These phenomena may be connected with the greater stability of the ammonium ion as compared with the oxonium ion.



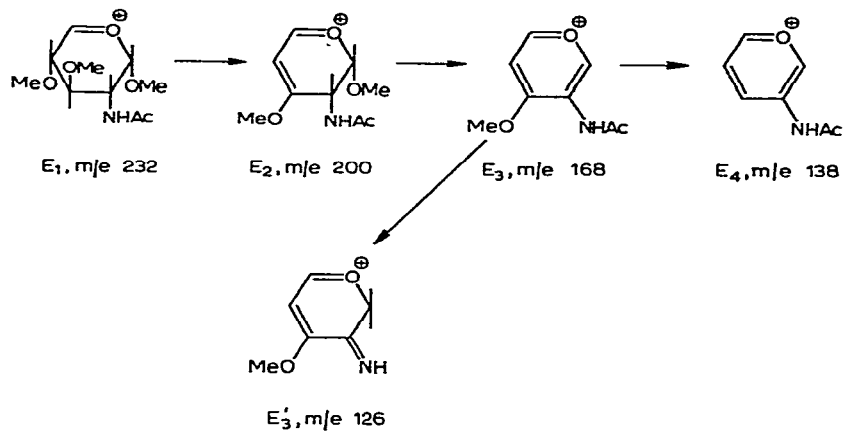
$(C_2^2)'$, m/e 185 $(C_2^2)''$, m/e 143 $(C_3^2)'$, m/e 98

E-series. The ions having m/e 232 (E_1), 200 (E_2), 168 (E_3), 126 (E_3'), and 138 (E_4) belong to this series. Loss of the CH_3O-group from C-4 (*cf.* the loss of the CH_3O-group from C-3 for neutral methyl hexopyranosides[2-4]), and the presence of an intense peak for the E_3-fragment (which is unstable for neutral monosaccharides and does not give the corresponding peak in their mass spectra) are characteristic for E-series fragments of permethylated methyl hexosaminides. These features of the fragmentation of compound (I) are difficult to explain.

The most intense peaks are those of the fragments belonging to the F, G, H, and J series. Splitting of ketene from ions having m/e 128 (F_1^2, G_1^1, G_1^2) and m/e 115 (H_1^1, H_1^2) leads to fragments having m/e 86 and 73, the latter being the most intense in the mass spectrum. The metastable peak at m/e 46.3 (calc. 46.3) corresponds to this transformation.

Elucidation of the structures and contributions of ions produced from compound

(I) during fragmentation permits calculation of the mass spectra of the trideuteromethyl analogues having CD_3-groups in all of the possible positions (*cf.* ref. 2,3).



These calculated and experimental data for the mass spectra of compounds (II) and (III) are given in Table I, from which it is seen that each isomer has a characteristic

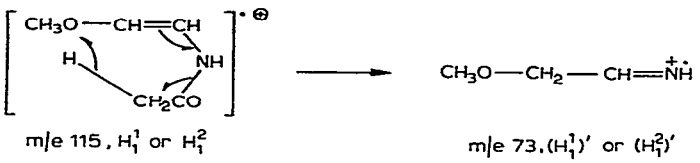


TABLE I

MASS SPECTRA OF METHYL 2-ACETAMIDO-2-DEOXY-3,4,6-TRI-*O*-METHYL-α-D-GLUCOPYRANOSIDE (I) AND ITS DEUTERATED ANALOGUES[a]

	Position of CD_3 groups					
I	*3*	*4*	*6*	*3,4*	*3,6*	*4,6*
246	249	249	249	252	252	252
232	235	235	232	238	235	235
214	214	217	217	217	217	220
203	206	206	203	209	206	206
172	172	175	172	175	172	175
168	171	168	168	171	171	168
128	128 (79)	128 (21)	128	131	128 (79)	128 (21)
	131 (21)	131 (79)			131 (21)	131 (79)
115	115 (17)	115	115	115 (17)	115 (17)	115
	118 (83)			118 (83)	118 (83)	
101	101 (62)	101 (38)	101 (38)	104 (62)	104	104 (38)
	104 (38)	104 (62)	104 (62)	107 (38)		107 (62)
88	91	91	88	94	91	91
75	78	75	75	78	78	75
73	73 (24)	73 (93)	73	73 (17)	73 (24)	73 (93)
	76 (76)	76 (7)		76 (83)	76 (76)	76 (7)

[a]m/e-values; in parentheses, relative intensities.

mass spectrum. Thus, the new approach to identification of partially methylated monosaccharides[1] can be applied to derivatives of *N*-acetyl-D-glucosamine and, bearing in mind the close similarity of fragmentation patterns of monosaccharides of the same type, to those of other hexosamines.

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ALKALINE DEGRADATION OF 6-THIO DERIVATIVES OF D-GLUCOSE AND D-GALACTOSE*

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(Received December 21st, 1965)

There is good evidence[1,2] that the conversion of phenyl β-D-glucopyranoside into 1,6-anhydro-β-D-glucopyranose by hot alkali involves the reactive, intermediate 1,2-anhydro-α-D-glucopyranose. A similar intermediate is probably involved in the conversion[3] of phenyl β-D-galactopyranoside into 1,6-anhydro-β-D-galactopyranose.

Neighboring-group participation by an oxy anion on C-2 is sterically unfavorable in phenyl α-D-glucopyranoside and phenyl α-D-galactopyranoside, as, for both, the hydroxyl group on C-2 is *cis* to the phenoxy group on C-1. Thus, phenyl α-D-glucopyranoside is stable in hot aqueous alkali whereas the D-galactose derivative is slowly converted by alkali into 1,6-anhydro-β-D-galactopyranose[3]. It has been postulated[4,5] that the formation of 1,6-anhydro-β-D-galactopyranose from phenyl α-D-galactopyranoside involves direct participation of the oxygen atom on C-6. Replacement of the oxygen atom on C-6 with sulfur as the nucleophile might be expected to enhance[6,7] the direct displacement of the α-D anomeric group in a D-glucopyranose or D-galactopyranose derivative, and to yield an analog of 1,6-anhydro-β-D-glucopyranose or 1,6-anhydro-β-D-galactopyranose wherein a sulfur atom replaces the oxygen atom in the 1,6-anhydro ring. For this reason, and because of the recent interest in sulfur-containing sugars, the alkaline degradation of 6-thio derivatives of D-glucose and D-galactose has been examined.

Phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranoside (II) was prepared by a modification[8] of an earlier[9] synthesis by using zinc chloride as the catalyst in a melt of phenol with 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose. Visual observation on thin-layer chromatograms showed that conversion into glycoside was complete in 2 h with the formation of a 1:1 mixture of phenyl α- and β-D-glucosides. The α-D anomer crystallized from the reaction mixture in 48% yield. By use of the same technique, 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranose also gave, after 6 h, an equimolar mixture of phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α- and β-D-galactosides, but neither anomer crystallized until the reaction mixture had been separated by preparative, thin-layer chromatography.

Fusion of 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose with *p*-nitrophenol, with zinc chloride as the catalyst, did not give the expected *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranoside (III). Instead a large propor-

*Journal paper No. 2674 of the Purdue Agricultural Experiment Station, Lafayette, Indiana.

tion of insoluble material was formed, and a yellow, crystalline, sugar derivative was isolated in low yield. However, compound III was synthesized by thiolacetate displacement of the *p*-tolylsulfonyloxy group from *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside, which was obtained in 36% yield by the Helferich reaction[9] on 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose. An attempt to prepare 2,4-dinitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranoside from the β-D anomer (V), using an anomerization reaction described by Lindberg[10], was unsuccessful. The reaction gave a brown tar that resisted attempts at crystallization.

Synthesis of the aryl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranosides was accomplished by one of several techniques. The *p*-nitrophenyl glycoside (VII) was obtained in 27% yield by treatment[11] of silver *p*-nitrophenoxide with 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl bromide. A modification[12] of the classical synthesis of aryl β-D-glucosides gave a 17% conversion into 2,4-dinitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranoside (V).

Although phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranoside (VI) could be prepared by the Helferich reaction[9] on 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose, VI was obtained in higher yield by thiolacetate displacement from phenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranoside. The latter compound was isolated from the reaction of molten phenol with 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose when *p*-toluenesulfonic acid or zinc chloride was used as catalyst (yield, 69 and 76%, respectively). The formation of the β-D anomer in the zinc chloride-catalyzed reaction contrasts with the formation of the phenyl α-D-glycoside in a similar treatment of D-glucopyranose pentaacetate. Apparently, phenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranoside, which is formed first, is difficult to anomerize, and the kinetically controlled product was isolated, instead of the thermodynamically more-stable α-D-glucopyranoside. When molten *p*-nitrophenol was substituted for phenol in the reaction, the anomerization reaction was faster than for phenol, and *p*-nitrophenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside crystallized (yield, 57%).

Alkaline degradation of phenyl and *p*-nitrophenyl 6-*S*-acetyl-6-thio-β-D-glucopyranosides (VI and VII) gave, as expected, 1,6-anhydro-6-thio-β-D-glucopyranose (VIII) in 73 and 56% yield, respectively (see Table I). Presumably, formation of the 1,6-anhydro ring proceeds by way of the 1,2-anhydro intermediate (B), as shown in Scheme I. The overall conversion of D-glucose into VIII by utilizing the degradation of phenyl 6-thio-β-D-glucopyranoside was 9.2%. By an independent method, Akagi and co-workers[13] reported an 8.5% overall conversion, by way of VIII triacetate, into VIII. The specific optical rotations obtained for our compounds, VIII and VIII triacetate, do not agree with previously recorded values[13].

Compound VIII was not formed when either II or III was heated in alkali at 100°. On following the reactions by paper chromatography, it was observed that the phenyl derivative is consumed after 96 h and the *p*-nitrophenyl derivative after 18 h (see Table I). Compound VIII could not be detected on paper chromatograms at any

time in either reaction mixture, and the triacetate of VIII could not be found on thin-layer chromatograms after the mixtures had been acetylated. Decomposition of the phenyl thioglycoside was extensive, as evidenced by the dark reaction product and the smell of phenol and hydrogen sulfide on acidification of the reaction mixture. The possibility that VIII was formed and then decomposed during the reaction was discredited, since the 1,6-anhydro derivative is only slightly degraded when treated for 96 h with hot alkali.

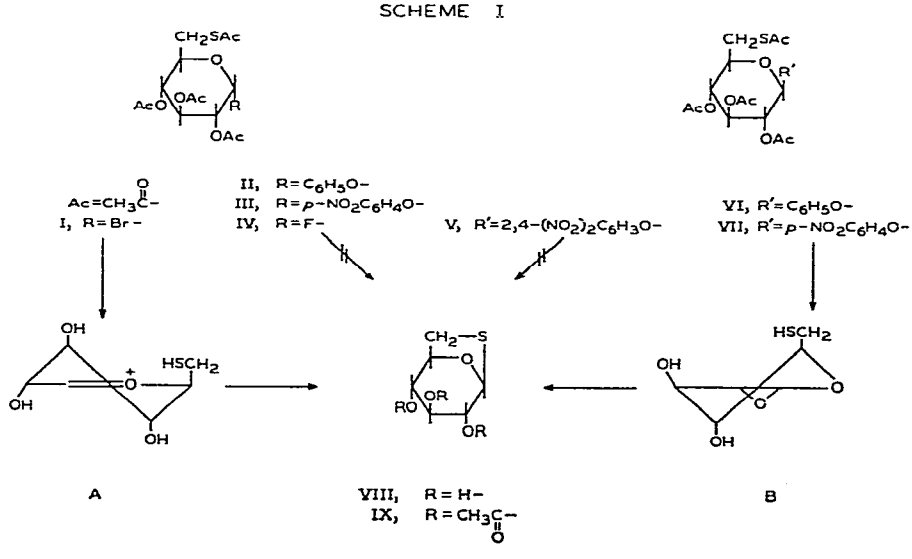
TABLE I

SUMMARY OF REACTIONS OF D-GLUCOSE AND D-GALACTOSE DERIVATIVES[a] IN ALKALI

Compound[a] of	*m.p., °C*	*$[\alpha]_D^{25}$, degrees (c~1, $CHCl_3$)*	*Reaction time[b], h*	*Yield of 1,6-anhydro derivative, %*
Phenyl β-D-glucopyranoside	97–98	− 10.3	7	73
Phenyl β-D-galactopyranoside	110	+ 47.1	6	70
p-Nitrophenyl β-D-glucopyranoside	169–170	− 10.9	5	56
2,4-Dinitrophenyl β-D-glucopyranoside	180–182	+ 61.0	0.5	—
Phenyl α-D-glucopyranoside	128	+161	96	—
Phenyl α-D-galactopyranoside	84–85	+242	124	—
p-Nitrophenyl α-D-glucopyranoside	136–137	+230	18	—
α-D-Glucopyranosyl bromide[c]	101–102	+182	0.5	55
α-D-Glucopyranosyl fluoride	133–134	+126	30	—
α-D-Galactopyranosyl bromide	syrup	+197	0.5	35

[a]All of the compounds have 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio substituents.
[b]Reaction at 100° in 1.3 N potassium hydroxide containing 33.3% of 2-methoxyethanol.
[c]All reactions of glycosyl halides were conducted at 25°.

SCHEME I



2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl bromide (I) underwent rapid reaction at room temperature in alkaline solution to produce VIII in 55% yield, whereas tetra-*O*-acetyl-α-D-glucopyranosyl bromide gave an 18% yield of 1,6-anhydro-β-D-glucopyranose. The higher yield of VIII is best explained by a solvolysis reaction at C-1, with the formation of a reactive intermediate (A) in a half-chair conformation (see Scheme I). Solvolysis of tetra-*O*-acetyl-α-D-glucopyranosyl bromide in alkaline solution gives an intermediate similar to A, but the oxy anion on C-6, which competes with solvent for the C-1 carbonium ion, does not participate as well as the larger and more polarizable sulfur anion. It is conceivable, then, that an α-D anomeric substituent such as bromide, in contrast to phenoxy and *p*-nitrophenoxy, leads to 1,6-anhydro formation, since solvolysis provides the driving force for the reaction by causing proximity of the C-6 anion to the anomeric carbonium ion, through formation of the half-chair conformer A.

Intramolecular displacement of the bromide ion by the sulfur anion, to form VIII, probably does not occur, since VIII is not obtained in the alkaline treatment of IV. Although displacement[7] of fluoride ion by a sulfur nucleophile occurs at a rate higher than that for displacement of bromide ion, solvolysis of α-D-glucopyranosyl fluoride is much slower[14,15] than solvolysis of tetra-*O*-acetyl-α-D-glucopyranosyl bromide. If the group on C-1 is displaced, it follows that IV should react faster than I, and produce more VIII. However, IV reacts slowly in alkali, without the formation of VIII. Paper-chromatographic examination of the reaction mixture indicated that 6-thio-α-D-glucopyranosyl fluoride is consumed after 30 h, with the production of a compound (R_G 0.62) that gave a reducing-sugar test with *p*-anisidine hydrochloride spray reagent, and no thiol activity with tetrazolium spray reagent.

Although phenyl α-D-galactopyranoside is slowly converted[3] by hot alkali into 1,6-anhydro-β-D-galactopyranose, phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranoside was degraded in 124 h with the formation of many unidentified compounds that reduced silver nitrate spray on paper chromatograms. 1,6-Anhydro-6-thio-β-D-galactopyranose was formed in alkali from phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranose and also from 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranosyl bromide (see Table I). Synthesis of the 1,6-anhydro-D-galactose derivative was confirmed by isolation of the same compound from the reaction of 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-galactopyranosyl ethylxanthate with sodium methoxide in methanol.

EXPERIMENTAL

All melting points were determined with a Fisher–Johns melting point apparatus, and are corrected. Paper chromatography was performed by the descending technique, on Whatman No. 1 paper at 25° by using as the irrigant 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water, or 10:4:3 (v/v) ethyl acetate–pyridine–water. Chromatograms were developed by using the following reagents: A, an aqueous acetone solution of silver nitrate, followed by a methanolic sodium hydroxide solution[16]; B, a 0.5%

chloroform solution of 2,3,5-triphenyl-2*H*-tetrazolium chloride, followed by methanolic sodium hydroxide solution[16]; or C, *p*-anisidine hydrochloride[17] in butyl alcohol.

Thin-layer chromatography was performed on 20 × 100 mm plates coated* with silica gel G. After two irrigations with isopropyl ether saturated with methyl sulfoxide, components were located by spraying with a 5% solution of sulfuric acid in methanol and then charring on a hot plate. Evaporations were conducted under diminished pressure below 50°.

*1,2,3,4-Tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-*D*-glucopyranose*

Ten g of crystalline 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose[18], in 125 ml of dry acetone containing 4.4 g of potassium thiolacetate was refluxed for 6 h, the mixture was cooled, and the potassium *p*-toluenesulfonate was removed by filtration. The filtrate was evaporated to dryness, the residue dissolved in chloroform, and the chloroform solution washed twice with water and dried (sodium sulfate). Evaporation of the solution gave 6.1 g (76%) of a solid that was recrystallized from ethanol, m.p. 127–128°, $[\alpha]_D^{25}$ −14.7° (*c* 3.4, chloroform); lit.[19], m.p. 130-131, $[\alpha]_D^{24}$ −19° (*c* 1, chloroform).

*Phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-*D*-glucopyranoside (II)*

In a 50-ml flask were placed 5 g of 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose, 5 g of phenol, and 0.2 g of freshly fused zinc chloride dissolved in 5 ml of a 5% solution of acetic anhydride in acetic acid[8,9]. The mixture was heated under diminished pressure on a steam bath, and agitated frequently; after 2 h the mixture was cooled and dissolved in benzene. Thin-layer chromatographic examination showed that all of the starting material had reacted, to give a 1:1 mixture of phenyl α- and β-D-glucopyranosides. The benzene solution was washed with water, N sodium hydroxide, and water, and dried (sodium sulfate). The benzene was removed by evaporation, and the resulting syrup was dissolved in ethanol, giving 2.6 g (48%) of crude crystals; recrystallized several times from a 19:1 (v/v) mixture of petroleum ether (b.p. 60–68°) and acetone, it had m.p. 128°, $[\alpha]_D^{25}$ +161° (*c* 0.09, chloroform).

Anal. Calc. for $C_{20}H_{24}O_9S$: C, 54.53; H, 5.49; S, 7.28. Found: C, 54.27; H, 5.60; S, 7.46.

*2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-*D*-glucopyranosyl fluoride (VI)*

Finely powdered 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose (5 g) was added in small portions to 10 ml of hydrogen fluoride at −63° (Dry Ice–chloroform). After 9 h, the solution was slowly poured into a mixture of ice and chloroform. The chloroform layer was washed with 5% sodium bicarbonate solution, and water, and dried (sodium sulfate). After removal of chloroform, the sugar derivative was crystallized from ethanol, and gave 2.2 g (49%) of product. After three recrystalli-

*Brinkman Instruments, Inc., Great Neck, N.Y.

zations from ethanol at room temperature, pure material was obtained; long needles, m.p. 133–134°, $[\alpha]_D^{25}$ +126° (*c* 1.0, chloroform). Calculations according to Hudson's rules of isorotation give, for the α-D anomer $[\alpha]_D$ +110°, and for the β-D anomer, $[\alpha]_D$ +73°.

Anal. Calc. for $C_{14}H_{19}FO_8S$: C, 45.89; H, 5.23; F, 5.18; S, 8.75. Found: C, 45.86; H, 5.33; F, 5.00; S, 8.76.

p-Nitrophenyl 2,3,4-tri-O-acetyl-6-S-acetyl-6-thio-α-D-glucopyranoside (III)

To 10 g of 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose were added 10 g of *p*-nitrophenol and 0.4 g of zinc chloride dissolved in 5 ml of a 5% solution of acetic anhydride in acetic acid. The mixture was heated for 10 min under diminished pressure in an oil bath at 125°. The mixture was cooled and treated (benzene solution) in the usual manner. After removal of the benzene, the syrup (7.2 g, 63%) was dissolved in 30 ml of acetone, the solution decolorized with carbon, and the glycoside (4.1 g, 36%) crystallized as needles from ethanol. Two recrystallizations from 5:1 (v/v) ethanol–acetone gave pure III, m.p. 178°, $[\alpha]_D^{25}$ +173° (*c* 1.04, chloroform).

Anal. Calc. for $C_{25}H_{27}NO_{13}S$: C, 51.65; H, 4.68; S, 5.51. Found: C, 51.89; H, 4.99; S, 5.69.

p-Nitrophenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-glucopyranoside was converted into III by refluxing in acetone with potassium thiolacetate for 14 h. Crystallization from ethanol gave pure material, m.p. 136–137°, $[\alpha]_D^{25}$ +230° (*c* 1.1, chloroform).

Anal. Calc. for $C_{20}H_{23}NO_{11}S$: C, 49.50; H, 4.75; S, 6.60. Found: C, 49.80; H, 5.02; S, 6.70.

Phenyl 2,3,4-tri-O-acetyl-6-thio-β-D-glucopyranoside (VI)

1,2,3,4-Tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose (5 g) with 3 g of phenol and 0.05 g of *p*-toluenesulfonic acid as catalyst was fused for 1 h on a steam bath. The mixture was treated as usual, and gave 3.4 g (69%) of crude crystals. Two recrystallizations from ethanol gave pure phenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranoside, m.p. 158–160°, $[\alpha]_D^{25}$ −29.0° (*c* 3.8, chloroform). The same glycoside was prepared in 76% yield by a melt reaction catalyzed by zinc chloride (1.3 g), by using 5 g of 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranose in 10 g of phenol and heating for 1 h at 100°. Helferich and Strauss[20] *p*-toluenesulfonated phenyl β-D-glucopyranoside and acetylated the product, to obtain phenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranoside, m.p. 161–162°, $[\alpha]_D^{25}$ −26° (chloroform).

Thiolacetate displacement with phenyl 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-glucopyranoside gave a 65% conversion into VI. Compound VI was also obtained in 40% yield when 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranose was fused with phenol, using *p*-toluenesulfonic acid as catalyst. The glycoside was obtained

as long needles by two recrystallizations from petroleum ether (b.p. 60–68°); m.p. 97–98°, $[\alpha]_D^{25}$ −10.3 (*c* 1.0, chloroform).

Anal. Calc. for $C_{20}H_{24}O_9S$: C, 54.53; H, 5.49; S, 7.28. Found: C, 54.60; H, 5.73; S, 7.49.

p-Nitrophenyl and 2,4-dinitrophenyl 2,3,4-tri-O-acetyl-6-S-acetyl-6-thio-β-D-glucopyranosides (VII and V)

In an adaptation of the procedure described by Goebel and Avery[11], 2 g of 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl bromide[13] (I) dissolved in 20 ml of *p*-xylene was added to 0.7 g of silver *p*-nitrophenoxide. Several glass beads were added and the mixture was shaken for 30 min. The addition of silver *p*-nitrophenoxide, followed by a 30-min reaction period, was repeated twice, and the mixture was filtered through a sintered-glass funnel. The filtrate was concentrated to dryness, and the syrup was dissolved and evaporated three times from ethanol. The syrup crystallized from ethanol, to give 0.60 g (27%) of product. Recrystallization from ethanol gave pure VII, m.p. 169–170°, $[\alpha]_D^{25}$ −10.9° (*c* 1.0, chloroform).

Anal. Calc. for $C_{20}H_{23}NO_{11}S$: C, 49.50; H, 4.75; S, 6.60. Found: C, 48.95; H, 4.94; S, 6.98.

2,4-Dinitrophenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranoside (V) was prepared according to the general procedure described by Mosettig and associates[12]. A mixture of 12 g of I, 6.3 g of 2,4-dinitrophenol, and 6.3 g of potassium carbonate in 150 ml of acetone was refluxed for 20 h. After the mixture had been cooled, 100 ml of water was added, and the solution was poured into 500 ml of cold water. The gummy precipitate was dissolved in 50 ml of 5:1 (v/v) acetone–ethanol, and the solution was decolorized with carbon. The filtrate was concentrated to dryness, and the solid was crystallized from ethanol to give 2.7 g (17%) of crude crystals. Two recrystallizations from ethanol gave pure material; m.p. 180–182°, $[\alpha]_D^{25}$ +61° (*c* 1.1, chloroform).

Anal. Calc. for $C_{20}H_{22}N_2O_{13}S$: C, 45.20; H, 4.17; S, 6.04. Found: C, 45.20; H, 4.48; S, 6.18.

Phenyl 2,3,4-tri-O-acetyl-6-S-acetyl-6-thio-α and β-D-galactopyranosides

Acetolysis, using the method of Reist *et al*[21] of 1,2:3,4-di-*O*-isopropylidene-6-*O*-(*p*-tolylsulfonyl)-α-D-galactopyranose gave only 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-galactopyranose[22], in 17% yield. This compound was treated with sodium iodide in acetone solution for 12 h at 110°. 1,2,3,4-Tetra-*O*-acetyl-6-deoxy-6-iodo-α-D-galactopyranose was formed: it was crystallized from ethanol; m.p. 138–139°, $[\alpha]_D^{25}$ +114° (*c* 1.5, chloroform); lit.[23] m.p. 107°, $[\alpha]_D^{18}$ + 70.2 (*c*. 0.94, chloroform).

Anal. Calc. for $C_{14}H_{19}IO_9$: C, 36.69; H, 4.18; I, 27.69. Found: C, 36.97; H, 4.38; I, 27.35.

1,2,3,4-Tetra-*O*-acetyl-6-deoxy-6-iodo-α-D-galactopyranose was converted into the 6-*S*-acetyl-6-thio derivative by refluxing for 12 h with potassium thiolacetate in acetone solution; m.p. 113°, $[\alpha]_D^{25}$ +130° (*c* 1.03, chloroform).

Anal. Calc. for $C_{16}H_{22}O_{10}S$: C, 47.28; H, 5.46; S, 7.89. Found: C, 47.30; H, 5.50; S, 7.71.

In an attempt to prepare the anomeric phenyl glycosides from 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranose by the Helferich technique[9], thin-layer chromatography showed that the α-D acetate reacted too slowly to be useful, and degradative reactions became prominent with prolonged heating of the reaction melt. For this reason, 1,2,3,4-tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-galactopyranose[22] was converted, as described above, into 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-iodo-β-D-galactopyranose, m.p. 116–118°, $[\alpha]_D^{25}$ +24.0° (*c* 1.0, chloroform), lit.[23] m.p. 113–114°, $[\alpha]_D^{18}$ +10.4° (*c* 0.96, chloroform). Displacement with thiolacetate anion gave 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranose, m.p. 157–158°, $[\alpha]_D^{25}$ +58.5° (*c* 1.2, chloroform).

Anal. Calc. for $C_{16}H_{22}O_{10}S$: C, 47.28; H, 5.46; S, 7.89. Found: C, 47.21; H, 5.32; S, 7.90.

1,2,3,4-Tetra-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranose (4.57 g) with 10 g of phenol was fused at 100°, using 0.2 g of zinc chloride as catalyst. Thin-layer chromatographic examination revealed that all of the starting material had been consumed after 6 h. The mixture was heated for a total of 7 h, and treated as usual. After separation by preparative, thin-layer chromatography, 1.8 g (35%) of each glycoside was obtained and crystallized from ethanol. Phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-thio-α-D-galactopyranoside had m.p. 84–85°, $[\alpha]_D^{25}$ +242° (*c* 1.0, chloroform), and the β-D anomer had m.p. 110° and $[\alpha]_D^{25}$ +47.1° (*c* 1.0, chloroform).

Anal. Calc. for $C_{20}H_{24}O_5S$: C, 54.53; H, 5.49; S, 7.28. Found for the α-D-anomer: C, 54.46; H, 5.38; S, 7.04. Found for the β-D anomer: C, 54.29; H, 5.28; S, 7.12.

Alkaline degradation

Reactions of all of the aryl glycosides with alkali were conducted as for the following example; the results are summarized in Table I. Compound VI (50 mg), in a small, glass-stoppered test-tube, was dissolved in 1.0 ml of 2-methoxyethanol, and 2.0 ml of 1.95 N potassium hydroxide was added. The gaseous oxygen in the 2-methoxyethanol, and in the potassium hydroxide solutions, had been previously displaced by passing oxygen-free nitrogen through each solution for 2 h. The tube was heated at 100°, and aliquots of the reaction mixture were taken periodically, neutralized with acetic acid, and examined by paper chromatography, using sprays A and B. After 7 h, all of the aryl 6-thio-β-D-glucoside had been consumed, and this reaction time was used, in a separate experiment, in determining the yield of 1,6-anhydro derivative. Compound VI (1 g) was treated with 10 ml of 2-methoxyethanol and 20 ml of 1.95 N potassium hydroxide in a sealed tube. The mixture was cooled, neutralized with acetic acid, and concentrated to dryness at 70°. To the residue was added 5 ml of acetic anhydride and 10 ml of pyridine. After being kept overnight, the mixture was poured onto 20 ml of ice water. After 1 h, the solution was extracted with chloroform, and the extract was washed with 5% sulfuric acid solution, N sodium hydroxide, and water, and dried (sodium sulfate). After evaporation of the chloroform, ethanol was

added, and the solution was clarified with carbon. On being cooled to 5°, the solution gave two crops of crystals; total yield, 0.51 g (74%). Recrystallization from ethanol gave IX, as platelets, m.p. 79–81°, $[\alpha]_D^{25}$ −55.0° (*c* 1.3, chloroform); lit.[12] m.p. 93–94°, $[\alpha]_D^{23}$ −25.2° (*c* 1.1, chloroform). Deacetylation of IX with sodium in methanol gave VIII, m.p. 180°, $[\alpha]_D^{25}$ −54.0° (*c* 1.0, water); lit.[13], m.p. 180°, $[\alpha]_D^{16}$ −5.1° (*c* 0.8, water). The preparation of VIII and IX was also performed as described by Akagi and co-workers[13], and the physical constants of these compounds agreed with those found for the compounds isolated after alkaline degradation of VI.

Reactions of glycosyl halides with alkali were conducted at 25°. Compound I was treated (10% concentration) with 1.3 N potassium hydroxide containing 33.3% of 2-methoxyethanol; or (20% concentration) with N sodium methoxide in methanol. In each case, the solution was neutralized with acetic acid, and the product was acetylated with acetic anhydride in pyridine, to afford a 55% yield of IX. When tetra-*O*-acetyl-α-D-glucopyranosyl bromide was treated in a similar way in potassium hydroxide solution, 2,3,4-tri-*O*-acetyl-1,6-anhydro-α-D-glucopyranose could not be crystallized after acetylation. By separating the reaction components on a paper chromatogram, developing with spray A, and determining the intensity of the spots of D-glucose and 1,6-anhydro-β-D-glucopyranose with a recording densitometer, the proportion of 1,6-anhydro-β-D-glucopyranose in the reaction mixture was found to be 18%.

2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl fluoride reacted slowly with potassium hydroxide at 25°. Paper-chromatographic examination, using sprays A, B, and C, showed the disappearance of starting material after 30 h, with the production of a sugar (R_G 0.62) which gave a reducing-sugar test with spray C, and no thiol activity with spray B. Treatment of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl fluoride with base, under the same conditions as had been used for compound IV, was complete in 24 h, and gave only a trace of 1,6-anhydro-β-D-glucopyranose as evidenced by visual observation on a paper chromatogram. D-Glucose was the only other product.

2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-galactopyranosyl bromide was prepared by the action of 35% hydrogen bromide in acetic acid on 1,2,3,4-tetra-*O*-acetyl-6-*S*-acetyl-6-thio-α(or β)-D-galactopyranose. The product, which contained bromine, was homogeneous, as shown by thin-layer chromatography, but all attempts at crystallization failed. Therefore, the syrup, $[\alpha]_D^{25}$ +197° (*c* 1.1, chloroform), was immediately treated, as before, with potassium hydroxide solution. Following acetylation, 2,3,4-tri-*O*-acetyl-1,6-anhydro-6-thio-β-D-galactopyranose was isolated, m.p. 126–127°, $[\alpha]_D^{25}$ +33.8° (*c* 1.0, chloroform).

Anal. Calc. for $C_{12}H_{16}O_7S$: C, 47.36; H, 5.29; S, 10.54. Found: C, 47.58; H, 5.47; S, 10.46.

The triacetate was deacetylated with sodium in methanol; yield, 80%; and the product was recrystallized twice from absolute ethanol to give pure 1,6-anhydro-6-thio-β-D-galactopyranose, m.p. 230–232° (dec., in a sealed tube heated in an oil bath), $[\alpha]_D^{25}$ +41.7° (*c* 1.0, water).

Anal. Calc. for $C_6H_{10}O_4S$: C, 40.44; H, 5.66; S, 17.99. Found: C, 40.40; H, 5.70; S, 18.2.

Alternative preparation of 1,6-anhydro-6-thio-β-D-galactopyranose

1,2,3,4-Tetra-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-galactopyranose was treated with hydrogen bromide in acetic acid in the usual way, to give a 64% yield of 2,3,4-tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-galactopyranosyl bromide; this was recrystallized twice from 1:9 (v/v) absolute chloroform–isopropyl ether, m.p. 143–145°, $[\alpha]_D^{25}$ +185° (*c* 1.1, chloroform).

Anal. Calc. for $C_{19}H_{23}BrO_{10}S$: C, 43.60; H, 4.43; Br, 15.27; S, 6.13. Found: C, 43.36; H, 4.43; Br, 15.30; S, 6.00.

2,3,4-Tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-α-D-galactopyranosyl bromide (6.25 g) and 3.7 g of potassium ethylxanthate in boiling acetone was heated with stirring for 30 min. The mixture was cooled and poured into water, and the solid (4.8 g, 71%) was collected by filtration and recrystallized twice from ethanol, to give pure material, m.p. 133–134°, $[\alpha]_D^{25}$ +17.9° (*c* 1.0, chloroform).

Anal. Calc. for $C_{22}H_{28}O_{11}S_3$: C, 46.79; H, 5.00; S, 17.04. Found: C, 46.76; H, 5.29; S, 17.29.

2,3,4-Tri-*O*-acetyl-6-*O*-(*p*-tolylsulfonyl)-β-D-galactopyranosyl ethylxanthate (1.5 g) was dissolved in 20 ml of methanol containing 0.3 g of sodium. After 24 h at 25°, the mixture was neutralized with acetic acid and evaporated to dryness. After acetylation in the usual manner, 2,3,4-tri-*O*-acetyl-1,6-anhydro-6-thio-β-D-galactopyranose (0.42 g, 52%) was obtained by crystallization from ethanol; m.p. and mixed m.p. with the compound obtained by alkaline degradation of phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranoside was 126-127°, $[\alpha]_D^{25}$ +34.0 (*c* 1.0, chloroform).

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SUMMARY

Phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-glucopyranoside and phenyl 2,3,4-tri-*O*-acetyl-6-*S*-acetyl-6-thio-β-D-galactopyranoside react with hot alkali to give approximately 70% conversions into 1,6-anhydro-6-thio-β-D-glucopyranose (VIII) and 1,6-anhydro-6-thio-β-D-galactopyranose, respectively. The corresponding α-D anomers are also decomposed in alkali, but without the formation of the corresponding 1,6-anhydro sugars. 2,3,4-Tri-*O*-acetyl-6-*S*-acetyl-6-thio-α-D-glucopyranosyl bromide was converted into compound VIII by alkaline solution, presumably by solvolysis, with participation by the sulfur anion on C-6.

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STUDIES ON URONIC ACID MATERIALS

PART XV*. THE USE OF MOLECULAR-SIEVE CHROMATOGRAPHY IN STUDIES ON *Acacia senegal* GUM (GUM ARABIC)

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INTRODUCTION

Lathe and Ruthven[2], and Andrews and Roberts[3], have suggested the possibility of applying molecular-sieve chromatography (gel "filtration") to molecular-weight estimations on polysaccharides. Some of the experiments described in this paper on the application of this technique to *Acacia senegal* gum have been reported in a preliminary communication[4].

MATERIALS AND METHODS

The nodules of *A. senegal* (syn. *verek*) were collected by (the late) Mr. M. P. Vidal-Hall, Gum Research Officer, Republic of the Sudan, at Qala en Nahal, Kassala Province, as the first collection of the 1960 gum season.

Nitrogen, ash, and moisture determinations

Nitrogen was determined by a semi-micro Kjeldahl method, moisture by heating to constant weight at 105°, and ash by heating (muffle furnace) to constant weight at 550°.

Viscosity measurements

Determinations were made in M sodium chloride, in a suspended-level, dilution viscometer at 25° (flow time for M sodium chloride, 189.9 sec).

Uronic acid determinations

Uronic acid content was determined by a vapour-phase, i.r. method after decarboxylation with hydriodic acid[5].

Methoxyl determinations

A vapour-phase, i.r. method was used[5].

*For Part XIV see ref. 1.

Polysaccharide hydrolyses

Polysaccharides were hydrolysed with N sulphuric acid for 7 h at 100°. These conditions do not cause any extensive hydrolysis of the uronic acid linkages in *A. senegal* gum; this was taken into account when determining proportions of galactose. Hydrolysates were neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+ form) and concentrated at *ca.* 35° on a rotary evaporator.

Sugar ratios

These were determined by chromatographic separation on Whatman 3MM paper, followed by elution and colorimetric estimation by the phenol–sulphuric acid method[6]. After periodate oxidation, rhamnose was also determined, as acetaldehyde, by a vapour-phase, i.r. method[7]. Sugar compositions were calculated as anhydro-sugar residues.

Periodate oxidations

Unless otherwise stated, these were carried out at room temperature in darkness using excess of sodium metaperiodate. Formic acid was estimated potentiometrically[8]. Formaldehyde was estimated colorimetrically with chromotropic acid[9].

Paper chromatography of sugars

Whatman No. 1 and 3MM papers were used with the following solvent systems (v/v): (*a*) benzene–butan-1-ol–pyridine–water (1:5:3:3, upper layer); (*b*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*c*) butan-1-ol–ethanol–water (4:1:5, upper layer); (*d*) butan-1-ol–acetic acid–water (4:1:5, upper layer); (*e*) ethyl acetate–pyridine–water (10:4:3); (*f*) butanone–acetic acid–water (9:1:1, saturated with boric acid). Chromatograms were developed with aniline oxalate, *p*-anisidine hydrochloride, alkaline silver nitrate, or the periodate–permanganate reagent.

Thin-layer chromatography of sugars

This was carried out on "Chromagram" sheets (Kodak Ltd., Kirkby, Liverpool) of polycarbonate or silica gel[10], using the following solvent systems (v/v): (*g*) propan-1-ol–ethyl acetate–water (10:3:1) with polycarbonate sheets; and (*h*) butan-1-ol–acetone–water (4:5:1), or (*i*) butanone–acetic acid–water (3:1:1) with silica-gel sheets.

Molecular-sieve chromatography

This was carried out on columns (6.0 × 50 cm) of "Bio-Gel P300" (Bio-Rad Laboratories, Richmond, California) using M sodium chloride as eluant[4]. To prevent deformation by "wall effects", columns were pre-treated with 1% dichlorodimethylsilane in benzene at 60°. After oven-drying, columns were packed with gel that had been allowed to swell in M sodium chloride for 2 days. The gel slurry was added continuously to the column; a thin layer of glass beads supported the gel and kept the "dead space" to a minimum. To stabilise the soft top-surface of the P300 gel, 1-cm

layers of "Bio-Gel P200" and "Bio-Gel P10" were applied successively to the column. Eluant was allowed to flow for 2 days before the columns were calibrated with dextran fractions (Pharmacia Ltd., Uppsala) of known, number-average, molecular weight ($\overline{M}_n$). Polysaccharide (*ca.* 10 mg), dissolved in 1.5M sodium chloride (1 ml), was applied to the column by careful layering beneath the M sodium chloride. Fractions, collected from a 2-ml siphon by an automatic collector, were screened by the phenol–sulphuric acid method[6]. Elution volumes (V_e) were estimated to the nearest ml from peak maxima.

RESULTS

Fractional precipitation of A. senegal *gum with sodium sulphate*

The gum (40 g) was dissolved in water (800 ml), filtered, and electrodialysed. Analyses on the freeze-dried product are shown in Table I.

A solution of the purified gum (25.4 g) in water (500 ml) was maintained at 28°. Anhydrous sodium sulphate was added in small portions with constant stirring. Precipitation commenced at concentrations approaching 40% (w/v); at 40%, a pale-brown material rose to the surface and was removed (Fraction I). Two further fractions, II and III, much lighter in colour, were obtained on continued, slow, stepwise addition of very small portions of sodium sulphate; eventually, the supernatant solution contained polysaccharide material which was not precipitated from a saturated solution of sodium sulphate, and this yielded Fraction IV. The fractions were dialysed against tap water until free of sulphate and were then electrodialysed to ensure complete removal of inorganic ions. Analytical data for the freeze-dried fractions are given in Table I.

Autohydrolysis of A. senegal *gum*

A sample (4 g) of electrodialysed gum was dissolved in water to give a 2% solution (pH, 2.8). Autohydrolysis on a boiling water-bath was followed polarimetrically[11]. After 50 h, the solution was cooled, filtered (to remove denatured protein), and dialysed against water (3 × 2 l). Dialysis was completed against running tap-water, and freeze-drying gave the degraded gum (2 g), $[\alpha]_D$ −11° (*c* 1.0, water) (Found: moisture, 9.7; uronic acid, 19.2; galactose, 68; arabinose, 2%). Hydrolysis of the degraded gum indicated the presence of two aldobiouronic acids, which had R_{Gal} values of 0.22 (major component) and 0.59 (minor component) in solvent (*b*), and were chromatographically identical with 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose and 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, respectively. Chromatographic examination of the diffusate from the degraded gum showed it to contain galactose, arabinose, rhamnose, three (major) neutral disaccharides, traces of the two aldobiouronic acids, and oligosaccharide material. Further hydrolysis of a portion of the diffusate yielded more of the same aldobiouronic acids found for the degraded gum.

TABLE I

ANALYTICAL DATA FOR ELECTRODIALYSED *A. senegal* GUM AND FRACTIONS OBTAINED BY PRECIPITATION WITH SODIUM SULPHATE

	A. senegal gum	*Fractions*			
		I	*II*	*III*	*IV*
Yield, %	—	23.6	29.5	33.7	1.2
Moisture, %	11.0	14.7	7.3	8.6	—
Ash, %	0.01	0.01	0.02	0.01	—
N, %	0.33	1.01	0.12	0.02	—
Protein, % [N % × 6.25]	2.1	6.3	0.75	0.13	—
$[\eta]$, cm^3g^{-1}	20.0	33.5	14.8	10.8	—
Rhamnose, %[a]	12(14)	10(13)	12(13)	12(13)	—
Arabinose, %[a]	25(28)	24(30)	26(28)	23(25)	—
Galactose, %[a]	34(39)	29(37)	37(40)	40(44)	—
Uronic acid, %[a,b]	16.7(19)	16.0(20)	17.5(19)	16.7(18)	15.5
Methoxyl, %	0.23	0.23	0.23	0.23	0.22
$[\alpha]_D$ (*c* 1.0, water)	−31.5°	−32.7°	−32.7°	−31.5°	—
Equiv. wt.[c]	1290	—	—	—	—
Formic acid released on periodate oxidation (mole/g) × 10^3	1.58	1.56	1.59	1.60	—
Ratio of galactose/arabinose[d]	1.40	1.23	1.43	1.76	—

[a]Values in parentheses are corrected for all non-carbohydrate material.
[b]Calculated as the anhydride of glucuronic acid.
[c]By direct titration (potentiometric) with 0.02N sodium hydroxide.
[d]Calculated from the values corrected for non-carbohydrate material.

Borohydride reduction of degraded gum

Degraded gum (500 mg) was dissolved in water (100 ml), and sodium borohydride (400 mg) was added. The solution was kept for 24 h at room temperature before further sodium borohydride (100 mg) was added. After the solution had been stirred for 6 h, it was dialysed against running tap-water for 2 days. The freeze-dried product was hydrolysed to yield the same aldobiouronic acids and neutral disaccharides found in the degraded gum. In addition, paper chromatography in solvent (*f*), and t.l.c. on silica gel with solvent (*h*) indicated the presence of galactitol. No arabinitol was detected.

Periodate oxidation of degraded and reduced, degraded gum

Degraded gum did not give detectable amounts of formaldehyde on periodate oxidation. The production of formaldehyde with time from reduced, degraded gum (34.32 mg, dry wt.) was as follows: 0.25 h, 160 μg; 0.5 h, 200 μg; 1 h, 215 μg; 2 h, 220 μg; 6 h, 220 μg; 24 h, 235 μg. Assuming production of one formaldehyde molecule per average polymer unit, a value for $\overline{M}_n$ of 4,400 was calculated for the degraded gum. Taking into account its composition, this corresponds to a number-average degree of polymerisation ($\overline{P}_n$) of 27.

Controlled Smith-degradation[12] *of degraded gum*

Periodate oxidation was carried out at 2°. Degraded gum (1 g) was dissolved in water (25 ml), 50% (w/w) periodic acid (1.75 ml) was added, and the solution made up to 50 ml. After 2 days, the reaction was stopped by addition of excess of ethylene glycol. Following dialysis against running tap-water for 2 days, the solution was treated with sodium borohydride (250 mg) for 36 h. Further dialysis for 2 days was followed by hydrolysis of the acetal linkages with N sulphuric acid for 2 days at 18°. The acidic solution was neutralised with barium carbonate, filtered, treated with Amberlite resin IR-120 (H^+ form), and concentrated on a rotary evaporator. Chromatographic examination revealed the presence of glycerol and glycolic aldehyde. Molecular-sieve chromatography on a column (2.5 × 75 cm) of "Bio-Gel P10" was used to separate such low molecular-weight materials from the Smith-degraded product (180 mg). Hydrolysis of a small portion of the latter product, with examination by paper chromatography, gave galactose, arabinose (a trace) and arabinitol [solvent (*f*)], but no galactitol or erythritol.

Molecular-sieve chromatography

Figure 1 shows the calibration plot of elution volume (V_e) against log $\overline{M}_n$ obtained with dextran fractions of known $\overline{M}_n$. For "Bio-Gel P300", this relationship[13–15] is approximately linear for values of $\overline{M}_n$ from 5,000 to 125,000; although the useful working range may extend slightly beyond these values, the exclusion limit of "Bio-Gel P300" *for the polysaccharides investigated* is apparently less than 300,000.

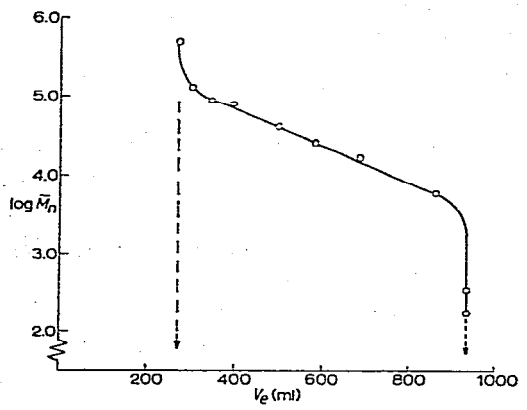

Fig. 1. Plot of elution volume (V_e) against log $\overline{M}_n$ for dextran fractions of known $\overline{M}_n$ values. ["Bio-Gel P300" column (5.0 × 50 cm), elution with M sodium chloride]. The arrows shown correspond with those on Fig. 2.

Sucrose and glucose have the same elution volume, which is defined as being equal to $V_o + V_i$, where V_o is the void volume and V_i the internal volume[14,16]. The elution volume of "blue dextran" (Pharmacia Ltd., Uppsala) was taken as the void volume, and values for the distribution coefficient (K_d) were calculated from the relationship[17], $K_d = (V_e - V_o)/V_i$. Figure 2 shows the elution patterns for *A. senegal*

gum, for the fractions (I–IV) precipitated by sodium sulphate, and for the degraded gum obtained by autohydrolysis of *A. senegal* gum. Table II gives the values found for K_d and $\overline{M}_n$; estimation of $\overline{M}_n$ for the whole gum was rendered difficult by the asymmetric nature of its elution curve.

TABLE II

ESTIMATION OF $\overline{M}_n$ BY MOLECULAR-SIEVE CHROMATOGRAPHY

	V_e	K_d	$\overline{M}_n$
A. senegal gum	(276)	—	—
Fraction I	270	0.00	—
Fraction II	294	0.04	140,000±20,000
Fraction III	351	0.12	99,000±10,000 (105,000)[a]
Fraction IV	532	0.40	35,000± 3,000 (37,000)[a]
Degraded gum[b]	884	0.92	4,800± 500 (4,400)[c]

[a]By osmometry; the authors thank Mr. S. Rahman for these determinations.
[b]Obtained by autohydrolysis.
[c]Periodate end-group analysis, as formaldehyde.

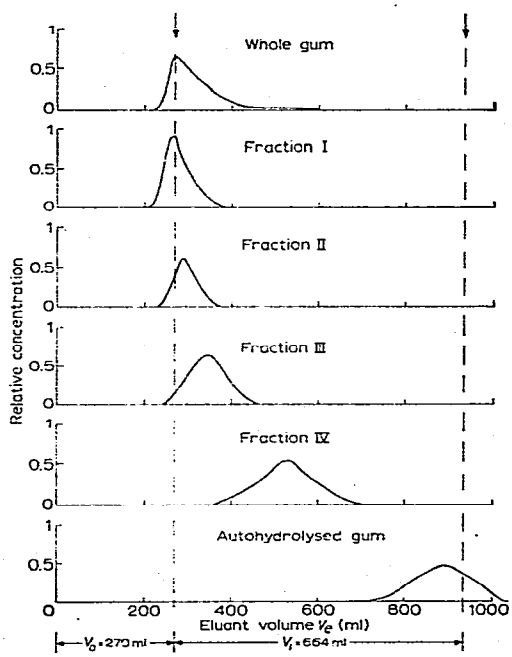

Fig. 2. Elution patterns for *A. senegal* gum, sodium sulphate fractions, and autohydrolysed gum. ["Bio-Gel P300" column (6.0 × 50 cm), elution with M sodium chloride].

DISCUSSION

Fractional precipitation of gum arabic with propan-2-ol[18] and acetone[19] has been reported. Van Beek[19] has suggested that there is a correlation between the limiting-viscosity numbers of the fractions precipitated by acetone and their content of divalent cation. In spite of the fact that metal ions were reported[20] to cause aggregation of complex acidic polysaccharides in solution, all attempts in this laboratory to repeat the experiments of Van Beek have failed.

Prolonged contact of *A. senegal* gum with organic solvents leads to insolubility difficulties, and the possibility of using salt as a fractional precipitant was therefore examined. Since the feasibility of fractional precipitation depends upon the polysaccharide in question having a broad molecular-weight distribution, *A. senegal* gum, and fractions obtained by fractional precipitation with sodium sulphate, were studied by molecular-sieve chromatography. With such fractional precipitations, the number of fractions isolated is arbitrary and is usually governed by the amount of material required for the analyses necessary to characterise the fractions. The elution patterns obtained on molecular-sieve chromatography (Fig. 2), and the limiting-viscosity numbers (Table I) of the fractions, clearly demonstrate that fractionation by "molecular" size was effected. Careful electrodialysis eliminated the possibility of traces of di- and poly-valent cations causing aggregation. Molecular-sieve chromatography and viscosity measurements were carried out in solutions having a constant concentration of univalent cations (*i.e.*, M sodium chloride). Aggregation by metal ions cannot, therefore, explain our results. Aggregation of the polysaccharide by protein is also unlikely to occur in M sodium chloride, since coacervates are broken down on addition of simple electrolytes[21]. Our results can, however, be explained by fractionation according to the molecular size of the polysaccharide.

The chemical composition of the fractions was investigated, and the results are summarised in Table I. The similar yields of formic acid released on periodate oxidation indicate that there is little variation in the degree of branching of the polysaccharides in the fractions, but the varying proportions of galactose to arabinose indicate that the gum is chemically heterogeneous. Previous evidence of chemical heterogeneity in commercial gum arabic was obtained by Heidelberger and Adams[22]; the small fraction of gum precipitated by Type II antipneumococcal horse-serum was depleted in rhamnose.

Confusion has arisen over the use of the terms *homogeneous* and *heterogeneous* in relation to plant gums and other polysaccharides. Smith and Lewis[23] claimed that the *heterogeneity* of *A. senegal* gum is revealed by electrophoresis on glass-fibre paper, whilst Jermyn[24] observed no sharp discontinuity in the properties of the molecular species after chromatography on DEAE-cellulose. *Combretum leonense* gum has been described by Aspinall and Bhavanandan[25] as *micro-heterogeneous*, *i.e.*, "a mixture of polysaccharides composed of the same structural units, which are linked in a similar manner, but are in slightly differing proportions". Norman[26] has stated that *A. senegal* gum is "not a substance of constant composition, but is con-

structed in a particular pattern from varying amounts of constituent units", and Hirst[27] has referred to it as "a mixture of closely related, molecular species". Other terms, such as *grossly heterogeneous*[25], *polydisperse*[3,28], and *polymolecular*[29], have also been employed. Unfortunately, their usage has not always been in accordance with their accepted definitions; *polydisperse* describes polymer systems containing more than one component; *polymolecular* denotes a *homogeneous* polymer having a variation in molecular weight (*cf.* ref. 20).

There is no evidence from our present investigations, nor from those of Jermyn[24], that *A. senegal* gum is *polydisperse*. On the other hand, if the gum is claimed to be *polymolecular*, the above definition of this term implies that it is a *homogeneous* polymer. The term *homogeneous* has been used[30,31] to indicate that polysaccharides are not *polydisperse*, even although, chemically, they are undoubtedly *heterogeneous*. To avoid this ambiguity, it is suggested that the term *polymolecular* be reserved for the description of those polymer systems having only a distribution in molecular weight, and the term *heteropolymolecular* be used to describe polymer systems having either a variation in monomer composition and/or a variation in the mode of linking and branching of the monomer units, in addition to a distribution in molecular weight. Defined in this way, the term *heteropolymolecular* conveys a more comprehensive description of the spectrum of related polysaccharides that comprise *A. senegal* gum.

Molecular-sieve chromatography of the degraded gum obtained on autohydrolysis (Fig. 2) indicates that $\bar{M}_n = 4{,}800$ (Table II). Degradation of the whole gum to produce units of this small size is much greater than would be expected to result from removal of labile sugar-residues (such as L-arabinofuranose and L-rhamnopyranose) from the periphery of the molecule. This observation was made by Smith and Montgomery[32], and it led them to suggest that some labile sugarresidues were present in the interior of the gum molecule. They postulated that blocks of degraded units might have been interconnected by labile residues of arabinofuranose. If this were so, it should be possible to show that some, if not all, of this arabinose is sited at the reducing end of the degraded molecules resulting from autohydrolysis. Arabinose was not, however, reported by Smith[11] to be present in the degraded gum.

This investigation shows that autohydrolysis of *A. senegal* gum results in the release of galactose residues, in addition to arabinose and rhamnose residues, with the formation of a degraded gum containing 2% of arabinose. Although autohydrolysis is sufficient to break galactopyranosidic bonds to give galactose, arabinose was not completely removed from the degraded portion that remained behind after dialysis (*cf.* ref. 33). In order to discover whether arabinose was present as the reducing end-group, the autohydrolysed, degraded gum was reduced with borohydride. The presence of galactitol, and the absence of arabinitol, in the hydrolysate of this reduced material shows that galactose occupies the reducing end-group. The 2% of arabinose in the degraded gum appears, therefore, to be sited other than at the reducing end.

Autohydrolysis is not very selective as a means of degradation. In our autohydrolysis experiments, traces of aldobiouronic acids are released, together with oligosaccharides which are small enough to pass through cellophane dialysis-tubing

(Kalle Aktiengesellschaft, Wiesbaden). Acidic material of low molecular-weight has also been obtained from autohydrolysis of gums from *A. karroo*[34] and *A. cyanophylla*[35], and this led Hirst[36] to suggest that acidic residues may occur in labile side-chains.

The methoxyl content of *A. senegal* gum has already received comment in a preliminary communication[37]. The methoxyl group is not present as the ester of D-glucuronic acid, since the methoxyl content does not decrease on treatment with N sodium hydroxide. Methoxyl groups are now known to occur commonly in plant gums in residues of 4-*O*-methyl-D-glucopyranosyluronic acid. A careful, chromatographic re-examination of the degraded gum from *A. senegal* resulted in the detection of the aldobiouronic acid, 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose, the presence of which had not been recognised by earlier investigators. The sample of *A. senegal* gum used in the present study has a methoxyl content of 0.23%; this corresponds to a content of 1.4% of 4-*O*-methyl-D-glucopyranosyluronic acid. The presence of this residue in *A. senegal* gum accounts for some of the 2,3,4-tri-*O*-methyl-D-glucuronic acid obtained after hydrolysis of the methylated whole-gum[38].

Periodate oxidation of the degraded gum produces no formaldehyde, so it may be concluded, in conjunction with methylation evidence[39], that the reducing galactose residue is substituted at C-6. As a result, borohydride-reduced, degraded gum was assumed to produce one formaldehyde molecule per average unit on periodate oxidation. On this basis, a value of 4,400 for $\overline{M}_n$ was calculated for the degraded gum.

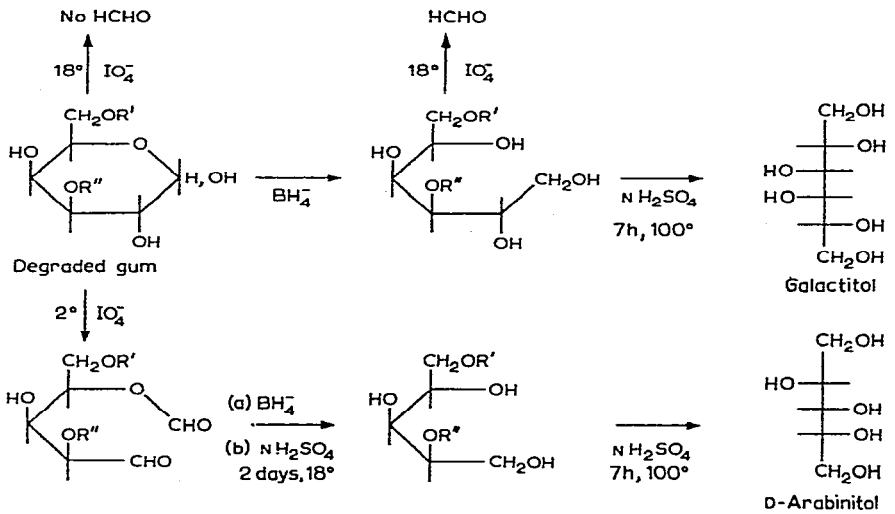

Fig. 3. Scheme of reactions carried out on autohydrolysed gum from *A. senegal*. R′ and R″ represent the remainder of the degraded gum.

The reactions carried out on the degraded gum are summarised in Fig. 3. A controlled Smith-degradation (*cf.* ref. 40) carried out at 2° provides additional

information that galactose constitutes the reducing end-group. The series of reactions proposed[32] to account for the appearance of arabinitol is evidence that the reducing galactose residues are also substituted at C-3.

If the extensive degradation of the macromolecule observed on autohydrolysis is not due to the presence of internal, labile, arabinofuranosidic bonds, certain galactopyranosidic bonds must be unusually reactive towards very mild conditions of hydrolysis, which would not normally be expected to cleave such bonds. As a result of studies on *Virgilia oroboides* gum, Stephen[41,42] has suggested that the carboxyl groups of the uronic acid residues may be responsible for "deep-seated decomposition". This fact, and the overall geometry of the *A. senegal* gum molecule, may provide the explanation for the unexpected lability of some pyranosidic bonds. More knowledge is required on the degree of branching within the molecular framework of *A. senegal* gum before a theory of more heuristic value may be advanced.

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SUMMARY

Investigations involving fractional precipitation of *A. senegal* gum by sodium sulphate lead to a discussion on the type of heterogeneity exhibited by the gum. Molecular-sieve chromatography is used to estimate number-average molecular weights. Results obtained using this chromatographic technique on the degraded gum produced on autohydrolysis indicate that such mild conditions of hydrolysis are not always very selective as a means of degradation. The degraded gum is shown to have galactose residues as reducing end-groups. There is no evidence for labile, internal, arabinofuranosyl linkages in the whole gum. In addition, chromatographic evidence is obtained for the presence of 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose residues in *A. senegal* gum.

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1,3:2,4-DI-*O*-ISOPROPYLIDENE-L-RIBITOL AND RELATED COMPOUNDS

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INTRODUCTION

Acetonation of ribitol (catalysed by mineral or Lewis acids) affords a mixture of two di-*O*-isopropylidene derivatives (*A* and *B*, isomer *B* having the longer retention time in gas–liquid chromatography). In the experiments previously described[1], catalysis by zinc chloride or hydrogen chloride afforded a mixture in which the *A*,*B*-ratio was *ca.* 2.3:1. The major isomer (*A*) was shown to possess an unblocked primary hydroxyl group and gave a benzoate (*C*) having m.p. 73–74°. Syrupy ribitol (obtained by borohydride reduction of D-ribose and possibly contaminated with boric acid) was used in these experiments; subsequent use[2] of crystalline ribitol invariably gave *A*,*B*-mixtures in which isomer *B* was markedly preponderant, although the proportion of isomers was dependent on the catalyst. Isomer *B* is[2] 1,2:4,5-di-*O*-isopropylidene-ribitol and affords a benzoate having m.p. 69–71°. The nature of the factor controlling the *A*,*B*-ratio in the earlier experiments was not discovered. We now report on isomer *A*.

RESULTS AND DISCUSSION

Since isomer *A* possesses an unblocked, primary hydroxyl group, it must contain a 1,2:3,4- (α, *α-erythro*[3]), 1,3:2,4- (*β*, *β-erythro*), or 1,4:2,3- (*γ*, *α-erythro*) distribution of the isopropylidene rings; each of these distributions constitutes a hitherto unknown type of condensation pattern. When this work was begun, there was no authenticated example of an *α-erythro-*, *β-erythro-*, or *γ*-ketal formed by direct condensation of acetone with an acyclic, polyhydric alcohol, although *α-erythro*-ketals may be obtained indirectly[4], and simple *erythro*-vicinal diols will condense with acetone[5]. A *β-erythro*-[6] and a *β*-isopropylidene derivative[7] have recently been reported**, and there are numerous examples[3,9] of the condensation of acetone with cyclic, polyhydric alcohols to give six-membered ketals as parts of fused-ring systems. Recent work[10] suggests that *γ*-ketals could be formed by the reaction of polyhydric

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**The compound described[8] as 1,2:3,5-di-*O*-isopropylidenexylitol, *i.e.* containing *α*- and *β*-ketals, is, in fact, a 1,2:4,5-diketal (ref. 15).

alcohols with a 2,2-dihalogenopropane in the presence of base. 2,2′, 5,5′-Tetrahydroxybiphenyl forms a seven-membered, 2,2′-*O*-isopropylidene derivative[11].

The retention times of isomers *A* and *B* in gas–liquid chromatography were too similar to permit their separation on a preparative scale, and although the *p*-phenylazobenzoates showed slightly different mobilities in thin-layer chromatography the ΔR_F was too small for a large-scale column separation. Tritylation of the *A,B*, mixture enabled isomer *B* to be removed subsequently by distillation and gave a crystalline trityl derivative of isomer *A*. It proved impracticable to isolate workable quantities of isomer *A* from *A,B*-mixtures derived from crystalline ribitol, and an alternative approach was used.

Acetonation of D-ribose diethyl dithioacetal gave a di-*O*-isopropylidene derivative, and demercaptalation of this compound followed by borohydride reduction of the product afforded 1,2,3,4-di-*O*-isopropylidene-L-ribitol (*A′*), which had the same retention time as isomer *A* in gas–liquid chromatography. Benzoylation of isomer *A′* gave the 5-benzoate (*C′*, m.p. 79–80°) the infrared spectrum of which, in carbon tetrachloride or carbon disulphide, was identical with the corresponding spectrum of benzoate *C*. Therefore, isomers *A* and *A′* contain the same distribution of ketal rings. The enantiomorph of compound *A′* was prepared by acetonation (zinc chloride catalysis) of 1-*O*-benzyl-L-ribitol followed by debenzylation using sodium and liquid ammonia. Benzoylation then gave 1-*O*-benzoyl-2,3,4,5-di-*O*-isopropylidene-L-ribitol. 1-Deoxy-D-ribitol also readily gave a di-*O*-isopropylidene derivative.

The infrared spectrum of a 0.005M solution of isomer *A′* in carbon tetrachloride (conditions where intermolecular hydrogen-bonding is negligible, and absorptions in the hydroxyl-stretching region may be assigned[12] to free and intramolecularly bonded hydroxyl groups) showed bands at 3637 (ε 14), 3595 (ε 55, $\Delta\nu$ 42), and 3538 cm^{-1} (ε 32, $\Delta\nu$ 99). The first band is assigned to free, primary hydroxyl groups, and, from the magnitude of the $\Delta\nu$ values[12,13], the latter absorptions are assigned to primary hydroxyl groups which are intramolecularly hydrogen-bonded to form five- and six-membered rings, respectively. On the basis of reasonable analogy, these data rule out a 1,2:3,4-diketal distribution for isomer *A′*, since, in such a molecule, the C-3–C-5 portion is closely related in structure to 1,2-*O*-isopropylideneglycerol [bands at 3647 (ε 25) and 3608 cm^{-1} (ε 49, $\Delta\nu$ 39)], which does not form an intramolecular hydrogen bond involving a six-membered ring[14]. Moreover, because the 3,4-ketal is *α-erythro*, C-2 and C-5 are *cis*-disposed and a hydrogen bond O-5-H....O-2 (seven-membered ring) should form resulting[13] in a band having $\Delta\nu$ *ca.* 140. The infrared spectral data for isomer *A′* do not permit a distinction between the 1,3:2,4- and 1,4:2,3-diketal distributions. It is of interest to note the similarity of the bands for bonded hydroxyl groups for isomer *A′* and for 1,4-dimethoxybutan-2-ol[13] [3598 ($\Delta\nu$ 31) and 3538 cm^{-1} ($\Delta\nu$ 91)].

The n.m.r. spectrum (methanol–water, 3:1) of isomer *A′* showed, *inter alia*, signals for isopropylidene methyl protons at τ 8.75, 8.79, and 8.83, having integrated areas in the ratio 1:1:2. When toluene-*p*-sulphonic acid was added to this solution at

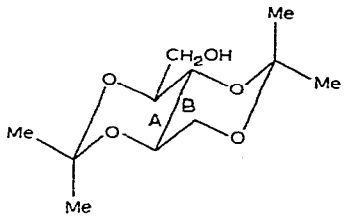
ca. 25°, the signal pattern for the methyl protons subsided and simplified during 3 h to two signals (τ 8.83 and 8.90) of similar integrated areas and having a combined area corresponding to *ca.* 15% of that of the original signals. At this stage, the hydrolysate contained ribitol, a small amount of starting material, and a mono-*O*-isopropylidene derivative (characterised as the tri-*p*-phenylazobenzoate) which was not oxidised by periodate. The latter derivative is therefore a 2,4-ketal, and it follows that, providing there is no rearrangement during the graded hydrolysis, compounds *A* and *A'* and the enantiomorph of *A'* are 1,3:2,4-di-*O*-isopropylidene-DL-, -L- (I), and -D-ribitol, respectively. The absence of ketal migration under hydrolysing conditions has been established for several isopropylidene derivatives[15].

Similar hydrolysis of the benzoate (*C'*) of compound *A'* resulted in a simplification of the initial signal pattern (τ 8.81, 8.88, and 8.98; ratio of signal strengths, 1:2:1) for the isopropylidene methyl protons to two signals (τ 8.73 and 8.79), consistent with the occurrence of graded hydrolysis. However, no such simplification occurred on hydrolysis of 1-deoxy-2,3,4,5-di-*O*-isopropylidene-D-ribitol, and the pattern of *C*-methyl proton signals (τ 8.67, 8.72, and 8.88; ratio of signal strengths, 4:5:1) subsided to leave a doublet at τ 9.00 due to the terminal methyl group.

Compound *A'* contains a ring system related to *trans*-decalin, and, although it is the first example of this type of ring system formed from two cyclic ketals, examples are known of a related ring system involving a cyclic ketal and a tetrahydropyran ring, *viz.*, methyl 4,6-*O*-isopropylidene-2,3-di-*O*-methyl-α-D-glucopyranoside[15] (signals for isopropylidene methyl protons at τ 8.81 and 8.70 in methanol–water, 3:1) and methyl 4,6-*O*-isopropylidene-α-D-altropyranoside* (8.80, 8.72). The corresponding *cis*-fused system is exemplified by methyl 4,6-*O*-isopropylidene-2,3-di-*O*-methyl-α-D-galactopyranoside[9] (8.80, 8.71), and methyl 4,6-*O*-isopropylidene-α-D-gulopyranoside* (8.90, 8.79) and its 3-acetate* (8.88, 8.77). The two signals for methyl protons shown by each of the above compounds must be due to equatorial and axial differentiation. Although the differences in chemical shift (0.08–0.11 p.p.m.) for these pairs of signals are slightly greater than that (0.05 p.p.m.) for 1-deoxy-2,3,4,5-di-*O*-isopropylidene-D-ribitol, the signal pattern for the methyl protons in the latter compound is consistent only with a 2,4:3,5-distribution of the ketal rings (*cf.* the results of Baggett *et al.*[15]). The signal pattern (τ 8.75, 8.79, and 8.83; ratio of signal strengths, 1:1:2) for the methyl protons of compound *A'* may be accounted for if one of the methyl groups is deshielded. It is possible for the primary hydroxyl group to approach significantly closer to the axial methyl group in ring B (I) than to any of the other three methyl groups. Such a close approach could cause deshielding[16]. The low-field signal (8.75) is therefore provisionally assigned to the axial methyl group in ring B. It follows that the high-field signal (8.83) may be assigned to the two, equatorial methyl groups and the remaining signal (8.79) to the axial methyl group in ring A. This assignment is tentative since the differences in chemical shift are low (<10 c.p.s.) and solvent effects have not been established. Robinson[17] has shown that, for cyclohexane derivatives

*Samples kindly provided by Dr. J. G. Buchanan.

containing *gem*-dimethyl groups, the signal for the axial methyl protons does not invariably appear at higher field. In these compounds, the long-range coupling (four σ-bonds) involving vicinal, *trans* hydrogen atoms results in a characteristic broadening of the signal for the axial methyl protons and permits reliable signal assignment. Such coupling cannot occur in the isopropylidene ketals; the signals for the isopropylidene methyl protons in, for example, methyl 4,6-*O*-isopropylidene-2,3-di-*O*-methyl-α-D-glucopyranoside had closely similar half-band widths.



I

EXPERIMENTAL

Unless otherwise stated, paper chromatography was performed on Whatman No. 1 paper by downward irrigation with the organic phase of butan-1-ol–ethanol–water (4:1:5), and detection was effected with alkaline silver nitrate[18]. Thin-layer chromatography (t.l.c.) was carried out on silica gel with detection by iodine vapour and/or vanillin–sulphuric acid[19]. Gas–liquid chromatography was effected using a Pye Argon instrument (β-ionization detection) with a column packing of polyethyleneglycol adipate and a gas pressure of 8 lb/sq in. N.m.r. spectra were determined on *ca.* 20% solutions by using a Varian A60 spectrometer under normal working conditions and a 6% solution of tetramethylsilane in chloroform as external reference. Infrared spectra in the hydroxyl-stretching region were obtained as previously described[13].

Acetonation of ribitol

(*a*) Commercial, fused zinc chloride (55 g) was shaken with acetone (500 ml) for 4 h, and the resulting mixture was stored overnight at room temperature and then decanted. Ribitol (15 g, m.p. 104–106°) was added to the supernatant solution, and, when dissolution had occured, the mixture was stored at room temperature overnight and then poured, with vigorous stirring, into 15% aqueous sodium hydroxide (3 l). The solution was extracted with chloroform in the usual manner to give a product which, on distillation, afforded the di-*O*-isopropylideneribitol mixture (14.4 g, 63%), b.p. 140–160°/30 mm. Examination by g.l.c. at 160° revealed components *A* and *B* having retention times of 15 and 18.5 min, and in the ratio *ca.* 1:4.

(*b*) When the procedure described in (*a*) was repeated, but the isolation was effected immediately after dissolution of the ribitol, the *A*,*B*-ratio was *ca.* 1:2. A similar product ratio was obtained when a rapidly prepared (*ca.* 5 min) solution of zinc chloride in acetone was used.

(*c*) When ribitol was dissolved in acetone containing 1% hydrogen chloride, and the solution was stored overnight at room temperature, the *A*,*B*-ratio for the product was *ca.* 1:2. A similar ratio of products was obtained when ribitol (0.9 g), acetone (20 ml), anhydrous copper sulphate (2 g), and conc. sulphuric acid (0.02 ml) were shaken overnight at room temperature.

1,3:2,4-Di-O-isopropylidene-5-O-trityl-DL-ribitol

Trityl chloride (7 g) was added to a solution of the di-*O*-isopropylideneribitol mixture [5 g, obtained by method (*a*) above] in pyridine (40 ml), and the mixture was stored for 1 h at 100°. Water was added to produce a turbid solution, and, after 1 h, the mixture was poured into an excess of water. Extraction with chloroform in the usual manner gave a mixture from which was distilled 1,2:4,5-di-*O*-isopropylideneribitol (*B*, 3.54 g), b.p. 140–160°/30 mm, contaminated with *ca.* 3% of isomer *A* (established by g.l.c.). The 3-benzoate had m.p. 69–71° (from methanol) (Found: C, 64.1; H, 7.4. $C_{18}H_{24}O_6$ calc.: C, 64.3; H, 7.2%).

T.l.c. (benzene–methanol, 97:3) of the residue in the still showed two components having R_F 0.88 (tritylcarbinol) and 0.94. Elution of the mixture from neutral alumina with benzene–light petroleum (b.p. 60–80°) (4:1) gave, as the first fraction, the title compound having m.p. 114–115°, R_F 0.94 (Found: C, 75.7; H, 6.9. $C_{30}H_{34}O_5$ calc.: C, 75.9; H, 7.2%).

1-Deoxy-2,3,4,5-di-O-isopropylidene-D-ribitol

A solution of D-ribose diethyl dithioacetal[20] (10 g) in 70% aqueous ethanol (700 ml) was heated under reflux with Raney nickel[21] (70 ml). The course of the reaction was followed by paper chromatography, and no starting material (R_F 0.76; *cf.* product, 0.45) remained after 4.5 h. Insoluble material was removed, and the filtrate was concentrated to give 1-deoxy-D-ribitol (1.7 g, 32%), m.p. 74–75° (from ethyl acetate), $[\alpha]_D$ +9° (*c* 1.2, water) (Found: C, 43.8; H, 9.1. $C_5H_{12}O_4$ calc.: C, 44.1; H, 8.9%). Hough *et al.*[22] recorded m.p. 77–80° for 1-deoxy-L-ribitol methanolate, and 65–69° for the solvent-free compound, $[\alpha]_D$ −10.6° in water.

A mixture of the foregoing compound (1.49 g), acetone (75 ml), anhydrous copper sulphate (3.75 g), and conc. sulphuric acid (0.04 ml) was shaken overnight at room temperature and then poured into an excess of conc. ammonia. Inorganic material was removed, and the crude product was isolated from the filtrate by extraction with ether in the usual manner. Distillation gave the title compound (1.25 g, 53%), b.p. 104-106°/28 mm, $[\alpha]_D$ +19° (*c* 1.9, chloroform) (Found: C, 61.2; H, 9.3. $C_{11}H_{20}O_4$ calc.: C, 61.1; H, 9.3%).

1-O-Benzyl-L-ribitol

A solution of 5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribitol[23] (3 g) in ethanol (47.5 ml) and N sulphuric acid (2.5 ml) was heated under reflux for 50 min. The hydrolysate was neutralised with Dowex-1 (CO_3^{2-} form), filtered, and concentrated. Examination of the syrupy residue (2.5 g) by paper chromatography [organic phase of butan-

1-ol–ethanol–water–ammonia (density 0.88), 40:10:49:1, Whatman No. 4 paper] revealed one component having $R_{Ribitol}$ 3.2. On storage, the syrup crystallized, and recrystallization from chloroform–light petroleum (b.p. 60–80°) gave the title compound (1.3 g), m.p. 69–70°, $[\alpha]_D$ +10° (*c* 2.5, water) (Found: C, 59.4; H, 7.3. $C_{12}H_{18}O_5$ calc.: C, 59.5; H, 7.4%).

1,3:2,4-Di-O-isopropylidene-D-ribitol

Zinc chloride (3 g) was shaken with acetone (15 ml), and the supernatant liquid, on cooling, was decanted onto the foregoing benzyl ether (1 g). The mixture was shaken until dissolution was complete and then stored at room temperature for 10 h. The di-*O*-isopropylidene derivative was isolated as a syrup (1.1 g) by the use of ether[24]. A solution of this product in ether (12 ml) was added to liquid ammonia (300 ml), and sodium (1 g) was added in small pieces with stirring during 1 h. The solvent was then allowed to evaporate at room temperature, and water (80 ml) was carefully added to the residue. The solution was extracted with chloroform, and the dried (K_2CO_3) extract was concentrated. A solution of the residue (0.45 g) in benzene was added to alumina (12 g), and elution was effected with chloroform to give 1,3:2,4-di-*O*-isopropylidene-D-ribitol (0.35 g), which afforded a benzoate having m.p. 82° (from methanol) and $[\alpha]_D$ +33° (*c* 2.3, chloroform) (Found: C, 64.3; H, 7.0. $C_{18}H_{24}O_6$ calc.: C, 64.3; H, 7.2%). 1-*O*-Benzoyl-2,4:3,5-di-*O*-isopropylidene-D-ribitol[1] had m.p. 82° (from methanol) and $[\alpha]_D$ −32° in chloroform. The infrared spectra of the two benzoates were indistinguishable.

Graded, acid hydrolysis of 1,3:2,4-di-O-isopropylidene-L-ribitol

A solution of the title compound (57.3 mg) in 75% aqueous methanol (4 ml) containing toluene-*p*-sulphonic acid (78 mg) was stored at room temperature, and the n.m.r. spectrum was recorded at suitable intervals. When the original signal pattern (τ 8.75, 8.79, and 8.83) for the isopropylidene methyl protons had simplified to two signals (τ 8.83 and 8.90) of approximately equal intensity (180 min), the solution was neutralised with Amberlite IRA-400 (HO^- form) and concentrated. T.l.c. (dichloromethane–methanol, 9:1) of the residue (*Z*, 202 mg) from a similar experiment revealed components having R_F values of 0.0, 0.59, and 1.0, and paper chromatography (detection with Tollens reagent[24]) revealed components having R_F values of 0.18 (ribitol) and 0.68. Extraction of the residue with hot chloroform gave a mixture of the faster-moving components (t.l.c.), leaving ribitol (50 mg), m.p. 102–104°. The chloroform extract was added to a column (1 × 9.5 cm) of silica gel (5 g, Hopkin and Williams). Elution with ether (50 ml) afforded starting material (50 mg), and a further amount (50 ml) of the same solvent gave the mono-*O*-isopropylidene derivative (20 mg, R_F 0.59), which was *p*-phenylazobenzoylated in the usual manner[25] to give 2,4-*O*-isopropylidene-1,3,5-tri-*O*-*p*-phenylazobenzoylribitol, m.p. 165–167° (from chloroform–ethanol) (Found: C, 69.3; H, 5.3; N, 10.6. $C_{47}H_{40}N_6O_8$ calc.: C, 69.0; H, 4.9; N, 10.3%).

A solution of the residue *Z* in an excess of 0.02M sodium metaperiodate contain-

ing sodium hydrogen carbonate was stored for 2 days at room temperature. The solution was then concentrated, and the residue was extracted with hot ethyl acetate. Concentration of the combined extracts and examination of the residue by t.l.c. revealed unaltered proportions of the components having R_F values of 0.59 and 1.0.

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SUMMARY

Acetonation of ribitol (catalysed by mineral or Lewis acids) affords a 1,2:4,5- and a 1,2,3,4-di-*O*-isopropylidene derivative. Evidence is presented which indicates that the latter isomer has a 1,3:2,4-distribution of the ketal rings.

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OBSERVATIONS ON ESTERIFICATION REACTIONS

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INTRODUCTION

A variety of reactions has been reported[1] in which intramolecular hydrogen-bonding has been invoked to account for rate enhancement and/or reaction pattern. In previous papers[2], we have examined the validity of a correlation drawn between patterns of intramolecular hydrogen-bonding, observable[3] for alcohols in dilute solutions in inert solvents, and reactivity effects which these alcohols display in various reactions carried out in the usual range of experimental conditions. For example, the ratio of the rate constants for the esterification of the 5-hydroxy-2-phenyl-1,3-dioxans with *p*-phenylazobenzoyl chloride in pyridine[2] is *cis*:*trans*, *ca.* 5.6:1, whereas for the 4-phenylcyclohexanols, under similar conditions, the *cis*:*trans* ratio is *ca.* 1:6.6, as would be expected[4] on conformational grounds. In dilute solution in carbon tetrachloride, no intramolecular hydrogen-bonding is possible for the 4-phenylcyclohexanols, but complete bonding occurs[5] in *cis*-5-hydroxy-2-phenyl-1,3-dioxan, and only limited bonding in the *trans*-isomer. For 1,4:3,6-dianhydro-D-glucitol (I), the apparently more sterically-hindered *endo*-5-hydroxyl group was found to be more reactive than the *exo*-2-hydroxyl group on esterification with toluene-*p*-sulphonyl chloride[6] or *p*-phenylazobenzoyl chloride[2] in pyridine. Only the *endo*-5-hydroxyl group can form an intramolecular hydrogen-bond.

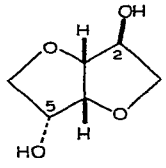
RESULTS AND DISCUSSION

In seeking clarification of the above and related reactivity differences, the rate constants for the esterification of the alcohols listed in Table I, using acetic anhydride in pyridine at 25°, were measured. Within the limits of experimental error, the esterifications showed second-order kinetics although, in some cases, small deviations were observed beyond 60% reaction. The rate constants were measured by essentially Eliel and Lukach's method[7] and calculated graphically. The values for cyclohexanol and *cis*- and *trans*-4-phenylcylohexanol are in reasonably good agreement with those previously reported[7].

The results in Table I show that, in accordance with expectation based[4] on conformational grounds, the *trans*-isomers of the *cis*,*trans*-pairs of 4-phenylcyclohexanol and 5-hydroxy-2-phenyl-1,3-dioxan are the more rapidly esterified. It was

noted above that the order of reactivity of the latter pair of isomers is reversed when esterification is effected with *p*-phenylazobenzoyl chloride in pyridine. The values for the rate constants were reflected in competition experiments. When a solution of 1 mol. of acetic anhydride in pyridine was allowed to react with a mixture containing 1 mol. each of *cis*- and *trans*-5-hydroxy-2-phenyl-1,3-dioxan, the ratio of *cis*- to *trans*-acetates formed was *ca.* 1:5.8 (determined by n.m.r. spectroscopy). Comparison of the rate constants for *trans*-5-hydroxy-2-phenyl-1,3-dioxan and *trans*-4-phenylcyclohexanol, and for 5-hydroxy-1,3-dioxan and cyclohexanol, indicates that replacement of ring methylene groups by oxygen atoms causes an increase in the reactivity of the hydroxyl group towards acetic anhydride in pyridine.

For the esterification of 1,4:3,6-dianhydro-D-glucitol (I), the relative reactivities of the two hydroxyl groups are dependent on the reaction conditions. Thus, when the dianhydride was treated with 1 mol. of acetic anhydride in pyridine at 25°, the ratio of 2- to 5-acetate was *ca.* 1.7:1, whereas, when a similar reaction was carried out in the presence of pyridine hydrochloride, the ratio was 1:3.6. The latter result parallels that observed when esterification was effected with *p*-phenylazobenzoyl chloride in pyridine[2]. Related results were obtained with the 2-methoxycyclopentanols. The hydroxyl groups in the *cis*- (II) and *trans*-isomer (III) are, respectively, in similar steric environments to the *endo*-5- and *exo*-2-hydroxyl groups in 1,4:3,6-dianhydro-D-glucitol (I). The rate constants in Table I show that the *trans*-isomer (III) is more reactive. It appears that, in these five-membered ring compounds, esterification by acetic anhydride in pyridine is sterically hindered by a substituent vicinal and *cis* to the hydroxyl group, and that the orders of reactivity are the reverse of those to be expected for activation by intramolecular hydrogen-bonding. Only the hydroxyl group at C-5 in the dianhydride[2] (I), and in *cis*-2-methoxycyclopentanol[8] (II), can form an intramolecular hydrogen-bond.



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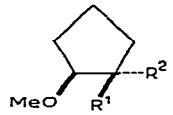


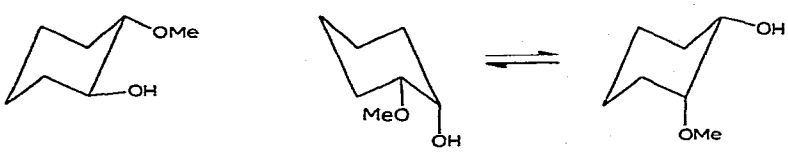
II R^1=OH, R^2=H

III R^1=H, R^2=OH

In the 2-methoxycyclohexanol series, the *trans*-isomer is esterified more rapidly (Table I). Since the *trans*-isomer should adopt the chair conformation (IV) having the hydroxyl group equatorial, while the *cis*-isomer is likely to be conformationally unstable with the hydroxyl group moving between axial (V) and equatorial positions (VI), the observed order of reactivity is not surprising. In dilute solution in carbon tetrachloride, the hydroxyl groups in both *cis*- and *trans*-2-methoxycyclohexanol[8] are completely intramolecularly hydrogen-bonded.

In another group of experiments, the rates of acetylation of ethanol, propan-1-ol, butan-1-ol, 2-methoxyethanol, 3-methoxypropan-1-ol, and 4-methoxybutan-1-ol

were measured (Table I). With the exception of 2-methoxyethanol, the magnitudes of the rate constants were similar, and there was no rate-enhancement effect. The slightly higher value for 2-methoxyethanol is probably due to the inductive effect of the methoxyl group. In dilute solution in carbon tetrachloride, the ω-methoxyalkan-1-ols show[9] characteristic and significantly different patterns of intramolecular



IV V VI

hydrogen-bonding, and bond formation decreases in the order 2-methoxyethanol> 3-methoxypropan-1-ol>4-methoxybutan-1-ol. Thus, it may be concluded that, within the series of alcohols studied, there is no correlation between the patterns of intramolecular hydrogen-bonding observed for dilute solutions in carbon tetrachloride and the rate constants measured for esterification with acetic anhydride and pyridine.

It has been severally observed that the use of acid anhydrides in place of acid chlorides during esterification can cause differences in the order of reactivity of hydroxyl groups. Thus, the 2-ester was the preponderant product when methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[10] was treated with one equivalent of a carboxylic acid chloride, or a sulphonic acid chloride or anhydride, in pyridine, whereas use of a carboxylic anhydride gave a preponderance of the 3-ester. However, it is possible that, in each case, esterification occurs selectively at position 2, but is followed by an acyl migration in the latter case. In another example[11], treatment of benzyl 4-*O*-methyl-β-D-xylopyranoside with one equivalent of acetyl chloride in pyridine gave the 2- and 3-esters in the ratio of 1.1:1, whereas, with acetic anhydride in pyridine, the ratio was 1.7:1. Furthermore, on partial esterification with acetic anhydride–perchloric acid and acetic anhydride–sodium acetate, the ratios were 1:3 and 2:1, respectively.

These and the above differences in reactivity indicate the operation of at least two different esterification mechanisms. It has been shown[12] that, in the esterification of ethanol with acetic anhydride, the observed rate of reaction falls between the theoretical values calculated on the assumption that the reaction involves (*a*) unionised molecules of ethanol and acetic anhydride (1), or (*b*) un-ionised acetic anhydride and ethoxide ion (2). Although the catalytic effect of pyridine on the esterification of

$$EtOH + Ac_2O \rightarrow \text{products} \quad (1)$$

$$EtOH \rightleftharpoons EtO^- + H^+ \quad (2)$$

$$EtO^- + Ac_2O \rightarrow \text{products}$$

alcohols has long been known, the precise mechanism remains to be elucidated. The evidence for different pathways has recently been reviewed[13]. Since carboxylic and

sulphonic acid chlorides [14], and sulphonic anhydrides [15,16], are known to form complexes with pyridine, probably of the type R·CO·N$^+$⟨⟩Cl$^-$ and R·SO$_2$.N$^+$⟨⟩X$^-$, it has been suggested[17] that the higher reactivity of these pyridinium salts is responsible for the catalytic activity of pyridine. On the other hand, base enhanced ionisation of alcohols has been invoked[18] to explain the catalytic effect of pyridine, but it fails to explain the low catalytic activity of 2- and 2,6-di-substituted pyridine derivatives[17] in the acetylation of water.

Efforts to detect complex formation between acetic anhydride and pyridine have failed[17], although the presence of very small amounts of such an intermediate has been invoked[19] to explain the catalytic effect of pyridine on the acetylation of water by acetic anhydride in acetic acid–acetate buffers. The mechanism formulated is shown in equations (3) and (4), and the latter stage was found to be rate determining.

$$Ac_2O + \text{pyridine} \rightleftharpoons \text{pyridineAc}^+ + AcO^- \quad (3)$$

$$\text{pyridineAc}^+ + H_2O \rightarrow AcOH + \text{pyridineH}^+ \quad (4)$$

Thus, it is probable that esterification by acyl halides and anhydrides in pyridine involves a rate-determining attack by an alcohol (5), or an alkoxide ion (6), on an acyl pyridinium salt, but the available evidence is not sufficient to confirm either of

$$R\cdot OH + \text{pyridineAc}^+ \rightarrow \text{products} \quad (5)$$

$$R\cdot OH \rightleftharpoons R\cdot O^- + H^+ \quad (6)$$

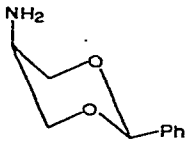
$$\text{pyridineAc}^+ + R\cdot O^- \rightarrow \text{products}$$

these possibilities. If the overall rate of reaction were dominated by process (5), it might be enhanced by intramolecular hydrogen-bonding and decreased by vicinal electronegative substituents in the alcohol[20], whereas, if process (6) were involved the reverse would apply. The results in Table I, together with previous results[2], are in accord with the assumption that esterification with *p*-phenylazobenzoyl chloride in pyridine occurs mainly by process (5), and that esterification with acetic anhydride and pyridine occurs mainly by process (6), but do not establish these processes to be the reaction mechanisms. As far as we are aware, the occurrence of intramolecular hydrogen-bonding in suitable alcohols in pyridine solution has not been established experimentally. Findlay and Kidman[21] have shown that, for solutions of alcohols (<0.1 M) and pyridine (>0.1 M) in carbon tetrachloride, intramolecular hydrogen-bonding between alcohol molecules occurs to a significant extent, although bonding mainly involves alcohol and pyridine molecules. It is possible that, for suitable alcohols, intramolecular hydrogen-bonding could also occur to a limited extent.

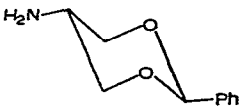
In extending the above study, the rates of acetylation of *cis*- and *trans*-5-amino-2-phenyl-1,3-dioxan with acetic anhydride were examined, since, in this case, prior

ionization of the amine is unlikely, and it might be predicted that the reactivity of the *cis*-amine would be enhanced by intramolecular hydrogen-bonding.

trans-5-Amino-2-phenyl-1,3-dioxan was prepared by treatment of the *cis*-5-methanesulphonate[22] with sodium azide in *N,N*-dimethylformamide, followed by reduction of the resultant *trans*-5-azido compound with lithium aluminium hydride. The *cis*-5-amine was prepared likewise from the *trans*-5-methanesulphonate. In dilute solution (<0.005M), the *cis*-amine (VII) had ν_{max} at 3389 (ε 13) and 3328 cm^{-1} (ε 3.1), while the *trans*-amine (VIII) had ν_{max} at 3397 (ε 8) and 3331 cm^{-1} (ε 2.8) in the N–H stretching region. The absorption patterns for the two amines and for cyclohexylamine[23] [ν_{max} at 3375 (ε 3.3) and 3311 cm^{-1} (ε 2.1)] were closely similar, which suggests that the absorption maxima in each isomer are due to asymmetric and symmetric stretching vibrations of the NH_2 group. Intramolecular hydrogen-bonding would be expected[24] to lower the stretching frequencies.



VII



VIII

When a 1:1 mixture of *cis*- (VII) and *trans*-5-amino-2-phenyl-1,3-dioxan (VIII) was allowed to react with 1 mol. of acetic anhydride in pyridine, the *cis*- and *trans*-acetates were formed in the ratio *ca.* 1.9:1. A similar order of reactivity was found by Eliel *et al.*[25] for the 4-*tert*-butylcyclohexylamines. The *cis*-isomer reacted with 2,4-dinitrochlorobenzene at twice the rate of the *trans*-compound. Unusual stabilisation of the equatorial amino group in the *trans*-isomer by solvation was invoked to explain the reactivity difference.

EXPERIMENTAL

Preparation of alcohols

5-Hydroxy-1,3-dioxan, prepared by saponification of the benzoate[26], had b.p. 85–87°/15 mm. Cyclohexanol and cyclopentanol were fractionally distilled through a 30-cm column packed with glass helices. The *cis*- (m.p. 75–76°) and *trans*-forms (m.p. 119°) of 4-phenylcyclohexanol[27], the *cis*[28] (m.p. 82–83°) and *trans*-forms[22] (m.p. 63–64°) of 5-hydroxy-2-phenyl-1,3-dioxan, and the *cis*- and *trans*-forms of 2-methoxycyclohexanol and 2-methoxycyclopentanol[8] were prepared by the relevant literature methods.

Determination of rate constants

The rates of esterification of alcohols with acetic anhydride and pyridine at 25.0 ± 0.05° (corr.) were measured by essentially Eliel and Lukach's method[7]. Rate constants were obtained from the slopes of graphs of either $1/a-x$ against time, or $\log_e b(a-x)/a(b-x)$ against time, where a and b are the initial concentrations of

TABLE I

RATE CONSTANTS FOR THE ESTERIFICATION OF CERTAIN ALCOHOLS WITH ACETIC ANHYDRIDE IN PYRIDINE AT 25°

	$k \times 10^5$ ($l.mole^{-1}sec^{-1}$)
Secondary alcohols	
cis-4-Phenylcyclohexanol	4.0
trans-4-Phenylcyclohexanol	11.9
Cyclohexanol	8.9
cis-5-Hydroxy-2-phenyl-1,3-dioxan	6.0
trans-5-Hydroxy-2-phenyl-1,3-dioxan	49.5
5-Hydroxy-1,3-dioxan	31.8
Cyclopentanol	10.6
cis-2-Methoxycylopentanol	4.2
trans-2-Methoxycyclopentanol	15.2
cis-2-Methoxycyclohexanol	2.9
trans-2-Methoxycyclohexanol	9.7
Primary alcohols	
Ethanol	55.9
2-Methoxyethanol	68.0
Propan-1-ol	55.7
3-Methoxypropan-1-ol	55.6
Butan-1-ol	55.6
4-Methoxybutan-1-ol	58.3

alcohol and acetic anhydride, and x is the concentration of ester formed. Good, straight-line plots were obtained up to *ca.* 60% reaction but, in some cases, deviation from linearity was observed when the reaction was studied from 60–90% completion. The cause of this deviation was not investigated.

All of the reactions proceeded to >97% completion, as determined from infinity titrations, and examination by t.l.c. indicated that the ester was the only product formed. In the case of *cis*- and *trans*-5-hydroxy-2-phenyl-1,3-dioxan, the respective acetates were isolated in yields of >95% from infinity samples. The results are shown in Table II.

Selective acetylation of 1,4:3,6-dianhydro-D-glucitol

(*a*) *With acetic anhydride and pyridine.*—A solution of the dianhydride (5.53 g) and acetic anhydride (4 g, 1 mol.) in pyridine (50 ml) was stored at 25° for 23.5 h and then poured into 2% aqueous sodium hydrogen carbonate (200 ml). The solution was extracted exhaustively with chloroform, and the dried ($MgSO_4$) extract was concentrated. Fractionation of the resulting syrup (5.89 g) on a column (50 × 3.5 cm) of silica gel (Davison 950) by elution with benzene–ether mixtures gave the 2,5-di-acetate (2.46 g, 56.5%), m.p. 59–60° (from ethanol), and then 2-*O*-acetyl-1,4:3,6-dianhydro-D-glucitol (1.54 g, 28.6%), m.p. 77.5–78.5° [from benzene–light petroleum (b.p. 60–80°)], ν_{max} (*c* 0.004M, carbon tetrachloride) 3562 cm^{-1} (bonded[2] OH) (Found: C, 51.1; H, 6.5. $C_8H_{12}O_5$ calc.: C, 51.1; H, 6.5%). Finally, 5-*O*-acetyl-1,4:3,6-dianhydro-

TABLE II

EXPERIMENTAL RATE CONSTANTS FOR ACETYLATION OF CERTAIN ALCOHOLS WITH ACETIC ANHYDRIDE IN PYRIDINE AT 25°

Alcohol	*Alcohol concentration (mole/l)*	*Acetic anhydride concentration (mole/l)*	$k \times 10^5$ ($l.mole^{-1}sec^{-1}$)
Cyclohexanol	0.5550	0.5550	8.9
	1.1571	0.5246	9.0
cis-4-Phenylcyclohexanol	0.5310	0.5310	4.0
trans-4-Phenylcyclohexanol	0.4936	0.4936	11.9
cis-5-Hydroxy-2-phenyl-1,3-dioxan	0.5474	0.5474	6.0
	0.2709	0.5916	5.5
	0.5595	1.1545	5.6
	0.5588	0.2758	6.1
	0.9031	0.4563	6.8
trans-5-Hydroxy-2-phenyl-1,3-dioxan	0.5471	0.4571	49.6
	0.5369	0.5369	49.5
5-Hydroxy-1,3-dioxan	0.5657	0.5657	31.9
	0.6602	0.6602	31.7
	0.6756	1.3279	30.1
	0.2113	0.6068	31.1
	1.2915	0.5341	34.7
	0.6708	0.3198	34.1
Cyclopentanol	0.5555	0.5555	10.6
	0.5562	0.5562	10.6
cis-2-Methoxycyclopentanol	0.5136	0.5136	4.23
	0.4872	0.4872	4.26
trans-2-Methoxycyclopentanol	0.5136	0.5136	15.3
	0.4840	0.4840	15.2
cis-2-Methoxycyclohexanol	0.4684	0.4684	2.9
trans-2-Methoxycyclohexanol	0.4152	0.4152	9.7
	0.4311	0.4311	9.7
Ethanol	0.6061	0.6061	56.2
	0.6061	0.6061	55.5
Propan-1-ol	0.6073	0.6073	55.7
	0.6075	0.6075	55.6
Butan-1-ol	0.5961	0.5961	56.3
	0.5941	0.5941	54.9
	0.5894	1.1788	52.2
	0.5852	1.1736	51.3
2-Methoxyethanol	0.5964	0.5964	67.2
	0.5945	0.5945	68.7
	0.3076	0.6153	66.8
	0.3076	0.6150	68.1
3-Methoxypropan-1-ol	0.6016	0.6016	55.8
	0.6008	0.6008	55.4
4-Methoxybutan-1-ol	0.5939	0.5929	58.7
	0.5929	0.5929	57.9

D-glucitol (1.20 g, 16.9%) was obtained, having b.p. 125–126°/0.2 mm, $\nu_{max}^{CCl_4}$ 3625 (free OH) and 3596 cm^{-1} (shoulder, due to contaminant dianhydride) (Found: C, 51.25; H, 6.3%).

In a duplicate experiment, the yields (calculated on acetic anhydride) were 2,5-di-acetate, 57.8%; 2-acetate, 27.6%; and 5-acetate, 17.1%.

(*b*) *With acetic anhydride, pyridine, and pyridine hydrochloride.* A solution of the dianhydride (5.67 g), acetic anhydride (3.88 ml, 1 mol.), and pyridine hydrochloride (2 g) in pyridine (50 ml) was stored at 25° for 24 h. The acetates were isolated and fractionated as in (*a*) to give the 2,5-diacetate (2.10 g, 47%), the 2-acetate (0.88 g, 12%), and the 5-acetate (3.14 g, 42.9%).

When either of the mono-acetates was dissolved in pyridine, or pyridine containing pyridine hydrochloride, and then isolated as in (*a*), acyl migration did not occur.

5-O-Acetyl-1,4:3,6-dianhydro-2-O-p-phenylazobenzoyl-D-glucitol

Acetylation of 1,4:3,6-dianhydro-2-*O*-*p*-phenylazobenzoyl-D-glucitol[2] with acetic anhydride and pyridine, in the usual manner, gave the title compound, m.p. 95–96° (from ethanol) (Found: C, 63.55; H, 5.1; N, 6.9. $C_{21}H_{20}N_2O_6$ calc.: C, 63.6; H, 5.1; N, 7.1%).

The same product was formed on *p*-phenylazobenzoylation of the 5-acetate described above.

2-O-Acetyl-1,4:3,6-dianhydro-5-O-p-phenylazobenzoyl-D-glucitol

Acetylation of 1,4:3,6-dianhydro-5-*O*-*p*-phenylazobenzoyl-D-glucitol[2] with acetic anhydride and pyridine, in the usual manner, gave the title compound, m.p. 145–146° (from ethanol) (Found: C, 63.7: H, 5.1: N, 7.0%).

The same product was obtained on *p*-phenylazobenzoylation of the 2-acetate described above.

5-Azido- and 5-amino-2-phenyl-1,3-dioxans

A mixture of sodium azide (3.9 g) and *cis*-5-methanesulphonyloxy-2-phenyl-1,3-dioxan[23] (5 g) in *N*,*N*-dimethylformamide was boiled under reflux for 6 h. The cooled mixture was diluted with chloroform (200 ml) and washed with water (80 ml). The aqueous washings were washed with chloroform (2 × 40 ml), and the combined chloroform solutions were washed with water (60 ml), decolourised with charcoal, dried ($MgSO_4$), and concentrated. Recrystallisation of the residue from light petroleum (b.p. 60–80°) gave *trans*-5-azido-2-phenyl-1,3-dioxan (2.2 g, 55%), m.p. 65.5–66.5°, $\nu_{max}^{CHCl_3}$ 2123 cm^{-1} (azide) (Found: C, 58.9; H, 5.4; N, 20.3. $C_{10}H_{11}N_3O_2$ calc.: C, 58.6; H, 5.4; N, 20.5%).

By essentially the same method, *trans*-5-methanesulphonyloxy-2-phenyl-1,3-dioxan[23] was converted into the *cis*-5-azido derivative (69%), b.p. 120–124°/0.15 mm, $\nu_{max}^{CHCl_3}$ 2121 cm^{-1} (azide) (Found: C, 58.9; H, 5.7; N, 20.3%).

A solution of the *trans*-5-azido compound (1.6 g) in ether (25 ml) was added dropwise to lithium aluminium hydride (0.6 g) at such a rate that gentle boiling occurred, and boiling was continued for an additional hour. Ethyl acetate (1.8 ml), ether (25 ml), and water (1.3 ml) were then added successively, and the mixture was boiled for 10 min.

Insoluble material was collected and washed with ether. Concentration of the combined filtrate and washings, and recrystallisation of the residue from light petroleum (b.p. 60–80°) gave *trans*-5-amino-2-phenyl-1,3-dioxan (1.14 g, 82%), m.p. 53–54° (Found: C, 66.9; H, 7.5; N, 7.8. $C_{10}H_{13}NO_2$ calc.: C, 67.1; H, 7.3; N, 7.8%). The *N*-acetyl derivative, prepared by the standard procedure, had m.p. 200–200.5°, $\nu_{max}^{CHCl_3}$ 1680 cm^{-1} (C=O) (Found: C, 64.8; H, 6.8; N, 6.0. $C_{12}H_{15}NO_3$ calc.: C, 65.1; H, 6.8; N, 6.3%).

By essentially the above method, the *cis*-5-azido compound was converted into the *cis*-5-amino derivative (58%), b.p. 130–134°/0.6 mm (Found: C, 66.8; H, 7.0; N, 7.8%) and thence into the *cis*-5-acetamido compound, m.p. 152–153°, $\nu_{max}^{CHCl_3}$ 1666 cm^{-1} (C=O) (Found: C, 65.1; H, 7.0; N, 6.2%).

Competitive acetylation of the 5-amino-2-phenyl-1,3-dioxans

Freshly distilled acetic anhydride (0.055 ml, 1 mol.) was added to a solution of *cis*- and *trans*-5-amino-2-phenyl-1,3-dioxan (100 mg of each) in pyridine (0.5 ml) and, after storage of the mixture overnight, saturated aqueous sodium hydrogen carbonate was added. Extraction with chloroform (3 × 10 ml), and concentration of the combined and dried ($MgSO_4$) extracts gave a residue which was analysed by n.m.r. spectroscopy, as described below. A similar, competitive acetylation was carried out using a mixture of *cis*- and *trans*-5-hydroxy-2-phenyl-1,3-dioxan.

The n.m.r spectra were obtained by using a Varian A60 spectrometer on *ca.* 10% solutions in chloroform with a 6% solution of tetramethylsilane in chloroform as external reference. Resonance for the acetyl protons in the *cis*- and *trans*-5-acetamido derivatives occurred at τ 7.98 and 8.03, respectively, and in the *cis*- and *trans*-5-acetoxy compounds at 7.86 and 7.96, respectively. Integration of the peak areas (expanded scale, 100-sec sweep-time) gave the proportions of individual acetates. The validity of the method was established by using standard mixtures. In the competitive acetylation of the 5-amino compounds, the *cis,trans* ratio of the resulting acetates was 1.45, and for the 5-hydroxy compounds, 0.17.

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SUMMARY

The rate constants for the esterification of a series of alcohols with acetic anhydride at 25° have been determined. No rate-enhancement effect attributable to intramolecular hydrogen-bonding was observed, in contrast to esterifications with acid chlorides and sulphonyl chlorides in pyridine. With acetic anhydride in pyridine, 1,4:3,6-dianhydro-D-glucitol is selectively acetylated at position 2. If pyridine hydrochloride is also present in the mixture, selective esterification occurs at position 5. The mechanisms of these and related reactions is considered.

cis-5-Amino-2-phenyl-1,3-dioxan is more rapidly esterified with acetic anhydride in pyridine than is the *trans*-isomer; parallel results have been observed in the cyclohexane series.

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BRÄUNUNGSREAKTIONEN UND FRAGMENTIERUNGEN VON KOHLENHYDRATEN

TEIL I. DIE FLÜCHTIGEN ABBAUPRODUKTE DER PYROLYSE VON D-GLUCOSE

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(Eingegangen den 3. Dezember, 1965)

EINLEITUNG

Beim Erhitzen von D-Glucose oberhalb des Schmelzpunktes beginnt ab 150–160° unter Gelbfärbung ein pyrolytischer Abbau, der bei steigender Temperatur und fortlaufender Erhitzungsdauer zu stark dunkel gefärbten polymeren Verbindungen als Rückstand führt. Während des Erhitzens entweicht ein Gemisch von Wasser, CO_2 und organischen Abbauprodukten. In der vorliegenden Untersuchung wurde D-Glucose unter Standardbedingungen rasch auf 300° erhitzt und die dabei gebildeten flüchtigen Abbauprodukte untersucht.

Gaschromatographische Untersuchungen über den thermischen Abbau von Kohlenhydraten wurden bisher an Cellulose[1,2,3], Hydroxyäthylstärke[4] und insbesondere von Bryce und Greenwood[5,6] an Stärke und verschiedenen Mono- und Disacchariden durchgeführt, nachdem schon Cerniani[7] mit Hilfe der konventionellen Gasanalyse die Zusammensetzung einiger dabei gebildeter, nicht kondensierbarer Gase aufgeklärt hatte. Ferner sind im Aroma des gerösteten Kaffees eine Reihe von Abbauprodukten identifiziert worden, deren Entstehung zum grossen Teil auf den Gehalt an Kohlenhydraten in der Kaffeebohne zurückzuführen ist[8,9,17,18].

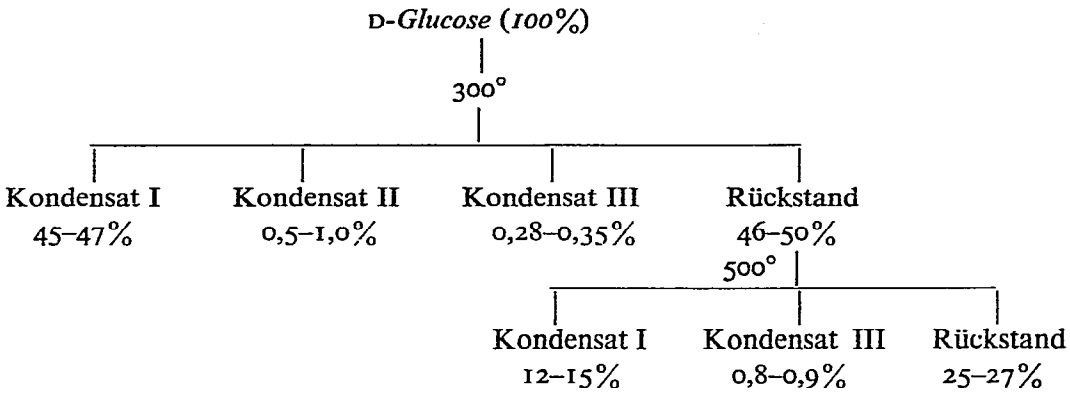
Eine Zuordnung der Substanzpeaks in den Gaschromatogrammen allein durch Vergleich der Retentionszeiten, wie sie von Bryce und Greenwood durchgeführt wurde, kann nach unseren Erfahrungen zu Fehlschlüssen führen. Bei der thermischen Fragmentierung von Zuckern entstehen Stoffe aus verschiedenen Substanzklassen in so grosser Zahl, dass auch mit Kapillarsäulen hoher Trennleistung eine hinreichende Auftrennung nicht für alle Komponenten erreicht werden kann. Weitere chemische oder physikalische Methoden, denen spezielle Anreicherungs- und Nachweisverfahren vorausgehen, müssen zur sicheren Identifizierung herangezogen werden. So erfolgte in dieser Untersuchung nach gaschromatographischer Trennung die Identifizierung durch eine Kombination mikroanalytischer Methoden mit der Massenspektrometrie.

METHODEN UND RESULTATE

Pyrolyseapparatur und Ausführung der Pyrolyse

Das Erhitzen von Kohlenhydraten auf höhere Temperaturen ist mit einer

heftigen Gasentwicklung verbunden, die zum Aufblähen der Schmelze führt. Da der Anteil flüchtiger organischer Stoffe gegenüber Wasser und dem nichtflüchtigen Rückstand klein ist, müssen jeweils 100 g D-Glucose unter gleichbleibenden Bedingungen eingesetzt werden, wenn man präparativ trennbare Mengen erhalten will. Die Schwierigkeiten, reproduzierbare Ergebnisse bei diesen Mengen zu erreichen, bestehen in einer exakten Wärmeführung. Ein möglichst schneller Temperaturanstieg ist anzustreben, da Umwandlungsprodukte, die bei tiefer Temperatur entstehen, den Verlauf der Zersetzung bei höherer Temperatur beeinflussen und Sekundärreaktionen liefern können. Ferner soll das Temperaturgefälle innerhalb der abzubauenden Probe möglichst gering sein[10]. Die Pyrolyse erfolgte daher in 2–6 cm weiten Glasrohren bei 300° in einem Stickstoffstrom, der entsprechend vorgeheizt wurde. Die Temperaturen der Rohrwand und des Gasstromes wurden durch Thermoelemente gemessen. Inhomogenitäten der Beheizung und ein Verstopfen des Rohres bei der Pyrolyse lassen sich durch Vermischen der D-Glucose mit grobkörnigem Quarzsand (Verhältnis 1:15) vermeiden. In drei hintereinander geschalteten Kühlfallen wurden 3 Fraktionen erhalten.



Das *Kondensat I* (Menge 45–47% bezogen auf D-Glucose) enthält eine bei +60° am absteigenden Kühler kondensierte Fraktion, die sauer reagiert (pH 2,5–3,0) und eine violette Eisen(III)Chlorid-Reaktion zeigt; sie enthält viel Wasser und schwerer flüchtige, meist wasserlösliche Anteile.

Das *Kondensat II* (Menge 0,5–1,0%) wurde bei 0°C im Eisbad kondensiert und besteht ebenfalls überwiegend aus Wasser, in dem leichtflüchtige wasserlösliche Stoffe enthalten sind. Diese Fraktion wurde nach gaschromatographischer Prüfung zur Aufarbeitung mit dem Kondensat I vereinigt.

Das *Kondensat III* (Menge ~ 0,3%) wurde durch Kühlung auf −80° erhalten und enthält die leichtflüchtigen und wasserunlöslichen Stoffe. Die nichtkondensierbaren Gase wurden nicht untersucht, da ihre Zusammensetzung bereits bekannt ist[7,11].

Der Rückstand der Pyrolyse ist braun-schwarz und fest mit dem Quarzsand verklebt. Er wurde einer Nacherhitzung auf 500° unterworfen, wobei ein staubförmiger kohleartiger Rückstand gebildet wird, und sich wiederum 3 entsprechende

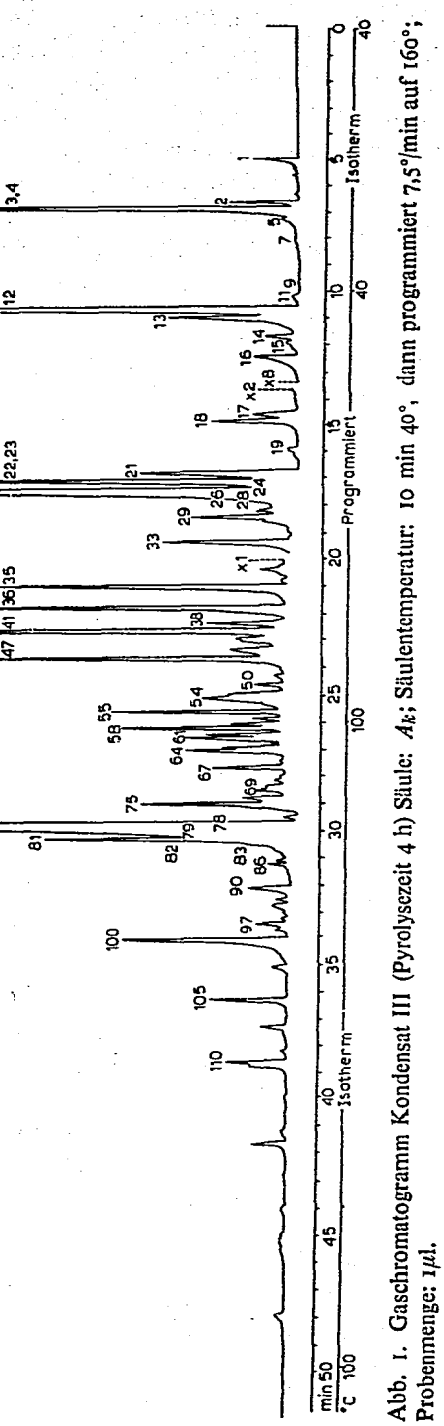


Abb. 1. Gaschromatogramm Kondensat III (Pyrolysezeit 4 h) Säule: A_K; Säulentemperatur: 10 min 40°, dann programmiert 7,5°/min auf 160°; Probenmenge: 1 µl.

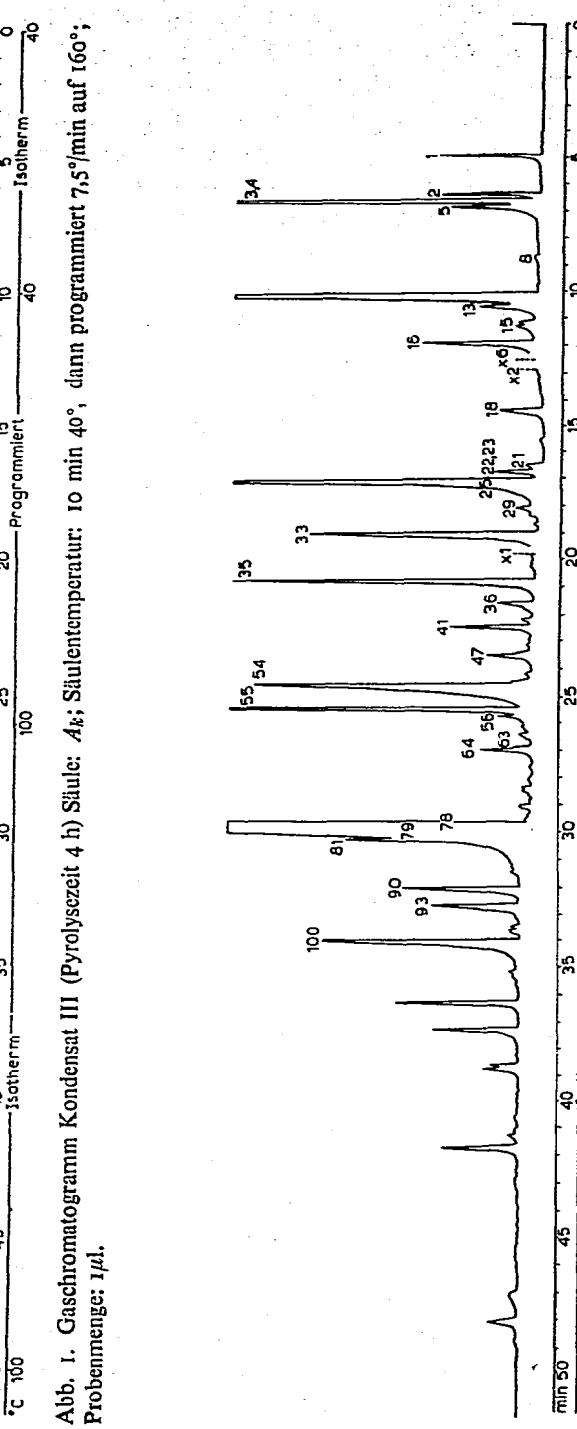


Abb. 2. Gaschromatogramm Kondensat III (Pyrolysezeit 30 min) Säule: A_K; Säulentemperatur: 10 min 40°, dann programmiert 7,5°/min auf 160°; Probenmenge: 1 µl.

Kondensate gewinnen lassen. Das Kondensat III/500° zeigt qualitativ eine ähnliche Zusammensetzung wie das Kondensat III/300°. Es konnte daher für Parallelversuche verwendet werden. Das Kondensat III/500° enthält einen vergleichsweise höheren Anteil an aromatischen Kohlenwasserstoffen und weniger Furane als das Kondensat III/300°. Die Kondensate III sind praktisch wasserfrei und können ohne vorhergehende Aufarbeitung der gaschromatographischen Trennung zugeführt werden.

Trennung und Identifizierung der leichtflüchtigen Pyrolyseprodukte (Kondensat III)

Ein Gaschromatogramm des Kondensats III auf einer Kapillarsäule zeigt bei Anwendung eines Temperaturprogramms über 130 verschiedene Substanzpeaks, von denen etwa 30 Substanzen in einer Konzentration über 1% vorliegen (Abb. 1). Weitere 30 Substanzen in einer Konzentration unter 1% lassen eine sichere Identifizierung noch zu. Genaue Untersuchungen zeigten, dass auch an Kapillarsäulen scharfe Peaks oft aus 2 oder 3 Substanzen bestehen. Die Retentionszeit reicht somit zur Charakterisierung eines Stoffes nicht aus.

Ein derartig komplexes Gemisch, wie es im Kondensat III vorliegt, erfordert zur gaschromatographischen Trennung eine Vortrennung an verschiedenen präparativen Säulen mit stationären Phasen unterschiedlicher Selektivität. So liessen sich an der präparativen Säule B_p (Trikresylphosphat) bzw. C_p ($\beta\beta'$-Oxydipropionitril) die im Kondensat III auftretenden Substanzen nach Stoffklassen auftrennen. Die so erhaltenen Einzelfraktionen von Homologen waren dann an der Kapillarsäule A_k (Polypropylenglykol) vollständig zerlegbar.

Bei der Trennung auf der präparativen Säule C_p liessen sich Acetaldehyd, Propionaldehyd, Aceton, Butanon-2, Furan, 2-Methylfuran, 2,5-Dimethylfuran, und 2-Methyl-5-äthylfuran rein in einer Menge isolieren, die eine Identifizierung über Massen- und IR-Spektren bzw. über Mischschmelzpunkte der 2,4-Dinitrophenylhydrazone zuliess. Die restlichen 3 Fraktionen bestehen aus 2 bis 7 Komponenten, die vorteilhafter durch eine unabhängige Trennung auf der präparativen Säule B_p zu gewinnen sind, wobei auf die Abtrennung der bereits bekannten Verbindungen verzichtet wird.

Die Säule B_p besitzt eine um 50° höhere maximale Arbeitstemperatur und eignet sich besser zur Trennung der höher siedenden Komponenten. Durch Probetrennungen wurden 14 Fraktionen als günstigste Schnitte festgelegt. 3 Fraktionen waren einheitlich und bestanden aus 2,3,5-Trimethylfuran, 1,3,5-Trimethylbenzol, und Furfurol. Die übrigen 11 Fraktionen enthielten 2 oder mehr Komponenten, die gaschromatographisch an Kapillarsäulen weiter aufgetrennt und mit Hilfe der „syringe-reactions" und der Massenspektrometrie identifiziert werden konnten. Die aus der Kapillarsäule austretenden reinen Substanzen wurden dabei direkt ins Massenspektrometer eingelassen. Selbst wenn sich die Retentionszeiten nur wenige Sekunden unterscheiden, kann man bei schnellem Massendurchlauf und Photoregistrierung das Massenspektrum jeder einzelnen Substanz aufnehmen.

Substanzen, deren Vergleichsspektren bekannt sind, lassen sich auf diese Weise leicht identifizieren, obwohl durch die schnelle Registrierung mit kontinuier-

lichem Massendurchlauf merkliche Verschiebungen in den relativen Peakintensitäten auftreten. Isomere Verbindungen wie z.B. *o*-, *m*-, und *p*-Xylol unterscheiden sich bei gleicher Peakverteilung häufig nur wenig in den Peakintensitäten und lassen sich nur dann sicher zuordnen, wenn gleichzeitig mit der Aufnahme des Massenspektrums die gaschromatographische Retentionszeit bestimmt wird. Für unbekannte Substanzen liefert das Massenspektrum in der Regel das Molekulargewicht und die für die Stoffklasse und die Art der Substituenten charakteristische Peakverteilung.

Welcher Stoffklasse die unbekannten Stoffe in den Gemischen angehören und welche funktionellen Gruppen sie enthalten, wurde mit der Methode der „ syringe-reactions" nach Hoff und Feit[12] bestimmt. Bei diesem Verfahren wird das Substanzgemisch in Dampfform in einer gasdichten Spritze mit speziellen Gruppenreagenzien, wie z.B. Hydroxylaminhydrochloridlösung als Reagenz auf Carbonylverbindungen, behandelt. Die kapillargaschromatographische Untersuchung des Restdampfes zeigte dann, welche Substanzen nach dieser Behandlung infolge Reaktion ihrer funktionellen Gruppen aus dem Gemisch verschwinden. Alle 11 Fraktionen wurden nach diesem Verfahren auf die verschiedensten funktionellen Gruppen geprüft.

Wie in besonders schwierigen Fällen eine Kombination beider Verfahren angewendet wird, zeigt das Beispiel der ungesättigten Substanz Nr. 35 (und deren Homologen Nr. 55, 63, 64, 78, 81), für die auf Grund der Massenspektren und der „syringe-reaction" die Struktur eines Vinylfurans oder Äthylcyclopentadiens in Frage kommt. Führt man mit dieser Substanz eine Syringe-Hydrierungsreaktion durch und nimmt das Massenspektrum der an der Kapillarsäule getrennten hydrierten Verbindung auf, so findet man eine Zunahme des Molekulargewichts um 6 Einheiten. Nur Vinylfuran, welches eine Doppelbindung mehr enthält als das Äthylcyclopentadien, kann bei der Hydrierung 6 Wasserstoffatome aufnehmen.

Besonders schwierig gestaltete sich die Identifizierung der zahlreichen, im Kondensat III enthaltenen substituierten Furane, deren Massenspektren und gaschromatographisches Verhalten nicht bekannt war. Für Vergleichszwecke mussten daher 32 Furanderivate synthetisiert werden. Die massenspektrometrische Untersuchung dieser Verbindungen lieferte detaillierte Vorstellungen über den massenspektrometrischen Zerfallsmechanismus substituierter Furane[19], welche wiederum entscheidende Hinweise auf die Struktur der unbekannten Substanzen ergab.

Die Aussagen der Massenspektrometrie, der „ syringe-reactions" und eine sich aus der Retentionszeit ergebende Siedepunktabschätzung führten zu der wahrscheinlichsten Struktur der unbekannten Verbindung. Die nunmehr abschliessend gezielt ausgeführte Synthese erlaubte dann eine sichere Identifizierung.

Insgesamt 56 verschiedene Stoffe konnten auf diese Weise im Kondensat III nachgewiesen und identifiziert werden, welche in Tab. I einschliesslich der zur Identifizierung benutzten Methoden angegeben sind. Die Verfahren der Identifizierung gelingen nur nach gaschromatographischer Vortrennung, da sonst infolge Peaküberlappungen, die auch an Kapillarsäulen auftreten, Massenspektrometrie und „syringe-reactions" keine eindeutigen Ergebnisse liefern. Nur beim Acrolein, Methacrolein, *n*- und iso-Butyraldehyd stellt der Vergleich der Retentionszeiten

TABELLE I

LEICHTFLÜCHTIGE ZERSETZUNGSPRODUKTE DER D-GLUCOSE

Peak-Nr. in Abb. 1 u. 2	*Substanz*[6]	*Säulentyp u. Fraktion der Vortrennung*	*Methoden der Identifizierung* t_r	*SR*	*MS*	*IR*	*DNPH*[a]
1	Acetaldehyd	C_p, Fra. 2	+	+		+	+
2	Propionaldehyd	C_p, Fra. 4	+	+			+
3	Furan	C_p, Fra. 1	+	+	+	+	
4	Aceton	C_p, Fra. 6	+	+	+	+	+
5	Acrolein	K III[b]	+	+			
7	iso-Butyraldehyd	K III	+	+			
9	Methacrolein	K III	+	+			
11	*n*-Butyraldehyd	K III	+	+			
12	2-Methylfuran	C_p, Fra. 3	+	+	+	+	
13	Butanon-2	C_p, Fra. 8	+	+	+		+
14	3-Methylfuran	C_p, Fra. 3	+	+	+		
15	Buten-3-on-2	C_p, Fra. 8	+	+	+		
16	Butandion-2,3	C_p, Fra. 10	+	+	+	+	
17	3-Methylbutanon-2	B_p, Fra. 2	+	+	+		
18	Benzol	C_p, Fra. 5	+	+	+		
21	3-Methylbuten-3-on-2	B_p, Fra. 3; C_p, Fra. 9	+	+	+		
22	Pentanon-2	B_p, Fra. 3; C_p, Fra. 9	+	+	+		
23	2-Äthylfuran	B_p, Fra. 2	+	+	+		
24	Crotonaldehyd	B_p, Fra. 4; C_p, Fra. 11	+	+	+		
25	2,5-Dimethylfuran	B_p, Fra. 2; C_p, Fra. 9	+	+	+	+	
26	Pentanon-3	B_p, Fra. 3; C_p, Fra. 9	+	+	+		
33	Pentandion-2,3	B_p, Fra. 4; C_p, Fra. 11	+	+	+		
35	2-Vinylfuran	B_p, Fra. 4	+	+	+		
36	Toluol	B_p, Fra. 5	+	+	+		
38	2-*n*-Propylfuran	B_p, Fra. 5	+	+	+		
41	2-Methyl-5-äthylfuran	B_p, Fra. 5	+	+	+	+	
42	Hexanon-3	B_p, Fra. 6	+	+	+		
	Penten-3-on-2	B_p, Fra. 7	+	+	+		
44	Hexanon-2	B_p, Fra. 7	+	+	+		
47	2,3,5-Trimethylfuran	B_p, Fra. 6					
	Penten-2-al-1	B_p, Fra. 8	+	+	+		
50	2-Methyl-5-isopropylfuran	B_p, Fra. 6	+	+	+		
54	Pentadien-1,3-al-5	B_p, Fra. 10	+	+	+		
55	2-Methyl-5-vinylfuran	B_p, Fra. 8; C_p, Fra. 10	+	+	+		
57	Äthylbenzol	B_p, Fra. 9; C_p, Fra. 10	+	+	+		
58	2-Methyl-5-*n*-propylfuran						
	2,5-Diäthylfuran	B_p, Fra. 8	+	+	+		
60	*p*-Xylol	B_p, Fra. 9, C_p, Fra. 10	+	+	+		
61	*m*-Xylol	B_p, Fra. 9, C_p, Fra. 10	+	+	+		
63/64	*cis*-2-Propenyl-furan	B_p, Fra. 10	+	+	+		
	trans-2-Propenyl-furan						
67	*o*-Xylol	B_p, Fra. 11	+	+	+		
69	Cyclopentanon	K I[c]	+	+	+		
75	Furan-3-aldehyd	K I		+	+		
78	*cis*-2-Methyl-5-propenylfuran	B_p, Fra. 13	+	+	+		
79	Furfurol	B_p, Fra. 14, K I	+	+	+	+	+
80	1,3,5-Trimethylbenzol	B_p, Fra. 12	+	+	+		

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TABELLE I *(Fortsetzung)*

Peak-Nr. in Abb. 1 u. 2	*Substanz*[e]	*Säulentyp u. Fraktion der Vortrennung*	*Methoden der Identifizierung* t_r	*SR*	*MS*	*IR*	*DNPH*[a]
81	*trans*-2-Methyl-5-propenylfuran	B_p, Fra. 13	+	+	+		
82	2-Methyl-3(furyl-2)propen-2	B_p, Fra. 13	+	+	+		
86	1,2,4-Trimethylbenzol	B_p, Fra. 13	+	+	+		
90	2-Acetylfuran	K I	+	+	+	+	+
91	1,2,3-Trimethylbenzol	direkt	+	+	+		
99	2,3-Benzofuran	K I	+		+		
100	5-Methylfurfurol	K I	+	+	+	+	+[d]

[a]SR = Gruppenzuordnung durch „syringe-reaction". t_r = Identifizierung durch Prüfung auf gaschromatographisch identisches Verhalten. MS = Identifizierung über Massenspektrum. IR = Identifizierung über IR-Spektrum. DNPH = Identifizierung über Mischschmelzpunkt des 2,4-Dinitrophenylhydrazons.
[b]Direkt im Kondensat III nachgewiesen.
[c]Isoliert aus Kondensat I.
[d]Mischschmelzpunkt des *p*-nitrophenylhydrazons
[e]Inzwischen wurden auch die Verbindungen Nr 34 und 56 sicher identifiziert. Es handelt sich um 2-Isopropylfuran (Nr 34) und 2-Isopropenylfuran (Nr 56).

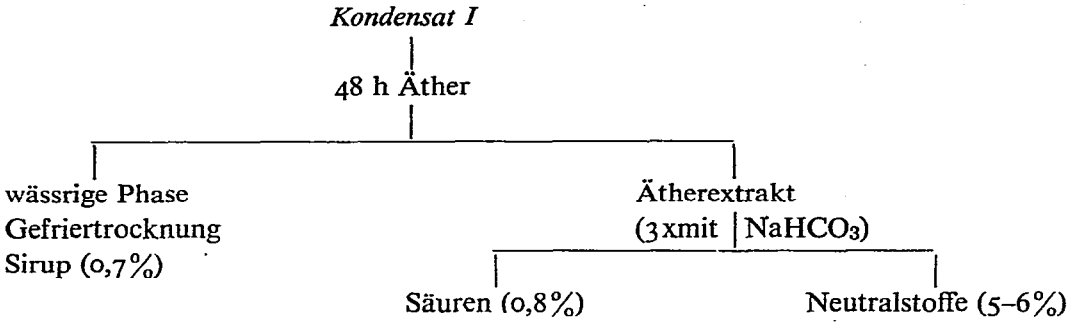
und der Nachweis, dass es sich um Aldehyde handelt, eine hinreichend sichere Identifizierung dar, weil bei so niedrigen Siedepunkten nur diese Aldehyde in Frage kommen. Die Substanz Nr. 75 besitzt ein mit dem Furfurol identisches Massenspektrum und dürfte auf Grund des im Vergleich zum Furfurol niedrigeren Siedepunktes Furan-3-aldehyd sein. Von den Substanzen Nr. 29 und 37, die in Tab. I fehlen, wurden bisher keine Vergleichssubstanzen hergestellt. Es handelt sich um Furane mit dem Molekulargewicht 96.

Eine Reihe von Verbindungen, deren Bildung bisher bei der Zersetzung von Kohlenhydraten angenommen wurde, konnte bei der D-Glucose durch die Anwendung der genannten Methoden sicher ausgeschlossen werden. Hierzu gehören Methanol, Isopropylalkohol[1,3,5,10], Furfurylalkohol[9], Valeraldehyd[2,6] und Benzaldehyd[10]. Ebenso sind Ester und andere Alkohole, die u.a. beim Kaffeerösten[9,17,18] gebildet werden, im Pyrolysat der D-Glucose sicher nicht vorhanden. Bemerkenswert ist die grosse Anzahl der im Pyrolysat gefundenen Furane; von den insgesamt 24 nachgewiesenen Furanen konnten 122 identifiziert werden. Bisher waren nur Furan, 2-Methylfuran, und 2,5-Dimethylfuran[4,5,6,13] als Produkte der Kohlenhydratpyrolyse bekannt. Auch ungesättigte Aldehyde, wie das Pentadien-1,3-al-5 liessen sich erstmals in Pyrolyseprodukten von Kohlenhydraten nachweisen.

Trennung und Identifizierung der schwerer flüchtigen Pyrolyseprodukte (Kondensat I)

Das Kondensat I, welches die Hauptmenge des Pyrolysats ausmacht, lässt sich gaschromatographisch nicht direkt untersuchen, da es einen zu hohen Wassergehalt besitzt. Durch vorsichtige Destillation im Vakuum bei 0° lässt sich ein Destillat gewinnen, welches sich als organische Phase vom Wasser abscheidet.

Eine Analyse dieser wasserdampfflüchtigen Stoffe, die ähnlich wie beim Kondensat III über gaschromatographische Vortrennung, Untersuchung an Kapillarsäulen, Massenspektrometrie und „syringe-reactions" durchgeführt wurde, zeigte folgende Substanzen: Penten-3-on-2, Penten-2-al-1, Pentadien-1,3-al-5, Cyclopentanon, Furan-3-aldehyd, Furfurol, 2-Acetylfuran, und 5-Methylfurfurol, die alle im Kondensat III bereits gefunden wurden. Ferner wurden 2-Methyltetrahydrofuranon-3, Cyclopenten-2-on-1, und 2-Methyl-5-acetylfuran identifiziert; sie wurden erstmals in Kohlenhydratpyrolysaten nachgewiesen. Zwei Substanzen sind auch Bestandteile des Kaffeearomas[8,9,18]. Eine weitere Aufarbeitung der wässrigen Phase des Kondensat I musste durch vorsichtige Ätherextraktion erfolgen, da beim Erwärmen schnelle Braunfärbung eintrat. Die Aufarbeitung wurde nach folgendem Schema durchgeführt:



Die wässrige Phase ergab nach dem Einengen durch Gefriertrocknung einen gelben Sirup (0,7% bezogen auf D-Glucose), der, wie sich dünnschichtchromatographisch zeigen lässt, überwiegend aus 1,6-Anhydro-β-D-glucopyranose neben sehr wenig 5-Hydroxymethylfurfurol besteht.

Aus dem Ätherextrakt liessen sich durch Ausschütteln mit $NaHCO_3$ die folgenden Carbonsäuren erhalten, die durch Gaschromatographie der freien Säuren, der Methyl- und *n*-Amylester und durch Dünnschichtchromatographie der *p*-Bromphenacylester nachgewiesen wurden: Ameisensäure 28%, Essigsäure 64%, Propionsäure 7%, iso-Buttersäure <0,3%, *n*-Buttersäure <0,3%. Die Gesamtkonzentration der Säuren beträgt etwa 0,8% bezogen auf D-Glucose. In geringer Menge treten Lävulinsäure und Brenztraubensäure auf.

Die Neutralfraktion des Ätherextraktes enthielt mit etwa 5% die Hauptmenge der organischen Substanz. Sie riecht charakteristisch nach Caramel und zeigt eine intensiv violette $FeCl_3$-Reaktion, was auf die Anwesenheit von Maltol hindeutet. Durch vorsichtige Destillation im Vakuum bis 50° lassen sich Destillate gewinnen, die Furfurol, 2-Acetylfuran, 5-Methylfurfurol, und Cyclopenten-2-on-1 als Haupt-

komponenten enthalten. Eine weitere Auftrennung der Neutralfraktion durch Destillation ist wegen der oberhalb 50° beginnenden Abbaureaktionen nicht möglich. Eine dünnschichtchromatographische Untersuchung der Neutralfraktion zeigte neben wenig 1,6-Anhydro-β-D-glucopyranose, 5-Hydroxymethylfurfurol, Brenzcatechin und Maltol als Hauptkomponente 1,4:3,6-Dianhydro-D-glucopyranose (farblose Kristalle, F = 126°), die durch präparative Dünnschichtchromatographie isoliert wurde. Die Struktur dieser Verbindung ergibt sich aus Elementaranalyse, Fragmentierungsschema des Massenspektrums[14], und einer vollständigen Analyse der Kopplungskonstanten des NMR-Spektrums; sie ist identisch mit der von Tishchenko[15] bei der Holzvergasung und Bedford[16] beim Erhitzen von Amylose erhaltenen Substanz.

Um einen Überblick über die quantitative Zusammensetzung der Neutralfraktion zu gewinnen, wurde die Gesamtfraktion mit Hexamethyldisilazan und Trimethylchlorsilan in die Trimethylsilyläther überführt und an 3 verschiedenen Säulen gaschromatographisch untersucht. Die Auswertung der Peakflächen lieferte folgende Verteilung: 1,4:3,6-Dianhydro-D-glucopyranose 75%, 1,6-Anhydro-β-D-glucopyranose 3%, 5-Hydroxymethylfurfurol 18%, Brenzcatechin 3%, Maltol 2%. Einwertige Phenole liegen bei einer Pyrolysetemperatur von 300° in nachweisbarer Menge nicht vor. Resorcin und Hydrochinon sind vermutlich in geringer Menge vorhanden. Ob Iso- und Allo-maltol vorkommen, lässt sich nicht entscheiden, da sie gaschromatographisch von 1,4:3,6-Dianhydro-D-glucopyranose nicht ausreichend getrennt werden.

1,4:3,6-Dianhydro-D-glucopyranose stellt somit den überwiegenden Bestandteil der Neutralfraktion dar. Da diese, wenn man vom Wasser absieht, mit 5% die Hauptmenge des Gesamtpyrolysats ausmacht, ist 1,4:3,6-Dianhydro-D-glucopyranose mit einem Anteil von schätzungsweise 2% am Pyrolysat die mengenmässig wichtigste gebildete Substanz. Sie übertrifft unter den gegebenen Versuchsbedingungen den Anteil der 1,6-Anhydro-β-D-glucopyranose.

DISKUSSION

Die Karamelisation unter fortschreitender Entstehung von Bräunungsprodukten und Entweichen flüchtiger Abbauprodukte ist bei allen Kohlenhydraten zu beobachten, wenn sie höheren Temperaturen ausgesetzt werden. Die beim Erhitzen von Kohlenhydraten in Nahrungsmitteln unter ähnlichen Bedingungen bei Bräunungsreaktionen entstehenden Umwandlungsprodukte sind als Geschmacks- und Aromastoffe von Bedeutung.

Die vorstehend bei der thermischen Fragmentierung von D-Glucose gewonnenen Ergebnisse sind im Hinblick auf die prozentuale Verteilung der enstehenden Reaktionsprodukte von den Versuchsbedingungen abhängig. Temperatur, Erhitzungszeit, Erhitzungsfläche, und Strömungsgeschwindigkeit des Inertgases haben einen Einfluss auf den Verlauf der Pyrolyse. Mengen und Konzentrationsangaben sind daher nur für die jeweils gewählten Reaktionsbedingungen repräsentativ.

Beim 4stündigen Erhitzen von D-Glucose auf 300° verbleiben unter den hier

gewählten Bedingungen 47–50% als schwarzbrauner Zersetzungsrückstand; 45–48% der eingesetzten Menge werden in den Kondensaten I, II, und III als flüchtige Verbindungen aufgefangen. Die nicht-kondensierbaren Gase CO_2, CO, CH_4 usw. werden mit dieser Versuchsanordnung nicht erfasst. Bryce und Greenwood[6] haben D-Glucose 18 Stunden im Vakuum bei 300° erhitzt und erhielten einen Rückstand von 20% und damit einen wesentlich höheren Anteil an flüchtigen Stoffen.

Das Hauptprodukt des thermischen Abbaus ist mit etwa 40% durch Dehydratisierungsreaktionen gebildetes Wasser. Unter den organischen Verbindungen im Pyrolysedestillat ist 1,4:3,6-Dianhydro-D-glucopyranose (etwa 2%) mengenmässig am stärksten vertreten, es folgen 1,6-Anhydro-β-D-glucopyranose (etwa 0,5–1,0%) dann ist nur noch Furfurol in vergleichbarer Menge vorhanden. Für die Bildung der beiden Anhydroglucopyranosen ist eine direkte Dehydratisierung der monomeren D-Glucose anzunehmen.

Das Kondensat III, welches die leichtflüchtigen Substanzen enthält, ist am Gesamtpyrolysat nur mit 0,3% beteiligt, die Einzelkomponenten liegen also in Konzentrationen unter 0,1% vor. Trotzdem ist die Kenntnis gerade dieser Produkte von Interesse, weil sie offensichtlich in einer fortgeschrittenen Phase der Pyrolyse enstehen, in der bereits Dehydratisierungs-, Polymerisierungs-, und Spaltungsreaktionen parallel ablaufen. Dementsprechend ist das Kondensat III ein komplexes Gemisch der verschiedensten Stoffe.

Mengenmässig und zahlenmässig vorherrschende Substanzklasse des Kondensats III sind die Furane, von denen 20 verschieden substituierte Verbindungen identifiziert werden konnten. Mengenmässig folgten als nächstes Diketone und Ketone, die stärker als Aldehyde vertreten sind. Klein ist der Anteil der aromatischen Kohlenwasserstoffe, er steigt bei Erhöhung der Pyrolysetemperatur stark an.

Innerhalb jeder Substanzklasse ist die Verteilung der Produkte stets so, dass die thermisch stabilen Anfangsglieder einer Reihe in grösserer Konzentration als die nächstfolgenden Glieder auftreten. So ist die Menge an Furan grösser als die der substituierten Furane. Aceton tritt bevorzugt vor Butanonen und Pentanonen auf, während Hexanone nur in Spuren vorhanden sind. Entsprechend überwiegt Acetaldehyd vor Propionaldehyd und Butyraldehyd. Auch Butandion-2,3 ist in grösserer Menge vorhanden als Pentandion-2,3; Benzol und Toluol verhalten sich entsprechend. Eine derartige Produkten-Verteilung deutet darauf hin, dass die Bildung dieser Substanzen des Kondensats III durch thermische Spaltung bereits gebildeter oligomerer und nicht aus monomeren Zersetzungsprodukten erfolgt, da bei dieser Bildungsweise kleine Bruchstücke statistisch bevorzugt sind.

Besonderheiten in der Produktenverteilung zeigen 2,5-substituierte Furane und Furane mit ungesättigten Seitenketten, die zwar in geringerer Menge als Furan selbst auftreten, doch gegenüber den anderen Furanhomologen deutlich bevorzugt sind. Dieser Befund ist ein Hinweis darauf, dass derartige Strukturen hinsichtlich der Verknüpfung und der Seitenketten im Polymerkörper in gewissem Masse preformiert sind.

Ungesättigte Furane und Carbonylverbindungen dürften insbesondere in der

Anfangsphase der Gesamtreaktion eine wichtige Rolle spielen. Dies zeigt eine Untersuchung der Produktenverteilung im Kondensat III bei verschiedenen Pyrolysezeiten. Eine starke Pyrolysezeitabhängigkeit wurde gefunden bei 2-Vinylfuran (Nr. 35), Pentadien-1,3-al-5 (Nr. 54), 2-Methyl-5-vinylfuran (Nr. 55), 2-Isopropenylfuran (Nr. 56), *cis-trans*-2-Propenylfuran (Nr. 63/64), und Furfurol (Nr. 79). Diese ungesättigten Verbindungen treten im Gaschromatogramm eines nach 30 Min. Pyrolysezeit erhaltenen Kondensats III stark hervor. (Abb. 2.) In Kondensaten, die nach 4stündigem Erhitzen gewonnen wurden, hat ihre Menge erheblich abgenommen (vgl. Abb. 1). Umgekehrt sind Aceton (Nr. 4), Butanon-2 (Nr. 13), 3-Methyl-butanon-2 (Nr. 17), 2-Methyl-5-äthylfuran (Nr. 41), 2,3,5-Trimethylfuran (Nr. 47), und 2-Methyl-5-*n*-propylfuran (Nr. 58) bei der Kurzzeitpyrolyse nur in geringer Menge vorhanden. Ihr Anteil steigt bei längerem Erhitzen stark an, 3-Methyl-butanon-2 (Nr. 17) und 2-Methyl-5-*n*-propylfuran (Nr. 58) treten dann neu auf.

Der Gehalt an Benzol (Nr. 18) und anderen aromatischen Kohlenwasserstoffen ist weniger von der Pyrolysezeit als von der Pyrolysetemperatur abhängig. Benzol ist bereits im Kurzzeitpyrolysat enthalten und nimmt auch bei langen Pyrolysezeiten (bis 20 Stunden) nicht wesentlich zu. Erst bei Erhöhung der Pyrolysetemperatur steigt der Anteil der Kohlenwasserstoffe stark an, so dass bei 500° Benzol, Toluol, und Xylole Hauptkomponenten des Pyrolysats werden. Die Vinylfurane und Furfurol sind unter diesen Bedingungen nur in geringer Menge vorhanden, Pentadien-1,3-al-5 fehlt völlig.

Die Untersuchungen über die Pyrolysezeitabhängigkeit lassen erkennen, dass ungesättigte Verbindungen wie 2-Vinylfuran, Pentadien-1,3-al-5 und 2-Methyl-5-vinylfuran bereits in der Primärphase des Abbaus der D-Glucose entstehen, wobei die Bevorzugung des 2-Methyl-5-vinylfurans besonders hervorzuheben ist. Die letztere Verbindung hat mehr als 6 C-Atome und kann somit nicht aus monomerer D-Glucose enstehen. Es scheint daher vernünftig, zwei generell unterschiedliche Wege der Wasserabspaltung anzunehmen. Der eine Weg führt vom Monomeren zur 1,6-Anhydro-β-D-glucopyranose und 1,4:3,6-Dianhydro-D-glucopyranose; der andere Weg ist eine dehydratisierende Polymerisation unter möglicher Beteiligung des 5-Hydroxymethylfurfurols. Die Mehrzahl der im Kondensat III aufgefundenen Pyrolyseprodukte dürften bereits thermische Spaltprodukte der auf dem letzten Weg gebildeten Oligomeren oder Polymeren sein.

Das bevorzugte Auftreten der Furane im Kondensat III weist darauf hin, dass im Polymerkörper der Furanring als Bauelement eine wichtige Rolle spielen sollte. Die grosse Zahl der nachgewiesenen 2,5-substituierten Furane deutet auf eine vorherrschende 2,5-Verknüpfung hin neben einer 3-Verknüpfung, die sich aus den gefundenen 3-substituierten Furanen ergibt. Für diese Annahme spricht der Befund, dass beim Nacherhitzen des bei 300° erhaltenen polymeren Rückstandes auf 500° neben unspezifischen Kohlenwasserstoffen und Carbonylverbindungen die gleichen substituierten Furane wie unter Standardbedingungen erhalten werden, wobei in geringer Menge auch ungesättigte Furane und Furfurol, aber kein Pentadien-1,3-al-5 nachweisbar sind.

Mit den hier geschilderten Untersuchungen wurden somit erstmalig experimentelle Befunde erhalten, die Hinweise auf die mögliche Entstehung und die Struktur des beim Erhitzen von D-Glucose gebildeten Polymerkörpers geben.

EXPERIMENTELLER TEIL

Ausführung der Pyrolyse

D-Glucose (Merck DAB 6), bei 90° i.Vak. getrocknet, wird mit grobkörnigem Quarzsand 1:15 vermischt und in 50-cm lange, 2-cm weite Pyrolyserohre eingefüllt. Glaswollepfropfen vor und hinter der Zersetzungszone sorgen für eine feste Füllung. Das Pyrolyserohr wird in starkwandige Metallhüllen eingepasst, mit nachgereinigtem Stickstoff (O_2<0,001 %) ausgespült und in den auf 300° vorgeheizten Ofen geschoben. Gleichzeitig wird die Heizpatrone H (60 W/220 V) eingeschaltet und die Verbindung zu den Kühlfallen hergestellt (vgl. Abb. 3). Die Heizpatrone H ist so in das Rohr ein-

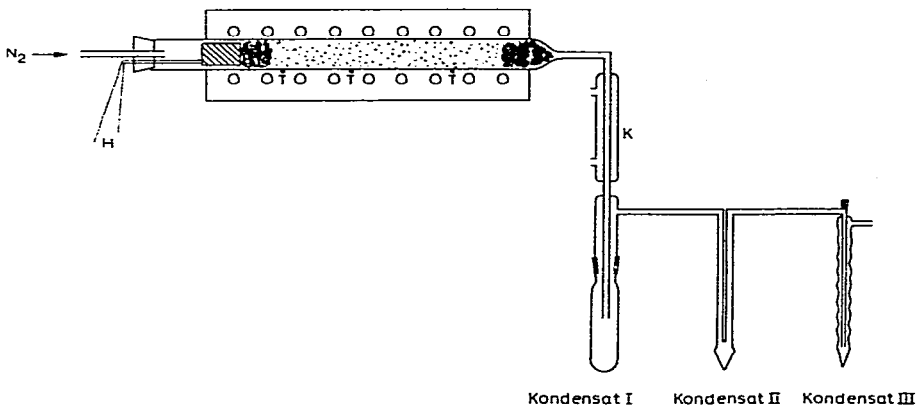


Abb. 3. Pyrolyse-Apparatur. H, Heizpatrone; T, Thermoelemente; K, Kühler (60°).

gefügt, dass der Stickstoff durch die Patrone hindurchströmt und dabei auf 300° vorgeheizt wird. Die Metallhüllen sorgen beim Einführen des Zersetzungsrohres für einen reproduzierbaren Temperaturanstieg der Rohrwand, die Vorheizung des Stickstoffes für ein möglichst geringes Temperaturgefälle im Innern des Rohres. Die Temperaturmessung erfolgte durch Thermoelemente (Isabellin-Konstantan) an der Rohrwand und im Stickstoffstrom hinter der Heizpatrone. Die Strömungsgeschwindigkeit des Stickstoffs wurde auf 150 ml/min eingestellt.

Gut reproduzierbare Ergebnisse erhält man nur mit kleinen Mengen. Es können 6 cm-Rohre und ein Mischungsverhältnis D-Glucose : Quarzsand von 1:6 benutzt werden, wenn innerhalb des Rohres durch Schichten aus Glaswolle mehrere gleichmässig gepackte Zersetzungszonen geschaffen werden, da sonst Verstopfen und Platzen des Rohres eintritt. Das so erhaltene Kondensat ist qualitativ ähnlich wie das bei kleinen Mengen erhaltene Pyrolysat, zeigt jedoch quantitative Differenzen.

Wird nur die Gewinnung einer grösseren Menge des Kondensats angestrebt, so können grössere Mengen D-Glucose zersetzt werden.

Nach 4 Stdn. wurden die Kühlfallen gewechselt und das Rohr 3 Stdn. auf 500° erhitzt. Der Pyrolyserückstand ist dann staubförmig und kann aus den Rohren ausgestossen werden. Die Zersetzung von je 80 g D-Glucose ergab in 10 aufeinanderfolgenden Pyrolysen etwa 3 ml Kondensat III/300° und 9 ml Kondensat III/500°. Beide Kondensate wurden für die präparative gaschromatographische Vortrennung, die Isolierung und Identifizierung der Komponenten verbraucht. Der Einfluss der Temperatur und der Zersetzungszeit auf die Pyrolyse wurde an der Zersetzung kleinerer Mengen (11 g in 225 g Quarz) in 2-cm weiten Rohren studiert.

Geräte und Säulen

Für analytische Trennungen wurde ein Perkin–Elmer-Fraktometer F6/4HF mit Flammenionisationsdetektor benutzt, Trägergase: Helium und nachgereinigter Stickstoff. Filter aus Aktivkohle oder Molekularsieb 5 A (Merck) verhinderten, dass Staub und Öl aus den Vorratsflaschen auf die Säule oder in den Detektor gelangen.

Die Trägergasgeschwindigkeit betrug für gepackte 2m-Säulen (4,65 mm i.D.) 60 ml/min, für 50 m-Kapillarsäulen (0,25 mm i.D.) 1,0 ml/min bei einem Teilungsverhältnis von 1:100. Die Temperatur des Einspeisblocks betrug 230°, die Temperatur der Ausgangsleitungen 220° und die des Detektorblocks 250°.

Präparative Trennungen wurden mit einem F & M-Modell 770 ausgeführt. Trägergas war Helium oder ein Gasgemisch (20% Helium, 80% Stickstoff); die Strömungsgeschwindigkeit betrug 600–900 ml/min bei den 3/4"-Säulen, und 60 ml/min bei den 1/4"-Säulen. Die Temperatur des Einspeisblocks betrug 175°, die Temperatur des Fraktionssammlers 150°, die des Detektorblocks 240°; der Brückenstrom wurde auf 150 mA eingestellt. Typ, Eigenschaften, und Anwendungsbereich der für diese Untersuchungen benutzten Säulen sind in Tab. II angegeben.

Auswahl geeigneter Säulenkombinationen

Acetaldehyd, Aceton, Butandion-2,3, Benzol, und Furan sind Vertreter der im Kondensat III auftretenden 5 Stoffklassen und daher ein repräsentatives Testgemisch zur Prüfung von Säulenkombinationen. Stationäre Phasen, die eine dieser Testsubstanzen selektiv abtrennen, werden auch deren höhere Homologen abtrennen. Die dann vorliegenden Homologengemische sind an Kapillarsäulen vollständig trennbar und über Massenspektren und „syringe-reactions" sicher zuzuordnen.

Von 16 untersuchten Phasen erwiesen sich zur Säulenbelegung die stationären Phasen, *A*, *B*, *C* als besonders geeignet. An den Säulen A_a, B_a, C_a unterscheiden sich die absoluten Retentionszeiten t der 5 Testsubstanzen beträchtlich (Tab. III), z.B. besitzt Butandion-2,3 an der Säule C_a eine extrem hohe Retentionszeit. Die relativen Retentionszeiten t_r, bezogen auf Acetaldehyd, zeigen jedoch, dass an keiner dieser Säulen eine selektive Abtrennung einer der 3 Carbonylverbindungen Acetaldehyd, Aceton, oder Diacetyl erfolgt. Furan dagegen wird auf Säule A_a nicht von Aceton getrennt, auf Säule B_a erscheint es zwischen Acetaldehyd und Aceton, und auf Säule C_a

noch vor Acetaldehyd. Ähnlich verschiebt sich Benzol, das auf Säule A_a als letzte Substanz und auf Säule C_a vor Aceton austritt. Da innerhalb einer homologen Reihe die Retentionszeit vom Siedepunkt abhängt, kann durch Kombination dieser 3 Säulen eine vollständige Trennung der Furane und aromatischen Kohlenwasserstoffe von den Carbonylverbindungen erreicht werden.

TABELLE II

GASCHROMATOGRAPHISCHE SÄULE

Typ[a]	*Stationäre Phase*	*Länge*	*i.D., mm*	*Max. Temp., °C*	*Anwendung*
A_a	Polypropylenglykol (Ucon LB-550X) 15% auf Celite 545	2 m	4,65	175	Vorversuche zur präparativen Trennung
B_a	Trikresylphosphat 15% auf Diatoport W,	2 m	4,65	125	Vorversuche zur präparativen Trennung
C_a	$\beta\beta'$-Oxydipropionitril 15% auf Diatoport W,	2 m	4,65	75	Vorversuche zur präparativen Trennung
C_p	$\beta\beta'$-Oxydipropionitril 20% auf Diatoport W	8 ft	16	75	Präparative Vortrennung Kondensat III
B_p	Trikresylphosphat 20% auf Diatoport W	8 ft	16	125	Präparative Vortrennung Kondensat III
A_k	Polypropylenglykol (Ucon LB-550X)	50 m	0,25	175	Analytische Säule für alle Untersuchungen am Kondensat III, der wasserdampfflüchtigen Stoffe im Kondensat I, Methyl- u. Amylester von Carbonsäuren, Trimethylsilyläther
D_p	Di-iso-decylphthalat 20% auf Diatoport W	8 ft	16	175	Präparative Vortrennung der wasserdampfflüchtigen Stoffe aus Kondensat I
E_a	Diäthylhexylsebacinat + Sebacinsäure 20% auf Celite 545	2 m	4,65	150	Carbonsäuren C_1–C_6
F_a	Silikongummi SE 52 5% auf Celite 545	2 m	4,65	300	Trimethylsilyläther, direkte Untersuchung Kondensat I, u.a. Bestimmung Dianhydro-D-glucopyranose, 5-Hydroxymethylfurfurol

[a]Index *a* : gepackte, analytische Säule; *p* : gepackte präparative Säule; *k* : Kapillarsäule.

Präparative Trennungen

Nach Festlegung der optimalen Trennbedingungen durch Vorversuche mit dem Kondensat III/500° wurde das Kondensat III in möglichst eng geschnittene Fraktionen zerlegt. 7 Fraktionen an der Säule C_p enthielten die niedrig siedenden Bestandteile. Die übrigen Komponenten wurden auf der Säule B_p in 14 Fraktionen zerlegt. Auch Spurenbestandteile wurden mit der Säulenkombination selektiv abgetrennt und liessen sich sicher nachweisen. Wie gut die Trennung durch Säulenkombination gelingt, zeigt Abb. 4 am Beispiel der Fraktion 2 und 3 der präparativen-Vortrennung an B_p.

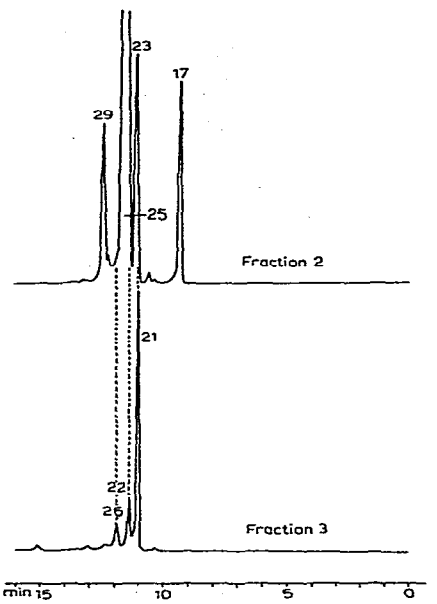


Abb. 4. Gaschromatogramm Fraktion 2 und 3 der Vortrennung an Säule C_p. Säule: A_k; Säulentemperatur: 50°; Probenmenge: 0,2 µl.

TABELLE III

RETENTIONSZEITVERSCHIEBUNGEN AN BESONDERS SELEKTIVEN STATIONÄREN PHASEN[a]

	Säule A_a		*Säule B_a*		*Säule C_a*	
	t	t_r	t	t_r	t	t_r
Acetaldehyd	10,5	1,0	15,0	1,0	62,5	1,0
Furan	29,5	2,80	25,5	1,70	54,0	0,86
Aceton	29,5	2,80	44,0	2,94	178,0	2,85
Diacetyl	81,5	7,75	116,5	7,75	495,0	7,95
Benzol	114,5	10,90	116,5	7,75	159,0	2,54

[a]Fraktometer F6/4 mit Hitzdrahtdetektor; Säulentemperatur, 50°; Probenmenge, 1 µl.

Im Gaschromatogramm des Gesamt-Kondensats III (vgl. Abb. 1 und 2) werden 3-Methylbuten-3-on-2 (Nr. 21); Pentanon-2 (Nr. 22); Pentanon-3 (Nr. 26); 2-Äthyl-

furan (Nr. 23); 2,5-Dimethylfuran (Nr. 25); Crotonaldehyd (Nr. 24), ein noch unbekanntes isomeres Furan (Nr. 29) und eine Reihe von Spurenbestandteilen nur unvollkommen getrennt. Durch die Selektivität der Trennsäule B_p gelingt jedoch die vollständige Abtrennung der Furane Nr. 23, 25, und 29 in Fraktion 2, von den Carbonylverbindungen Nr. 21, 22, und 25 in Fraktion 3. Crotonaldehyd (Nr. 24) wird als ungesättigte Verbindung stärker zurückgehalten und erscheint in der 4. Fraktion der Vortrennung. Das dem Pentanon-2 und Pentanon-3 isomere 3-Methylbutanon-2 (Nr. 17) tritt wegen seines niedrigeren Siedepunktes bereits in der Fraktion 2 zusammen mit dem Furanen auf.

Verbindung von Gaschromatographie und Massenspektrometrie

Der Ausgang der Kapillarsäule und der Einlassteil EC2 eines CH Massenspektrometers (Atlas-Werke) wurden durch ein 120-cm langes, indirekt beheiztes Kapillarrohr verbunden. Der Einlassteil EC2 am Massenspektrometer besteht aus einer auf wenige μ Innendurchmesser ausgezogenen, am oberen Ende plangeschliffenen Glaskapillare, von der ein beheizbares Rohr zur Ionenquelle führt. Die Verbindungskapillare vom Gaschromatographen her endet ebenfalls in der Mitte einer planen Fläche. Beide Flächen werden fest und möglichst zentrisch gegeneinander gepresst. Bei schnellem Massendurchlauf (maximal 1 Massenoktave/0,6 sec) wurden die Massenspektren über einen UV-Lichtpunktschreiber (ABEM-Ultragraph) auf Photopapier registriert. Bei direkter Verbindung von Gaschromatograph und Massenspektrometer wurde ein parallel geschalteter Oszillograph (Oscilloscope 130 C, Hewlett-Packard) als Detektor benutzt. Bei gaschromatographisch vollständig getrennten leichtflüchtigen Substanzen können noch getrennte Massenspektren erhalten werden, wenn der Unterschied in der Retentionszeit 5 bis 10 sec beträgt. Höher siedende Stoffe, vor allem polare Verbindungen wie z.B. 5-Methylfurfurol werden in den Zuleitungen zur Ionenquelle festgehalten und werden häufig auch dann noch im Massenspektrometer angezeigt, wenn bereits längere Zeit keine Substanz mehr aus der gaschromatographischen Säule austritt.

Die quantitative Reproduzierbarkeit der Massenspektren ist bei kurzen Meßzeiten recht mangelhaft, weil durch den steilen Anstieg und Abfall der gaschromatographischen Peaks die Konzentration der ins Massenspektrometer eintretenden Stoffe sich während der Messung ändert. Die gaschromatographische Trennung wurde daher bei möglichst niedriger Säulentemperatur ausgeführt, bei der die Peakbreite gross ist.

Ausführung der „Syringe-reactions"

1–10 μl des zu untersuchenden Gemisches werden in einen mit Serumkappe verschlossenen, mit nachgereinigtem Stickstoff gefüllten 100 ml-Erlenmeyer-Kolben eingespritzt. Man entnimmt 2 ml dieses sehr verdünnten Dampfes mit einer Spritze, die durch einen dünnen Teflonschlauch mit einer zweiten Spritze verbunden wird. Die zweite Spritze enthält 20-100 μl einer Reagenzlösung. Durch mehrfaches Hin- und Herpumpen wird der Dampf mit dem Reagenz in Berührung gebracht und der Rest-

dampf gaschromatographisch untersucht. Bei höher siedenden Stoffgemischen werden Vorratsbehälter und Spritzen im 120° heissen Trockenschrank erwärmt. Auf diese Weise lassen sich Stoffe mit Siedepunkten bis zu 175° noch gut untersuchen.

Die Reagenzlösungen wurden nach Hoff und Feit[12] hergestellt. Bewährt haben sich: H_2O zur Entfernung wasserlöslicher Stoffe; Hydroxylaminhydrochloridlösung zur Entfernung von Carbonylverbindungen; $KMnO_4$-Lösung zur Oxydation von Alkoholen und Aldehyden zu Ketonen; $NaBH_4$-Lösung zur Hydrierung von Carbonylverbindungen zu Alkoholen; H_2SO_4–H_2O 7:3 zur Entfernung ungesättigter Kohlenwasserstoffe und teilweise Entfernung von Furanen; H_2SO_4 konz. zur Entfernung aller Verbindungen bis auf gesättigte Kohlenwasserstoffe; $PtO_2/H_2/H_2O$ zurHydrierung ungesättigter Kohlenwasserstoffe. Die Umsetzung zu gaschromatographisch trennbaren Verbindungen, wie im Falle der Hydrierung mit $NaBH_4$ oder PtO_2/H_2, wird bei unbekannten Stoffen erst wertvoll durch die Verknüpfung der Gaschromatographie mit der Massenspektrometrie.

Darstellung des 2-Methyl-5-n-propenylfuran

10 g 3-(5-Methylfuryl-2)-2-methyl-acrylsäure wird bei 250–270° am Rückfluss erhitzt. Das überdestillierende Furan wird mit verd. $NaHCO_3$-Lösung und mit Wasser gewaschen, über Na_2SO_4 getrocknet und rektifiziert. Ausbeute 2,3 g (31%), Kp_{760} = 155–160° *cis*- und *trans*-Verbindung wurden gaschromatographisch getrennt. Das *cis*-2-Methyl-5-*n*-propenylfuran besitzt die kürzere Retentionszeit und unterscheidet sich im IR-Spektrum durch 2 charakteristische Banden bei 1385 und 788 cm^{-1} von der *trans*-Verbindung, bei der diese Banden fehlen, und 2 Banden bei 1290 und 965 cm^{-1} auftreten. 2-Methyl-5-vinylfuran und 2-*n*-Propenylfuran, welche bereits bekannt sind, wurden zweckmässigerweise analog durch Decarboxylierung von 3-(5-Methylfuryl-2)acrylsäure bzw. 2-Methyl-3-(furyl-2)acrylsäure hergestellt.

ZUSAMMENFASSUNG

Die durch Erhitzen von D-Glucose auf 300° erhaltenen flüchtigen Pyrolyseprodukte wurden untersucht. 1,4:3,6-Dianhydro-D-glucopyranose ist das Hauptprodukt. Die leichtflüchtigen Verbindungen wurden durch wiederholte gaschromatographische Trennung vollständig zerlegt. Mit Hilfe der Massenspektrometrie, der „syringe-reaction" und durch Vergleich mit Testsubstanzen liessen sich 56 Verbindungen identifizieren. Als Abbauprodukte entstehen hauptsächlich Furanderivate sowie Aldehyde, Ketone, Diketone und aromatische Kohlenwasserstoffe. Siebzehn verschieden substituierte Furane, einige ungesättigte Carbonylverbindungen und eine Reihe aromatischer Kohlenwasserstoffe wurden erstmals bei Kohlenhydratpyrolysen aufgefunden. Zu Beginn der Pyrolyse treten als charakteristische Zersetzungsprodukte Vinylfurane, Pentadien-1,3-al-5, und Furfurol auf. Ein Teil der gefundenen Furane, wie z.B. 2-Methyl-5-vinylfuran enthalten mehr als 6 C-atome. Dies spricht dafür, dass Furane nicht aus monomerer D-Glucose, sondern durch Fragmentierung von Polymeren entstehen.

SUMMARY

Volatile products from the thermal degradation of D-glucose at 300° were analysed. 1,4:3,6-Dianhydro-D-glucopyranose is the main product. The most-volatile compounds were separated by repeated gas–liquid chromatography. Fifty-six compounds were identified by mass spectrometry, "syringe-reactions", and comparison with synthetic, standard substances. Furans are formed as the principal decomposition products, along with aldehydes, ketones, diketones, and aromatic hydrocarbons. Seventeen alkylfurans, some unsaturated, carbonyl-containing compounds, and some aromatic hydrocarbons were isolated for the first time from carbohydrate pyrolysis. Typical degradation products at the start of pyrolysis are vinylfurans, penta-1,3-dien-5-al, and furfural. Some compounds containing more than 6 C-atoms (for instance, 2-methyl-5-vinylfuran) were found. This suggests that furans are formed not from D-glucose itself, but by fragmentation of polymers.

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Chondroitin 4-sulfate was isolated from bovine cartilage powder, obtained from Wilson Laboratories, Chicago, Illinois. The powder was stirred with 10 parts of 0.05 N NaOH (w/v) at 4° for 2 days and the mixture centrifuged. The supernatant fraction was neutralized with acetic acid and thrichloroacetic acid was added until a concentration of 4% was reached. After being kept for 30 min at room temperature, the mixture was centrifuged.

In order to purify the polysaccharide, 1 g of Darco G-60 was added per 3 g of starting material. The relatively small amounts of polysaccharide adsorbed by charcoal were eluted by treating the charcoal at 4° for 4 or 5 h with 30% ethanol containing 0.5% NH_4OH. The charcoal supernatants and eluates were dialyzed and purified further by means of chromatography on Dowex-1.

Anal.: Uronic acid, 29.0%; hexosamine, 24.5%; N, 2.1%.

Dermatan sulfate was isolated from heparin by-products (obtained through the courtesy of the late Dr. A. Winterstein, Hoffmann-La Roche, Basel, Switzerland, and Dr. H. H. R. Weber, Wilson Laboratories, Chicago, Illinois) by use of a copper precipitation method[18], using two precipitations for the final product. A low color yield is normally obtained in the uronic acid estimation of dermatan sulfate when using the carbazole method[19].

Anal.: Uronic acid, 14.6%; hexosamine, 29.8%; N, 2.4%.

Chondroitin 6-sulfate obtained from shark cartilage was a product of the Kaken-Yaku Kako Co., Ltd., Tokyo, Japan, and used without further purification.

Anal.: Uronic acid, 31.0%; hexosamine, 28.3%; N, 2.5%.

Keratosulfate was isolated from human nucleus pulposus. The acetone-dried tissue was digested with papain and the extract, kindly provided by Dr. A. Saunders, purified by use of charcoal and chromatography on Dowex-1.

Anal.: Uronic acid, 1.4%; carbohydrate, 22.7%; hexosamine, 22.8%; N, 2.6%.

Heparin and heparitin sulfate were generously supplied by Dr. L. L. Coleman of the Upjohn Co., Kalamazoo, Michigan, and Dr. H. H. R. Weber, Wilson Laboratories, Chicago, Illinois, and were purified by the use of cetylpyridinium chloride and fractionation on Dowex-1 as described previously[3]. High color yields for uronic acid estimations by the carbazole method are normally obtained for both heparin and heparitin sulfate.

Anal.: Uronic acid, 38.3%; hexosamine, 22.1%; total sulfate, 28.1%; N, 1.8%. Heparitin sulfate: Uronic acid, 39.6%; hexosamine, 23.9%; N, 2.05%; total sulfate, 14.0%; *N*-sulfate, 7.4%.

N-Acetylheparin was prepared from 600 mg of heparin which had been desulfated by heating in 0.04 N HCl (100 ml) for 100 min. After adjusting the pH to 8.0 by addition of 1 N NaOH, acetic anhydride (1.0 ml) and saturated $NaHCO_3$ (5 ml) were added and the solution stirred occasionally over a 30-min period. The pH was again adjusted to 8.0–8.5 and the acetylation procedure repeated with half the quantities of acetic anhydride and $NaHCO_3$. Finally, the solution was concentrated in a flash evaporator at 35° and the *N*-acetylated heparin (550 mg) was

precipitated by the addition of 3 vol. of alcohol. The nitrous acid–indole reaction[14] indicated complete *N*-acetylation.

Anal.: Uronic acid, 29.8%; hexosamine, 22.8%; N, 1.6%; sulfate, 19.4%.

Preparation of oligosaccharides

Oligosaccharides from chondroitin 4-sulfate and chondroitin 6-sulfate were obtained after exhaustive digestion with testicular hyaluronidase[20] and purification on Sephadex or Dowex-1 columns. An oligosaccharide preparation from chondroitin 4-sulfate, which had been fractionated on a Sephadex G-25 column, was kindly provided by Dr. A. Saunders.

Anal.: Uronic acid, 35.2%; hexosamine, 28.0%; reducing sugar, 11.4%.

The products of chondroitin 6-sulfate digestion were fractionated on a Dowex-1 column. The material eluted with 1.0 and 1.5 M LiCl was concentrated and precipitated with 4 vol. of ethanol.

Anal.: Uronic acid, 36.8%; hexosamine, 30.0%; *N*-acetylhexosamine, 16.5%.

N-Acetylchondrosine was prepared by hydrolysing of chondroitin 4-sulfate with 1 N HCl for 2 h, adsorption of the chondrosine on charcoal, washing the adsorbent several times with water to remove acid and free hexosamine, and eluting of the chondrosine with hot 30% ethanol. Acetylation was performed directly on the alcohol eluate by the addition of acetic anhydride and $NaHCO_3$ as described for *N*-acetylheparin.

Anal.: Uronic acid, 36.3%; hexosamine, 30.8%; *N*-acetylhexosamine, 31.0%.

Hydrolysis conditions

The conditions used for determining the hydrolysis of mucopolysaccharides as illustrated in Figs. 1 and 2 were as follows: solutions (0.1%) of the substances in 0.02 to 0.2 N HCl were heated in screw cap tubes with Teflon liners. After heating

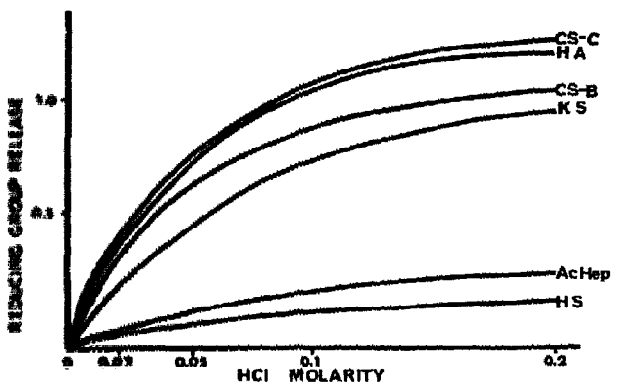

Fig. 1. Release of reducing groups during hydrolysis of mucopolysaccharides. Values are expressed as ratios of reducing sugar to total hexosamine content. Conditions of hydrolysis as described in EXPERIMENTAL. Abbreviations: HS, heparitin sulfate; AcHep, *N*-acetylheparin; KS, keratan sulfate; CS-B, dermatan sulfate; HA, hyaluronic acid; CS-C, chondroitin 6-sulfate.

for 1 h, the hydrolyzates were cooled under tap water and neutralized by the addition of equivalent amounts of Na_2CO_3. Analyses were carried out on the neutralized solutions.

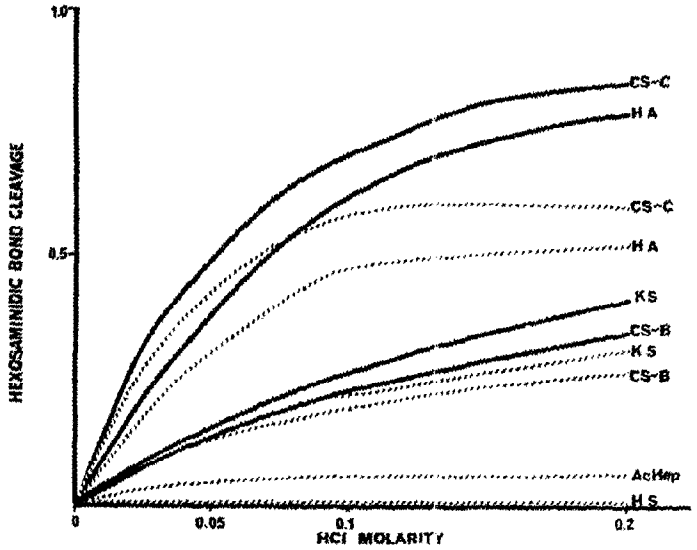

Fig. 2. Liberation of hexosamine reducing units determined by the Morgan–Elson reaction after hydrolysis of mucopolysaccharides as described in EXPERIMENTAL. Values are expressed as ratios of *N*-acetylhexosamine to total hexosamine content. The solid line refers to results obtained after acetylation of the hydrolyzates as described by Levvy and McAllan[13], and the broken lines represent values obtained by direct analysis of the hydrolyzates. For abbreviations, see Fig. 1.

Following hydrolysis of mucopolysaccharides with 0.04 N HCl, free amino groups were estimated (Fig. 3) as described by Foster *et al*[21]. One ml of 0.04 N hydro-

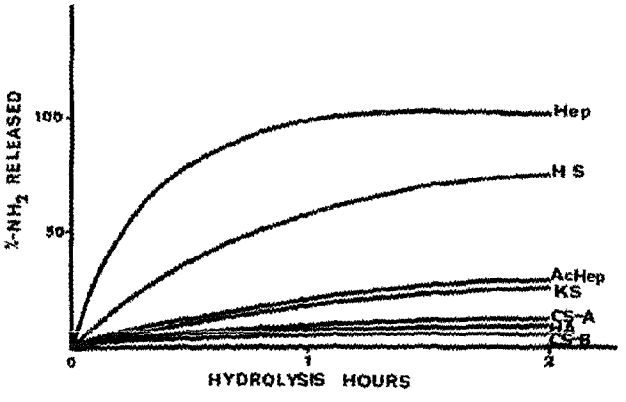

Fig. 3. Release of free amino groups from hexosamine during hydrolysis of mucopolysaccharides with 0.04 N HCl for periods up to 2 h. Estimation of the liberated amino groups was accomplished by application of the 1-fluoro-2,4-dinitrobenzene method[21]. All results were related to heparin used as a standard. For abbreviations, see Fig. 1; CS-A, chondroitin 4-sulfate.

chloric acid containing 1 mg of mucopolysaccharides in a 10 ml volumetric flask was heated for 90 min in a boiling water bath. To the cooled solution 0.4 ml of saturated $NaHCO_3$ solution, 0.1 ml of 1-fluoro-2,4-dinitrobenzene and 1.5 ml of ethanol were added. The mixture was shaken vigorously for 4 h and then extracted twice with ether to remove excess 1-fluoro-2,4-dinitrobenzene reagent. After being acidified with 1.0 N HCl, the solution was again extracted with ether and diluted to 10 ml with water. The optical densities at 355 mμ of the reaction products were compared with that from a standard heparin preparation.

Gel filtration

Hydrolyzates of mucopolysaccharides were obtained by heating 80–100 mg of mucopolysaccharides in 25 ml of 0.04 N HCl for 4 h. After being cooled, the hydrolyzates were neutralized with 1 N NaOH, concentrated to 2 ml and applied to a 1.1 × 200 cm column of Sephadex G-25 (30–80 microns, beaded form). Elution was carried out with 1 N NaCl. Fractions of 3 ml were collected at 30-min intervals and, after analysis for uronic acid content, were pooled as indicated in Fig. 5. Results of the analyses of the pooled material are shown in Table 1.

RESULTS

Release of reducing groups

Figure 1 illustrates the release of reducing groups, as determined by the alkaline-ferricyanide method[20], after hydrolysis of mucopolysaccharides at 100° for 1 h with 0.02 to 0.2 N HCl. The mucopolysaccharides, hyaluronic acid, chondroitin 6-sulfate, dermatan sulfate, and keratan sulfate, in which the 2-amino-2-deoxyhexopyranosyl residues are β-D-linked appear to be cleaved to appreciable extents (approximately average disaccharide length with 0.2 N HCl), whereas the mucopolysaccharides having α-D-linked residues, *N*-acetylheparin and heparitin sulfate, are much more resistant to hydrolysis. Separate experiments with chondroitin 4-sulfate indicate that hydrolysis of this substance parallels that of chondroitin 6-sulfate and hydrolysis of heparin is similar to that of heparitin sulfate.

Appraisal of the actual extent of hydrolysis, however, must take note of the fact that partial degradation of oligosaccharides may occur under the alkaline conditions of the reducing sugar method. This degradation increases as the oligosaccharide size decreases and is most pronounced with (1→3) linked reducing units[23]. When the alkaline-ferricyanide method is applied to *N*-acetylchondrosine, *N*-acetylhyalobiuronic acid, and turanose, reducing values are obtained which indicate extensive cleavage of the (1→3) linkages, whereas maltose and melibiose which contain 1,4- and 1,6-linked units, respectively, have been found to give reducing values only one-third or less above theoretical. Considered on this basis, the results shown in Fig. 1 should be taken to indicate that the hydrolysis products obtained are larger than suggested by the reducing-sugar data. This is borne out by fractionation of the hydrolyzates by gel filtration on Sephadex G-25 as described later.

2-Amino-2-deoxyglucoside bond cleavage

An additional approach for evaluating the extent of the 2-amino-2-deoxy-β-D-hexopyranosyl bond cleavage in mucopolysaccharides was based on the formation of hexosamine reducing groups during hydrolysis. Figure 2 shows the *N*-acetylhexosamine values obtained after hydrolysis of mucopolysaccharides as described under "hydrolysis conditions". The broken lines refer to the results of the Morgan–Elson reaction carried out directly on the hydrolyzate and the solid lines represent the results found with the same method applied after acetylation as described by Levvy and McAllan[13]. The latter technique measures both hexosamine and *N*-acetylated hexosamine reducing units present after hydrolysis while the former measures only acetylated hexosamine. Disaccharides from the β-D and α-L-linked mucopolysaccharides have been found to give color yields in the Morgan–Elson reaction equivalent to 80–90% of those obtained with standard 2-acetamido-2-deoxyglucose. On this basis, the data in Fig. 2 indicate that up to one-third of the hexosamine reducing end groups are deacetylated after hydrolysis with 0.2 N acid for 1 h.

Factors which affect the estimation of 2-amino-2-deoxyglucoside bond cleavage by the Morgan–Elson method include the extent of both monosaccharide formation and desulfation during hydrolysis. Substitution by sulfate at C-4 of the 2-amino-2-deoxygalactose unit in chondroitin 4-sulfate and dermatan sulfate causes inhibition of color production in the colorimetric reaction[9,24]. Consequently, the presence of sulfated oligosaccharides in hydrolyzates of these mucopolysaccharides results in decreased ratios of *N*-acetylhexosamine to reducing sugar. A similar decreased ratio results from the formation of free 2-acetamido-2-deoxygalactose, since this amino sugar yields only a third the color obtained from an equivalent amount of 2-acetamido-2-deoxyglucose used as a standard in the Morgan–Elson method. A comparison of the relatively low *N*-acetylhexosamine values with the reducing sugar values for dermatan sulfate, as noted in Figs. 1 and 2, suggests that this factor is of significance in the estimation of hexosaminidic bond cleavage for this mucopolysaccharide.

Keratan sulfate, which is reported to contain *O*-galactosyl-(1→4)-2-acetamido-2-deoxyglucose linkages[25], produces oligosaccharides which are not expected to react in the *N*-acetylhexosamine method. The findings in Fig. 2, therefore, indicate the extent of monosaccharide formation from keratan sulfate. Approximately one-third of the keratan sulfate is cleaved to monosaccharides with 0.2 N HCl. The low values shown in Fig. 2 for the mucopolysaccharides with α-D-linked residues conform with their relative resistance to acid hydrolysis and to the formation of products which contain principally uronic acid at the reducing ends.

Deacetylation of mucopolysaccharides

The above described methods were not applicable for estimating *N*-deacetylation of internal hexosamine units. Liberation of such amino groups was estimated by reaction of the hydrolyzates with 1-fluoro-2,4-dinitrobenzene[21], using heparin as a standard or by use of the indole–nitrous acid method[14]. Figure 3 shows the results

TABLE I

ANALYSES OF HYDROLYSIS FRACTIONS OF CHONDROITIN 4-SULFATE, CHONDROITIN 6-SULFATE, AND DERMATAN SULFATE ISOLATED BY GEL FILTRATION[a]

	Chondroitin 4-sulfate			*Chondroitin 6-sulfate*			*Dermatan sulfate*		
Fraction	*Uronic acid*	N-*Acetyl-hexosamine*	*Sulfate*	*Uronic acid*	N-*Acetyl-hexosamine*	*Sulfate*	*Uronic acid*	N-*Acetyl-hexosamine*	*Sulfate*
I	20	0	—	20	—	—	4.0	0	—
II	1.04	0.26	0	1.20	0.44	—	0.56	0.07	—
III	0.97	0.83	(–)[b]	1.06	0.87	(–)[b]	0.40	0.56	(–)[b]
IV	1.10	0.51	0.32	1.11	0.43	0.49	0.56	0.25	—
V	1.17	0.33	0.30	1.08	0.29	0.52	0.55	0.24	0.17
VI	1.04	0.17	0.29	1.09	0.19	—	0.53	0.14	0.12

[a]All values expressed as ratios to total hexosamine; [b]Contains inorganic sulfate.

hydrolysis of mucopolysaccharides with 0.04 N HCl for up to 2 h, conditions used in the determination of *N*-sulfate groups of heparitin sulfate and heparin[14,21].

It is noteworthy that *N*-acetylheparin, though resistant to glycosidic cleavage, is deacetylated approximately 30% after hydrolysis for 2 h. Similar results were found for keratan sulfate, but hyaluronic acid, dermatan sulfate, and chondroitin 4-sulfate were more resistant to deacetylation. The progressive release of amino groups from heparitin sulfate indicates that appreciable deacetylation occurs along with *N*-desulfation.

Hydrolysis of oligosaccharides

In order to study the course of acid hydrolysis of oligosaccharides, certain of these were subjected to the action of 0.04 N HCl for up to 6 h. Figure 4 illustrates the results obtained with oligosaccharides from chondroitin 4-sulfate (CSA-O), chondroitin 6-sulfate (CSC-O), and *N*-acetylated chondrosine (CS-O).

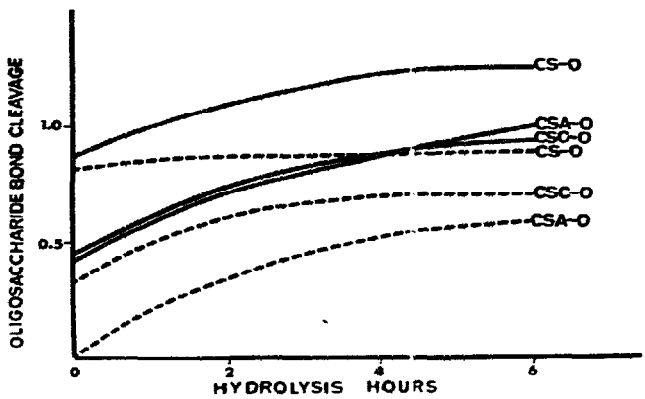

Fig. 4. Hydrolysis of oligosaccharides with 0.04 N HCl for up to 6 h as described in EXPERIMENTAL. The solid lines refer to reducing sugar values and the broken lines represent *N*-acetylhexosamine results obtained after acetylation. Values are expressed in terms of ratios to hexosamine content. For abbreviations see Text.

Preparation CSA-O, obtained after exhaustive digestion of chondroitin 4-sulfate with testicular hyaluronidase, appeared by analysis to be of average hexasaccharide size. As noted earlier, the presence of ester sulfate at C-4 of the acetylhexosamine reducing unit inhibits color formation in the Morgan–Elson reaction. This analytical method therefore provides a convenient means for determining the extent of desulfation of CSA-O during acid hydrolysis.

The results shown in Fig. 4 indicate that hydrolysis of CSA-O yields an approximately parallel increase of reducing sugar (solid line) and *N*-acetylhexosamine values (broken lines). This suggests that desulfation occurs at approximately the same rate as glycosidic bond cleavage. After hydrolysis for 6 h the ratio of reducing sugar to total hexosamine is approximately 1.0 suggesting the formation of disaccharide units to a large extent. This is supported by the increase in *N*-acetylhexosamine

content after hydrolysis for 6 h to two-thirds that of CS-O, considered to be largely disaccharide in nature. Since 10% or less difference was found between the *N*-acetylhexosamine values obtained before or after acetylation of hydrolyzates, it is concluded that the primary product of hydrolysis was a desulfated and *N*-acetylated disaccharide.

The hydrolysis of an oligosaccharide fraction from chondroitin 6-sulfate (CSC-O) is also shown in Fig. 4. The results are found to be similar to those for CSA-O, suggesting that disaccharide formation from this oligosaccharide occurs also under mild acid conditions. The extent of desulfation cannot be estimated in the same way as CSA-O since the Morgan-Elson reaction is not affected by the C-6 sulfate in this substance.

Hydrolysis of *N*-acetylchondrosine (CS-O), the desulfated repeating unit of both chondroitin 4- and 6-sulfate, indicates that cleavage beyond the stage of disaccharide does not readily occur. This is indicated by the results in Fig. 4 which show relatively small increases in reducing sugar values and essentially unchanged *N*-acetylhexosamine values during hydrolysis.

Isolation of hydrolysis fractions

Chondroitin 4- and 6-sulfate and dermatan sulfate were hydrolyzed with 0.04 N HCl for 4 h and the hydrolyzates subjected to gel filtration on Sephadex G-25 columns[26]

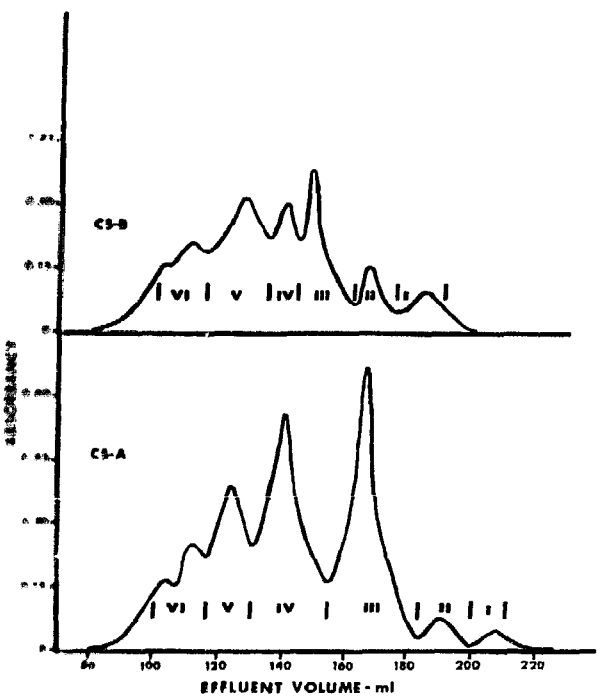

Fig. 5. Fractionation of hydrolyzates from chondroitin 4-sulfate and dermatan sulfate by gel filtration on Sephadex G-25. Curves shown are based on uronic acid contents.

The fractionation patterns obtained from hydrolyzates of chondroitin 4-sulfate and dermatan sulfate are shown in Fig. 5. Chondroitin 6-sulfate yielded a pattern, not shown in Fig. 5, which was similar to that from chondroitin 4-sulfate.

Analyses of the gel filtration fractions are shown in Table 1. Data are given as molar ratios to hexosamine. As indicated above, *N*-acetylhexosamine estimations of chondroitin 6-sulfate fractions are a direct measure of molecular size, but results for the chondroitin 4-sulfate and dermatan sulfate products cannot be used in this way except when reducing end hexosamine units are totally devoid of sulfate.

Results of the Morgan–Elson method for chondroitin 4-sulfate indicate that the main fractions in decreasing proportions are di-, tetra- and hexa-saccharides, respectively (fractions III, IV and V of Fig. 5). Reduction with borohydride of fractions IV and III gave losses in hexosamine contents of 44 and 91%, respectively, with no loss in uronic acid, in agreement with the Morgan–Elson data. The slowest fraction (peak I) contains almost entirely uronic acid. When this was examined by paper chromatography, only substances moving similar to glucuronolactone and glucuronic acid were noted. Fraction II showed, after paper chromatography, the presence of 2-amino-2-deoxygalactose, 2-acetamido-2-deoxygalactose, and glucuronolactone.

The fractions obtained from chondroitin 4-sulfate appear similar to those from chondroitin 6-sulfate except for the lower *N*-acetylhexosamine values which probably are attributable to the sulfate remaining after hydrolysis. Hydrolysis of dermatan sulfate produces a relatively large proportion of free uronic acid (fraction I), amounting to possibly 10% of the total uronic acid, when allowance is made for the lower color yield[19] of the free uronic acid compared to that bound in the oligosaccharide fractions. Paper chromatography showed the presence only of iduronolactone and iduronic acid in this fraction.

DISCUSSION

Partial hydrolysis of mucopolysaccharides for the production of oligosaccharides has been generally carried out under relatively vigorous acidic conditions. Thus, the preparation of disaccharides from hyaluronic acid and chondroitin 4-sulfate has been accomplished by heating at 100° in 1 N HCl for several h[4,5]. Under these conditions, complete deacetylation and desulfation occurs. The present study indicates that mild acid conditions result in the extensive hydrolysis of β-D and α-L-linked mucopolysaccharides to produce mixtures of oligosaccharides and monosaccharides which to a major extent remain acetylated and which, except for hyaluronic acid products, are partially sulfated as well. The possibility of isolating such fragments is of obvious interest for structural studies. The results from reducing sugar and *N*-acetylhexosamine determinations illustrated in Figs. 1 and 2 suggest that small oligosaccharides preponderate after hydrolysis of the β-D-linked mucopolysaccharides with 0.1 or 0.2 N HCl for 1 h.

As expected, the α-D-linked mucopolysaccharides, heparitin sulfate and

N-acetylheparin, showed only a slight tendency for glycosidic bond cleavage under mild conditions. Despite this resistance to hydrolysis, *N*-acetylheparin was 30% deacetylated (Fig. 3) when heated in 0.04 N HCl for 2 h, conditions used for the determination of *N*-sulfate groups in heparin and heparitin sulfate[21]. The finding that heparitin sulfate, which contains both *N*-sulfate and *N*-acetyl groups, shows a significant increase in release of amino groups beyond the time required for complete *N*-desulfation indicates that the *N*-acetyl bonds present in heparitin sulfate are as labile to acid as those found in *N*-acetylheparin and is another indication of the similarity of the two substances. It is evident from the above results that determinations of *N*-sulfate contents by the methods generally used should be interpreted with caution.

Sulfated oligosaccharides from chondroitin 4- and 6-sulfate, produced after exhaustive digestion of the mucopolysaccharides with testicular hyaluronidase were found to follow a course of hydrolysis similar to the parent mucopolysaccharide, shown in Fig. 4. The hydrolysis yielded preponderantly disaccharide which were relatively resistant to further cleavage under the conditions used.

The isolation of oligosaccharide fractions from mucopolysaccharide hydrolyzates obtained by heating with 0.04 N HCl for 4 h served to support the suggestions from Figs. 1 and 2 of the occurrence of extensive hydrolysis. As indicated in Table 1, hydrolyzates from chondroitin 4- and 6-sulfate and dermatan sulfate contain disaccharides as major components, with larger oligosaccharides comprising successively smaller proportions of the product (Fig. 5). Free uronic acids were demonstrated from each mucopolysaccharide. It is noted that the fractions have not been deacetylated significantly. Desulfation was found to be more extensive in chondroitin 4-sulfate and dermatan sulfate, which retained only a third or less of that originally present, than in chondroitin 6-sulfate, which lost approximately half its sulfate.

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SUMMARY

The hydrolysis of mucopolysaccharides with dilute hydrochloric acid was studied. Glycosidic bond cleavage of the β-D- and α-L-linked mucopolysaccharides occurred with unexpected facility and produced partially sulfated and *N*-acetylated disaccharides as well as higher oligosaccharides and smaller proportions of monosaccharides. These products were isolated by means of gel filtration with Sephadex G-25.

The α-D-linked substances, heparin, *N*-acetylheparin, and heparitin sulfate,

remained relatively resistant to hydrolysis under the conditions used although *N*-deacetylation occurred more readily with *N*-acetylheparin than with the other polysaccharides examined.

Oligosaccharides of chondroitin 4- and 6-sulfate were found to undergo hydrolysis readily to *N*-acetylated and partially sulfated disaccharides.

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STUDIES ON URONIC ACID MATERIALS

PART XIV*. METHYLATION WITH THE SODIUM HYDRIDE–METHYL IODIDE–DIMETHYL SULPHOXIDE SYSTEM

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(Received December 2nd, 1965)

INTRODUCTION

Recently, there has been renewed interest in methods for methylating polysaccharides. Attempts have been made to improve yields and also to reduce the number of repetitive treatments required with the classical Haworth[2] and Purdie[3] techniques. Kuhn[4] and his co-workers used *N,N*-dimethylformamide as solvent with silver oxide and methyl iodide for permethylations and have since proposed[5] the use of *N,N*-dimethylformamide and dimethyl sulphoxide, separately or in admixture, as solvents for methylations with barium hydroxide and methyl iodide or dimethyl sulphate.

Our attempts to methylate *Acacia* gum polysaccharides, using barium salts, gave reasonable methoxyl contents, but emulsions, which were extremely difficult to break, tended to form. The use of dimethyl sulphoxide, with powdered sodium hydroxide and dimethyl sulphate, has been reported[6] to give high yields of almost fully methylated, neutral polysaccharides, but, in our experience, this technique is less successful with acidic materials.

We have investigated the use of sodium hydride and methyl iodide for the methylation of acidic *Acacia* polysaccharides dissolved in dimethyl sulphoxide. (A similar technique has been used to methylate glycoproteins and neutral polysaccharides[7], but its use with acidic materials has not, to our knowledge, been reported previously). We have also used this reaction for the rapid methylation of mono- and di-saccharides; sodium hydride has been used in the methylation of monosaccharide derivatives in ether-type solvents[8,9] or in *N,N*-dimethylformamide[9].

In the presence of acidic groups, the sodium hydride reaction might lead to the following side-reactions, with the formation of artifacts: (*a*) condensation of ester groups with the methylsulphinyl carbanion to give a sulphoxide; (*b*) in the presence of ester groups, β-elimination could occur to give 4,5-unsaturated acids[10] (esters of both glucuronic and galacturonic acids can undergo[11] such eliminations); (*c*) the product from (*b*) could react further to give a variety of products.

The methylated products were therefore examined to ascertain whether such artifacts had arisen.

*For Part XIII see ref. 1

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 paper with the following solvent systems (v/v): (*a*) butan-1-ol–ethanol–water (4:1:5, upper layer); (*b*) butan-1-ol–acetic acid–water (4:1:5, upper layer); (*c*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4).

Gas–liquid chromatography (g.l.c.) was carried out on columns (3 ft × 0.25 in) of polyethyleneglycol adipate (15% by weight on acid-washed Celite, 80–100 mesh) at 150°; the carrier gas was nitrogen at a flow rate of 100 ml/min. The chromatograph (Model S3A, Gas Chromatography Ltd, Maidenhead) was fitted with flame-ionisation detectors. Retention times are given relative to that of methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside.

Weights recorded are those corrected for moisture content (by drying to constant weight at 105°). Hydrolyses and methanolyses were effected with N sulphuric acid for 8 h at 100° and with 5% methanolic hydrogen chloride for 6 h at 100° (sealed tube), respectively. Reagent-grade dimethyl sulphoxide and methyl iodide were redistilled before use. Methoxyl contents were determined by a specific, vapour-phase, infrared method[12]. Absorption spectra were taken with a Perkin–Elmer 137 UV Spectrophotometer.

Experimental precautions. Powdered sodium hydride was used without difficulty throughout our experiments; due precautions were observed when handling this reagent. Where preferred, the commercial dispersion of sodium hydride in oil may be used. A convenient method is to add the dispersion to dry dimethyl sulphoxide; the layer containing sodium hydride dissolved in dimethyl sulphoxide may then be added to a solution, in dimethyl sulphoxide, of the material to be methylated.

RESULTS

(a) Acidic polysaccharides

Methylation of the gum from Acacia nubica *(Benth.)*

The gum (4.64 g) was dissolved in dimethyl sulphoxide (250 ml), and powdered sodium hydride (2 g) was added in small portions, with gentle stirring, during 1 h. The solution turned yellow and, ultimately, became semi-solid. Methyl iodide (5 ml) was added dropwise with stirring during 2 h. The solution was stirred overnight, and one drop then gave a neutral reaction when added to water. A further three additions of sodium hydride and methyl iodide were made to the reaction mixture on successive days, as described for the first addition. The mixture was then poured into water (1.5 l) to precipitate the methylated polysaccharide, and any excess of methyl iodide was removed by aspiration. The precipitate was collected (centrifuge) and dissolved in chloroform. The supernatant solution (aqueous dimethyl sulphoxide) was extracted with chloroform, and the extract was combined with the solution of precipitated gum. The chloroform solution was washed with water to remove dimethyl sulphoxide.

dried ($MgSO_4$), concentrated (rotary evaporator at less than 30°), and then added to light petroleum (b.p. 60–80°) to precipitate the methylated polysaccharide, which was removed by centrifugation and dried at room temperature under diminished pressure: yield, 3.98 g (85%) (Found: OMe, 40.8%).

Single-step methylation of a degraded gum from Acacia nubica

Degraded gum (1.86 g), prepared from *A. nubica* gum by autohydrolysis, was dissolved in dimethyl sulphoxide (50 ml). Sodium hydride (2 g) was added in small portions, followed by methyl iodide (5 ml), as for the whole gum. The mixture was stirred overnight, water (200 ml) was added, and the mixture was extracted with chloroform. The isolation of the methylated, degraded gum (1.62 g, 87%) then followed the sequence already described for the whole gum (Found: OMe, 37.1%).

Tests for the formation of artifacts

The following tests were made on the methylated whole-gum and methylated, degraded gum.

The methylated gum, dissolved in spectroscopic grade ethanol, showed no absorption at 235 mμ, indicating the absence of β-elimination products.

The methylated gum was tested for the presence of unsaturation by the thiobarbituric acid method[10]. Methylated gum (10 mg) was shaken with water (1 ml), and hydrochloric acid (5 ml, 0.5M) and thiobarbituric acid (10 ml, 0.01M) were then added. The solution was immersed in a boiling water-bath for 30 min and cooled, and the absorption spectrum was examined. There was no absorption at 547 mμ, indicating the absence of 4,5-unsaturated derivatives of D-glucuronic acid.

Small-scale methylation of degraded A. nubica *gum*

To degraded gum (30 mg) in dimethyl sulphoxide (10 ml) was added, as described above, sodium hydride (500 mg) followed by methyl iodide (1.3 ml), and the mixture was stirred gently overnight. A second addition of reagents was then made, the mixture was stirred overnight, and the methylated product (20 mg) was isolated as described for the methylation of whole gum (Found: OMe, 40.0%).

A half-portion of the product was dissolved in chloroform and examined by g.l.c. The remainder of the product was hydrolysed and then examined by paper chromatography in solvents (*a*) and (*b*). In all of these examinations, the chromatograms were identical with those obtained from the product of the large-scale methylation of degraded *A. nubica* gum.

(b) Acidic mono- and di-saccharides

Methylation of D-*glucuronic acid.*

D-Glucuronic acid [200 mg, chromatographically homogeneous in solvent (*c*) and containing no D-glucurone] was dissolved in dimethyl sulphoxide (10 ml), and sodium hydride (500 mg) was added in small portions with gentle stirring during 1 h. Methyl iodide (1.4 ml) was added dropwise, and the mixture was stored overnight.

The methylated product was then isolated by pouring into water, followed by extraction with chloroform at room temperature.

The solution of the product in chloroform was divided into four parts. Portion 1 was examined directly by g.l.c. and gave only three peaks, having relative retention times of 0.17, 2.14, and 2.81. The first component had the same retention time as dimethyl sulphoxide. The other two peaks had the same retention times as were given by an authentic specimen of methyl (methyl 2,3,4-tri-*O*-methyl-$\alpha\beta$-D-glucopyranosid)-uronate.

Portion 2 was concentrated to dryness and subjected to methanolysis, and the neutralised (silver carbonate) solution was concentrated to dryness. A solution of the residue in chloroform was examined by g.l.c. as for portion 1. The same peaks were obtained, but with a decreased proportion of dimethyl sulphoxide.

Portion 3 was concentrated to dryness and hydrolysed. The product was neutralised (barium carbonate) and examined by paper chromatography in solvents (*a*) and (*b*). Only one component, chromatographically identical with 2,3,4-tri-*O*-methyl-D-glucuronic acid, was detected.

Portion 4 was concentrated to dryness and warmed with water (1 ml), and then hydrochloric acid (5 ml, 0.5 *M*) and thiobarbituric acid (10 ml, 0.01 M) were added. The solution was kept for 30 min at 100° and then cooled; there was no absorption at 547 mμ.

Methylation of methyl α-D-*glucopyranoside and methyl* α-D-*galactopyranoside*

Quantities and procedure were as for D-glucuronic acid, except that the mixtures were stirred for only 1 h after addition of the methyl iodide. Examination by g.l.c. of the products from each glycoside showed that only the fully methylated sugars were present.

Methylation of an aldobiouronic acid

6-*O*-(β-D-Glucopyranosyluronic acid)-D-galactose (60 mg) was dissolved in dimethyl sulphoxide (10 ml). Reaction with sodium hydride (500 mg) and methyl iodide (1.4 ml) was then carried out as described above for the small-scale methylation of the degraded gum from *A. nubica*.

A half-portion of the product was hydrolysed and examined by paper chromatography in solvents (*a*) and (*b*) Only two components, chromatographically identical with reference samples of 2,3,4-tri-*O*-methyl-D-glucuronic acid and 2,3,4-tri-*O*-methyl-D-galactose, were detected. The remainder of the product was hydrolysed in methanolic hydrogen chloride and neutralised (silver carbonate), and the methanol was removed by evaporation. The product was dissolved in dry chloroform and examined by g.l.c. Components having retention times identical with those of the methyl glycosides of 2,3,4-tri-*O*-methyl-D-galactose and of the methyl ester of 2,3,4-tri-*O*-methyl-D-glucuronic acid were found*.

**Note added in proof.* In some reactions, it has been observed that 2,3,5-tri-*O*-methyl-D-galactose may also be formed, presumably through reaction of the disaccharide in the furanose form.

DISCUSSION

The methylation technique described gives complete methylation of monosaccharides and acidic disaccharides in one stage, and methoxyl contents exceeding 36% have consistently been achieved for acidic polysaccharides by making 2–4 additions of the reagents. Careful tests have failed to indicate the formation of artifacts. The products are given in high yield, and satisfactory results have been obtained with 30-mg samples. Yields of only 20–30% were obtained when classical methods were used, in multi-stage processes, to methylate acidic polysaccharides from *A. seyal*[13] and *A. nilotica*[14]. In the light of this direct comparison, the sodium hydride method clearly offers distinct advantages.

It is hoped that this communication will lead other investigators to assess the performance of the method with a wider range of polysaccharides.

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SUMMARY

Reaction of acidic poly-, mono-, and di-saccharides with sodium hydride and methyl iodide, in dimethyl sulphoxide as solvent, requires very few repetitive treatments to give highly methylated products in very good yield. Artifacts could not be detected in the products.

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Notes

Alkylation of carbohydrates using sodium hydride

The preceding paper[1], describing the use of sodium hydride and dimethyl sulphoxide in the methylation of acidic polysaccharides and some mono- and di-saccharides, prompts us to report on our experience with this reagent. Other reports have appeared on the use of sodium hydride in the methylation of monosaccharides[2], neutral polysaccharides[3], and glycoproteins[3], but few examples have been reported[4] of its use in other alkylations.

A search for new ethers of sucrose, having possible commercial interest, caused us to examine a number of alkylation procedures. In our hands, sucrose and other carbohydrates were conveniently alkylated by using an alkyl halide (usually the bromide) in either *N*,*N*-dimethylformamide, or *N*-methyl-2-pyrrolidone, containing suspended sodium hydride. Previously, tetrahydrofuran had been employed[2], but this is unsuitable for general use since it is not a good solvent for unsubstituted sugars. Both *N*,*N*-dimethylformamide and *N*-methyl-2-pyrrolidone are good solvents for most carbohydrates and are likely to enhance formation of the nucleophilic alkoxide ion. Although both solvents possess a carbonyl group, neither reacts significantly with sodium hydride at room temperature. At higher temperatures (*ca.* 70–100°), *N*,*N*-dimethylformamide reacted with sodium hydride to give a product which afforded formaldehyde (identified as its 2,4-dinitrophenylhydrazone) on treatment with water, and tetramethylammonium iodide (identified by its m.p. and infrared spectrum) on treatment with methyl iodide.

Some alkylations using sodium hydride in the aforementioned, aprotic solvents are recorded in the Experimental section. The method is easily applied, provided that due care is exercised in the handling of the hydride and, on completion, the excess of reagent is rapidly removed by addition of dry methanol. Thus, methylation, butylation, octylation, allylation, and benzylation of methyl 4,6-*O*-benzylidene-α- and -β-D-glucopyranoside were accomplished under mild conditions and in high yield. *N*,*N*-Dimethylformamide could not be used in the propargylation of the foregoing compounds as a vigorous reaction occurs between propargyl bromide and sodium hydride in this solvent. In this case, tetrahydrofuran was the solvent of choice. Allylation of sucrose was complete in one stage using this procedure, whereas, previously, repetitive treatments were required[5]. Applications of this procedure to other carbohydrate derivatives have been reported in recent papers[6,7] from our laboratories.

EXPERIMENTAL

N,N-Dimethylformamide and *N*-methyl-2-pyrrolidone were shaken with potassium hydroxide pellets and then with calcium oxide, and distilled on to calcium hydride for storage. Organic halides were shaken with phosphorus pentoxide, decanted, and distilled. Sodium hydride was received as a dispersion in mineral oil. The oil was removed by washing the dispersion with ether under nitrogen on a sintered-glass crucible (No. 3 porosity). Evaporations were usually performed at $< 45°/0.5$ mm.

Methyl 4,6-O-benzylidene-2,3-di-O-butyl-α-D-glucopyranoside

Sodium hydride powder (1.17 g, 49 mmole) was added to a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (1.9 g, 6.7 mmole) in *N,N*-dimethylformamide (60 ml), and the suspension was swirled for 20 min before the addition, with cooling (0°), of 1-bromobutane (10 ml, 95 mmole). The reaction mixture became solid within 40 min and was set aside at room temperature for 24–48 h. Methanol (10 ml) was added carefully to the resulting clear solution and, when effervescence had ceased, the solution was concentrated to dryness. The residue was partitioned between chloroform (50 ml) and water (50 ml), and the separated organic layer was further washed with water (3 × 40 ml), filtered, and concentrated, to yield a crystalline residue. Two recrystallizations from aqueous ethanol afforded *the product* (2.58 g, 97%), m.p. 89–90°, $[\alpha]_D^{21}$ + 50° (*c* 0.6, chloroform). (Found: C, 67.3; H, 8.5. $C_{22}H_{34}O_6$ calc.: C, 67.0; H, 8.7%).

Other alkylations

Essentially the same procedure was used in the preparation of the following compounds; the percentage yield from the reaction is given in parenthesis. In many cases, the reaction could be carried out in *N*-methyl-2-pyrrolidone, without diminution of yield.

Methyl 4,6-O-benzylidene-2,3-di-O-methyl-β-D-glucopyranoside (82%), m.p. 133–134°, $[\alpha]_D^{18}$ −61° (*c* 0.5, ethanol) {lit.[8], m.p. 134°, $[\alpha]_D$ −61° (ethanol)}.

Octa-O-methyl sucrose (69%), b.p. 162–165°/0.4 mm, $[\alpha]_D^{20}$ + 62° (*c* 1.1, chloroform), n_D^{20} 1.4570. {lit.[9], b.p. 140–145° (bath)/0.001 mm, $[\alpha]_D$ + 72° (*c* 0.76, methanol), n_D^{21} 1.4559–1.4570}.

Methyl 4,6-O-benzylidene-2,3-di-O-octyl-α-D-glucopyranoside (91%), m.p. 58–59° (from aqueous ethanol), $[\alpha]_D^{19}$ + 35° (*c* 0.6, chloroform). (Found: C, 71.0; H, 9.9. $C_{30}H_{50}O_6$ calc.: C, 71.1; H, 9.95%).

Methyl 2,3-di-O-allyl-4,6-O-benzylidene-α-D-glucopyranoside (95%), m.p. 62–63° (from light petroleum, b.p. 40–60°), $[\alpha]_D^{18}$ + 54° (*c* 0.45, chloroform). (Found: C, 66.1; H, 7.2. $C_{20}H_{26}O_6$ calc.: C, 66.25; H, 7.2%). Isomerisation[10] of the foregoing compound with potassium *tert*-butoxide in dry dimethyl sulphoxide gave only low yields (*ca.* 4%) of methyl 4,6-*O*-benzylidene-2,3-di-*O*-(prop-1-enyl)-α-D-glucopyranoside, m.p. 94–95° (from aqueous ethanol), $[\alpha]_D^{23}$ + 41 ± 5° (*c* 0.024, chloroform). (Found: C, 65.75; H, 6.8. $C_{20}H_{26}O_6$ calc.: C, 66.25; H, 7.2%). Hydrolysis

of the latter ether with 2N sulphuric acid for 1 h at 100° yielded a mixture of methyl α-D-glucopyranoside and D-glucose (identified by paper chromatography).

Methyl 4,6-O-benzylidene-2,3-di-O-propargyl-α-D-glucopyranoside (85%, with tetrahydrofuran as solvent), m.p. 75–76° (from aqueous methanol), $[\alpha]_D^{21}$ + 27° (*c* 0.3, chloroform) (Found: C, 66.9; H, 6.0. $C_{20}H_{22}O_6$ calc.: C, 67.0; H, 6.2%).

Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside (93%), m.p. 118–119° (from light petroleum, b.p. 60–80°), $[\alpha]_D^{21}$ −37° (*c* 1.4, chloroform) {lit.[11], m.p. 119–120°, $[\alpha]_D^{20}$ −35.8° (*c* 3, chloroform)}.

Methyl 2,3,4,6-tetra-O-allyl-α-D-glucopyranoside (64%), b.p. 152–153°/0.5 mm, $[\alpha]_D^{20}$ + 114° (*c* 9, ethanol), n_D^{20} 1.4710 {lit.[12], b.p. 160–162°/1.5 mm, $[\alpha]_D^{25}$ + 115.6° (*c* 8, ethanol, n_D^{20} 1.4710}.

Octa-O-allyl sucrose (96%), $[\alpha]_D^{19}$ + 45.4° (*c* 1.4, chloroform), n_D^{21} 1.4818 (lit.[5], n_D^{20} 1.4822).

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The infrared spectra of some furans related to the aldofuranoses

Infrared spectra can be used to distinguish between the anomers of the aldopyranoses[1], as well as of their acetates[2]. The deoxy sugars also have characteristic absorption peaks[3]. Since, in general, the conformation of the aldopyranose and, more specifically, the presence of an equatorial or axial hydrogen atom on C-1 determines the location of absorption bands diagnostic of the anomeric form[4], it might be expected that the furanose sugars, which have essentially a planar ring[5], would not show bands characteristic of the anomeric forms. Barker and Stephens[5] have, however, been able to correlate the presence of a furanoid or hydrofuranol ring with certain specific absorption bands of types *A*, *B*, *C*, and *D* (at 924 $\pm$13, 879 $\pm$7, 858 $\pm$7, and 799 $\pm$17 cm^{-1}, respectively). Furthermore, these absorption bands were assigned as follows[5,6]: type *A*, the symmetrical ring-breathing frequency; types *B* and *C*, modes of vibration involving the skeletal stretching of the substituents; and type *D*, a carbon–hydrogen deformation mode, where the hydrogen atom is present on a carbon atom directly attached to a ring-oxygen atom of a furanoid or hydrofuranol ring.

It was considered of interest to prepare a number of chloro-, dihydro-, and tetrahydro-furans having a methoxy or ethoxy group on C-1. Such compounds would be analogous to the methyl and ethyl aldofuranosides. Indeed, they may be considered to be the simplest form of the aldofuranosides; and, therefore, it was expected that they would show the infrared absorption bands characteristic of the anomeric hydrogen atom, the ring breathing, and the ring vibrations of the furanoses. It was anticipated, however, that the exact location of the peaks would be determined by the presence of (*a*) such groups as methoxy or ethoxy on C-1 and (*b*) a double bond or chlorine or hydrogen atom on C-2; and that there would be no influence of hydroxyl groups on C-2 and C-3, as for the sugars.

EXPERIMENTAL

2,3-Dichlorotetrahydrofuran was prepared by the chlorination of tetrahydrofuran[7], and converted into the 3-chloro-2-(methoxy or ethoxy)-tetrahydrofuran by treatment with sodium methoxide or sodium ethoxide, respectively[7]. Further treatment of the appropriate 3-chlorotetrahydrofuran with sodamide yielded 2-methoxy- or 2-ethoxy-dihydrofuran[8]. The corresponding tetrahydrofurans were prepared by reduction of the dihydrofuran with hydrogen at 20 lb/sq in in the presence of a platinum

catalyst at room temperature. The physical constants agreed with those reported in the literature[6,8].

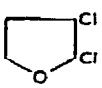
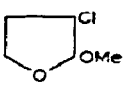
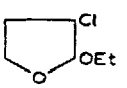
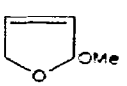
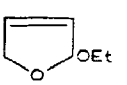
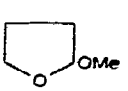
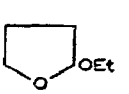
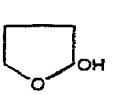
The infrared spectra were recorded for the neat liquids (no solvent) on a Beckman IR 8 infrared spectrophotometer in cells 0.025 mm long.

RESULTS AND DISCUSSION

All of the spectra were essentially the same in the 4000 to 1000 cm^{-1} region, and showed absorption bands at approximately the positions reported for hydrofuranols[6]. The compounds containing methoxyl groups showed, in addition, a band at 2825 cm^{-1} characteristic[4,9] of the methoxyl group. The compounds containing a double bond showed characteristic absorption[10] at 3077, 1610, and 692 cm^{-1}, and those compounds containing chlorine in the molecule had an absorption band at 727 cm^{-1}.

TABLE I

FREQUENCIES (IN CM^{-1}) OF ABSORPTION PEAKS[a]

Compound	*A*	*B*	*C*	*D*
	924 ± 13 921 (s) 905 (s)	875 ± 7	858 ± 7 834 (w)	799 ± 17 776 (w)
	910 (s)		848 (m)	816 (w)
	921 (s) 906 (s)	889 (w)	837 (vw)	813 (w)
	910 (s)		848 (vw)	807 (s)
	917 (s) 906 (s)	889 (w)	837 (vw)	803 (s)
	928 (m)		848 (m)	764 (m)
	928 (s) 910 (m)	889 (w)	840 (s)	791 (m)
	923 (vs)[6]		852 (s)[6]	760 (m)[6]

[a] v, very; s, strong; m, medium; w, weak.

Absorption bands in the region corresponding to absorption of types *A*, *B*, *C*, and *D* are shown in Table I, from which it may be seen that the ring-breathing frequencies (type *A* absorption) of the series fall within the reported range[5] of 924 ± 13 cm^{-1}. A second peak at 905 to 910 cm^{-1}, was found for those compounds having a chlorine atom or ethoxyl group on C-1.

Type *B* absorption at 879 ± 7 cm^{-1} has been considered to be due either to (*a*) modes of vibration involving the skeletal stretching of such substituents on the ring as $-CH(OH)-CH_2OH$ or (*b*) rocking vibrations of the methylene group in the case of the hydrofuranol ring[5]. Since, in our series, type *B* absorption is only found associated with ethoxy derivatives, it is tentatively suggested that type *B* absorption is, in some cases at least, due to the methylene hydrogen atoms of the ethoxyl group.

Type *C* absorption at 858 ± 7 cm^{-1} is considered to be due to the methylene-rocking vibration[6]. In agreement with this assignment, our series of compounds show type *C* absorption at 834 to 848 cm^{-1}. Since the methylene group attached to the oxygen atom of the ring is the only methylene group common to all of our compounds, it is suggested that this group is responsible for type *C* absorption. The attachment to oxygen is probably responsible for the observation that the frequency is slightly lower than normal.

Type *D* absorption at 799 ± 17 cm^{-1} is considered to be associated with a carbon–hydrogen deformation mode[5,6]. In agreement with the assignment, each compound of our series absorbs in the region 816 to 760 cm^{-1}. In addition, it should be noted that the substituent on C-2 affects the location of the absorption peak. With a methoxyl group on C-1, a saturated C-2 gives a type *D* frequency of 764 cm^{-1}, a double bond gives a frequency of 807 cm^{-1}, and a chlorine atom attached to C-2 gives a frequency of 816 cm^{-1}. With an ethoxyl group on C-1, the frequency values are: saturated C-2, 791 cm^{-1}, double bond on C-2, 803 cm^{-1}, and a chlorine atom on C-2, 813 cm^{-1}. In general, the frequency of the type *D* absorption is increased by substituents on C-2, as follows: $-CH_2- < = CH- < -CHCl-$.

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Synthesis of β-D-xylopyranosides of cyclic and aromatic alcohols

In the present work, we prepared a series (Table I) of tri-*O*-acetyl-β-D-xylopyranosides of cyclic and aromatic alcohols through the reaction of tri-*O*-acetyl-α-D-xylopyranosyl bromide (acetobromoxylose)[1] with the appropriate alcohol in the presence of silver oxide[2] or mercuric acetate as catalyst. Catalytic deacetylation (sodium methoxide)[3] yielded the corresponding β-D-xylopyranosides (Table II).

EXPERIMENTAL

The purity of the products was tested by t.l.c. on silicagel G (Merck) in acetic acid–water–ethyl acetate (1:1 3; v/v) for xylosides, and ethyl acetate–benzene (3:7; v/v) for the acetates; spray reagent, 5% sulfuric acid in ethanol (10 min at 120°).

Silver oxide method. Absolute chloroform (200 to 300 ml), silver oxide (0.1 mole), acetobromoxylose (0.1 mole), and the appropriate alcohol (0.5 to 1 mole) were stirred in a closed vessel until the supernatant liquid was free of bromide ions (several hours). Silver salts were filtered off, and the clear solution was concentrated *in vacuo* to yield the tri-*O*-acetyl-β-D-xylopyranoside (Table I).

Mercuric acetate method. Mercuric acetate (0.1 mole), absolute chloroform (200 ml), Sikkon (calcium sulfate, Fluka) (0.5 mole), and the alcohol (1 to 1.5 mole) were stirred for 15 min at room temperature. With stirring, acetobromoxylose (0.2 mole) was added, and the mixture was boiled under reflux on a water bath for 15 min. After the solution had been cooled and filtered, it was thoroughly washed with water, dried (Na_2SO_4), and concentrated *in vacuo*: the following tri-*O*-acetyl-β-D-xylopyranosides were prepared in this way: cyclopentyl (50%), cyclohexyl (60%), 2-phenylethyl (65%), and 3-phenylpropyl (17%).

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EXPERIMENTAL

The purity of the products was tested by t.l.c. on silicagel G (Merck) in acetic acid–water–ethyl acetate (1:1 3; v/v) for xylosides, and ethyl acetate–benzene (3:7; v/v) for the acetates; spray reagent, 5% sulfuric acid in ethanol (10 min at 120°).

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Mercuric acetate method. Mercuric acetate (0.1 mole), absolute chloroform (200 ml), Sikkon (calcium sulfate, Fluka) (0.5 mole), and the alcohol (1 to 1.5 mole) were stirred for 15 min at room temperature. With stirring, acetobromoxylose (0.2 mole) was added, and the mixture was boiled under reflux on a water bath for 15 min. After the solution had been cooled and filtered, it was thoroughly washed with water, dried (Na_2SO_4), and concentrated *in vacuo*: the following tri-*O*-acetyl-β-D-xylopyranosides were prepared in this way: cyclopentyl (50%), cyclohexyl (60%), 2-phenylethyl (65%), and 3-phenylpropyl (17%).

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TABLE I

METHYLATED β-D-XYLOPYRANOSIDES

Aglycon group	M.p. (°C)	Crystallisation solvent	$[\alpha]_D^{22}$, (°) in chloroform	Yield, %	Found, % C	Found, % H	Formula	Calc., % C	Calc., % H
Cyclohexyl	116–117	ethanol	−55.2 *c*, 4	64	57.0	7.4	$C_{17}H_{28}O_8$	57.0	7.3
Cyclopentyl	150	ethanol	−69.3 *c*, 2	54	55.6	7.1	$C_{16}H_{24}O_8$	55.8	7.0
2-Methylcyclohexyl	105	methanol–water	−27.0 *c*, 1	30	58.0	7.4	$C_{18}H_{28}O_8$	58.1	7.5
3-Methylcyclohexyl	109	methanol–water	−53.7 *c*, 1.5	24	58.2	7.4			
4-Methylcyclohexyl	87	methanol–water	−47.9 *c*, 1	25	58.1	7.3			
Benzyl	90–91	ethanol–water	−85.1 *c*, 4	40	58.9	6.1	$C_{18}H_{22}O_8$	59.0	6.1
2-Phenylethyl	93–94	methanol	−57.7 *c*, 4	43	59.9	6.3	$C_{19}H_{24}O_8$	60.0	6.3
3-Phenylpropyl	77–78	dioxan–water or methanol	−50.4 *c*, 2	40	60.8	6.7	$C_{20}H_{26}O_8$	60.9	6.6

TABLE II

β-D-XYLOPYRANOSIDES

Aglycon group	M.p. (°C)	Crystallisation solvent	$[\alpha]_D^{22}$, (°) in methanol	Yield, %	Found, % C	Found, % H	Formula	Calc., % C	Calc., % H
Cyclohexyl	151–152	methanol	−56.6 *c*, 2 (water)	71	56.5	8.7	$C_{11}H_{20}O_5$	56.9	8.6
Cyclopentyl	125–126	ethyl acetate	−58.4 *c*, 0.6	75	54.9	8.3	$C_{10}H_{18}O_5$	55.0	8.3
2-Methylcyclohexyl	syrup		−13 (?) *c*, 1	—	—	—	$C_{12}H_{22}O_5$	58.5	8.9
3-Methylcyclohexyl	160	ethyl methyl ketone	−40.0 *c*, 1	60	58.4	8.8			
4-Methylcyclohexyl	154	ethyl methyl ketone	−51.9 *c*, 2	52	58.5	8.7			
Benzyl	116–117	water	−64.8 *c*, 2	70	59.5	6.9	$C_{12}H_{16}O_5$	60.0	6.7
2-Phenylethyl	107–108	ethyl methyl ketone or water	−42.5 *c*, 4	89	61.3	7.2	$C_{13}H_{18}O_5$	61.4	7.2
3-Phenylpropyl	96–97	ethyl acetate	−39.3 *c*, 4	74	62.3	7.5	$C_{14}H_{20}O_5$	62.7	7.5

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Oxidation of D-glucose by persulfate

A study performed in this Department[1], of the kinetics of oxidation of D-glucose by persulfate, showed that constant rate coefficients could not be obtained for the reaction. This is contrary to the findings of Wood and Walker[2], who obtained fairly constant and concordant rate coefficients for the oxidation of a number of aldoses with persulfate. Acid products were formed which were assumed[2] to be aldonic acids. In view of the discrepancy, it was thought necessary to identify the products of the oxidation. D-Glucose was oxidized in aqueous solution by an equal weight of potassium persulfate for five days at 60°. Distillation of the aqueous solution gave a volatile organic acid, together with volatile non-acidic compounds, one of which was identified as formaldehyde. The non-volatile part of the reaction mixture was acetylated under acidic conditions, and the acetylated product was resolved, by column[3], and by thin-layer chromatography, into four products: penta-*O*-acetyl-α-D-glucopyranose (from unchanged D-glucose), tetra-*O*-acetyl-α-D-glucopyranuronic acid, penta-*O*-acetyl-D-gluconic acid, and 2,3,5,6-tetra-*O*-acetyl-D-glucono-1,4-lactone.

The presence of tetra-*O*-acetyl-α-D-glucopyranuronic acid in the products arises from the acetylation of D-glucuronic acid. The latter is undoubtedly formed by oxidation of the $-CH_2OH$ group in D-glucose.

The oxidation of primary alcohols by persulfate is known to proceed by way of an aldehyde [4], but unless the alcohol is in excess, oxidation proceeds further to the carboxylic acid. Differences in rate of oxidation of the aldehydes probably account for the observed differences in rate coefficients for the oxidation of such alditols as D-mannitol, galactitol, D-glucitol, erythritol, and glycerol, by persulfate[4].

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Oxidation of D-glucose by persulfate

A study performed in this Department[1], of the kinetics of oxidation of D-glucose by persulfate, showed that constant rate coefficients could not be obtained for the reaction. This is contrary to the findings of Wood and Walker[2], who obtained fairly constant and concordant rate coefficients for the oxidation of a number of aldoses with persulfate. Acid products were formed which were assumed[2] to be aldonic acids. In view of the discrepancy, it was thought necessary to identify the products of the oxidation. D-Glucose was oxidized in aqueous solution by an equal weight of potassium persulfate for five days at 60°. Distillation of the aqueous solution gave a volatile organic acid, together with volatile non-acidic compounds, one of which was identified as formaldehyde. The non-volatile part of the reaction mixture was acetylated under acidic conditions, and the acetylated product was resolved, by column[3], and by thin-layer chromatography, into four products: penta-*O*-acetyl-α-D-glucopyranose (from unchanged D-glucose), tetra-*O*-acetyl-α-D-glucopyranuronic acid, penta-*O*-acetyl-D-gluconic acid, and 2,3,5,6-tetra-*O*-acetyl-D-glucono-1,4-lactone.

The presence of tetra-*O*-acetyl-α-D-glucopyranuronic acid in the products arises from the acetylation of D-glucuronic acid. The latter is undoubtedly formed by oxidation of the $-CH_2OH$ group in D-glucose.

The oxidation of primary alcohols by persulfate is known to proceed by way of an aldehyde [4], but unless the alcohol is in excess, oxidation proceeds further to the carboxylic acid. Differences in rate of oxidation of the aldehydes probably account for the observed differences in rate coefficients for the oxidation of such alditols as D-mannitol, galactitol, D-glucitol, erythritol, and glycerol, by persulfate[4].

The isolation of penta-*O*-acetyl-D-gluconic acid undoubtedly indicated oxidation of the reducing group of D-glucose by persulfate. The fourth product, 2,3,5,6-tetra-*O*-acetyl-D-glucono-1,4-lactone, can arise through dehydration of D-gluconic acid.

These results clearly show that oxidation of D-glucose by persulfate is not confined to one group and oxidation of the aldehydic as well as the primary hydroxyl group occurs concurrently. There is also oxidative cleavage of the carbon–carbon bonds, giving rise to volatile compounds of low molecular weight.

EXPERIMENTAL

Oxidation of D-*glucose with potassium persulfate*

A mixture of D-glucose (50 g) and potassium persulfate (50 g) in water (500 ml) was heated for five days at 60°, and then evaporated to dryness under diminished pressure. The resulting distillate was titrated in 25 ml portions with 0.10 N sodium hydroxide, and with acidified 0.10 N potassium permanganate solutions; the titers were 15.00 ml and 19.00 ml respectively. Formaldehyde was determined colorimetrically[5] by the chromotropic acid method; 25 ml of the distillate was found to contain 34 μmoles of formaldehyde. The residual brown solid was finely ground and acetylated as described below.

Acetylation of the non-volatile oxidation products

Concentrated sulfuric acid (5 ml) was added dropwise to a stirred suspension of the solid product (*ca.* 100 g), from the preceding experiment, in acetic anhydride (500 g). Stirring was continued for 24 h, and then the mixture was poured into ice and water (*ca.* 2 l). The dark, sticky material which separated was extracted with chloroform (300 ml), and the chloroform extract was washed five times with water, dried over sodium sulfate, and concentrated under diminished pressure. The residual material was dissolved in ethanol (200 ml), heated with charcoal (*ca.* 10 g), and filtered through a pad of Micro-Cel C (Johns–Manville Co., New York, N.Y.). The straw-colored filtrate was concentrated to dryness under diminished pressure; yield 20 g. Thin-layer chromatographic examination on silica gel, with 10:1 v/v benzene–methanol as solvent, showed at least four components, having R_F values: 0.10, 0.33, 0.77 and 0.98.

Separation of the acetylated products

Portions (1.5 g) of the acetylated material were dissolved in chloroform (10-ml portions) and placed at the top of a 10 × 3-in. column of Micro-Cel C[3]. The column was developed with 1.1 l of 10:1 v/v benzene–methanol. Indication with 1% potassium permanganate in 2.5 N potassium hydroxide revealed two zones, A and B. The zones were extracted with acetone, and the extracts were evaporated. Zone A yielded crystalline penta-*O*-acetyl-α-D-glucopyranose (3.2 g from 15 g of the acetylated material) having m.p. and mixed m.p. 110–111°, R_F 0.89. Zone B yielded a colorless syrup, which was fractionated by thin-layer chromatography on silica gel G with

10:1 v/v benzene–methanol as eluent. Zones were located on the plates by spraying with water[6], the different fractions were extracted from the appropriate zones with acetone, and the resulting syrupy products were rechromatographed on silica gel G as before. Three or four successive fractionations were performed for each zone, before chromatographically pure compounds were obtained.

The zone, R_F 0.77, crystallized upon refrigeration to give tetra-*O*-acetyl-α-D-glucopyranuronic acid, yield 0.135 g from 15 g of the acetylated material. Recrystallization from ethanol gave pure material, m.p. 118°, $[\alpha]_D^{20}$ −110° (*c* 1.0, chloroform) [lit.[7] m.p. 118–119°, $[\alpha]_D^{19}$ −111° (chloroform)]. The m.p. was undepressed on admixture with an authentic specimen of tetra-*O*-acetyl-α-D-glucopyranuronic acid. X-Ray powder diffraction patterns of the two samples were identical.

Anal. Calc. for $C_{14}H_{18}O_{11}$: C, 46.40; H, 4.97. Found: C, 46.60; H, 5.10.

The zone, R_F 0.33, was crystalline penta-*O*-acetyl-D-gluconic acid (yield 0.37 g from 15 g of the acetylated material). After two recrystallizations from ethanol it had m.p. 112°, $[\alpha]_D^{20}$ +10° (*c* 1.2, chloroform). The m.p. was not depressed on admixture with an authentic sample prepared according to Major and Cook[8], who reported m.p. 110–111° $[\alpha]_D^{20}$ +11.5° (chloroform).

Anal. Calc. for $C_{16}H_{22}O_{12}$: C, 47.34; H, 5.42. Found: C, 47.51; H, 5.53.

The zone, R_F 0.10, was crystalline 2,3,5,6-tetra-*O*-acetyl-D-glucono-1,4-lactone; yield 85 mg from 15 g of the acetylated material. After recrystallization from ethanol it had m.p. 115°, $[\alpha]_D^{20}$ −2.3° (*c* 1.0, chloroform) [lit.[9] m.p. 114–117° $[\alpha]_D$ −1.21° (chloroform)]. The m.p. was undepressed on admixture with an authentic sample of 2,3,5,6-tetra-*O*-acetyl-D-glucono-1,4-lactone kindly supplied by Professor M. L. Wolfrom.

Anal. Calc. for $C_{14}H_{18}O_{10}$: C, 48.52; H, 5.20. Found: C, 48.50, H, 5.40.

ACKNOWLEDGMENT

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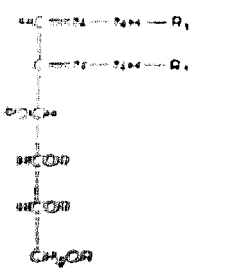
Preliminary Communication

Carbohydrate derivatives of 1-substituted 1,2,3-triazole

Recently, Curtin and Alexandrou[1] have studied the structure of the oxidation products obtained by the action of iodine and mercuric oxide on the bis(benzoylhydrazones) of dimethylglyoxal, pyruvaldehyde, and benzil. They found that the products possessed ester bands at 1750 cm^{-1} but not the amide band that would be expected on the basis of the tetrazine structures previously[2] ascribed to them. This fact and other experiments led the authors to consider these compounds to be derivatives of 1-benzoylamino-1,2,3-triazole enol benzoate.

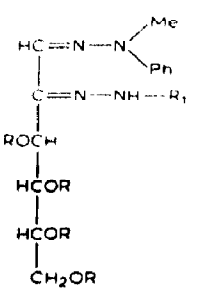
For a similar oxidation in the carbohydrate series, the D-*arabino*-hexosulose bis(acylhydrazones) (Ia) shown in the Table were prepared from D-*arabino*-hexosulose (glucosone) and acyl hydrazines, by the procedure used for the prepration of carbamoyl osazones[3]. The colorless bis(acylhydrazones) obtained showed two CONH bands between 1640–1690 cm^{-1}, in addition to a C=N band which appeared between 1595–1615 cm^{-1} (see Table I).

Acetylation of the bis(acylhydrazones) gave the tetraacetates (Ib) which showed, in addition to the amide bands, an ester band at 1750 cm^{-1}. Oxidation of these acetyl derivatives with iodine and mercuric oxide gave products (III) having two hydrogen atoms fewer than the bis(acylhydrazone) acetates.

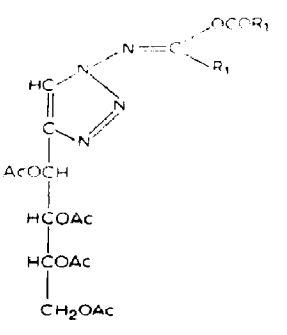


(I)

a, B = H; b, B = Ac



(II)



(III)

The i.r. spectra of the oxidation products revealed no CONH bands but showed large ester bands at 1750 cm^{-1} (see Table) probably arising from the *O*-acetyl and the

TABLE I[a]

BIS(ACYLHYDRAZONE) DERIVATIVES

Formula R_1	*M.p., degrees*	*Color*	*Yield, %*	$[\alpha]_D$ (°)	ν_{max}^{KBr}			λ^{EtOH}			
					$\nu_{C=N}$	ν_{CONH}	ν_{OH}	λ_{max}	log ε	λ_{min}	log ε
Ia –$SCNH_2$	232	Yellow	60	−27.2 (P)	1612	[b]	3220	[c]	[c]	[c]	[c]
Ia –OCNHPh	245	Pale yellow	55	−114.9 (P)	1600	1640, 1665	3300	[c]	[c]	[c]	[c]
Ia –OCMe	216	Colorless	60	−18.1 (W)	1610	1675, 1695	3290	296	4.49	225	3.62
Ia –OCpyridyl-3	202	Colorless	50	−52.8 (P)	1595	1665, 1685	3360	251, 320	1.87, 4.36	217, 286	3.86, 4.18
Ia –OCPh	188	Colorless	70	−89.8 (P)	1605	1665, 1675	3450	256, 330	4.35, 4.24	225, 295	3.08, 3.97
Ia –OCC_6H_4Me-*m*	167	Colorless	70	−35.0 (A)	1605	1645, 1690	3360	258, 332	4.26, 4.08	226, 302	3.95, 3.87
Ia –OCC_6H_4Me-*p*	189	Colorless	75	−22.1 (A)	1610	1645, 1690	3400	262, 332	4.40, 4.40	230, 298	4.23, 4.12
IIa –$OCNH_2$	180	Pale yellow	45	−48.2 (P)	1605	1690	3280	242, 285	4.17, 3.64	220, 264	4.00, 3.64
IIa –$SCNH_2$	203	Yellow	80	−27.6 (P)	1600	[b]	3160	254, 302	4.40, 4.15	226, 274	4.07, 3.96
IIa –OCMe	187	Yellow	75	−50.9 (P)	1600	1652	3350	244, 352	3.04, 3.19	218, 304	2.75, 2.49
IIa –OCpyridyl-3	178	Yellow	40	−69.2 (P)	1598	1666	3350	278, 370	4.10, 4.37	260, 315	4.06, 3.66
IIa –OCpyridyl-4	190	Yellow	45	−29.8 (P)	1595	1660	3260	278, 360	4.10, 4.32	260, 312	4.00, 4.62
IIa –OCPh	188	Yellow	50	−69.3 (P)	1600	1665	3370	235, 275	4.02, 3.90	260, 310	3.88, 3.40
IIa –OCC_6H_4Me-*o*	145	Yellow	50	−47.6 (A)	1600	1660	3440	246, 363	4.22, 4.28	222, 310	4.08, 3.47
IIa –OCC_6H_4Me-*p*	187	Yellow	50	−16.6 (A)	1610	1660	3480	248, 314	4.24, 3.51	224, 274	4.07, 4.27
							$\nu_{(COO)}$				
Ib –OCPh	232	Colorless	90	−40.9 (A)	1605	1670, 1690	1750	256, 336	4.42, 4.18	226, 298	4.15, 3.96
Ib –OCC_6H_4Me-*m*	181	Colorless	90	−40.7 (A)	1595	1675, 1690	1750	258, 342	4.31, 4.13	228, 306	3.95, 3.84
Ib –OCC_6H_4Me-*p*	153	Colorless	90	−7.8 (A)	1610	1665, 1690	1750	262, 342	4.36, 4.19	234, 304	4.12, 3.83
IIb –OCMe	144	Pale yellow	80	−91.0 (A)	1600	1670	1750	242, 288	4.14, 3.63	218, 272	3.35, 3.61
IIb –OCPh	132	Yellow	90	−34.2 (A)	1600	1690	1750	285, 370	4.00, 3.98	260, 320	3.60, 3.40
III –Ph	198	Colorless	20	−3.7 (P)	1612	[d]	1750	255, 315	4.10, 4.40	235, 268	4.00, 4.07
III –C_6H_4Me-*m*	160	Colorless	30	−8.2 (C)	1614	[d]	1750	227, 272	4.27, 4.00	252	3.94
III –C_6H_4Me-*p*	169	Colorless	30	−21.8 (C)	1605	[d]	1750	232	4.36	216	4.22

[a]All compounds gave proper C, H, and N analyses. Specific rotations were determined in: A, ethanol; C, chloroform; P, pyridine; W, water. [b]C=S band at 1500 cm^{-1}. [c]Not determined [d]No absorption at this frequency.

enol benzoate groups. These data accord with the general structure of Curtin and Alexandrou[1] for the pyruvaldehyde derivative, and the products are, therefore, tentatively formulated as 4-substituted 1-benzoylamino-1,2,3-triazole enol benzoates (III). The reaction may be exemplified by the conversion of D-*arabino*-hexosulose bis-(benzoylhydrazone) (Ia; R_1 = COPh) into the tetraacetate (Ib; R_1 = COPh), and the oxidation of the latter to the 1-benzoylamino-4-(D-*arabino*-tetraacetoxybutyl)-1,2,3-triazole enol benzoate (III; R_1 = Ph). Mixed osazones of the type (IIa) were also prepared, from D-*arabino*-hexosulose 1-(2-methyl-2-phenylhydrazone) and acylhydrazines. Their acetates (IIb) displayed the expected resistance toward oxidation to enol acylates of the type (III).

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REACTIONS OF FURANOSIDE EPOXIDES: THE PREPARATION OF BROMOHYDRINS FROM METHYL 5-*O*-ACETYL-2,3-ANHYDRO-α(AND β)-D-LYXOFURANOSIDE

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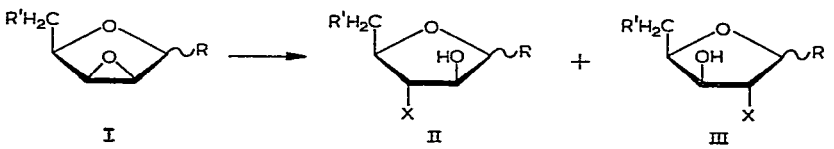
Nucleophilic attack of 2,3-anhydrofuranosides has generally been observed to give oxirane ring opening by attack of the nucleophile, predominantly at C-3. Thus, the reaction of a number of substituted 2,3-anhydro-α(and β)-D-lyxofuranosides (I)[1–5] with such nucleophiles as ammonia, sodium methoxide, sodium ethanethioxide, or sodium benzoate gave 3-substituted D-arabinofuranosides (II) almost exclusively, with, at most, only trace amounts of the isomeric 2-substituted D-xylofuranosides (III).

a = α–D series ; b = β–D series



I II III

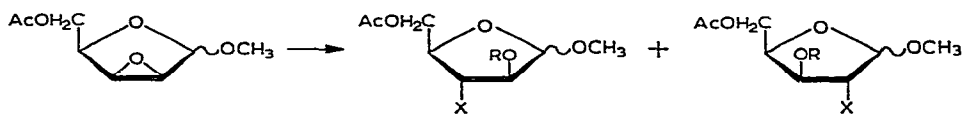
One early exception to this rule was the observation by Percival and Zobrist[2] that methyl 2,3-anhydro-α(and β)-D-lyxofuranoside (I, R = OCH_3, R′ = OH), when treated with methanolic sodium methoxide, gave a 2:1 mixture of methyl 3-*O*-methyl-D-arabinofuranoside (II, R = X = OCH_3, R′ = OH) and methyl 2-*O*-methyl-D-xylofuranoside (III, R = X = OCH_3, R′ = OH), respectively. A recent example was reported[6] by Casini and Goodman, in which treatment of methyl 2,3-anhydro-β-D-lyxofuranoside (Ib, R = OCH_3, R′ = OH) with sodium α-toluenethioxide gave a 3:2 mixture of 2-substitution over 3-substitution, in contrast to the behavior of the α-D anomer (Ia, R = OCH_3, R′ = OH), in which only a 3-substitution product was isolated[7]. An interesting comparison of acid-catalyzed and base-catalyzed epoxide-opening was reported by Doerr, Codington, and Fox[8], wherein treatment of 1-[2,3-anhydro-5-*O*-(methylsulfonyl)-β-D-lyxofuranosyl]uracil (Ib, R = uracil, R′ = OMs) with sodium benzyloxide apparently gave only attack at C-3, although treatment of this same epoxide with aqueous sulfuric acid gave a 5:1 mixture of C-3 over C-2 opening.

It seemed interesting at this point to investigate other acid-catalyzed openings of 2,3-anhydro-D-lyxofuranosides, to check for possible attack at C-2. An α,β-D series which was particularly interesting to investigate was that of the simple glycosides (I, R = OCH_3, R′ = OH), both from the standpoint of ready availability[1] and from

the fact that the α- and β-D anomers react so differently with sodium α-toluenethioxide[6,7] although there is apparently little difference in their reaction towards sodium methoxide[2]. The reaction examined was that of anhydrous magnesium bromide with methyl 5-*O*-acetyl-2,3-anhydro-α(and β)-D-lyxofuranosides, (I, R = OCH_3, R′ = OAc), because the resulting bromohydrins II and III, (R = OCH_3, R′ = OAc, X = Br) would be useful in another connection.

The reaction of anhydrous magnesium bromide with methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-mannopyranoside proceeds in the same fashion[9] as with such basic nucleophiles as ammonia[10] or sodium methanethioxide[11], to give methyl 4,6-*O*-benzylidene-3-bromo-3-deoxy-α-D-altropyranoside. When a similar reaction was attempted with methyl 5-*O*-acetyl-2,3-anhydro-β-D-lyxofuranoside (IVb), a syrup was obtained which consisted of a mixture of 2 components, presumably Vb and IXb, in the ratio 1:2, as shown by vapor-phase chromatography (v.p.c.) of the trimethylsilyl ether[12]. Preparative separation of these components as their trimethylsilyl ethers by v.p.c. gave 38% of the major and 15% of the minor component. De(trimethylsilyl)ation of the separated components gave a 15% yield of methyl 5-*O*

a = α-D series b = β-D series



IV

V X = Br, R = H
VI X = Br, R = $Si(CH_3)_3$
VII X = Br, R = Ac
VIII X = H, R = Ac

IX X = Br, R = H
X X = Br, R = $Si(CH_3)_3$
XI X = Br, R = Ac
XII X = H, R = Ac

acetyl-3-bromo-3-deoxy-β-D-arabinofuranoside (Vb) and 38% of methyl 5-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranoside (IXb). These structural assignments were made tentatively, on the basis of the n.m.r. spectra, which showed H-1 as a singlet at τ 4.90, suggestive of an H-1–H-2 *trans*-relationship for the major component, whereas the minor component showed H-1 as a doublet (*J* 4 c.p.s.) at τ 5.21, compatible with an H-1–H-2 *cis*-relationship[13]. These coupling constants are best accommodated by assigning the D-xyloside structure (IXb) to the major component and the D-arabinoside structure (Vb) to the minor component. That these assignments are correct was demonstrated in the following way. Acetylation of the bromohydrins (Vb and IXb) gave the syrupy diacetates (VIIb and XIb), which were hydrogenated directly, to give methyl 2,5-di-*O*-acetyl-3-deoxy-β-D-arabinofuranoside (VIIIb) and methyl 3,5-di-*O*-acetyl-2-deoxy-β-D-xylofuranoside (XIIb). The n.m.r. and i.r. spectra of VIIIb and XIIb were identical with the spectra of authentic samples of these compounds prepared by Casini and Goodman[6], and proved beyond question our structural assignments for the bromohydrins Vb and IXb.

Treatment of methyl 5-*O*-acetyl-2,3-anhydro-α-D-lyxofuranoside (IVb) with magnesium bromide in 1,2-dimethoxyethane gave again a syrup which, when converted

into its trimethylsilyl ether and submitted to v.p.c., proved to consist of 1 major component of about 85% purity, either the 3-bromo-D-arabinoside (VIa) or the 2-bromo-D-xyloside (Xa). Conversion of this bromohydrin into the deoxypentoside diacetate (VIIIa or XIIa) as described for the β-D series, followed by acetolysis and deacylation, gave 3-deoxy-D-*threo*-pentose[6], characterized as its crystalline 2-benzyl-2-phenylhydrazone, which was identical with the authentic benzylphenylhydrazone[6]. The origin of the 3-deoxy-D-*threo*-pentose must be methyl 5-*O*-acetyl-3-bromo-3-deoxy-α-D-arabinofuranoside (Va); hence, the epoxide (IVa) must react with magnesium bromide by almost exclusive attack at C-3, as no 2-bromo compound (IXb) could be detected.

The epoxide (IVb) reacts with magnesium bromide, predominantly at C-2, a reaction which represents the second example of a predominant attack at C-2 of an anhydro-D-lyxofuranoside. The overall course of the reaction of magnesium bromide with methyl 5-*O*-acetyl-2,3-anhydro-α(and β)-D-lyxofuranoside is qualitatively similar to the reaction of the anhydro-D-lyxosides with sodium α-toluenethioxide[6], not to that with sodium methoxide[2]. To the best of the authors' knowledge, the reaction of methyl 2,3-anhydro-α-D-lyxofuranoside with sodium methoxide represents the only example of a nucleophilic attack of the anhydro-α-D-lyxoside at C-2.

EXPERIMENTAL

Vapor-phase chromatograms, both analytical and preparative, were performed with a Wilkens, Autoprep 700, gas chromatograph, using a 5′ × 3/8″ stainless-steel column packed with 15% phenyl diethylaminosuccinate on Chromosorb W (60–80 mesh). Helium was used as the carrier, at a flow rate of 340 ml/min. The column temperature was 170–175°.

Melting points were determined with a Fisher–Johns apparatus, and are corrected. Specific rotations were determined with a Rudolph photoelectric polarimeter. Thin-layer chromatograms were run on silica gel HF (E. Merck A.-G. Darmstadt). Spots were detected by means of iodine vapor. Organic solutions were dried with anhydrous magnesium sulfate. N.m.r. spectra were recorded for solutions in deuteriochloroform, using tetramethylsilane as the internal standard, with either a Varian A-60 or HA-100 spectrometer.

Methyl 5-O-acetyl-2,3-anhydro-α-D-lyxopyranoside (IVa)

To a solution of 5.0 g (34.2 mmole) of methyl 2,3-anhydro-α-D-lyxofuranoside[2] in 50 ml of pyridine was added 6.5 ml (68.8 mmole) of acetic anhydride, dropwise with stirring. The resulting solution was kept for 18 h at room temperature and then the excess acetic anhydride was decomposed by the addition of 30 ml of ice-water. The aqueous mixture was extracted with three 40-ml portions of chloroform. The chloroform extracts were dried, and evaporated to dryness *in vacuo*, to give 6.6 g of crystalline product (IVa). Recrystallization from 50 ml of petroleum ether (b.p. 62–70°) gave 5.1 g (78%) of material, m.p. 71.5–72.5° in 2 crops.

An analytical sample was obtained by sublimation, and had m.p. 72.0–73.5°; $[\alpha]_D$ + 65° (*c* 1, chloroform); λ_{max}^{Nujol} 5.70 (C=O), 8.05 (acetate C–O–C); 11.33 μ (epoxide).

Anal. Calc. for $C_8H_{12}O_5$: C, 51.1; H, 6.43. Found: C, 51.2; H, 6.43.

Methyl 5-*O*-acetyl-2,3-anhydro-β-D-lyxopyranoside (IVb) was prepared in 89% yield by the same procedure, as a colorless oil, b.p. 80–92° (0.02 Torr), $[\alpha]_D$ --80° (*c* 1, chloroform), λ_{max}^{film} 5.70 (C=O), 8.05 (acetate C–O–C) 11.3 μ (epoxide).

Anal. Calc. for $C_8H_{12}O_5$: C, 51.1; H, 6.43. Found: C, 51.1; H, 6.70.

Methyl 5-O-acetyl-2-bromo-2-deoxy-β-D-xylofuranoside (IXb) and methyl 5-O-acetyl-3-bromo-3-deoxy-β-D-arabinofuranoside (Vb)

A solution of magnesium bromide[9], prepared from 2.0 g (82.4 mmole) of magnesium and 11.0 ml (127.4 mmole) of 1,2-dibromoethane in 100 ml of ether was evaporated to dryness *in vacuo*. To the solid residue was added a solution of 2.0 g (10.6 mmole) of methyl 5-*O*-acetyl-2,3-anhydro-β-D-lyxofuranoside (IVb) in 100 ml of dry 1,2-dimethoxyethane. The resulting mixture was heated for 3.5 h under reflux (nitrogen atmosphere); then the mixture was cooled and 40 ml of water was added. The pH of the mixture was adjusted to pH 2 with 5 N hydrochloric acid, and the aqueous solution was extracted with two 40-ml portions of chloroform. The combined chloroform extracts were washed successively with 15 ml of 5% aqueous sodium thiosulfate and 15 ml of water, dried, and evaporated to dryness *in vacuo*, to yield 2.57 g (89%) of a yellow syrup. Vapor-phase chromatography of the trimethylsilyl ether[12] of the crude product showed it to contain two main components in the ratio of 2:1.

To a solution of the crude product (Vb and IXb) in 8 ml of dry pyridine were added 13 ml of hexamethyldisilazane and 1.3 ml of chlorotrimethylsilane. The reaction mixture was stirred at room temperature for 5 min, 30 ml of dry benzene was added, and the solution was centrifuged to remove the precipitated salts. The supernatant liquor was purified by means of preparative gas-chromatography at 170°, to separate the trimethylsilyl ether VIb from Xb. A recovery of 1.24 g (38%) of Xb (retention time, 6.23 min) and 0.49 g (15%) of VIb (retention time, 7.18 min), both as their trimethylsilyl ethers, was effected.

The trimethylsilyl ether Xb (1.24 g) was mixed with 30 ml of 50% aqueous methanol and kept for 16 h at room temperature. The trimethylsilyl ether had not entirely dissolved, so the mixture was heated for an additional 4 h at 55°, by which time, dissolution was complete. The solvent was removed *in vacuo*, and the resulting syrup was dried by the addition, and removal *in vacuo*, of 5 ml of absolute ethanol, to give 0.96 g (98%) of methyl 5-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranoside (IXb) as a colorless oil; $[\alpha]_D^{22}$ −35° (*c* 0.97, chloroform); λ_{max}^{film} 2.82 (OH), 5.69 μ (acetate C=O).

Anal. Calc. for $C_8H_{13}BrO_5$: C, 35.7; H, 4.87; Br, 29.7. Found: C, 35.4; H, 4.73; Br, 29.6.

The n.m.r. spectrum showed a singlet at τ 4.89 which was assignable to H-1

of IXb, together with a trace of a doublet, τ 5.23, due to some contamination by the 3-bromohydrin (VIb). That this contamination was present was borne out by repeating the v.p.c. of the trimethylsilyl ether of purified IXb; there was a second peak, representing the presence of about 10% of Vb in the 2-bromohydrin IXb.

Treatment of the trimethylsilyl ether (VIb) (0.49 g) in 30 ml of 50% aqueous methanol as described above gave 0.39 g (100%) of methyl 5-*O*-acetyl-3-bromo-3-deoxy-β-D-arabinofuranoside (Vb) as a colorless oil, $[\alpha]_D^{22}$ −89° (*c* 0.91, chloroform).

Anal. Calc. for $C_8H_{13}BrO_5$: C, 35.7; H, 4.87; Br, 29.7. Found: C, 36.1; H, 5.06; Br, 29.6.

The n.m.r. spectrum of Vb showed a doublet (*J* 4.5 c.p.s.), assignable to H-1 of Vb. There was no evidence for contamination by the 2-bromohydrin (IXb), either in the n.m.r., spectrum or by v.p.c. of the trimethylsilyl ether.

*Methyl 2,5-di-*O*-acetyl-3-deoxy-β-*D*-threo-pentofuranoside (VIIIb)*

Acetylation of methyl 5-*O*-acetyl-3-bromo-3-deoxy-β-D-arabinofuranoside (Vb), (0.32 g, 1.19 mmole) in 5 ml of pyridine with 0.5 ml (5.29 mmole) of acetic anhydride at room temperature gave 0.35 g (95%) of the diacetate (VIIb) as a yellow oil which was free of hydroxyl i.r. absorption at 2.9 μ and which was used directly for the next step.

A solution of 0.29 g (0.93 mmole) of the diacetate (VIIb) in 30 ml of 2-methoxyethanol was treated with Norit, and filtered through a Celite pad. To the filtrate was added 0.14 g (2.1 mmole) of anhydrous sodium acetate and 0.15 g of 5% palladium-on-carbon. The resulting mixture was stirred under an atmosphere of hydrogen for 22 h, at room temperature, by which time, hydrogen uptake had ceased. The mixture was filtered through a Celite pad, and the filtrate was evaporated to dryness *in vacuo*. The residue was partitioned between 40 ml of ether and 40 ml of water. The ether layer was washed with saturated aqueous sodium bicarbonate and water, dried, and evaporated to dryness *in vacuo*, to yield 0.17 g (79%) of product (VIIIb) as colorless crystals, m.p. 53–56.5°. Recrystallization from petroleum ether (b.p. 62–70°) gave 0.13 g of material having m.p. 60–64°. Methyl 2,5-di-*O*-acetyl-3-deoxy-β-D-xylofuranoside (VIIIb), prepared by Casini and Goodman[6], had m.p. 63–64°. The i.r. and n.m.r. spectra were identical for the compound (VIIIb) prepared by either route.

*Methyl 3,5-di-*O*-acetyl-2-deoxy-β-*D*-threo-pentofuranoside (XIIb)*

Acetylation of 0.136 g (0.51 mmole) of methyl 5-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranoside (IXb), followed by hydrogenolysis of the resulting diacetate (XIb) as described for the preparation of (VIIIb), gave 78 mg (64%) of product (XIIb) as a colorless oil that had i.r. and n.m.r. spectra identical with that of XIIb prepared by Casini and Goodman[6].

*Methyl 5-*O*-acetyl-3-bromo-3-deoxy-α-*D*-arabinofuranoside (Va)*

A solution of 1.0 g (5.32 mmole) of methyl 5-*O*-acetyl-2,3-anhydro-α-D-lyxofuranoside (IVa) in 50 ml of 1,2-dimethoxyethane was heated with magnesium

bromide at reflux as described for the β-D anomer (Vb), except that refluxing was conducted for 24 h, to give 1.06 g (74%) of bromohydrin (Va). V.p.c. of the trimethylsilyl ether of the reaction product showed a major peak (retention time, 5.2 min), representing 85% of the volatile components, along with 2 minor components.

Anal. Calc. for $C_8H_{13}BrO_5$: C, 35.7; H, 4.78; Br, 29.7. Found: C, 37.3; H, 5.26; Br, 28.9.

The n.m.r. spectrum of the reaction product showed H-1 at τ 5.19 ($J_{1,2} = 2$ c.p.s.), suggestive of a *trans*-relationship between H-1 and H-2.

Characterization of Va was accomplished by acetylation, to give the bromohydrin diacetate (VII a), followed by hydrogenation to the deoxypentoside diacetate (VIIIa). The n.m.r. spectrum of VIIIa showed H-1 as a singlet (τ 5.04), H-2 as a quartet ($J_{2,3'}$ 2 c.p.s.; $J_{2,3''}$ 6 c.p.s.; τ 4.95), H-3′ as a multiplet (τ 8.3), H-3″ as a multiplet (τ 7.5), and H-4 and H-5 as a multiplet (τ 5.7).

Acetolysis[14] of VIIIa with acetic anhydride–acetic acid–sulfuric acid, followed by methanolysis of the resulting triacetate with methanolic sodium methoxide, gave 3-deoxy-D-*threo*-pentose, characterized as the 2-benzyl-2-phenylhydrazone, m.p. 81.5–83.0°. The i.r. spectrum of this material was identical with the spectrum of the benzylphenylhydrazone, m.p. 86.0–86.5°, prepared by Casini and Goodman[6].

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SUMMARY

The reaction of anhydrous magnesium bromide with methyl 5-*O*-acetyl-2,3-anhydro-β-D-lyxofuranoside results in a mixture of methyl 5-*O*-acetyl-2-bromo-2-deoxy-β-D-xylofuranoside and methyl 5-*O*-acetyl-3-bromo-3-deoxy-β-D-arabinofuranoside, with the D-xyloside preponderating. These results contrast with the results of normal nucleophilic opening of an anhydro-β-D-lyxofuranoside, where attack at C-3 predominates to give a D-arabinofuranoside; the reaction represents the second example of predominant attack at C-2, to give a D-xyloside.

The reaction of anhydrous magnesium bromide with methyl 5-*O*-acetyl-2,3-anhydro-α-D-lyxofuranoside follows the normal course, and methyl 5-*O*-acetyl-3-bromo-α-D-arabinofuranoside was the only product observed.

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SUBSTITUTED CEREBROSIDES

PART I. 1-*O*-[6-*O*-(β-D-HEXOPYRANOSYL)-β-D-HEXOPYRANOSYL]DIHYDROCERAMIDES

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INTRODUCTION

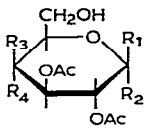
Complex glycosphingolipids, containing more than one sugar residue attached to the ceramide moiety have been known for some time. They include the gangliosides[1] and various disaccharide-, trisaccharide-, and glycosamino-lipids found in different organs, both under normal[2-5] and pathological[6,7] conditions. In some cases, the disaccharide-lipids may comprise an appreciable proportion of the total neutral glycolipids[3] and have important immunological properties[6].

The lactosylceramide, cytolipin H, has been completely characterised[6] and recently synthesized[8], and it would appear that the lactosyl moiety is common to most of the complex glycosphingolipids. However, the structure of some of these complex compounds has not yet been elucidated, and the possibility exists of naturally occurring dihexosides containing sugars other than lactose. In the case of the ceramide trihexosides, the question arises of the site of linkage of the galactosyl moiety on the lactoside[2]. It is of interest, then, to devise a method for the preparation of cerebrosides having suitable hydroxyl groups free for the unambiguous selective attachment of further glycosyl units. This would provide a means of synthesizing the more complex glycosphingolipids, and would offer an alternative approach to the direct attachment of oligosaccharide units to a suitably protected ceramide. It would further provide a convenient possibility of preparing a common monoglycosyllipid to which could be added, at will, specified glycosyl units or other substituents. Owing to the known reactivity of the primary hydroxyl group at C-6 in hexopyranose sugars[9], attention was first directed towards the preparation of a suitably substituted cerebroside, having a free hydroxyl group at C-6, and the attachment of various glycosyl units to this compound.

In order to synthesize ceramide dihexosides, the preparation of the key intermediate – (2,3,4-tri-*O*-acetyl-β-D-hexopyranosyl)ceramide – was investigated. The presence of acetyl groups precludes the removal by alkaline or mineral acid hydrolysis of substituents at C-6, while application of this work to the unsaturated series (sphingosine) precludes hydrogenolysis.

Consequently, it seemed that a more promising approach (and a more direct one) would be to utilize suitable acylglycosyl halides possessing an unsubstituted hydroxyl group at C-6. Such derivatives of both D-glucopyranose and D-galactopyranose have

already been described. The glucose compound, 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide[10] (I) condenses with itself in the presence of silver oxide to give (1→6)-linked oligosaccharides[11]. It might be expected, then, that the reaction of 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine* (II) with such a halide might provide a mixture of mono- and oligo-saccharides of II together with (1→6)-linked glucose polymers. Careful selection of reaction conditions would minimise the production of oligosaccharide derivatives of II, and the separation of the glycosphingolipids produced, from non-lipid material, should be relatively simple. Indeed, it was found that a reasonable yield of the required substituted cerebroside (III) was obtained by adding the halide (I) portionwise to an excess of II in a vigorously stirred nitromethane–benzene solution. When, however, excess of the halide was used, an appreciable proportion of the substituted disaccharide was formed. Saponification of the reaction product, followed by chromatography on silicic acid, enabled the isolation of the ceramide dihexoside (IV).



I. $R_1 = H$, $R_2 = Br$, $R_3 = H$, $R_4 = OAc$

II. $HOCH_2-CH(C_{17}H_{35}CONH)-CH(OBz)-(CH_2)_{14}-CH_3$

III. $R_1 = -O-CH_2-CH(C_{17}H_{35}CONH)-CH(OBz)-(CH_2)_{14}-CH_3$, $R_2 = H$, $R_3 = H$, $R_4 = OAc$

V. $R_1 = H$, $R_2 = Br$, $R_3 = OAc$, $R_4 = H$

VI. $R_1 = H$, $R_2 = Cl$, $R_3 = OAc$, $R_4 = H$

VII. $R_1 = -O-CH_2-CH(C_{17}H_{35}CONH)-CH(OBz)-(CH_2)_{14}-CH_3$, $R_2 = H$, $R_3 = OAc$, $R_4 = H$

The corresponding galactosyl bromide (V) has not been obtained in crystalline form, but the oily product resulting from the treatment of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose with titanium bromide reacted with II to give the desired compound. The chloride (VI) has already been characterized as a crystalline compound[11] and this was also found to react with II, to give, in slightly higher yield, the identically substituted cerebroside (VII). The optical rotation and n.m.r. spectrum of VII indicated the β-D-glycosidic configuration.

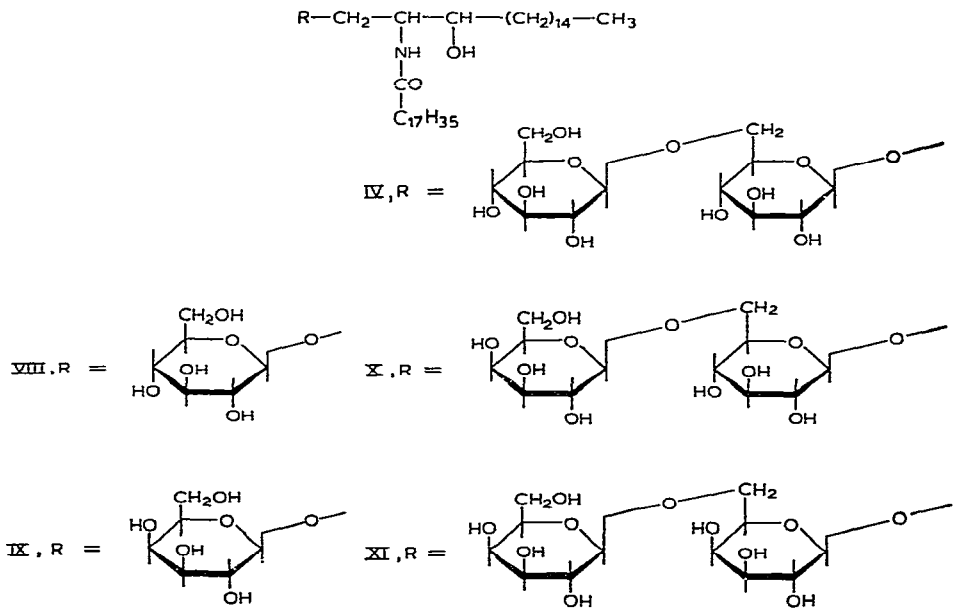
A proof that there had been no migration of acetate groups under the glycosidation conditions was afforded by the preparation of pure methyl 2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-β-D-glucopyranoside in good yield by reaction of I with methanol, under reaction conditions similar to those used for the cerebrosides,

*Sphingosine is the trivial name for D-*erythro*-2-amino-*trans*-4-octadecene-1,3-diol.

followed by *p*-toluenesulfonation of the reaction mixture and crystallization from methanol. Likewise, methyl 2,3,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-β-D-galactopyranoside was isolated from the product of reaction of VI with methanol followed by tritylation.

The substituted cerebrosides thus prepared could be brought into reaction with tetra-*O*-acetylglycosyl halides to give disaccharide esters. Reaction was only partial, and, following transesterification to remove the ester groups, separation by chromatography from monoglycosides was feasible. The glycosyl- or galactosyl-dihydroceramides (monohexosides) obtained as by-products were shown to be identical with those resulting from the direct reaction of II and the corresponding tetra-*O*-acetylglycosyl bromide, thus proving that the glycosyldihydrosphingosine linkages in both the mono- and di-saccharides had the same anomeric configuration. One of the dihexosides (IV) prepared by this method of stepwise addition showed the same speed of migration on t.l.c. in two solvent systems as the compound prepared in our laboratory[12] by direct interaction of II with hepta-*O*-acetyl-α-D-gentiobiosyl bromide, and the identity of melting point and optical rotation proved that the linkage between the two sugar moieties in compound IV is of the β-D configuration.

All the ceramide monohexosides (cerebrosides) and ceramide dihexosides prepared showed a similar complex behaviour on heating between glass slides. A translucent glass formed at temperatures varying between 110 and 120°, and this glass cleared sharply and reproducibly at the temperature indicated as m.p. The prevalent β-D anomeric configuration obtained in these reactions is in accordance with previous results of glycosidations by using mercuric cyanide in nitromethane[13–17].



EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and were corrected. Rotations were determined in semimicro-tubes, using a Perkin–Elmer 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer using tetramethylsilane as internal standard and deuteriochloroform as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used, and elution was stepwise, in order of increasing polarity of the solvents. The proportion of weight of substance added to the column to weight of adsorbent was 1 to 50–100. The fractions eluted were 2 ml/g of the column. "Silicic acid" refers to Bio-Rad silicic acid, used after preliminary heating for 60 min at 120°, and the chromatographic columns were prepared using chloroform–methanol 19:1. Thin-layer chromatograms were performed on Kieselgel G. (E. Merck, Darmstadt).

3-O-Benzoyl-N-octadecanoyl-DL-dihydrosphingosine (II)

This compound was prepared, in 90% yield, by the method described in the literature[14], m.p. 73–75°.

Anal. Calc. for $C_{43}H_{77}NO_4$: C, 76.83; H, 11.55; N, 2.08. Found: C, 76.90; H, 11.35; N, 2.01.

2,3,4-Tri-O-acetyl-α-D-glucopyranosyl bromide (I)

A solution of titanium bromide (5 g) in dry, alcohol-free chloroform (5 ml) was added to a stirred solution of 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose[18] (2 g) in chloroform (20 ml), and the mixture boiled under reflux for 30 min, with careful exclusion of moisture. After being cooled, the organic layer was washed successively with a cold sodium bicarbonate solution and with water, and was dried with calcium chloride. It was concentrated *in vacuo* to a few ml, and crystallization was induced by the addition of hexane to turbidity. The yield was 1.10 g (52%), m.p. 120–122° (raised to 123° on recrystallization), $[\alpha]_D^{15}$ + 219° (*c* 0.60, chloroform). This product was identical with that produced by the treatment of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-glucopyranose with titanium bromide[11].

2,3,4-Tri-O-acetyl-α-D-galactopyranosyl bromide (V)

Treatment of 2,3,4-tri-*O*-acetyl-1,6-anhydro-β-D-galactopyranose[19] (1.0 g) with titanium bromide in chloroform solution, as described for I, gave an oil (1.1 g), $[\alpha]_D^{25}$ + 147.4° (*c* 1.53, chloroform). This oil could not be crystallized, but was used directly for glycosidation.

1-O-(β-D-Glucopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine (VIII)

A stirred solution of 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine (II, 670 mg, 0.001 mole) in nitromethane (30 ml) and benzene (20 ml) was boiled until approx. 20 ml of the solvent mixture had distilled, to ensure complete dehydration

and then cooled to 70°. Mercuric cyanide (250 mg, 0.001 mole) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (410 mg, 0.001 mole) were added, and the reaction was continued overnight at 70° under rigidly anhydrous conditions.

After being cooled, the mixture was diluted with benzene (50 ml) and washed successively with a cold, saturated, solution of sodium bicarbonate and water, dried over sodium sulfate, and concentrated *in vacuo*. The residue was dissolved in hexane and passed through a column of silica gel (50 g). Hexane (200 ml) eluted 780 mg (80%) of material, which was contaminated with a small proportion of compound II. Part of this product (400 mg) was saponified overnight, at room temperature, with a catalytic amount of sodium in anhydrous methanol[20]. The addition of a little ice precipitated an amorphous solid (295 mg). This was dissolved in chloroform–methanol, 19:1, and chromatographed on silicic acid. This solvent mixture removed dihydroceramide, and chloroform–methanol 9:1 eluted fractions which crystallized from acetone–pyridine–water 9:1:1, in clusters of tiny needles (200 mg, 54% yield from II), m.p. 180° (after previous sintering), $[\alpha]_D^{27}$ −2.3° (*c* 1.0, pyridine).

Anal. Calc. for $C_{42}H_{83}NO_8$: C, 69.01; H, 11.47. Found: C, 69.21; H, 11.56.

1-O-(β-D-Galactopyranosyl)-N-octadecanoyl-DL-dihydrosphingosine (IX)

Treatment of II (670 mg) with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (410 mg) and chromatography under the conditions previously described for (VIII), eluted a waxy solid which crystallized from cold methanol; yield 700 mg (73%), m.p. 42–43°, $[\alpha]_D^{25}$ −8.2° (*c* 1.47, chloroform). N.m.r. $H_{1a,2a}$ at τ 5.45 ($J_{1,2}$ 8 c.p.s.).

Anal. Calc. for $C_{57}H_{95}NO_{13}$: C, 68.30; H, 9.55. Found: C, 68.60; H, 9.63.

Catalytic saponification, followed by chromatography on silicic acid and recrystallization from 90% aq. acetone–pyridine, gave 300 mg (41% yield from II), m.p. 192–193° (after previous sintering), $[\alpha]_D^{26}$ −0.3° (*c* 1.33, pyridine).

Anal. Calc. for $C_{42}H_{83}NO_8$: C, 69.01; H, 11.47. Found: C, 69.18; H, 11.40.

3-O-Benzoyl-N-octadecanoyl-1-O-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-DL-dihydrosphingosine (III)

To a stirred solution of II (1.30 g, 0.0020 mole), in a mixture of nitromethane and benzene (50 ml, 3:2), (dried as previously described) at 70°, was added mercuric cyanide (500 mg), and four separate portions of I, totalling 700 mg (0.0019 mole), spaced 2 h apart, the last portion being accompanied by a further addition of 200 mg of mercuric cyanide. The reaction was allowed to continue overnight at 70° and the product was isolated and purified in the usual manner. Crystallization from cold methanol gave 770 mg (40%), m.p. 46–48°, $[\alpha]_D^{20}$ −1.1° (*c* 1.20, chloroform). N.m.r. showed $H_{1a,2a}$ at τ 5.55 ($J_{1,2}$ 8 c.p.s.), and 9 protons (OAc) at τ 7.95, using the five aromatic protons at τ 2.4 as a standard. T.l.c. in benzene–ether, 2:1 showed R_F 0.15 and R_{II} 0.5.

Anal. Calc. for $C_{55}H_{93}NO_{12}$: C, 68.79; H, 9.76; Found: C, 68.69; H, 9.95.

3-O-Benzoyl-N-octadecanoyl-1-O-(2,3,4-tri-O-acetyl-β-D-galactopyranosyl)-DL-dihydrosphingosine (VII)

(A) Treatment of II (0.97 g) with 2,3,4-tri-*O*-acetyl-α-D-galactopyranosyl chloride[10] (VI) and mercuric cyanide in nitromethane–benzene, and purification as described previously, gave 520 mg (40%), m.p. 47–48°, $[\alpha]_D^{20}$ + 5.0° (*c* 1.03, chloroform). N.m.r. showed $H_{1a,2a}$ at τ 5.55 ($J_{1,2}$ 8 c.p.s.) and 9 protons (OAc) at τ 7.8–8.1. T.l.c. in benzene–ether, 2:1, showed R_{II} 0.4.

Anal. Calc for $C_{55}H_{93}NO_{12}$: C, 68.79; H, 9.76 Found: C, 68.77; H, 9.57.

(B) A solution of the oily bromide, V (1.0 g) in dry benzene (5 ml) was added dropwise to a nitromethane–benzene solution of II (1.40 g), over a period of 60 min at 70°, and the mixture was kept overnight at 70°. After purification in the usual way, 0.65 g (35%), m.p. 48–50°, $[\alpha]_D^{18}$ + 4.70 (*c* 1.0, chloroform), was obtained by crystallization from cold methanol. This product was pure on t.l.c. using benzene–ether 2:1 or benzene–methanol 9:1, and identical with the product obtained by method (A) (mixed m.p. and i.r. spectrum).

Methyl 2,3,4-tri-O-acetyl-6-O-p-tolylsulfonyl-β-D-glucopyranoside

A stirred mixture of I (300 mg), anhydrous methanol (0.05 ml), and mercuric cyanide (300 mg) in nitromethane (20 ml) and benzene (10 ml), was kept at 50° for 18 h. The usual work-up and evaporation of the organic layer gave a residue weighing 250 mg. To this was added 1.1 mole *p*-toluenesulfonyl chloride in pyridine solution and the clear solution was kept for 24 h at room temperature. On addition of excess cold water a crystalline solid precipitated. Recrystallization from methanol gave needles (200 mg, 52% overall yield), m.p. 170–171°, $[\alpha]_D^{25}$ + 7.3° (*c* 1.02, chloroform) (reported[21], m.p. 170–171°, $[\alpha]_D$ + 7.2°). The product was homogeneous on t.l.c. with benzene–methanol 9:1 as developing solvent, R_F 0.7.

Anal. Calc. for $C_{20}H_{26}O_{11}S$: C, 50.62; H, 5.52; S, 6.76. Found: C, 50.58; H, 5.45; S, 6.74.

Methyl 2,3,4-tri-O-acetyl-6-O-triphenylmethyl-β-D-galactopyranoside

Treatment of VI (100 mg) with methanol (0.02 ml) and mercuric cyanide (100 mg) in nitromethane–benzene 2:1 (10 ml) under similar conditions gave a sirup (100 mg). This was dissolved in pyridine (1 ml), chlorotriphenylmethane (90 mg) was added, and the solution was kept at room temperature for 24 h. On addition of cold water (20 ml), a sticky solid precipitated; this was collected, washed thoroughly with water, dried, and crystallized from ether–hexane. The product (79 mg, m.p. 136–138°, 51%) was homogeneous on t.l.c., R_F 0.84 (benzene–methanol 15:1) and indistinguishable from an authentic specimen[22] (m.p. and t.l.c.).

1-O-[6-O-(β-D-Galactopyranosyl)-β-D-glucopyranosyl]-N-octadecanoyl-DL-dihydrosphingosine (X)

To a solution of III (480 mg, 0.005 mole) in a stirred mixture of nitromethane and benzene at 70° (dried as described previously), was added mercuric cyanide

(125 mg, 0.005 mole) and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (205 mg, 0.005 mole). After 18 h at 70°, a further portion of mercuric cyanide (60 mg) and of bromide (100 mg) was added, and the mixture was kept for a further 24 h at 70°. The solution was processed in the usual way, and passed through a column of silica gel. Hexane–benzene 1:1 eluted 520 mg of substance which gave two spots on t.l.c. in benzene–ether 2:1, R_F 0.15 and R_F 0.0. Catalytic saponification in methanol, followed by the addition of a little ice, precipitated an amorphous solid (350 mg). This was dissolved in chloroform–methanol 19:1 and chromatographed on silicic acid. Fractions totalling 200 mg (51% yield) were eluted with chloroform–methanol 19:1 and crystallized from acetone–pyridine–water 9:1:1 in tiny rosettes, m.p. 181–183°, $[\alpha]_D^{28}$ −2.0° (*c* 1.03, pyridine). This material was identical with VIII and gave a single spot on t.l.c., R_F 0.67 in chloroform–methanol–water, 80:20:3. Chloroform–methanol 9:1 eluted fractions which crystallized from acetone–pyridine–water; yield 140 mg (32%), m.p. 235° $[\alpha]_D^{28}$ −4.3° (*c* 0.6 pyridine).

Anal. Calc. for $C_{48}H_{93}NO_{13}\cdot H_2O$: C, 63.32; H, 10.52; N, 1.54. Found: C, 63.35; H, 10.25; N, 1.82.

1-O-[*6*-O-(*β*-D-*Glucopyranosyl*)-*β*-D-*glucopyranosyl*]-N-*octadecanoyl*-DL-*dihydrosphingosine* (*IV*)

(A) Glycosidation of III (960 mg) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide under conditions similar to those described for the preparation of X, and catalytic saponification of the crude glycoside ester mixture gave a powder weighing 420 mg. Purification was achieved by chromatography on silicic acid. Chloroform–methanol 19:1 eluted a product weighing 200 mg (28% yield) which crystallized from acetone–pyridine–water 9:1:1 to give a substance identical with VIII as shown by m.p., optical rotation, i.r. spectrum, and t.l.c. determinations. Chloroform–methanol 9:1 eluted a second component, also weighing 200 mg (23% yield). After crystallization from acetone–pyridine–water, it melted at 223°, $[\alpha]_D^{26}$ −8.6° (*c* 0.70, pyridine).

Anal. Calc. for $C_{48}H_{93}NO_{13}\cdot H_2O$: C, 63.32; H, 10.52. Found: C, 63.67; H, 10.58.

This product was chromatographically homogeneous on t.l.c. on which it could not be distinguished from a product obtained by the direct reaction of hepta-*O*-acetyl-α-D-gentiobiosyl bromide with II[12], followed by saponification [(m.p. 220°, $[\alpha]_D^{26}$ −9.3°); thin-layer chromatography in chloroform–methanol–water, 80:20:3, showed R_F 0.39, and in chloroform–methanol–ammonia, 80:20:0.4, R_F 0.22. The i.r. spectra of both products were identical.

(B) After 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (300 mg, 1.1 mole-equiv.) was added to II (500 mg) in the presence of mercuric cyanide (220 mg), the mixture was kept at 70°. After 20 h, further portions of bromide (300 mg) and mercuric cyanide (220 mg) were added, and the reaction was prolonged for another 24 h. The usual purification procedure and crystallization from cold methanol gave 200 mg, m.p. 48–50°, $[\alpha]_D^{25}$ −6.9° (*c* 0.6, chloroform). Thin-layer chromato-

graphy, using benzene–ether 2:1 as developing solvent, showed two spots, one of which was indicative of III, and the other remained at the point of origin. Catalytic saponification of 150 mg of this mixture, followed by chromatographic separation on silicic acid, gave 39 mg of VIII and 14 mg of IV, *i.e.* the ratio of disaccharide isolated to monosaccharide was 0.35:1.

1-O-[*6*-O-(*β*-D-*Galactopyranosyl*)-*β*-D-*galactopyranosyl*]-N-*octadecanoyl*-DL-*dihydrosphingosine* (*XI*)

A solution of VII (700 mg) in nitromethane and benzene was treated with mercuric cyanide and 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide under the conditions described for X. After catalytic saponification, the product was purified by chromatography on silicic acid. Elution with chloroform–methanol 19:1 gave 125 mg (24%), m.p. 190°, $[\alpha]_D^{26}$ −0.7° (*c* 1,00, pyridine), R_F 0.15, identical with that of VII. Chloroform–methanol 9:1 eluted 100 mg (15%), m.p. 215°, $[\alpha]_D$ −1.8° (*c* 0.73, pyridine), R_F 0.28. The developing solvent for the t.l.c. was in both cases chloroform–methanol–ammonia 80:20:0.4.

Anal. Calc. for $C_{48}H_{93}NO_{13}\cdot H_2O$: C, 63.33; H, 10.52: Found: C,63.56; H, 10.79.

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SUMMARY

The condensation of 2,3,4-tri-*O*-acetyl-α-D-gluco- or -galacto-pyranosyl halides (I, V, VI) with 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine (II) gave the dihydrocerebroside derivatives, 3-*O*-benzoyl-*N*-octadecanoyl-1-*O*-(2,3,4-tri-*O*-acetyl-*β*-D-glucopyranosyl)-DL-dihydrosphingosine (III) and its galactose analog (VII). From the reaction mixture obtained by treatment of these esters with 2,3,4,6-tetra-*O*-acetyl-α-D-gluco-(or galacto)-pyranosyl bromide, *β*-D-(1→6)-linked dihydroceramide dihexosides could be isolated. One of these compounds was identical with that obtained by direct reaction of hepta-*O*-acetyl-α-D-gentiobiosyl bromide with II.

Treatment of I with methanol, followed by *p*-toluenesulfonylation, gave a good yield of methyl 2,3,4-tri-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-*β*-D-glucopyranoside. Treatment of VI with methanol, followed by reaction with chlorotriphenylmethane gave in good yield methyl 2,3,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-*β*-D-galactopyranoside.

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DERIVATE DER ZUCKER-THIOACETALE

XXXVII. MITTEILUNG[1]. BENZYLIDEN-VERBINDUNGEN DER PENTOSE-DIALKYLACETALE

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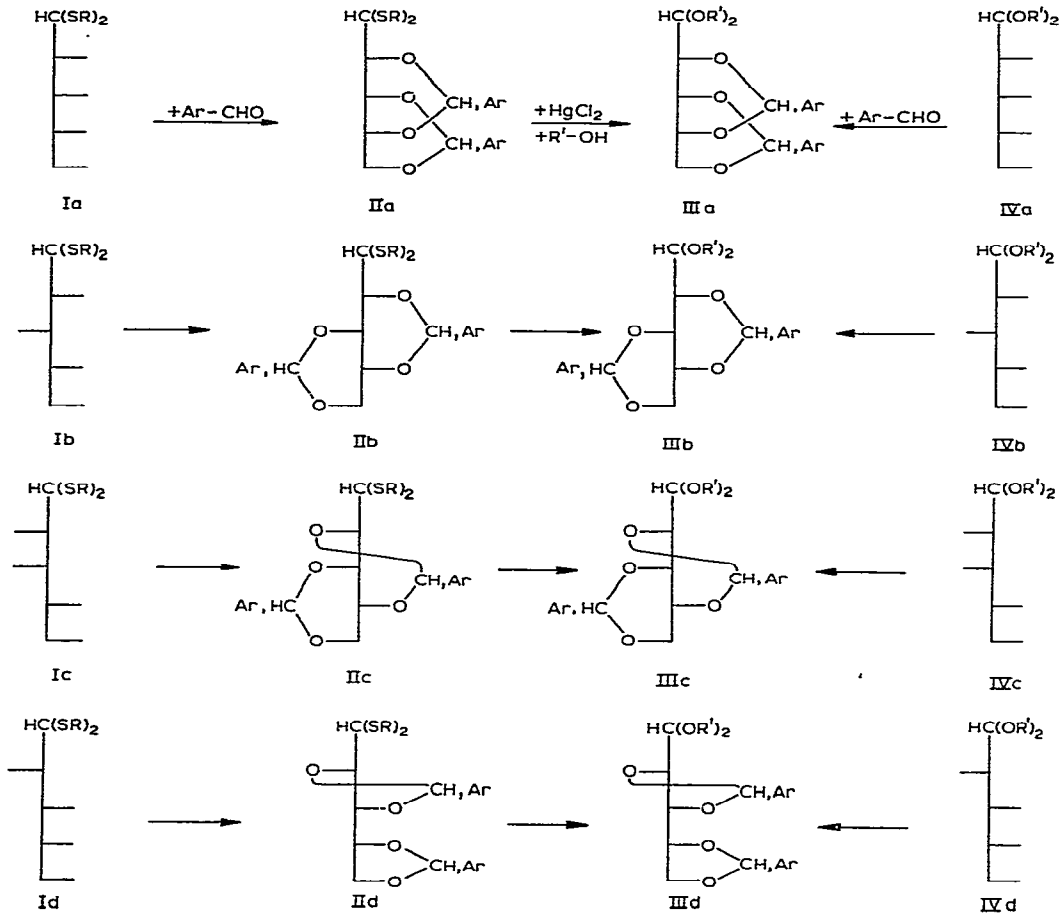
Zuckeralkohole bilden mit Aldehyden bei Gegenwart von sauren Katalysatoren cyclische Acetale, die unterschiedliche Ringweite aufweisen können. Die Regeln von Hann und Hudson[2] gestatten Aussagen über die Bildungstendenz und Stabilität der Verbindungen. Je nach Ringweite und sterischer Anordnung der acetalbildenden Hydroxylgruppen werden die Acetalringe als βC-, β-, αT-, α-, βT-, oder γT- Ringe bezeichnet[3]. In dieser Reihenfolge nimmt bei den Benzyliden-Verbindungen die Bildungstendenz der Acetalringe ab. αC-, γ- und γC-Ringe werden nicht gebildet, wenn andere Möglichkeiten vorhanden sind.

In den letzten Jahren wurde gezeigt[4-7], daß die Regeln von Hann und Hudson nicht nur für die Benzyliden-Verbindungen der Zuckeralkohole, sondern auch für die der Aldose-thioacetale Gültigkeit haben. Bei den D-Ribose-thioacetalen (Ia) und auch bei den D-Xylose-thioacetalen (Ib) ist die Bildung eines nach den genannten Regeln bevorzugten βC-Ringes (= 2,4-Stellung) möglich. Weiterhin kann ein β-Ring (= 3,5-Stellung) enstehen, der die zweite Position in der Bildungsreihe einnimmt. In Übereinstimmung damit bilden die D-Ribose-[4] und D-Xylose-thioacetale[5] 2,4:3,5-Di-*O*-benzyliden-Verbindungen. Bei den D-Lyxose-thioacetalen (Ic) ist ein βC-Ring nicht möglich, wohl aber ein β-Ring. Dieser bildet sich in Kombination mit einem βT-Ring. Es entstehen also ebenfalls 2,4:3,5-Di-*O*-benzyliden-D-lyxose-thioacetale[6] (IIc). Die Kombination α- und αC-Ring tritt hier nicht auf, weil letzterer sehr ungünstig ist. Bei den D-Arabinose-thioacetalen (Id) stehen β- und βT-Ring einerseits sowie αT- und α-Ring anderseits zur Diskussion. Die Umsetzung von L-Arabinose-diäthylthioacetal[7] mit Benzaldehyd hat ergeben, daß die letztgenannte Kombination bevorzugt ist, es wird das 2,3:4,5-Di-*O*-benzyliden-L-arabinose-diäthylthioacetal erhalten. Wir führten die analogen Untersuchungen auch mit dem D-Arabinose-diäthylthioacetal durch und kamen erwartungsgemäß zum gleichen Ergebnis, wir isolierten das 2,3:4,5-Di-*O*-benzyliden-D-arabinose-diäthylthioacetal (IId).

Es ist anzunehmen, daß die Regeln von Hann und Hudson nicht nur für die cyclische Acetalbildung der Zuckeralkohole und Pentose-thioacetale zutreffen, sondern auch für die cyclische Acetalbildung aller Zucker-Derivate, die eine gestreckte, nicht cyclische Form aufweisen. Mit den Umsetzungen von Pentose-dialkylacetalen (IVa–d) mit Benzaldehyd zu Di-*O*-benzyliden-pentose-dialkylacetalen (IIIa–d) soll ein experimentelles Beispiel für die Verallgemeinerung der Regeln gebracht werden.

Die als Ausgangsmaterial benötigten Pentose-dialkylacetale (IVa–d) stellten wir aus den Tetra-*O*-acetyl-pentose-diäthylthioacetalen[8-10] dar. Durch Behandeln der Verbindungen mit Methanol oder Äthanol und Quecksilber(II)-chlorid bei Gegenwart von Quecksilberoxyd nach Green und Pacsu[11] erhält man zunächst die acetylierten Dimethyl- bzw. Diäthylacetale der Pentosen. Von diesen konnten wir lediglich das Tetra-*O*-acetyl-D-arabinose-dimethylacetal zur Kristallisation bringen,



R = C_2H_5 ; R' = CH_3,C_2H_5 ; Ar = C_6H_5

es wird durch Umkristallisieren aus Äther–Petroläther gereinigt. Alle übrigen Tetra-*O*-acetyl-pentose-dialkylacetale fallen als Sirupe an, die sich nur schwer von anhaftenden Quecksilber-Verbindungen befreien lassen. Die restlose Abtrennung der Quecksilber-Verbindungen gelingt weder durch Ausschütteln der in Chloroform gelösten Tetra-*O*-acetyl-pentose-dialkylacetale mit wässriger Kaliumjodid-Lösung noch durch Ausfällen mit Pyridin als Quecksilber–Pyridin-Komplex. Eine weitgehende Reinigung der acetylierten Dialkylacetale wird durch mehrfache Destillation im

Hochvakuum erreicht; die meisten Verbindungen konnten so nahezu analysenrein gewonnen werden. Unerwarteter Weise bereitete die Entacetylierung der Tetra-*O*-acetyl-pentose-dialkylacetale mit Bariummethylat in Methanol zu den Pentose-dialkylacetalen Schwierigkeit. Das durch Neutralisation des Bariummethylats mit Schwefelsäure entstandene Bariumsulfat fiel kolloidal aus und ließ sich durch Filtrieren nur schwer abtrennen. Nach dem Einengen der Filtrate erhält man die Dialkylacetale der D-Arabinose als reine, kristallisierte Verbindungen. Die Dialkylacetale der anderen Pentosen sind jedoch nicht zur Kristallisation zu bringen; die anfallenden sirupösen Verbindungen sind nicht analysenrein. Wegen ihrer Empfindlichkeit lassen sie sich nicht durch Destillation im Hochvakuum reinigen.

Obwohl die meisten Pentose-dialkylacetale nicht ganz rein waren, setzten wir sie mit Benzaldehyd bei Gegenwart von Zinkchlorid um. Die besten Ergebnisse wurden mit den Dimethylacetalen nach einer dreistündigen Reaktionszeit erzielt. In Übereinstimmung mit den Regeln von Hann und Hudson erhielten wir von den Dimethylacetalen der D-Ribose, D-Xylose, und D-Lyxose 2,4:3,5-Di-*O*-benzyliden-Verbindungen (IIIa–c). Das D-Arabinose-dimethylacetal bildete ein 2,3:4,5-Di-*O*-benzyliden-Derivat (IIId). Die analoge Umsetzung der Pentose-diäthylacetale verlief teilweise unter Komplikationen. Die Verbindungen sind gegen Zinkchlorid empfindlicher, die Alkylacetal-Gruppierungen werden während der Reaktion teilweise abgespalten.

Die Struktur der Di-*O*-benzyliden-pentose-dialkylacetale läßt sich durch Synthesen aus den Di-*O*-benzyliden-pentose-dialkylthioacetalen (IIa–d) beweisen. Wenn man diese Verbindungen nach Green und Pacsu[11] mit einem Alkohol und Quecksilber(II)-chlorid bei Gegenwart von gelbem Quecksilberoxyd behandelt, werden die Alkylthio- gegen Alkoxy-Gruppen ausgetauscht. Es enstehen Di-*O*-benzyliden-pentose-dialkylacetale (IIIa–d), in denen sich die beiden Benzyliden-Gruppen in den gleichen Stellungen befinden müssen wie in den Di-*O*-benzyliden-pentose-dialkylthioacetalen, aus denen sie hergestellt wurden. Die so gewonnenen Di-*O*-benzyliden-pentose-dialkylacetale sind identisch mit denen, die durch Umsetzung der Pentose-dialkylacetale mit Benzaldehyd und Zinkchlorid erhalten wurden.

EXPERIMENTELLER TEIL

*2,3:4,5-Di-*O*-benzyliden-*D*-arabinose-diäthylthioacetal*

Man löst 2.56 g (0.01 Mol) D-Arabinose-diäthylthioacetal[10] unter Erwärmen in 10 ml frisch destilliertem Benzaldehyd, kühlt die Lösung auf 0° ab, leitet 90 Sek. einen langsamen Strom (2 Blasen pro Sek.) trocknen Chlorwasserstoff ein, schüttelt dann unter Eintauchen in ein Eisbad um, bis die Kristallisation beginnt (5–10 Minuten), gibt sofort 40 ml Äthanol hinzu, kühlt in einer Eis–Kochsalz-Mischung ab, filtriert, wäscht mit wenig Äther nach, trocknet im Vakuum über Kaliumhydroxyd und kristallisiert aus Äthanol um. Ausbeute 1.90 g (44%), Nadeln, Schmp. 103–105°, $[\alpha]_D^{20}$ + 12.6° (*c* 2.21, Chloroform). ($C_{23}H_{28}O_4S_2$ Ber.: C, 63.86; H, 6.52. Gef.: C, 63.62; H, 6.57%).

2,3-O-Benzyliden-D-arabinose-diäthylthioacetal

Vorstehende Verbindung (3.0 g) wird partiell hydrolysiert, wie für die Darstellung der entsprechenden L-Verbindung beschrieben[7]. Ausbeute 0.70 g (29%), Schmp. 102–103°, $[\alpha]_D^{20}$ + 24.6° (*c* 2.06, Chloroform). ($C_{16}H_{24}O_4S_2$ Ber.: C, 55.78; H, 7.02. Gef.: C, 55.65; H, 6.90%). Die Verbindung verbraucht bei der Oxydation mit Bleitetraacetat in Benzol nach 30 Min. 1.0 Mol des Oxydationsmittels. Der dabei gebildete Formaldehyd wird als Dimedon-Verbindung mit einer Ausbeute von 41% nachgewiesen.

Darstellung der Di-O-benzyliden-pentose-dialkylacetale (IIIa–d)

(*a*) *Aus Di-O-benzyliden-pentose-dialkylthioacetalen*

4.32 g (0.01 Mol) eines Di-*O*-benzyliden-pentose-diäthyl-thioacetals[4–6] werden mit 130 ml eines absoluten Alkohols, 4.0 g Quecksilber(II)-chlorid, und 6.0 g gelbem Quecksilberoxyd 3 Stdn. auf dem siedenden Wasserbad unter Rühren und Rückfluß erhitzt. Dann wird heiß filtriert, der Rückstand mehrmals mit dem betreffenden Alkohol gewaschen. Die vereinigten Filtrate dampft man bei Gegenwart von Quecksilberoxyd ein, extrahiert den Rückstand viermal mit je 30 ml Chloroform, schüttelt die vereinigten Extrakte viermal mit je 40 ml 10-proz. Kaliumjodid-Lösung sowie mit Wasser, trocknet mit Natriumsulfat und dampft ein. Das zurückbleibende Rohprodukt kristallisiert oft erst beim Stehenlassen im Eisschrank, es wird bis zur Reinheit umkristallisiert.

(*b*) *Aus Pentose-dialkylacetalen*

0.01 Mol eines Pentose-dialkylacetals schüttelt man mit 15 ml frisch destilliertem Benzaldehyd und 2.0 g wasserfreiem Zinkchlorid 3 Stdn. bei 20°, versetzt dann die Lösung mit 100 ml Äther, schüttelt die Lösung zum Entfernen des Zinkchlorids dreimal mit je 20 ml Wasser und dampft den Äther ab. Zum Entfernen des überschüssigen Benzaldehyds gibt man 40 ml Wasser hinzu und destilliert dieses im Vakuum bei 40° Badtemperatur ab. Diese Operation wird zweimal wiederholt. Den Rückstand kristallisiert man um.

2,4:3,5-Di-O-benzyliden-D-ribose-dimethylacetal, Ausbeute nach (*a*) 1.72 g (46%), nach (*b*) 1.00 g (27%), Nädelchen aus Aceton–Wasser, Schmp. 155–156°, $[\alpha]_D^{20}$ −44.5° (*c* 2.30, Chloroform). Lit.[12]; Schmp. 155–156°.

2,4:3,5-Di-O-benzyliden-D-ribose-diäthylacetal, Ausbeute nach (*a*) 1.88 g (47%), Nadeln aus Äthanol–Wasser, Schmp. 144–145°, $[\alpha]_D^{20}$ −44.2° (*c* 1.12, Chloroform). ($C_{23}H_{28}O_6$ Ber.: C, 68.98; H, 7.05. Gef.: C, 69.02; H, 6.98%).

2,4:3,5-Di-O-benzyliden-D-xylose-dimethylacetal, Ausbeute nach (*a*) 2.20 g (59%), nach (*b*) 1.15 g (31%), Nadeln aus Methanol, Schmp. 211°, $[\alpha]_D^{20}$ −8.3° (*c* 1.41, Chloroform). Lit.[13], Schmp. 211°.

2,4:3,5-Di-O-benzyliden-D-xylose-diäthylacetal, Ausbeute nach (*a*) 2.60 g (65%), nach (*b*) 1.22 g (30%), Nadeln aus Äthanol, Schmp. 179°, $[\alpha]_D^{20}$ −2.8° (*c* 2.12, Chloroform). ($C_{23}H_{28}O_6$ Ber.: C, 68.98; H, 7.05. Gef.: C, 68.79; H, 7.37%).

2,4:3,5-Di-O-benzyliden-D-lyxose-dimethylacetal, Ausbeute nach (*a*) 2.39 g (64%), nach (*b*) 0.71 g (19%), Nädelchen aus Methanol–Wasser, Schmp. 129°, $[\alpha]_D^{20}$ −42.6° (*c* 2.64, Chloroform). Lit.[6], Schmp. 129°.

2,4:3,5-Di-O-benzyliden-D-lyxose-diäthylacetal, Ausbeute nach (*a*) 2.00 g (50%), Nädelchen aus Äthanol–Wasser, Schmp. 109°, $[\alpha]_D^{20}$ −45.4° (*c* 2.32, Chloroform). ($C_{23}H_{28}O_6$ Ber.: C, 68.98; H, 7.05. Gef.: C, 68.93; H, 7.17%).

2,3:4,5-Di-O-benzyliden-D-arabinose-dimethylacetal, Ausbeute nach (*a*) 2.20 g (59%), nach (*b*) 0.92 g (24%), Nadeln aus Methanol–Wasser, Schmp. 69–70°, $[\alpha]_D^{20}$ −19.7° (*c* 0.68, Chloroform). ($C_{21}H_{24}O_6$ Ber.: C, 67.73; H, 6.50. Gef.: C, 67.83; H, 6.74%).

2,3:4,5-Di-O-benzyliden-D-arabinose-diäthylacetal, Ausbeute nach (*a*) 2.72 g (68%), Nadeln aus Äthanol–Wasser, Schmp. 68–69°, $[\alpha]_D^{20}$ −22.4° (*c* 1.80, Chloroform). ($C_{23}H_{28}O_6$ Ber.: C, 68.98; H, 7.05. Gef.: C, 69.18; H, 6.93%).

Darstellung der Tetra-O-acetyl-pentose-dialkylacetale

4.24 g (0.01 Mol) eines Tetra-*O*-acetyl-pentose-diäthylthioacetals[9–11] werden in der gleichen Weise behandelt, wie oben unter (*a*) zur Darstellung der Di-*O*-benzyliden-pentose-dialkylacetale beschrieben, man erwärmt jedoch 8 Stdn. im siedenden Wasserbad. Die sirupösen Rohprodukte werden bei 10^{-3} Torr destilliert; die Dimethylacetale destilliert man bei einer Badtemperatur von 120–130°, die Diäthylacetale bei 115–125°.

Tetra-O-acetyl-D-ribose-dimethylacetal, nach mehrmaliger Destillation im Hochvakuum erhält man 0.81 g (22%) eines Sirups, der nicht analysenrein ist. $[\alpha]_D^{20}$ + 18.0° (*c* 2.72, Chloroform).

Tetra-O-acetyl-D-xylose-dimethylacetal, Ausbeute 1.75 g (48%), Sirup, $[\alpha]_D^{20}$ + 30.6° (*c* 1.29, Chloroform). ($C_{15}H_{24}O_{10}$ Ber.: C, 49.44; H, 6.64. Gef.: C, 49.43; H, 6.70%).

Tetra-O-acetyl-D-xylose-diäthylacetal, Ausbeute 3.42 g (87%), Sirup, $[\alpha]_D^{20}$ + 32.5° (*c* 2.38, Chloroform). ($C_{17}H_{28}O_{10}$ Ber.: C, 52.04; H, 7.19. Gef.: C, 51.78; H, 6.83%).

Tetra-O-acetyl-D-lyxose-dimethylacetal, Ausbeute 2.33 g (64%), Sirup, $[\alpha]_D^{20}$ + 21.6° (*c* 3.35, Chloroform). ($C_{15}H_{24}O_{10}$ Ber.: C, 49.44; H, 6.64. Gef.: C, 49.48; H, 7.04%).

Tetra-O-acetyl-D-lyxose-diäthylacetal, Ausbeute 2.94 g (75%), Sirup, $[\alpha]_D^{20}$ + 30.5°, (*c* 2.87, Chloroform). ($C_{17}H_{28}O_{10}$ Ber.: C, 52.04; H, 7.19. Gef.: C, 51.21; H, 7.17%).

Tetra-O-acetyl-D-arabinose-dimethylacetal, Ausbeute 2.50 g (69%), Prismen aus Äther–Petroläther, Schmp. 77°, $[\alpha]_D^{22}$ + 23.0° (*c* 1.31, Chloroform). Lit.[14], Schmp. 80°.

Tetra-O-acetyl-D-arabinose-diäthylacetal, Ausbeute 2.70 g (69%), Sirup, $[\alpha]_D^{20}$ + 16.4° (*c* 1.41, Chloroform). ($C_{17}H_{28}O_{10}$ Ber.: C, 52.04; H, 7.19. Gef.: C, 52.18; H, 7.27%).

Darstellung der Pentose-dialkylacetale (IVa–d)

0.01 Mol eines Tetra-*O*-acetyl-pentose-dialkylacetals wird in 50 ml absolutem Methanol gelöst, die Lösung mit 100 ml 0.1 N $Ba(OCH_3)_2$ in Methanol versetzt und 36 Stdn. bei 20° stehengelassen. Dann neutralisiert man unter Umschütteln mit 0.1 N H_2SO_4. Kurz vor Erreichen des Neutralpunktes gibt man etwas Bariumcarbonat hinzu, um zu verhindern, daß die Lösung vorübergehend sauer wird. Dann schüttelt man mit etwas Aktivkohle, filtriert durch ein Kieselgurfilter und dampft ein. Die Dialkylacetale der D-Arabinose kristallisieren, die der anderen Pentosen fallen als nicht ganz reine Sirupe an, sie werden bei 64° und 10^{-3} Torr getrocknet.

D-*Ribose-dimethylacetal*, Ausb. 1.26 g (64%), $[\alpha]_D^{20}$ + 6.3° (*c* 1.08, Wasser).

D-*Xylose-dimethylacetal*, Ausb. 1.88 g (96%), $[\alpha]_D^{20}$ + 19.3° (*c* 1.81, Wasser).

D-*Xylose-diäthylacetal*, Ausb. 2.18 g (97%), $[\alpha]_D^{20}$ + 20.7° (*c* 1.92, Wasser).

D-*Lyxose-dimethylacetal*, Ausb. 1.92 g (98%), $[\alpha]_D^{20}$ + 8.4° (*c* 2.18, Wasser).

D-*Lyxose-diäthylacetal*, Ausb. 2.17 g (97%), $[\alpha]_D^{20}$ + 24.7° (*c* 1.89, Wasser).

D-*Arabinose-dimethylacetal*, Ausb. 1.74 g (90%), Prismen aus Methanol, Schmp. 121°, $[\alpha]_D^{20}$ −18.1° (*c* 0.69, Wasser). Lit.[14], Schmp. 122°.

D-*Arabinose-diäthylacetal*, Ausb. 1.28 g (57%), Nadeln aus Äthanol–Wasser, Schmp. 108–109°, $[\alpha]_D^{20}$ −16.9° (*c* 1.88, Wasser). ($C_9H_{20}O_6$ Ber.: C, 48.20; H, 8.99. Gef.: C, 48.00; H, 8.75%).

ZUSAMMENFASSUNG

Die Regeln von Hann und Hudson haben nicht nur Gültigkeit für die Bildung von Benzyliden-Verbindungen der Zuckeralkohole und Pentose-thioacetale, sondern

auch für die Bildung der Di-*O*-benzyliden-pentose-dialkylacetale. Bei der Umsetzung von Dimethylacetalen der D-Ribose, D-Xylose, und D-Lyxose mit Benzaldehyd bei Gegenwart von Zinkchlorid erhält man 2,4:3,5-Di-*O*-benzyliden-Verbindungen. Das D-Arabinose-dimethylacetal liefert ein 2,3:4,5-Di-*O*-benzyliden-Derivat. Die Struktur der Verbindungen wird durch Synthesen aus zugehörigen Di-*O*-benzyliden-pentose-diäthylthioacetalen bewiesen.

SUMMARY

The rules of Hann and Hudson apply not only to the formation of benzylidene derivatives of sugar alcohols and pentose dithioacetals, but also to the formation of di-*O*-benzylidenepentose dialkyl acetals. Treatment of the dimethyl acetals of D-ribose, D-xylose, and D-lyxose with benzaldehyde and zinc chloride yields 2,4:3,5-di-*O*-benzylidene derivatives. D-Arabinose dimethyl acetal gives a 2,3:4,5-di-*O*-benzylidene derivative. The structures of these compounds were proved by their synthesis from the appropriate di-*O*-benzylidenepentose diethyl dithioacetals.

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PHENYLHYDRAZONO–PHENYLAZO TAUTOMERISM

PART I*. *xylo*-4,5,6-TRIHYDROXY-2-OXO-1,3-BIS(PHENYLHYDRAZONO)CYCLOHEXANE AND 4-OXO-1-PHENYL-5-PHENYLAZO-3-PYRIDAZINE DERIVATIVES

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INTRODUCTION

In an earlier paper[1], we described the preparation of *xylo*-4,5,6-trihydroxycyclohexane-1,2,3-trione (I) from *myo*-inositol and showed that it forms a crystalline bis(phenylhydrazone) (II) of unknown structure. In continuation of the study, we have found that the bis(phenylhydrazone) exists in two forms, one yellow-orange and the other red. The structure of phenylhydrazono compounds in general is a subject of high current interest[2], because they exist in several tautomeric forms which may be studied by physical methods of analysis recently developed. N.m.r. spectral studies by Mester and co-workers[3], by Wolfrom and co-workers[4], and by Chapman and co-workers[5], have confirmed the presence of the N···H–N chelate structure originally proposed by Fieser and Fieser[6] for the phenylosazones of sugars in solution. However, very little is known concerning the molecular structure of phenylhydrazono compounds having an oxo group in proximity to a phenylhydrazono group. Hence, we have investigated the structure of the oxo-bis(phenylhydrazono) compound in considerable detail.

From the method of synthesis, it would be expected that this compound (II) is either a 1-oxo-2,3-bis(phenylhydrazono) or a 2-oxo-1,3-bis(phenylhydrazono) derivative**. To determine the positions of the phenylhydrazono groups in II, we first sought to obtain a phenylosotriazole derivative. In accord with the absence of a 2,3-bis(phenylhydrazono) group, the compound did not produce a phenylosotriazole when treated with cupric sulfate in the usual manner[7]. Definite proof for the 2-oxo-1,3-bis(phenylhydrazono) structure of II was obtained by oxidation of the compound with sodium metaperiodate and identification of the reaction product.

From the periodic acid oxidation of phenylosazones of cyclitols reported by prior workers[8], it would be expected that a 2-oxo-1,3-bis(phenylhydrazono) derivative of I would, on oxidation with sodium metaperiodate, yield 3-oxo-2,4-bis(phenylhydrazono)glutardialdehyde (III). However, we did not obtain III, but instead

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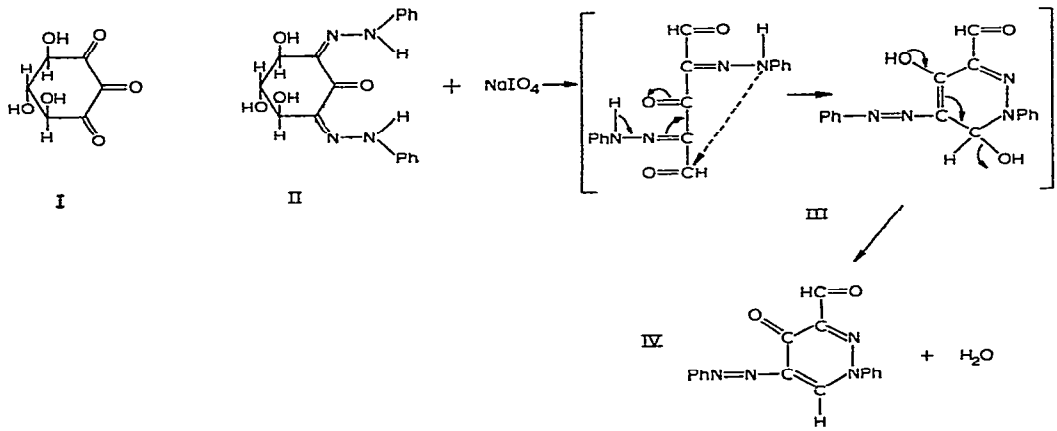
**This name is based on the premise that the phenylhydrazono group at C-1 is the principal function. If the oxygen atom is considered to be the principal function, the compound is named *xylo*-3,4,5-trihydroxy-1-oxo-2,6-bis(phenylhydrazono)cyclohexane.

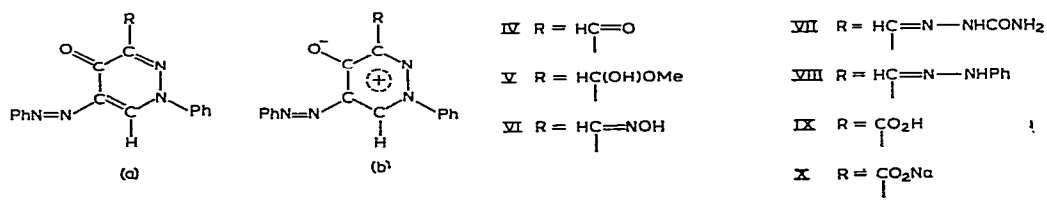
obtained a compound having the molecular formula $C_{18}H_{16}N_4O_3$. This substance was found to be the methyl hemiacetal of 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde (IV). Presumably, this compound was formed by cyclization of III, followed by dehydration as illustrated. Formation of IV from II proved that II is, in fact, *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane*.



The results of periodate oxidation of II not only proved the structure of II, but also provided a synthetic route to a new series of pyridazine derivatives.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazine derivatives

Compound IV was conveniently separated as the methyl hemiacetal (V). Surprisingly, dissolution of V in cold, concentrated sulfuric acid, followed by addition of ice-water, produced IV in good yield; the aldehyde IV was also obtained by heating V under diminished pressure. Slow crystallization of IV from aqueous methanol regenerated V. The presence of the aldehyde group in IV was confirmed by preparation of oxime (VI), semicarbazone (VII), phenylhydrazone (VIII), and carboxylic acid (IX). Examination of molecular models shows that the compounds may have a resonance structure involving a quinonoid form (a) and an aromatic "zwitter" ion (b). In the latter form, electrons are shifted from the pyridazine ring to the oxygen atom on C-4. This electron shift satisfies the electrophilic property of the 4-oxo group and accounts for the fact that this group does not react with phenylhydrazine or similar carbonyl reagents.



*See note ** preceding.

TABLE I

INFRARED ABSORPTION BANDS (CM^{-1}) FOR PYRIDAZINE DERIVATIVES[a]

Assignment	*Compound number*							
	IV	*V*[a]	*VI*	*VII*	*VIII*	*IX*	*X*	*XII*
OH,NH		3401s 3030w	3333s	3508w 3333m	3333m	3174w	3448s	3279m
C=O (non-conjugated)	1712s			1700s		1742s		
C–O (conjugated)	1628s	1623s	1636s	1623m	1618s	1639sh	1626s	1618s
Phenyl ring	1592m 1492s	1592m 1519s 1492s	1592m 1510sh	1589s 1492m	1592w	1600s 1492m	1608w 1492m	1603s 1492s
N=N (azo)	1572m	1567sh	1572m	1562m	1572m	1584s 1563w	1582w	
N–H (bending)					1538s			1550s 1529s
Fingerprint region	1408w 1329m 1298s 1282s 1262m 1204s 1169sh 1149m 1098sh 1081sh 1030m 1020sh 1000w 971s 935w 909s 893w 877m 826s 766s 735s 690s	1466s 1398s 1333m 1315m 1298s 1259s 1257m 1273s 1169sh 1250m 1149w 1204s 1075m 1176w 1025w 1149m 990s 1098s 943w 1075s 926m 1041s 893w 1030sh 840w 1000sh 794sh 990w 763s 962m 741s 935s 699m 909s 685s 896sh 866sh 826m 806m 769s 746s 735w 687s	1333sh 1298m 1282w 1259s 1257m 1169sh 1149w 1116w 1075m 1149w 1025w 1104m 990s 1081sh 943w 1025w 926m 1000w 893w 850m 840w 888w 794sh 840w 763s 817w 741s 797w 699m 772s 685s 732m 694w 686s	1408sh 1333m 1298w 1282s 1256w 1204m 1116w 1149w 1104m 1081sh 1025w 1000w 850m 888w 840w 817w 797w 772s 732m 694w 686s	1333sh 1315m 1298s 1272s 1242s 1204w 1176m 1149sh 1142s 1081m 1058sh 1030m 990m 948m 926m 917sh 909sh 893s 885sh 870s 847w 813m 800w 772s 758s 709sh 690s	1324m 1299m 1282s 1204m 1190w 1176w 1162m 1142m 1086w 1075w 1063m 1020w 995w 966sh 926m 901m 840sh 820m 787w 778s 711w 704w 694sh 685s	1329w 1299m 1278m 1234s 1204m 1162m 1015w 1047m 1018w 1000m 971m 926sh 909m 881m 833s 806m 763sh 758s 746m 685s	1408w 1366w 1330m 1302m 1262s 1219s 1165m 1121s 1098sh 1086sh 1063s 1022w 1005m 985w 971w 962w 935s 921w 901s 882s 862m 847s 826m 816w 803s 782m 769s 758s 752sh 690s

[a]All spectra with the exception of that of V were measured in Nujol mulls; that of V was measured in a potassium chloride pellet.

The chemical stability of the pyridazine ring and its resistance to oxidation are compatible with the aromatic character depicted in (b). However, the i.r. and u.v. spectra indicate that the compounds also partake of the characteristics of quinonoid structure (a). Thus, all of the compounds show an i.r. band at 1639–1618 cm^{-1} (see Table I) which is in accord with the conjugated carbonyl stretching frequencies reported for certain γ-pyridones and dihydropyridazine derivatives[9,10]. All compounds, with the exception of hydrazo compound XII, show absorption at 1584–1562 cm^{-1}, which may be ascribed to the azo (–N=N–) group[11].

The i.r. spectra of acid IX and salt X are noteworthy. The spectrum of IX shows a band at 1742 cm^{-1} (C=O of a CO_2H group), and a doublet at 1299 and 1282 cm^{-1}. These bands are characteristic of carboxylic acids[12], but surprisingly, the compound does not show strong absorption in the region (3333 cm^{-1}) indicative of the carboxyl OH group. The hydrated sodium salt X, obtained by careful neutralization of acid IX, shows a rather broad band in the region of 1626 cm^{-1} corresponding to a conjugated carbonyl group and the carboxylate ion. The displacement of the carbonyl frequency from 1742 cm^{-1} in the acid IX to about 1626 cm^{-1} in the salt X is typical of carboxylic acids and their salts[13]. Thus, IX seems to have a normal carboxylic acid structure rather than a "zwitter ion" structure like that of nicotinic acid[14].

In the ultraviolet region, the spectra (see Table II) show: (1) a strong band at about 230 mμ, which is attributable to conjugation of a double bond with a carbonyl group, and (2) a strong band at about 300 mμ. The relatively high intensity of this

TABLE II

ULTRAVIOLET AND VISIBLE ABSORPTION BANDS[a] AND THEIR INTENSITIES, FOR PYRIDAZINE AND PHENYLAZO–PHENYLHYDRAZONO COMPOUNDS

Compound No.	λ_{max} (*in mμ*) *and* ε_{max}[b]				
	Pyridazine derivatives				
IV	228 (11,200)	300 (17,500)	366sh (11,400)	376 (11,600)	462sh (1,050)
V	227 (16,700)	301 (24,900)	367sh (16,700)	376 (17,000)	465sh (1,600)
VI	225 (20,750)	306 (23,750)		394 (17,750)	475sh (2,000)
VII	233 (21,100)	305 (21,700)		403 (21,000)	
IX	228 (14,200)	301 (23,500)	368sh (15,300)	377 (16,000)	470sh (1,550)
X	228 (14,000)	304 (19,750)	369sh (13,200)	386 (14,200)	420sh (6,800)
VIII	243 (19,700)	292 (20,900)			467 (23,300)
XII	242 (26,650)	292sh (12,700)	360 (18,700)	422 (20,600)	485sh (5,700)
	Phenylazo–phenylhydrazono derivatives				
II	251 (18,800)	286sh (6,000)			487 (34,300)
XI	257 (16,700)	292sh (7,200)			441 (18,500)

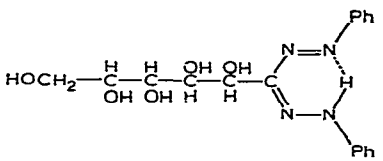
[a]The spectra were recorded 30 min after dissolving the sample in ethyl alcohol, except for compound VIII, which was dissolved in methanol. [b]Molecular extinction coefficient, given in parentheses.

band ($\varepsilon > 10{,}000$) can be explained on the basis of the reduced symmetry[15], which is in agreement with the quinonoid character of the above pyridazine derivatives. The

band at 300 mμ is close to a band (λ_{max} 308 mμ) reported for certain pyridazinone derivatives[16].

In the visible region, aldehyde IV, hemiacetal V, acid IX, and salt X show high-intensity bands with maxima at 366–369 mμ and at 376–386 mμ, with a shoulder at longer wavelengths. The bands are far more intense than the bands (300–350 mμ) reported[17] for certain pyridazine derivatives, and presumably arise from the quinonoid structure (a). No appreciable change in the u.v. and visible spectra of compounds IV,V,IX, and X was observed on changing the pH. In the visible region, the principal absorption band of the oxime (VI), the semicarbazone (VII), and the phenylhydrazone (VIII) occurs at 394, 403, and 467 mμ, respectively.

A comparison of the u.v. and visible spectra of the phenylhydrazone (VIII) with those of II and of D-mannose diphenylformazan (XI) is of interest. All three of



XI

these compounds show absorption bands in the regions 243–257, 286–292, and 441–487 mμ. This similarity appears to arise from the presence in each compound of a phenylazo and a phenylhydrazono chromophore, in accord with the known structure[18] of formazan XI and the structures proposed for II and VIII.

The absorption spectra of compounds IV to X, inclusive, are in accord with the resonance structure arising from forms (a) and (b). The n.m.r. spectra of the compounds measured (those of IV and V) are also consistent with this structure.

The n.m.r. spectrum of the aldehyde (IV) in deuteriochloroform shows signals corresponding to three kinds of protons: a singlet at 10.25 ±0.1 p.p.m. (aldehydic proton); a singlet at 8.5 ±0.1 p.p.m. (vinyl proton of the pyridazine ring)[19]; and a multiplet centered at 7.55 p.p.m. (phenyl ring protons).

The n.m.r. spectrum of the hemiacetal (V) (Fig. 1) in deuteriochloroform shows signals for five types of protons, consistent with the proposed structure. The signals are: a singlet at 8.8 ±0.1 p.p.m. (vinyl proton of the pyridazine ring); a multiplet centered at 7.5 ±0.1 p.p.m. (phenyl ring protons); a sharp singlet at 3.62 ±0.1 p.p.m. (methoxyl protons); and two, equally intense doublets centered at 6.3 ±0.1 p.p.m. (hydroxyl proton) and at 5.55 ±0.1 p.p.m. (methine proton). Presumably, the doublets arise from the first-order, AB pattern[20] (CH_AOH_B); $J_{A,B}$ 11.5–12.0 c.p.s.; $\Delta\nu$ 45 c.p.s. Upon the addition of 0.1 ml of deuterium oxide, and mixing for a few seconds at room temperature, the hydroxyl proton (6.3 p.p.m.) is exchanged readily and the doublet collapses to a singlet (5.6 p.p.m.). Treatment of IV in deuteriochloroform with methanol results in slow disappearance of the aldehyde signal at 10.25 p.p.m. with appearance of the hemiacetal signals at 6.3 and 5.55 p.p.m.

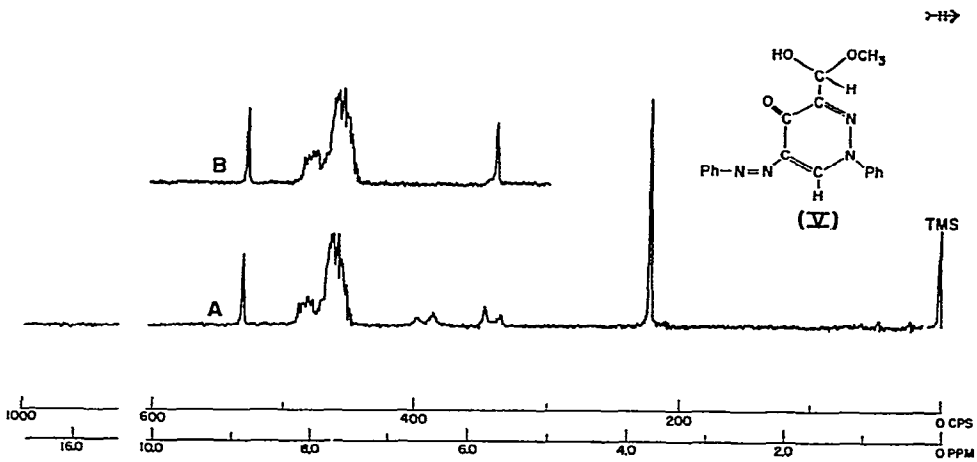


Fig. 1. N.m.r. spectra of 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde methyl hemiacetal (V) at 60 Mc.p.s. and 1000 c.p.s. sweep width; tetramethylsilane internal standard; spectrum A in deuteriochloroform; spectrum B after exchange with deuterium oxide.

The large value of the coupling constant ($J_{A,B}$ 11.5–12.0 c.p.s.) observed for V may indicate that the geometry of the adjacent protons (CH_AOH_B) is influenced by the neighboring carbonyl group of the heterocyclic ring. So far, we have been unable to find a compound of similar structure having so large a value for the coupling constant[21].

The behavior of phenylhydrazone VIII on treatment with excess phenylhydrazine was found to be unusual. Heating of VIII with an excess of phenylhydrazine did not cause substitution of the potential carbonyl group on C-4; instead, reduction of the azo group occurred. The elementary analysis and the visible, u.v., i.r., and n.m.r. spectra suggest that the reaction product is 4-oxo-1-phenyl-5-phenylhydrazo-3-pyridazinecarboxaldehyde phenylhydrazone (XII).

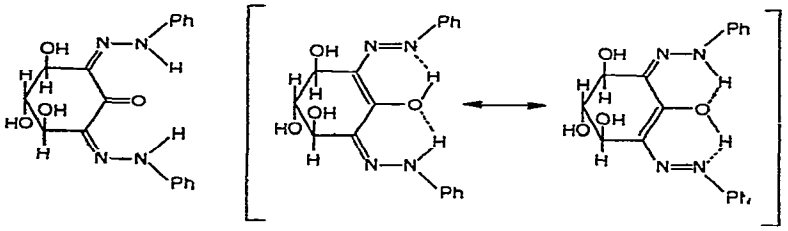
The i.r. spectrum of XII shows the following characteristic absorption bands: at 3279 cm^{-1} (N–H group); a shoulder at 1618 cm^{-1} (conjugated C=O); 1603 cm^{-1} (phenyl ring); 1550 and 1529 cm^{-1} (N–H bending)[22]. The u.v. spectrum of XII is similar to that of VIII, with bands at 242 mμ and 292 mμ. In the visible region, however, XII shows strong bands at 360 mμ and 422 mμ, and a shoulder at 485 mμ. Presumably, the band at 422 mμ does not arise from a simple azo chromophore because of its high intensity; the source of the band at 422 mμ and the shoulder at 485 mμ is not yet clear. A compound having the composition of XII might exist in several tautomeric forms. The structure of XII is being investigated further.

Tautomeric forms of xylo-*4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane*

As already mentioned, II exists in a red and a yellow form. When freshly prepared II is mixed with toluene plus water, it is partitioned between the two phases, coloring the organic phase bright-red and the aqueous phase yellow. On warming the mixture, the yellow (more-polar) form gradually changes to the red form, which

passes into the toluene. This observation is consistent with a phenylhydrazono–phenylazo tautomerism.

A possible tautomeric change from the yellow to the red form is depicted in formulas IIa and IIb. This isomerization has been confirmed by a study of the visible,



IIa IIb

u.v., i.r., and n.m.r. spectra of IIa and IIb, as compared with the spectra of model compounds which possess similar structures. The spectrometric study will be presented in a future publication, in which it will be shown that 2-oxo-1,3-bis(phenylhydrazono) compounds in general yield red modification having enolic phenylhydrazono–phenylazo structures and, in some instances, yellow keto forms.

As indicated in structure IIb, the red, enolic phenylhydrazono–phenylazo compound does not have a free carbonyl group, a true phenylhydrazono group, or a true phenylazo group. The potential phenylhydrazono and phenylazo groups are joined in a resonance structure, and, consequently, they cannot be allocated to specific carbon atoms. In view of this situation, the red form of compound II cannot be given a definitive name, but is best described as the enolic hybrid of *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane.

EXPERIMENTAL

Prior to analysis, all compounds were dried for 4 h at 25°/0.1 mm over phosphorus pentaoxide, unless stated otherwise. Melting points were taken in Pyrex capillary tubes and are uncorrected. U.v. and visible spectra were measured with a Beckman DK-2 recording spectrophotometer*. I.r. spectra were determined in potassium chloride pellets or in Nujol mulls, with a Perkin–Elmer Infracord spectrophotometer, Model 137*; the spectra are not corrected for hydroxyl bands arising from moisture in the potassium chloride. The abbreviations for i.r. bands are s (strong), m (moderate), w (weak), and sh (shoulder). The n.m.r. spectra were measured with a Varian A-60 spectrometer*, using tetramethylsilane (δ = 0.00 p.p.m.) as the internal reference standard.

*Certain commercial instruments are identified in this paper in order to specify the experimental procedure adequately. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the equipment identified is necessarily the best available for the purpose.

xylo-4,5,6-Trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (II)

To a solution of 3 g of DL-*xylo*-4,5,6-trihydroxycyclohexenediolic acid[1] in 300 ml of 4% aqueous acetic acid was added a saturated solution of iodine (prepared from iodine and a 1:4 (v/v) mixture of glacial acetic acid and methanol) until a trace of free iodine remained. The excess iodine was removed by cautious addition of a small amount of the parent compound, and the solution was filtered on a thin layer of decolorizing carbon.

The resulting, colorless filtrate was mixed with 400 ml of crushed ice, and 10 ml of phenylhydrazine in 10 ml of methanol was added with continuous stirring, followed by 6 g of sodium acetate trihydrate in 50 ml of water. After 60 min at 15° and 2 h at 0°, the resulting, orange-red crystals were collected on a filter, washed with 4% aqueous acetic acid, and dried (5 g).

The product was recrystallized from methanol; dark-red needles, m.p. 185–187°. The sample for analysis was purified by column chromatography (neutral alumina with benzene–methanol as the eluant).

Anal. Calc. for $C_{18}H_{18}N_4O_4$; C, 61.0; H, 5.1; N, 15.8. Found: C, 61.3; H, 5.0; N, 15.9.

The inability of II to form a phenylosotriazole was established by treatment of II in aqueous *p*-dioxane with cupric sulfate in the conventional manner[7] for obtaining a phenylosotriazole. After removal of copper salts, I was recovered in good yield, and no phenylosotriazole could be separated.

A sample of freshly prepared II was mixed with toluene and water; this gave a red toluene phase and a yellow aqueous phase. When the mixture was heated with mixing, the color disappeared from the aqueous phase and the red color of the toluene phase became more intense.

Periodate oxidation of II and preparation of 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde methyl hemiacetal (V)

A solution of 1.1 g (0.003 mole) of *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane (II) in 250 ml of methanol was mixed at 15–20° with a solution of 1.3 g (0.006 mole) of sodium metaperiodate in 100 ml of water. After 1 h, the reaction mixture was filtered. The filtrate was concentrated and the sirupy residue was extracted with dichloromethane (4 × 80 ml). The extract was dried with sodium sulfate, treated with 0.1 g of decolorizing carbon, filtered, and concentrated to a thick sirup. The residue was diluted with 4 ml of methanol and filtered with a small amount of a decolorizing carbon. The solution was kept for 24 h at 0°, diluted with a cold mixture of 3 ml of ether and 7 ml of pentane, and stirred in an ice-bath. When crystallization began, the mixture was diluted with 3 ml of pentane and, ultimately, the crystalline product was separated and washed with cold 1:1 ether–pentane. Recrystallization from aqueous methanol at 0°, with stirring, gave 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde methyl hemiacetal (V), 250–300 mg; orange needles; m.p. 134–136°. The sample for analysis was dried in a vacuum desiccator to constant weight.

Anal. Calc. for $C_{18}H_{16}N_4O_3$; C, 64.3; H, 4.8; N, 16.1; Found: C, 64.1; H, 4.9; N, 16.4.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde (IV)

Aldehyde IV was obtained by heating purified hemiacetal V for 6 h at 110°/0.1 mm. The product was recrystallized from acetone–pentane; orange-red needles, m.p. 172–174°. For analysis, the sample was dried for 3 h at 110°/0.1 mm.

Anal. Calc. for $C_{17}H_{12}N_2O_2$; C, 67.1; H, 4.0; N, 18.4. Found: C, 67.0; H, 4.2; N, 18.3.

Aldehyde IV was also obtained by dissolving hemiacetal V in ice-cold, concentrated sulfuric acid, followed by dilution of the dark solution with ice water. Product IV was reconverted into V by slow crystallization from aqueous methanol. Rapid crystallization of IV from aqueous methanol gave, in some instances, a hydrated species, m.p. 66–68°, which could be converted into V by slow crystallization. The i.r. spectrum of the compound was almost identical with that of V, except for a band at 3546 cm^{-1} which may indicate water of crystallization. A solution of IV in methanol gave a positive aldehyde test with Schiff reagent in 15 min. Aldehyde IV and hemiacetal V gave the same semicarbazone VII and phenylhydrazone VIII, thus excluding any structural change during the removal of the elements of methanol.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldoxime (VI)

A filtered solution of hydroxylamine acetate, prepared from 1.5 g of hydroxylamine hydrochloride and 1.5 g of potassium acetate in 15 ml of methanol, was mixed with 75 mg of V dissolved in 3 ml of methanol. The mixture was stirred for 10 min at 50°, and for 1 h at room temperature. The mixture was diluted with 10 ml of water, cooled for 30 min in an ice bath, and filtered.

The crude oxime (80–90 mg) was recrystallized from methanol by concentration and cooling, to give VI as long, orange needles; m.p. 253–255°. The sample for analysis was dried for 2 h at 78°/0.1 mm.

Anal. Calc. for $C_{17}H_{15}N_5O_2$; C, 63.5; H, 4.7; N, 21.8. Found: C, 63.5; H, 4.4; N, 21.3.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde semicarbazone (VII)

A filtered solution of semicarbazide acetate (prepared from 1.5 g of semicarbazide hydrochloride in 7 ml of 70% aqueous methanol and 1.5 g of potassium acetate in 5 ml of methanol) was added to 75 mg of V in 3 ml of methanol. The mixture was stirred for 10 min at 55°, diluted with 10 ml of water, and stirred for 1 h at room temperature. The crude, crystalline oxime (90 mg) was separated, and recrystallized from methanol; m.p. 241–243° (dec.). The sample for analysis was dried for 2 h at 78°/0.1 mm.

Anal. Calc. for $C_{18}H_{15}N_7O_2$; C, 59.8; H, 4.2; N, 27.1. Found: C, 59.7; H, 4.1; N, 27.1.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxylic acid (IX) and its sodium salt (X)

A solution of 100 mg of V in 20 ml of methanol was added at 0° to a solution of 1.2 g of silver nitrate in 3 ml of water, and 9 ml of 10% sodium hydroxide solution was added dropwise, with stirring, during 15 min. The mixture was stirred for 30 min, filtered through paper pulp and decolorizing carbon, and the filtrate was acidified (pH 5) with dilute sulfuric acid. Lustrous, orange crystals of IX separated (55–60 mg); after recrystallization from methanol, it had m.p. 235–236°. The sample for analysis was dried for 4 h at 78°/0.1 mm.

Anal. Calc. for $C_{17}H_{12}N_4O_3$; C, 63.7; H, 3.8; N, 17.5. Found: C, 63.6; H, 3.9; N, 17.4.

A sample (11.58 mg) of IX in aqueous ethyl alcohol required 4.15 ml of 0.0087 N sodium hydroxide for neutralization; this corresponds to an equivalent weight of 321; calc. for $C_{17}H_{12}N_4O_3$, eq. wt. 320.

Neutralization of IX with sodium hydroxide, concentration of the solution, and storage in a desiccator gave orange crystals of the sodium salt (X) which was recrystallized from 95% ethyl alcohol and ether. The salt X was hydrated; it melted at 85°, and then solidified and decomposed at 204–206°.

Anal. Calc. for $C_{17}H_{12}N_4NaO_3 \cdot 2.5H_2O$; C, 52.8; H, 4.2; N, 14.5. Found: C, 53.0; H, 4.1; N, 14.2.

4-Oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxyaldehyde phenylhydrazone (VIII)

A 50-mg sample of V in 10 ml of methanol was mixed at 50° with five drops of phenylhydrazine, followed by 2 drops of glacial acetic acid. Compound VIII crystallized almost immediately. The mixture was cooled in an ice bath and filtered, and the crystals were washed with methanol*. The compound separated in dark-red, lustrous prisms (55 mg), which, after recrystallization from hot nitromethane, had m.p. 244–246°.

The sample for analysis was dried for 2 h at 110°/0.1 mm.

Anal. Calc. for $C_{23}H_{20}N_6O$; C, 69.7; H, 5.1; N, 21.2. Found: C, 69.7; H, 5.0; N, 21.3.

4-Oxo-1-phenyl-5-phenylhydrazo-3-pyridazinecarboxaldehyde phenylhydrazone (XII)

A mixture of crude VIII (150 mg) and 2 ml of phenylhydrazine was heated for 1 h at 130 to 135°; the solid dissolved, and the solution changed from red to light-brown. The mixture was cooled, diluted with 2 ml of ethyl alcohol, and poured, with stirring, into 150 ml of cold, 4% aqueous acetic acid. After 1 h, the bright-yellow compound XII was separated, washed with 25% aqueous ethyl alcohol, and dried (100–120 mg). The product was recrystallized by dissolving in warm 95% ethyl alcohol (85 ml), diluting with warm water, and cooling overnight. After drying for 2 h at 78°/0.1 mm, it melted at 182–184°.

*When the filtrate (after isolation of VIII) was poured into cold, 4% aqueous acetic acid, a small amount (30 mg) of orange-yellow compound XII was precipitated.

Anal. Calc. $C_{23}H_{20}N_4O$; C, 69.7; H, 5.1; N, 21.2. Found: C, 69.7; H, 5.2; N, 20.9.

When the yellow XII was exposed to the atmosphere in the light, it gradually changed to phenylazo compound VIII; oxidation of yellow XII with mercuric oxide in methanol gave VIII also. The reaction appears analogous to the oxidation of hydrazobenzene to azobenzene[23].

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SUMMARY

The positions of the phenylhydrazono groups in *xylo*-4,5,6-trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane were established by degradation of the compound with sodium metaperiodate and identification of the reaction product. The dialdehyde initially formed by periodate oxidation (but not isolated) cyclized to 4-oxo-1-phenyl-5-phenylazo-3-pyridazinecarboxaldehyde, which gave a crystalline methyl hemiacetal, an oxime, a semicarbazone, and, by oxidation, the corresponding monocarboxylic acid and sodium salt. Upon treatment with phenylhydrazine at 50°, the carboxaldehyde gave a red, crystalline phenylhydrazone which, with phenylhydrazine at 130°, gave a product that appears to be 4-oxo-1-phenyl-5-phenylhydrazo-3-pyridazinecarboxaldehyde phenylhydrazone.

The reactions provide a route to a series of pyridazine derivatives which may prove valuable for the synthesis of unusual compounds for biological and medical research.

xylo-4,5,6-Trihydroxy-2-oxo-1,3-bis(phenylhydrazono)cyclohexane exists in a yellow keto form and in a red enolic form. On warming in toluene, the yellow form gives the red form, which has an enolic phenylhydrazono–phenylazo structure.

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FURTHER OBSERVATIONS ON THE ACID-CATALYSED BENZALDEHYDE–GLYCEROL REACTION

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INTRODUCTION

The acid-catalysed condensation of aldehydes with tetritols and higher, acyclic, polyhydric alcohols yields derivatives of 1,3-dioxan in preference to those of 1,3-dioxolan[1]. Glycerol is exceptional in that 1,3-dioxolan derivatives (1,2-acetals) are reported to be preponderant at equilibrium[2a]. In extending a study of benzylidene acetals[3], we have re-examined the acid-catalysed condensation of benzaldehyde and glycerol in order to clarify the apparently anomalous situation.

The benzylidenation of glycerol was first described by Fischer[4], and, subsequently, Irvine *et al.*[5] concluded that the product was preponderantly, if not exclusively, *cis,trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan. However, Hibbert and his co-workers[2a] found that a crystalline 5-hydroxy-2-phenyl-1,3-dioxan (later shown[6] to be *cis*) formed a significant percentage of the *O*-benzylideneglycerol mixture. On the basis of the isolation of the crystalline *cis*-1,3-dioxan derivative from various equilibrium mixtures of the *O*-benzylideneglycerols, the Canadian workers[2a] concluded that, in each case, the 4-hydroxymethyl-2-phenyl-1,3-dioxolans were markedly preponderant, although the inaccuracy of the method was recognised.

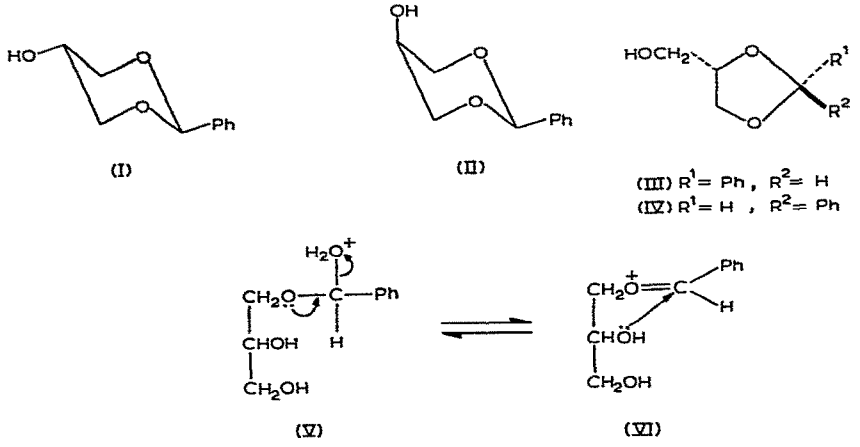
The observation[7] that each component of an *O*-benzylideneglycerol mixture may be identified by the chemical shift of the benzyl proton signal in the n.m.r. spectrum allows an easy and reasonably accurate analysis of an equilibrium mixture.

RESULTS AND DISCUSSION

The benzyl proton signals for *trans*- (I) and *cis*-5-hydroxy-2-phenyl-1,3-dioxan (II) and *cis*- (III) and *trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan (IV), present in an equilibrium mixture [*A*, acetal ratio (I)–(II)–(III)–(IV), *ca.* 1:1:3:2.7, see below], occurred at τ 4.84, 4.72, 4.41, and 4.25, respectively*, for a *ca.* 25% solution (total solute) in carbon tetrachloride. The signals were readily assigned since the acetals (I)[6], (II)[6], and (III)[8] have been isolated, and their structures determined.

*In dioxan (internal tetramethylsilane), the corresponding signals (τ 4.78, 4.66, 4.42, and 4.27) appeared at appreciably lower field than those previously recorded[7] relative to an external reference (6% tetramethylsilane in chloroform).

The differences in chemical shift for the isomer pairs (I)–(II) and (III)–(IV) have been rationalised[7,9]. The chemical shift for the benzyl proton of the *cis*-1,3-dioxan (II) was



markedly less dependent on concentration (Table I) than were the corresponding signals for the acetals (I), (III), and (IV). This may be due, in part at least, to intramolecular hydrogen-bonding which tends to diminish molecular association of the *cis*-1,3-acetal (II). Verkade and van Roon[10] have observed that, for solutions of the *cis*- (II) and *trans*-acetal (I) separately in benzene, molecular weight values (determined by cryoscopy) rise above 180 with increase in concentration above 0.1M, but the rate of increase was appreciably greater for the *trans*-isomer (I).

TABLE I

EFFECT OF CONCENTRATION ON THE CHEMICAL SHIFTS FOR THE BENZYL PROTONS OF THE *O*-BENZYLIDENEGLYCEROLS IN CARBON TETRACHLORIDE

Concentration[a] (%)	*Downfield shift (c.p.s.) relative to* CH_2Cl_2			
	cis-*1,3* (*II*)	trans-*1,3* (*I*)	cis-*1,2* (*III*)	trans-*1,2* (*IV*)
100[b]	16.5	15.25	37.5	50
85.7	16.5	14.25	37	49
75	16.75	13.5	36.75	48
60	16	11.75	35.5	46.5
50	15.25	10	34.5	45

[a]Refers to total solute; mixture *A* was used with a (I)–(II)–(III)–(IV) acetal ratio of *ca.* 1.0:1.0:3.0:2.7
[b]Neat liquid, containing *ca* 2% of dichloromethane.

The hydroxyl proton signals for *ca.* 20% solutions of the *cis*-1,3- (II) and *trans*-1,3-acetal (I) in dimethyl sulphoxide were doublets[11] at τ 5.13 (*J ca.* 5 c.p.s.) and 4.90 (*J ca.* 4 c.p.s.). The appearance of the signals for the axial hydroxyl group [*cis*-acetal (II)] at higher field accords with the observations of Casu *et al.*[12] for α- and β-D-glycopyranoses. In the latter series, the coupling constants were 4.0–4.5 and

6.5–7.0 c.p.s. for the axial (α) and equatorial (β) anomeric hydroxyl groups, respectively. The same trend, although not so marked, was shown by the 4-phenylcyclohexanols [*cis* (axial OH), 5.74 (*J ca.* 3 c.p.s); *trans* (equatorial OH), 5.53 (*J ca.* 4 c.p.s.)]. The reverse trend is observed for the 1,3-acetals, in that the coupling constant for the *cis*-isomer (II) is the larger, and this difference may be due to a hydroxyl-group orientation effect. Thus, for *cis*-4-phenylcyclohexanol, in the preferred chair conformation (equatorial phenyl group[13]), the hydrogen atom of the axial hydroxyl group is likely (*cf.* the results of Cole and Jefferies[14]) to be oriented away from the *syn*-axial[15] hydrogen atoms in the cyclohexane ring, so that the dihedral angle H-O-C-1-H is *ca.* 0°. On the other hand, if the axial hydroxyl group in the *cis*-5-hydroxy-2-phenyl-1,3-dioxan (II) is oriented to allow hydrogen bonding to the ring oxygen atoms[6], in addition to solvent molecules, the dihedral angle O-H–C-5-H is *ca* 180°.

In the literature, various methods are given for the condensation of benzaldehyde and glycerol. The compositions of the acetal mixtures obtained by repetition of three of these methods are given in Table II. Thus, the mixture (*A*) obtained[2a] by

TABLE II

COMPOSITION OF VARIOUS *O*-BENZYLIDENEGLYCEROL MIXTURES

Mixture	*Acetal*(%)				*1,3-Dioxolan-1,3-dioxan ratio*	
	cis-*1,3* (*II*)	trans-*1,3* (*I*)	cis-*1,2* (*III*)	trans-*1,2* (*IV*)	*This work*	*Hibbert et al.*[6]
A	13.1	13.0	38.7	35.2	*ca.* 2.8:1	*ca.* 7.5:1
B	20.5	14.1	34.9	30.5	*ca.* 1.9:1	—
C	36.6	22.8	22.0	18.6	*ca.* 0.68:1	5.5:1

A, Gerhardt method[16] (CO_2 sweep at *ca.* 150°); *B*, hydrogen chloride catalysis for 1 h at 100°; *C*, storage of mixture *B* (without neutralisation) for 12 h at room temperature.

sweeping out the water of condensation from a benzaldehyde–glycerol mixture at *ca.* 150° with carbon dioxide (Gerhardt method[16]), and that (*B*) obtained by hydrogen chloride-catalysed equilibration[2a] at 100°, contain preponderantly *cis,trans*-1,3-dioxolan derivatives (73.9 and 65.4%, respectively). When mixture *B* was subsequently stored[2a] (without neutralisation) at room temperature for 12 hours, a new mixture (*C*) was obtained in which *cis,trans*-1,3-dioxan derivatives are preponderant (59.4%). The effect of temperature is further illustrated by equilibration of an *O*-benzylideneglycerol mixture at *ca.* 180° in the presence of hydrogen chloride. The product (*E*) is mainly *cis,trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan containing <20% of the 1,3-acetals (I) and (II). The 1,3-dioxan–1,3-dioxolan ratios recorded by Hibbert *et al.*[2a] for various *O*-benzylideneglycerol mixtures are seriously in error (Table II). Presumably, all of the *cis*-1,3-acetal (II) was not isolated from a particular mixture, and the substantial proportion of the *trans*-1,3-acetal (I), now shown to be present, was included with the 1,3-dioxolan fraction. van Roon[17] observed a temperature

effect in the acid-catalysed ethylidenation of glycerol; above 0°, 1,3-dioxolan derivatives were preponderant and, at 179°, were the exclusive products.

By comparison with other benzaldehyde–diol reactions, the presence of a substantial proportion of 1,3-dioxolan derivatives in mixture *C* suggests the operation of an effect additional to that of temperature. Thus, in the competition reaction[2b] of equimolar proportions of ethane-1,2-diol and propane-1,3-diol with one molecular proportion of benzaldehyde, 2-phenyl-1,3-dioxan is the preponderant product. Also, benzylidenation of butane-1,2,4-triol[18] yields the 2,4-acetal as the major product, with only 5–10% of the 1,2-acetal. The benzaldehyde–glycerol reaction pattern may be rationalised by a consideration of intermolecular hydrogen-bonding. In the above, competition experiment and in the butane-1,2,4-triol reaction, intermolecular hydrogen-bonding would not be a significant factor since, after formation of 1,3-dioxan or 1,3-dioxolan derivatives, only primary hydroxyl groups remain unsubstituted. For the glycerol derivatives, however, the five- and six-membered acetals contain primary and secondary hydroxyl groups, respectively. It is possible that the stronger acidity and greater steric accessibility of the primary hydroxyl groups facilitate more effective, intermolecular hydrogen-bonding for *cis*- and *trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan, so that their thermodynamic stabilities, relative to those of the *cis*- (II) and *trans*-1,3-dioxan derivative (I), are increased. It follows that, on acid-catalysed equilibration of an *O*-benzylideneglycerol mixture in an inert solvent (*i.e.*, a solvent which is a weak proton-acceptor in hydrogen bonding), as concentration diminishes, intermolecular hydrogen-bonding will diminish, and intramolecular hydrogen-bonding will become dominant. Hence, as the dilution is increased, and if the latter effect is important, *cis*-5-hydroxy-2-phenyl-1,3-dioxan (II) should begin to preponderate at equilibrium since this is the only member of the acetal series (I)–(IV) in which complete, intramolecular hydrogen-bonding can occur[6a]. Preliminary results[6b], which suggested that such a trend was, indeed, operative, are now amply confirmed.

A series of equilibrations was effected in carbon tetrachloride, which was *ca.* 0.015N with respect to hydrogen chloride, at 3°, 37°, and 70°, with total solute concentrations in the range 0.001–5.0M. The equilibria were approached from two sides using *cis*-5-hydroxy-2-phenyl-1,3-dioxan (II) and the mixture *E* which contained mainly *cis,trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan. The proportions of the acetals (I)–(IV) in the neutralised, equilibrated mixtures were determined by integration of the benzyl proton signals in the n.m.r. spectra. The results are depicted in Fig. 1, which clearly shows certain trends. Thus, as the temperature of equilibration increases, and for a given molarity of total solute, the combined proportions of six-membered acetals decreases. For example, at 0.05M and at 3°, 37°, and 70°, the percentages of acetals were as follows: *cis*-1,3 (II), 57.5, 45.5, 32.5; *trans*-1,3 (I) 11, 10.5, 12; *cis*-1,2 (III), 18, 25, 30.5; *trans*-1,2 (IV), 13.5, 19, 25. Little has been reported[1,19] on the effect of temperature on the pattern of acid-catalysed acetalations of polyhydric alcohols other than glycerol, and this aspect of cyclic acetal chemistry merits further investigation.

Another trend, especially noticeable at 3° and 37°, is that, as the concentration of total solute diminishes, the proportion of the *cis*-1,3-acetal (II) increases at the expense of the *trans*-isomer (I), whereas the proportions of the *cis*- (III) and *trans*-1,2-acetals (IV) remain relatively constant. At 37°, for example, the dilution effect is

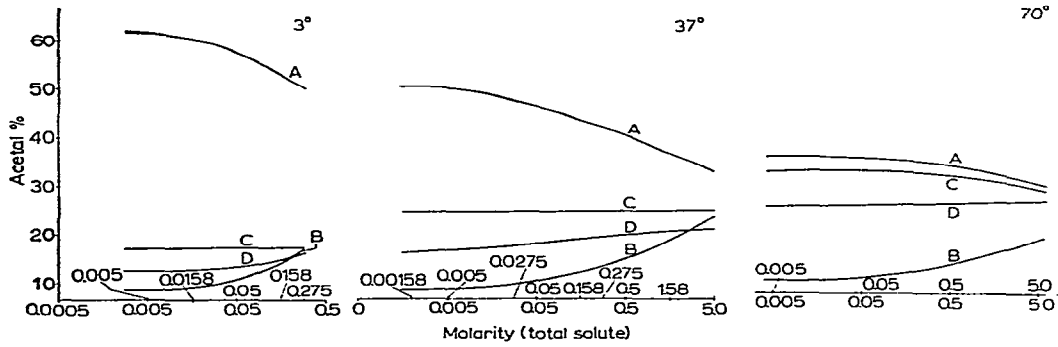


Fig. 1. Composition of equilibrium *O*-benzylideneglycerol mixtures with variation in temperature and molarity of total solute. Equilibrations were performed at the molarities recorded above the ordinate. *A*, *cis*-5-hydroxy-2-phenyl-1,3-dioxan; *B*, *trans*-isomer; *C*, *cis*-4-hydroxymethyl-2-phenyl-1,3-dioxolan; *D*, *trans*-isomer.

minimal at concentrations (<0.025M) at which it is well known[20] that, in carbon tetrachloride, intermolecular hydrogen-bonding is normally insignificant. Moreover, the proportions of acetals in the equilibrium mixture at this concentration parallel the extent of intramolecular hydrogen-bonding. Thus, for ≯0.005M solutions in carbon tetrachloride, the hydroxyl group in the *cis*-1,3-acetal is completely bonded[6], those in the *cis,trans*-1,2-acetals are extensively bonded[21], and that in the *trans*-1,3-acetal is mainly free[22]. Evaluation of the thermodynamic parameters relevant to the equilibria described above will be published elsewhere.

On benzylidenation of glycerol under homogeneous conditions in *N,N*-dimethylformamide (catalysis by toluene-*p*-sulphonic acid) at room temperature, the benzyl proton signals for the *cis*- (III, τ 4.55) and *trans*-1,2-acetal (IV, 4.36) appeared rapidly (<2 min); the former signal developed slightly faster. After 5 min, weak signals for the *cis*- (II, 4.77) and *trans*-1,3-acetal (I, 4.86) could be detected and, after 12 min, the ratio of acetals (I)–(II)–(III)–(IV) was *ca.* 1.1:1.0:6.2:4.0. After two days, equilibrium had apparently been reached, and the corresponding ratio was *ca.* 1.8:1.8:1.2:1.0, with the 1,3-acetals clearly being preponderant. During the reaction, a weak signal at τ 4.93, which developed and subsided, was probably due to a small concentration of hemi-acetal or acyclic acetal. The above results are consistent with protonated hemi-acetal formation (V) involving primary hydroxyl groups, followed by cyclisation of the subsequent oxonium ion (VI) to give the 1,2-acetals (III) and (IV). The initial, kinetic phase of the reaction is followed by slow equilibration to give an ultimate preponderance of the thermodynamically more-stable 1,3-acetals. This type of kinetic phase has not hitherto been observed in the benzylidenation of acyclic polyhydric alcohols (*cf.* the results obtained[23] on benzylidenation of cyclic vicinal

cis-diols), and other examples are being studied. Evidence and argument has been presented[8] which indicate that hemi-acetal formation preferentially involves the primary hydroxyl group of terminal vicinal diols, and further evidence was sought from the n.m.r. spectra of diols in dimethyl sulphoxide. In this solvent, the signals[11] for the primary (triplet, τ 5.60) and secondary hydroxyl groups (doublet, 5.64) of propane-1,2-diol were partially superimposed and gave an unsymmetrical triplet. Glycerol gave a more complex signal pattern [5.61 (2-proton triplet), 5.53 (1-proton doublet)]. It was hoped that addition of a suitable aldehyde to a solution of propane-1,2-diol in dimethyl sulphoxide would result in diminution in intensity of the primary hydroxyl signal as hemi-acetal formation proceeded.

However, trichloroacetaldehyde and pentafluorobenzaldehyde, which readily form hemi-acetals, could not be freed from traces of acid, and their separate addition caused coalescence of the hydroxyl proton signals. Coalescence occurred gradually when acetaldehyde was used, and was complete when equimolar amounts of the diol and aldehyde were present.

At equilibrium in *N,N*-dimethylformamide at room temperature, the *cis*- (II) and the *trans*-1,3-acetal (I) are present in comparable proportions, in contrast to the situation in carbon tetrachloride (*e.g.*, Fig. 2) where the *cis*-1,3-acetal was preponderant. When equilibrium was effected in sulpholane, using the *cis*-1,3-acetal (II) or the mixture *E* as starting material, the acetal ratio [(I)–(II)–(III)–(IV), *ca.* 1.4:1.7:1.2:1.0] was not significantly different from the ratio noted above for *N,N*-dimethylformamide, but, with dimethyl sulphoxide at room temperature, the ratio was *ca.* 2.4:1.3:1.2:1.0, showing a preponderance of the *trans*-1,3-acetal (I). The ratio was little different when the equilibration was effected at 0°. Hydrogen bonding effects may be responsible, at least in part, for these differences. In carbon tetrachloride, significant hydrogen-bonding can occur intramolecularly or between solute molecules, and the favoured component (*cis*-1,3-acetal) is that which can undergo the most extensive, intramolecular hydrogen-bonding, whereas, in dimethyl sulphoxide, strong hydrogen-bonding to the solvent occurs, and the preponderant isomer (*trans*-1,3-acetal) is that which would be expected on conformational grounds. The effect of solvent on the equilibration of other cyclic acetal systems is being investigated.

EXPERIMENTAL

Unless otherwise stated, n.m.r. spectra were obtained on *ca.* 20% solutions (internal tetramethylsilane) by using a Varian A60 spectrometer under normal working conditions.

Equilibration experiments

A series of separate solutions of *cis*-5-hydroxy-2-phenyl-1,3-dioxan[6] (0.45 g) and *cis,trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan (0.45 g, mixture *E*, see DISCUSSION) in carbon tetrachloride (volume determined by the desired molarity of solute), which was *ca.* 0.015N with respect to hydrogen chloride, was stored for suitable

periods at 3° (5 days), 37° (24 h), and 70° (5 h). The solutions were then neutralized with ammonia gas and, after storage at 37° for 30 min, filtered, concentrated (to *ca.* 0.5 ml) under diminished pressure, and analysed by n.m.r. spectroscopy. Control experiments showed that no change in the composition of an equilibrium mixture occurred after the ammonia treatment.

The benzyl proton signals for *cis*- and *trans*-5-hydroxy-2-phenyl-1,3-dioxan and *cis*- and *trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan occur at τ 4.72, 4.84, 4.41, and 4.25, respectively, in carbon tetrachloride. The relative proportions of each acetal were determined from the average of several integrations of the relevant peak areas, using a sweep width of 100 c.p.s. The results are shown in Fig. 1.

Preparation of equilibrium mixtures

(*a*) Water was removed during 1 h from a mixture of benzaldehyde (150 g) and glycerol (120 g) at 145–155° by a stream of carbon dioxide (Gerhardt method[17]) and then for a further 30 min at 165°. Distillation of the residue gave the acetal mixture[2a] *A* (173 g), b.p. 152–160°/*ca.* 12 mm.

(*b*) The procedure of Hibbert *et al.*[2a] was repeated as exactly as possible. Thus, *cis*-5-hydroxy-2-phenyl-1,3-dioxan was equilibrated by heating for 1 h at 100° in the presence of hydrogen chloride (mixture *B*, after neutralisation with conc. ammonia) and then stored at room temperature for 12 h (mixture *C*, after neutralisation). An ethereal solution of the product was neutralised with solid potassium carbonate and concentrated (mixture *D*). The procedure was repeated with *cis*,*trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan (mixture *E*) to give the corresponding products, *B'*, *C'*, and *D'*. Some of the results are recorded in Table II. The composition of the mixture pairs *B*,*B'*, *C*,*C'*, and *D*,*D'* were closely similar, as were mixtures *C* and *D*.

(*c*) The equilibrations in sulpholane and dimethyl sulphoxide involved, in each case, a mixture of the solvent (0.5 ml), *cis*-1,3-acetal (II, 0.45 g) or mixture *E* (0.45 g), and toluene-*p*-sulphonic acid (5 mg). The homogeneous benzylidenation involved *N*,*N*-dimethylformamide (2 ml), benzaldehyde (3 g), glycerol (2 g), and toluene-*p*-sulphonic acid (5 mg).

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SUMMARY

The proportions of *cis*- and *trans*-5-hydroxy-2-phenyl-1,3-dioxan and *cis*- and *trans*-4-hydroxymethyl-2-phenyl-1,3-dioxolan in acid-catalysed, equilibrated, *O*-benzylideneglycerol mixtures are critically dependent on solvent and temperature. In carbon tetrachloride, elevation of temperature increases the proportion of dioxolan

derivatives and, at a given temperature, diminishing concentration increases the proportion of the *cis*-1,3-acetal at the expense of the *trans*-isomer. At equilibrium in dimethyl sulphoxide, the *trans*-1,3-acetal is preponderant.

On acid-catalysed benzylidenation of glycerol under homogeneous conditions (*N*,*N*-dimethylformamide), the 1,2-acetals form rapidly in the initial, kinetic phase, and the 1,3-acetals are preponderant at equilibrium.

A rationalisation of some of these observations is presented.

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STRUCTURAL STUDIES ON THE CAPSULAR POLYSACCHARIDE OF *Pneumococcus* TYPE V

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INTRODUCTION

Pneumococcus Type V, first classified[1] in 1929, derives its immunological specificity, as do all *Pneumococci*, from its capsular polysaccharide, designated SV. The alkali-labile polysaccharide, which has been purified by precipitation with cetyltrimethylammonium bromide[2] and by chromatography on diethylaminoethyl-Sephadex[3], liberates D-glucose, D-glucuronic acid, and two amino sugars, identified[4,5] as 2-amino-2,6-dideoxy-L-galactose (L-fucosamine) and 2-amino-2,6-dideoxy-L-talose (L-pneumosamine), on hydrolysis with acid[2]. The intact polysaccharide contains *N*-acetyl, but no *O*-acetyl, groups. Barker *et al.*[2] tentatively identified a disaccharide and a trisaccharide, produced on partial hydrolysis of SV by acid, as *O*-(D-glucopyranosyluronic acid)-(1→3)-L-fucosamine and *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-[D-glucopyranosyl-(1→4)]-L-fucosamine, respectively.

Preliminary studies[2] showed that both SV and reduced SV are oxidised by periodate, with liberation of formic acid. In both cases, only L-fucosamine and a trace of D-glucose resisted oxidation. All of the L-pneumosamine residues were considered to be present as non-reducing end-groups, and the L-fucosamine residues to be 1,3-linked and/or involved in 1,3,4-branch points.

In this work, further details of the structure of the polysaccharide have been elucidated by methylation studies and by oxidation with periodate.

RESULTS AND DISCUSSION

Methylation. Polysaccharide SV was methylated, to constant methoxyl content, by the method of Srivastava *et al.*[6], which has been recommended[7] for the methylation of polysaccharides having a high hexosamine-content. Prior to methylation, the reducing end-group was converted into an alditol residue by reaction with sodium borohydride at a neutral pH. A similar technique has been employed[8] to reduce an alkali-labile, capsular polysaccharide of *Klebsiella pneumoniae*. Despite a careful control of pH, only 80% of the polysaccharide was non-dialysable after treatment with sodium borohydride, and this probably reflects the extreme lability of SV towards alkali. Heidelberger *et al.*[9] showed that the precipitating power of SV towards homologous rabbit-antiserum fell to 18 and 7% of the original value after treatment,

at room temperature, with 0.1N sodium hydroxide for 3 and 6 days, respectively. The products from methanolysis and hydrolysis of methylated SV (OMe, 18%; because of the extreme alkali-lability of SV, further methylation by more drastic methods was not attempted) were separated into neutral, acidic, and basic fractions on ion-exchange resins.

The neutral fraction was further separated into one major and two minor components by preparative paper chromatography. The major component was characterised as 2,3,6-tri-*O*-methyl-D-glucose, and the two minor components were tentatively identified as a di- and tetra-*O*-methyl-D-glucose, respectively, on the basis of their paper-chromatographic mobilities. Methylation studies thus show that the majority of the D-glucose residues in SV are 1,4-linked.

The acidic fraction from methylated and hydrolysed SV was esterified and reduced to give a trace amount of a component, tentatively identified as 2,3,4-tri-*O*-methyl-D-glucose, and a major component, characterised as 3,4-di-*O*-methyl-D-glucose. The identification of the latter component establishes that the majority of the D-glucuronic acid residues in SV are 1,2-linked, although a small proportion are probably present as non-reducing end-groups. Residues of 1,2-linked D-glucuronic acid have recently been found[10] in the minor polysaccharide component of *Khaya senegalensis* gum.

The basic fraction from the methylated SV was a complex mixture of at least eleven components which were separated by chromatography on Dowex-50 (H^+ form) and designated, *A*–*K*, in order of elution from the column. Chromatographic and electrophoretic analyses indicated that several of the components were inhomogeneous. This fact, together with the small amounts of material available, permitted tentative assignment of structure to only one of the components, *A*. This was considered to be a methylated tetrasaccharide of the type [(D-glucosyluronic acid)-L-fucosamine]$_2$, since hydrolysis by acid yielded a methylated disaccharide [(D-glucosyluronic acid)-L-fucosamine] and a methylated trisaccharide [(D-glucosyluronic acid)-L-fucosaminyl-D-glucuronic acid], together with L-fucosamine hydrochloride, 3,4-di-*O*-methyl-D-glucuronic acid, and a trace of 2,3,4-tri-*O*-methyl-D-glucuronic acid. Fraction *F* was unequivocally identified as L-fucosamine hydrochloride. The recovery of L-fucosamine from the methylated and hydrolysed polysaccharide supports the suggestion that L-fucosamine is present in SV as 1,3,4-branch points, as indicated earlier[2] by the isolation of the trisaccharide *O*-(β-D-glucopyranosyluronic acid)-(1→3)-*O*-[D-glucopyranosyl-(1→4)]-L-fucosamine from SV by mild hydrolysis with acid.

Oxidation with periodate. Previous workers[2] have shown that oxidation of SV is maximal with 0.04M sodium metaperiodate after 70.5 h at room temperature. Oxidised SV was reduced with sodium borohydride in neutral solution, since both SV and the aldehydic products of glycol cleavage are alkali-labile. However, the yield (50%) of non-dialysable polysaccharide, following oxidation and reduction, indicates that considerable degradation occurred during reduction, and the results

of such experiments must be interpreted with reservations. The products of hydrolysis of the reduced, oxidised SV were separated into neutral, basic, and acidic components by ion-exchange chromatography.

Paper chromatography and electrophoresis of the neutral fraction indicated the presence of erythritol, together with a small amount of glucose, estimated to be *ca.* 0.1% of the weight of the polysaccharide before oxidation. Erythritol can only arise from D-glucose residues in SV, and it is therefore concluded that these are either 1,4-linked, or constitute 1,4,6-branch points; however, the latter possibility was discounted by methylation studies. Glycerol was not detected in the neutral fraction, but the presence of glycolic aldehyde, which would be produced concomitantly with erythritol from 1,4- or 1,4,6-linked glucose residues, was indicated. Only D-glucuronic acid, estimated to be less than 0.1% of the weight of the polysaccharide before oxidation, was detected in the acid fraction. The destruction of D-glucuronic acid residues on oxidation with periodate was expected from the results of methylation studies which demonstrated that the majority of these residues are involved in 1,2-linkages. The absence of glyceric acid in the acid fraction may reflect over-oxidation of the 1,2-linked residues of D-glucuronic acid in SV.

The presence of small proportions of D-glucose and D-glucuronic acid in the hydrolysate of reduced, oxidised SV might reflect incomplete oxidation of the polysaccharide. However, Barker *et al.*[2] showed that oxidation of SV was complete under the conditions described, so that a small proportion of the D-glucose and D-glucuronic acid residues in SV may be either 1,3-linked, or, involved in branching.

Chromatography of the basic fraction on Dowex-50 (H^+ form) suggested that most of the *N*-acetyl-L-pneumosamine residues had been destroyed, whereas the *N*-acetyl-L-fucosamine residues resisted oxidation. These results are in agreement with earlier observations[2] that the former residues are present in SV as non-reducing end-groups, and the latter are either 1,3-linked, or constitute branch points.

A third basic component was eluted from the Dowex-50 just before L-fucosamine and the simultaneous disappearance of L-pneumosamine and appearance of the new component suggested that there might be a correlation between these components. The structure of the third component, which moved slightly faster than pneumosamine on paper chromatography and moved as a basic monosaccharide on paper electrophoresis, was not defined. Terminal, non-reducing residues of *N*-acetyl-L-pneumosamine would be degraded by sequential oxidation with periodate, reduction, and acid hydrolysis to give 1,2-dihydroxypropane and 2-amino-3-hydroxypropanal. The former component was not detected by gas–liquid chromatography, and the latter would be expected to have chromatographic and electrophoretic properties different from those of the unidentified basic component, which may therefore arise from condensation reactions of products from the hydrolysis of reduced, oxidised SV.

In summary, we conclude that polysaccharide SV contains multiple residues of *O*-(D-glucopyranosyluronic acid)-(1→3)-*N*-acetyl-L-fucosamine, to which are attached residues of 1,4-linked D-glucose and *N*-acetyl-L-pneumosamine. It is not possible to define the types of linkage for all of the aminosugar residues in SV since most of the

complex mixture of oligosaccharides from methylated SV were not characterised. However, the results confirm earlier suggestions[2] that most of the *N*-acetyl-L-pneumosamine residues are present as non-reducing end-groups and that those of *N*-acetyl-L-fucosamine constitute branch points.

The immunological specificity of Types II and V *Pneumococcus*, and the chemical basis of the long-known immunological relationship between the two types[11] have been extensively investigated by Heidelberger[12], who concluded that the II–V relationship is due to the multiple occurrence of similarly linked residues of D-glucuronic acid in the respective capsular polysaccharides, SII and SV. The cross-reaction of SII and gums of known constitution in antipneumococcal-V, horse and rabbit sera indicated that SV would be found to contain either fewer non-reducing end-groups of D-glucuronic acid than SII, or (and possibly also) 1,4- or 1,2-linked D-glucuronic acid. Reinforcement of the II–V relationship by similarly linked D-glucose residues was considered unlikely.

Methylation of SII indicated[13] that one third of the D-glucuronic acid is present as terminal non-reducing residues, and that the remaining residues are 1,4-linked. The present work shows that in SV, D-glucuronic acid residues are mainly 1,2-linked, with a few present as non-reducing end-groups. It is unlikely that the latter residues are solely responsible for the II–V cross-reaction, and the discovery of 1,2-linked D-glucuronic acid residues endorses Heidelberger's original suggestion[12] that such residues acquire, in part, the antigenic properties usually associated with end-groups. The cross-reaction of certain polysaccharides in anti-pneumococcal Type VI serum has been attributed[14] to the presence, in those polysaccharides, of multiple, non-reducing end-groups of D-galactose. Polysaccharide SVI contains multiple residues of D-galactose, solely 1,2-linked[15], and Heidelberger and Rebers[14] consider that these residues function like an end-group in the animal stimulated to produce anti-SVI serum.

EXPERIMENTAL

Polysaccharide SV was kindly supplied by Dr. Michael Heidelberger, and was purified by precipitation with alcohol and with cetyltrimethylammonium bromide, essentially as described by Barker *et al.*[2].

General methods

Paper chromatographic analyses were carried out on Whatman No. 1 and 3MM papers by using the following solvent systems (v/v): (*a*) butan-1-ol–ethanol–water (4:1:5); (*b*) butan-1-ol–acetic acid–water (4:1:5). Electrophoretic analyses were performed on Whatman 3MM paper in one of the following electrolytes: 0.2M acetate, pH 5; 0.75M formate, pH 2; 0.2M borate, pH 10. Chromatograms and electrophoretograms were developed with alkaline silver nitrate, aniline hydrogen phthalate, ninhydrin, or the Elson–Morgan reagents. The elution volumes of basic components from Dowex-50, relative to that of glucosamine, are expressed as E_{GN} values.

Methoxyl content was determined by the method of Belcher and Godbert[16]. Amino sugars were determined by means of the Elson–Morgan reaction[17], as modified by Rondle and Morgan[18], and hexuronic acids by a modification[19] of the Dische[20] carbazole reaction. *N*-Methyl sugars were determined by the method of Scudi *et al*[21].

Methylation of SV

Polysaccharide SV (1.17 g) in water (300 ml) was reduced with sodium borohydride (1.17 g), added during 8.5 h. Carbon dioxide was bubbled continuously through the solution, during the addition of borohydride, in order to maintain neutral pH. The solution was adjusted to pH 4, by addition of 50% acetic acid, dialysed against several changes of distilled water for 2 days at 0–5°, and freeze-dried to yield reduced SV (939.4 mg) which was dried to constant weight *in vacuo* over P_2O_5. Dry barium oxide (5 g) and dry ($CaCl_2$), redistilled methyl iodide (20 ml) were added to a solution of reduced SV (939 mg) in dimethyl sulphoxide (20 ml), which had been dried and purified by azeotropic distillation with benzene. The mixture was stirred at room temperature under anhydrous conditions for 24 h, water was added, and the resulting turbid solution was thoroughly dialysed against distilled water for 3.5 days at 0–5°. The dialysed solution was centrifuged, and the supernatant liquor was freeze-dried to give a product (941 mg) which was methylated to constant methoxyl content by two further treatments as described above. The product (930 mg) had OMe, 17.9%.

Methanolysis and hydrolysis of methylated SV

A solution of dry, methylated SV (913 mg) in 3% methanolic hydrogen chloride (119 ml) was heated under reflux, under anhydrous conditions, for 6 h, during which time $[\alpha]_D$ changed from −89.7 to −21.6°. The solution was concentrated to dryness under diminished pressure, with intermittent addition of methanol, and the syrupy residue was hydrolysed with 2N hydrochloric acid (115 ml) for 4 h at 100°. The hydrolysate had $[\alpha]_D$ −8.6° (calculated on the weight of methylated polysaccharide before methanolysis). Hydrochloric acid was removed from the hydrolysate by repeated co-distillation with water, and, finally, by neutralisation with silver carbonate. The resulting mixture was centrifuged, the supernatant solution was concentrated to dryness, and the residue was dissolved in water (25 ml). The components of the hydrolysate were separated on ion-exchange resins.

Basic fraction. The hydrolysate of methylated SV was applied to a column (3.9 × 45 cm) of Dowex-50 (H^+ form), previously calibrated with D-glucosamine hydrochloride, and the resin was washed with water (1.5 l). The aqueous eluate (neutral and acidic fraction) was concentrated to 25 ml. The resin was eluted with 0.3N hydrochloric acid, and fractions (25 ml) were collected automatically and analysed for amino sugars. The component present (*A*–*K*) had E_{GN} values of 0.89, 1.02, 1.23, 1.50, 1.66, 1.86, 2.18, 2.35, 2.52, 2.83, and 3.15, respectively. Fractions containing each component were combined, and hydrochloric acid was removed by repeated

co-distillation with methanol under diminished pressure. The syrupy products were dried *in vacuo* over phosphoric oxide and analysed (with the exceptions of *B* and *C*) for amino sugar and *N*-methylamino sugar. Each component analysed gave a negligible colour in the latter assay. Component *C* was present in negligible amount and was not further investigated. The yields of other components, together with the optical rotation, methoxyl content (of some), and reaction in the Elson–Morgan assay are recorded in Table I. Several components were shown to be inhomogeneous on analysis by paper chromatography in solvent (*a*), and by paper electrophoresis, and only two components (*A* and *F*) were investigated further. Component *A* (R_{GN} 1.66) was immobile on electrophoresis in acetate buffer, and component *F* (R_{GN} 1.16; M_{GN} 1.05 in acetate and formate buffers) was chromatographically and electrophoretically identical with authentic L-fucosamine hydrochloride.

TABLE I

BASIC COMPONENTS FROM THE HYDROLYSATE OF METHYLATED SV

Component	*Weight, mg*	$[\alpha]_D$ *in water,°*	OMe, %	*Colour intensity*[a] *in Elson-Morgan assay,%*	λ_{max} *of chromogen, mμ*
A	81.4	−81.3		20.4	512
B	9.1	−64.8			
D	49.3	+16.6	4.53	43.3	530
E	16.7	−13.8		16.0	530
F	64.0	−61.1		66.7	530
G	38.5	−30.9		15.9	510
H	31.5	+ 7.9		19.1	530
I	33.3	+18.9	8.16	23.3	530
J	38.4	+22.4	7.49	13.1	530
K	68.5	+18.1	7.04	10.5	520

[a]As compared with that given by an equal weight of D-glucosamine hydrochloride (λ_{max} 530 mμ).

(*a*) *Hydrolysis of component* A. Component *A* (73.1 mg) was hydrolysed with 2N hydrochloric acid (3 ml) for 4 h at 100°, and the cooled solution was neutralized with silver carbonate. The neutralised hydrolysate was concentrated to dryness under diminished pressure, and a solution of the residue in 0.3N hydrochloric acid (5 ml) was applied to a column of Dowex-50 (H^+ form), previously calibrated with D-glucosamine hydrochloride. Fractions were eluted with 0.3N hydrochloric acid and analysed for content of amino sugars. Four components, designated 1–4, respectively, were obtained and had E_{GN} values of 0.60, 0.85, 1.26, and 1.75. Components 1–3 contained uronic acid, and the ratio of uronic acid to hexosamine was highest for component 1. The position of elution of free acidic material (component 5) from the column was also indicated. The colours given by components 1–3 and 5 in the carbazole assay had the same λ_{max} (520mμ) as that given by D-glucuronic acid. In the Elson–Morgan reaction, component 4 gave a chromogen having the same λ_{max}

(530mμ) as that given by D-glucosamine hydrochloride, whereas components 1 and 3 had λ_{max} 525mμ, and component 2 had λ_{max} 518mμ.

Components 1–5 were analysed by paper chromatography and paper electrophoresis. The major component of 1 moved towards the anode (M_{GA} 0.46) in acetate buffer and towards the cathode (M_{GN} 0.29) in formate buffer. Components 2 and 3 were immobile in acetate buffer, but component 3 moved towards the cathode (M_{GN} 0.48) in formate buffer. The paper-chromatographic and -electrophoretic mobilities of component 4 and its E_{GN} value on Dowex-50 (H^+ form) were identical with those of authentic L-fucosamine hydrochloride. Component 5 moved as an acid (M_{GA} 1.01) on electrophoresis in acetate buffer, and paper-chromatographic analysis showed that it contained no 2,3,6-tri-*O*-methyl-D-glucose. It showed two components having mobilities similar to those of 3,4-di-*O*-methyl-D-glucuronic acid (major) and 2,3,4-tri-*O*-methyl-D-glucuronic acid (minor).

(*b*) *Identification of component* F. Component *F* was crystallized from methanol–ether to give a product, $[\alpha]_D^{22}$ −98.7 (5 min)→−80.4° (equilibrium; *c* 0.41, water), which decomposed at 185°. Similar behaviour has been reported for L-fucosamine hydrochloride[22] {$[\alpha]_D^{24}$ −93.4 ± 2° (*c* 1.29, water)} isolated from a bacterial source. The infrared spectrum (KBr disc) of the crystalline product was identical with that of authentic L-fucosamine hydrochloride.

Separation of neutral and acidic components from hydrolysate of methylated SV

The concentrated solution containing neutral and acidic components was applied to a column of De-acidite FF (CO_3^{2-} form) which was washed with water to elute the neutral components. The solution was concentrated to dryness under diminished pressure.

Acidic components were eluted from the column with N ammonium carbonate solution, and the eluate was stirred with Amberlite IR 120 (H^+ form) and left to stand overnight. The solution was filtered and concentrated to dryness under diminished pressure.

Neutral fraction. The dry syrup {167 mg, $[\alpha]_D$ + 59.1° (*c* 3.34, water)} showed three components on paper chromatography. The major component (R_G 4.75) corresponded to 2,3,6-tri-*O*-methyl-D-glucose (R_G 4.79). The other two components (R_G 3.43 and 5.47, respectively) were present in small proportions only. No component of the neutral fraction formed a complex when examined by paper electrophoresis in borate buffer.

The three components were separated and isolated by preparative, paper chromatography of an aliquot of the neutral fraction. The major component was crystallised from dry ether to give a product, m.p. 110–115° (undepressed on admixture with authentic 2,3,6-tri-*O*-methyl-D-glucose), $[\alpha]_{5461}^{25}$ + 63.4 (2 min)→ + 57.7 (10 min)→−37.4° (18 h, equilibrium; *c* 0.44, 2% methanolic hydrogen chloride). An authentic sample of 2,3,6-tri-*O*-methyl-D-glucose had $[\alpha]_{5461}^{25}$ + 65.7 (2 min)

→+ 56.6 (10 min)→—38.5° (18 h, equilibrium; *c* 0.44, 2% methanolic hydrogen chloride). The infrared spectrum (KBr disc) and X-ray powder photograph were identical with those of authentic 2,3,6-tri-*O*-methyl-D-glucose.

Acidic fraction. The dry syrup (75.5 mg) {$[\alpha]_D$ + 29.9° (*c* 1.51, water)} showed only one component (M_{GA} 0.98) on paper-electrophoretic analysis in acetate buffer. Paper-chromatographic analysis indicated the presence of one major and two minor components (R_G 7.37, 2.56, and 15.0, respectively).

The dry, acidic fraction (75.4 mg) was heated under reflux with a solution of 3% methanolic hydrogen chloride for 6 h. Hydrochloric acid was removed by repeated co-distillation with methanol under diminished pressure, and the residue (79 mg) was dried *in vacuo* over phosphoric oxide and pellets of potassium hydroxide. A solution of the esterified fraction in ether was treated with lithium aluminium hydride for 90 min at room temperature, and the mixture was stirred for a further 1 h. The excess of lithium aluminium hydride was decomposed by cautious addition of water, the resulting suspension was stirred and filtered, and the filtrate was concentrated, under diminished pressure, to 10 ml. The solution was deionised by successive treatments with Amberlite IR 120 (H^+ form) and De-acidite FF (CO_3^{2-} form), and the solution was concentrated to dryness under diminished pressure. The product was hydrolysed with 2N sulphuric acid for 3 h at 100°, and the acid was neutralised with barium carbonate. Paper-chromatographic analysis of the hydrolysate of the reduced fraction showed the presence of a small proportion of a tri-*O*-methyl-D-glucose (R_G 4.49), which differed from authentic 2,3,6-tri-*O*-methyl-D-glucose (R_G 4.36), and a major component (R_G 3.22), which gave a pink colour with aniline hydrogen phthalate. The major component had a mobility (M_G 0.11) similar to that (M_G 0.12) of authentic 3,4-di-*O*-methyl-D-glucose on electrophoresis in borate buffer, pH 10. The minor component did not form a complex with borate ion.

The major component was isolated by preparative, paper chromatography and crystallised from dry ethyl acetate to give a product, m.p. 119–121°, $[\alpha]_D^{25}$ + 80.8 (2 min)→ + 76.8° (20 h, equilibrium; *c* 0.25, water), identical with authentic 3,4-di-*O*-methyl-α-D-glucopyranose {m.p. 114–118.5°; $[\alpha]_D$ + 80.0 (initial) → + 76.0° (final, *c* 5.0, water)[23]}.

Oxidation of SV with periodate, reduction, and hydrolysis with acid

An aqueous solution of SV (392 mg) was oxidised at room temperature with 0.04M sodium metaperiodate for 70.5 h; ethylene glycol was added to destroy excess of periodate, and the solution was dialysed and freeze-dried. The oxidised polysaccharide (347 mg) was treated with sodium borohydride (1 g), during which time (7 h) the solution was maintained at neutral pH by passage of carbon dioxide. The excess of borohydride was decomposed with dilute acetic acid, and the solution was dialysed and freeze-dried to give a product (184 mg) which was hydrolysed with 2N sulphuric acid for 4 h at 100°. The products of acid hydrolysis were separated on ion-exchange resins.

Acidic fraction. Acidic components in the mixture were separated by absorption on De-acidite FF (CO_3^{2-} form), which was then washed with water, and the aqueous eluate (neutral and basic fraction) was concentrated. The resin was eluted with N ammonium carbonate solution, and the eluate was treated overnight with Amberlite IR 120 (H^+ form) to remove cations. After filtration, the solution was freeze-dried to give a small amount of a hygroscopic solid which showed the presence of glucuronic acid on paper-electrophoretic analysis in acetate buffer. The amount of glucuronic acid present was estimated, by a modification[19] of the Dische[20] carbazole reaction, to be about 0.06% of the original weight of the polysaccharide before oxidation.

Basic fraction. The concentrated solution containing neutral and basic components of the hydrolysate was applied to a calibrated column of Dowex-50 (H^+ form), which was then eluted with water to give the neutral fraction.

The column was eluted with 0.3N hydrochloric acid, and fractions were analysed for aminosugar content. Three components, having E_{GN} values of 1.37, 1.65, and 2.15, respectively, were obtained. The first component, which was absent from the hydrolysate of intact SV, had R_{GN} 2.03 on paper chromatography, and its mobility (M_{GN} 1.04) on paper electrophoresis in acetate buffer was typical of a basic monosaccharide. It was not further characterised.

The paper-chromatographic and -electrophoretic mobilities of the second component, and its E_{GN} value (1.65) on Dowex-50 (H^+ form), were identical with those of authentic L-fucosamine hydrochloride. Similar analytical methods indicated the third component, which was present in trace amount, to be L-pneumosamine.

Neutral fraction. Paper chromatography of the neutral components indicated the presence of glucose, erythritol, and glycolic aldehyde. The presence of erythritol was confirmed by paper electrophoresis in molybdate buffer (pH 5)[24], in which it was clearly distinguished from authentic D-threitol. The amount of residual D-glucose in the neutral fraction was estimated, by reaction with glucose oxidase[25], to be about 0.1% of the weight of the polysaccharide before oxidation. 1,2-Dihydroxypropane was not detected in a portion of the neutral fraction analysed by gas–liquid chromatography on a column of polyethyleneglycol adipate.

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SUMMARY

Further details of the structure of the capsular polysaccharide (SV) of *Pneumococcus* Type V have been elucidated by methylation studies and by oxidation with periodate. It is proposed that the polysaccharide contains multiple residues of 2-

acetamido-2,6-dideoxy-3-*O*-(D-glucopyranosyluronic acid)-L-galactose, to which are attached residues of D-glucose and 2-acetamido-2,6-dideoxy-L-talose (*N*-acetyl-L-pneumosamine). Residues of 2-acetamido-2,6-dideoxy-L-galactose (*N*-acetyl-L-fucosamine) constitute branch points, and pneumosamine residues are present mainly as non-reducing end-groups. The majority of the D-glucose and D-glucuronic acid residues are 1,4- and 1,2-linked, respectively. The immunochemistry of SV is reviewed in the light of these findings.

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THE DIMERIC FORMS OF SOME α-HYDROXYCARBONYL COMPOUNDS

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INTRODUCTION

It has long been known that the crystalline forms of glycolaldehyde[1,2], DL-glyceraldehyde[3], and 1,3-dihydroxyacetone[4,5] are dimeric. Wohl and Neuberg[6] suggested formulae based on 1,4-dioxan for these dimers. It is also known[1–6] that the dimers dissociate into the monomeric forms in aqueous solution, but the free monomer has only been isolated for 1,3-dihydroxyacetone[7,8]. Formation of isomers of the crystalline dimers is also possible, but has received little attention. The existence of an alternative dimer of glycolaldehyde, derived from 1,3-dioxolan[9], and the occurrence of *cis*- and *trans*-forms of 2,5-diethoxy-1,4-dioxan[10], have been reported. The formation of 2,3-dihydroxy-1,4-dioxan[11], by an addition reaction between glyoxal and ethylene glycol, is also of interest.

RESULTS AND DISCUSSION

The structures of crystalline samples of dimeric glycolaldehyde, DL-glyceraldehyde, and 1,3-dihydroxyacetone have now been established by p.m.r. spectroscopy of their per-*O*-trimethylsilyl (per-*O*-trisyl) ethers. The results are summarised in Table I, which includes, for comparison, results for the anomeric protons of penta-*O*-trisyl-α- and β-D-glucopyranoses. The behaviour of the anomeric protons in these D-glucose derivatives was similar to that reported for the corresponding acetates by Lemieux *et al.*[12]. Thus, the anomeric proton in the β-D-glucopyranose derivative showed a greater, vicinal coupling constant ($J_{1,2}$) than that in the α-D-glucopyranose derivative, the relevant protons being *trans*-diaxial rather than *gauche*. The same effect occurs in 1,4-dioxan for which Cohen *et al.*[13] found J_{trans} 9.4 c.p.s. and J_{gauche} 2.7 c.p.s. The signals for the anomeric protons in the spectra of glycolaldehyde and DL-glyceraldehyde dimers were identified by their low chemical shifts. DL-Glyceraldehyde, like β-D-glucopyranose, showed a value of $J_{1,2}$ corresponding to a *trans*-diaxial conformation, while glycolaldehyde showed both *trans*-diaxial and *gauche* couplings and resembled the β-anomer of 2-deoxy-D-*arabino*-hexopyranose reported by Lenz and Heeschen[14]. Thus, both these dimers are derivatives of 1,4-dioxan having the substituents disposed symmetrically and in equatorial conformation (I and II).

For glycolaldehyde dimer (I), the structural assignment is supported by the behaviour of the methylenic protons, which showed a relative chemical shift of 0.52 p.p.m. The axially disposed proton, *i.e.*, that showing the higher vicinal coupling,

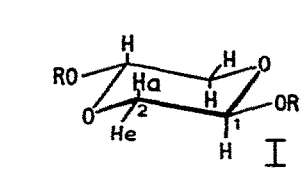
TABLE I

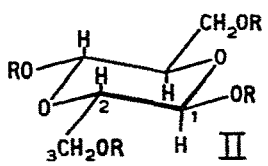
PROTON CHEMICAL SHIFTS AND COUPLING CONSTANTS

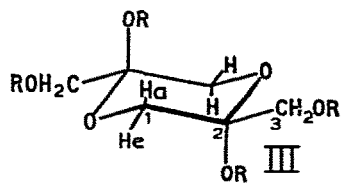
Crystalline dimer of	*Proton*[c]	*Appearance*	*τ value*	*J(c.p.s.)*
Glycolaldehyde (I)[a]	H-1	quartet	5.23	$J_{1,2a}$ 6.0
	H-2_a	quartet	6.76	$J_{1,2e}$ 2.4
	H-2_e	quartet	6.24	$J_{2a,2e}$ 11.2
DL-Glyceraldehyde (II)[a]	H-1	doublet	5.32	$J_{1,2}$ 7.4
	H-2	sextet	6.80	$J_{2,3}$ 3.0
	H-3	doublet[d]	6.35	
1,3-Dihydroxyacetone (III)[a]	H-1_a	doublet	6.56	$J_{1a,1e}$ 11.2
	H-1_e	doublet	6.09	
	H-3	singlet[d]	6.50	
1,3-Dihydroxyacetone (IV)[b]	H-1_a	doublet	6.51	$J_{1a,1e}$ 11.0
	H-1_e	doublet	5.99	
	H-3_x	doublet	6.38	$J_{3x,3y}$ 8.7
	H-3_y	doublet	6.12	
α-D-Glucopyranose[a]	H-1	doublet	5.05	$J_{1,2}$ 3.0
β-D-Glucopyranose[a]	H-1	doublet	5.55	$J_{1,2}$ 6.5

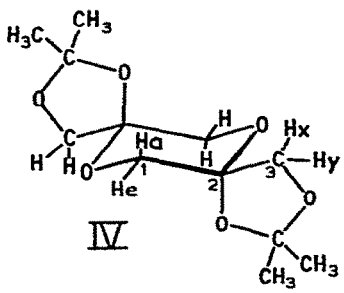
[a]Measured as per-*O*-trisyl ether in CCl_4 solution.
[b]Measured as di-*O*-isopropylidene derivative in CCl_4 solution.
[c]For numbering of protons see the formulae.
[d]Signal integrates for two protons.

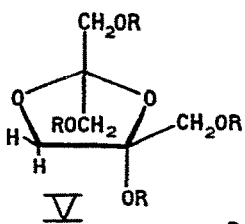
was the more shielded. This effect, an account of which is given by Jackman[15], is likewise seen in the relative chemical shift of the anomeric protons of the penta-*O*-trisyl ethers of α- and β-D-glucopyranose (0.50 p.p.m.).

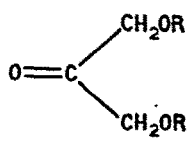












R = Si $(CH_3)_3$

The spectra of the tetra-*O*-trisyl and di-*O*-isopropylidene derivatives of the 1,3-dihydroxyacetone dimer were appropriate for symmetrically substituted derivatives of 2,5-dihydroxy-2,5-di(hydroxymethyl)-1,4-dioxan (III and IV, respectively). Both compounds showed pairs of doublets, having relative chemical shifts of 0.47 and

0.52 p.p.m., respectively, *cf.* glycolaldehyde dimer (I). The exocyclic methylenic protons of dimer (III) were equivalent, whereas the isopropylidene derivative (IV) showed a second pair of doublets attributed to the methylenic protons of the 1,3-dioxolan rings. The relative chemical shift of this pair of protons (0.26 p.p.m.) is not necessarily characteristic of a five-membered ring, but the rather low geminal coupling resembles that found for the H-6 protons in certain 3,6-anhydro-D-glucose derivatives[16]. For the structurally related "di-D-fructose anhydride I", Lemieux and Nagarajan[17] argued that the ketal oxygens are probably axially disposed about the dioxan ring; the formulae show the corresponding conformations for compounds (III) and (IV).

The *O*-trisyl derivatives (I), (II), and (III) were analysed by g.l.c. (*cf.* Sweeley *et al.*[18]); hydrocarbon (column *A*) and polyester (columns *B* and *C*) stationary phases were used. The retention volumes observed on columns *A* and *B* are summarised in Table II. Fresh, crystalline samples of glycolaldehyde, DL-glyceraldehyde, and 1,3-dihydroxyacetone each showed one major component on g.l.c., when trisylated under

TABLE II

SPECIFIC RETENTION VOLUMES (V_g^0) OF *O*-TRISYL ETHERS

Parent Compound	*Column* A	*Column* B
Glycolaldehyde dimer (I)[a]	1110	724
DL-Glyceraldehyde dimer (II)[a]	17800	6530
1,3-Dihydroxyacetone monomer (VI)	727	676
1,3-Dihydroxyacetone dimer (V)	14450	6060
1,3-Dihydroxyacetone dimer (IV)[a]	19300	7540
Glycolaldehyde/1,3-dihydroxyacetone[b]		2010
Glycolaldehyde/DL-glyceraldehyde[b]		3160
α-D-Glucopyranose	42000	11500
β-D-Glucopyranose	94000	26300

[a]Crystalline, dimeric form.
[b]Mixed, dimeric form?

carefully controlled conditions. However, complex chromatograms were often obtained, showing the presence of dimers other than the crystalline forms. This behaviour is illustrated in Fig. 1 for DL-glyceraldehyde that had been subjected to (a) partial and (b) complete mutarotation, and for D-glyceraldehyde (c). Comparison of these chromatograms, obtained on column *A*, with those for the same samples on column *B* indicated the presence of at least eight dimeric forms. Chromatograms obtained from freshly distilled DL-glyceraldehyde were similar to those from the completely mutarotated sample. There was no evidence for the presence of monomeric glyceraldehyde in any of these products, but its presence in aqueous solutions or in the vapour phase is not precluded.

By contrast, trisylated, mutarotated samples of 1,3-dihydroxyacetone clearly

showed the presence of the monomeric form as a peak of low retention volume in the chromatograms, and by the presence of carbonyl absorption in the i.r. spectra. Vacuum distillation of the crystalline material gave almost pure monomer, confirming the finding of Fischer and Mildbrand[7].

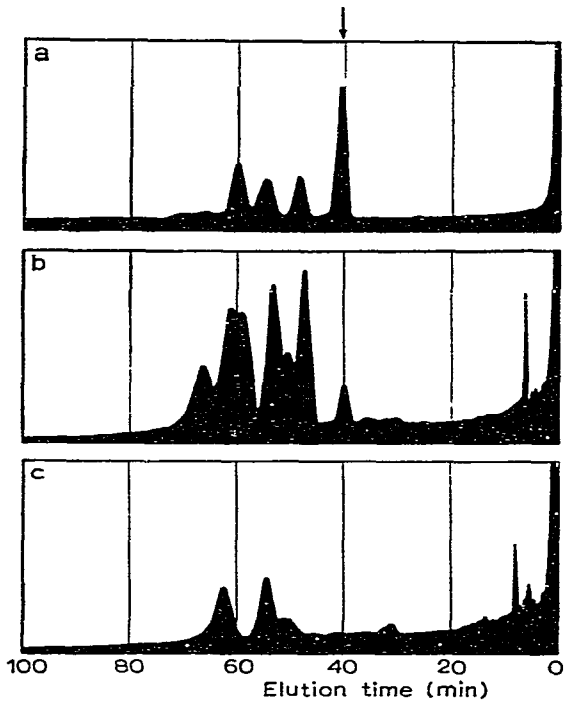


Fig. 1. G.l.c. (column *A*, 125°) of the trisylated products from (a) partially mutarotated DL-glyceraldehyde, (b) completely mutarotated DL-glyceraldehyde, and (c) D-glyceraldehyde. The arrow shows the peak characteristic of crystalline DL-glyceraldehyde dimer.

Chromatograms on columns *A* and *B* of 1,3-dihydroxyacetone, which had been subjected to partial mutarotation and then trisylated, showed a third peak, often as the major component, attributable to a second dimeric form. One such product had the following approximate composition (by g.l.c. on column *B*): monomer, 25%; crystalline dimer, 15%; second dimer, 60%. In addition, g.l.c. on column *C* at 175° showed the presence, in small proportions, of at least sixteen components of higher retention volume, which may have been trisyl derivatives of oligomers or condensation polymers such as those reported by Levene and Walti[19]. The main feature of the p.m.r. spectrum of this mixture was a group of three singlets (τ 6.32, 6.37, and 6.44), of equal intensities, close to the singlet arising from the exocyclic methylene groups of dimer (III). In addition, two doublets (τ 6.01, 6.24. J 8.0 c.p.s.), similar to those observed for the methylenic protons of the 1,3-dioxolan rings of compound (IV), were present. It was tentatively concluded that the principal component of this mixture was a dimer of 1,3-dihydroxyacetone possessing a structure (V) related to 1,3-dioxolan. The signal from the methylenic protons of the monomer (VI) was observed as a sharply resolved singlet (τ 5.70).

Equimolar mixtures of glycolaldehyde and DL-glyceraldehyde, and of glycol-

aldehyde and 1,3-dihydroxyacetone, prepared under conditions of partial mutarotation, were trisylated and examined by g.l.c. on column *B*. Extremely complex chromatograms were obtained. In addition to peaks characteristic of the individual components, new major components were observed, whose retention volumes (Table II) suggested that they were mixed dimers. The mixture of glycolaldehyde and DL-glyceraldehyde also showed peaks attributable to traces of aldopentoses.

EXPERIMENTAL

The glycolaldehyde, DL-glyceraldehyde, D-glyceraldehyde, and 1,3-dihydroxyacetone were fresh, commercial samples. The di-*O*-isopropylidene derivative of dihydroxyacetone dimer was prepared by the method of Fischer and Taube[8], using anhydrous copper sulphate. G.l.c. on column *B* showed two components (85% and 15% by peak area); the structure of the major component (IV) only was established.

'*Partial mutarotation*' implies that which occurred when a freshly prepared aqueous solution of a hydroxycarbonyl compound (1% w/v) was concentrated to dryness under diminished pressure at 40°. '*Complete mutarotation*' implies that which occurred when an aqueous solution of a hydroxycarbonyl compound (1% w/v) was shaken for 24 h at 20° with Amberlite IR-120 resin (H^+ form), filtered, and concentrated to dryness as above.

Trisylation. This was performed in the following way, which minimised mutarotation during reaction. To the hydroxycarbonyl compound (0.05–0.5 g) was added sufficient, dry pyridine to dissolve it without heating (*cf.* ref. 18). When dissolution was complete, chlorotrimethylsilane (>50% excess) was added. After 30 sec, light petroleum (b.p. 100–120°; twice the volume of pyridine used) was added. The *O*-trisyl ethers were isolated at once by concentration of the reaction mixture to dryness, and extraction of the residue with light petroleum (b.p. 60–80°). P.m.r. spectra of the *O*-trisyl or *O*-isopropylidene derivatives were measured for carbon tetrachloride solution, with tetramethylsilane as an internal standard, at room temperature with a Varian A-60 spectrometer. Chemical shifts are expressed on the τ-scale and represent the centres of gravity of the appropriate signals. Coupling constants were obtained by direct measurement of the spectra.

G.l.c. Analyses of the trisyl derivatives, suitably diluted with chloroform, were carried out on a modified Pye Argon Chromatograph. The columns were Pyrex tubes of 4-mm internal diameter and 122-cm length. Columns *A* and *B* were packed with pretreated glass microbeads (105–125 μ diameter) and maintained at 125°. Column *C* was packed with Kieselguhr (M. and B. 'Embacel', acid-washed, 150–250 μ diameter) and maintained at 175°. Column *A* contained hexatriacontane, 0.20% by weight, (0.0441 g). Column *B* contained poly(decane-1,10-diol succinate), 0.21% by weight, (0.0496 g). Column *C* contained poly(ethyleneglycol succinate), 10.7% by weight, (0.326 g). Specific retention volumes were corrected for column dead-volume and calculated at column temperature.

ACKNOWLEDGEMENTS

The author thanks Mr. B.F. Sagar, B.Sc., for g.l.c. measurements, and Dr. G.R. Bedford of Imperial Chemical Industries Ltd., Pharmaceuticals Division, for the p.m.r. spectra. This research was conducted under a project authorised by U.S. Public Law No. 480, 85th Congress, and administered by the U.S. Department of Agriculture.

SUMMARY

The crystalline, dimeric forms of glycolaldehyde, DL-glyceraldehyde, and 1,3-dihydroxyacetone, converted into their pertrimethylsilyl ethers and then analysed by g.l.c., showed one major component in each case. P.m.r. spectroscopy of their trimethylsilyl ethers showed these crystalline dimers to be symmetrically substituted derivatives of 1,4-dioxan. The same conclusion held for the major isopropylidene derivative obtained from the condensation of 1,3-dihydroxyacetone with acetone. Samples of glyceraldehyde and 1,3-dihydroxyacetone, partially or completely equilibrated in aqueous solution, recovered by evaporation, trimethylsilylated, and analysed by g.l.c., gave complex chromatograms. At least eight dimeric forms of glyceraldehyde were detectable. For 1,3-dihydroxyacetone, the free monomer and a second dimer were observed. This second dimer is thought, from p.m.r. evidence, to be a derivative of 1,3-dioxolan. Both glyceraldehyde and 1,3-dihydroxyacetone formed mixed dimers with glycolaldehyde.

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ACETYLENIC SUGAR DERIVATIVES FROM GLYCALS

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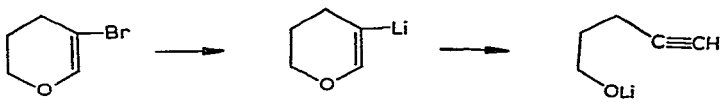
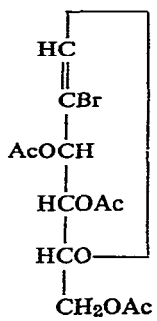
(Received December 29th, 1965; revised version, March 2nd, 1966)

INTRODUCTION

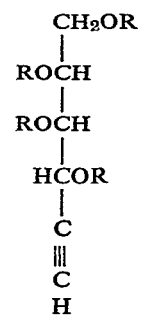
Paul and Tchelitcheff[1] reported the synthesis of 4-pentyn-1-ol, $HC{\equiv}CCH_2CH_2CH_2OH$, from 5-bromo-2,3-dihydro-4*H*-pyran and butyllithium or butylsodium. The simultaneous production of butyl bromide was taken as evidence that the initial step was replacement of halogen by metal:



Similarly, it would be expected that D-*lyxo*-5-hexyne-1,2,3,4-tetrol (II) should be obtainable from 3,4,6-tri-*O*-acetyl-2-bromo-D-glucal (I). Such a synthesis would provide a novel route to carbohydrates containing acetylenic functionality.



I

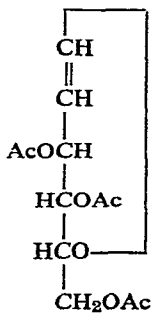


II: R = H
II-Ac: R = Ac

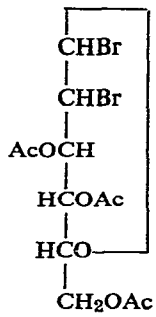
Recently, a quite different synthesis of related sugar derivatives was announced[2]. A Grignard method was employed, using ethynylmagnesium bromide with an aldehyde, to form a 1-substituted propargyl alcohol: $HC{\equiv}CMgBr + RCHO \rightarrow HC{\equiv}C{-}CHR{-}OH$. The R in RCHO was a carbohydrate residue.

*American Viscose Corporation Research Fellow, 1957-1959.

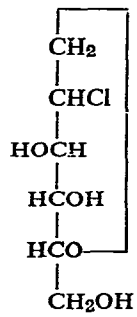
To synthesize I, tri-*O*-acetyl-D-glucal (III) was prepared by way of Fischer's synthesis[3] from tetra-*O*-acetyl-α-D-glucopyranosyl bromide, zinc dust, and aqueous acetic acid. Yields were irregular, ranging from 0 to 85%, and appeared to depend on the nature of the zinc dust that was used. Copper salts or platinum salts were ineffectual promoters for our samples of inert zinc dust, although they have been reported by others[4] as being helpful. Experiments with magnesium turnings in place of zinc dust, either in cold acetic acid or in refluxing tetrahydrofuran, were unsuccessful; most of the glycosyl bromide was recovered.



III

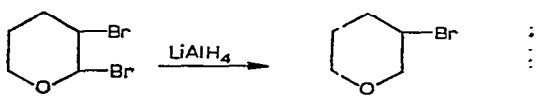


IV



V

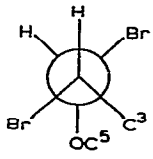
Compound III was converted into both its dichloride and dibromide (IV). On reduction with lithium aluminum hydride, the dichloride was changed smoothly into a monochloride. Structure V, 1,5-anhydro-2-chloro-2-deoxy-D-glucitol (and -mannitol)*, is assigned to it because, of the two chlorine atoms, that on C-2 behaves as a not particularly reactive alkyl chloride, and that on C-1 is a hemiacetal chloride which is in a class known to be very reactive: thus, whereas alkyl chlorides or bromides (comparable to the chlorine on C-2) are not affected[5] by lithium aluminum hydride, tetra-*O*-acetyl-α-D-glucopyranosyl bromide (a hemiacetal bromide comparable to the chlorine on C-1 of the dichloride) is reduced, as well as deacetylated, by lithium aluminum hydride to 1,5-anhydro-D-glucitol[6]. Furthermore, 2,3-dibromotetrahydropyran, of analogous structure, gave rise to 3-bromotetrahydropyran in good yield when treated comparably with lithium aluminum hydride:



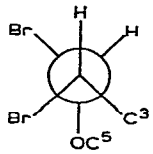
The dichloride or the dibromide, as formed from the glucal, may exist in four

*In this name, the substitutional prefix "chloro" is alphabetized among the two operational prefixes "anhydro" and "deoxy" to conform with Rule 14 of the Rules of Carbohydrate Nomenclature. If the operational prefixes were directly attached (alphabetically) to the stem name that they operate on, to create a new stem name, 1,5-anhydro-2-deoxyglucitol, a more defensible, complete name for V would result, namely, 2-chloro-D-1,5-anhydro-2-deoxyglucitol (and -mannitol). Compare (*a*) the parallel example of hexane and cyclohexane, wherein cyclo is the operational prefix, and (*b*) the non-alphabetization of cyclo and nitro in the name nitrocyclohexane.

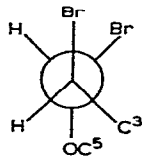
isomeric structures, the pertinent parts of which are depicted below in a projection that was first employed by Böeseken[7] in 1928, but which has become popularized in the last decade as a "Newman projection".



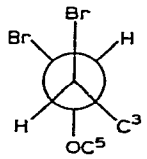
α-D-manno



β-D-manno



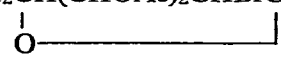
α-D-gluco



β-D-gluco

It has been established recently[8] for the dibromide that 60% of the mixture is the α-D-*gluco* isomer, 30% is the α-D-*manno* isomer, and no more than 10% is a mixture of the two β-D isomers. Thus, the major product is the one that seemingly involves cis addition at the double bond, differing from the usual trans path that is followed in the bromination of alkenes. This apparent conflict, however, is readily resolved by assuming an initial trans addition, followed by anomerization of the reactive C-1 halogen atom from a β-D-*gluco* to the more favorable α-D-*gluco* structure with its axial halogen atom.

Triethylamine was used as dehydrohalogenating agent to convert the dibromide into (I). The combined effects of greater reactivity of Br at C-1 and the relatively high acidity (because of the inductive effect of the Br at C-2) of the H at C-2 set the stage for the elimination reaction. Triethylammonium bromide was isolated in yields of 60 to 90%. Whether or not the elimination was momentarily preceded by quaternary salt formation (*i.e.*, $AcOCH_2CH(CHOAc)_2CHBrCH\overset{+}{N}Et_3\ ^-Br$), as is known[9]

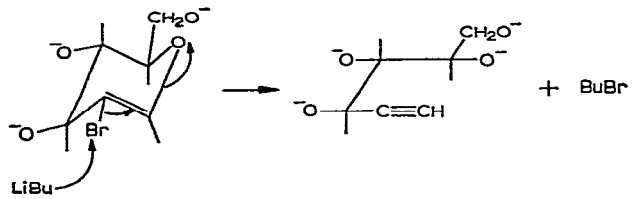


to occur with tetra-*O*-acetyl-α-D-glucopyranosyl bromide and triethylamine, cannot be answered; none was isolated.

The acetylated bromoglucal (I) was obtained as a viscous, orange oil. Its formation from the dibromide in an E2 type of elimination reaction calls for coplanarity of the atoms involved, namely, H–C–C–Br. For this to occur with the pyranose ring, both H and Br must be axial. Consideration of the Newman projections shows that, of the four possibilities, only the α-D-*gluco* structure meets this requirement if Br on C-1 is the departing halogen. It is evident, therefore, that conversion of the total dibromide into (I) cannot be expected.

3,4,6-Tri-*O*-acetyl-2-bromo-D-glucal (I) is a new compound, although there is one erroneous reference[10] in *Chemical Abstracts* to "2-bromotriacetylglucal". Actually, the compound therein reported was the glycose, not a glycal, namely, 3,4,6-tri-*O*-acetyl-2-bromo-2-deoxy-D-glucose which had been made from the dibromide of 3,4,6-tri-*O*-acetyl-D-glucal and moist silver oxide. 2-Bromoglycals are not mentioned by Helferich in his extensive review[11] of glycals.

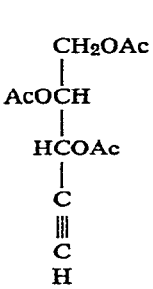
Treatment of one mole of (I) with 12 moles of butyllithium yielded 5-methyl-5-nonanol (37%) as anticipated by reaction of butyllithium with the acetate groups, and the hexynetetrol II was obtained in low yields as its tetraacetate II-Ac. The following mechanism is favored, omitting details for removal of ester groups:



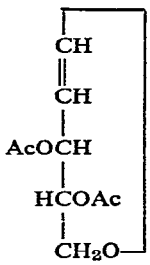
Compound II was not formed from tri-*O*-acetyl-D-glucal dibromide and butyllithium without the prior dehydrobromination step, nor was II formed from I by use of either isopropylmagnesium bromide or lithium aluminum hydride.

The structure of II-Ac was supported by elemental analysis, by infrared absorption bands at 3.04 and 4.66 μ which are so characteristic of the –C≡CH grouping, and by its saponification followed by hydrogenation to form D-*lyxo*-1,2,3,4-hexanetetrol, a known crystalline compound[12].

Just as II-Ac was made from tri-*O*-acetyl-D-glucal, so also was D-*threo*-4-pentyne-1,2,3-triol triacetate (VI) synthesized comparably from 3,4-di-*O*-acetyl-D-xylal (VII). In contrast to II-Ac, which was crystalline, VI was obtained as



VI



VII

a clear yellow oil. Its infrared spectrum supports the assigned structure. It showed bands for the acetylenic function at 3.04 and 4.68 μ of intensity comparable to that of the same bands for II-Ac.

The tetraacetate of this hexanetetrol has not been reported previously. We prepared it in two ways: (1) by hydrogenation of the hexynetetrol tetraacetate, and

(2) by acetylation of the hexanetetrol. This tetraacetate exhibits dimorphism, one form melting unsharply at about 65° and the other at 77–78.5°.

Ozonolysis of II-Ac gave rise to the known tetra-*O*-acetyl-D-arabinonic acid in good yield. This was the expected product of the cleavage of the acetylenic triple bond by ozone[13]. There are not many ways to prepare fully acetylated aldonic acids[14], and this synthesis from aglycal provides a new general method.

Both the hydrogenation and ozonolysis reactions proved that there was retention of the stereochemical structure in forming II. Such retention of configuration follows the known behavior of other carbohydrate derivatives towards organometallic reagents[15].

Hydration of the triple bond of II-Ac proceeded normally in acetic acid solution when catalyzed by mercury(II) ion. The product was 1-deoxy-*keto*-D-fructose tetraacetate, $AcOCH_2$–$(CHOAc)_3$–$COCH_3$, the acyclic ketose derivative.

Tetra-*O*-acetyl-D-arabinononitrile, $AcOCH_2$–$(CHOAc)_3$–$C{\equiv}N$, to which II-Ac has a formal structural relationship, is known to be split into D-erythrose and cyanide ion in basic media (Wohl degradation). However, deacetylation of II-Ac to afford II was readily effected, with no evident formation of erythrose and acetylene, by treatment with ammonia in aqueous methanol. With sodamide in liquid ammonia, three-fifths of the II-Ac was recovered. No doubt, conditions may yet be found for this cleavage; if they should be, the reaction would provide a means of degrading sugars by 2-carbon units, *e.g.*, from hexose to tetrose. Analogy for such a cleavage is known with simpler acetylenic alcohols, as in the smooth decomposition of propargyl alcohol[16] into acetylene and potassium formate on heating with potassium hydroxide, or of 2,5-dimethyl-3-hexyne-2,5-diol[17] into acetylene and acetone by heating with a variety of basic reagents.

Oxidative coupling of copper(I) acetylides is a well known reaction. An example[18] is the high-yield, oxidative coupling of the copper(I) salt of 4-hexen-1-yn-3-ol into 5,7-dodecadiyne-4,9-diol. It was hoped to adapt the procedure to the synthesis of a dodecadiynoctol, $HOCH_2$–$(CHOH)_3$–$C{\equiv}C$–$C{\equiv}C$–$(CHOH)_3$–CH_2OH. This reaction, however, was not realized; nine-tenths of II was recovered as its acetate. From this result, it would be expected that oxygen consumption would have been negligible also. Unaccountably, however, a fair amount of oxygen was consumed.

EXPERIMENTAL

Microanalyses reported below were performed by Miss Hilda Beck.

Glycals

Tri-*O*-acetyl-D-glucal was made by treatment of tetra-*O*-acetyl-α-D-glucopyranosyl bromide with zinc dust and aqueous acetic acid, following Fischer's synthesis[3]. Our experience showed that the yield depended primarily on the particular zinc dust employed. Sample SS-3416 of No. 1 zinc dust, Federated Metals Division, American Smelting and Refining Co., was effective, and gave rise to an 85% yield

after recrystallization from aqueous alcohol; m.p. 52.5–55°. Some inactive samples of zinc dust could be activated by preliminary etching with dilute hydrochloric acid, but others could not. The zinc could be either reagent or technical grade; some samples in either category worked well, whereas others failed.

Di-*O*-acetyl-D-xylal, b.p. 67–68°/0.1 mm, was made by the procedure of Levene and Mori[19].

3-*Bromotetrahydropyran*

To 20 ml of dihydropyran was added dropwise a 20% solution of bromine in chloroform until a yellow color persisted. Solvent was removed, leaving a sirupy dibromide, a product previously described by Woods and Sanders[20]. It was dissolved in 50 ml of dry ether and treated during 30 min with 3.0 g of lithium aluminum hydride in 50 ml of dry ether. After 30 min of stirring at 20°, water was added, and the solution was acidified (HCl). The ether layer was separated, washed with water, dried, and concentrated. The sirupy residue was distilled; b.p. 38–40°/4 mm. The colorless distillate was redistilled, giving 26 g (70% yield) of product, b.p. 67–68° /15 mm, n_D^{25} 1.4907. It did not decolorize bromine (in CCl_4), and it only gave a precipitate with alcoholic silver nitrate on standing for a time.

Anal. Calc. for C_5H_9BrO: C, 36.38; H, 5.48. Found: C, 36.88; H, 5.53.

1,5-*Anhydro-2-chloro-2-deoxy-*D*-glucitol* (*and -mannitol*) *triacetate*

Ten grams of tri-*O*-acetyl-D-glucal was converted into its dichloride by using Fischer's directions[21]. The sirupy product in 50 ml of absolute ether was slowly added, with stirring, to a solution of 4.8 g of lithium aluminum hydride in 200 ml of absolute ether during 30 min. A white precipitate formed. The suspension was refluxed for one h, and then cooled, and about 100 g of water was added incrementally. The water layer was separated, acidified with acetic acid, and evaporated to dryness. Acetic anhydride (50 ml) was added, and the mixture was heated to boiling. After the vigorous reaction had subsided, the mixture was refluxed for 30 min, and then about 30 ml of solvent was distilled off. The concentrate was cooled and poured into 100 ml of ice water. A light-brown oil separated, and was extracted into chloroform. The solution was washed with water, sodium bicarbonate solution, and again with water, dried ($MgSO_4$), and the solvent evaporated; the residue was distilled; yield, 8.1 g (71%); b.p. 155–165°/0.05 mm. The distillate was a clear, very viscous oil that resisted crystallization. Although the halogen in the compound was not readily removed by treatment with sodium bicarbonate, and was only slowly responsive towards silver nitrate, the compound gave a strong halogen test after sodium fusion.

Anal. Calc. for $C_{12}H_{17}ClO_7$: C, 46.77; H, 5.54. Found: C, 46.36; H, 5.53.

D-lyxo-5-*Hexyne*-1,2,3,4-*tetrol tetraacetate* (*II-Ac*)

A solution of 5.01 g of bromine in carbon tetrachloride (20 ml) was added during 20 min to an ice-cold solution of 7.75 g of tri-*O*-acetyl-D-glucal in 40 ml of carbon tetrachloride. After a further 30 min, the solvent was removed under

diminished pressure, leaving a clear, pale-yellow oil. This was dissolved in 30 ml of triethylamine, and the solution was heated for 2 h at 60–70°, cooled, diluted with 40 ml of ether, and filtered to remove a 75% yield of triethylammonium bromide (m.p. and mixed m.p., 240–241°). Evaporation of the filtrate left 10.8 g of a viscous, orange oil which was, presumably, mostly I.

The above oil (I), dissolved in 50 ml of absolute ether, was added dropwise, during 30 min, to a well stirred solution of butyllithium[22] (0.5 mole) in 300 ml of dry ether. There was much foaming, but no precipitation. The mixture was refluxed for 2 h, and then treated with 100 ml of water. The ether layer contained 5.0 g (37%) of 5-methyl-5-nonanol*, b.p. 61–62°/0.2 mm.

The water layer was acidified with acetic acid, and evaporated to a thick gum. Absolute alcohol (50 ml) was added and distilled off, and the process was repeated (to remove traces of water). Acetic anhydride (150 ml) was added, and the mixture was warmed on a steam bath. A vigorous, exothermic reaction occurred, and the solution became black. (No catalyst was added, since much lithium acetate was present.) The solution was heated on a steam bath for one h, and then the solvent was distilled off. The black, gummy residue was poured into 100 ml of ice water, producing a black tar, essentially insoluble in water or ether; it was slowly dissolved by shaking it with a mixture of these solvents. The water layer was extracted with ether, and the (black) ether extracts were combined, dried ($CaCl_2$), decolorized with Norit, filtered, and evaporated to a yellow oil. If the solution was insufficiently decolorized by this treatment, it was evaporated, dissolved in 40 ml of alcohol and 10 ml of water, and decolorized by Norit, and re-evaporated.

The clear, yellow oil was induced to crystallize by chilling a 5% solution in butanol to −78°, while scratching the insides of the vessel. Alternatively, crystallization could be effected by adding water to the alcohol solution of the oil to turbidity and scratching the inner walls of the container. The yield of white crystals varied from zero to 0.90 g (0 to 10% of the theoretical), m.p. 62–64°. The product could be crystallized from a solution in water and alcohol, but larger crystals were obtained from a solution in ether and pentane. Several recrystallizations gave large crystals, m.p. 64–66°, $[\alpha]_D$ −10.9 ± 0.7° (*c* 6, $CHCl_3$).

Anal. Calc. for $C_{14}H_{18}O_8$: C, 53.52; H, 5.74. Found: C, 53.85; H, 5.85.

A solution of the above product in aqueous alcohol gave an immediate, white precipitate with ammoniacal silver nitrate, but the precipitate turned black within a few seconds. A pale-green precipitate was formed with ammoniacal copper(I) chloride, but only at fairly high concentrations of the reactants.

Strong infrared absorption bands were observed for II-Ac at 5.75, 7.27, 8.10, 9.25, 9.40, and 9.62 μ, and medium bands at 3.03, 4.65, 6.81, 6.95, 10.25, 10.50, 11.78, and 13.80 μ.

*Whitmore *et al.*[23] list the b.p. as 100°/24 mm.

Ozonation of II-*Ac*

A cold solution (0°) of 0.80 g of II-Ac in 100 ml of carbon tetrachloride was treated for 6 h with a 5% ozone–in–oxygen stream at the rate of 0.01 ft^3 per min. After processing (to hydrolyze the ozonide[13]), 0.5 g of crystalline product was obtained. This was recrystallized from toluene, to yield 0.33 g of tetra-*O*-acetyl-D-arabinonic acid as fine, white needles, m.p. 132.5–133.5°, $[\alpha]_D$ +32.9° (*c* 5, $CHCl_3$); lit.[24] m.p. 135–136°, $[\alpha]_D$ +32.5°; and for the L-isomer[14], m.p. 135–135.5°, $[\alpha]_D$ −32°.

Hydration of II-*Ac*

A solution of 0.50 g of II-Ac in 5 ml of acetic acid was added to 10 ml of acetic acid containing about 0.1 g of catalyst (HgO, $BF_3{\cdot}Et_2O$, Cl_3CCO_2H)[25]. The clear mixture was warmed at 40–50° for 3 h, and then diluted with 25 ml of chloroform. The solution was washed with water, sodium hydrogen carbonate solution, and water, dried (Drierite), and evaporated, giving a light-yellow oil which crystallized, m.p. 75–79°. The product was recrystallized from absolute ethanol by the addition of hexane, and then from isopropyl ether, after filtration through Norit. The sandy, white crystals of 1-deoxy-*keto*-D-fructose tetraacetate had m.p. 80.5–82.5°, $[\alpha]_D$ +56.4° (*c* 11, $CHCl_3$); yield 0.35 g (63%).

Wolfrom[26] *et al.* synthesized this compound, m.p. 81–83°, $[\alpha]_D$ +56° (*c* 3, $CHCl_3$), from D-arabinonaldehyde tetraacetate plus diazomethane, and also by reduction of the diazo ketone formed from tetra-*O*-acetyl-D-arabinonoyl chloride and diazomethane.

Hydrogenation of II-Ac to D-lyxo-1,2,3,4-*hexanetetrol tetraacetate*

A solution of 0.50 g of II-Ac in 10 ml of absolute alcohol was hydrogenated at 25° in a Parr apparatus with hydrogen in the presence of 0.05 g of 10% palladium-on-charcoal catalyst. The hydrogen consumed during 40 min was 97% of the theoretical. After removal of catalyst and solvent, the residue was crystallized from 25% alcohol; yield of white crystals, 0.42 g. The m.p. was about 63–65° when the sample was heated rapidly. The melt resolidified when held at 70° for about 15 min, and the new crystals melted at 75–76.5°. After being melted at the higher temperature, the product was quickly cooled; it again showed m.p. about 63–65°. This material was reconverted into the higher-melting form, as before. The substance is dimorphic. If the sample is heated very slowly (about 0.5° per min.), it melts at the higher temperature without noticeable change between 60 and 70°.

Anal. Calc. for $C_{14}H_{22}O_8$: C, 52.84; H, 6.97. Found: C, 53.12; H, 6.66.

Deacetylation of II-*Ac*

Two g of II-Ac was dissolved in 20 ml of methanol containing about 20% of ammonia, and kept at 25° overnight. Solvent was removed under diminished pressure and the residual oil was extracted twice with 20-ml portions of warm ethyl acetate (to remove acetamide). The crystalline residue was recrystallized from 20 ml of absolute ethanol, giving 0.81 g (87% of theory) of white needles, m.p. 134–135°.

A sample, recrystallized from ethanol, melted at 134.5–135.5°, $[\alpha]_D$ −10.1° (*c* 7, water). Very small quantities of II could be recrystallized efficiently from ethyl acetate.

Anal. Calc. for $C_6H_{10}O_4$: C, 49.30; H, 6.89. Found: C, 49.36, 49.40; H, 6.99, 6.74.

Acetylation of II

A mixture of 0.50 g of II, 5 ml of pyridine, and 5 ml of acetic anhydride was heated at 100° for 2 h, the solution turning light-orange in color. Removal of solvents, and crystallization from 50% aqueous alcohol, gave 0.81 g of white crystals, m.p. 64–66°; mixture m.p. with II-Ac, 64–66°.

Hydrogenation of II

D-*lyxo*-5-Hexyne-1,2,3,4-tetrol (97 mg) was dissolved in 10 ml of absolute alcohol, and reduced at 27° with hydrogen, using 0.05 g of platinum oxide as catalyst. The quantity of hydrogen consumed was 96% of the theoretical. The catalyst was filtered off and the solution was evaporated to about 3 ml. Cooling and scratching the walls induced crystallization; then, 3 ml of ethyl acetate and 5 ml of isopropyl ether were added, the solution was cooled at 0° for one h, and the crystals were collected; yield, 60 mg; m.p. 121–122.5°; $[\alpha]_D$ +6.8 ±2° (*c* 4, water). Karrer and Davis[12] reported, for D-*lyxo*-1,2,3,4-hexanetetrol, m.p. 122° and $[\alpha]_D$ +4.2°.

Acetylation of D-lyxo-1,2,3,4-*hexanetetrol*

The hexanetetrol prepared in the preceding experiment was warmed, on a steam bath, with 0.5 ml of acetic anhydride and 0.5 ml of pyridine. The solvents were evaporated, and the crystalline residue was recrystallized from 2 ml of aqueous alcohol (1:1). The white crystals obtained melted at 65–67°. When held at about 70–72° for 30 min, it resolidified and then melted at 77–78.5°. This melting-point behavior is the same as that of the substance prepared by the catalytic reduction of D-*lyxo*-5-hexyne-1,2,3,4-tetrol tetraacetate. A mixture of the two products showed the same sort of melting-point behavior.

Attempted oxidative coupling of II

The method used was essentially that of Armitage[18]. An aqueous solution of II (0.20 g) was added to the copper(I) chloride solution (in concentrated HCl, buffered to pH 6.5 with NH_4OH); a green precipitate formed. At first, the vigorously stirred suspension took up oxygen rapidly, then at the rate of 0.4 ml per h after 4 h, and finally, at 0.05 ml per h after 22 h. Processing, which included acetylation, yielded 0.37 g of II-Ac (theory, 0.42 g), m.p. and mixture m.p. 64-66°. No other product was observed.

Non-reaction of II with sodamide

A solution of II-Ac (50 mg) in liquid ammonia containing about 170 mg of

sodamide, under a slight pressure of nitrogen to exclude moisture, was maintained at −78° for one h. Ammonium chloride (250 mg) was added, the ammonia was evaporated, and 20 ml of water was added. The solution was boiled for 5 min, cooled, and passed through a column of Amberlite IR-120 (a sulfonic acid, cation-exchange resin.) The effluent was evaporated at 100° to about 5 ml and was then kept in a desiccator over sodium hydroxide pellets. The residual 35 mg of white crystals melted at 124–129°; mixture m.p. with II, 125–133°. About 70% of the starting material was recovered.

D-threo-4-*Pentyne*-1,2,3-*triol triacetate*

A solution of bromine in chloroform was slowly added at 0° to a solution of 9.6 g of D-xylal diacetate, b.p. 67–68°/0.1 mm, in 50 ml of chloroform, until the brown color persisted. After evaporation of the chloroform, 100 ml of triethylamine was added to the residue and the mixture was refluxed for 3 h. The crystalline triethylammonium bromide [4.7 g (54%), m.p. and mixed m.p. 240–241°] that separated was filtered off, and the filtrate was evaporated. The residue was dissolved in 50 ml of dry ether, and treated during 30 min with 0.75 mole of butyllithium in 400 ml of dry ether. The procedure, from this point, was adapted from that given above for II-Ac. The product was 4 g of clear, yellow oil. Attempts to crystallize it were fruitless; hence, the substance was not analyzed for C and H by combustion. The infrared spectrum of this oil showed strong bands at 5.70 to 5.80, 7.27, 8.0, 9.40, and 9.60 μ, and medium bands at 3.03, 3.30, 3.40, 4.65, 6.90, 8.34, 10.25, 10.50, and 13.8 μ.

One gram of the sirupy triacetate was dissolved in 25 ml of 20% ammonia in methanol. After 16 h at 25°, the solvents were removed, and the clear, yellow, sirupy residue was extracted twice with warm, 10-ml portions of ethyl acetate to remove the acetamide. The yellow residue could not be induced to crystallize.

SUMMARY

D-*lyxo*-5-Hexyne-1,2,3,4-tetrol (II) was synthesized by reaction of 3,4,6-tri-*O*-acetyl-2-bromo-D-glucal (I) with butyllithium. Compound I was made from tri-*O*-acetyl-D-glucal *via* its dibromo adduct and treatment with triethylamine. The α-D-*gluco* form of the dibromo adduct is regarded as being the active participant in the dehydrobromination. D-*threo*-4-Pentyne-1,2,3-triol was similarly prepared from di-*O*-acetyl-D-xylal. II was acetylated to its tetraacetate (II-Ac), and hydrogenated to D-*lyxo*-1,2,3,4-hexanetetrol. II-Ac was hydrogenated to the hexanetetrol tetraacetate, which exhibited dimorphism. Ozonolysis gave tetra-*O*-acetyl-D-arabinonic acid and thus provides a new general method for preparing fully acetylated aldonic acids. The $-C\equiv CH$ part of II-Ac was readily hydrated to $-COCH_3$, thus yielding an acyclic ketose derivative; but conditions were not found for effecting oxidative coupling of the copper(I) acetylide. Saponification of II-Ac (to II) was not accompanied by further cleavage of II into acetylene and D-erythrose. Lithium aluminum hydride converts 2,3-dibromotetrahydropyran into 3-bromotetrahydropyran, and

changes the dichloride of tri-*O*-acetyl-D-glucal to 1,5-anhydro-2-chloro-2-deoxy-D-glucitol (and -mannitol). In the reduction of tetra-*O*-acetyl-α-D-glucopyranosyl bromide to tri-*O*-acetyl-D-glucal, some samples of zinc dust gave excellent yields, whereas other samples failed completely, even in the presence of some favored promoters.

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1,6-ANHYDRO-2,3-*O*-ISOPROPYLIDENE-β-D-*lyxo*-HEXOPYRANOS-4-ULOSE
STUDIES ON OXIDATION OF CARBOHYDRATE DERIVATIVES

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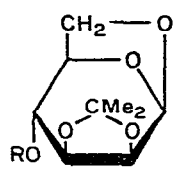
INTRODUCTION

A number of procedures have been reported for oxidation of "isolated"[1] secondary alcohol groups to ketones in blocked derivatives of sugars. Oxidations with oxygen–platinum oxide[2], and with chromium trioxide–pyridine[3] and related procedures[4], have been used, but yields are often low. Ruthenium tetroxide[5,6] has proved effective with a number of carbohydrates[7,8]. The Pfitzner–Moffatt reagent[9] (methyl sulfoxide–*N*,*N*′-dicyclohexylcarbodiimide–pyridinium phosphate), and methyl sulfoxide–phosphorus pentaoxide[10], are also effective with carbohydrates[11], but isolation of the product may present difficulties, especially if the product is sensitive to aqueous acid. As part of a program in this laboratory on the synthesis of certain deoxy, branched-chain, and amino sugars, especially 4-amino-4-deoxy sugars[12], we have evaluated a number of oxidation procedures with regard to effectiveness, convenience, and cost of reagents. Methyl sulfoxide–acetic anhydride, an oxidant used in the steroid field[13], is shown to be effective with carbohydrate derivatives[13a], and it has the particular advantage, for a product that is water-sensitive, that all reagents



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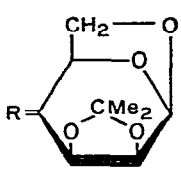
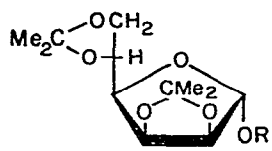
II R = Ac



III R = O

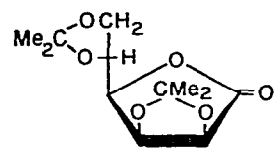
IV R = N–OH

V R = 



VI R = H

VII R = Ac



VIII

can be removed after the oxidation by simple lyophilization. In the present report, the methyl sulfoxide–acetic anhydride procedure is compared with a number of other oxidation procedures for the synthesis of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (III), and for oxidation of 2,3:5,6-di-*O*-isopropylidene-D-

mannose (VI). Lead tetraacetate–pyridine[14] did not prove to be a useful oxidant for these conversions.

Treatment of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[15] (I) with methyl sulfoxide–acetic anhydride for 3–4 days at room temperature, with subsequent lyophilization, gave the ketone III, isolated crystalline in 77% yield. Oxidation of I with ruthenium tetroxide in carbon tetrachloride[5–7] gave crystalline III in over 90% yield. However, oxidation of I with the Pfitzner–Moffatt reagent[9] gave a product which was difficult to purify, giving III in only low yield. Lead tetraacetate in pyridine[14] had no detectable effect on I under anhydrous conditions; addition of a trace of water led to partial conversion into another substance, but the latter proved to be the 4-acetate (II) of I, instead of the desired ketone III.

The "isolated" hemiacetal group of 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose[16] (VI) underwent oxidation by methyl sulfoxide–acetic anhydride to give 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (VIII) in essentially quantitative yield; the product was identical with the substance prepared[17] by *O*-isopropylidenation of D-mannono-1,4-lactone or by oxidation of VI with alkaline permanganate, followed by lactonization. Oxidation of VI with the Pfitzner–Moffatt reagent[9] gave VIII, but the yield was only 35%. No oxidation of VI was observed with lead tetraacetate–anhydrous pyridine; a trace of added water caused partial transformation into two products – apparently, the lactone VIII and the 1-acetate (VII) of VI. The acetate VII was independently prepared from VI for comparison.

Progress of the oxidation reactions was followed, with aliquot samples, by observation of the appearance of carbonyl absorption and disappearance of hydroxyl absorption in the infrared spectrum. Thin-layer chromatography was also employed, and was particularly useful in detecting formation of acetic esters from the starting materials.

The 4-ketone III was characterized by elemental analysis, and by infrared and n.m.r. spectroscopy. It gave a crystalline oxime IV, which was evidently a mixture of the *syn* and *anti* isomers, since it gave two closely migrating zones on thin-layer chromatograms, and repeated recrystallization did not give a product having a sharp melting point or constant specific rotation. Treatment of the 4-ketone III with diazomethane led to methylene insertion into the carbonyl group, to give a crystalline, spiro epoxide V. The latter was characterized by elemental analysis, and by infrared and n.m.r. spectral data. The configuration at C-4 in V was not definitively established, but steric considerations suggest that the favored product would be the D-*talo* isomer, and this assignment is supported by the n.m.r. data.

Details of all n.m.r. spectral measurements, first-order analyses, and assignments, are given in the Experimental section. It is noteworthy that, in the ketone III, the H-2 and H-3 protons have almost identical chemical shifts, and the spectrum (see Fig. 1) shows coupling of the H-2 and H-3 signals to that of H-1 as an AA'X system (virtual long-range coupling)[18], with the result that the H-1 signal (τ 4.37) is observed as a one-proton triplet, and the H-2,H-3 signal (τ 5.52) is observed as a two-proton doublet. The assignment was confirmed by spin-decoupling experiments. Irradiation

at the frequency of the H-1 signal caused the signal for H-2,H-3 to collapse to a singlet, and irradiation at the frequency of the latter signal caused the H-1 signal to collapse to a singlet (see Fig. 1).

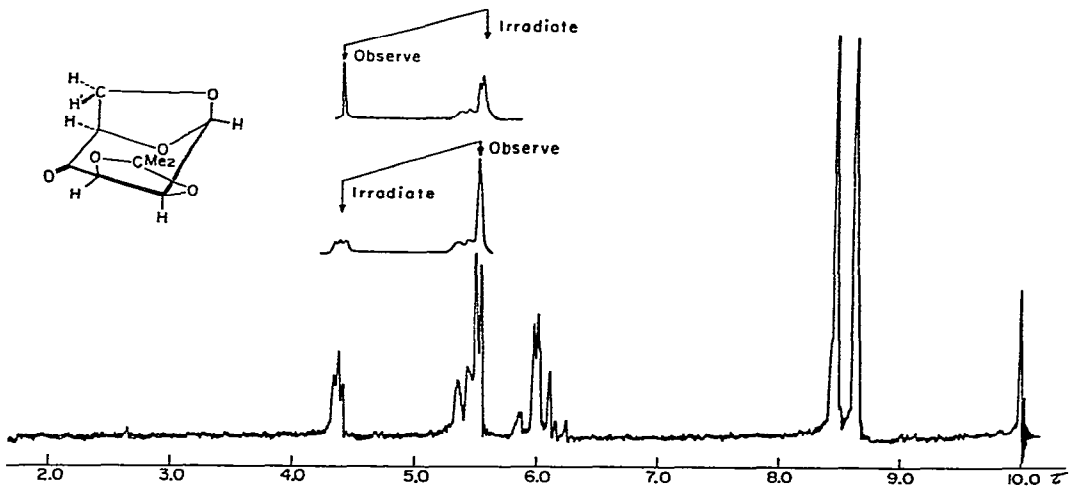


Fig. 1. The n.m.r. spectrum of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-*lyxo*-hexopyranos-4-ulose (III) at 60 Mc.p.s. in deuteriochloroform, with spin-decoupling of the H-1 and H-2,3 signals.

The quartet at τ 6.13 in the spectrum of III may be assigned to the *exo* proton (H-6) at C-6, and the quartet at τ 5.94 to the less-shielded *endo* proton (H-6′) at C-6; both protons are coupled to H-5 (ABX system), and the signal for H-5 is observed as a multiplet at τ 5.40. The projected valence angle between H-6 and H-5 is small (~20°), that between H-6′ and H-5 is ~100°, and the observed first-order coupling constants ($J_{5,6}$ 5.2 c.p.s., $J_{5,6'}$ 1.6 c.p.s.) are in agreement[19] with these assignments.

Complete analyses of the other n.m.r. spectra were not made. The spectra of the anhydro sugar derivative I and its 4-acetate II (see Fig. 2 and Experimental) show the H-1 signal as a broadened, unresolved singlet, presumably because of long-range coupling[20] with H-3 (diequatorial protons) in addition to the normal small coupling of H-1 with H-2. The signal of the *exo* proton (H-6) at C-6 is observed at higher field than that of the *endo* proton (H-6′) in I and II. Comparison of the spectra of I, II, and III, and consideration of the anticipated deshielding of H-3 and H-5 in III by the carbonyl group at C-4, permitted assignment of the signal near τ 5.4 in I and II to H-2. Although the H-3, 4, and 5 signals in I were not resolved, acetylation of I caused deshielding of H-4, so that, in the spectrum of II, the H-4 signal could be observed independently, at τ 4.89, as a broad, apparent singlet.

The nonequivalence of the protons on C-6 in I, II, and III is to be expected, because the *endo* proton is in a magnetic environment different from that of the *exo* proton. In the spiro epoxide V, a similar nonequivalence might be expected for the C-4′ methylene protons if C-4′ were *endo* (D-*manno* configuration), because one of the protons would be much closer to O-3 than the other. The fact that the signal

for the C-4′ methylene group is observed as a singlet indicates that the two protons must be equivalent, or very nearly so, as would be the case if the group were *exo* (D-*talo* configuration). This evidence, therefore, provides further support for the D-*talo* configuration, and degradative studies are in progress for definitive confirmation.

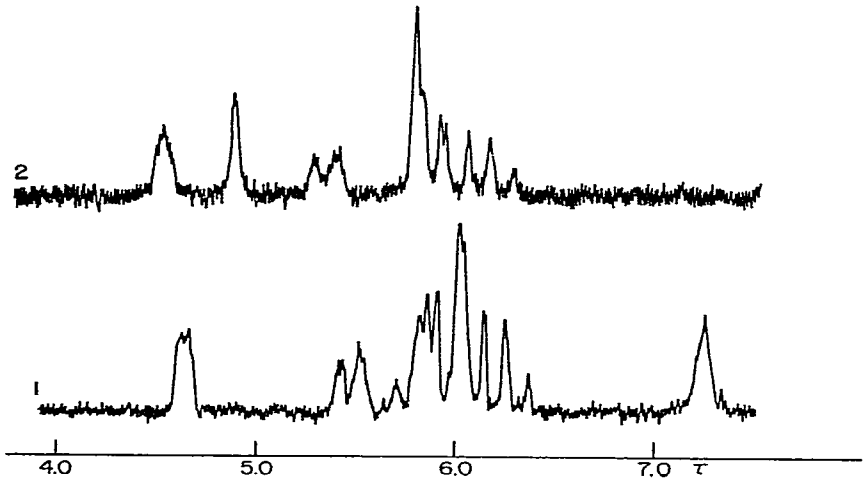


Fig. 2. The low-field portion of the n.m.r. spectra of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (1) and 4-*O*-acetyl-1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (2) at 60 Mc.p.s. in deuteriochloroform.

The n.m.r. spectrum of the oxime IV showed a low-field signal, τ 0.52, which disappeared on deuteration, as anticipated for the oxime structure. Reduction of this oxime provides a high-yielding route to 4-amino-4-deoxy sugar derivatives[21].

EXPERIMENTAL

General methods

Solutions were evaporated below 40° under diminished pressure. Melting points were determined with a Thomas–Hoover melting point apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania), and are uncorrected. Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin–Elmer Model 137 "Infracord" infrared spectrometer. Ultraviolet spectra were measured with a Bausch and Lomb "Spectronic 505" spectrometer. Nuclear magnetic resonance spectra were measured at 60 Mc.p.s. with a Varian A-60 n.m.r. spectrometer; spin-decoupling experiments were performed with a Varian HR-60 n.m.r. spectrometer by the field-sweep, double-sideband technique, with a Varian integrator. Chemical shifts are given on the τ-scale, and, unless otherwise stated, all spectra were measured at *ca* 30° with solutions (*ca* 10%) in deuteriochloroform, with tetramethylsilane (τ 10.00) as the internal standard. Spectra were analyzed on a first-order basis. W_h denotes the width at half-height. Microanalyses were determined

by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuK_α radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest), and double numbers indicate approximately equal intensities. Thin-layer chromatography was performed with Desaga equipment, with Silica Gel G (E. Merck, Darmstadt, Germany), activated at 110°, as the adsorbent. Unless otherwise stated, the adsorbent thickness was 0.25 mm, and the developing solvent was 3:1 chloroform–ether. Indication was effected with acid molybdate[22] or with sulfuric acid.

Preparation of 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose (I)

Ivory-nut mannan shavings (Pfanstiehl Laboratories, Waukegan, Illinois) were pyrolyzed, and the pyrolyzate was treated with acetone according to the procedure of Hudson and co-workers[15], except that crystallization and recrystallization were effected from butyl alcohol; the product had m.p. 160–161°, $[\alpha]_D^{20}$ −57 ± 2° (*c* 2.1, water) [lit.[15] m.p. 161–162°, $[\alpha]_D^{20}$ −58.8° (*c* 2.08, water)]; R_F 0.33; λ_{max}^{KBr} 2.90 (OH), 7.30 μ (CMe_2); n.m.r. data: τ 8.67, 8.46 (3-proton singlets, CMe_2), τ 7.25 (1-proton broadened singlet, disappears on deuteration, OH), τ 6.20 (1-proton quartet, $J_{6,6'}$ 13.7 c.p.s., $J_{5,6}$ 7.5 c.p.s., H-6), τ 5.96 (1-proton quartet, $J_{6,6'}$ 13.7 c.p.s., $J_{5,6'}$ 1.3 c.p.s., H-6'), τ 5.69–6.08 (3-proton multiplet, H-3,4,5), τ 5.48 (1 -proton broadened doublet, $J_{2,3}$ 6.0 c.p.s., H-2), τ 4.64 (1-proton broadened singlet, W_h 5.5 c.p.s., H-1); X-ray powder diffraction data: 9.82 w, 7.03 vs (1), 5.75 m, 5.20 vw, 4.67 vs (2), 4.22 s (3), 3.97 w, 3.55 m, 3.35 s (4), 3.22 vw, 3.03 m, 2.90 w, 2.73 m, 2.64 w, 2.57 m, 2.46 w, 2.37 m, 2.32 m, 2.25 m.

Preparation of 4-O-acetyl-1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose (II)

Acetylation of I according to the procedure of Hudson and co-workers[15] gave II, m.p. 99–101°, $[\alpha]_D^{20}$ −70 ± 2° (*c* 0.5, chloroform) [lit.[15] m.p. 101–102°, $[\alpha]_D^{20}$ −72.2° (*c* 1.6, chloroform)]; R_F 0.48; λ_{max}^{KBr} 5.81 (OAc), 7.30 μ (CMe_2); n.m.r. data: τ 8.66, 8.45 (3-proton singlets, CMe_2), τ 7.85 (3-proton singlet, OAc), τ 6.15 (1 -proton quartet, $J_{6,6'}$ 13.5 c.p.s., $J_{5,6}$ 7.0 c.p.s., H-6), τ *ca.* 5.85 (3-proton multiplet, H-3,5,6'), τ 5.40 (1-proton broadened doublet, $J_{2,3}$ 6 c.p.s., H-2), τ 4.94 (1-proton broadened singlet, W_h 2.8 c.p.s., H-4), τ 4.58 (1-proton broadened singlet, W_h 5.5 c.p.s., H-1); X-ray powder diffraction data: 9.00 m, 6.88 s (3), 6.28 m, 5.54 vs (1), 5.16 s (3), 4.93 vw, 4.53 m, 4.35 w, 3.87 s (2), 3.60 w, 3.52 m, 3.43 vw, 3.30 m.

1,6-Anhydro-2,3-O-isopropylidene-β-D-lyxo-hexopyranos-4-ulose (III)

(*a*) *Oxidation of* I *with methyl sulfoxide–acetic anhydride.* To a mixture of dried methyl sulfoxide (40 ml) and acetic anhydride (2 ml) was added I (200 mg), and the mixture was stirred under nitrogen for 3–4 days at 30°. The resultant yellow solution was lyophilized, and the partially crystalline residue was dissolved in carbon tetrachloride. Addition of petroleum ether (b.p. 30–60°), followed by refrigeration, gave the ketone III as needles; yield 152 mg (77%), m.p. 82.5–83.0°; $[\alpha]_D^{20}$ −76 ± 1° (*c* 1, chloroform); R_F 0.34; λ_{max}^{KBr} 5.68 (C=O), 7.30 μ (CMe_2); $\lambda_{max}^{CHCl_3}$ 300 mμ (ε 50), no

absorption in aqueous solution over the range 250–500 mμ; n.m.r. data (see Fig. 1): τ 8.65, 8.49 (3-proton singlets, CMe_2), τ 6.13 (1-proton quartet, $J_{6,6'}$ 8.2 c.p.s., $J_{5,6}$ 5.2 c.p.s., H-6), τ 5.94 (1-proton quartet, $J_{6,6'}$ 8.2 c.p.s., $J_{5,6'}$ 1.6 c.p.s., H-6'), τ 5.52 (2-proton doublet, J 2.2 c.p.s., H-2,3), τ 5.40 (1-proton multiplet, width between outer peaks ~7 c.p.s., H-5), τ 4.37 (1-proton triplet, J 2.2 c.p.s., H-1); X-ray powder diffraction data: 8.59 w, 7.56 vs (2), 6.03 w, 5.61 w, 5.44 s (3), 4.98 m, 4.80 vs (1), 4.60 m, 4.40 vw, 4.23 s (4), 3.97 m, 3.82 m, 3.59 m.

Anal. Calc. for $C_6H_{12}O_5$: C, 54.00; H, 6.04. Found: C, 54.07; H, 6.10.

The substance was readily soluble in water, the lower alcohols, acetone, ether, chloroform, benzene, and carbon tetrachloride, and moderately soluble in petroleum ether (b.p. 30–60°). It sublimed readily at 40°/0.2 mm. It was stable on storage in a tightly closed container, but liquefied on prolonged exposure to air. The specific rotation, measured in alcohol-free chloroform, did not change with time, but addition of a few drops of ethanol to the solution caused the specific rotation to decrease to $[\alpha]_D^{20}$ −51 ± 1° after 2 h. On thin-layer chromatograms, with the molybdate detecting-reagent[22], the substance gave an intense, dark-blue zone after 1 min at 130°, whereas the precursor (I) gave a pale-blue zone after 8 min at 130°.

(*b*) *Oxidation of I with ruthenium tetroxide.* To a solution of I (1.00 g) in carbon tetrachloride (250 ml) was added a solution of ruthenium tetroxide in carbon tetrachloride (450 ml). The latter had been prepared[6] by stirring ruthenium dioxide (Engelhard Industries, Inc., Newark, New Jersey) (2.4 g) in 5% aqueous sodium periodate (100 ml), followed by extraction of the solution with three 150-ml portions of carbon tetrachloride. The mixed solutions were stirred for 2 h at room temperature, the black precipitate of ruthenium dioxide was removed by filtration, the green filtrate was stirred a further 30 min and refiltered, and the colorless filtrate was evaporated at 25–30° to give the ketone III as a white solid; yield 0.87 g (88%); m.p. 82.5–83.0°. This product was analytically pure without recrystallization, was free from starting material, and was identical, in all physical characteristics examined, with the product prepared by method (*a*).

In ten preparations, under the above conditions, the yields of III were in the range of 85–92%.

(*c*) *Oxidation of I with methyl sulfoxide–N,N'-dicyclohexylcarbodiimide–pyridinium phosphate.* Treatment of I with methyl sulfoxide–*N,N'*-dicyclohexylcarbodiimide–pyridinium phosphate (under the general conditions of Pfitzner and Moffatt[9]) during 18 h at room temperature, followed by lyophilization and extraction of the residue with carbon tetrachloride, gave a solution of the ketone III. The ketone was obtained crystalline from the solution in 10–20% yield, but most of the product was obtained as a gum which was free from I, but which contained *N,N'*-dicyclohexylurea in addition to III, and was difficult to resolve.

Treatment of 1,6-anhydro-2,3-O-isopropylidene-β-D-mannopyranose (I) with lead tetraacetate in pyridine

To a solution of I (1.00 g) in anhydrous pyridine (20 ml) was added ~5 g of lead

tetraacetate (G. Frederick Smith Co., Columbus, Ohio). After 1 week at room temperature, or 3 days at ~60°, recovery of starting material was almost quantitative when the solution was poured into water and the mixture was extracted with ether. However, when the original solution of I in pyridine with lead tetraacetate was treated with 1 drop of water, a bright-red color developed. After 8 h at room temperature, by which time the solution was pale yellow, the reaction mixture was added to ice and water (25 ml) and the mixture was extracted with three 25-ml portions of dichloromethane. The dried (magnesium sulfate) solution was evaporated to a syrup which showed infrared absorption at 5.81 μ, but not at 5.68 μ. This syrup, which was shown by t.l.c. to contain components of R_F 0.48 and 0.33, was resolved on 10 chromatoplates (20 × 20 mm, with 1.25-mm coating) to give the component of R_F 0.33 as needles (from chloroform), yield 723 mg (72%), m.p. 159–160°, identical with the starting material (I), and the component of R_F 0.48 as plates (from methanol), yield 178 mg (50%, based on unrecovered starting material), m.p. 99–101°, identical by X-ray powder diffraction pattern with an authentic sample of 4-*O*-acetyl-1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (II).

1,6-Anhydro-2,3-O-isopropylidene-β-D-lyxo-hexopyranos-4-ulose oxime (IV)

To a solution of hydroxylamine hydrochloride (2.0 g) in methanol (50 ml) were added water (0.15 ml), potassium hydrogen carbonate (2.5 g), and the ketone III (820 mg). The mixture was refluxed on a steam bath for 15 min, and evaporated, and the residue was extracted with dichloromethane (100 ml). The extract was evaporated to a syrup which, on t.l.c., showed two components, of R_F 0.82 and 0.74, in approximately 4:3 ratio. Addition of carbon tetrachloride to the syrup gave a solution which, on refrigeration, gave a solid product; yield 494 mg (56%), m.p. 119–123°; λ_{max}^{KBr} 3.19 (OH), 7.25 (CMe_2); n.m.r. data: τ 8.44, 8.62 (3-proton singlets, CMe_2), τ 4.24–4.82, 5.08–5.45, 5.73–6.31 (multiplets, 6 protons, H-1,2,3,5,6,6'), τ 0.52 (1-proton broad singlet, disappears on deuteration, OH).

Anal. Calc. for $C_9H_{13}NO_5$: C, 50.21; H, 6.09; N, 6.51. Found: C, 50.49; H, 6.25; N, 6.53.

The product was crystalline by X-ray powder diffraction pattern, and showed no carbonyl absorption at 5.68 μ in the infrared region. Repeated isolation of the substance from carbon tetrachloride solution gave solid products having specific rotations in the range of $[\alpha]_D^{20}$ −60 to −71° (*c* 1, chloroform) and melting points within the range of 110–119°, but isolation of a product giving a single zone on t.l.c. was not achieved.

1,6:4,4'-Dianhydro-4-C-(hydroxymethyl)-2,3-O-isopropylidene-β-D-talopyranose (-mannopyranose) (V)

To a solution of III (350 mg) in 200 ml of ether at 0° was added an excess of diazomethane in ether. The solution was stirred for 2 h at 0°, and was then kept for 18 h at room temperature. Evaporation gave a syrup which crystallized from ether to give the epoxide V, yield 153 mg (46%), m.p. 132–135°, $[\alpha]_D^{20}$ −74 ± 2° (*c* 2.3, chloroform); R_F 0.88; λ_{max}^{KBr} 7.29 (CMe_2), 11.75 (epoxide)[23], 13.68 μ (CH_2 rock)[23];

n.m.r. data: τ 8.67, 8.37 (3-proton singlets, CMe_2), τ 7.00 (2-proton singlet, 4'-CH_2), τ 5.48–6.28 (5-proton multiplet, H-2,3,5,6,6'), τ 4.56 (1-proton broadened doublet, W_h 5 c.p.s., H-1); X-ray powder diffraction data: 8.53 w, 7.52 m, 6.04 m, 5.54 s (1,1), 5.19 m, 4.87 s (1,1), 4.47 w, 4.26 vw, 4.05 w, 3.71 m, 3.58 vw, 3.24 w, 3.10 m, 2.86 m, 2.72 m.

Anal. Calc. for $C_{10}H_{14}O_5$: C, 56.04; H, 6.59. Found: C, 55.84; H, 6.46.

Oxidation of 2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (VI)

(a) With methyl sulfoxide–acetic anhydride. A solution of VI (400 mg) in anhydrous methyl sulfoxide (30 ml) containing acetic anhydride (4 ml) was stirred under nitrogen for 18 h at room temperature and lyophilized, and the residue was crystallized and recrystallized from petroleum ether (b.p. 95–110°) to give 2,3:5,6-di-*O*-isopropylidene-D-mannono-1,4-lactone (VIII) as flakes, yield 350 mg (96%), m.p. 126°, $[\alpha]_D^{20}$ + 50 ± 2° (*c* 1.5, chloroform) [lit.[7] m.p. 126°, $[\alpha]_D$ + 50.6° (*c* 1, chloroform)]; R_F 0.95; λ_{max}^{KBr} 5.55 (γ-lactone[24]), 7.25 μ (CMe_2), no OH absorption; no u.v. maximum (carbon tetrachloride) between 250–500 mμ; n.m.r. data: τ 8.60, 8.55, 8.51 (3, 3, and 6-proton singlets, respectively, CMe_2), τ 5.88 (2-proton multiplet, H-6,6'), τ 5.55 (2-proton multiplet, H-4,5), τ 5.10 (2-proton apparent singlet, H-2,3); X-ray powder diffraction data: 12.33 w, 8.93 s (1), 5.75 s (2,2), 5.32 w, 4.89 m, 4.50 m, 4.28 s (2,2).

Anal. Calc. for $C_{12}H_{18}O_6$: C, 55.78; H, 7.03. Found: C, 56.00; H, 7.00.

(b) With methyl sulfoxide–N,N'-dicyclohexylcarbodiimide–pyridinium phosphate To a solution of VI (1.0 g) in 12 ml of anhydrous methyl sulfoxide were added pyridinium phosphate (0.2 g) and *N,N'*-dicyclohexylcarbodiimide (4.3 g). The mixture was stirred for 21 h at room temperature and filtered, and the filtrate was poured into water (20 ml). The solution was extracted with three 75-ml portions of ether, the dried (magnesium sulfate) extract was evaporated, and the residue was crystallized from petroleum ether to give VIII, yield 0.346 g (35%), m.p. 122–124°, identical, by mixed m.p., specific rotation, and infrared spectrum, with the product prepared by method (*a*).

(c) With lead tetraacetate in pyridine. To a solution of VI (1.0 g) in 20 ml of anhydrous pyridine was added lead tetraacetate (~7 g), and the mixture was stirred at room temperature. Aliquots examined by t.l.c. indicated no evidence of conversion of starting material after 3 days. Addition of 3 drops of water caused the solution to turn red, and, after 12 h, it became yellow. At this point, the solution was poured into ice and water (20 ml), the resulting solution was extracted with three 30-ml portions of dichloromethane, and the dried (magnesium sulfate) extract was evaporated to a syrup, yield 780 mg. Examination of the syrup by t.l.c. revealed three zones, R_F 0.47, 0.73, and 0.95, in the approximate intensity ratio 4:1:1. The zone of R_F 0.47 was chromatographically indistinguishable from the starting material VI, the zone of R_F 0.95 was chromatographically indistinguishable from the lactone VIII, and the

zone of R_F 0.73 was chromatographically indistinguishable from 1-*O*-acetyl-2,3:5,6-di-*O*-isopropylidene-α-D-mannopyranose (VII) (see below).

1-O-Acetyl-2,3:5,6-di-O-isopropylidene-α-D-mannofuranose (VII)

To a solution of VI (1.00 g) in 5 ml of anhydrous pyridine was added acetic anhydride (3 ml). The solution was kept for 18 h at room temperature, and was poured into ice and water (50 ml). The mixture was extracted with dichloromethane (300 ml), and the extract was washed with water, dried (magnesium sulfate), evaporated, and distilled at 0.04 mm, to give VII as a chromatographically homogeneous, viscous oil; yield 0.69 g (60%), b.p.$_{0.04}$ 140–142°, $[\alpha]_D^{20}$ + 45 ± 1° (*c* 2.7, chloroform); R_F 0.73; λ_{max}^{film} 5.80 (OAc), 7.25 μ (CMe_2); n.m.r. data: τ 8.67, 8.63, 8.52 (3-, 3-, and 6-proton singlets, respectively, CMe_2), τ 7.95 (3-proton singlet, OAc), τ 5.02–6.18 (multiplets, 6 protons, H-2,3,4,5,6,6′), τ 3.88 (1-proton singlet, H-1).

Anal. Calc. for $C_{14}H_{22}O_7$: C, 55.58; H, 7.33. Found: C, 55.19; H, 7.69.

The starting material VI gave the following n.m.r. data: τ 8.68, 8.60, 8.53 (3-, 3-, and 6-proton singlets, respectively, CMe_2), τ 5.08–6.05 (multiplets, 7 protons, H-2,3,4,5,6,6′, OH), τ 4.43 (1-proton singlet, H-1).

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SUMMARY

Methyl sulfoxide–acetic anhydride is shown to be an effective reagent for the oxidation of an "isolated" secondary alcohol group in a carbohydrate derivative to the corresponding ketone; the reagents are removed by lyophilization to give the product in high yield. The procedure is compared with oxidations employing ruthenium tetroxide, and methyl sulfoxide–*N*,*N*′-dicyclohexylcarbodiimide–pyridinium phosphate (Pfitzner–Moffatt reagent). Oxidation of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (I) gave the 4-ketone (III), which was obtained crystalline in high yield and was characterized as the crystalline oxime (IV) and epoxide (V). The methyl sulfoxide–acetic anhydride reagent was also effective for conversion of the "isolated" hemiacetal derivative VI into the lactone VIII. No significant oxidation of I or VI could be effected with lead tetraacetate in pyridine. The n.m.r. spectra of a number of the products are discussed, and spin-decoupling techniques were applied for analysis of the n.m.r. spectrum of the ketone III.

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Announcement

CARBOHYDRATE GROUP

The inaugural meeting of the Carbohydrate Group will take place on Wednesday, September 28th, 1966, at Burlington House, London. The meeting will be devoted to a business meeting and to the reading of short papers. Membership of the Group, which covers all aspects of carbohydrate chemistry, is open to all. For further details, please write to Dr. R.D. Guthrie, The Chemical Laboratory, University of Sussex, Falmer, Brighton, marking the envelope "Carbohydrate Group".

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OPTICAL ROTATORY DISPERSION OF SUGARS*

II. RELATIONSHIP TO CONFIGURATION AND CONFORMATION OF THE RING FORMS OF KETOHEXOSES AND HIGHER-CARBON SUGARS

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INTRODUCTION

The extensive nuclear magnetic resonance[2] and infrared spectroscopic[3] analyses of sugars in solution have recently been supplemented by optical rotatory dispersion studies[4]. Thus, sugar derivatives containing various chromophores, such as lactones[5], *C*-nitro alcohols[6], and free aldehydo groups[7], exhibit Cotton effects in the spectral region of the absorption bands. Cotton effects have also been observed for nucleosides [8–10] and nucleotides [11,12], but these are in the region of absorption of the purine or pyrimidine ring.

Since the simple sugars in the ring forms contain functional groups that absorb only in the far and vacuum ultraviolet regions, relatively few optical rotatory dispersion studies of the more common sugars were reported until the recent extensions of the spectral range of commercially available spectropolarimeters.

Anomalous optical rotatory dispersion curves in the far-ultraviolet region have been described for a number of sugars[13–15]. In a previous report from this laboratory[1], a set of rules relating configurations and conformations to rotations in the far-ultraviolet region was suggested on the basis of a study of a series of aldopyranoses. The present report represents an extension of these principles to ketoses, some higher-carbon sugar analogs of aldo- and keto-pyranosides, and the furanoid forms of sugars.

RESULTS AND DISCUSSION

The optical rotary dispersion curves of sugars in the various spectral regions reflect the diverse structural features of the ring forms. Those portions of the curves distant from the region of absorption of asymmetric chromophores are usually plain, but become anomalous as the region of absorption is approached. It was suggested[1]

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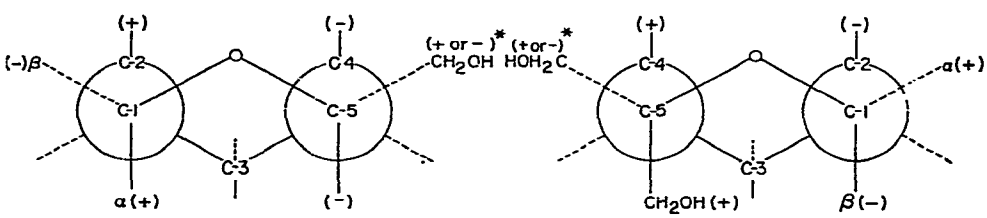
that the optical rotatory data in the 200-mμ spectral region may be interpreted in terms of the stereochemistry of individual asymmetric centers of the pyranoid ring. A sharp transition in the direction of rotation (suggesting the first peak of a Cotton effect) was observed for many sugars at wavelengths below 210 mμ. Evidence was presented, however, that these breaks in the dispersion curves are a consequence of the combined rotational contributions of the individual asymmetric configurations. Therefore, in these cases, the observed transition in the direction of rotation was not the first peak of a Cotton effect, but represented an approach to a Cotton effect which has its peak at wavelengths below 185 mμ.

The ring oxygen atom, which has its absorption maximum[16] below 180 mμ, was considered to be the chromophore having the greatest effect on the shape of the curves in the 200-mμ spectral region. Other chromophores present, such as the hydroxyl group, have their main absorption peaks in regions well below this wavelength. The contribution to the total rotation near 200 mμ by each asymmetric carbon atom is determined primarily by the stereochemical relationship of that atom to the oxygen atom of the ring. In addition, the sign of the first peak of the Cotton effect, which develops below 185 mμ, would be determined primarily by the configuration about the two carbon atoms adjacent to the ring oxygen atom.

On the basis of these considerations, the direction of the rotations in the far-ultraviolet region could be related to individual features of the carbohydrates. The contributions to the direction of the rotation near 200 mμ by substituents in various spatial alignments on the D-aldohexopyranoid ring are summarized in Fig. 1. (See also, Table I in Ref. 1). In this representation, axial components to the left of the ring oxygen atom contribute to the rotation in the positive direction, and those to the right contribute in the negative direction. Equatorial substituents at each carbon atom generally contribute in the direction opposite to that of their axial counterparts.



C1 CONFORMATION *1C* CONFORMATION

Fig. 1. *C1* and *1C* conformations of the D-aldohexopyranoses. Solid and dashed bonds in projections denote axial and equatorial positions, respectively. (+) or (—) represents the contribution of the indicated group to the direction of the optical rotations in the far-ultraviolet region. An asterisk indicates that a positive or negative directional contribution of the group depends on the presence or absence of an axial hydroxyl group on C-4.

These contributions are, however, not necessarily of the same sign in other spectral regions[1]. A similar approach was considered in analyzing the optical rotary dispersion curves of the sugars discussed in the present communication.

The ketoses

The rules for the direction of rotations in the far-ultraviolet region for keto-pyranosides is similar to that for aldopyranosides. Here, C-2 and C-6 are proximal to the ring oxygen atom, compared to C-1 and C-5 in the aldopyranoid series (see Fig. 1). The anomeric carbon atom (C-2) of the 2-ketopyranoses has both a hydroxyl and a hydroxymethyl substituent. According to predictions based on the instability factors of Reeves[17], the hydroxymethyl group at C-2 generally assumes an equatorial disposition.

The optical rotatory dispersion curves for selected ketoses are shown in Figs. 2 and 3. At mutarotational equilibrium, D-fructose exists as the β-D anomer preponder-

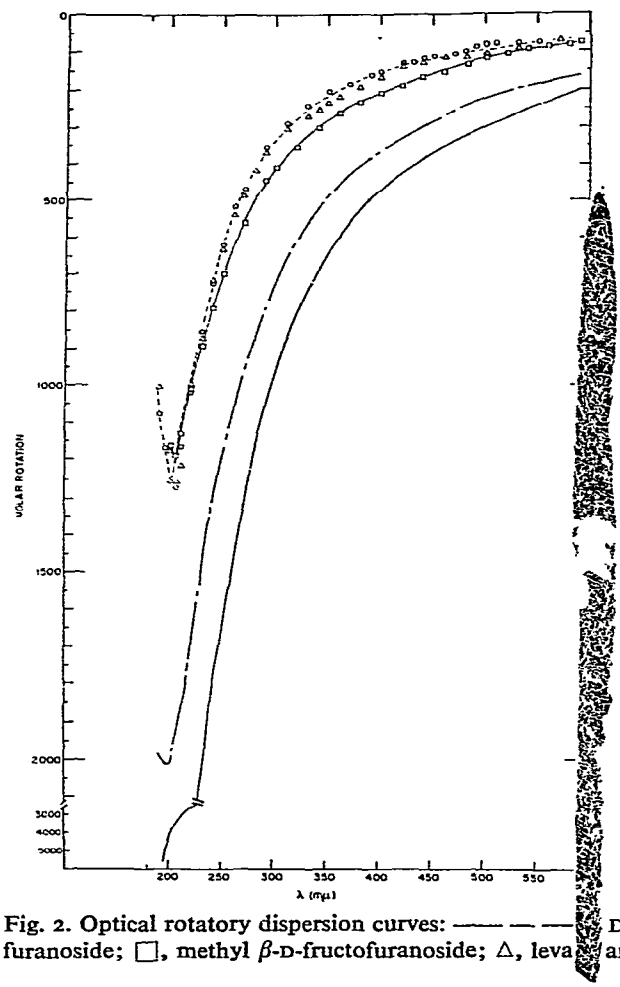


Fig. 2. Optical rotatory dispersion curves: —— — —, D-fructose; ————, ethyl β-D-galactofuranoside; □, methyl β-D-fructofuranoside; △, levan and ○, inulin.

antly in the pyranoid form, and about 20% in the furanoid form[18–20]. The *IC* conformation is most stable for D-fructose, and, therefore, as a first approximation, it may be compared to methyl β-D-arabinopyranoside and methyl α-L-galactopyrano-

side. D-Fructose has lower levorotations than the arabinopyranoside and galactopyranoside throughout the spectral region studied. In the far-ultraviolet region, a very slight shift to the positive direction is evident (see Fig. 2). This is a result of the positive rotatory contribution of the equatorial hydroxymethyl group on C-2 of D-fructose, as predicted from the rule summarized in Fig. 1.

The rotations of D-sorbose (see Fig. 3) gradually increase in the positive direction to about 220 mμ, at which point a broad transition to the negative direction commences. Conformationally, α-D-sorbopyranose is comparable to methyl α-D-xylopyranoside

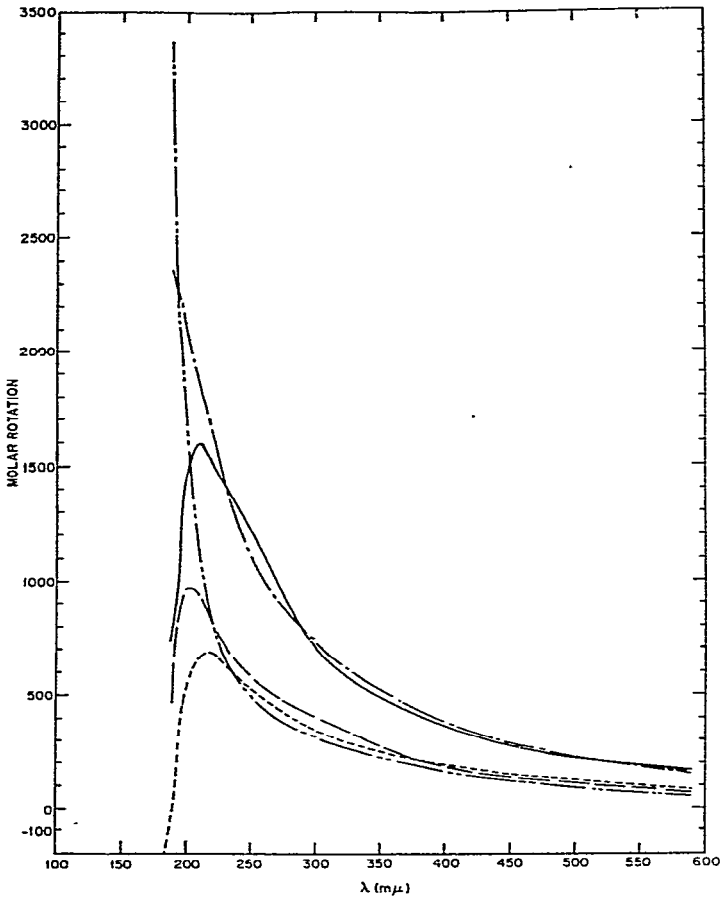


Fig. 3. Optical rotary dispersion curves for some ketoses: ——— — — ———, D-*manno*-heptulose; ——— — ———, D-*gluco*-heptulose; ——————, D-*galacto*-heptulose; ——— ——— ——— ——— ———, D-*glycero*-D-*gulo*-octulose; — — — — , D-sorbose. The molar rotations of D-*galacto*-heptulose are those obtained experimentally for L-*galacto*-heptulose with the rotation sign reversed.

in the *CI* conformation[21,22], but it has an additional hydroxymethyl group on C-2. This hydroxymethyl group contributes to the negative direction of rotation (see Fig. 1), and accounts for the levorotations of D-sorbose in the far-ultraviolet region,

in contrast to the shallow, positive increase in rotations observed with methyl α-D-xylopyranoside[1].

The D-heptulopyranoses (Fig. 3) have equatorial hydroxymethyl groups on both C-2 and C-6. The rotatory contributions of these substituents are, however, not necessarily equal and opposite. It has been shown previously[1] that the configuration of the asymmetric carbon atom adjacent to the carbon atom bearing the hydroxymethyl group (corresponding to C-3 and C-5 in the heptulose series) has a profound influence on the rotatory contribution of the hydroxymethyl group. In addition, the hydroxyl substituent on C-2 of the heptuloses would also affect the rotatory contribution of the hydroxymethyl group at this carbon atom. Thus, because of the differences in the rotatory contributions of the hydroxymethyl groups on C-2 and C-6, curves for the individual heptulopyranoses are not necessarily comparable to those of the corresponding α-(D or L)-aldopentopyranoses.

Optical rotatory dispersion curves for a number of higher-carbon D-ketopyranoses are also shown in Fig. 3. The inflection point and change in rotation toward the negative direction at 210 mμ, observed with D-*galacto*-heptulose and with D-*glycero*-D-*gulo*-octulose, are consistent with the predicted effect of the adjacent, axial, C-5 hydroxyl group on the rotatory contribution of the hydroxymethyl [and D-(1,2-dihydroxyethyl)] group at C-6. For these sugars, both the hydroxymethyl group at C-2 and the substituent at C-6 contribute in the negative direction in the far-ultraviolet region, whereas the axial hydroxyl group at C-2 contributes in the positive direction. For D-*galacto*-heptulose, this results in rotations very similar to those of a mutarotational equilibrium mixture of α- and β-D-galactopyranoses. Throughout the entire spectral range studied, D-*glycero*-D-*gulo*-octulose has greater positive rotations than D-gulose, which exists preponderantly in the β-D form (80%)[23,24]. The effect of replacing the hydroxymethyl group at C-6 by a (1,2-dihydroxyethyl) group will be discussed below.

The hydroxymethyl groups at C-2 and C-6 of D-*gluco*-heptulose are both adjacent to an equatorial hydroxyl group, and thus have almost equal but opposite rotatory contributions. Accordingly, the optical rotatory dispersion curve of D-*gluco*-heptulose (see Fig. 3) is very similar to that of methyl α-D-xylopyranoside, and is distinct from the curve obtained with methyl α-D-glucopyranoside[1]. The hydroxymethyl groups at C-2 and C-6 of D-*manno*-heptulose are both adjacent to axial hydroxyl groups and should have compensatory effects on the rotation. The rotatory dispersion curve of D-*manno*-heptulose should, therefore, be similar to that of methyl α-D-lyxopyranoside.

The rotatory contributions of various positions on the ketopyranose ring can be approximated from an examination of the molar rotation difference curves presented in Fig. 4. The rotations of D-*gluco*-heptulose over the spectral region studied were subtracted from those of D-*galacto*-heptulose (curve A, Fig. 4) to evaluate the rotatory contribution of the configuration at C-5. The sharp decline toward the negative direction near 210 mμ is analogous to that in the curves obtained from the rotational differences of the methyl D-glucopyranosides and methyl D-galactopyran-

osides[1]. This tendency toward negative rotation below 210 mμ for aldopyranosides was considered to result from the interaction of the axial C-4 hydroxyl group with the C-5 hydroxymethyl group[1].

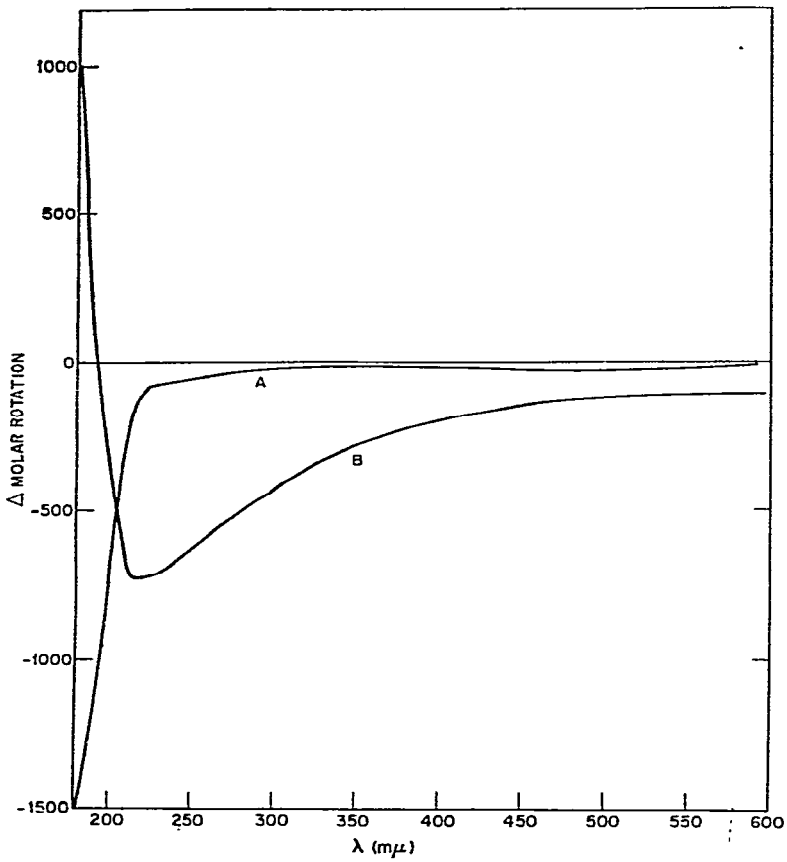


Fig. 4. Molar rotation difference curves. Curve A, D-*galacto*-heptulose minus D-*gluco*-heptulose; Curve B, D-*manno*-heptulose minus D-*gluco*-heptulose.

The effect of an axial hydroxyl group at C-3 of the ketopyranoses was determined by subtracting the rotations of D-*gluco*-heptulose from those of D-*manno*-heptulose (see curve B, Fig. 4). The difference curve is negative, with an inflection point near 210 mμ and a sharp increase toward the positive direction at lower wavelengths. This curve is similar to that obtained by subtracting the curves for methyl α-D-glucopyranoside from those for methyl α-D-mannopyranoside[1]. It is, therefore, evident that an axial hydroxyl group at C-3 of the ketopyranoses contributes in a direction opposite from that contributed by an axial hydroxyl group at C-5. The magnitudes are not equal, however, because of different neighboring substituents at C-2 and C-6, respectively.

The higher-carbon sugars

By replacement of the C-5 hydroxymethyl group in the formulas depicted in Fig. 1 by a *glycero* or *erythro* group, the structures of the corresponding higher-carbon sugars are obtained. The freely rotating (1,2-dihydroxyethyl) or *erythro* group assumes a sterically favored alignment in which the hydrogen atoms at C-5 and C-6 are *trans* to each other (see Fig. 5). Thus, unlike the C-5 hydroxymethyl group in the aldohexopyranose series[1], the alignment of the (1,2-dihydroxyethyl) group is relatively

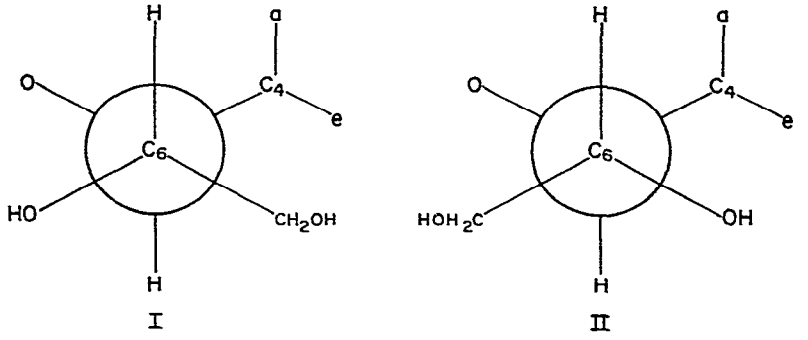


Fig. 5. Alignment of the *glycero* substituent of the higher-carbon sugars. Structure I represents the preferred alignment of a D-(1,2-dihydroxyethyl) group, and Structure II represents the preferred alignment of an L-(1,2-dihydroxyethyl) group.

independent of the configuration at C-4. If it is assumed that, for the higher-carbon sugars studied, the conformations and the percentage distribution of anomeric forms at equilibrium do not differ significantly from those of the corresponding hexoses, the optical rotatory dispersion curves of the heptoses and octoses may be related to those of the corresponding hexoses.

The curve for D-*glycero*-L-*galacto*-heptose (see Fig. 6), although having the same inflection point as the curve for L-galactopyranose[1], does not show so great a tendency toward the positive direction below 205 mμ. The curve for D-*erythro*-L-*galacto*-octose shows no transition to the positive direction, but continues with a shallow, negative slope. Indeed, the curves for other D-*glycero*-L-heptoses shown in Fig. 6 and that for D-*glycero*-L-*gluco*-heptose, not shown in the Figure ($[M]_{190}$ −4100), are all more negative than the curves of the corresponding L-hexoses. Also, the curve for D-*glycero*-D-*gulo*-heptose is more negative than the curve obtained with D-gulose. Thus, for (D or L) sugars, an equatorial D-(1,2-dihydroxyethyl) group at C-5 contributes to the negative direction of rotation in the far-ultraviolet region to a greater extent than a hydroxymethyl group. It follows from the foregoing considerations that an L-(1,2 dihydroxyethyl) group contributes to the positive direction of rotation.

The furanosides

The assessment of the rotatory contribution of a specific configuration at a given carbon atom in the furanoid forms of sugars is not greatly complicated by

interactions with neighboring substituents. On the other hand, the furanoid ring in many sugars[2,25–27] has been shown to be nonplanar, and the position and magnitude of the deviation from planarity must be known before accurate relationships between rotations and overall structure can be inferred. Nevertheless, a rule can be formulated for the direction of the rotatory contribution in the 200-mμ spectral region associated

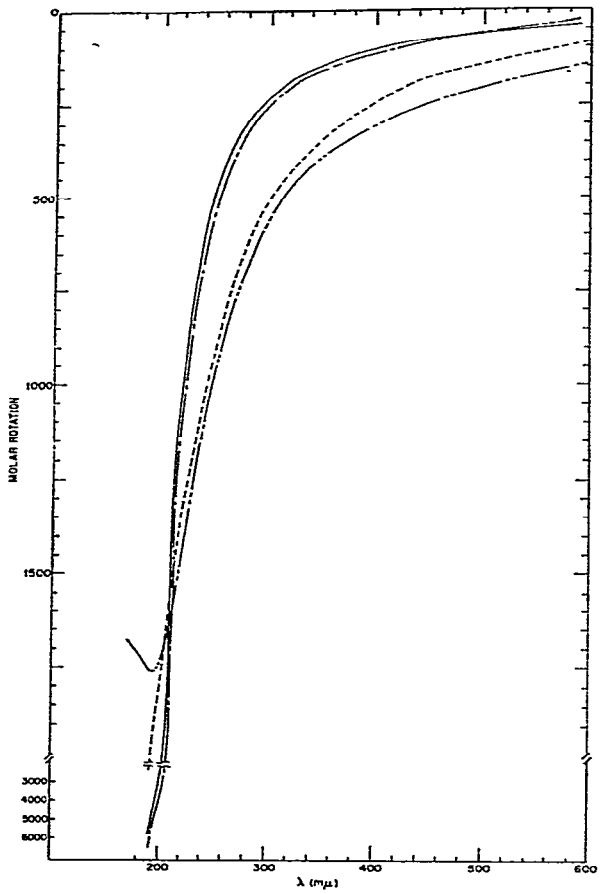


Fig. 6. Optical rotatory dispersion curves for the higher-carbon sugars: ————, D-*glycero*-D-*gulo*-heptose; —— — ——, D-*glycero*-L-*manno*-heptose; —— — — ——, D-*glycero*-L-*galacto*-heptose; — — — —, D-*erythro*-L-*galacto*-octose.

with substituents at carbon atoms adjacent to the furanose ring-oxygen atom. The symmetrical representation in Fig. 7 shows the direction of rotational contribution of substituents at the indicated positions in the furanoid form of a (D- or L-) aldo- or keto-furanose. The similarity of this rule to that devised for pyranoses is evident from Fig. 7. Deviations from planarity would slightly affect the magnitude of rotation, but not the direction of rotation, in the far-ultraviolet region.

In accordance with the rule shown in Fig. 7, methyl α-D-fructofuranoside

([M]$_{200}$ +3410, [M]$_{190}$ +4850), methyl β-D-fructofuranoside, inulin, and levan (see Fig. 2) exhibit rotations which go toward the positive direction in the far-ultraviolet region. Since these fructofuranosides have a hydroxymethyl group at C-5 contributing in the positive direction of rotation, and substituents at C-2 contributing

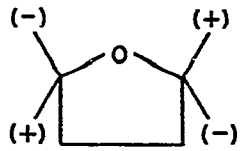


Fig. 7. The furanose ring of sugars: (+) and (—) represent the contribution to the direction of the optical rotations in the far-ultraviolet region for a substituent at the indicated positions.

in both positive and negative directions, their overall rotations are positive. The striking similarities in the rotations of the β-D-fructofuranose derivatives (see Fig. 2), particularly in the far-ultraviolet region, suggest that there is little conformational alteration of the furanose rings in their polymeric forms.

The curve for ethyl β-D-galactofuranoside (see Fig. 2) is negative throughout the spectral range examined. This circumstance may be attributed to the negative rotatory contribution of the (1,2-dihydroxyethyl) substituent at C-4 (see Fig. 7). This effect is also evident in the curve obtained for 1,6-anhydro-α-D-galactofuranose (not shown), where a transition from positive to negative rotation occurs near 210 mμ. The same negative, rotatory contribution of the (1,2-dihydroxyethyl) substituent at C-4 may be predicted for the D-talo-, D-gulo-, and D-ido-furanosides. On the other hand, the (1,2-dihydroxyethyl) substituent at C-4 of the D-gluco-, D-manno-, D-altro-, and D-allo-furanosides should have a positive rotatory contribution.

CONCLUSIONS

A general rule relating overall structure to optical rotation in the far-ultraviolet region, postulated previously for aldopento- and aldohexo-pyranosides[1] (see Fig. 1), has been shown to be applicable to other sugars—in particular, to a number of keto-pyranoses, aldoheptopyranoses, and aldo-octopyranoses. A similar rule describes the optical rotatory behavior of sugars in the furanoid form (see Fig. 7). The direction of rotations in the far-ultraviolet region may also be related to specific configurational features of the ring. For both the pyranoid and furanoid forms (as shown in Figs. 1 and 7, with the ring oxygen atom facing the viewer), substituents to the right and above the ring, and those to the left and below the ring, contribute to the positive direction of rotation. On the other hand, rotatory contributions in the negative direction result from substituents that are to the right and below the ring, or to the left and above the ring.

A change in direction of rotation in the spectral region near 210 mμ frequently occurs prior to the appearance of the first peak of a Cotton effect at lower wavelengths. This inflection point occurs in instances in which the rotational directions associated

with certain portions of the molecule become more prominent, thus influencing the direction of the total molecular rotation at these wavelengths. Since the chromophore having major influence on the shape of the curve in the 200-mμ region is a ring oxygen atom, the configurations at positions adjacent to this oxygen atom have the greatest effect on the direction of rotation. In addition, the configuration at the carbon atom adjacent to a hydroxymethyl group has a pronounced effect on the magnitude and direction of rotation. Thus, the difference curves between ketopyranoses containing axial and equatorial substituents at C-3 and C-5 have shapes similar to those of the corresponding curves obtained for aldopyranoses having axial and equatorial substituents at C-2 and C-4 (see Fig. 4). Because of asymmetric interactions between neighboring positions on a ring, it is difficult to evaluate the rotatory contribution of a specific configuration at a given carbon atom. In this regard, a study of deoxy sugars may prove useful, since certain specific interactions between positions on a ring are precluded. Despite inherent difficulties in quantitation of optical rotation data, information concerning the structure of a sugar may be derived from an analysis of the optical rotatory dispersion curves as shown in this study. Clear distinctions can be made between sugars having specific configurational and conformational differences from an analysis of the dispersion curves in various spectral regions.

EXPERIMENTAL

All the higher-carbon sugars and 1,6-anhydro-α-D-galactofuranose were generous gifts from Dr. N.K. Richtmyer. The methyl β-D-fructofuranoside and bacterial levan were prepared by standard procedures, and all other sugars were commercial samples of high purity. To establish the purity of samples studied, sodium D-line rotations were compared to those reported in the literature.

A Cary model 60 spectropolarimeter was used for the optical rotatory dispersion measurements. The experimental conditions were the same as those reported in the previous paper of this series[1]. The slit widths for these experiments were programmed for a resolution of better than ± 0.75 mμ throughout the entire spectral region. Mutarotations were followed polarimetrically, and the measurements in the spectral range between 600 and 185 mμ were made after mutarotational equilibrium had been achieved.

For most of the sugars, plots of $\frac{I}{[M]_\lambda{}^2}$ *vs* $\frac{I}{\lambda^2}$ throughout the entire spectral region (prior to any transition in direction of rotation) were essentially linear, suggesting that a single-term Drude equation[28] was applicable. The λ_c values calculated from the single-term Drude equation were near 150 mμ for most of the sugars studied.

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SUMMARY

The direction of optical rotatory dispersion curves in the far-ultraviolet spectral region has been related to configuration and conformation of the ring forms of sugars. Rules have been proposed for the study of ketoses, higher-carbon sugars, and furanoid forms of sugars. Agreement has been established with previous treatments of optical rotatory dispersion data for aldopyranoses. Changes in the direction of rotation or inflection points near 210 mμ have been observed for some additional sugars. These transitions did not represent the first peak of a Cotton effect, but were related to specific structural aspects of the ring. Because of neighboring-group interaction, it was not possible to sum, accurately, the rotational contributions of individual configurations on the ring. Nevertheless, it was possible to estimate the magnitude and direction of rotations associated with certain configurational alignments.

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THE ISOLATION OF SOME HEPTOSES, HEPTULOSES, OCTULOSES, AND NONULOSES FROM *PRIMULA OFFICINALIS* JACQ*

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When Nordal and Öiseth[1] examined an aqueous extract of the dried roots of *Primula elatior* (L.) Hill, they established the presence of sedoheptulose (I; D-*altro*-heptulose) by isolating the crystalline di-*O*-benzylidene acetal of sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose). This was the first reported occurrence of sedoheptulose in a plant outside the family Crassulaceae (except for some concurrent studies on photosynthesis by Benson *et al.*[2]) since the discovery of that sugar in *Sedum spectabile* Bor. by LaForge and Hudson[3] in 1917. Nordal and Öiseth obtained paper-chromatographic evidence for the presence of a *manno*-heptulose also. This was the first reported occurrence of that sugar since the discovery by LaForge[4] of D-*manno*-heptulose (II) in the fruit of the avocado (*Persea gratissima* Gaertn.) in 1917; it was isolated in 1954 by Nordal and Benson[5] from avocado leaves.

Although sedoheptulose is now believed to occur extensively in nature (although usually in relatively small amounts), the report of the finding of *two* heptuloses in a single plant seemed novel enough to warrant the effort to obtain a definitive proof of the configuration of the second heptulose. Therefore, with the encouragement of Professor Nordal, we undertook a large-scale study of the higher-carbon sugars in *Primula officinalis* Jacq. Before the completion of this study, however, reports from some other workers were published. Nordal and co-workers[6] detected, by paper chromatography, both sedoheptulose and *manno*-heptulose in the capsules of *Papaver somniferum* L. (opium poppy). Next, Rendig and McComb[7] stated that *manno*-heptulose occurs in alfalfa (*Medicago sativa* L.), and Rendig, McComb, and Hu[8] identified both sedoheptulose and *manno*-heptulose in the leaf-petiole fraction of alfalfa by paper chromatography; the *manno*-heptulose was also identified by its X-ray powder diffraction pattern. Bevenue *et al.*[9] obtained evidence, by paper chromatography, of the presence of sedoheptulose and *manno*-heptulose in both the fruit and the leaf of the fig tree (*Ficus carica* L.), and confirmed this finding through the microscopic identification of a crystalline osazone from each of the isolated sugars[10]. Esau and Amerine[11] examined the residual sugars in a grape wine and, after a paper-

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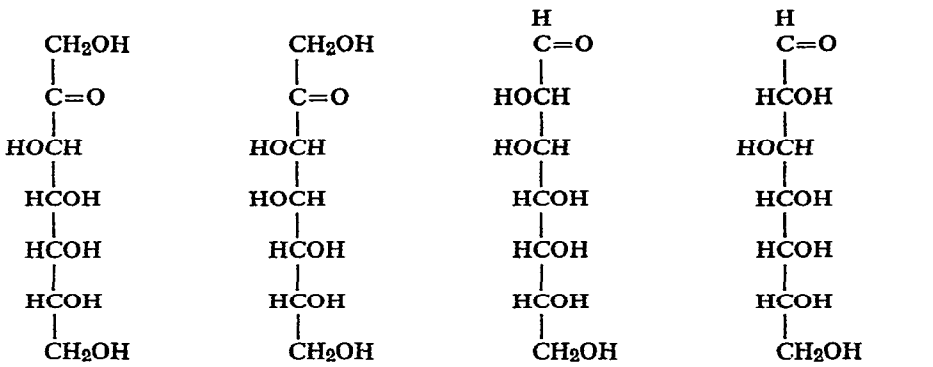
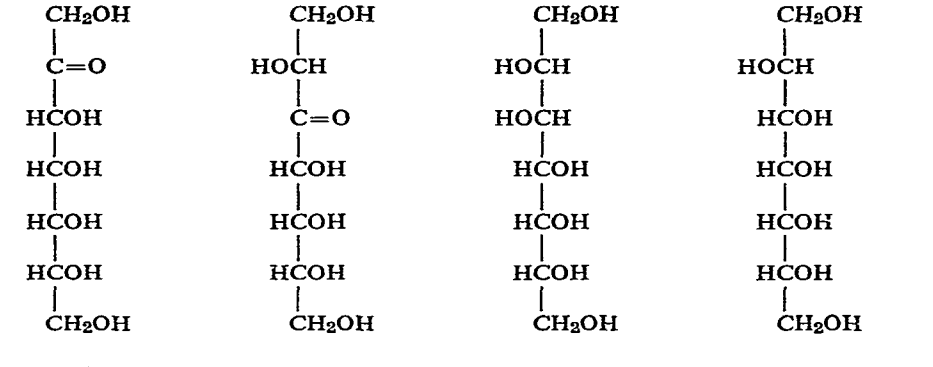
chromatographic study, assumed that the two heptuloses present were *altro*-heptulose and *manno*-heptulose.

Although it seems highly probable that the sugars identified above by paper chromatography and other methods actually are D-*altro*-heptulose and D-*manno*-



I II III IV



V VI VII VIII

heptulose, there is no positive evidence, either from optical rotations or from mixed melting points, that the heptuloses belong to the D series. We have now remedied this lack of evidence, in one case at least, by isolating two heptuloses from the roots of *Primula officinalis* Jacq. and proving, by optical rotations and by mixed melting points with authentic samples, that they are, indeed, D-*altro*-heptulose (sedoheptulose) and D-*manno*-heptulose.

In addition to these two heptuloses, other C_7 sugars have been isolated from natural products in recent years. Several aldoheptoses, for example, have been found

as components of various bacterial polysaccharides[12], and D-*glycero*-D-*galacto*-heptose has been reported as the first aldoheptose found in higher plants[13]. Now, by fractionation of an aqueous extract of *P. officinalis* Jacq., first on a column of cellulose and then on a column of Dowex 50W-X8 (Ba^{2+}), we have isolated sirupy D-*glycero*-D-*manno*-heptose (III) and crystalline D-*glycero*-D-*gluco*-heptose (IV) as the β anomer [m.p. 156–157°; $[\alpha]_D^{20}$ +17 → +46.2° (in water)*]. Identification of these two heptoses was accomplished by conversion into the known[14] D-*glycero*-α-D-*manno*- and D-*glycero*-α-D-*gluco*-heptose hexaacetates. Low-temperature acetylation of the new crystalline heptose has furnished the previously unknown D-*glycero*-β-D-*gluco*-heptose hexaacetate in crystalline form.

The isolation of these two heptoses that are so closely related to D-*altro*-heptulose recalls the isolation, by Ginsburg, O'Brien, and Hall[15], of D-*glycero*-D-*manno*-heptose from a guanosine pyrophosphate nucleotide found in bakers' yeast; they suggested that there may be a parallelism between the heptoses and hexoses with regard to their biosynthesis and incorporation into polysaccharides.

In addition to the long-known D-*manno*- and D-*altro*-heptuloses, one other has recently been reported to occur naturally**, namely, D-*talo*-heptulose from the avocado[19]. Although crystalline D-*talo*-heptulose[20] was not isolated, considerable evidence was obtained to establish the identity of the sugar.

We have now isolated crystalline D-*allo*-heptulose (V) from *P. officinalis* extract, and have identified it conclusively by direct comparison with a sample of the synthetic sugar[20]. Another heptulose that we have obtained in crystalline form from the fractionation of the *P. officinalis* extract has been identified as D-*altro*-3-heptulose (VI) through direct comparison with a product obtained from the rearrangement of sedoheptulose (I) in boiling pyridine; the structure of that product was based upon its reduction with borohydride to D-*glycero*-D-*manno*-heptitol (VII) and D-*glycero*-D-*altro*-heptitol (VIII). This is the first reported isolation of a 3-heptulose from a plant source.

Although compounds III to VI have, at C-4 to C-7, the same configuration as sedoheptulose (I), and although it is conceivable that they were formed by isomerizations occurring at C-1 to C-3 during the course of their isolation, we do not believe them to be artifacts but, rather, that their formation was caused by the action of enzymes within the plant itself.

Two octuloses have recently been discovered in nature, namely, D-*glycero*-

*In a personal communication, Professor Donald L. MacDonald, of Oregon State University, has informed us that he and Mr. Roger Wong have crystallized this sugar independently; they found m.p. 150–151° (dec.) and $[\alpha]_D^{20}$ +18 (5 min)→+47° (in water).

**Still other heptuloses have been obtained from plants by deliberately feeding them with precursors. McComb and Rendig (Ref. 16) fed L-sorbose to alfalfa shoots, and isolated L-*galacto*-heptulose. When Rendig and McComb (Ref. 17) fed D-ribose, they found that D-*altro*-heptulose accumulated; L-arabinose similarly gave L-*gluco*-heptulose, D-xylose gave D-*ido*-heptulose, and L-lyxose gave L-*galacto*-heptulose. The same authors (Ref. 18) later showed that each of the four tetroses induces an accumulation of a specific heptulose that could be identified through paper-chromatographic examination of the extract of the alfalfa leaves to which the tetroses had been fed.

D-*manno*-octulose in the avocado[13,19,21], in *Sedum*[19], and, probably, in a red wine[11]; and D-*glycero*-L-*galacto*-octulose in the avocado[13] and, probably, in *Sedum*[22]. We have now isolated the same two octuloses from the *P. officinalis* extract, and have identified them by direct comparisons of paper chromatograms, opticai rotations, infrared spectra, and crystalline derivatives with those of the octuloses from the avocado and with those of the same octuloses previously synthesized in this laboratory[13,19]. Degradations modeled on those employed in their original characterization by Charlson and Richtmyer[19,21] and by Sephton and Richtmyer[13] have furnished confirmatory evidence for these structures.

Two nonuloses also have been reported to occur naturally, namely, D-*erythro*-L-*gluco*-nonulose[22] and D-*erythro*-L-*galacto*-nonulose[23], both in the avocado, and both, probably, in *Sedum*[22]. We have now isolated the same two nonuloses from *P. officinalis*, and identified them by direct comparisons and by degradations modeled on those employed in their original characterization by Sephton and Richtmyer[22,23].

In addition to volemitol (D-*glycero*-D-*manno*-heptitol, VII), which was first isolated from the roots of *Primula* species (including *P. grandiflora* Lam., *P. elatior* Jacq., and *P. officinalis* Jacq.) by Bougault and Allard[24], following its earlier discovery in the mushroom *Lactarius volemus* Fr. by Bourquelot[25], we have isolated β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol) from the *P. officinalis* extract. Although the latter heptitol had previously been reported as accompanying sedoheptulose in *Sedum* species[19], this appears to be the first example of two heptitols occurring in the same plant.

Primeverose (6-*O*-β-D-xylopyranosyl-D-glucose) is the carbohydrate constituent of a number of naturally occurring phenolic glycosides, including primeverin and primulaverin from the fresh roots of *Primula officinalis* Jacq.[26]. We were not surprised, therefore, when we found the free sugar in the aqueous extract of the dried roots of that plant, even though primeverose has been isolated directly only once previously: Wallenfels and Lehmann[27] obtained it from ripe carob beans (St. John's bread; *Ceratonia siliqua* L.). A portion of the primeverose was reduced with borohydride, to yield the new, crystalline primeveritol, which was characterized further as its crystalline octaacetate.

The other substances that we isolated from the *P. officinalis* extract and then identified were glycerol, erythritol, D-xylose, xylitol, and *myo*-inositol. Although xylitol had been detected previously[28] in the edible mushroom *Psalliota campestris* by means of a specific enzyme called TPN-xylitol (L-xylulose) dehydrogenase[29], we believe that this is the first time that xylitol has been isolated in crystalline form from a plant source.

EXPERIMENTAL

General methods

Paper chromatography was carried out with Whatman No. 1 filter paper by the descending method at room temperature. The following solvent systems were

used: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); B, ethyl acetate–pyridine–water (10:4:3); C, the same as A, except that it contained 0.5% of benzeneboronic acid[30]; and D, butyl alcohol–pyridine–water (6:4:3). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, alkaline hydroxylamine–ferric chloride for lactones, and silver nitrate (ammoniacal, or followed by sodium hydroxide in ethanol) or 0.25% sodium periodate in 5% aqueous acetic acid followed by 2% *p*-anisidine in 5% acetic acid for alditols, sugars, and other polyhydroxy compounds. The values for R_{Rha}, R_{Sed}, and R_{Fru} refer to the rate of movement of the compounds on paper chromatograms (developed in solvent A, unless otherwise noted) relative to that of rhamnose, sedoheptulosan, and fructose. All concentrations were carried out at temperatures below 50°, and final drying of sirups was effected over granular calcium chloride *in vacuo* or by lyophilization. Unless otherwise specified, melting points were determined on a Kofler micro hot-stage, and infrared spectra were measured in Nujol mulls.

Preparation of the extract

Twenty kg of the dried roots of *Primula officinalis* Jacq.* was ground to a fine powder and extracted in 500-g batches. Each 500 g was suspended in 8 l of distilled water, 50 g of calcium carbonate was added to neutralize any acidity that might develop, and the mixture was digested for 8 h on a steam bath. About 150 g of a filter aid (Johns–Manville Celite 535) was stirred into the mixture, which was then filtered through a large Büchner funnel precoated with about 100 g of the same filter aid. The filtration was very slow. The residue on the funnel was extracted a second time with 8 l of water. The combined, dark-colored extracts were deproteinized by dissolving 200 g of zinc sulfate heptahydrate in the solution and adding slowly, with mechanical stirring, a saturated aqueous solution of barium hydroxide until the main solution was nearly alkaline to phenolphthalein paper. About 150 g of decolorizing carbon (Darco G-60) was stirred into the mixture, and the mixture, after being kept for about 2 h to let the precipitated material coagulate and settle, was filtered through a large Büchner funnel. The clear, almost colorless filtrate was deionized by successive passage through columns of Amberlite IR-120(H^+) and Duolite A-4 (OH^-) ion-exchange resins. Unless the aqueous solution was quite dilute (volume at least 30 l), a fine, white precipitate formed in the Amberlite column, and retarded or even stopped the flow of solution through the resin; the precipitate was suspected to be a rather insoluble, acidic substance, but no attempt was made to establish its identity.

When the deproteinized, deionized extracts from ten batches of *P. officinalis* were combined, and concentrated *in vacuo* to a thick sirup, the volemitol (D-*glycero*-D-*manno*-heptitol ≡ D-*glycero*-D-*talo*-heptitol, VII) crystallized spontaneously. It was isolated by redissolving the mixture in hot water, diluting with methanol, cooling, and filtering off the prismatic needles. Identification was made through its m.p. of

*This material was purchased from S.B. Penick & Company, 100 Church Street, New York, N.Y., through the special cooperation of Mr. Hans R. Schmidt.

154–155° (alone, and when mixed with authentic material[31]), by comparison of infrared spectra, and through the preparation of its heptaacetate[31], having m.p. 62–63° and $[\alpha]_D^{20}$ +36.0° (*c* 1.3, dichloromethane). The total yield of 344 g from 20 kg (1.7%) is comparable to the 1.5% recorded by Bougault and Allard[24].

Concentration *in vacuo* of the volemitol filtrates yielded 152 g of a sirup whose paper-chromatographic examination in solvents A and B indicated the presence of at least twelve components, including relatively large proportions of D-glucose, D-fructose, and sucrose. These last three were removed by dissolving the sirup in 3.2 l of water, adding 1.25 cakes of bakers' yeast and 75 g of D-glucose as a primer, and allowing the mixture to ferment for 3 days at 37°. Successive deproteinization, deionization, and concentration *in vacuo* yielded 84 g of sirup in which xylose, *manno*-heptulose, sedoheptulose, and primeverose could be tentatively identified through their mobilities and color reactions on paper chromatograms. In a separate experiment, a mixture of D-glucose, D-fructose, D-xylose, D-arabinose, and D-ribose was fermented with bakers' yeast; no higher-carbon aldose or ketose could be detected. Bevenue *et al.*[32] had similarly fermented pure sucrose, but could detect no formation of heptulose.

Fractionation of the extract on a cellulose column

A glass tube (100 × 10 cm) was packed with 2 kg of Whatman standard-grade, cellulose powder (effective height, 68 cm) and the 84 g of sirupy extract, made into a smooth slurry with 150 g of cellulose powder and 750 ml of quarter-saturated aqueous butyl alcohol, was put on top of the cellulose column, by the procedure described earlier[13]. Elution was begun with quarter-saturated aqueous butyl alcohol, continued in a stepwise manner with half- and three-quarter-saturated aqueous butyl alcohol, and concluded with fully saturated aqueous butyl alcohol. With the aid of an automatic fraction-collector, the eluate was distributed among 14,200 tubes, each containing 21 ml; the contents of these tubes were combined on the basis of their paper-chromatographic assays, and concentrated to give 66 fractions. The constituents of these fractions, insofar as they have been identified, are reported below.

Glycerol

Fractions 9–11 (tubes 331–550; 4.68 g) appeared to contain glycerol (R_{Rha} 1.20) as the principal constituent. Accordingly, a small portion of the thick, mobile liquid of fraction 10 was heated with *p*-nitrobenzoyl chloride and pyridine for 2 h at 80–90°, and the product isolated; it was identified as glycerol tris-*p*-nitrobenzoate, m.p. 195–196° (capillary tube) both alone and when mixed with authentic material[33]. The infrared spectra of the two substances were identical.

Erythritol

Fraction 12 (tubes 551–700; 1.04 g) crystallized in part, and furnished 0.78 g of stout prisms (R_{Rha} 0.96). After one recrystallization from aqueous ethanol, the erythritol melted at 120–122°, both alone and when mixed with authentic material; a comparison of the infrared spectra confirmed the identification.

*2,7-Anhydro-β-*D-altro*-heptulofuranose*

Fraction 13 (tubes 701–885; 0.52 g) yielded 24 mg of stout prisms (R_{Sed} 1.37 and R_{Fru} 1.23 in solvent D) that melted at 195–196°, alone, and at 195–197° when mixed with authentic anhydro compound[34] of m.p. 198-200° (all in capillary tubes). Identification was confirmed through paper-chromatographic and infrared-spectral comparisons.

*α-*D*-Xylose*

Fractions 15 and 16 (tubes 1011–1130; 1.02 g) showed strong evidence for xylose (R_{Rha} 0.74) as the only carbohydrate constituent, and prisms of α-D-xylose were separated from the sirup and also from fraction 14. After recrystallization from aqueous methanol, the compound had m.p. 151–152° and $[\alpha]_D^{20}$ +95° (3 min)→ +20.5° (*c* 1.5, water), in good agreement with the recorded values[35] of m.p. 153° and $[\alpha]_D^{25}$ +96° → +20°. A mixture of the compound with authentic α-D-xylose melted at 151–153°, and the infrared spectra of the two samples were identical.

Subfractionation on Dowex 50W-X8 (Ba^{2+}) resin

After the initial separation of the *Primula officinalis* constituents on a cellulose column, some of the fractions were resolved further on a column of Dowex 50W-X8 (Ba^{2+}) ion-exchange resin[36]. To this end, the resin (200–400 mesh) was converted into its barium form by treatment with several portions of aqueous barium chloride (barium acetate would probably be better, to avoid possible release of hydrogen chloride that might occur later because of incomplete washing), and washed thoroughly with water. The resin was poured, as a slurry, into a glass tube (2.5 cm, inside diameter) equipped with a needle-valve stopcock and with a plug of polyurethane foam in the bottom to retain the resin; after the resin had settled, the effective height of the column was 116 cm. The fraction to be investigated was dissolved in the minimum amount of water, and the solution was added to the top of the column; after the solution had passed into the resin at the top of the column, elution was effected with water at a flow rate of 4–8 ml/h, and the eluate was collected automatically in 1- to 1.5-ml portions. In many cases, these fractions were chromatographically pure. Tubes were combined according to their chromatographic behavior, and the solutions were treated with small amounts of Amberlite IR-120 and Duolite A-4 ion-exchange resins to insure that they would be free from ionic material before being concentrated.

Xylitol

Fractions 17–19 (tubes 1131–1370; 3.09 g) showed the presence of three orcinol-positive compounds having R_{Fru} 0.88, 0.99, and 1.09 on paper chromatograms. The first and third spots corresponded to sedoheptulose and sedoheptulosan, but for the complete identification of the intermediate spot we resorted to subfractionation on Dowex 50W-X8 (Ba^{2+}) as described above. During that procedure, an intermediate fraction (420 mg) crystallized very slowly from its solution in methanol. The m.p. of 92–93°, and the chromatographic mobility, suggested xylitol, and the infrared spec-

trum was indistinguishable from that of an authentic sample of xylitol. Upon acetylation with acetic anhydride and fused sodium acetate, the compound afforded xylitol pentaacetate as hexagonal plates (from aqueous ethanol); the m.p. was 62–63° alone (as well as when mixed with authentic material), and the infrared spectra of the two specimens of pentaacetate were identical.

D-altro-*3-Heptulose* (VI)

After the xylitol had been eluted in the subfractionation of fractions 17–19 on Dowex 50W-X8 (Ba^{2+}) resin, the next tubes yielded D-*altro*-3-heptulose (VI; m.p. *ca.* 165°, $[\alpha]_D^{20}$ *ca.* +20°). On paper chromatograms sprayed with orcinol–hydrochloric acid, it appeared as an orange-brown spot that sometimes faded to a grayish-brown color; the isomeric D-*manno*-3-heptulose has been described by Schaffer[37] as forming a gray-brown spot with the orcinol–trichloroacetic acid spray reagent. The infrared spectrum of the compound isolated from fractions 17–19 was indistinguishable from that of a compound isolated following the rearrangement of sedoheptulose (D-*altro*-heptulose) in boiling pyridine; their chromatographic mobilities were the same, and the m.p. of a mixture of the two substances was not depressed. The sodium borohydride reduction of the sample isolated from the latter source yielded D-*glycero*-D-*manno*-heptitol (VII) and D-*glycero*-D-*altro*-heptitol, which were identified by comparison of melting points and infrared spectra with those of known specimens of these heptitols. Further details of the synthesis and properties of D-*altro*-3-heptulose will be included in a later publication.

D-allo-*Heptulose* (V)

The final tubes from the subfractionation of fractions 17–19 on Dowex 50W-X8 (Ba^{2+}) resin contained a heptulose that was readily separated from the other constituents through its relatively high retention-volume. Crystals were obtained by the slow evaporation of a solution of the sirupy sugar in methanol. Recrystallization from 95% ethyl alcohol yielded long needles whose melting-point behavior (75–80° on rapid heating, 90–97° on slow heating, and 128—130° after being dried at 75° *in vacuo* for 24 h) was characteristic of an *allo*-heptulose hydrate[38,39]. The *allo*-heptulose structure received confirmation through paper chromatography and by comparison of an infrared spectrum of the hydrate with that of a sample prepared by crystallization of authentic, anhydrous D-*allo*-heptulose* from 95% ethyl alcohol. A mixture of the two anhydrous forms showed no depression of the melting point. Finally, a phenylosazone was prepared by heating 35 mg of the sirupy heptulose (obtained by concentrating the mother liquor from which the crystalline D-*allo*-heptulose had separated) with 0.08 ml of phenylhydrazine and 0.04 ml of glacial acetic acid in 1.3 ml of 2-methoxyethanol[40] for 3 h at 100°. The reaction mixture was poured onto ice, and the yellow precipitate was collected on a sintered-glass funnel and washed successively with 10% acetic acid and water. After one recrystal-

*Kindly supplied by Dr. Robert Schaffer.

lization from ethyl alcohol, the yellow needles (24 mg) of D-*allo*-heptosulose bis(phenylhydrazone) melted at 164–167°, a value that was not significantly different from that of a mixture of this compound and the phenylosazone prepared similarly from authentic* D-*glycero*-D-*allo*-heptose[38]. The infrared spectra of the two phenylosazones were identical.

Sedoheptulose (D-altro-*heptulose*, I)

Paper chromatograms indicated that fractions 18–27 contained both sedoheptulose and sedoheptulosan (2,7-anhydro-β-D-*altro*-heptulopyranose). To verify this conclusion, fraction 23 (tubes 1731–1850; 0.89 g) was dissolved in 25 ml of water containing 0.4 ml of concentrated sulfuric acid, and the mixture was heated for 6 h on the steam bath, cooled, neutralized with barium carbonate, and filtered, and the filtrate deionized by successive treatments with Amberlite IR-120(H^+) and IR-45(OH^-) resins, and concentrated to a sirup. When the sirup was dissolved in a small amount of hot methanol, and the solution cooled and inoculated with a seed crystal, 0.62 g of the anhydrous modification of sedoheptulosan was obtained. After three recrystallizations from methanol, the clear, chunky prisms melted at 155–156° both alone and when mixed with authentic sedoheptulosan; $[\alpha]_D^{20}$ −145° (*c* 1, water); and the infrared spectrum was indistinguishable from that of authentic material. For further identification, a portion of the crystalline product obtained from fraction 23 was benzoylated, to give a substance having m.p. 164–165° and $[\alpha]_D^{20}$ −185° (*c* 0.5, dichloromethane), in good agreement with the reported values[41] of 165–166° and −188°, respectively. A mixture with authentic sedoheptulosan tetrabenzoate melted at 164–165°.

D-manno-*Heptulose* (II)

Fraction 28 (tubes 2331–2570; 0.72 g) deposited chunky prisms of D-*manno*-heptulose from its concentrated solution in methanol. The sugar had m.p. 152–153° alone, as well as when mixed with authentic material; its rotation, $[\alpha]_D^{20}$ +29° (*c* 1, water), agreed with the reported rotation; and infrared spectra of the two specimens were identical. The identity was confirmed by treating the mother liquor (from which the crystals had been separated) with a mixture of phenylhydrazine and 1-benzyl-1-phenylhydrazine according to the directions of White and Secor[42]. The resulting D-*manno*-heptosulose 1-(2-benzyl-2-phenylhydrazone) 2-phenylhydrazone, after two recrystallizations from absolute ethyl alcohol, melted at 199–200° when heated at a rate of 1.5°/min up to 180° and then at 1°/min. A mixture with authentic material showed no significant depression of the melting point, and the identity of the infrared spectra of the two samples confirmed their identity. Analyses for C, H, and N were also confirmatory.

*Dr. J.W. Pratt had prepared the same phenylosazone from D-*glycero*-D-*allo*-heptose, but its description had been omitted from the publication (Ref. 38).

Anal. Calc. for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.43. Found: C, 59.00; H, 6.15; N, 14.27.

The infrared spectrum of the phenylosazone prepared by Dr. Pratt was indistinguishable from the other two spectra. (N.K.R.)

Volemitol (VII)

In addition to the 344 g of volemitol that had crystallized before the *Primula officinalis* extract was fractionated on cellulose, 8 g crystallized later from fractions 32–38 (tubes 3171–6300; 9.6 g).

D-glycero-D-manno-*Heptose* (III)

The subfractionation, on Dowex 50W-X8 (Ba^{2+}) resin, of 1.31 g of fractions 29–33 (tubes 2571–4100; 4.04 g, from which 3.40 g of crystalline volemitol had been removed) yielded a middle fraction of 363 mg of sirup that appeared, from paper-chromatographic evidence, to contain a heptose, but no heptulose or other ketose. Since the product did not crystallize, an 86-mg portion of it was heated with 43 mg of fused sodium acetate in 5 ml of acetic anhydride for 5 h at 110°, and 114 mg of crude, sirupy acetate was isolated in the usual way. After decolorization with Darco X, and reconcentration, a solution of the sirup in a small amount of 60% aqueous methanol began to deposit crystals after standing at 0° for 2 weeks. After one recrystallization from aqueous methanol, the prisms were identified as D-*glycero*-α-D-*manno*-heptose hexaacetate through a melting point of 137–138° alone, and 137–139° when mixed with authentic material[14] of m.p. 138–139°. The identity of the two hexaacetates was confirmed by comparison of their infrared spectra.

Crystalline D-glycero-β-D-gluco-*heptose*

Fractions 34–36 (tubes 4101–5810; 5.12 g, from which 4.08 g of crystalline volemitol had been removed) appeared to contain an octulose. To isolate it, the remaining 916 mg of these fractions was chromatographed on a Dowex 50W-X8 (Ba^{2+}) resin column, as described earlier. Of the 8 subfractions thus obtained, subfractions 2–4 were richest in octulose; these were combined (530 mg), and rechromatographed on a cellulose column (90 × 2.2 cm) with 92.5% aqueous acetone as eluent. Of the 12 new fractions thus obtained, fractions 7 and 8 deposited 43 mg of crystals when their concentrated solutions in methanol were kept in a refrigerator for about 10 days. The compound, after recrystallization from methanol, melted at 156–157° (unchanged after a second recrystallization) and showed $[\alpha]_D^{20}$ +17° (extrapolated)→ +46.2° (6 h, constant; *c* 2.4, water). Its eventual identification as D-*glycero*-β-D-*gluco*-heptose was based on the evidence that follows.

Anal. Calc. for $C_7H_{14}O_7$: C, 40.00; H, 6.71. Found: C, 40.15; H, 6.87.

On a paper chromatogram sprayed with aniline hydrogen phthalate, the compound gave the brown color that is characteristic of hexoses and higher aldoses; its mobility was less than that of any of the hexoses. When oxidized with 1.25 molecular equivalents of lead tetraacetate*, a paper chromatogram showed ribose as the only

*Perlin and Brice (Ref. 43) used 2 molecular equivalents of oxidant. We used less than that, in an attempt to identify, also, the hexose that should be an intermediate in the degradation. Failure to obtain any chromatographic evidence for such an intermediate here, and in other oxidations to be described later, may be the result of a very rapid oxidation of the intermediate, together with the use of too small an amount of substrate.

degradation product. For acetylation, 25 mg of sirupy heptose (recovered from measurements of optical rotation) was dissolved in 5 ml of acetic anhydride containing 7 mg of freshly fused zinc chloride, and the mixture was kept for 6 h at room temperature. The product, isolated in the usual way, was identified as D-*glycero*-α-D-*gluco*-heptose hexaacetate through (*a*) its m.p. 180–182°, alone as well as when mixed with authentic material[14], (*b*) its rotation $[\alpha]_D^{20}$ +105° (*c* 1, dichloromethane), and (*c*) a comparison of infrared spectra. Finally, deacetylation of a sample of authentic α-hexaacetate[14] with methanolic sodium methoxide yielded crystalline D-*glycero*-β-D-*gluco*-heptose, indistinguishable in m.p. and infrared spectrum from those of the substance isolated from the subfractionations of fractions 34–36 as described above.

D-glycero-β-D-gluco-*Heptose hexaacetate*

The acetylation of 200 mg of D-*glycero*-β-D-*gluco*-heptose at 0° with a mixture of 1 ml of acetic anhydride and 1.5 ml of pyridine yielded 405 mg of the corresponding β-hexaacetate. After recrystallization from aqueous ethyl alcohol, and then several times from dichloromethane–pentane, the clusters of small prisms of the new hexaacetate melted at 133–134° and showed $[\alpha]_D^{20}$ +19.6° (*c* 1, chloroform).

Anal. Calc. for $C_{19}H_{26}O_{13}$: C, 49.35; H, 5.67; CH_3CO, 55.9. Found: C, 49.45; H, 5.39; CH_3CO, 56.3.

β-Sedoheptitol (D-glycero-D-gluco-*heptitol*; L-glycero-D-talo-*heptitol*)

As described above, the further separation of fractions 34–36 on Dowex 50W-X8 (Ba^{2+}) yielded 8 subfractions. Of these, subfractions 5–7 deposited, on standing, 38 mg of a crystalline substance that was identified as β-sedoheptitol through direct comparison (m.p., mixed m.p., paper chromatography, and infrared spectra) with an authentic sample of m.p. 129–130°, prepared by the reduction of sedoheptulose.

D-glycero-D-manno-*Octulose*

As described above, fractions 34–36 were fractionated on Dowex 50W-X8 (Ba^{2+}) resin, and the octulose-rich subfractions 2–4 were rechromatographed on a cellulose column. The new fractions 7–8 (from which 43 mg of crystalline D-*glycero*-D-*gluco*-heptose had been removed) and 9–11 were combined (288 mg of sirup) and dissolved in 14 ml of water, and the remaining aldoheptose was oxidized in the dark with 0.3 ml of bromine in the presence of an excess of barium carbonate. After 18 h, the excess of bromine was removed by aeration, and the solution was filtered and then deionized with a mixture of Amberlite IR-120 (H^+) and IR-45 (OH^-) ion-exchange resins. Concentration of the solution gave 98 mg of a yellowish sirup that contained an octulose, contaminated (as determined by paper chromatography) with lactones and a small proportion of a hexulose-containing disaccharide. These impurities were removed by chromatography on sheets of Whatman 3MM filter paper developed in solvent A. In this manner, there was obtained 40 mg of chromatographically pure, sirupy D-*glycero*-D-*manno*-octulose having R_{Sed} 0.46 and $[\alpha]_D^{20}$ +25.2° (*c* 2, 90% aqueous methanol); the latter value is comparable to the value of +26.5° (*c* 5,

methanol) reported previously[13] for the same octulose isolated from the avocado, and to the value of +27° (*c* 1, methanol) measured for the same octulose synthesized by the diazomethane method[44].

The infrared spectrum of the D-*glycero*-D-*manno*-octulose from *Primula officinalis* roots, obtained for a dried film from methanol, was indistinguishable from those of the same octulose isolated from two avocado varieties and from *Sedum* species, as well as from the spectrum of the synthetic specimen[44]. The (2,5-dichlorophenyl)hydrazone prepared from this *Primula* octulose, after recrystallization from aqueous methanol as yellowish needles, melted at 171–172°; a mixed melting point with the product prepared from the same octulose from the avocado[19,21] was undepressed, and the infrared spectra of the two compounds were identical. Degradation of this *Primula* octulose with two molecular equivalents of lead tetraacetate, as described previously[19,21], yielded, as reported, a single pentose whose mobility on paper chromatograms (developed in solvents A and B) was the same as that of ribose.

Finally, by the procedures described previously[22,45], 4.7 mg of the sirupy D-*glycero*-D-*manno*-octulose from *Primula officinalis* roots was converted into a mixture of the corresponding methyl octulosides; oxidation with one molecular equivalent of sodium metaperiodate, followed by reduction of the aldehyde group with sodium borohydride and hydrolysis of the methyl glycosides, yielded 2.5 mg of a reducing sugar. Its chromatographic mobility in solvents A, B, and C was, as previously reported, identical with that of *manno*-heptulose; and, on a paper chromatogram sprayed with orcinol–hydrochloric acid, it gave the characteristic greenish-blue color reaction of *manno*-heptulose instead of the blue color that is given by the other heptuloses.

D-glycero-L-galacto-*Octulose*

The original fractions 34–36 had been fractionated further on Dowex 50W-X8 (Ba^{2+}) resin as described above, and the subfractions 5–7 (after removal of 38 mg of crystalline β-sedoheptitol) were then found to contain a second octulose. This sirup (140 mg) was fractionated further, on sheets of Whatman 3MM filter paper developed in solvent A, and 13.5 mg of a (chromatographically) practically pure octulose (R_{Sed} 0.42) was thus obtained. Its mobility on paper chromatograms developed in solvents A, B, and C was the same as that of the known D-*glycero*-L-*galacto*-octulose[13,45], and its infrared spectrum, obtained for a dried film from methanol, was very closely similar to that of the previously described octulose[13]. Degradation of 1.8 mg of this second octulose from *Primula* roots, with somewhat more than one molecular equivalent of lead tetraacetate in glacial acetic acid, furnished xylose as the only pentose detectable on a paper chromatogram (solvents A and B). This second octulose yielded a (2,5-dichlorophenyl)hydrazone whose melting point of 176–178° (unrecrystallized) was not depressed significantly (175–179°) when it was mixed with the corresponding derivative of D-*glycero*-L-*galacto*-octulose from the avocado[13].

D-erythro-L-gluco-*Nonulose*

From their behavior on paper chromatograms, fractions 37–41 (tubes 5811–7130; 2.39 g) appeared to contain two nonuloses; these were separated relatively easily on a column of Dowex 50W-X8 (Ba^{2+}) resin. The first nonulose was purified further by chromatography on a cellulose column, with elution by 92.5% aqueous acetone (which removed much non-nonulosic material) followed by methanol. Final purification of the 148 mg thus obtained was effected on Whatman 3MM filter paper developed with solvent A. The middle zone from this chromatogram yielded 59 mg of chromatographically pure D-*erythro*-L-*gluco*-nonulose. Its rotation of $[\alpha]_D^{20}$ −42.8° (*c* 1.3, water) is intermediate between the values of −40.0° for the same nonulose isolated from the avocado[22] and −47.2° for the synthetic nonulose[22,46]. The infrared spectrum of this nonulose, obtained for a dried film from methanol, was indistinguishable from that of the synthetic D-*erythro*-L-*gluco*-nonulose[22]. In confirmation of earlier findings[22], degradation of 13.9 mg of this nonulose with 2 molecular equivalents of lead tetraacetate in 98% acetic acid, by the procedure described earlier, yielded 5.7 mg of a sirup identified by chromatography in solvents A and B, and by its rotation of $[\alpha]_D^{20}$ +10° (*c* 0.57, water), as D-mannose; neither a pentose nor a heptose was detectable. For confirmation, 3.7 mg of the sirup containing D-mannose was dissolved in 0.075 ml of water; to the solution was added 0.055 ml of redistilled phenylhydrazine, 0.055 ml of glacial acetic acid, and 0.105 ml of water and the mixture was kept overnight at 0°; 4 mg of white needles of D-mannose phenylhydrazone was deposited. The m.p. was 198–199° (dec.), both alone and when mixed with authentic material, and the infrared spectra of the two phenylhydrazones were identical.

Another portion (16.5 mg) of this *Primula* root nonulose was converted into a mixture of methyl glycosides, and this mixture was oxidized with 2 molecular equivalents of sodium metaperiodate for 30 min at 0°. After reduction of the product with sodium borohydride, followed by acid hydrolysis, the 5.8 mg of sirup that was obtained was identified as a *gluco*-heptulose by paper chromatography in solvents A, B, and C, and as belonging to the L series because of its rotation of $[\alpha]_D^{20}$ −41° (*c* 0.3, water). This result is in agreement with the earlier degradation studies on the same nonulose isolated from the avocado[22].

Finally, 14.6 mg of this nonulose was refluxed for 14.5 h with 63.5 mg of (2,5-dichlorophenyl)hydrazine in 1.25 ml of ethyl alcohol containing 0.125 ml of glacial acetic acid. The quite insoluble, yellow needles obtained weighed 7.1 mg and melted at 242–245° (dec.); this value was not depressed when the compound was mixed with a sample of the (2,5-dichlorophenyl)osazone derived from synthetic D-*erythro*-L-*gluco*-nonulose[22]. The infrared spectra confirmed the identity of the two samples.

D-erythro-L-galacto-*Nonulose*

The second nonulose from *Primula* roots had a relatively high retention-time on the Dowex 50W-X8 (Ba^{2+}) column, and the material thus obtained from fractions 37–41 weighed 203 mg. A final purification on Whatman 3MM filter paper, developed in

solvent A, gave 100 mg of chromatographically pure D-*erythro*-L-*galacto*-nonulose as a sirup having $[\alpha]_D^{20}$ −37° (*c* 3.7, water). This value is very close to that of −36.2° (*c* 5.2, 90% methanol) for the synthetic nonulose[23]. Its identity was confirmed by repeating the two degradation procedures by which it was originally characterized. Thus, treatment of 8.3 mg of this second nonulose with 2 molecular equivalents of lead tetraacetate in 15 ml of glacial acetic acid for 20 min at room temperature afforded 3.5 mg of a sirup that contained a hexose having the same mobility as D-glucose on paper chromatograms developed in solvents A, B, and C. A trace of a pentose having a mobility corresponding to that of an arabinose was also observed. That the hexose was D-glucose was shown by digesting a solution of 1.5 mg of the sirupy oxidation product in 1 ml of water with 0.1 ml of a 4% solution of D-glucose oxidase*; subsequent paper chromatography showed that the hexose had been completely destroyed.

Secondly, 3 mg of this second nonulose from *Primula* roots was converted into a mixture of methyl nonulosides that was oxidized with 2 molecular equivalents of sodium metaperiodate for 30 min in an ice bath; borohydride reduction and acid hydrolysis, as described previously[23], yielded 2 mg of a sirup that contained a heptulose having the mobility of a *galacto*-heptulose on paper chromatograms developed in solvents A, B, and C.

α-Primeverose (*6*-O-*β*-D-*xylopyranosyl*-*β*-D-*glucopyranose*)

Fractions 47–50 (tubes 8106–9200; 8.24 g) yielded 5.76 g of crystalline primeverose. When recrystallized from methanol, it separated as clusters of flat, wedge-shaped prisms that melted at 191–192° with browning when heated slowly, and at about 210° when heated rapidly. It showed mutarotation $[\alpha]_D^{20}$ +24 → −3.5° (*c* 2.5, water). All these values are in accord with those recorded for this compound in the literature[26]. Analyses for C and H were also confirmatory.

In addition, a sample of the sugar was acetylated with acetic anhydride and fused sodium acetate; the *β*-primeverose heptaacetate melted at 215–216° and showed $[\alpha]_D^{20}$ −20.1° (*c* 1, chloroform). These values are comparable to the m.p. 216° and $[\alpha]_D^{20}$ −23.4° reported by Helferich and Rauch[47] for the *β*-heptaacetate of synthetic primeverose. The infrared spectrum of our primeverose heptaacetate and that of a sample derived from *Ceratonia siliqua* L.** were identical when obtained in chloroform solutions; and a mixture of our heptaacetate with a synthetic sample*** showed no depression of melting point (capillary tube).

Primeveritol (*6*-O-*β*-D-*xylopyranosyl*-D-*glucitol*)

A 310-mg portion of primeverose was reduced with 125 mg of sodium borohydride in the usual way. Upon crystallization and recrystallization from aqueous

*Worthington Biochemical Corporation's "Glucostat", in a phosphate buffer.
**Kindly supplied by Dr. Jochen Lehmann (Ref. 27).
***Kindly supplied by Dr. George H. Coleman (Ref. 48).

ethyl alcohol, the small needles of primeveritol melted at 141–142° and showed $[\alpha]_D^{20}$ —34.0° (*c* 1, water).

Anal. Calc. for $C_{11}H_{22}O_{10}$: C, 42.04; H, 7.06. Found: 42.10; H, 7.08.

Primeveritol octaacetate (*6-O-β-D-xylopyranosyl-D-glucitol octaacetate*)

The acetylation of a sample of primeveritol with acetic anhydride and fused sodium acetate, at 110° overnight, yielded a sirup that crystallized when its solution in aqueous ethyl alcohol was kept at 0° for 2 weeks. The primeveritol octaacetate was recrystallized from aqueous methanol, and then from dichloromethane by the addition of pentane; the fine needles melted at 119–120° and showed $[\alpha]_D^{20}$ —25.8° (*c* 1, chloroform).

Anal. Calcd. for $C_{27}H_{38}O_{18}$: C, 49.84; H, 5.89. Found: C, 50.18; H, 6.07.

myo-*Inositol*

Fractions 51 and 52 (tubes 9201–10,055; 2.84 g) yielded the rather insoluble *myo*-inositol in the first crop of crystals. After recrystallization from aqueous methanol, identification was effected by paper chromatography, by a melting point and mixed melting point of 222–224°, and finally by a comparison of infrared spectra.

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SUMMARY

An aqueous extract of 20 kg of the dried roots of *Primula officinalis* Jacq. has been deproteinized, deionized, fermented with bakers' yeast, and fractionated by chromatography on cellulose columns, Dowex 50W-X8 (Ba^{2+}) resin columns, and sheets of filter paper. The following higher-carbon sugars were isolated: D-*altro*-heptulose (sedoheptulose), D-*manno*-heptulose, D-*allo*-heptulose, D-*altro*-3-heptulose, D-*glycero*-D-*manno*-heptose, D-*glycero*-D-*gluco*-heptose, D-*glycero*-D-*manno*-octulose, D-*glycero*-L-*galacto*-octulose, D-*erythro*-L-*gluco*-nonulose, and D-*erythro*-L-*galacto*-nonulose. Two higher-carbon polyhydric alcohols — volemitol (D-*glycero*-D-*manno*-heptitol) and β-sedoheptitol (D-*glycero*-D-*gluco*-heptitol) — were also isolated, as well as glycerol, erythritol, xylitol, *myo*-inositol, D-xylose, and primeverose. Of all these substances, only volemitol had previously been isolated from *P. officinalis*, although primeverose was known to occur there as a constituent of the glycosides primeverin

and primulaverin. In addition, primeverose has been reduced to primeveritol(6-*O*-β-D-xylopyranosyl-D-glucitol), which was characterized further as the crystalline octaacetate; and the crystalline D-*glycero*-β-D-*gluco*-heptose has been converted into the new, crystalline D-*glycero*-β-D-*gluco*-heptose hexaacetate.

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THE ISOLATION OF D-*erythro*-L-*galacto*-NONULOSE FROM THE AVOCADO, TOGETHER WITH ITS SYNTHESIS AND PROOF OF STRUCTURE THROUGH REDUCTION TO D-*arabino*-D-*manno*-NONITOL AND D-*arabino*-D-*gluco*-NONITOL*

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The application of chromatographic separation techniques has, in recent years, resulted in the addition of several higher-carbon sugars to those previously isolated from natural sources. D-*manno*-Heptulose (I)[1] and sedoheptulose (D-*altro*-heptulose, II) as the crystalline 2,7-anhydro-β-D-*altro*-heptulopyranose[2] had been isolated from plant sources by crystallization techniques before the era of chromatography. An investigation of the higher-carbon sugars of the avocado fruit (*Persea gratissima* Gaertn., family Lauraceae) has resulted in the isolation of D-*talo*-heptulose (III)[3], D-*glycero*-D-*galacto*-heptose (IV)[4], D-*glycero*-D-*manno*-octulose (V)[3–5], D-*glycero*-L-*galacto*-octulose (VI)[4], D-*erythro*-L-*gluco*-nonulose (VII)[6], and now D-*erythro*-L-*galacto*-nonulose (VIII). These octuloses and nonuloses have also been found in *Sedum* species (family Crassulaceae)[3,6], have recently been isolated from the roots of the primrose (*Primula officinalis* Jacq., family Primulaceae)[7], and have been indicated, by paper chromatography, to occur, with D-*manno*-heptulose and sedoheptulose, in at least five other genera of the Crassulaceae[8]. Sedoheptulose occurs widely in nature, and its role as an intermediate, both in carbohydrate metabolism and photosynthesis, is well understood. D-*manno*-Heptulose has been discovered in several plant families, but the mechanism of its biosynthesis is still obscure. The biosynthesis and possible role of the octuloses and nonuloses in carbohydrate metabolism are unknown, although several octuloses have been synthesized enzymically from aldoses[4,9–11].

It is interesting that the higher-carbon ketoses that have been shown to occur in plants can be divided into two well-defined groups with reference to their structures and possible modes of biosynthesis. The first group includes those that have a D-*threo* configuration at C-3 and C-4 (the "D-xylulose" configuration at C-1 to C-4); these should be capable of being synthesized by the enzymes transaldolase, transketolase, or aldolase. In this group belong sedoheptulose (II), D-*glycero*-L-*galacto*-octulose (VI)

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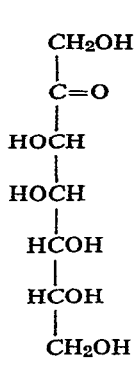
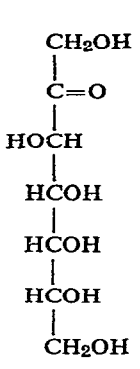
(which has been synthesized *in vitro* by an aldolase[4,9]), D-*erythro*-L-*gluco*-nonulose(VII), and D-*erythro*-L-*galacto*-nonulose (VIII). The configurations of the naturally occurring D-xylose, D-mannose, and D-glucose can be recognized in the lower portions of the octulose and two nonulose formulas, respectively, and this suggests that aldolase or



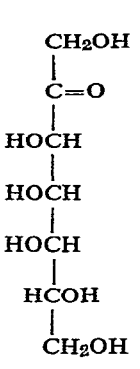
D-*manno*-Heptulose

I



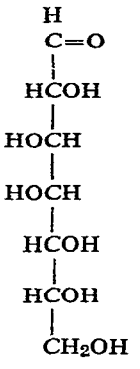
D-*altro*-Heptulose

II



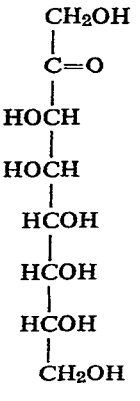
D-*talo*-Heptulose

III



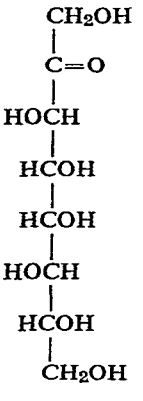
D-*glycero*-D-*galacto*-Heptose

IV



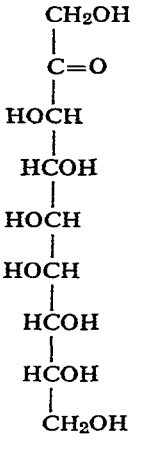
D-*glycero*-D-*manno*-Octulose

V



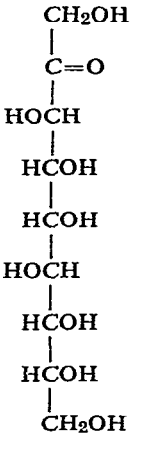
D-*glycero*-L-*galacto*-Octulose

VI



D-*erythro*-L-*gluco*-Nonulose

VII



D-*erythro*-L-*galacto*-Nonulose

VIII

transaldolase reactions are involved in their biosynthesis. The second group includes those higher-carbon ketoses that have an L-*erythro* configuration at C-3 and C-4, and whose mode of biosynthesis is at present obscure. D-*manno*-Heptulose (I), D-*talo*-heptulose (III), and D-*glycero*-D-*manno*-octulose (V) belong to this group.

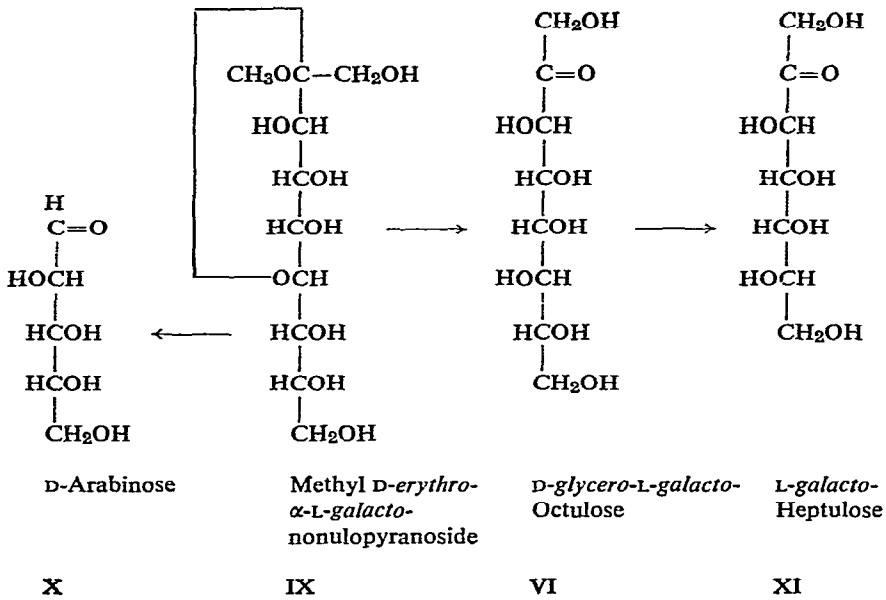
D-*erythro*-L-*galacto*-Nonulose (VIII), the primary subject of this article, was

isolated from the avocado fruit (Calavo, Hass variety) in very low yield, as a hygroscopic sirup, by repeated chromatography on cellulose columns. The structure of the nonulose was determined by two methods of degradation similar to those that had been used for D-*erythro*-L-*gluco*-nonulose[6], previously isolated from the avocado fruit. Oxidation from the reducing end with two molecular equivalents of lead tetraacetate, according to the procedure of Perlin and Brice[12], yielded as the main product a reducing sugar having the paper-chromatographic mobility of a glucose; it was shown to be D-glucose by its complete oxidation in the presence of D-glucose oxidase. The configurations of C-5 to C-8 of the nonulose were thus indicated to be the same as those of D-glucose. In agreement with this conclusion, minor components detected in the lead tetraacetate oxidation mixture corresponded to D-*glycero*-D-*gulo*-heptose and D-arabinose. The configurations of C-3 to C-6 were determined by application of the degradation procedure of Jones and Sephton[10] in which the methyl nonulopyranoside IX (or, probably, a mixture of the anomeric forms) was oxidized with two molecular equivalents of periodate at 0° and at 25°. At 0°, oxidation occurred mainly at the C-7, C-8 glycol outside the ring, producing a heptosuloside that, after reduction with potassium borohydride and hydrolysis with acid, gave a heptulose indistinguishable by paper chromatography from L-*galacto*-heptulose (perseulose, XI). An octulose, not separable from D-*glycero*-L-*galacto*-octulose (VI)[4], was also produced in small proportion by oxidation at C-8, C-9. When the borohydride reduction was omitted, the main aldose product (after acid hydrolysis) cochromatographed with D-arabinose (X); this indicated that a substantial portion of the nonuloside had been oxidized within the pyranose ring, with the lower portion of the nonulose being left intact. This observation indicated a relatively labile *cis*-glycol[13] at C-4, C-5, and is in agreement with the *galacto*-heptulose configuration indicated above for C-1 to C-6. When the periodate oxidation was carried out at 25°, followed by borohydride reduction and hydrolysis, the major product was a polyhydric alcohol cochromatographing with D-arabinitol; this result is consistent with the pyranoside structure ascribed to IX.

Since degradation from the reducing end of the nonulose produced D-glucose, the *galacto*-heptulose obtained by degradation from the nonreducing end must belong to the L series (so as to overlap with D-glucose). The complete structure of the nonulose is, thus, indicated to be D-*erythro*-L-*galacto*-nonulose (VIII).

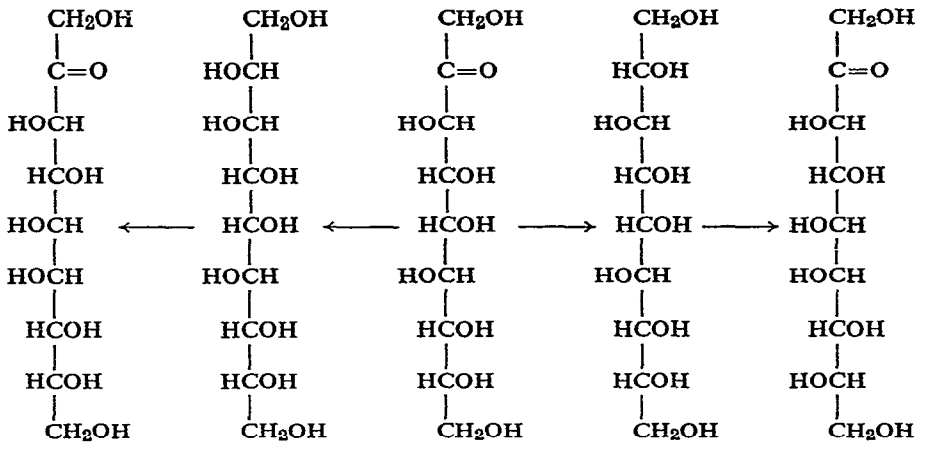
To confirm the structure thus determined by degradation, D-*erythro*-L-*galacto*-nonulose (VIII) was synthesized by (a) the nitroethanol method of Sowden[14] and (b) the diazomethane method of Wolfrom[15]. The addition of nitroethanol to D-*glycero*-D-*gulo*-heptose was carried out under a variety of conditions, all of which were found to give the desired nonulose, but in very low yield. The diazomethane synthesis from sodium D-*erythro*-L-*galacto*-octonate was somewhat better, giving VIII in 4.8% yield as a sirup having $[\alpha]_D^{20}$ −36.2° in water. A crystalline (2,5-dichlorophenyl)osazone of this sugar was prepared. The synthetic nonulose was found to have the same chromatographic mobility as the second avocado nonulose by paper chromatography and by gas–liquid chromatography. The identity of the synthetic

D-*erythro*-L-*galacto*-nonulose with the avocado nonulose was established by reduction of each with borohydride and isolation of corresponding, pairs of identical nonitols. Thus, each nonulose reduction mixture was chromatographed on a



column of Dowex 50W-X8 (Ba^{2+}) resin[16], and completely separated into fractions from which D-*arabino*-D-*manno*-nonitol (XII) and D-*arabino*-D-*gluco*-nonitol (XIII) were obtained in crystalline form. The identity of these two pairs of nonitols with each other was established by infrared and melting-point data. The assignment of configuration to the two nonitols was determined by oxidizing them to nonuloses with *Acetobacter suboxydans*; this organism oxidizes the penultimate hydroxyl group of a *cis* glycol when it is of the D configuration and next to a primary hydroxyl group. The main product thus obtained from the higher-melting nonitol (192–193°) was chromatographically inseparable from D-*erythro*-L-*gluco*-nonulose (VII), the first nonulose isolated from the avocado[6]; hence, this nonitol was D-*arabino*-D-*manno*-nonitol (XII). D-*erythro*-L-*galacto*-Nonulose (VIII), the second oxidation product to be expected, was, however, not detected. Oxidation of the second nonitol (m.p. 180–181°) gave a new nonulose (XIV) that was readily separable by chromatography from the two avocado nonuloses (VII and VIII); hence, the second nonitol was indicated to be D-*arabino*-D-*gluco*-nonitol (XIII). Emil Fischer[17] had obtained the higher-melting of these two nonitols by reduction of a synthetic nonose ("D-glucononose" or "D-α,α,α-glucononose"). C.S. Hudson[18] concluded that Fischer's nonose probably had the D-*arabino*-D-*manno* configuration because of its preponderance in the cyanohydrin synthesis, its weak dextrorotation, and the low solubility of its crystalline phenylhydrazone. The higher-melting of the above pair of nonitols was

shown to be identical with Fischer's nonitol by mixed melting point, by X-ray diffraction patterns, and by *Acetobacter suboxydans* oxidation. Hudson's assignment of the D-*arabino*-D-*manno* configuration was thus confirmed.



VII XII VIII XIII XIV

EXPERIMENTAL

Paper chromatography was carried out on Whatman No. 1 filter paper by the descending method at room temperature. The following solvent systems were used: A, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); B, butyl alcohol–ethyl alcohol–water (40:11:19); C, butyl alcohol–pyridine–water(6:4:3); and D, ethyl acetate–pyridine–water saturated with boric acid (12:5:4). Spray reagents used were aniline hydrogen phthalate for aldoses, orcinol–hydrochloric acid for ketoses, and silver nitrate (ammoniacal, or in conjunction with sodium hydroxide in ethyl alcohol) for alditols, sugars, and other polyhydroxy compounds in general. With the orcinol–hydrochloric acid spray and heating at 100–110°, heptuloses give a pinkish orange color changing to blue (or greenish blue with *manno*-heptulose), octuloses give a pink to red color changing to a brownish gray, and nonuloses give a similar pink to red changing to a greenish gray. The octulose and nonulose spots fluoresce bluish white under ultraviolet light. All concentrations were carried out *in vacuo* at temperatures not over 50°; the final drying of sirups was completed in evacuated desiccators over granular calcium chloride. Melting points were determined on a Kofler micro hot-stage.

Isolation of D-erythro-L-galacto-*nonulose from the avocado*

A sirupy fraction (0.420 g) containing principally this nonulose was isolated

from the fruit pulp (95 kg) of 400 ripe avocados (Californian Calavo, Hass variety) by repeated chromatography on cellulose-powder columns as described in a previous paper[4]. The sirup was dissolved in a small volume of methanol, and polyhydric alcohols were removed from it by crystallization at low temperature. The mother liquor was concentrated to a sirup (0.282 g) that was dissolved in water (5 ml), the solution was filtered through a layer of decolorizing carbon (Darco X), and the filtrate was concentrated to a dry sirup (0.262 g). The presence of oligosaccharides in this sirup was indicated when acid hydrolysis of a small portion of it (5 mg) produced sugars that cochromatographed with D-glucose, D-xylose, D-*manno*-heptulose, and D-fructose, in addition to the nonulose. Oligosaccharides were removed from a portion (150 mg) of the nonulose fraction by chromatography on thick filter-paper (Whatman No. 3MM) with solvent D. A nonulose fraction (103 mg) having $[\alpha]_D^{20}$ −9.7° (*c* 1.24, water) was thus obtained. This product still contained a small amount of oligosaccharide contaminants.

Lead tetraacetate oxidation of the avocado D-erythro-L-galacto-*nonulose to* D-glycero-D-gulo-*heptose*, D-*glucose, and* D-*arabinose*

The nonulose (6.5 mg) was dissolved in glacial acetic acid (5 ml), and treated with a solution of two molecular equivalents of lead tetraacetate in glacial acetic acid (0.75 ml of a 0.116 N solution) at 22°. After 15 min, sufficient oxalic acid (10%) in glacial acetic was added to the solution to complete the precipitation of lead oxalate, which was removed by centrifugation. The supernatant liquor was concentrated to a sirup that was dissolved in aqueous 0.1 N sulfuric acid (5 ml), and the solution was heated on the steam bath for 5 h (to hydrolyze formyl and glycolyl esters), deacidified with Duolite A-4 ion-exchange resin, and concentrated to a sirup. The product was examined by paper chromatography with solvent systems A, B, and C, which indicated the following sugar components: a heptose not separable from D-*glycero*-D-*gulo*-heptose, a hexose (the major product) not separable from D-glucose, and a pentose not separable from D-arabinose. After incubation of a portion of the degradation products with D-glucose oxidase, the hexose component that had previously cochromatographed with D-glucose was completely absent, whereas the heptose and pentose were still detectable on paper chromatograms.

Periodate oxidation of the avocado methyl D-erythro-L-galacto-*nonuloside to* D-glycero-L-galacto-*octulose*, L-galacto-*heptulose*, D-*glucose, and* D-*arabinose*

The nonulose (6.0 mg) was refluxed with 2.5% methanolic hydrogen chloride (5 ml) for 5 h. The solution was cooled, neutralized to pH 5 by the gradual addition of a methanolic solution of potassium hydroxide, and concentrated *in vacuo* without heating. The sirupy residue was dissolved in water (5 ml), the solution was cooled to 0°, and a cold aqueous solution of 2 molecular equivalents of sodium metaperiodate (9.3 mg) was added to it. The solution was kept at 0° overnight, and divided into two equal volumes. One portion was treated with an excess of potassium borohydride for 4 h at 22°, decationized (Dowex 50W-X8), freed from boric acid by the usual

procedure, hydrolyzed in a 0.1 N aqueous sulfuric acid solution on the steam bath for 2 h, deionized with ion-exchange resins (Dowex 50W-X8 and Duolite A-4), and concentrated to a sirup. Upon examination by paper chromatography with solvent systems A, B, and C, it was found to contain some residual nonulose, an octulose not separable from D-*glycero*-L-*galacto*-octulose[4], and, as the major product, a heptulose not separable from L-*galacto*-heptulose. The second portion of the periodate oxidation mixture was hydrolyzed with aqueous 0.1 N sulfuric acid (5 ml) on the steam bath for 2 h, deionized with ion-exchange resins (Dowex 50W-X8 and Duolite A-4), concentrated to a sirup, and examined by paper chromatography with solvent systems A, B, and C. The principle aldose in this hydrolyzate was inseparable from D-arabinose. Minor components, inseparable from D-*glycero*-D-*gulo*-heptose and D-glucose, were also detected.

The methyl glycosides obtained from the avocado nonulose (5 mg) were also oxidized at 25° with two molecular equivalents of sodium metaperiodate, and the products were reduced with borohydride and hydrolyzed as described above. Paper chromatography revealed a main constituent that cochromatographed with D-arabinitol. Minor products (not separable from D-glucitol and erythritol), as well as some nonulose, were also detected.

Synthesis of D-erythro-L-galacto-*nonulose*

(*a*) *From* D-glycero-D-gulo-*heptose*

2-Nitroethanol (150 ml; Commercial Solvents Corporation) was purified by codistillation with phenyl ether (150 ml) under diminished pressure, with the precautions suggested by Noland[19]. The purified reagent was kept in the refrigerator.

In preliminary experiments, several methods of addition of 2-nitroethanol to D-*glycero*-D-*gulo*-heptose were tried, on a small scale, with different solvents and basic catalysts. These included sodium methoxide in methanol, aqueous sodium hydroxide, Amberlyst XN-1002 anion-exchange resin in pyridine, powdered potassium hydroxide in pyridine, sodium methoxide in methanol plus pyridine, sodium methoxide in methanol plus glycerol, and sodium hydroxide in methanol–water. The yields of nonuloses obtained were low in all cases.

D-*glycero*-D-*gulo*-Heptose[20] (57 g) was dissolved in distilled water (500 ml) by stirring at 60°. To the solution, cooled to 20°, 2-nitroethanol (30 g) was added, followed by the slow addition, with stirring, of sodium hydroxide (15 g) in water (300 ml). Methanol (400 ml) was added to provide a homogeneous solution. Stirring was continued at room temperature for 30 min, and the solution was then neutralized by the addition of Dowex 50W-X8 cation-exchange resin, filtered, and the filtrate concentrated to a small volume. Unreacted heptose (51.8 g) crystallized during the concentration, and was recovered by filtration and washing with methanol. The combined mother liquor and wash methanol, examined by paper chromatography, showed the presence of two nonuloses and a heptulose. It was combined with similar products obtained by re-treating the recovered heptose (51.8 g) with 2-nitroethanol as described above. The crystalline heptose (43 g) recovered from the second reaction

was treated a third time, to yield unchanged heptose (37 g) and a mother liquor that, upon further concentration, decomposed exothermally with evolution of gas and total loss of the product. The recovered heptose (37 g) was treated a fourth time with 2-nitroethanol in aqueous methanolic sodium hydroxide. The residual heptose (30 g) was recovered as described above, and the mother liquor containing the mixture of ketoses, the excess of 2-nitroethanol, and degradation products was combined, in aqueous solution (200 ml), with the mother liquor products previously obtained, and extracted with ethyl ether (5 times, 200 ml) to remove the excess of 2-nitroethanol and some of the degradation products. The residue was precipitated onto cellulose powder, in a slurry, by means of quarter-saturated aqueous butyl alcohol; the slurry was transferred to the top of a packed cellulose column (5 × 100 cm); and the column was eluted with quarter- to half-saturated aqueous butyl alcohol as described in a previous publication[4]. Fractions were collected on the basis of their ketose content as indicated by paper chromatography. D-*gluco*-Heptulose (6.7 g) and two nonulose fractions (1.295 g and 0.367 g) were obtained by concentrating appropriate fractions. The second of these two nonuloses was not separable from the avocado nonulose (VIII) by paper chromatography with solvent systems A, B, or C.

(*b*) *From sodium* D-erythro-L-galacto-*octonate*

The procedure of Barker[21] was adapted for the preparation of D-*erythro*-L-*galacto*-octonic acid heptaacetate. Freshly fused zinc chloride (7.5 g) was dissolved in acetic anhydride (100 ml) by magnetic stirring at 22° in a three-necked flask fitted with a calcium chloride tube, a thermometer, and a gas-dispenser tube. The solution was cooled to −10°, and powdered sodium D-*erythro*-L-*galacto*-octonate* (25 g) was added. Cooled in a freezing mixture at −20°, the solution was treated with dry hydrogen chloride at a low rate for 1.5 h with continuous stirring, care being taken to maintain the temperature of the solution below 0°. The solution thus saturated with hydrogen chloride at −10° was set aside to warm slowly overnight to 22°. The solution was then cooled to 0°, and chipped ice (500 g) was added slowly, with stirring, during 1.5 h. The acetylated octonic acid was extracted from the solution with dichloromethane (5 times, 200 ml), and the extract was dried (sodium sulfate). The dried solution was concentrated to a sirup that was dissolved in dry benzene (200 ml), and thionyl chloride (20 g) was added. The solution was heated under reflux, with protection against atmospheric moisture (calcium chloride tube), for 4 h, kept overnight at 22°, and the excess of reagent and the solvent were removed by concentration, followed by drying of the resulting sirup *in vacuo*. A solution of diazomethane in ethyl ether [prepared by swirling *N*-methyl-*N'*-nitrosoguanidine (20 g) in cold ethyl ether (400 ml) over a layer of cold 50% aqueous potassium hydroxide solution (10 ml), and drying over potassium hydroxide pellets] was decanted onto the dry, sirupy, acetylated octonyl chloride. After the sirup had dissolved, the solution was kept at

* The sodium octonate was made by earlier workers in this Laboratory from the lactone, which was prepared according to Hockett and Hudson (Ref. 22).

room temperature for 4 h, concentrated, and the sirupy residue dried *in vacuo*. A solution of the sirup in glacial acetic acid (250 ml), to which powdered copper (20 mg) and cupric acetate (trace) were added, was carefully heated to the point of vigorous gas evolution (114°), just below the boiling point. After the evolution of gas had subsided, the solution was heated at its boiling point for several min, cooled, and concentrated, and the sirup was dried *in vacuo* over potassium hydroxide (pellets). The sirup was dissolved in dry methanol (500 ml), the solution cooled to −5°, a catalytic amount of sodium methoxide in methanol (5 ml of 2.9 M) was added, and the solution was kept at −5° until deacetylation of the nonulose was complete, as indicated by paper chromatography (48 h). Sodium ions were removed from the solution by passing it through a column of Dowex 50W-X8 (H^+) resin, and the eluate was concentrated to a thin sirup. The sirup was precipitated onto cellulose powder slurried in quarter-saturated aqueous butyl alcohol, and fractionated by chromatography on a cellulose-powder column by elution with quarter- to half-saturated aqueous butyl alcohol as described in a previous publication[4]. The nonulose fraction obtained (1.15 g; 4.8% overall yield) was decolorized by treatment with activated carbon (Darco X), and filtered. It had $[\alpha]_D^{20}$ −36.2° (*c* 5.16, 90% aqueous methanol), and was chromatographically inseparable from the avocado nonulose (VIII) by solvent systems A, B, or C. The trimethylsilyl derivatives of this synthetic nonulose and the avocado nonulose were prepared by mixing a small quantity (about 1 mg) of each with pyridine (4 drops), hexamethyldisilazane (4 drops), and chlorotrimethylsilane (2 drops)[23]. The reaction mixtures were subjected to gas–liquid chromatography on a packed column (0.6 × 183 cm) of 3% SE 52 on Gas-Chrom A by injecting small volumes (1 μl) of each and heating the column from 75 to 280° at a rate of 11° per min, with a nitrogen flow-rate through the column of 100 ml per min. Each of the reaction mixtures produced a single, main chromatographic peak eluting at 275°, detected by hydrogen flame ionization; these peaks were not separated when the two preparations were cochromatographed. A crystalline (2,5-dichlorophenyl)-osazone was obtained from the synthetic nonulose (25 mg) by heating it under reflux for several days with (2,5-dichlorophenyl)hydrazine (50 mg) in absolute ethyl alcohol (5 ml) containing glacial acetic acid (0.5 ml). The osazone crystallized from the boiling reaction-mixture as nodular aggregates of fine needles; m.p. 247–249°. When mixed with the (2,5-dichlorophenyl)osazone of D-*erythro*-L-*gluco*-nonulose[6], the m.p. was 238–240°.

Anal. Calc. for $C_{21}H_{24}Cl_4N_4O_7$: C, 43.02; H, 4.13; Cl, 24.19; N, 9.56. Found: C, 43.33; H, 4.14; Cl, 23.88; N, 9.79.

The infrared spectrum of the osazone, recorded in a Nujol mull, showed distinctive absorption maxima at 745, 792, 845, 873, 920, 1013, 1047, 1090, 1170, 1205, 1248, 1258, 1268, 1455, 1473, 1587, 3275, and 3400 cm^{-1}.

Reduction of D-erythro-L-galacto-*nonulose, and isolation of the products:* D-arabino-D-manno-*nonitol and* D-arabino-D-gluco-*nonitol*

Synthetic D-*erythro*-L-*galacto*-nonulose (0.060 g), dissolved in water (25 ml),

was treated with an excess of potassium borohydride (0.10 g), and the solution was kept overnight at 22°. Cations were removed from the solution by stirring it with an excess of Dowex 50W-X8 (H^+) resin, and the acidic solution was filtered and concentrated to a sirup. Boric acid was removed from the product as (volatile) methyl borate. The residual sirup was dissolved in water (2 ml), placed on a column (1.2 × 140 cm) of Dowex 50W-X8 (200–400 mesh) in the Ba^{2+} form, and eluted with distilled water at a low rate (5 ml per h) according to the method described by Jones and Wall[16]. Small fractions (1 ml) were collected, and were examined by paper chromatography. The first crystalline nonitol (0.017 g) was recovered from fractions 151 to 220. After recrystallization from aqueous methanol, it had m.p. 192–193°.

Anal. Calc. for $C_9H_{20}O_9$: C, 39.70; H, 7.41. Found: C, 39.99; H, 7.06.

A second crystalline nonitol (0.022 g) was recovered from eluate fractions 285 to 380. After recrystallization from aqueous methanol, it had m.p. 180–181°.

Anal. Calc. for $C_9H_{20}O_9$: C, 39.70; H, 7.41. Found: C, 39.17; H, 7.46.

Duplicate infrared spectra obtained on each of these two nonitols (KBr discs) showed the following distinctive absorption maxima: first nonitol–860, 880, 1030, 1090, 1215, 1390, 1435, 1630, 2855, 2925, and 3410 (broad) cm^{-1}; second nonitol–625, 685, 855, 875, 920, 955, 1020, 1030, 1045, 1090, 1200, 1250, 1320, 1445, 1630, 2850, 2920, 2970, and 3380 (broad) cm^{-1}.

The avocado nonulose (VIII; 0.050 g) was also reduced with potassium borohydride (0.10 g), and a pair of crystalline nonitols (5 mg each) was obtained by chromatography on the Dowex 50W-X8 (Ba^{2+}) resin column as described above. After recrystallization from aqueous methanol, the first of these two nonitols had m.p. 193–194°. A mixture of this nonitol with the first nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose melted at 192–193°. After recrystallization from aqueous methanol, the second nonitol obtained from the avocado nonulose had m.p. 180–181°. A mixture of this nonitol with the second nonitol from synthetic D-*erythro*-L-*galacto*-nonulose melted at 180–181°. The infrared spectrum of this second nonitol (KBr disc) clearly confirmed its identity with the second nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose.

Oxidation of D-arabino-D-manno-*nonitol to* D-erythro-L-gluco-*nonulose, and of* D-arabino-D-gluco-*nonitol to* L-threo-L-gluco-*nonulose with* Acetobacter suboxydans

The first nonitol (0.8 mg) obtained from synthetic D-*erythro*-L-*galacto*-nonulose was oxidized with *Acetobacter suboxydans* (A.T.C.C. No. 621), grown on a mannitol–agar slant and suspended in nutrient broth (1.5 ml) containing 0.5% of yeast extract, at 25° for 4 days, with gentle agitation. The solution was filtered through decolorizing carbon (Darco X), evaporated to a sirup, and examined by paper chromatography. The main product gave the characteristic nonulose reaction with the orcinol reagent, and was not separable from synthetic D-*erythro*-L-*gluco*-nonulose[6] in solvent systems A, B, or C.

The second nonitol (2 mg) obtained from synthetic D-*erythro*-L-*galacto*-nonulose was oxidized with *A. suboxydans* in the same manner. The main product was shown

by paper chromatography to be a nonulose readily distinguishable from both D-*erythro*-L-*galacto*-nonulose and D-*erythro*-L-*gluco*-nonulose.

Emil Fischer's "D-glucononitol"[17]* was found by us to have m.p. 192–194°, a value that was not depressed when this nonitol was mixed with the first nonitol obtained from synthetic D-*erythro*-L-*galacto*-nonulose. These two nonitol preparations gave closely similar X-ray diffraction spectra. When Fischer's nonitol (3.5 mg) was oxidized with *A. suboxydans* as described above, the main product obtained was chromatographically indistinguishable from D-*erythro*-L-*gluco*-nonulose.

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SUMMARY

D-*erythro*-L-*galacto*-Nonulose was isolated as a sirup from the avocado fruit, in very low yield, by chromatography. The structure of the sugar was determined by degradation from the reducing end with lead tetraacetate, and from the non-reducing end with sodium metaperiodate. The two sets of reaction products thus obtained were identified with known compounds whose structures could be "overlapped" to conform with the D-*erythro*-L-*galacto*-nonulose configuration. This sugar was synthesized, and its identity with that of the avocado nonulose was established by reduction, followed by isolation of crystalline D-*arabino*-D-*manno*-nonitol and D-*arabino*-D-*gluco*-nonitol from each nonulose. Assigment of configurations to the two pairs of nonitols was determined by oxidation with *Acetobacter suboxydans*.

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*A sample of this nonitol from the Fischer collection was kindly supplied by Professor Clinton E. Ballou of the University of California (Berkeley).

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SYNTHESIS OF 1,3-ANHYDRO-D-GLUCITOL AND SOME DERIVATIVES OF 1,5-ANHYDRO-D-GLUCITOL*

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INTRODUCTION

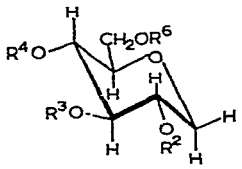
Gallic acid is found in Nature predominantly in association with D-glucose[2], but examples of its occurrence esterified to (—)-quinic acid (XII), D-hamamelose, 1,5-anhydro-D-glucitol (I), *epi*-catechin, and *epi*-gallocatechin have also been noted[3–6]. Acer tannin, first isolated by Perkin and Uyeda[7] from *Acer ginnale* and later identified in other *Acer* species, was shown by Kutani[5] to be 1,5-anhydro-3,6-di-*O*-galloyl-D-glucitol (II), but proof of this structural assignment by a rational synthesis of (II) has not been obtained. An important procedure in the selective galloylation of 1,5-anhydro-D-glucitol to give compound (II) is the preparation of a suitable derivative of the anhydrohexitol in which the hydroxyl functions at C-2 and C-4 are protected (*e.g.*, III or IV), and the work described here outlines some approaches towards the synthesis of this type of intermediate.

RESULTS AND DISCUSSION

A general procedure, which was successfully applied to the synthesis of substituted 1,5-anhydro-D-glucitol derivatives, including the required 2,4-di-*O*-benzyl (III) and 2,4-di-*O*-acetyl (IV) compounds, was based on the preparation[8] of 1,5-anhydro-D-glucitol by the lithium aluminium hydride reduction of tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Thus, 1,5-anhydro-3-*O*-benzyl-D-glucitol (V) resulted from the metal hydride reduction of 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl bromide[9] or the corresponding chloro-compound prepared from 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-β-D-glucose[9] by the methods of Baddiley and co-workers[10] or Pravdic and Keglevic[11]. Similar reaction sequences, commencing with 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucose[12] and 1,3,6-tri-*O*-acetyl-2,4-di-*O*-benzyl-D-glucose (*cf.* ref. 13), afforded the corresponding 2,3,4-tri-*O*-benzyl (VI) and 2,4-di-*O*-benzyl (III) ethers of 1,5-anhydro-D-glucitol; the latter compound has been utilised in a successful synthesis of the 3,6-di-*O*-galloyl ester, but complete comparison with the natural product has not been made.

*For a preliminary report of some of these results, see ref. 1.

Treatment of 1,5-anhydro-3-*O*-benzyl-D-glucitol (V) with one molecular proportion of triphenylmethyl chloride, and subsequent acetylation, gave compound (VII). The p.m.r. spectrum of this compound contained a 3-proton singlet, at unusually high field (τ 8.35), which was attributed to the C-4 acetate group. Examination

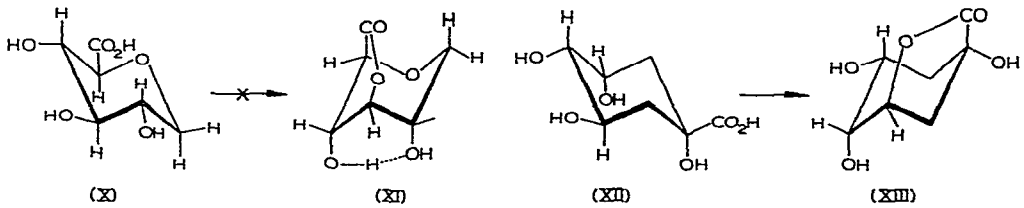


I	$R^2 = R^3 = R^4 = R^6 = H$
II	$R^2 = R^4 = H$, $R^3 = R^6 = CO{\cdot}C_6H_2(OH)_3$-3,4,5
III	$R^3 = R^6 = H$, $R^2 = R^4 = CH_2{\cdot}Ph$
IV	$R^3 = R^6 = H$, $R^2 = R^4 = Ac$
V	$R^2 = R^4 = R^6 = H$, $R^3 = CH_2{\cdot}Ph$
VI	$R^6 = H$, $R^2 = R^3 = R^4 = CH_2{\cdot}Ph$
VII	$R^2 = R^4 = Ac$, $R^3 = CH_2{\cdot}Ph$, $R^6 = C(Ph)_3$
VIII	$R^2 = R^3 = R^4 = Ac$, $R^6 = Ts$

of molecular models indicated that the acetate methyl group, due to its structural environment adjacent to the 3-benzyl and 6-trityl ether groups, was probably subject to a positive shielding by (on average) one of the aromatic nuclei of the trityl ether. Removal of the trityl and benzyl ether groups from compound (VII) was achieved by brief treatment with acid, followed by hydrogenation, and gave 2,4-di-*O*-acetyl-1,5-anhydro-D-glucitol (IV) as a syrup, which, however, still retained traces of unidentified impurities after several chromatographic separations. The structure of the product (IV) was confirmed by its deacetylation to give 1,5-anhydro-D-glucitol, its acetylation to give 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-glucitol, and its conversion by selective toluene-*p*-sulphonylation, followed by acetylation, into 2,3,4-tri-*O*-acetyl-1,5-anhydro-6-*O*-toluene-*p*-sulphonyl-D-glucitol (VIII), identical with the product previously described by Baker[14]. However, the use of the diacetate (IV) as an intermediate in the synthesis of compound (II) was rendered invalid by the observation that, in the presence of pyridine at 60° (conditions normally required for esterification with tri-*O*-benzylgalloyl chloride[15]), the compound (IV) underwent changes which were analysed using t.l.c. and tentatively interpreted as involving acetyl migration. This observation is in broad agreement with the recent studies of Angyal and Melrose[16], who have further emphasized the relative ease with which acyl migrations occur in partially acylated polyols under basic conditions.

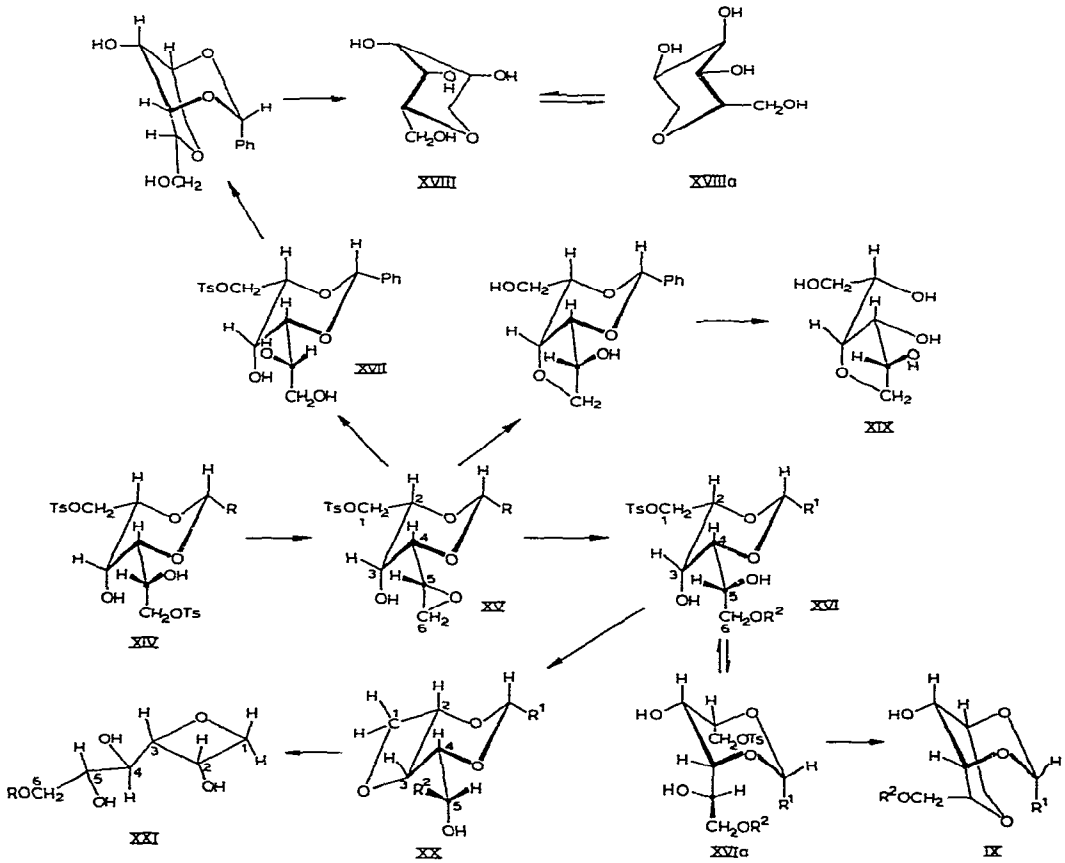
Some interesting results, which were not directly applicable to the synthesis of compound (II), followed attempts to prepare 1,5-anhydro-2,4-*O*-benzylidene-D-glucitol (IX, $R^1 = Ph$, $R^2 = H$). Catalytic oxidation[17] of 1,5-anhydro-D-glucitol (I) gave a product formulated as the uronic acid (X), but the projected transformation of this compound into 1,5-anhydro-2,4-*O*-benzylidene-D-glucitol (IX, $R^1 = Ph$, $R^2 = H$) was not possible since all attempts to form the 3,6-lactone (XI) failed. This resistance to lactonisation was unexpected since, although the conversion involves an unfavourable inversion of conformation, similar changes have been observed in other systems such as (−)quinic acid (XII to XIII), and the lactone (XI),

once formed, might be expected to be stabilised by intramolecular 1,3-hydrogen bonding.



(X) (XI) (XII) (XIII)

In 1954, Baker[14] prepared 1,5-anhydro-2,4-*O*-methylene-D-glucitol (IX, $R^1 = R^2 = H$) by the action of base upon the toluene-*p*-sulphonate (XVI, $R^1 = R^2 = H$), and it was envisaged that a similar reaction, commencing with the corresponding 2,4-*O*-benzylidene derivative (XVI, $R^1 = Ph$, $R^2 = H$), should produce the required compound (IX, $R^1 = Ph$, $R^2 = H$). Although it was noted that the conformational changes, which are necessary for cyclisation to occur in the desired manner, are energetically less favourable for the benzylidene than for the methylene compound



(XVI, $R^1 = Ph$, $R^2 = H$ and XVI, $R^1 = R^2 = H$, respectively), since the chair form (XVI, $R^1 = Ph$, $R^2 = H$) involves phenyl rather than hydrogen in 1,3-diaxial interaction with the participating groups, it was considered that, in this case, the required orientation of the toluene-*p*-sulphonate ester and hydroxyl group at C-5 might be more readily attained in a skew-boat conformation, with the phenyl group retaining its equatorial disposition. The ditoluene-*p*-sulphonate (XIV, R = Ph) was prepared according to the method of Vargha[18], and although the physical properties of this compound were at variance with previous data[18], the product was smoothly transformed into the epoxide (XV, R = Ph), and its diacetate gave a p.m.r. spectrum fully consistent with the structure shown. Since it was not found possible to prepare the diol (XVI, $R^1 = Ph$, $R^2 = H$) by preferential, acid-catalysed fission of the epoxide (XV, $R^1 = Ph$) (because of the lability of the benzylidene acetal grouping[18]), the latter was treated directly with base when it was assumed that the epoxide ring would open in the normal manner to give the diol (XVI, $R^1 = Ph$, $R^2 = H$) as an intermediate in the reaction. The final product *A*, $C_{13}H_{16}O_5$, was isolated and purified as its diacetate *B*. Hydrogenation of *B* gave compound *C* which, on deacetylation, afforded the crystalline anhydrohexitol *D*, $C_6H_{12}O_5$. Compound *D* formed a tetra-acetate, and the presence of a primary alcohol function in *A*, but not *C*, was shown by its conversion into a monotrityl acetate which could not be formed from *C*. The chromatographic characteristics of *D* were identical, in the systems investigated, with those of 1,5-anhydro-D-glucitol, but its other physical and spectroscopic properties were quite different, and it was evident that the cyclisation had taken an unexpected course. The formulation of *D* as 1,6-anhydro-D-glucitol or 3,6-anhydro-D-glucitol (XIX) was also ruled out, since these compounds, prepared respectively by Vargha and Kasztreiner[19] and Montgomery and Wiggins[20], also differed significantly from *D*; the fission of the epoxide ring in an abnormal manner (XVII) followed by cyclisation leading to 1,5-anhydro-L-iditol (XVIII), and the formation of an oxetan derivative, 1,3-anhydro-D-glucitol (XXI, R = H), appeared as plausible, alternative reaction pathways. Evidence which favours the last of these hypotheses is discussed below.

Hydrolysis of the ditoluene-*p*-sulphonate (XIV, R = Ph) with base in the presence of methyl, ethyl, or benzyl alcohol gave the corresponding methyl, ethyl, and benzyl ether analogues of *A*. In the case of the methyl and ethyl ethers, these could also be prepared by initial treatment of the epoxide (XV, R = Ph) with sodium methoxide or ethoxide to give[18] products (XVI, $R^1 = Ph$, $R^2 = Me$ and XVI, $R^1 = Ph$, $R^2 = Et$) which were then cyclised by base independently. The ethyl ether was identical with a product derived in a similar way by Vargha[18], but for which no structure was put forward. The structural relationship of these compounds to one another and to *A* was indicated by a comparison of the p.m.r. spectra of their acetates (Fig. 1), and by hydrogenation of the benzyl ether to give a compound identical with *D*. A comparison of the p.m.r. spectra of the methyl, ethyl, and benzyl ethers and their monoacetates also showed that the free hydroxyl group in these compounds was attached to a methine and not a methylene group, and hence that the epoxide ring had been cleaved

in the expected manner. An unusual, and as yet unexplained, long-range coupling phenomenon in the p.m.r. spectra of the ethyl and benzyl ethers concerned the methylene* group signal. For example, in the case of the ethyl ether (–O–CH_2*·CH_3), in the parent compound, this signal appeared as the usual sharp quartet (1:3:3:1), but, in its acetate, each signal of the quartet was subject to further splitting (J 2 c.p.s.). A similar phenomenon was present in the case of the benzyl ether, but the methyl ether signals in the parent methyl ether and its acetate appeared as sharp singlets.

For the methyl, ethyl, and benzyl ethers, it is clear therefore that compounds (XVI, R^1 = Ph, R^2 = Me), (XVI, R^1 = Ph, R^2 = Et), and (XVI, R^1 = Ph, R^2 = CH_2·Ph), respectively, must act as intermediates in their formation. With the structural relationship of these ethers to *A* in mind, it is thus reasonable to assume that compound (XVI, R^1 = Ph, R^2 = H) is very probably involved in the formation of *A*, and hence that *D* cannot be 1,5-anhydro-L-iditol (XVIII). The 1,5-anhydro-L-iditol structure (XVIII) was further, albeit less convincingly, excluded by a comparison of the p.m.r. spectrum of the tetraacetate of *D* and the acetates of some 1,5-anhydro-D-hexitols. A detailed discussion of these spectra is reserved for a later publication, but the preliminary analyses shown in Table I were obtained using the concepts arising from previous analyses[21,22] of carbohydrate acetates and the spectra of model compounds, such as the 1,5-anhydro-6-deoxy-6-iodo-hexitols. Although some doubt exists as to the conformation of the L-iditol derivative[23] (XVIII or XVIIIa), it was considered doubtful, even if the molecule exists entirely in the conformation (XVIIIa) in which the C-6 group is axially disposed, that this would account for the considerable differences between the spectrum of the acetate of *D* and those of the known 1,5-anhydro-D-hexitols.

These observations therefore permit the formulation of *D* as 1,3-anhydro-D-glucitol (XXI, R = H), and the course of the base catalysed cyclisation as shown (XV → XXI). The absence of a more extensive range of model compounds has, as yet, prevented an unequivocal elucidation of the p.m.r. spectra of compound *D* [Fig. 1(d)] and its various derivatives, and the assignments shown in this paper await verification by further, more-detailed work which will include an investigation of the chemistry of 1,3-anhydro-D-glucitol itself.

EXPERIMENTAL

P.m.r. spectra were recorded with a Varian A-60 spectrometer.

1,5-*Anhydro*-3-O-*benzyl*-D-*glucitol*

A solution of 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-glucopyranosyl bromide[9] (10 g) in anhydrous ether (100 ml) was added to a stirred suspension of lithium aluminium hydride (9 g) in the same solvent (150 ml). The mixture was stirred for 2 h at 20°, distilled water (150 ml) was added, and the ether layer was separated. The residual aqueous layer was filtered and de-ionised with Zeo Karb 215 (H^+ form) and Amberlite CG 400 (acetate form). Concentration of the resulting solution, and recrystalli-

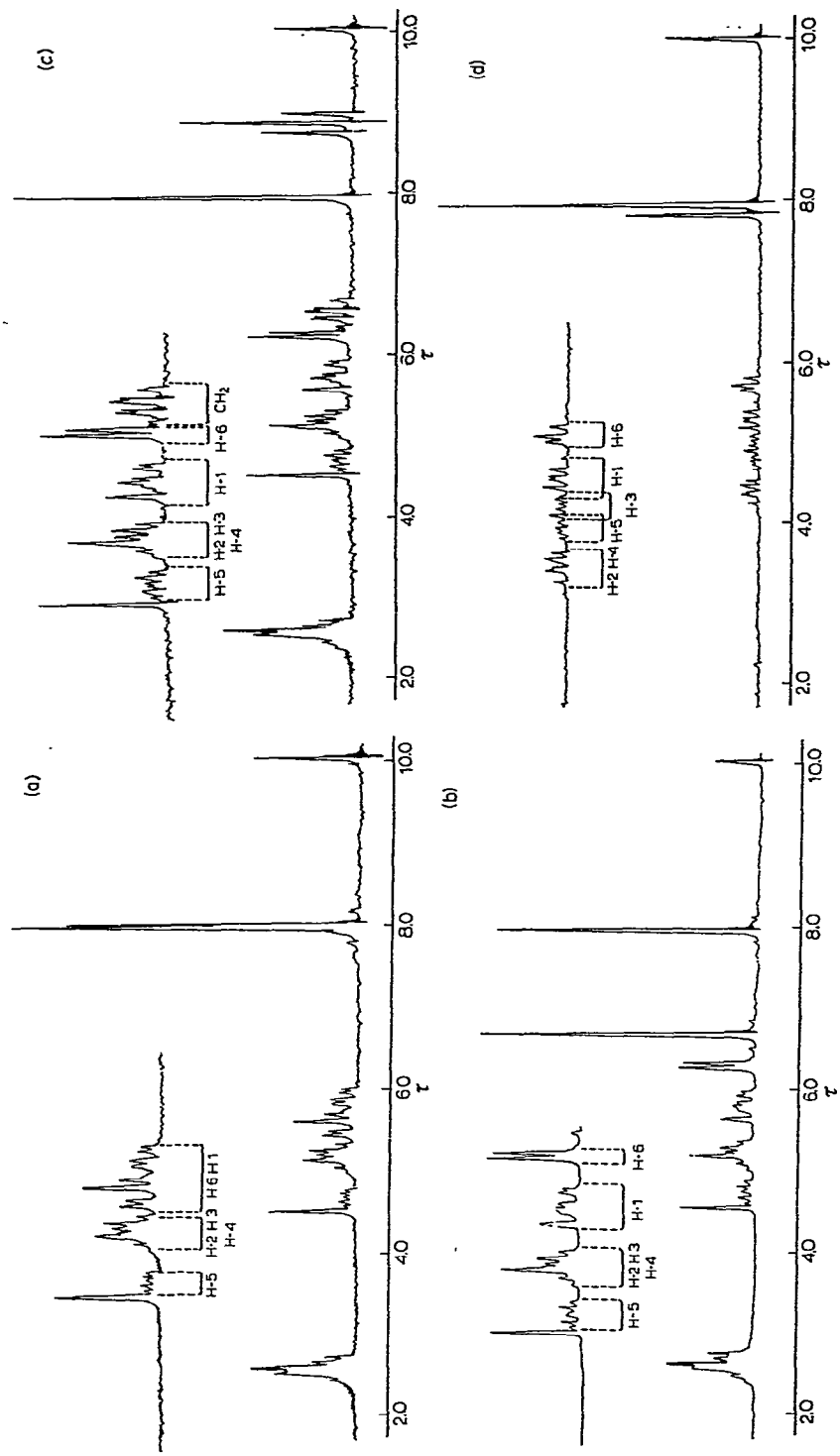


Fig. 1. The p.m.r. spectra ($CDCl_3$) of (a) 5,6-di-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-D-glucitol (Compound *B*); (b) 5-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-6-*O*-methyl-D-glucitol; (c) 5-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-6-*O*-ethyl-D-glucitol; (d) 2,4,5,6-tetra-*O*-acetyl-1,3-anhydro-D-glucitol (acetate of Compound *D*).

TABLE I

THE p.m.r. SPECTRA OF SOME 1,5-ANHYDROHEXITOL TETRA-ACETATES ($CDCl_3$)

Hexitol	*2*	*3*	*4*	*6*
Acetoxy resonances (τ *values*)				
D-Glucitol[8]	7.97	7.97	7.97	7.92
D-Mannitol[24]	7.84	7.96	8.00	7.91
D-Galactitol[25]	7.94	7.99	7.83	7.94
D-Talitol[26]	7.85	8.00	7.85	7.94
D-Altritol[27]	7.86	7.86	8.00	7.92

Hexitol	*H-1*		*H-2, H-3, H-4*	*H-5*	*H-6*
	eq	*ax*			
Proton resonances (τ *values*)					
D-Glucitol[8]	6.35	6.60	4.72–5.23	5.70–6.00	
D-Mannitol[24]	6.18	6.56	4.51–5.07	6.12	5.80
D-Galactitol[25]	6.10	6.75	4.46–4.93	5.62–5.93	
D-Talitol[26]	6.08	6.41	4.59–4.90	6.00	5.80
D-Altritol[27]	5.90	6.20	4.53–5.23	6.10	5.80

sation of the residue from aqueous ethanol, gave 1,5-anhydro-3-*O*-benzyl-D-glucitol (3.1 g) as prisms, m.p. 154–155°, $[\alpha]_D^{20}$ +34.6° (*c* 3.8, dioxan), R_F (silica gel, benzene–methanol, 19:1) 0.27 (Found: C, 60.7; H, 7.6. $C_{13}H_{18}O_5 \cdot \frac{1}{2}C_2H_5OH$ calc.: C, 60,6; H, 7.6%).

Acetylation of the product (pyridine–acetic anhydride) gave, after crystallisation from ethanol, 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-D-glucitol as needles, m.p. 101–102°, $[\alpha]_D^{20}$+15.0° (*c* 3.3, dioxan) (Found: C, 59.7, H, 6.2. $C_{19}H_{24}O_8$ calc.: C, 60.0; H, 6.3%).

Hydrogenation of 1,5-anhydro-3-*O*-benzyl-D-glucitol (100 mg) over palladium–charcoal (10%, 100 mg) in ethanol (5 ml) gave, on removal of the catalyst and solvents, a clear syrup which crystallised from ethanol to give 1,5-anhydro-D-glucitol (50 mg), m.p. and mixed m.p. 142–143°.

2,4-Di-O-acetyl-1,5-anhydro-3-O-benzyl-6-O-trityl-D-glucitol

A solution of 1,5-anhydro-3-*O*-benzyl-D-glucitol (1.0 g) and triphenylmethyl chloride (1.2 g) in pyridine (50 ml) was kept for 3 days at 20° and then acetic anhydride (30 ml) was added. After a further 24 h, the mixture was poured into ice–water, with vigorous stirring, and the precipitated solid was filtered off, washed well with water, and crystallised from ethanol. 2,4-Di-*O*-acetyl-1,5-anhydro-3-*O*-benzyl-6-*O*-trityl-D-glucitol (1.5 g) was obtained as needles, m.p. 163–165°, $[\alpha]_D^{20}$ +21.1° (*c* 2.7, in dioxan) (Found: C, 74.3; H, 6.2. $C_{39}H_{36}O_7$ calc.: C, 74.5; H, 6.2%).

2,4-Di-O-acetyl-1,5-anhydro-D-glucitol

To an ice-cold solution of the foregoing product (0.5 g) in acetic acid (3 ml), an ice-cold solution of hydrogen bromide in glacial acetic acid (50% w/v, 1 ml) was added. After 1 min, the solution was poured into ice-water, and the products were extracted with chloroform (50 ml). Removal of the chloroform gave a colourless syrup which was hydrogenated in ethanol (20 ml) over palladium–charcoal catalyst (10%, 200 mg) for 24 h. T.l.c. using benzene–methanol (4:1) on silica gel revealed one component (R_F 0.45), and purification by this means gave 2,4-di-*O*-acetyl-1,5-anhydro-D-glucitol as a syrup. (Found: C, 48.8; H, 6.6. $C_{10}H_{16}O_7$ calc.: C, 48.4; H, 6.4%).

Acetylation of the product (acetic anhydride–pyridine) gave 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-glucitol, m.p. and mixed m.p., with an authentic sample[8], 74–75°.

2,3,4-Tri-O-acetyl-1,5-anhydro-6-O-toluene-p-sulphonyl-D-glucitol

Toluene-*p*-sulphonyl chloride (0.15 g) was added in portions (0.025 g) to an ice-cold solution of 2,4-di-*O*-acetyl-1,5-anhydro-D-glucitol (0.2 g) in pyridine (3 ml). The mixture was stood for 3 h at 20° when it was cooled to 0° and acetic anhydride added. After 24 h at 20°, the solution was poured into water, and the products were extracted with chloroform. Evaporation of the chloroform gave a syrup which crystallised from ether–light petroleum (b.p. 40–60°) to give 2,3,4-tri-*O*-acetyl-1,5-anhydro-6-*O*-toluene-*p*-sulphonyl-D-glucitol, m.p. 144–145°, undepressed on admixture with an authentic[14] sample.

1,5-Anhydro-2,4-di-O-benzyl-D-glucitol

1,6-Anhydro-2,4-di-*O*-benzyl-D-glucopyranose[13] (2.5 g) was dissolved in acetic anhydride (15 ml), and sulphuric acid in acetic anhydride (4% v/v; 0.6 ml) was added to the solution which was then heated for 3 min at 100°. The reaction mixture was poured into ice–water and, after 1 h, the products were extracted with chloroform. After being washed with water and sodium hydrogen carbonate solution, the chloroform was evaporated to yield a gum, which was treated with a solution of dry hydrogen chloride in dioxan (5% w/v; 100 ml) for 30 h at 37°. Removal of the dioxan gave a gum which, after evaporation with toluene, was dissolved in anhydrous ether (50 ml) and added slowly to a stirred suspension of lithium aluminium hydride (2.5 g) in ether (50 ml). Further lithium aluminium hydride was added after 2 h and, after another hour, distilled water (100 ml) was cautiously added. The filtered aqueous layer was de-ionised (as above) and concentrated at 30°. Crystallisation of the residue from ethanol gave 1,5-anhydro-D-glucitol (10 mg), m.p. and mixed m.p. 142–143°. The dried (Na_2SO_4) ether solution yielded a gum which t.l.c. on silica gel using benzene–methanol (15:1) indicated to contain two components, R_F 0.36 and 0.52. Crystallisation from benzene gave 1,5-anhydro-2,4-di-*O*-benzyl-D-glucitol (0.26 g, R_F 0.36) as needles, m.p. 111–112°, $[\alpha]_D^{20}$ +23.2° (*c* 2.5, dioxan) (Found: C, 69.6; H, 6.7. $C_{20}H_{24}O_5$ calc.: C, 69.8; H, 7.0).

Hydrogenation of the product (as above) gave an uptake of 2.1 moles of hydrogen per mole and yielded 1,5-anhydro-D-glucitol, m.p. and mixed m.p. 142–143°.

1,5-*Anhydro*-2,3,4-*tri*-O-*benzyl*-D-*glucitol*

This compound was prepared in a manner analogous to that described above for 1,5-anhydro-2,4-di-*O*-benzyl-D-glucitol. 1,6-Anhydro-2,3,4-tri-*O*-benzyl-D-glucopyranose[12] gave 1,5-anhydro-2,3,4-tri-*O*-benzyl-D-glucitol (VI) as needles, m.p. 83–84°. (Found: C, 74.7; H, 6.8. $C_{37}H_{30}O_5$ calc.: C, 74.6; H, 6.9%).

2,4-O-*Benzylidene*-1,6-*di*-O-*toluene*-p-*sulphonyl*-D-*glucitol*

This compound was prepared according to Vargha[18] and was obtained as needles, m.p. 125–126°, $[\alpha]_D^{20}$ +16.7° (*c* 6.3, pyridine); Vargha[18] reported m.p. 148°, $[\alpha]_D$ +17.8°. The product was smoothly converted into 5,6-anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (XV, R = Ph) by the method of Vargha[18]. The p.m.r. spectrum of this compound is as shown below ($CDCl_3$, D_2O shake).

τ	*Multiplicity*	*Intensity*	*Assignment*
2.20	doublet (*J* 8 c.p.s.)	2	AB quartet
2.70	doublet (*J* 8 c.p.s.)	2	toluene-*p*-sulphonyl
2.60	singlet	5	benzylidene (aromatic)
4.48	singlet	1	benzylidene (proton)
5.68–6.0	multiplet	3	H-2, H-3, H-4
6.32	doublet	2	H-1
6.75	multiplet	1	H-5
7.20	doublet (*J* 4 c.p.s.)	2	H-6

5,6-*Di*-O-*acetyl*-1,3-*anhydro*-2,4-O-*benzylidene*-D-*glucitol* (*Compound B*)

5,6-Anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (5 g) was added to a solution of sodium hydroxide (0.9 g) in water (250 ml), and the mixture was heated with stirring for 5 h at 80°, cooled, and neutralised (2N sulphuric acid). Evaporation gave a crystalline residue which was extracted with chloroform; removal of the chloroform gave a gum which was dissolved in pyridine (30 ml) and treated with acetic anhydride (30 ml) for 24 h. The solution was poured into water, and the solid was collected and crystallised from pyridine–water to give 5,6-di-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-D-glucitol (0.8 g) as prisms, m.p. 81–82°, $[\alpha]_D^{20}$ +11.0° (*c* 3.0, dioxan) (Found: C, 66.6; H, 6.0. $C_{17}H_{20}O_7$ calc.: C, 67.1; H, 6.0%). The p.m.r. spectrum of this compound is shown in Fig. 1(a).

1,3-*Anhydro*-2,4-O-*benzylidene*-D-*glucitol* (*Compound A*)

The diacetate above (0.5 g) was added to methanol (25 ml) containing sodium (0.1 g) and, after 24 h, the solution was neutralised (2N sulphuric acid) and concentrated. The residue was extracted with chloroform, and removal of the organic solvent gave 1,3-anhydro-2,4-*O*-benzylidene-D-glucitol, which crystallised from chloroform–ethanol–light petroleum (b.p. 40–60°) as prisms, m.p. 121–123°, ν_{max} (Nujol) 3300 cm^{-1}, R_F [silica, benzene–methanol (4:1)] 0.45. (Found: C, 61.4; H, 6.4. $C_{13}H_{16}O_5$ calc.: C, 61.9; H, 6.3%).

5,6-*Di*-O-*acetyl*-1,3-*anhydro*-D-*glucitol* (*Compound C*)

A solution of 5,6-di-*O*-acetyl-2,4-*O*-benzylidene-1,3-anhydro-D-glucitol (0.5 g) in ethyl acetate (25 ml), containing palladium–charcoal (10%, 0.1 g), was hydrogenated until uptake of hydrogen (75 ml) was complete. Removal of the solvent and catalyst, and crystallisation from ethyl acetate–light petroleum (b.p. 40–60°) gave 5,6-di-*O*-acetyl-1,3-anhydro-D-glucitol (0.27 g) as needles, m.p. 88–89° (Found: C, 48.7; H, 6.6 $C_{10}H_{16}O_7$ calc.: C, 48.4; H, 6.4%).

1,3-*Anhydro*-D-*glucitol* (*Compound D*)

(*a*) After standing for 12 h at 20°, a solution of 5,6-di-*O*-acetyl-1,3-anhydro-D-glucitol (0.25 g) in methanol (5 ml) containing sodium (0.01 g) was neutralised (2N sulphuric acid) and concentrated to dryness. The residue was extracted with ethanol (10 ml), and the solution was filtered and concentrated. Crystallisation from ethanol–light petroleum (b.p. 40–60°) gave 1,3-anhydro-D-glucitol (0.13 g) as needles, m.p. 98–99°, $[\alpha]_D^{20}$ −1° (*c* 6.0, water) (Found: C, 43.7; H, 7.4. $C_6H_{12}O_5$ calc.: C, 43.9; H, 7.3%).

(*b*) 1,3-Anhydro-2,4-*O*-benzylidene-D-glucitol (0.25 g) in absolute alcohol (10 ml) was hydrogenated at room temperature in the presence of palladium–charcoal (10%, 0.05 g). Removal of the catalyst and solvent, and crystallisation of the residual gum from ethanol–light petroleum (b.p. 40–60°) gave 1,3-anhydro-D-glucitol, m.p. and mixed m.p. 98–99°.

5-O-*Acetyl*-1,3-*anhydro*-2,4-O-*benzylidene*-6-O-*trityl*-D-*glucitol*

After 3 days at 20°, a solution of 1,3-anhydro-2,4-*O*-benzylidene-D-glucitol (0.3 g) and triphenylmethyl chloride (0.36 g) in pyridine (3 ml) was treated with acetic anhydride (3 ml), stood for a further 2 days, and then poured into ice–water. The precipitated solid was separated, and crystallised from acetone–water, to give 5-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-6-*O*-trityl-D-glucitol (0.45 g) as prisms, m.p. 187–189°, $[\alpha]_D^{20}$ −45.5° (*c* 2.3, dioxan) (Found: C, 76.1; H, 6.2. $C_{34}H_{32}O_6$ calc.: C, 76.1; H, 6.0%). The p.m.r. spectrum of (XX; $R^1 = C_6H_5$, $R^2 = CH_2{\cdot}O{\cdot}C(C_6H_5)_3$) showed the following absorptions:

τ	*Multiplicity*	*Intensity*	*Assignment*
2.40–2.92	multiplet	20	Aromatic protons
4.45	singlet	1	Benzylidene proton
4.50–4.81	multiplet	1	H-5
4.89–5.37	multiplet	3	H-2, H-3, H-4
5.40–5.75	multiplet	2	H-1
6.55	doublet	2	H-6
7.93	singlet	3	Acetate methyl

2,4,5,6-*Tetra*-O-*acetyl*-1,3-*anhydro*-D-*glucitol*

1,3-Anhydro-D-glucitol (0.1 g) was treated in pyridine (3 ml) with acetic anhy-

dride (3 ml) for 24 h, and the solution was poured into ice–water. The product was extracted with chloroform and chromatographed, in the same solvent, on alumina to give 2,4,5,6-tetra-*O*-acetyl-1,3-anhydro-D-glucitol as a syrup, ν_{max}(Nujol) 1750 cm^{-1} (Found: C, 50.9; H, 6.4. $C_{13}H_{20}O_9$ calc.: C, 50.6; H, 6.0%). The p.m.r. spectrum of the product is shown in Fig. 1(d).

1,3-*Anhydro*-2,4-O-*benzylidene*-6-O-*methyl*-D-*glucitol*

(*a*) A mixture of 2,4-*O*-benzylidene-6-*O*-methyl-1-*O*-toluene-*p*-sulphonyl-D-glucitol[18] (2.0 g) and sodium hydroxide (0.5 g) in water (100 ml) was heated with stirring for 2 h, and the solution was then cooled, neutralised (2N sulphuric acid), and concentrated. The product was extracted with chloroform and, after evaporation of the solvent, crystallised from chloroform–light petroleum (b.p. 40–60°) to give the methyl ether as needles (0.9 g), m.p. 123–124°, $[\alpha]_D^{20}+32.9°$ (*c* 3.5, dioxan) (Found: C, 63.1; H, 6.6. $C_{14}H_{18}O_5$ calc.: C, 63.2; H, 6.8%). The p.m.r. spectrum of the product ($CDCl_3$, D_2O shake) showed the following absorptions:

τ	*Multiplicity*	*Intensity*	*Assignment*
2.30–2.73	multiplet	5	Benzylidene aromatic protons
4.52	singlet	1	Benzylidene proton
4.89–5.25	multiplet	3	H-2, H-3, H-4
5.44–6.25	multiplet	3	H-1, H-5
6.37	doublet	2	H-6
6.60	singlet	3	Methoxyl

Acetylation of the product (acetic anhydride–pyridine), with crystallisation from pyridine–water, gave 5-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-6-*O*-methyl-D-glucitol as needles, m.p. 109–110°, $[\alpha]_D^{20}$ +1.7° (*c* 3.7, dioxan) (Found: C, 62.7; H, 6.6. $C_{16}H_{20}O_6$ calc.: C, 62.3; H, 6.5%). The p.m.r. spectrum of the acetate is shown in Fig. 1(b).

(*b*) 2,4-*O*-Benzylidene-1,6-di-*O*-toluene-*p*-sulphonyl-D-glucitol or 5,6-anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (2.0 g) was added to a refluxing solution of methanol (20 ml) containing sodium hydroxide (2N, 5 ml). After 2 h, the solution was neutralised (2N hydrochloric acid), and concentrated, and the residue was extracted with chloroform. Removal of the chloroform gave a gum which crystallised on trituration with methanol. Recrystallisation from chloroform–light petroleum (b.p. 60–80°) gave 1,3-anhydro-2,4-*O*-benzylidene-6-*O*-methyl-D-glucitol (0.45 g), m.p. and mixed m.p. 123–124°.

1,3-*Anhydro*-2,4-O-*benzylidene*-6-O-*ethyl*-D-*glucitol*

(*a*) After being heated for 5 h at 100°, with stirring, a solution of 2,4-*O*-benzylidene-6-*O*-ethyl-1-*O*-toluene-*p*-sulphonyl-D-glucitol[18] (0.2 g) and sodium hydroxide (0.05 g) in water (10 ml) was cooled, neutralised (2N sulphuric acid), and

concentrated. The residual gum was extracted with chloroform, and evaporation of the solvent and crystallisation of the residue (light petroleum, b.p. 80–100°) gave 1,3-anhydro-2,4-*O*-benzylidene-6-*O*-ethyl-D-glucitol (0.05 g) as needles, m.p. 74–75°, $[\alpha]_D^{20}$ +33.2° (*c* 3.32, dioxan) (Found: C, 64.1; H, 7.2. $C_{15}H_{20}O_5$ calc.: C, 64.2; H, 7.2%). The compound was identical with the product described as "2,4-monobenzal-*X*,*X*'-anhydro-ethyl-hexitol" by Vargha[18]. The p.m.r. spectrum of the product ($CDCl_3$, D_2O shake) showed the following absorptions:

τ	*Multiplicity*	*Intensity*	*Assignment*
2.32–2.80	multiplicity	5	Benzylidene aromatic protons
4.55	singlet	1	Benzylidene proton
4.91–5.30	multiplet	3	H-2, H-3, H-4
5.49–6.28	multiplet	3	H-1, H-5
6.29–6.67	multiplet	4	H-6, methylene of ethoxyl
8.83	triplet (1:2:1)	3	Methyl of ethoxyl

Acetylation of the product (acetic anhydride–pyridine), with crystallisation from ethanol, gave 5-*O*-acetyl-1,3-anhydro-2,4-*O*-benzylidene-6-*O*-ethyl-D-glucitol as needles, m.p. 79–80°, $[\alpha]_D^{20}$ +3.3° (*c* 1.80, dioxan) (Found: C, 63.1; H, 6.4. $C_{17}H_{22}O_6$ calc.: C, 63.3; H, 6.5%). The p.m.r. spectrum of the product is shown in Fig. 1(c).

(*b*) 5,6-Anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (2.0 g) was added to a solution of sodium hydroxide (2N, 5 ml) in ethanol (20 ml), and the mixture was refluxed for 2 h. The resulting solution was neutralised (2N sulphuric acid), and concentrated, and the residue was extracted with chloroform. Removal of the chloroform, and crystallisation from light petroleum (b.p. 60–80°), gave the ethyl ether, m.p. and mixed m.p. 74–75°.

6-O-*Benzyl*-2,4-O-*benzylidene*-1-O-*toluene*-p-*sulphonyl*-D-*glucitol*

5,6-Anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (4.06 g) in benzyl alcohol (40 ml) was treated with sodium benzylate (prepared from 0.23 g of sodium) for 3 days at 20°. The solution was neutralised (2N sulphuric acid) and washed with water, and removal of the benzyl alcohol (66°, 1 mm) gave a gum which crystallised from ethanol to give 6-*O*-benzyl-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (2.51 g) as needles, m.p. 109°, $[\alpha]_D^{20}$ +29.3° (*c* 2.7, dioxan) (Found: C, 62.9; H, 6.1. S, 6.2. $C_{27}H_{30}O_8S$ calc.: C, 63.0; H, 5.8. S, 6.2%).

Acetylation (acetic anhydride–pyridine), with crystallisation from ethanol, gave 3,5-di-*O*-acetyl-6-*O*-benzyl-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol, as needles, m.p. 120–121°, $[\alpha]_D^{20}$ −8.5° (*c* 1.4, dioxan) (Found: C, 62.3; H, 6.0; S, 5.4. $C_{31}H_{34}O_{10}S$ calc.: C, 62.2; H, 5.7; S, 5.4%).

1,3-Anhydro-6-O-benzyl-2,4-O-benzylidene-D-glucitol

2,4-*O*-Benzylidene-1,6-di-*O*-toluene-*p*-sulphonyl-D-glucitol *or* 5,6-anhydro-2,4-*O*-benzylidene-1-*O*-toluene-*p*-sulphonyl-D-glucitol (2.0 g) was added to a solution of aqueous sodium hydroxide (2N, 5 ml) in benzyl alcohol (20 ml) and heated for 4 h at 80° with stirring. After cooling and neutralisation (2N sulphuric acid), the organic layer was concentrated, and the residue was crystallised from ethanol–light petroleum (b.p. 40–60°) to give 1,3-anhydro-6-*O*-benzyl-2,4-*O*-benzylidene-D-glucitol (0.6 g) as needles, m.p. 124°, $[\alpha]_D^{20}$ +44.4° (*c* 1.9, dioxan) (Found: C, 69.8; H, 6.5. $C_{20}H_{22}O_5$ calc.: C, 70.2; H, 6.4%). The p.m.r. spectrum of the product ($CDCl_3$, D_2O shake) showed the following absorptions:

τ	*Multiplicity*	*Intensity*	*Assignment*
2.40–2.90	multiplet	10	Aromatic protons
4.59	singlet	1	Benzylidene proton
4.91–5.35	multiplet	3	H-2, H-3, H-4
5.46	singlet	2	Benzyl methylene protons
5.53–6.24	multiplet	3	H-1, H-5
6.32	doublet	2	H-6

Acetylation of the product (acetic anhydride–pyridine), with crystallisation from ethanol, gave 5-*O*-acetyl-1,3-anhydro-6-*O*-benzyl-2,4-*O*-benzylidene-D-glucitol (0.31 g) as prisms, m.p. 117–118°, $[\alpha]_D^{20}$ +9.0° (*c* 2.4, dioxan) (Found: C, 68.7; H, 6.2. $C_{22}H_{24}O_6$ calc.: C, 68.8; H, 6.3%). The p.m.r. spectrum of the acetate ($CDCl_3$, D_2O shake) showed the following absorptions:

τ	*Multiplicity*	*Intensity*	*Assignment*
2.38–2.85	multiplet	10	Aromatic protons
4.54	singlet	1	Benzylidene proton
4.58–4.83	multiplet	1	H-5
4.97–5.37	multiplet	3	H-2, H-3, H-4
5.48	doublet	2	Benzyl methylene
5.55–5.90	multiplet	2	H-1
6.20	doublet	2	H-6
7.96	singlet	3	Acetate methyl

Hydrogenation of the titled compound (Pd·C) gave 1,3-anhydro-D-glucitol, m.p. and mixed m.p. 98-99°.

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SUMMARY

Syntheses of 1,5-anhydro-2,4-di-*O*-benzyl-D-glucitol and 2,4-di-*O*-acetyl-1,5-anhydro-D-glucitol are outlined. Some approaches to the synthesis of 1,5-anhydro-2,4-*O*-benzylidene-D-glucitol, which led to the alternative formation of the corresponding 1,3-anhydrohexitol, are discussed. Relevant properties of the parent 1,3-anhydro-D-glucitol and its derivatives are described, as is the evidence in favour of this novel structural formulation.

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EXTENSION OF SUGAR CHAINS THROUGH ACETYLENIC INTERMEDIATES

PART II[1]. DERIVATIVES OF 1-HEPTYNE-L-*gluco*- AND L-*manno*-3,4,5,6,7-PENTOL

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The acetylene function is a versatile group which can be employed in a wide range of synthetic reactions[2]. It has been utilized in the carbohydrate field for the total syntheses of meso and DL alditols[3,4], and related derivatives[5,6], from non-carbohydrate precursors. Hydroxylated, acetylenic derivatives are of interest as potential tranquilizers[7]. The present report* describes the synthesis of optically active, acetylenic sugar derivatives by the ethynylation of an aldehydo sugar derivative. Also described are methods for separation of the epimers formed in the reaction, procedures for configurational assignment at the newly formed asymmetric center, and applications of n.m.r. techniques for structural assignment.

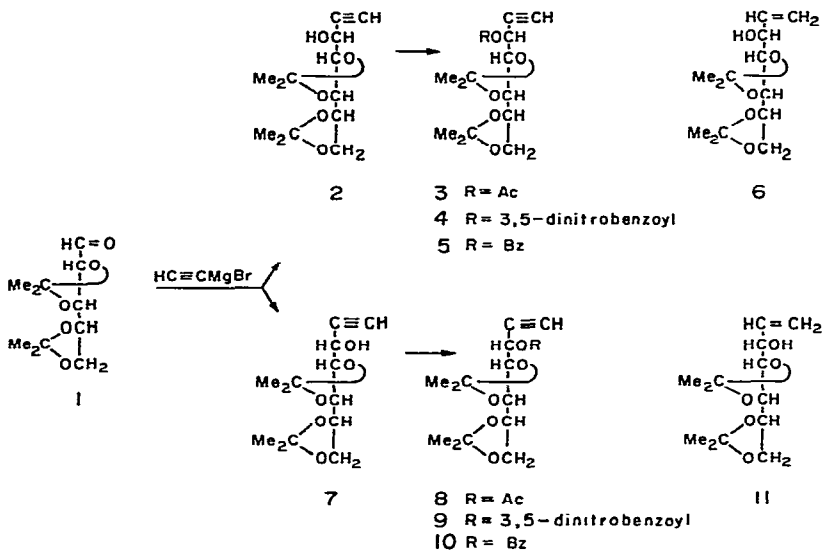
2,3:4,5-Di-*O*-isopropylidene-*aldehydo*-L-arabinose (**1**), prepared through L-arabinose diethyl dithioacetal by a standard procedure[8], underwent reaction with ethynylmagnesium bromide in tetrahydrofuran at room temperature to give the mixed 3-epimeric heptyne derivatives (**2** and **7**) in almost quantitative yield. It was essential to maintain a large excess of acetylene in the system during the reaction, as otherwise the yield of **2** and **7** was diminished by the condensation of two molecules of sugar derivative with one of acetylene, to give a high-boiling side-product. The product (**2** and **7**) showed the anticipated acetylenic hydrogen and C≡C stretching frequencies in its infrared spectrum, at 3.06 μ and 4.74 μ, respectively. The acetylenic hydrogen signal at $\tau \sim 7.50$ in the n.m.r. spectrum was very characteristic. The two epimers were resolved on thin-layer chromatograms, and the evidence of t.l.c. and integrated n.m.r. spectra provided the most reliable criteria of purity of the product.

The two epimers were effectively separated by preparative, gas–liquid chromatography (g.l.c.). The first component to be eluted, approximately 60% of the mixture, was obtained crystalline, m.p. 67–69°, $[\alpha]_D$ +6° (chloroform), and subsequent degradation studies showed it to be the L-*gluco* epimer (**2**). The slower-moving component, about 40% of the mixture, was obtained as a liquid, $[\alpha]_D$ −24° (chloroform), and this was subsequently shown to be the L-*manno* epimer (**7**).

A procedure was sought that would provide a convenient separation of the epimers on a larger scale. Acetylation of the epimers **2** and **7** gave the corresponding 3-acetates (**3** and **8**), which were characterized by physical methods, but they proved

*For a preliminary communication, see Ref. 1.

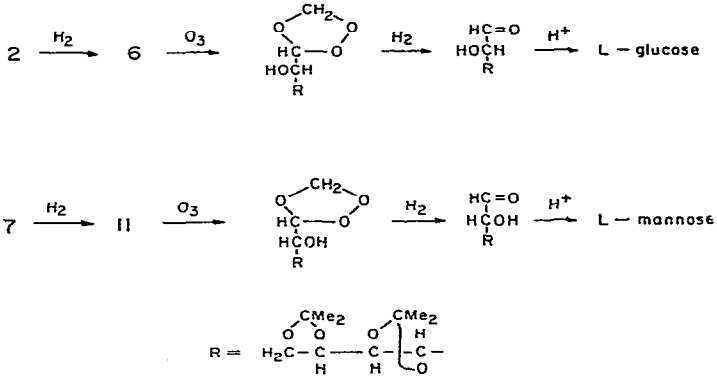
difficult to separate in admixture on a preparative scale. The corresponding 3-benzoates (**5** and **10**) were also prepared, and were characterized by physical methods, but a suitable method for separation in admixture was not found.



Acylation of the mixed epimers (**2** and **7**) with 3,5-dinitrobenzoyl chloride gave the crystalline, mixed, 3-epimeric 3,5-dinitrobenzoates **4** and **9** in essentially quantitative yield. Recrystallization of this mixture several times from ethyl alcohol gave the pure L-*manno* epimer (**9**) in 36% yield (32% overall from **1**); its melting point (167.0–167.5°) and specific rotation (+2.9°) did not change on further recrystallization, and it was identical with a sample of **9** which had been prepared by 3,5-dinitrobenzoylation of the pure epimer **7** obtained by g.l.c. of the mixture of **2** and **7**. The mother liquors from the crystallization of **9** contained the L-*gluco* epimer (**4**) almost exclusively (50% overall yield from **1**), and recrystallization gave pure **4**, m.p. 134.5–135.5°, $[\alpha]_D$ −19.5°.

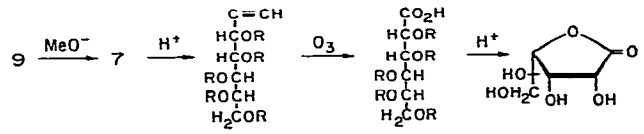
Proof of configuration of **2** and its derivatives (**3**, **4**, and **5**), and of **7** and its derivatives (**8**, **9**, and **10**), was achieved by two independent degradative routes. In the first route, the heptyne derivatives were degraded by C-1–C-2 cleavage to the corresponding hexoses. Conditions were established with the mixed heptyne derivatives (**2** and **7**) for hemihydrogenation to the corresponding heptenes (**6** and **11**), over a partially poisoned palladium catalyst (Lindlar[9] catalyst). N.m.r. spectroscopy provided a very sensitive index of the progress of the reaction; the sharp one-proton doublet at τ 7.50 for the acetylenic hydrogen disappeared, and was replaced by a three-proton multiplet in the vinyl region (τ 3.68–4.95). Over-reduction was indicated by the appearance of a triplet at τ 8.82 and a quartet at τ 6.38, due to an ethyl group, and a diminution in the intensity of the vinyl signals. Different batches of catalyst required different lengths of time for hemihydrogenation, but, in each instance, it was

possible to interrupt the hydrogenation when all of the acetylene had been reduced (but no reduction of the alkene had occurred). Each batch of catalyst was evaluated by reduction of the model alkynols 3-methyl-1-butyn-3-ol and 3-methyl-1-pentyn-3-ol to the corresponding alkenes; n.m.r. data for the model compounds and their reduction products are recorded in the Experimental section. The heptyne derivatives (**2** and **7**) were separately reduced to the heptenes (**6** and **11**), and each product was subjected to ozonolysis. The "ozonides" were cleaved by hydrogenolysis, and the resultant 3,4:5,6-di-*O*-isopropylidene-*aldehydo*-L-hexoses were hydrolyzed to the free hexoses. The hexose obtained from **2** was chromatographically indistinguishable from a glucose, and that from **7** was indistinguishable from a mannose, indicating that **2** and its derivatives have the L-*gluco* configuration, and **7** and its derivatives have the L-*manno* configuration. Some arabinose was present in each hydrolysis product, and it was probably formed by additional cleavage during the ozonolysis step. When the degradation was repeated with the corresponding 3-acetates **3** and **8**, the extent of degradation to arabinose was much diminished, and the hexoses formed were again glucose and mannose, respectively. The overall sequence of conversions from **1** constitutes a method for chain ascent in the sugars.

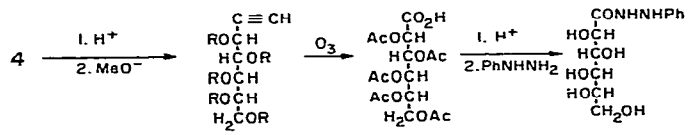


The second sequence for proof of the configuration of **2** and **7** sets out from the crystalline 3,5-dinitrobenzoates **4** and **9**, and the final products were characterized on a crystalline basis. The less-soluble isomer (**9**) of the two 3,5-dinitrobenzoates was saponified to give **7**, and the isopropylidene groups were removed by hydrolysis with 60% acetic acid to give the heptynepentol **12**, which was converted into the pentaacetate **13**. Ozonolysis of **13** for an extended period of time gave penta-*O*-acetyl-L-mannonic acid (**14**), in 41% overall yield from **9**. The latter reaction[10] was first applied in the carbohydrate field by Raphael, for the conversion of 1-pentyne-DL-*erythro*-3,4,5-triol into DL-erythronic acid[4]. Saponification of the pentaacetate **14** gave L-mannonic acid (**15**), which was converted into L-mannono-1,4-lactone (**16**). The latter was identified by chromatography, by its sign of rotation, and by the fact that the X-ray powder diffraction pattern of the crystalline material was identical with that of a sample of the authentic D enantiomorph. Similarly, the

3,5-dinitrobenzoate **4** was deacetonated, and the product was converted, by way of the heptynepentol **17** into the pentaacetate **18**. Ozonolysis of the latter gave penta-*O*-acetyl-L-gluconic acid (**19**), which, on hydrolysis followed by treatment with phenylhydrazine, gave crystalline L-gluconic acid phenylhydrazide (**20**). The X-ray powder diffraction pattern of **20** was identical with that of an authentic sample of the D enantiomorph. An alternative conversion of **4** into **20** was effected by ozonolysis of **4**, followed by hydrolysis, and conversion of the resultant D-glucono-1,4(and 1,5)-lactones into **20**.



12 R = H
13 R = Ac
14 R = Ac
15 R = H
16



17 R = H
18 R = Ac
19
20

It is possible to correlate the appearance of the n.m.r. signal for H-3 with the configuration at C-3 in the heptyne derivatives. The spectra of **2** and **7** are not suitable for exact analysis, owing to coupling of the hydroxyl proton with H-3, and because the H-3 signal occurs close to the multiplet "envelope" of signals for hydrogens on the rest of the chain. The H-3 signal is, however, shifted to low field in the acylated derivatives, and coupling of this proton to H-1 and H-4 can be observed. All of the derivatives showed coupling of H-1 with H-3, $J_{1,3}$ 2.2–2.3 c.p.s. The L-*gluco* derivatives **3**, **4**, and **5** all showed larger coupling of H-3 with H-4 ($J_{3,4}$ 3.6–4.0 c.p.s.) than the corresponding L-*manno* derivatives **8**, **9**, and **10** ($J_{3,4}$ 2.4–2.9 c.p.s.). This observation may be of general utility for assignment of configuration at the newly formed asymmetric center in derivatives of this type[11]. The data indicate that the stereoelectronic requirements of the various ester functions (OR') exceed those of

A B

the ethynyl group, so that the favored rotamer state of the L-*gluco* derivatives **3**, **4**, and **5** is that indicated in A, wherein the OR' group is antiparallel to C-5 and the

rest of the sugar chain (R), and the favored rotamer state in the L-*manno* derivatives **8**, **9**, and **10** is B, having a similar disposition of the R and OR' groups. The observed $J_{3,4}$ couplings, which will reflect a population-weighted time-average of the three rotamer states, are greater for **3**, **4**, and **5** (large contribution from rotamer A, with antiparallel H-3 and H-4) than for **8**, **9**, and **10** (large contribution from rotamer B, with gauche H-3 and H-4).

EXPERIMENTAL

General methods

Solutions were concentrated below 40° under diminished pressure. Melting points were determined with a Thomas–Hoover melting-point apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin-Elmer Model 137 "Infracord" infrared spectrometer. Nuclear magnetic resonance spectra were measured at 60 Mc.p.s. with a Varian A-60 n.m.r. spectrometer. Chemical shifts are given on the τ scale, and spectra were measured, unless otherwise stated, at *ca* 30° with solutions (10–20%) in deuteriochloroform, with tetramethylsilane (τ 10.00) as the internal standard. Deuteration was effected by shaking the prepared sample with one drop of deuterium oxide. Spectra were analyzed on a first-order basis. Microanalyses were determined by W. N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuK_α radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities. Thin-layer chromatography was performed with Desaga equipment, with silica gel G (E. Merck, Darmstadt, Germany) activated at 110° as the adsorbent. Indication was effected with sulfuric acid.

4,5:6,7-Di-O-*isopropylidene*-1-*heptyne*-L-gluco(*and* L-manno)-*3,4,5,6,7-pentol* (**2** *and* **7**)

A solution of ethylmagnesium bromide, prepared in dry tetrahydrofuran (300 ml) from magnesium (4.00 g) and ethyl bromide (20 ml), was added dropwise to tetrahydrofuran (600 ml) saturated with acetylene at room temperature. A stream of acetylene was passed through the solution throughout the addition, and for 1 h afterwards. To the resultant solution of ethynylmagnesium bromide was added slowly, with stirring, a solution of freshly prepared, freshly distilled 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose[8] (**1**, 22.0 g) in tetrahydrofuran (100 ml). A slow stream of acetylene was passed through the solution throughout the addition, and subsequently for a further 3 h. The solution was concentrated to 300 ml, ether (300 ml) was added, and the solution was washed at 0° with three 400-ml portions of 10% aqueous ammonium chloride, three 400-ml portions of water, and dried (magnesium sulfate). Evaporation of the solution gave the product as a syrup which crystallized partially upon refrigeration, yield 22.0 g (90%). This product contained none of the starting material, and was composed almost exclusively of the two 3-epimeric heptyne

derivatives, as revealed by t.l.c. (4:1 benzene–methanol) and g.l.c. (see below). A sample distilled at 0.1 mm Hg furnished the analytical sample.

Anal. Calc. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.86. Found: C, 61.02; H, 7.88.

The n.m.r. spectrum of this product gave only those signals anticipated for a mixture of **2** and **7**. In five such experiments, the yields were in the range 85–92%.

Separation of the 3-epimers

The product from the preceding preparation was resolved by preparative g.l.c. on a Beckman GC2A gas chromatograph (Beckman Instruments, Inc., Fullerton, California) equipped with a thermal conductivity detector and a 10 ft × 5/8 in stainless-steel column packed with Carbowax* 20M (15%) on Chromosorb* W (60–100 mesh). The carrier gas was helium at an inlet pressure of 30 lb. in^{-2}, and samples (0.3–0.6 g in 2–3 ml of chloroform) were injected manually, with the column temperature initially at 200°, rising to 240° during 80 min. Two components were eluted, having retention times 122 and 136 min, in the weight ratio of 3:2. The components were completely separated from one another, and no other components were detected in the samples. The component eluted first, identified (by subsequent conversions) as 4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**2**), crystallized spontaneously; it had m.p. 67–69°; $[\alpha]_D^{22}$ +6 ±1° (*c* 3, chloroform)**; R_F 0.80 (4:1 benzene–methanol); λ_{max}^{KBr} 2.91 (OH), 3.11 (C≡CH), 4.74 (C≡C), 7.25, 7.31 μ (CMe_2); n.m.r. data (deuteriochloroform): τ 8.52, 8.57, 8.64 (3-, 6-, and 3-proton singlets, CMe_2), τ 7.50 (1-proton doublet, shifts to τ 7.68 at lower concentration, $J_{1,3}$ 2.3 c.p.s., H-1), τ 6.53 (1-proton broad doublet, shifts to higher field at lower concentration, OH), τ 5.80–6.10, 6.27–6.39 (multiplets, 5 protons, H-4,5,6,7,7'), τ 5.41 (1-proton multiplet, H-3); X-ray powder diffraction data: 10.22 s (2,2), 7.90 s (2,2), 5.73 w, 5.54 vs (1,1), 4.95 vs (1,1), 4.64 m (3,3), 3.97 m (4), 3.75 m (3,3), 3.24 w, 3.09 vw, 2.97 vw, 2.48 vw, 2.40 vw, 1.99 w.

Anal. Calc. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.86. Found: C, 60.94; H, 7.94.

The product having retention time of 136 min, 4,5:6,7-di-*O*-isopropylidene-1-heptyne-L-*manno*-3,4,5,6,7-pentol (**7**) was obtained as a liquid, $[\alpha]_D^{22}$ −24 ±1° (*c* 2.3, chloroform)**; R_F 0.66 (4:1 benzene–methanol); λ_{max}^{KBr} 2.93 (OH), 3.06 (C≡CH), 4.72 (C≡C), 7.26, 7.32 μ (CMe_2); n.m.r. data (deuteriochloroform): τ 8.56, 8.65, 8.75 (singlets, 12 protons CMe_2), τ 7.50 (1-proton doublet, shifts to τ 7.67 at lower concentration, $J_{1,3}$ 2.3 c.p.s., H-1), τ 7.02 (1-proton broad singlet, shifts to higher field at lower concentration, OH), τ 5.76–6.11, 6.25–6.36 (multiplets, 5 protons, H-4,5,6,7,7'), τ 5.48 (1-proton multiplet, H-3).

Both products were homogeneous by t.l.c.

*3-O-Acetyl-4,5:6,7-di-O-isopropylidene-1-heptyne-*L-gluco(*and* L-manno)*-3,4,5,6,7-pentol* (**3** *and* **8**)

A solution of the mixed epimers (**2** and **7**, 9.1 g) in pyridine (20 ml) was treated

*Analabs, Inc., Hamden, Connecticut.

The signs of rotation of **2 and **7** were inadvertently reversed in Ref. 1.

with acetic anhydride (20 ml). After 18 h at room temperature, the mixture was poured into water, stirred for 30 min, and the product was extracted with dichloromethane. The extract was washed at 0° with N hydrochloric acid, water, dried (magnesium sulfate), evaporated, and the product distilled at 0.15 mm Hg to give the mixed epimeric 3-acetates (**3** and **8**), yield 8.14 g (76%); b.p./0.15 mm 108–109°; R_F 0.96 (4:1 benzene–methanol); λ_{max}^{film} 3.09 (C≡CH), 4.72 (C≡C), 5.72 μ (OAc).

Anal. Calc. for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 59.92; H, 7.74.

Acetylation of **2** under the above conditions gave the L-*gluco* epimer (**3**), R_F 0.96 (4:1 benzene–methanol); n.m.r. data (deuteriochloroform): τ 8.54, 8.58, 8.62 (6-, 3-, and 3-proton singlets, CMe_2), τ 7.87 (3-proton singlet, OAc), τ 7.49 (1-proton doublet, $J_{1,3}$ 2.2 c.p.s., H-1), τ 5.50–6.12 (5-proton multiplet, H-4,5,6,7,7'), τ 4.46 (1-proton quartet, $J_{3,4}$ 3.8 c.p.s., H-3). Acetylation of **7** gave the L-*manno* epimer (**8**), R_F 0.96 (4:1 benzene–methanol); n.m.r. data (deuteriochloroform): τ 8.53, 8.63, 8.72 (6-, 3-, and 3-proton singlets, CMe_2), τ 7.85 (3-proton singlet, OAc), τ 7.51 (1-proton doublet, $J_{1,3}$ 2.2 c.p.s., H-1), τ 5.45–6.15 (5-proton multiplet, H-4,5,6,7,7'), τ 4.36 (1-proton triplet, $J_{3,4}$ 2.4 c.p.s., H-3).

The mixture of **3** and **8** gave an n.m.r. spectrum corresponding to a 3:2 mixture of the two isomers.

*3-O-(3,5-Dinitrobenzoyl)-4,5:6,7-di-O-isopropylidene-1-heptyne-*L-manno*-3,4,5,6,7-pentol* (**9**)

To 3,5-dinitrobenzoyl chloride (20.5 g) in benzene (1 l) was added dropwise, with stirring at 0°, a solution of the mixed epimers **2** and **7** (15.3 g) in pyridine (50 ml). The mixture was kept 30 min at 0°, and 18 h at room temperature, 5 ml of water was added, and, after 1 h, ether (300 ml) was added. The solution was washed at 0° with water, N hydrochloric acid, 0.5N sodium hydroxide, and water, dried (magnesium sulfate), and evaporated, to give the mixed 3-epimeric 3-(3,5-dinitrobenzoates) (**4** and **9**) as a solid, yield 25 g (93.5%); m.p. 97–151°; R_F 0.82 (1:1 benzene–ether). Five recrystallizations from ethanol gave the pure L-*manno* epimer (**9**) as fine needles, yield 9.6 g (36%), m.p. 167.0–167.5°; $[\alpha]_D^{22}$ +2.9 ±0.8° (*c* 2, chloroform); R_F 0.82 (1:1 benzene–ether); λ_{max}^{KBr} 3.08 (C≡CH), 4.73 (C≡C), 5.76 (OAc), 6.14 (aryl), 6.46 (NO_2), 7.24, 7.28 (CMe_2), 7.44 μ (NO_2); n.m.r. data: τ 8.46, 8.50, 8.53, 8.62 (3-proton singlets, CMe_2), τ 7.28 (1-proton doublet, $J_{1,3}$ 2.3 c.p.s., H-1), τ 5.50–6.18 (5-proton multiplet, H-4,5,6,7,7'), τ 3.98 (1-proton triplet, $J_{3,4}$ 2.7 c.p.s., H-3), τ 0.60 (3 protons, aryl); X-ray powder diffraction data: 9.61 vw, 8.84 w, 8.12 w, 7.31 m, 6.37 s (6), 5.13 vs (2), 5.04 vs (1), 4.67 m, 4.52 vw, 4.21 s (5), 4.08 vw, 3.82 s (3,3) 3.63 s (3,3), 3.47 s (4), 3.38 vw, 3.18 vw, 3.01 w, 2.67 m, 2.55 w, 2.43 w, 2.35 vw.

Anal. Calc. for $C_{20}H_{22}N_2O_{10}$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.29; H, 5.03; N, 6.33.

A sample of **7**, which had been isolated by g.l.c. of the mixture of epimers, was acylated with 3,5-dinitrobenzoyl chloride by the foregoing procedure, to give **9**, m.p. 166.5–167.5°, identical with the product obtained from the mixed epimers, by mixed m.p., X-ray powder diffraction pattern, and i.r. and n.m.r. spectra.

3-O-(3,5-Dinitrobenzoyl)-4,5:6,7-di-O-isopropylidene-1-heptyne-L-gluco-*3,4,5,6,7-pentol* (**4**)

The mother liquors from the foregoing preparation from the mixed epimers (**2** and **7**) contained almost exclusively the L-*gluco* derivative (**4**), crude yield 15 g (56%), and it was obtained pure after several recrystallizations (from ethanol) to constant specific rotation; yield 4.16 g (15.5%); m.p. 134.5–135.5°; $[\alpha]_D^{22}$ −19.5 ±0.8° (*c* 2.1, chloroform); R_F 0.82 (1:1 benzene–ether); λ_{max}^{KBr} 3.11 (C≡CH), 4.71 (C≡C), 5.77 (C=O), 6.12 (aryl), 6.40 (NO_2), 7.23, 7.30 (CMe_2), 7.44 μ (NO_2); n.m.r. data: τ 8.46, 8.53, 8.62, 8.65 (3-proton singlets, CMe_2), τ 7.30 (1-proton doublet, $J_{1,3}$ 2.3 c.p.s., H-1), τ 5.48–6.19 (5-proton multiplet, H-4,5,6,7,7′), τ 4.10 (1-proton quartet, $J_{3,4}$ 4.0 c.p.s., H-3), τ 0.76 (3 protons, aryl); X-ray powder diffraction data: 10.69 m, 9.40 w, 8.08 m, 6.76 m, 5.59 vs (1), 5.04 w, 4.67 s (3,3), 4.35 s (3,3), 4.00 s (2), 3.76 vw, 3.65 vw, 3.44 w, 3.26 w, 3.12 vw, 2.91 vw, 2.83 vw.

Anal. Calc. for $C_{20}H_{22}N_2O_{10}$: C, 53.33; H, 4.92; N, 6.22. Found: C, 53.36; H, 5.06; N, 6.38.

A mixture of **4** and **9** melted unsharply over the range 114–125°.

3-O-Benzoyl-4,5:6,7-di-O-isopropylidene-1-heptyne-L-gluco (*and* L-manno)-*3,4,5,6,7-pentol* (**5** *and* **10**)

The heptyne derivatives **2** and **7**, which had been separated by g.l.c., were separately benzoylated. Each derivative (500 mg) in pyridine (10 ml) was treated with benzoyl chloride (0.5 ml), and the reaction mixture was processed by the procedure used for the 3,5-dinitrobenzoates, to give the 3-benzoates **5** and **10** as oils in essentially quantitative yield. The L-*gluco* isomer **5** had R_F 0.93 (4:1 benzene–methanol); n.m.r. data: τ 8.41, 8.49, 8.65, 8.68 (3-proton singlets, CMe_2), τ 7.27 (1-proton doublet, $J_{1,3}$ 2.3 c.p.s., H-1), τ 5.50–6.16 (5-proton multiplet, H-4,5,6,7,7′), τ 4.11 (1-proton quartet, $J_{3,4}$ 3.6 c.p.s., H-3), τ 1.91, 2.54 (multiplets, 5 protons, Ph). The L-*manno* isomer **10** had R_F 0.93 (4:1 benzene–methanol), λ_{max}^{film} 3,09 (C≡CH), 4.71 (C≡C), 5.80 (OBz), 7.25, 7.32 (CMe_2), 6.23 μ (aryl); n.m.r. data (deuteriochloroform): τ 8.52, 8.58, 8.68 (3-, 6-, and 3-proton singlets, CMe_2), τ 7.30 (1-proton doublet, $J_{1,3}$ 2.3 c.p.s., H-1), τ 5.58-6.06 (5-proton multiplet, H-4,5,6,7,7′), τ 4.10 (1-proton triplet, $J_{3,4}$ 2.9 c.p.s., H-3) τ 1.92, 2.56 (multiplets, 5 protons, Ph).

Anal. (for **10**): Calc. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.61, H, 6.79.

4,5:6,7-Di-O-isopropylidene-1-heptene-L-gluco(*and* L-manno)-*3,4,5,6,7-pentol* (**6** *and* **11**)

To a solution of the mixed epimeric heptynes (**2** and **7**, 4.0 g) in cyclohexane (100 ml) and ethyl acetate (100 ml) was added freshly prepared Lindlar catalyst[9] (2 g) and quinoline (1 g). The mixture was shaken in an atmosphere of hydrogen at a pressure of 15 lb.in^{-2} until 1 mole/mole of hydrogen had been absorbed (35–40 min). The catalyst was filtered off, the filtrate was washed at 0° with N hydrochloric acid, and water, and the dried (magnesium sulfate) organic layer was evaporated to give the mixed 3-epimeric heptenes (**6** and **11**) as an oil; yield, essentially quantitative;

λ_{max}^{film} 2.91 (OH), 6.07 (CH_2=CH), 7.26, 7.32 μ (CMe_2); n.m.r. data: τ 8.60, 8.63, 8.68 (singlets, 12 protons, CMe_2), τ 5.67–6.25 (7-proton multiplet, H-3,4,5,6,7,7′, OH), τ 3.68–4.95 (3-proton multiplet, H-1,1′,2).

Anal. Calc. for $C_{13}H_{22}O_5$: C, 60.44; H, 8.58. Found: C, 60.39; H, 8.59.

When the time of reduction was increased, the n.m.r. spectrum showed appearance of a triplet, τ 8.82, and a quartet, τ 6.38, having coupling J 7 c.p.s. and intensity ratios characteristic of the ethyl group. Different batches of catalyst required different reaction times for reduction to the alkene without further reduction to the saturated derivative.

Reduction of 3-methyl-1-butyn-3-ol and 3-methyl-1-pentyn-3-ol to the corresponding alkenes

The alkyne derivatives were reduced over Lindlar catalyst, essentially by the procedure of the preceding experiment, to establish the optimum time of reduction for each batch of catalyst, and the extent of conversion was determined by n.m.r. spectroscopy. The butyne derivative, n.m.r. data (neat liquid): τ 8.48 (6-proton singlet, Me), τ 7.43 (1-proton singlet, H-1), τ 5.63 (1-proton singlet, OH), was converted into 3-methyl-1-buten-3-ol, n.m.r. data (neat liquid): τ 8.78 (6-proton singlet, Me), τ 6.05 (1-proton singlet, OH), τ 5.10 (1 proton, $J_{1,1'}$ 1.7 c.p.s., $J_{1,2}$ 10.0 c.p.s., H-1), τ 4.87 (1 proton $J_{1',2}$ 18.0 c.p.s., H-1′), τ 4.02 (1 proton, H-2). Similarly, the pentyne derivative, n.m.r. data (neat liquid): τ 8.98 (3-proton triplet, $J_{4,5}$ 7 c.p.s., H-5), τ 8.53 (3-proton singlet, 3-Me), τ 8.30 (2-proton multiplet, H-4), τ 7.48 (1-proton singlet, H-1), τ 6.03 (1-proton singlet, OH), was converted into 3-methyl-1-penten-3-ol, n.m.r. data (deuteriochloroform): τ 8.85 (3-proton triplet, $J_{4,5}$ 7 c.p.s., H-5), τ 8.48 (3-proton singlet, 3-Me), τ 8.01–8.61 (3 protons, H-4, OH), τ 4.55 (1 proton, $J_{1,1'}$ 1.7 c.p.s., $J_{1,2}$ 10 c.p.s., H-1), τ 4.51 (1 proton, $J_{1,2}$ 17.5 c.p.s., H-1′), τ 3.77 (1 proton, H-2).

Conversion of **2** *(or* **3***) into a glucose and* **7** *(or* **8***) into a mannose*

The crystalline heptyne derivative **2**, m.p. 67–69°, $[\alpha]_D$ +6° (40 mg) in cyclohexane (50 ml), ethyl acetate (50 ml), and quinoline (1 drop) was hydrogenated over Lindlar[9] catalyst (0.1 g) for 1 h under 15 lb.in^{-2} pressure. The mixture was filtered, and a stream of ozonized oxygen was passed through the filtrate for 1 h at room temperature. Adams catalyst (platinum oxide) (0.1 g) was added, the mixture was hydrogenated for 1 h under 15 lb.in^{-2} pressure, and filtered, and the filtrate was washed at 0° with N hydrochloric acid, and water, dried (magnesium sulfate), and evaporated. The product, which gave a positive Schiff test, was heated in 1% aqueous sulfuric acid (50 ml) for 3 h at 95°, the solution was neutralized with barium carbonate, filtered, and the filtrate was treated with a 1:1 mixture (20 ml) of Dowex-50 (H^+) and Dowex-1 (OH^-) ion-exchange resins. The solution was evaporated, and the residue was compared by paper chromatography with reference samples of D-glucose, D-mannose, and L-arabinose. The product contained a major component and a minor component, as revealed by aniline hydrogen phthalate[12] or silver nitrate–sodium

hydroxide[13]. The major component was indistinguishable from a glucose, the minor component was indistinguishable from an arabinose, and no component corresponding to a mannose was observed, in each of the following solvent systems: 4:1:5 butyl alcohol–ethanol–water (upper phase), 45:5:49:1 butyl alcohol–ethanol–water–ammonium hydroxide (upper phase), 6:4:3 butyl alcohol–pyridine–water, and 5:7.2:2:2 propyl alcohol–benzyl alcohol–85% formic acid–water.

When the procedure was repeated with the 3-acetate **3**, an essentially similar result was obtained, except that the proportion of glucose to arabinose in the final product was greater.

The same degradation was applied to the noncrystalline heptyne derivative **7**, $[\alpha]_D$ −24°. Paper chromatography, with the same systems as previously used, revealed that the product contained a component indistinguishable from a mannose, and a component corresponding to an arabinose. No component corresponding to a glucose was observed. Degradation of the corresponding 3-acetate (**8**) gave an essentially similar result, except that a greater proportion of mannose to arabinose was observed.

Conversion of **9** *into* L-*mannono-1,4-lactone* (**16**)

A solution of the 3,5-dinitrobenzoate **9**, m.p. 166.5–167.5°, (1.00 g) in methanol (10 ml) was treated with a catalytic amount of sodium methoxide for 2 h at room temperature, the precipitated methyl 3,5-dinitrobenzoate was removed, the filtrate was deionized by treatment with Dowex-50 (H^+) ion-exchange resin, and the solution was evaporated. The residue was heated with 60% aqueous acetic acid (50 ml) for 1 h at 95°, the solution was concentrated to 20 ml, and water was added to precipitate residual methyl 3,5-dinitrobenzoate. The mixture was filtered, and the filtrate was evaporated to give 1-heptyne-L-*manno*-3,4,5,6,7-pentol (**12**) as a white solid. The latter was dissolved in pyridine (10 ml), acetic anhydride (10 ml) was added, the mixture was kept overnight at room temperature, poured into ice and water, and the mixture was extracted with chloroform. The extract was washed successively at 0° with water, N hydrochloric acid, and aqueous sodium bicarbonate, and dried (magnesium sulfate). Evaporation of the solution gave L-*manno*-3,4,5,6,7-penta-acetoxy-1-heptyne (**13**) as a syrup; λ_{max}^{film} 3.09 (C≡CH), 4.72 (C≡C), 5.75 μ (OAc).

A stream of ozonized oxygen was passed through a solution of the foregoing product (**13**), in carbon tetrachloride (100 ml), for 6 h at 0°. Water (100 ml) was added, the mixture was evaporated until the carbon tetrachloride had been removed, and the remaining aqueous solution was maintained for 15 min at 60–70°. The strongly acidic solution was neutralized with sodium hydrogen carbonate, and washed with dichloromethane (200 ml), and the aqueous layer was acidified with N hydrochloric acid, and extracted with dichloromethane (200 ml). The organic extract was washed with water, dried (magnesium sulfate), and evaporated, to give penta-*O*-acetyl-L-mannonic acid (**14**), yield 372 mg (41% from **9**); λ_{max}^{film} 3.42, 3.92 (CO_2H), 5.77 μ (OAc, CO_2H).

A solution of **14** (185 mg) in methanol (10 ml) was treated with 3 molar equiva-

lents of sodium methoxide; a white precipitate formed. After 2 h at room temperature, the mixture was decationized by shaking with an excess of Dowex-50 (H^+) ion-exchange resin, whereupon the precipitate redissolved. Evaporation of the methanolic solution gave L-mannonic acid (**15**) as a partially crystallized syrup, yield 51 mg (~60%). This product (10 mg) in N hydrochloric acid (0.5 ml) was heated for 30 min at 95°, and the solution was evaporated, and examined by paper chromatography (3:1:3 ethyl acetate–acetic acid–water) and by t.l.c. with "Avicel-Technical Grade" microcrystalline cellulose[14] (4:1:5 butyl alcohol–ethanol–water, upper phase), with hydroxylamine–ferric chloride[15] as the detecting reagent. Reference samples of D-mannonic acid and D-gluconic acid were submitted to a similar acid treatment. The product gave a single component (on the chromatograms) which was indistinguishable from a mannono-1,4-lactone (**16**). The remainder of the L-mannonic acid (40 mg) was evaporated four times with propyl alcohol containing a trace of hydrochloric acid, and the crystalline L-mannono-1,4-lactone (**16**) was washed with propyl alcohol; yield 30 mg (37%); $[\alpha]_D^{22}$ *ca* −40° (30 min, water) [lit.[16] $[\alpha]_D$ −51.8° (water)]; X-ray powder diffraction data: 15.06 w, 8.96 vw, 6.97 vw, 5.88 m, 5.11 s (3), 4.57 s (4), 4.20 vs (1), 3.98 vw, 3.75 m (6), 3.54 m, 3.21 s (2), 2.87 s (5), 2.71 w, 2.56 m, 2.43 vw, 2.38 vw, 2.24 w, 2.14 w, 2.07 vw. A sample of D-mannono-1,4-lactone, m.p. 151°, gave an identical X-ray powder diffraction pattern.

Conversion of **4** *into* L-*gluconic acid phenylhydrazide* (**20**)

A solution of the 3,5-dinitrobenzoate **4**, m.p. 134.5–135.5°, (0.85 g) in 60% aqueous acetic acid (50 ml) was heated for 1 h at 95°, and then evaporated. The residue was dissolved in methanol (10 ml), a trace of sodium methoxide was added, and, after 2 h at 22°, the solution was filtered, decationized by shaking with Dowex-50 (H^+) resin, and evaporated, to give syrupy 1-heptyne-L-*gluco*-3,4,5,6,7-pentol (**17**). The latter was acetylated, the resultant pentaacetate (**18**) was ozonized, and the product was purified, essentially by the procedure used for conversion of **12** into **14**, to give 2,3,4,5,6-penta-*O*-acetyl-L-gluconic acid (**19**) as a partially crystallized syrup, yield 200 mg. A solution of **19** in N hydrochloric acid (10 ml) was heated for 2 h at 96°, and then evaporated. Chromatographic examination of the product, with the systems used for characterization of the lactone **16**, revealed two components, whose chromatographic properties were indistinguishable from those of D-glucono-1,4-lactone and D-glucono-1,5-lactone. Subjection of calcium D-gluconate to the hydrolytic conditions used with **19** gave a mixture of the two D-gluconolactones, chromatographically indistinguishable from the hydrolyzate from **19**. The mixture of L-gluconolactones from **19** (ca 50 mg) was heated with acetic acid (0.05 ml) and phenylhydrazine (0.05 ml) for 2 h at 96°, and the product was purified by chromatography on microcrystalline cellulose[14], to give crystalline L-gluconic acid phenylhydrazide (**20**), R_F 0.47 (4:1:5 butyl alcohol–ethanol–water, upper phase); X-ray powder diffraction data: 13.10 vs (1,1,1), 7.56 m, 5.41 vw, 5.04 vs (1,1,1), 4.55 vs (1,1,1), 4.32 m (3), 4.00 m (2), 3.77 vw, 3.55 vw, 3.39 m (4), 3.20 w, 3.09 vw, 2.70 w, 2.59 w, 2.25 w.

A sample of D-gluconic acid phenylhydrazide[17] had the same R_F value, and gave identical X-ray powder diffraction data.

In a second experiment, a stream of ozonized oxygen was passed for 6 h at 0° through a solution of the 3,5-dinitrobenzoate **4** (0.76 g) in carbon tetrachloride (100 ml). Water (100 ml) was added, the carbon tetrachloride was removed by evaporation, and the remaining aqueous solution was kept for 15 min at 60–70°. The solution was neutralized with sodium hydrogen carbonate, and washed with ether (200 ml), and the aqueous layer was acidified with N hydrochloric acid, and the product extracted with ether (2 × 200 ml). The extract was washed with water, dried (magnesium sulfate), and evaporated, and the residue was heated for 2 h at 95° with N hydrochloric acid (10 ml). The mixture was filtered, and the filtrate was evaporated to give a syrup, yield 250 mg (83%), which, by chromatography with the systems used for the lactone **14**, was shown to consist, almost exclusively, of the glucono-1,4(and 1,5)-lactones. The product could be converted into crystalline L-gluconic acid phenylhydrazide (**20**), identical in all respects with the product prepared by the preceding route.

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SUMMARY

Ethynylation of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-L-arabinose (**1**) gave a high yield of the 3-epimeric heptyne derivatives **2** and **7**, separable by preparative g.l.c. or by fractional recrystallization of the derived 3,5-dinitrobenzoates **4** and **9**. Proof of configuration of **2** and **7** was effected by ozonolysis of the derived alkenes **6** and **11** to give derivatives of L-glucose and L-mannose, respectively. A second proof was afforded by conversion of **2** and **7** into the corresponding pentaacetoxyheptynes **18** and **13**, followed by ozonolysis to give derivatives of L-gluconic and L-mannonic acids, respectively. Differences in the n.m.r. spectra of the two series of heptyne derivatives were correlated with the difference in configuration at C-3.

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BRÄUNUNGSREAKTIONEN UND FRAGMENTIERUNGEN VON KOHLENHYDRATEN

TEIL III. ÜBER REAKTIONEN DES DIHYDROXYACETONS MIT AMINEN UND AMINOSÄUREN*

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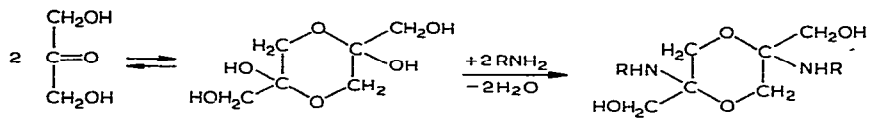
(Eingegangen den 4. Februar, 1966)

EINFÜHRUNG

Dihydroxyaceton reagiert bei erhöhter Temperatur mit Verbindungen, die primäre Aminogruppen besitzen, sehr rasch unter Bildung tiefbraun gefärbter polymerer Substanzen, die als Melanoidine bezeichnet werden. Diese Reaktion verläuft, ähnlich wie bei Hexosen[1], über eine komplizierte Reaktionsfolge, wobei zahlreiche verschiedenartige Zwischenprodukte auftreten können.

DISKUSSION

Setzt man Dihydroxyaceton (I) jedoch unter milden Bedingungen bei niedriger Temperatur mit primären Aminen um, so lassen sich labile, farblose, kristallisierte Primärprodukte abfangen. Diese Produkte stellen Ketosylamine (III) dar, die sich vom dimeren Dihydroxyaceton (II) ableiten[2]. Sie werden analog der *N*-Glycosidbildung der Hexosen auf folgendem Wege gebildet :



I II III

Die Struktur der Ketosylamine ergibt sich aus folgenden Eigenschaften: Die Elementaranalyse zeigt, dass sie aus den Reaktionspartnern unter Wasserabspaltung entstanden sind. Im I.r.-Spektrum frisch dargestellter Präparate tritt die Absorptionsbande der C=N-Doppelbindung[3] bei 1690–1640 cm^{-1} praktisch nicht auf. Es kann also nicht eine einfache Kondensation zur Schiffschen Base eingetreten sein. Beim Behandeln mit verdünnter Salzsäure werden die Ketosylamine leicht hydrolysiert und als Spaltprodukte lassen sich chromatographisch nur die Ausgangsverbindungen nachweisen. Die Ketosylamine sind so wenig stabil, dass beim Versuch der Umkristallisation oder beim Stehen bei Zimmertemperatur innerhalb kurzer Zeit Zerset-

*Teil II: K. HEYNS, R. STUTE, UND H. SCHARMANN, *Tetrahedron*, im Druck.

zung eintritt. Aus diesem Grunde liess sich das Molekulargewicht nicht sicher bestimmen.

Die Ketosylamine schmelzen unter Zersetzung. Eine Ketosylamin-Umlagerung zum entsprechenden Derivat des 2-Amino-2-desoxyglycerinaldehyds konnte in keinem Falle beobachtet werden. Statt dessen trat bei Einwirkung katalytischer Säuremengen stets Bräunung und gleichzeitige Hydrolyse ein.

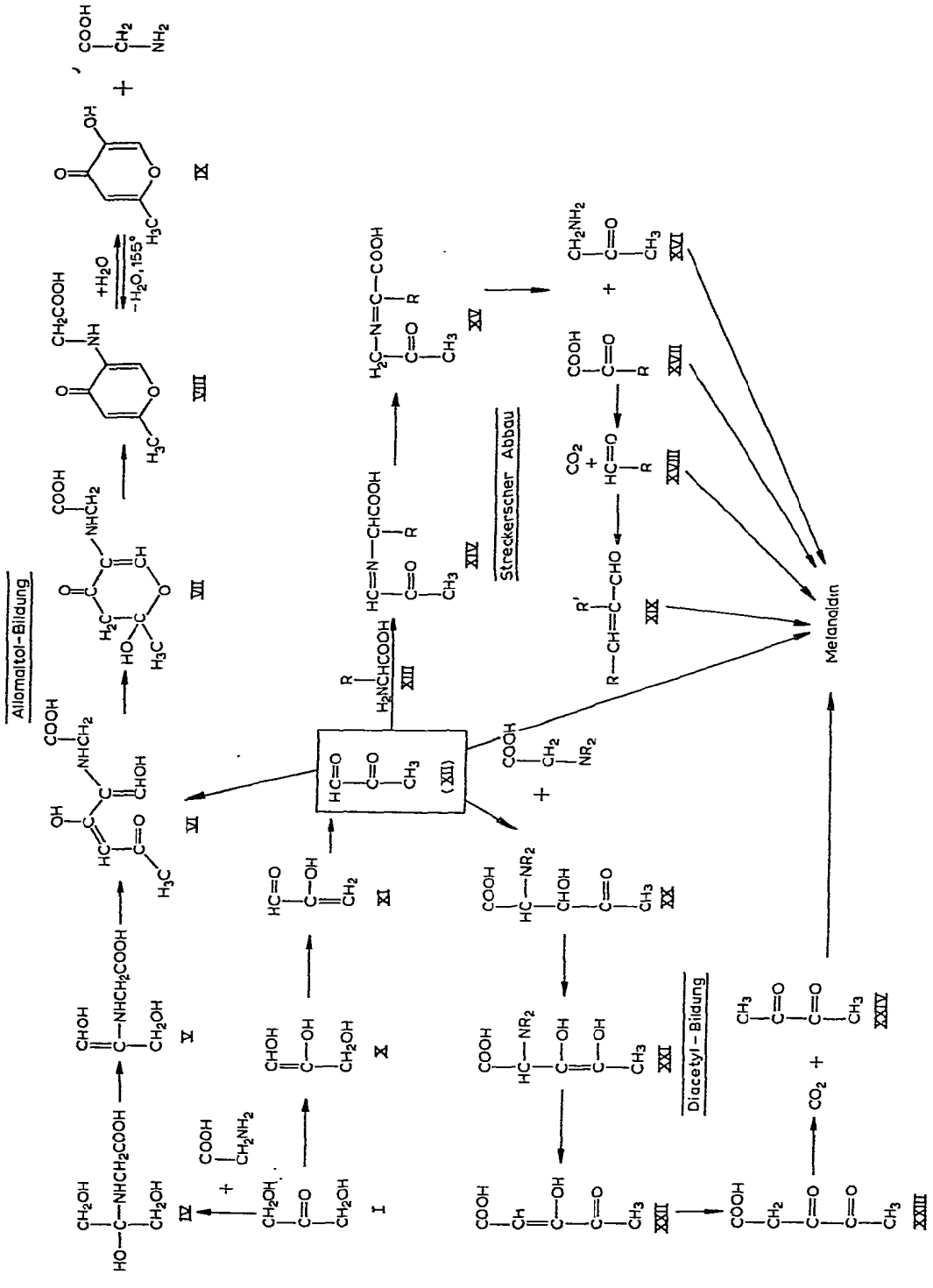
Prey und Unger[4] haben vermutlich ein ähnliches Ketosylamin aus Äthylendiamin und Dihydroxyaceton dargestellt. Das von ihnen erhaltene Reaktionsprodukt bestand jedoch aus zwei Komponenten und wurde als ein Gemisch aus monomerem Halbaminal und Schiffscher Base angesprochen.

Weiterhin wurde die Reaktion von Aminosäuren mit Dihydroxyaceton untersucht, wobei insbesondere folgende Aminosäuren eingesetzt wurden: Glycin, Sarcosin, Alanin, Phenylalanin, Tyrosin, Methionin, Lysinhydrochlorid, Argininhydrochlorid, Glutaminsäure; als Lösungsmittel wurden Wasser (50°, 90°), Methanol (50°), Äthanol (50°, 78°) und Dimethylformamid (50°, 90°) verwendet.

Eine papier- und dünnschichtchromatographische Untersuchung aller Reaktionslösungen ergab, dass Ketosylaminosäuren und durch Ketosylamin-Umlagerung daraus entstandene Aldoseaminosäuren (Glycerinaldehyd–Aminosäuren) im Reaktionsmilieu nicht nachweisbar sind. Schon bei 50° führt die Reaktion allgemein schnell zur Bildung von Melanoidinen, ohne dass eine Anreicherung eines Intermediärproduktes zu beobachten ist.

Aus einer wässerigen Reaktionslösung von Dihydroxyaceton und Glycin liessen sich jedoch durch Extraktion während der ablaufenden Bräunungsreaktion zwei interessante Zwischenprodukte isolieren : Allomaltol (IX) und *N*-(Methylpyron-4-yl-5)glycin (VIII). Die Extraktion erfolgte mit 15 l Chloroform, welche im Verlauf von 30 Stunden durch die Reaktionslösung getropft wurden. Mit dieser Arbeitsweise kann man die kontinuierliche Extraktion so schonend ausführen, dass die Abtrennung der empfindlichen Zwischenprodukte neben der schnell ablaufenden Bräunungsreaktion möglich ist.

Allomaltol (IX) wurde mit einer Ausbeute von *ca.* 1% aus dem Extrakt einer bei 55° ablaufenden Bräunungsreaktion isoliert. Es wurde mit einem nach Yabuta[5] und Brown[6] dargestellten Präparat durch Vergleich der *p*-Toluolsulfonsäureester identifiziert. Lässt man die gleiche Bräunungsreaktion von Dihydroxyaceton mit Glycin bei 40° ablaufen, so wird bei der Chloroformextraktion kein Allomaltol isoliert, sondern in ebenfalls etwa 1% Ausbeute *N*-(2-Methylpyron-4-yl-5)glycin (VIII). Die Struktur von VIII ergibt sich aus folgenden Befunden: Die wässrige Lösung der Substanz reagiert sauer. Bei der potentiometrischen Titration in Aceton erhält man eine Titrationskurve mit nur einem Wendepunkt; es liegt demnach eine einbasische Säure vor. Durch Umsetzung mit Diazomethan entsteht der Methylester von VIII. Bei der Hydrolyse von VIII durch Kochen mit verdünnter Salzsäure werden Allomaltol (IX) und Glycin als Spaltstücke erhalten. Demnach kann das durch Hydrolyse abgespaltene Glycin nur am Kohlenstoffatom 4 oder 5 des Methylpyronylringes gebunden gewesen sein. Diese Tatsache wird durch die N.m.r.-Spektro-


Allomaltol-Bildung
Strecker'scher Abbau
Diacetyl-Bildung
Melanoidin
+H2O
-H2O, 155°
CO2
CO2

skopie bestätigt : das Spektrum des Methylesters von VIII zeigt ebenso wie das des Allomaltols zwei verschiedene Vinylprotonen an, zu denen kein weiteres Proton benachbart angeordnet ist.

Im I.r.-Spektrum zeigt VIII eine scharfe N–H-Absorptionsbande bei 3400 cm^{-1}, der Methylester von VIII bei 3325 cm^{-1}. Umsetzung von VIII und des Methylesters von VIII mit Isoamylnitrit führt zu den entsprechenden Nitrosaminen, in deren I.r.-Spektren die Absorptionsbande der N–H-Valenzschwingung fehlt. Bei beiden Nitrosaminen ist die Liebermannsche Reaktion positiv. Die Extraktion des Reaktionsansatzes von Dihydroxyaceton und Glycin ergab somit bei 40° als Zwischenprodukt nur *N*-(Methylpyronyl)glycin (VIII), bei 55° dagegen nur Allomaltol. Ein bei 50° durchgeführter Versuch lieferte ein Gemisch von Allomaltol und VIII. Diese Befunde lassen sich damit erklären, dass VIII das primäre Zwischenprodukt darstellt, welches bei höherer Temperatur in wässriger Lösung sekundär hydrolysiert wird. Der umgekehrte Reaktionsverlauf – die Reaktion von Allomaltol mit Glycin zu VIII – lässt sich in wässriger Lösung nicht realisieren. Durch direktes Zusammenschmelzen von Allomaltol mit Glycin ist dagegen *N*-(Methylpyronyl)glycin in einer Ausbeute von 45% der Theorie darstellbar.

Auch bei der Reaktion von Alanin und Norvalin mit Dihydroxyaceton lässt sich Allomaltol isolieren, das in ähnlicher Weise wie beim Glycin über das *N*-(Methylpyronyl)alanin bzw. *N*-(Methylpyronyl)norvalin entstanden sein dürfte. Diese beiden Aminosäurederivate werden offenbar noch leichter hydrolysiert als VIII, denn durch Extraktionen bei niedriger Temperatur konnten beide Verbindungen nicht aufgefunden werden.

Bei der Reaktion von Alanin mit Dihydroxyaceton liess sich Methylglyoxal als Zwischenprodukt in geringer Menge nachweisen. Die Methylglyoxalbildung ist sowohl säure- als auch basen-katalysiert, so dass sein Auftreten in der Reaktionslösung zu erwarten ist[7,8]. Da es eine wesentlich höhere Reaktivität als das Dihydroxyaceton besitzt[9], kann es sich nicht in wesentlicher Menge anreichern. Der Nachweis des Methylglyoxals (XII) ist deswegen bemerkenswert, weil es eine zentrale Stellung in der Maillard-Reaktion der Triosen einzunehmen scheint. Insbesondere reagiert XII leicht mit Aminosäuren, wobei gemäss Schema XIII–XVIII ein Abbau nach Strecker[10] eintritt. Dabei geht XII in das wenig stabile Aminoaceton XVI über. Der aus der Aminosäure XIII entstandene Aldehyd XVIII wird zum Teil in das Melanoidin eingebaut, möglicherweise durch eine Mannich-Reaktion mit dem gleichzeitig entstehenden Aminoaceton.

Um einen Einblick in das Ausmass des Streckerschen Abbaus bei der Reaktion von Dihydroxyaceton mit Aminosäuren zu gewinnen, wurde der bei dieser Reaktion gebildete entsprechende Aldehyd quantitativ bestimmt: Alanin und Dihydroxyaceton (Molverhältnis 1:2) wurden zusammen in wässriger Lösung am Rückfluss gekocht. Durch das Reaktionsgefäss wurde ein schwacher Stickstoffstrom geleitet. Der Gasstrom trug den entstandenen Acetaldehyd in eine hinter dem Rückflusskühler angeschlossene Kühlfalle. Nach 5.5 Stunden wurde die Reaktion abgebrochen und 51% des eingesetzten Alanins zurückgewonnen. Verlängerung der Reaktionsdauer

vergrössert die Aldehydausbeute kaum, da das Dihydroxyaceton praktisch vollständig umgesetzt ist. Die in der Kühlfalle bestimmte Aldehydmenge entsprach 23% des eingesetzten Alanins. Demnach haben unter diesen Reaktionsbedingungen Alanin und Dihydroxyaceton im Molverhältnis 1:4 mit einander reagiert, wobei mindestens die Hälfte des umgesetzten Alanins zu Acetyldehyd abgebaut worden ist. Sicher ist der Anteil des Alanins, welcher über den Strecker-Abbau in Acetaldehyd überführt wurde, noch grösser, da mit der beschriebenen Versuchsanordnung der unmittelbar in das Melanoidin eingebaute Acetaldehyd im Destillat nicht mit erfasst wird.

Bei der Umsetzung anderer Aminosäuren mit Dihydroxyaceton wurden die entsprechenden, durch Strecker-Abbau gebildeten Aldehyde erhalten, wie aus Tabelle I ersichtlich. Daneben wurden in kleinen Mengen andere Carbonylverbindungen nachgewiesen. Identifiziert wurden davon die in Tabelle I als Nebenprodukte aufgeführten Acroleinderivate. Sie dürften in jedem Fall aus den als Hauptprodukt entstandenen Aldehyden durch Aldolkondensation und anschliessende Wasserabspaltung entstanden sein.

TABELLE I

STRECKERSCHER ABBAU

Aminosäure	*Hauptprodukt*	*Nebenprodukt*
Alanin	Acetaldehyd	
α-Amino-*n*-buttersäure	Propionaldehyd	α-Methyl-β-äthylacrolein
Valin	Isobutyraldehyd	
Norvalin	Butyraldehyd	α-Äthyl-β-propylacrolein
Leucin	β-Methylbutyraldehyd	
Norleucin	*n*-Valeraldehyd	α-Propyl-β-butylacrolein

Versucht man, bei der Umsetzung von Glycin mit Dihydroxyaceton mit der gleichen Versuchsanordnung die entstandenen Aldehyde abzudestillieren, so erhält man nur eine geringe Destillatmenge, die hauptsächlich aus Diacetyl neben wenig Methylglyoxal besteht.

Die beste Erklärung für das Auftreten des Diacetyls XXIV bietet eine Aldolisierungsreaktion, bei der sich das Glycin – vermutlich als Schiffsche Base – an Methylglyoxal anlagert zu (XX), welches dann gemäss Schema (XXI) – (XXIV) weiterreagiert. Die β-Eliminierung bei XXI sollte begünstigt sein, da die Doppelbindung in (XXII) zu einer Carbonyl- und einer Carboxylgruppe in Konjugation steht. XXII wird nach Umlagerung zurtautomeren β-Ketosäure (XXIII) zu Diacetyl (XXIV) decarboxyliert.

In dem Formelschema (IV–XXIV) sind die bei der Umsetzung von Dihydroxyaceton mit Aminosäuren aufgefundenen Reaktionen zusammengefasst. Die Übersicht lässt drei Reaktionswege erkennen: Vom Dihydroxyaceton dürfte nach Reaktion mit Glycin zu IV–V und ausschliessender Reaktion von V mit Methylglyoxal XII zu VI ein direkter Weg über das *N*-(Methylpyranyl)glycin VIII zum Allomaltol IX

führen (I–IV–IX). Auch bei den beiden anderen Wegen, dem Strecker-Abbau (XIII–XVIII) und der Diacetylbildung (XX–XXIV), sollte Methylglyoxal eine entscheidende Zwischenstufe sein.

EXPERIMENTELLER TEIL

Darstellung der Ketosylamine des Dihydroxyacetons

Man löst 0.11 Mol des umzusetzenden Amins in 10 ml absolutem Methanol oder Äthanol und setzt unter Rühren 0.1 Mol Dihydroxyaceton zu. Nach kurzer Zeit hat sich das Dihydroxyaceton gelöst, die Viskosität des blaßgelben Gemisches steigt langsam an und es bilden sich bereits Kristalle. Die Mischung wird rasch auf etwa 0° gekühlt und zur Vervollständigung der Kristallisation 24 Stunden lang bei −15° aufbewahrt. Danach wird das zum Kristallkuchen erstarrte Reaktionsgemisch dreimal in Methanol aufgeschlämmt und scharf abgesaugt. Man wäscht mit etwas Äther nach und trocknet das Produkt sofort im Vakuum über Blaugel. Die getrocknete Substanz ist in den meisten Fällen analysenrein und bei Aufbewahrung im Tiefkühlschrank wochenlang haltbar. Folgende Ketosylamine wurden dargestellt:

N-Dihydroxyacetonyl-äthanolamin aus 6.7 g Äthanolamin und 9.0 g Dihydroxyaceton. Farblose Kristalle, die sich relativ rasch unter Braunfärbung zersetzten, Fp. 71–73° (Zers.), Ausb. 8.2 g (62% d.Th.) (Gef. : C, 44.62; H, 8.10; N, 10.85. $C_{10}H_{22}N_2O_6$ ber. : C, 45.10; H, 8.33; N. 10.52%)

Dihydroxyacetonyl-*n*-butylamin aus 8.0 g *n*-Butylamin und 9.0 g Dihydroxyaceton. Farblose Kristalle, Fp. 66–67° (Zers.), Ausb. 7.5 g (52% d.Th.) (Gef. : C, 58.29; H, 10.45; N, 9.68. $C_{14}H_{30}N_2O_4$ ber. : C, 57.90; H, 10.41; N, 9.65%).

Dihydroxyacetonylcyclohexylamin aus 10.9 g Cyclohexylamin und 9.0 g Dihydroxyaceton. Farblose Kristalle, Fp. 79–81° (Zers.), Ausb. 8.2 g (48% d.Th.) (Gef. : C, 61.95; H, 9.89; N, 7.80. $C_{18}H_{34}N_2O_4$ ber. : C, 63.12; H, 10.01; N, 8.18%).

Dihydroxyacetonyl-*n*-octylamin aus 14.2 g *n*-Octylamin in 20 ml abs. Methanol und 9.0 g Dihydroxyaceton. Farblose Kristalle, Fp. 66–68° (Zers.), Ausb. 15.9 g (79% d.Th.) Gef. : C, 65.65; H, 11.42; N, 7.20. $C_{22}H_{46}N_2O_4$ ber. : C, 65.63; H, 11.52; N, 6.96%).

Dihydroxyacetonylbenzylamin: 9.0 g Dihydroxyaceton wurden in 20 ml abs. Methanol heiss gelöst, auf 10° abgekühlt und 11.8 g Benzylamin zugesetzt. In der Kälte farblose Kristalle, Fp. 77.5–79° (Zers.), Ausb. 14.1 g (79% d.Th.) (Gef.: C, 66.27; H, 7.21; N, 7.76. $C_{20}H_{26}N_2O_4$ ber. : C, 67.02; H, 7.31; N, 7.81%).

Dihydroxyacetonyl-*β*-phenyläthylamin aus 13.3 g *β*-Phenyläthylamin und 9.0 g Dihydroxyaceton. Farblose Kristalle, Fp., 73.5–74° (Zers.,) Ausb. 11.6 g (60% d.Th.) (Gef. : C, 67.92; H, 7.62; N, 7.19. $C_{22}H_{30}N_2O_4$ ber. : C, 68.35; H, 7.82; N, 7.25%).

Dihydroxyacetonylglycinäthylester aus 11.3 g Glycinäthylester und 9.0 g Dihydroxyaceton. Farblose Kristalle, Fp. 87–88° (Zers.), Ausb. 3.9 g (22% d.Th.) (Gef. : C, 47.63; H, 7.34; N, 7.71. $C_{14}H_{26}N_2O_8$ ber. : C, 47.99; H, 7.48; N, 8.00%).

Dihydroxyacetonylanilin aus 10.2 g Anilin und 9.0 g Dihydroxyaceton. Gelb-

liche Kristalle, die nach zwei Monate langem Stehen im Tiefkühlschrank isoliert wurden, Fp. 118–119.5° (Zers.), Ausb. 2.2 g (13% d.Th.) (Gef. : C, 63.83; H, 6.81; N, 8.24. $C_{18}H_{22}N_2O_4$ ber. : C, 65.44; H, 6.71; N, 8.48%).

Hydrolyse der Ketosylamine

Das Ketosylamin (0.2 g) wurde in 2 ml 2N Salzsäure oder 2N Essigsäure bei Raumtemperatur gelöst und mit 4 ml Methanol verdünnt, wobei sofort Hydrolyse eintrat. Das Hydrolysat wurde durch Papierchromatographie unter Verwendung der Lösungsmittelgemische Pyridin–*n*-Amylalkohol–Wasser (7:7:6) und *sec.* Butanol–Eisessig–Wasser (70:7:23) mit den Ausgangssubstanzen verglichen.

Allomaltol

Glycin (15 g) und Dihydroxyaceton (18 g) wurden in 180 ml Wasser gelöst und bei einer Reaktionstemperatur von 55° kontinuierlich mit Chloroform extrahiert (*ca.* 300 ml Chlf/Std.) Der Extrakt der ersten 4 Stunden, der ausser Dihydroxyaceton nur geringe Mengen Allomaltol enthielt, wurde verworfen. Die vereinigten Extrakte der nächsten 20 Stunden wurden im Vakuum zum Sirup eingeengt, der Rückstand viermal mit je 25 ml Methylenchlorid ausgekocht. Das Methylenchlorid wurde unter vermindertem Druck abdestilliert und ergab als Rückstand ein sirupartiges Rohprodukt von Allomaltol. Zur Reinigung wurde in gleicher Weise mit Methylenchlorid ausgekocht, wobei die Verunreinigungen unlöslich blieben. Nach Einengen und Sublimation im Vakuum (12 Torr/110° Badtemperatur) ergaben sich 180 mg (1.4% d.Th.). Gelbliche Kristalle, Fp. 152–153° (Lit. 153°). Das I.r.-Spektrum stimmte mit dem von synthetischem Allomaltol überein. (Gef. : C, 57.04; H, 4.89; $C_6H_6O_3$ ber. : C, 57.14; H, 4.80%).

p-*Toluolsulfonsäureester des Allomaltols*

Allomaltol (70 mg) wurde in 2 ml abs. Pyridin gelöst und bei 0° mit 0.3 g *p*-Toluolsulfochlorid versetzt. Nach einer Stunde wird mit 20 ml Wasser verdünnt. Der Toluolsulfonsäureester scheidet sich langsam kristallin ab. Farblose Kristalle, Fp. 129–130°, Ausb. 106 mg (68% d.Th.); Fp. 132–133° (M). (Gef. : C, 55.77; H, 4.38; S, 11.34. $C_{13}H_{12}O_5S$ ber. : C, 55.73; H, 4.32; S, 11.44%).

N-(*2-Methylpyron-4-yl-5*)*glycin durch Extraktion*

Glycin (15 g) und Dihydroxyaceton (18 g) wurden in 180 ml Wasser gelöst und bei einer Reaktionstemperatur von 40° kontinuierlich mit Chloroform (*ca.* 500 ml Chlf/Std.) extrahiert. Der Extrakt der ersten 5 Stunden wurde verworfen, da er praktisch ausschliesslich Dihydroxyaceton enthielt. Der Extrakt der nächsten 40 Stunden wurde zum Sirup eingeengt und in Methanol aufgenommen. Durch mehrfach fraktionierte Kristallisation aus abs. Methanol oder abs. Äthanol wurden 150 mg rohes Methylpyronylglycin erhalten. Das Rohprodukt wurde durch Sublimation bei 170–185°/0.01 Torr gereinigt. Fast farblose Kristalle, Fp. 205–208° (Zers.), Ausb. 130 mg (0.7% d.Th.) (Gef. : C, 52.63; H, 4.87; N, 7.82. $C_8H_9NO_4$ ber. : C, 52.45; H, 4.95; N, 7.65%).

N-(*2-Methylpyron-4-yl-5*)*glycin aus Allomaltol*

Allomaltol (20 g) und Glycin (20 g) wurden zusammen fein gepulvert 2.5 Stunden auf 155° erhitzt. Das Gemisch schmolz unter Braunfärbung und Gasentwicklung. Nach dem Abkühlen wurde die gepulverte erstarrte Schmelze zweimal mit je 50 ml kaltem Methanol extrahiert. Der ungelöste Filterrückstand wurde dreimal mit Methanol ausgekocht, das Ungelöste mit 20 ml Wasser ausgezogen und das in Wasser Unlösliche nach dem Trocknen nochmals mit Methanol ausgekocht. Aus den methanolischen Lösungen fiel beim Erkalten das Methylpyronylglycin in glänzenden Blättchen aus. Das Rohprodukt wurde bei 170–185°/0.01 Torr sublimiert. Fast farblose Kristalle, Fp. 208–212° (Zers.), Ausb. 13.2 g (45% d.Th.) (bezogen auf Allomaltol). (Gef. : C, 52.25; H, 4.94; N, 7.77 $C_8H_9NO_4$ ber. : C, 52.45; H, 4.95; N, 7.65%).

N-(*2-Methylpyron-4-yl-5*)*glycinmethylester*

Methylpyronylglycin (5 g) wurde in 100 ml siedendem Methanol gelöst. Die Lösung wurde rasch unter 25° gekühlt und, ehe die Kristallisation des Methylpyronylglycins begann, solange mit ätherischer Diazomethanlösung versetzt, bis die Stickstoffentwicklung beendet war. Dann wurde am Rotationsverdampfer zur Trockene eingedampft und der Rückstand zuerst aus Benzol–Petroläther, danach aus Tetrachlorkohlenstoff umkristallisiert. Gelbliche nadelförmige Kristalle, Fp. 86–87°, Ausb. 2.4 g (45% d.Th.) (Gef. : C, 54.44; H, 5.75; N, 7.11. $C_9H_{11}NO_4$ ber. : C, 54.82; H, 5.63; N, 7.10%).

N-*Nitroso*-N-(*2-methylpyron-4-yl-5*)*glycin*

Methylpyronylglycin (5.5 g) wurde in 55 ml Methanol aufgeschlämmt und in drei Portionen mit insgesamt 9.0 ml Isoamylnitrit versetzt. Nach einigem Stehen löste sich das gesamte feingepulverte Methylpyronylglycin auf. Die Lösung wurde im Vakuum bei 35° Badtemperatur zum Sirup eingeengt, der bald danach kristallisierte. Umkristallisation aus Methanol und Alkohol–Benzol. Blassgelbliche Kristalle, Fp. 136–137°, Ausb. 2.8 g (44% d.Th.) (Gef. : C, 45.29; H, 3.84; N, 13.17. $C_8H_{18}N_2O_5$ ber. : C, 45.29; H, 3.80; N, 13.21%).

N-*Nitroso*-N-(*2-methylpyron-4-yl-5*)*glycinmethylester*

N-Nitroso-N-(methylpyronyl)glycin (1.0 g) wurde in 20 ml abs. Methanol gelöst und mit einer äquivalenten Menge ätherischer Diazomethanlösung versetzt. Die erhaltene Lösung wurde im Vakuum zum Sirup eingeengt und aus Benzol–Petroläther kristallisiert. Blassgelbliche Kristalle, Fp. 75–76°, Ausb. 0.75 g (70% d.Th.) (Gef. : C, 47.82; H, 4.53; N, 12.24. $C_9H_{10}N_2O_5$ ber. : C, 47.79; H, 4.46; N, 12.39%).

Hydrolyse des N-(*2-methylpyron-4-yl-5*)*glycins*

Methylpyronylglycin (323 mg) wurde in 5 ml Wasser unter Zusatz von 0.1 ml 5N Salzsäure 2 Stunden am Rückfluss gekocht. Die bräunlichgelbe Lösung wurde viermal mit je 5 ml Chloroform ausgeschüttelt. Nach dem Abdampfen des Chloro-

forms wurden 210 mg Allomaltol (94% d.Th.) erhalten, Fp. 150–151°. Die extrahierte wässrige Lösung wurde zur Trockene eingedampft und das im Trockenrückstand enthaltene Glycin mit Benzoylchlorid in Hippursäure umgewandelt. Ausb. 95 mg Hippursäure (30% d.Th.).

Methylglyoxal

Alanin (18 g) und Dihydroxyaceton (18 g) wurden in 180 ml Wasser bei einer Reaktionstemperatur von 55° kontinuierlich mit 6 l Chloroform 20 Stdn. extrahiert, wobei in Fraktionen von je 1 Stunde Extraktionsdauer unterteilt wurde. Die Fraktionen 9–17 wurden bei 30° Badtemperatur im Vakuum auf *ca.* 10 ml eingeengt. Dünnschichtchromatographie auf Kieselgel G [Laufmittel: Essigester–2-Propanol (10:1), Sprühreagens: 2,4-Dinitrophenylhydrazin, gesättigte Lösung in 2N HCl] zeigt Methylglyoxal, welches bei 100° aus der Dünnschicht verdampft. Isolierung erfolgte durch Säulenchromatographie auf Kieselgel mit gleichem Lösungsmittelgemisch; Identifizierung als Bissemicarbazon und als Bis-2,4-dinitrophenylhydrazon durch I.r.-Spektrum und Mischschmelzpunkt.

Flüchtige Carbonylverbindungen

Die Aminosäure wurde mit dem Dihydroxyaceton im Molverhältnis 1:2 in wässriger Lösung am Rückflusskühler erhitzt, der aus einem Thermostaten mit 55° warmem Wasser gespeist wurde. In den Reaktionskolben wurde ein langsamer Stickstoffstrom eingeleitet, der die flüchtigen Carbonylverbindungen durch den Rückflusskühler austrug. An den Rückflusskühler angeschlossen waren zwei hintereinandergeschaltete Kühlfallen, von denen die erste durch ein Eisbad gekühlt wurde. In ihr befand sich nach Beendigung des Versuchs fast ausschliesslich Wasser. Die zweite Kühlfalle, die mit Trockeneis–Methanol gekühlt wurde, enthielt die flüchtigen Carbonylverbindungen.

Beispiel: Umsetzung von α-Aminobuttersäure zu Propionaldehyd und α-Methyl-β-äthylcrotonaldehyd

α-Amino-*n*-buttersäure (10.3 g) und Dihydroxyaceton (18.0 g) wurden in 150 ml Wasser am Rückfluss erhitzt. Der Inhalt der Kühlfallen wurde mit 225 ml der 2,4-Dinitrophenylhydrazinlösung gefällt. Das Hauptprodukt erwies sich als 2,4-Dinitrophenylhydrazon des Propionaldehyds. Bei der Dünnschichtchromatographie der Dinitrophenylhydrazone wurde eine weitere Substanz isoliert, die in roten Blättchen kristallisierte; sie wurde durch Vergleich der R_F-Werte, der I.r.-Spektren, Fp. und Misch-Fp. als 2,4-Dinitrophenylhydrazon des α-Methyl-β-äthylcrotonaldehyds identifiziert.

ZUSAMMENFASSUNG

Dihydroxyaceton reagiert mit primären Aminen bei niedriger Temperatur unter Bildung von dimeren Ketosylaminen, die nur als Kristalle stabil sind.

Methylglyoxal ist bei der Reaktion von Dihydroxyaceton mit Aminosäuren ein wichtiges Zwischenprodukt, welches über den Strecker-Abbau die Aminosäuren in Aldehyde überführt. Glycin bildet im Gegensatz zu den anderen Aminosäuren mit Dihydroxyaceton nur wenig flüchtige Carbonylverbindungen, unter denen Diacetyl bevorzugt auftritt. Allomaltol wurde als Nebenprodukt der Reaktion von Glycin mit Dihydroxyaceton erhalten, die hauptsächlich zu Melanoidinen führt. Die Vorstufe des Allomaltols ist *N*-(2-methylpyron-4-yl-5)-glycin, welches isoliert und mit einem direkt aus Maltol und Glycin synthetisierten Produkt identifiziert wurde.

SUMMARY

Dihydroxyacetone reacts with primary amines at low temperature to form dimeric ketosylamines, which are stable only in the crystalline state at low temperatures.

In the reaction of dihydroxyacetone with amino acids pyruvic aldehyde seems to be an important intermediate. This causes the Strecker degradation of amino acids to aldehydes. In contrast to other amino acids, glycine reacts with dihydroxyacetone to give small proportions of volatile carbonyl compounds, in which diacetyl predominates. Allomaltol is isolated as a by-product from the reaction of dihydroxyacetone with glycine, which leads primarily to melanoidins. In this case, the precursor of allomaltol, which is *N*-(2-methylpyron-4-yl-5)glycine, has been isolated. This compound was synthesised by melting allomaltol and glycine, and its structure proved by the preparation of derivatives.

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Preliminary communication

Migration of sulfate groups of chondroitin sulfates at elevated temperatures

In connection with structural studies on sulfated polysaccharides, we have found that a migration of sulfate groups occurs in chondroitin sulfates when they are heated in the free-acid form.

Chondroitin 4-sulfate calcium salt, isolated from whale cartilage[1], was dissolved in water, and the solution was poured onto a column of Dowex 50 (H^+ form), which was washed with water. The acidic fractions were collected and lyophilized. The free chondroitin 4-sulfuric acid thus obtained was dried at room temperature over P_2O_5 *in vacuo* overnight, and then heated at 100° for 6 h *in vacuo*. This heated free acid was

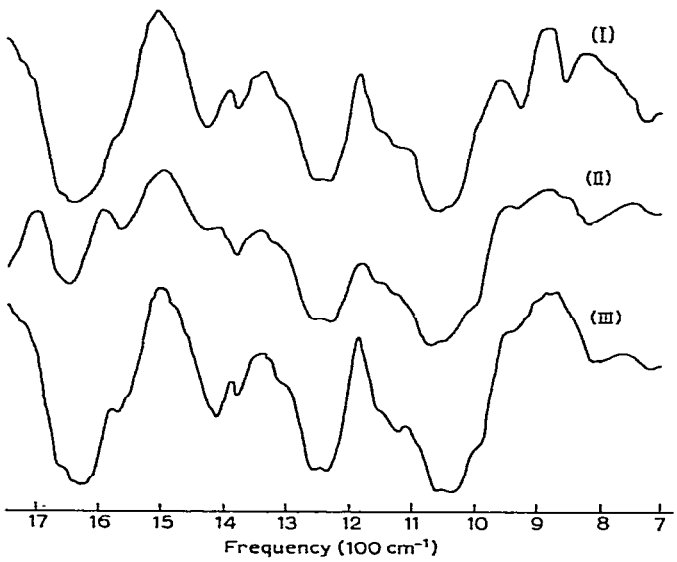

Fig. 1. Changes in the infrared spectrum of whale chondroitin 4-sulfate caused by elevated temperature. (I) Chondroitin 4-sulfate calcium salt; (II) chondroitin 4-sulfuric acid after heat treatment; (III) calcium salt of (II). These spectra were taken as potassium bromide discs, on 2.0 mg of substance, on a Hitachi EPI infrared spectrophotometer Model SII.

dissolved in water, and the calcium salt was precipitated with two volumes of ethanol, in the presence of calcium acetate, at pH 5.0.

The changes in the infrared spectrum of whale chondroitin 4-sulfate caused by elevated temperature are shown in Fig. 1. When the free acid was heated, the absorption bands at 850 cm^{-1} and 928 cm^{-1} corresponding to the axial sulfate group[2,3] disappeared, and a strong absorption band in the 820 cm^{-1} region, corresponding to an equatorial sulfate group[2,3], appeared. The spectrum, thus, became apparently identical with that of chondroitin 6-sulfate.

On the other hand, the spectra of the chondroitin 6-sulfate[4], that was treated in a similar manner, were the same before and after heat was applied.

The change of infrared spectra was not observed when chondroitin 4-sulfate was treated as follows: (1) the free acid was kept in the solid state or in water solution at room temperature for 10 days; (2) the solution of the free acid was immediately neutralized, after passing through Dowex 50 (H^+ form), and then was lyophilized. The salt form was stable when heated at 100° for 6 h. These experiments indicate that heat treatment causes the sulfate groups of chondroitin 4-sulfuric acid to migrate from an axial to an equatorial position.

As shown in Fig. 1, the strong absorption band in the 1240 cm^{-1} region, attributable to the S=O stretching vibration, persisted after heat treatment. Analysis of the chondroitin sulfates for ester sulfate content[5] indicated that about 90% of the sulfate groups of chondroitin 4-sulfate remained after heat treatment, whereas about 10% was removed. In the chondroitin 6-sulfuric acid, however, no change of ester sulfate content was observed after application of heat. Consequently, these results suggest that most of the 4-sulfate groups of chondroitin 4-sulfate are shifted to an equatorial position at elevated temperature, whereas the 6-sulfate groups are fairly stable, even in the free state.

Squid chondroitin polysulfate[6] has a molar ratio of sulfate to D-glucuronic acid of 1:6 and absorption bands at 850 cm^{-1} and 820 cm^{-1}, corresponding to the axial and equatorial sulfate groups. When it was treated as described above, only the absorption bands corresponding to axial sulfate groups disappeared after heat treatment and showed the 6-sulfate type of spectrum; the molar ratio of sulfate to uronic acid became approximately 1:1.

With dermatan sulfuric acid[7], heat caused the disappearance of the absorption bands corresponding to axial sulfate groups and the appearance of a strong absorption band at 820 cm^{-1}.

Similar phenomena were also observed for the oligosaccharides (free acid form) obtained from chondroitin 4- and 6-sulfate by testicular hyaluronidase digestion.

From these results, it can be concluded that, when chondroitin sulfates and the oligosaccharides derived from them are heated in the free form, a considerable proportion of axial sulfate groups migrates to an equatorial position. Since no trans-

formation of the amino sugar was detected[8] after heat treatment, the axial 4-sulfate group in the 2-amino-2-deoxy-D-galactose moiety may migrate to the stable equatorial 6-sulfate position through a cyclic 4,6-diester.

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Announcement

In view of the exceptionally good response to *Carbohydrate Research* by authors, we are now in a position to expedite publication of papers by initiating a monthly publication schedule ahead of the dates previously proposed. It has now been decided that no further bi-monthly issues will appear. Therefore Vol. 3 will commence with issue No. 1 in November 1966. Three volumes of 500 pages each will be published between November 1966 and December 1967.

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REACTION OF D-*glycero*-D-*gulo*-HEPTONO-γ-LACTONE WITH ACETONE*

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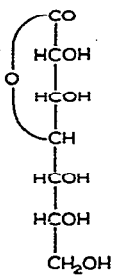
RESULTS AND DISCUSSION

As a possible route to isopropylidene derivatives of D-*glycero*-D-*gulo*-heptose, which were required in connection with other studies, we have examined the acid-catalysed reaction of acetone with D-*glycero*-D-*gulo*-heptono-γ-lactone (I). The reaction yielded a di-*O*-isopropylidenelactone (*A*) (*ca.* 80%) as the main product, together with a small proportion of a mono-*O*-isopropylidene derivative, which was readily separated on partitioning the product mixture between chloroform and water. A small proportion of a second di-*O*-isopropylidene derivative (*B*) was obtained by chromatography of the mother liquors of the diketal fraction on silica gel. The infrared spectra of the three products exhibited an absorption band at either 1758 or 1788 cm^{-1}, indicating[2] that the γ-lactone ring had remained intact during the acetonation.

The heptitol monohydrate derived from di-*O*-isopropylidenelactone *A* consumed 1 mol. of periodate which reasonably limits the structure to either a 2,3:6,7- or 3,5:6,7-distribution of the ketal groups in *A*, since any other arrangement would involve at least one seven-membered or larger ring. A distinction between these arrangements was readily afforded when periodate oxidation of the heptitol derivative yielded a crystalline diketal, which gave D-glucose (characterised as the β-D-pentaacetate) on acid hydrolysis. Di-*O*-isopropylidenelactone *A* is thereby identified as 3,5:6,7-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptono-γ-lactone (II), the heptitol as 1,2:3,5-di-*O*-isopropylidene-L-*glycero*-L-*gulo*-heptitol (III), and the product of periodate treatment as 2,4:5,6-di-*O*-isopropylidene-*aldehydo*-D-glucose (VI). Unequivocal evidence for the distribution of the ketal groups in compound (VI) followed from its conversion into 2,4-*O*-isopropylidene-D-glucitol[3] on reduction with sodium borohydride and selective removal of the α-ketal group**, and into 1,2:3,5-di-*O*-isopropylidene-4-*O*-methyl-L-gulitol (2,4:5,6-di-*O*-isopropylidene-3-*O*-methyl-D-glucitol[4]) (X) by sequential reduction, tritylation, methylation, and detritylation (VII→X). The latter diketal was identical with a minor product formed in the acid-catalysed acetonation[4] of 3-*O*-methyl-D-glucitol. Methylation of di-*O*-isopropylideneheptitol

*A preliminary communication on a part of this work has already been published[1].
**The significance of this observation will be discussed with related studies in a subsequent publication.

(III) afforded a syrupy trimethyl ether (V) which, after removal of the ketal groups, reduced 2.1 mol. of periodate; the heptitol (III) was further characterised as its crystalline triacetate (IV).



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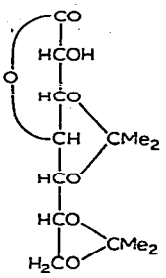

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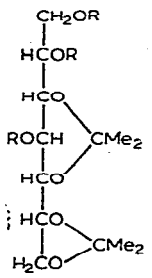
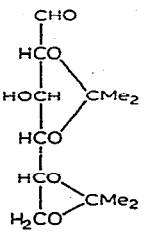
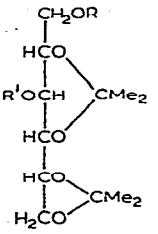


III R=H
IV R=Ac
V R=Me

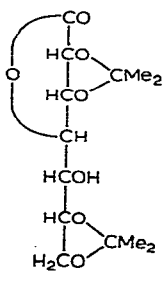


VI



VII R=R'=H
VIII R=Tr, R'=H
IX R=Tr, R'=Me
X R=H, R'=Me

The crystalline heptitol derived by reduction of di-*O*-isopropylidenelactone *B* also reduced 1 mol. of periodate and, from the preceding arguments, may be assigned the structure 1,2:5,6-di-*O*-isopropylidene-L-*glycero*-L-*gulo*-heptitol (XII), while that of diketal *B* is 2,3:6,7-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptono-γ-lactone (XI). These assignments of structure were further confirmed when treatment of heptitol (XII) with one equivalent of sodium metaperiodate, reduction with sodium borohydride and acid hydrolysis, gave glycerol and erythritol.



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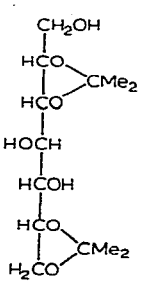
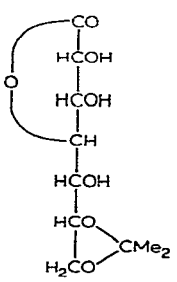
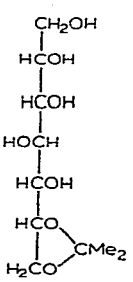


XII



XIII



XIV

The mono-*O*-isopropylidenelactone was obtained in substantially better yield by reaction of lactone (I) with acetone in the presence of either anhydrous copper sulphate or toluene-*p*-sulphonic acid. The monoketal rapidly consumed 1 mol. of periodate, followed by a slow uptake of another 3.2 mol. Successive alkaline and acid treatments of the product formed after the uptake of 1 mol. of periodate afforded crystalline D-arabinose, which clearly arises from scission between carbon atoms 2 and 3 of the γ-lactone ring and, hence, the location of the ketal group is narrowed to carbon atoms 5, 6, and 7. In the acid-catalysed reaction of acetone with acyclic,

polyhydric alcohols, condensation usually involves terminal vicinal (α) and α-*threo** groups, so that a 6,7-distribution (XIII) of the ketal group in the mono-*O*-isopropylidenelactone is most likely. Moreover, the same monoketal is formed in the reaction catalysed by copper sulphate, in which preferential reaction of the primary hydroxyl group with acetone would be expected. In agreement with this assignment of structure, the derived heptitol (XIV) and its methylated and de-isopropylidenated analogue consumed 4.2 and 1.1 mol. of periodate, respectively.

The ready condensation between acetone and vicinal, *cis*-diol groups attached to furanoid and γ-lactone rings is well exemplified[6,7], so that the 3,5-distribution in lactone (II), in preference to the formation of a 2,3-ketal, is unusual. 2,3:6,7-Di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptono-γ-lactone (XI) was largely converted into the isomeric diketal (II) on equilibration in acetone–sulphuric acid, thereby demonstrating that the latter is thermodynamically the more stable. The reason(s) for this preferred stability is (are) not clear, and the problem is being further investigated. An analogous case has been reported[3] recently, in that acid-catalysed acetonation of D-glucurono-δ-lactone diethyl dithioacetal yields a 2,4-*O*-isopropylidene derivative, presumably with contraction in size of the lactone ring.

EXPERIMENTAL

Thin-layer chromatography (t.l.c.) was performed on silica gel (Merck) using either pyridine or ethyl acetate as the mobile phase, unless otherwise indicated; detection was effected with vanillin–sulphuric acid[8]. Periodate oxidations were performed by Dixon and Lipkin's method[9], and formic acid was determined titrimetrically[10]. Infrared spectra were recorded on Nujol mulls with a Perkin–Elmer 125 spectrometer. Melting points are uncorrected.

Acetonation of D-glycero-D-gulo-*heptono-γ-lactone*

A mixture of acetone (500 ml), conc. sulphuric acid (0.9 ml), and D-*glycero*-D-*gulo*-heptono-γ-lactone[11] (10 g) was shaken for 12 h at room temperature and was then neutralised with conc. ammonia (sp. gr. 0.88, 2 ml). The filtered solution was concentrated to a syrup which was dissolved in chloroform (250 ml) and partitioned with water (250 ml). The aqueous layer was washed with chloroform (2 × 100 ml) and concentrated to a syrup which was taken up in chloroform (200 ml) and dried ($MgSO_4$). Removal of the solvent afforded a syrup which crystallised slowly and, on recrystallisation from ethanol–chloroform–light petroleum (b.p. 60–80°), gave a mono-*O*-isopropylideneheptono-γ-lactone (0.6 g), m.p. 167–168°, $[\alpha]_D^{25}$ −30° (*c* 2, ethanol), ν_{max} 1758 cm^{-1} (γ-lactone[2]). (Found: C, 48.0; H, 6.3. $C_{10}H_{16}O_7$ calc.: C, 48.4; H, 6.5%).

The combined chloroform extract and washings (from above) were dried ($MgSO_4$) and concentrated to *ca.* 250 ml, and light petroleum (b.p. 80–100°, 1.5 l)

*See reference 5 for an explanation of the nomenclature.

was added. The crystalline deposit was filtered off and recrystallised from the same solvent pair to give di-*O*-isopropylidenelactone *A* (5.5 g), m.p. 153–154°, $[\alpha]_D^{30}$ −76° (*c* 2, chloroform), ν_{max} 1788 cm^{-1} (γ-lactone[2]). (Found: C, 54.3; H, 7.1. $C_{13}H_{20}O_7$ calc.: C, 54.2; H, 7.0%). The combined mother liquors were concentrated and chromatographed on a column (3.5 × 55 cm) of silica gel by elution with ethyl acetate; 10-ml fractions were collected. Fractions 6–15 were combined and concentrated to give di-*O*-isopropylidenelactone *B* (0.61 g), m.p. 169–170° [from chloroform–light petroleum (b.p. 80–100°)], $[\alpha]_D^{30}$ −70° (*c* 1, chloroform), ν_{max} 1758 cm^{-1} (γ-lactone[2]). (Found: C, 54.4; H, 6.8%). Fractions 16–50 gave further quantities (2.5 g) of di-*O*-isopropylidenelactone *A*, m.p. 153–154°. In other reactions, the total yield of lactone *A* approached 80%.

1,2:3,5-Di-O-*isopropylidene*-L-glycero-L-gulo-*heptitol monohydrate* (*III*)

A solution of di-*O*-isopropylidenelactone *A* (1 g) in methanol (20 ml) was treated with sodium borohydride (0.4 g) for 4 h at room temperature. The solution was concentrated, and the residue was dissolved in water (20 ml) and deionized with Amberlite IRA-400 (OH^- form) and IR-120 (NH_4^+ form). Concentration of the eluate and washings yielded a syrup which slowly crystallised and on recrystallisation from chloroform–light petroleum (b.p. 60–80°) gave the title compound (0.75 g), m.p. 67–68°, $[\alpha]_D$ −6° (*c* 2, water), ν_{max} 1640 cm^{-1} (hydrate water). (Found: C, 50.0; H, 8.7. $C_{13}H_{26}O_8$ calc.: C, 50.3; H, 8.4%).

4,6,7-Tri-O-*acetyl-1,2:3,5-di*-O-*isopropylidene*-L-glycero-L-gulo-*heptitol* (*IV*)

To a solution of the foregoing heptitol (8 g) in dry pyridine (50 ml) was added acetic anhydride (30 ml), and the mixture was set aside for 24 h at 30°. Water (20 ml) was added and, after 30 min, the mixture was added to ice-cold water (1 l) which was extracted with chloroform (2 × 250 ml). Concentration of the dried ($MgSO_4$) extract afforded a syrup which was shown to be inhomogenous by t.l.c. Distillation afforded a product, b.p. 130–150°/0.1 mm, which showed no substantial increase in purity. A sample of the distillate crystallised after 3 months and, on recrystallisation from ethanol, gave the pure triacetate (2 g), m.p. 71–72°, $[\alpha]_D$ −18° (*c* 1, chloroform). (Found: C, 53.9; H, 7.0. $C_{19}H_{30}O_{10}$ calc.: C, 54.5; H, 7.2%).

1,2:3,5-Di-O-*isopropylidene-4,6,7-tri*-O-*methyl*-L-glycero-L-gulo-*heptitol* (*V*)

To a solution of compound (III) (0.3 g) in tetrahydrofuran (20 ml) was added sodium hydride powder (0.3 g) and redistilled methyl iodide (7 ml), and the mixture was set aside for 6 h at room temperature. Ethanol (5 ml) was added and, after the initial reaction had subsided, water (200 ml) was gradually added. The aqueous solution was extracted with chloroform (2 × 100 ml), and the dried ($MgSO_4$) extract was concentrated to a syrup which on distillation gave the title compound (0.32 g), b.p. 120° (bath)/0.1 mm, $[\alpha]_D^{25}$ −6° (*c* 3, chloroform). (Found: C, 57.6; H, 9.0. $C_{16}H_{30}O_7$ calc.: C, 57.5; H, 9.0%).

After hydrolysis of the tri-*O*-methyl ether with N sulphuric acid at 95–100° for

4 h, the neutralised and concentrated hydrolysate (R_F 0.7, pyridine) consumed 2.14 mol. of periodate.

*Periodate oxidation of 1,2:3,5-di-*O*-isopropylidene-*L*-glycero-*L*-gulo-heptitol monohydrate (III)*

Quantitative measurement showed that the title compound rapidly consumed 1 mol. of periodate.

A solution of the title compound (0.5 g) in water (25 ml) was treated with a solution of sodium metaperiodate (0.375 g) in water (25 ml) for 15 min at room temperature. The solution was concentrated to dryness, and the residue was extracted with chloroform (100 ml). Concentration of the dried ($MgSO_4$) extract afforded a crystalline product, which was recrystallised from chloroform–light petroleum (b.p. 60–80°) to give 2,4:5,6-di-*O*-isopropylidene-*aldehydo*-D-glucose (VI) (0.36 g, 84%), m.p. 88–89°, $[\alpha]_D^{20}$ −12° (*c* 2, chloroform). (Found: C, 56.1; H, 8.35. $C_{12}H_{20}O_6$ calc.: C, 55.4; H, 7.75%).

Hydrolysis of compound (VI) (0.1 g) in water (25 ml) for 6 h at 95–100° with Amberlite IR-120 (H^+ form) (25 ml), and concentration of the filtered solution yielded D-glucose (identified by paper chromatography), which was converted, in the usual manner, into the β-D-pentaacetate (0.038 g), m.p. and mixed m.p. 131°, $[\alpha]_D^{25}$ +5° (*c* 0.5, chloroform). The infrared spectrum of the pentaacetate was indistinguishable from that of an authentic sample.

*1,2:3,5-Di-*O*-isopropylidene-*L*-gulitol (VII)*

To a solution of the foregoing compound (5 g) in methanol (100 ml) was added sodium borohydride (2 g), and the mixture was set aside for 4 h at room temperature. Cations were removed on Amberlite IR-120 (NH_4^+ form) (250 ml), and the methanolic solution was concentrated to a syrup which rapidly crystallised. Recrystallisation from chloroform–light petroleum (b.p. 60–80°) gave the title compound (3.8 g), m.p. 117–118°, $[\alpha]_D$ +2° (*c* 1, methanol). (Found: C, 55.0; H, 8.3. $C_{12}H_{22}O_6$ calc.: C, 54.95; H, 8.45%).

*1,2:3,5-Di-*O*-isopropylidene-6-*O*-trityl-*L*-gulitol (VIII)*

A solution of compound (VII) (1 g) in dry pyridine (50 ml) containing triphenylmethyl chloride (2 g) was heated for 3 h at 95–100°. Dry methanol (5 ml) was then added, and the solution was heated under reflux for 1 h. The cooled solution was poured into chloroform (200 ml) and washed successively with water, dilute sulphuric acid, dilute ammonium hydroxide, and water. The dried ($MgSO_4$) chloroform layer was concentrated to a syrup, which was shown by t.l.c. to contain at least four components. Chromatography on a column (2 × 20 cm) of silica gel by elution with chloroform afforded the title compound (0.65 g), m.p. 61–63° (from aqueous methanol), $[\alpha]_D$ +5° (*c* 1, chloroform). (Found: C, 73.8; H, 6.9. $C_{31}H_{36}O_6$ calc.: C, 73.8, H, 7.2%).

1,2:3,5-Di-O-isopropylidene-4-O-methyl-6-O-trityl-L-gulitol (IX)

A solution of the foregoing compound (0.5 g) in methyl iodide (10 ml) containing suspended sodium hydride powder (0.5 g) was set aside for 12 h at room temperature. Work up in the usual manner gave a syrup, which was shown by t.l.c. (chloroform) to contain three components. Chromatography on silica gel (50 g) by elution with ethyl acetate–chloroform (1:19) afforded the title compound (0.31 g), m.p. 159–160° (from aqueous methanol), $[\alpha]_D$ +6° (*c* 1, chloroform). (Found: C, 74.2; H, 7.2. $C_{32}H_{38}O_6$ calc.: C, 74.1; H, 7.4%).

1,2:3,5-Di-O-isopropylidene-4-O-methyl-L-gulitol (X)

A solution of the foregoing trityl ether (0.15 g) in methanol (25 ml) containing suspended 5% palladium-on-charcoal (0.25 g) was shaken with a slight overpressure of hydrogen for 24 h at room temperature. The filtered solution was concentrated, and the residue was dissolved in chloroform and dried ($MgSO_4$). Removal of the solvent afforded a crystalline residue which, on recrystallisation (twice) from light petroleum (b.p. 40–60°), gave the title compound (0.05 g), m.p. 136°, undepressed on admixture with an authentic sample[4], $[\alpha]_D$ +12° (*c* 0.5, chloroform). The infrared spectrum of the product could not be distinguished from that of an authentic sample.

1,2:5,6-Di-O-isopropylidene-L-glycero-L-gulo-heptitol (XII)

Reduction of di-*O*-isopropylidenelactone *B* (0.25 g) with sodium borohydride (0.1 g) was accomplished as described for the isomeric compound (II). The product (0.13 g) had m.p. 99° [from ether–light petroleum (b.p. 60–80°)], $[\alpha]_D$ +7° (*c* 0.5, chloroform). (Found: C, 53.2; H, 8.5%).

Heptitol (XII) consumed 0.96 mol. of periodate over 5 min. Sodium metaperiodate (25 mg) was added to a solution of the heptitol (25 mg) in water (10 ml) and, after 5 min, the solution was concentrated. The residue was extracted with chloroform (4 × 10 ml), and the organic layer was dried ($MgSO_4$) and concentrated. A solution of the residue in methanol (5 ml) was treated with sodium borohydride (10 mg) for 1 h and was then processed in the usual manner. The resulting syrup was dissolved in water (25 ml) and shaken for 12 h with Amberlite IR-120 (H^+form, 5 ml). The filtered and concentrated hydrolysate contained two components indistinguishable in their electrophoretic mobilities (molybdate buffer[12], pH 5) from those of glycerol and erythritol.

6,7-O-Isopropylidene-D-glycero-D-gulo-heptono-γ-lactone (XIII)

The monoketal formed in the acid-catalysed acetonation was prepared in good yield by either of the following procedures.

(*a*) *Catalysis by anhydrous copper sulphate.* Suspensions of *D-glycero-D-gulo*-heptono-γ-lactone (10 g) and anhydrous copper sulphate (10 g) in dry acetone (500 ml) were shaken for 48 h at room temperature. The filtered solution was concentrated, and the residue was recrystallised from methanol to give the monoketal (6.9 g), m.p. and mixed m.p. 167–168°.

(*b*) *Catalysis by toluene*-p-*sulphonic acid.* The lactone (1 g) was shaken in dry acetone (50 ml) containing toluene-*p*-sulphonic acid (9.5 mg) for 20 h at room temperature, and the solution was neutralised with 1 drop of conc. ammonia and concentrated. Crystallisation of the resulting syrup from methanol, with recrystallisation from methanol–chloroform–light petroleum (b.p. 80–100°), gave the monoketal (1.05 g), m.p. 167–168°.

Periodate oxidation of mono-O-*isopropylidenelactone* (*XIII*)

Quantitive measurement of the uptake of periodate (mol.) by the monoketal was as follows: 0.94 (1 min), 1.2 (10 min), 1.6 (40 min), 4.2 (14 h).

A solution of the monoketal (0.1 g) in water (10 ml) was treated with 1 mol. of sodium metaperiodate (0.09 g) in water (10 ml) for 15 min. Conc. ammonia (1 ml) was added and, after 15 min, the solution was concentrated to dryness. The residue was extracted with acetone (4 × 10 ml), the extract was concentrated, and the syrupy product was hydrolysed by shaking with Amberlite IR-120 (H^+ form, 5 ml) in water (25 ml) for 24 h. Removal of the solvent afforded a crystalline residue which was recrystallised (twice) from methanol to give D-arabinose (0.015 g), m.p. 156–157° (lit.[13], m.p. 158.5–160°); m.p. on admixture with L-arabinose 138°. The chromatographic properties of the product could not be distinguished from those of L-arabinose.

1,2-O-*Isopropylidene*-L-glycero-L-gulo-*heptitol* (*XIV*)

A solution of monoketal (XIII) (0.7 g) in methanol (25 ml) was reduced with sodium borohydride (0.5 g) as described in previous experiments. The product was recrystallised from acetone to give the title compound (0.35 g), m.p. 114–115°, $[\alpha]_D^{25}$ +4° (*c* 1, methanol), R_F 0.9 (pyridine). (Found: C, 47.3, H, 8.1. $C_{10}H_{20}O_7$ calc.: C, 47.6; H. 8.0). The heptitol showed the following uptake of periodate: 3.1 mol. (20 min, 2 h), 3.9 mol. (18 h).

1,2-O-*Isopropylidene-3,4,5,6,7-penta*-O-*methyl*-L-glycero-L-gulo-*heptitol*

To a solution of the foregoing compound (0.2 g) in dry tetrahydrofuran (50 ml) containing suspended sodium hydride powder (0.5 g) was added methyl iodide (1.5 ml), and the mixture was set aside for 48 h. Work up in the usual manner gave an oil which was distilled to give the title compound (0.21 g), b.p. 120° (bath)/0.1 mm, $[\alpha]_D$ +9° (*c* 2, chloroform). (Found: C, 55.0; H, 8.5. $C_{15}H_{30}O_7$ calc.: C, 55.9; H, 9.4%). The infrared spectrum of the product exhibited no absorption at *ca.* 3600 cm^{-1} (hydroxyl), and the product was homogeneous on t.l.c. (R_F 0.5, ethyl acetate).

1,2,3,4,5-Penta-O-*methyl*-D-glycero-D-gulo-*heptitol*

The foregoing distillate (0.2 g) in methanol (50 ml) containing suspended Amberlite IR-120 (H^+form, 2.5 ml) was stirred for 12 h at room temperature. Concentration of the filtered solution afforded the title compound (0.17 g), $[\alpha]_D$ +26.5° (*c* 1.7, chloroform), as a syrup, which quantitatively consumed 1.09 mol. of periodate during 15 min.

Equilibration of 3,5:6,7-(II) and 2,3:6,7-diketals (XI)

A solution of the 2,3:6,7-diketal (XI) (50 mg) in acetone (2.5 ml) made 0.1N in sulphuric acid was set aside for 12 h at room temperature. The reaction was monitored by t.l.c. and showed the gradual conversion of diketal (XI) into the isomeric diketal (II), which was preponderant in the equilibrium mixture. A mixture of approximately the same composition (as judged by t.l.c.) was obtained when diketal (II) was treated in like manner.

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SUMMARY

The acid-catalysed reaction of D-*glycero*-D-*gulo*-heptono-γ-lactone (I) with acetone affords 3,5:6,7-di-*O*-isopropylidene-D-*glycero*-D-*gulo*-heptono-γ-lactone (II) as the principal product, together with small proportions of the 2,3:6,7-diketal (XI) and the 6,7-monoketal (XIII). The structures of these ketals have been established by periodate oxidation and by methylation of the derived heptitols.

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SYNTHESIS AND REACTIONS OF UNSATURATED SUGARS IV. METHYL 4,6-*O*-BENZYLIDENE-α-D-*erythro*-HEX-2-ENOPYRANOSIDE AND ITS HYDROLYSIS BY ACID*

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INTRODUCTION

A program in this laboratory is concerned with the conversion of selected diol groups in carbohydrate derivatives into alkene functions, and with reactions of the resultant, unsaturated sugar derivatives. Conversion of a *trans*-diol system in a cyclic sugar derivative into the corresponding alkene has been reported[1] briefly. Conversion of a terminal diol group of a sugar derivative into an alkene, by way of a cyclic thionocarbonate derivative[1], has been discussed in detail[2], and the conversion of a *cis*-diol in a cyclic system into the corresponding alkene has been noted[2]. The present report describes in detail the preparation of a cyclic-sugar 2,3-alkene, methyl 4,6-*O*-benzylidene-α-D-*erythro*-hex-2-enopyranoside (**6**), from a 2,3-*trans*-diol precursor, methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**), by way of disulfonic ester intermediates (**2**, **3**, **4**, or **5**); and from a corresponding *cis*-diol precursor, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**11**), by way of the cyclic thionocarbonate (**10**). Conversion of an epoxide precursor (**8**) into the alkene **6** is also described, and the alkene **6** is shown to undergo facile hydrolysis to give 2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**).

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside[3,4] (**1**) was converted into the known 2,3-di-*p*-toluenesulfonate[4,5] (**2**) and 2,3-dimethanesulfonate[6] (**3**); the 2,3-di-*p*-nitrobenzenesulfonate (**4**) and 2,3-di-*p*-bromobenzenesulfonate (**5**) were prepared crystalline, in high yield, by sulfonylation of **1** in pyridine solution. Treatment of each of the disulfonic esters with an excess of potassium ethylxanthate in boiling butyl alcohol gave the corresponding 2,3-alkene, methyl 4,6-*O*-benzylidene-α-D-*erythro*-hex-2-enopyranoside [7–12] (**6**), in 40–45% yield in most cases (see Table I and Experimental); colored side-products were removed most readily by chromatography on partially deactivated alumina. Under similar conditions, methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside[4,13] (**8**) and its 2,3-dideoxy-2,3-epithio analog[10] (**9**) were also converted into **6**.

Conversion of the sulfonic ester derivatives **2**, **3**, **4**, and **5** into the alkene **6** was also achieved by treatment of the derivatives with sodium iodide and an excess

*For part III, see ref. 2.
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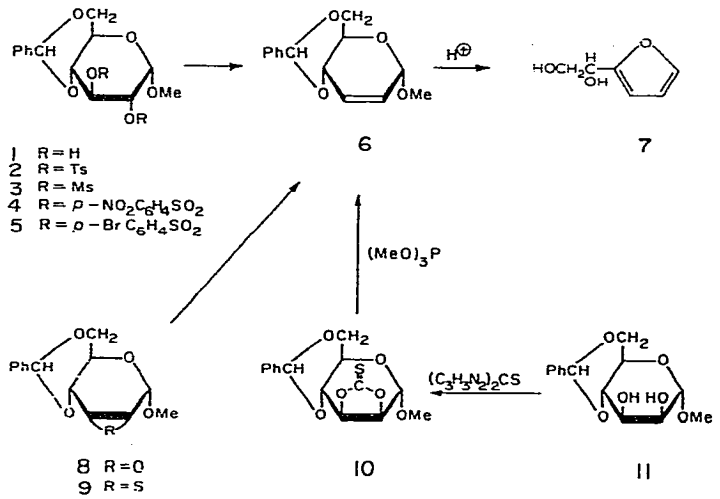
of zinc dust in boiling *N*,*N*-dimethylformamide, a procedure[14] used for the introduction of 3,4-unsaturation into 1,2:5,6-di-*O*-isopropylidene-3,4-di-*O*-(*p*-tolylsulfonyl)-D-mannitol[15]. Yields of **6** by this method (see Table II and Experimental) were 45–55%, except for the case of the di-*p*-nitrobenzenesulfonate **4**, which gave a lower yield. The results indicate that the sodium iodide–zinc dust–*N*,*N*-dimethylformamide procedure (Tipson–Cohen procedure) is not limited to precursors wherein free rotation about the carbon–carbon bond of the diol is possible, and the method provides an excellent alternative procedure for synthesis of the alkene **6**.



The two foregoing methods provide a route for the conversion of the *trans* diol system in **1** into the corresponding alkene **6**, in 35–50% overall yield, in two facile reaction steps. The route is probably of general applicability for alkene synthesis from vicinal diol groups, in carbohydrate derivatives otherwise protected with alkali-stable substituents. Methods in the literature[7–12] for the conversion of **1** into **6** give very low net yields, and most of them involve a large number of steps. The present routes make the alkene **6** readily available as an intermediate for synthesis.

Treatment of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[16] (**11**) with bis(imidazol-1-yl)thione[2,17] in boiling acetone gave the crystalline 2,3-thionocarbonate (**10**) of **11**, indicating that this procedure[18] can be used to prepare cyclic thionocarbonates of nonterminal, as well as terminal[1,2], diols in carbohydrate derivatives. The thionocarbonate **10** was converted by trimethyl phosphite into the alkene derivative **6**. The conversion **11**→**6** further illustrates the usefulness of the Corey–Winter alkene synthesis[18] in carbohydrate systems [1,2,19], and establishes that the procedure can be used in the presence of an *O*-benzylidene group.

Treatment of 1,2-*O*-isopropylidene-5,6-di-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose[20] with sodium iodide–zinc dust in acetone gave the terminal alkene, 1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose[1,2], as predicted[15], but this product was accompanied by a considerable proportion of 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-

(*p*-tolylsulfonyl)-α-D-glucofuranose[21]. The result indicates that, under the reaction conditions, intramolecular attack at C-6 by the C-3 hydroxyl group competes with attack at C-6 by iodide ion[22]. The proportion of alkene formed was increased, at the expense of the anhydro derivative, when the reaction was conducted under strictly anhydrous conditions.

The crystalline alkene derivative **6** had a melting point and specific rotation in agreement with literature values [8–12]. Additional data, including infrared, ultraviolet, and n.m.r. spectral measurements, chromatographic behavior, and X-ray powder diffraction data, are recorded in the Experimental section. The ultraviolet absorption data differ somewhat from values earlier reported[8]. The substance is quite stable under basic conditions, and may be purified conveniently by column chromatography on basic alumina. Reaction mixtures containing **6** were analyzed by thin-layer chromatography on alumina or silica gel. In the cold, the substance gives an intense black spot immediately when the chromatoplate is sprayed with sulfuric acid, in contrast to the precursors and side-products of the reactions used in the synthesis of **6**.

Under acidic conditions, the alkene **6** is very labile; this is to be expected because C-1 is in the allylic position. Prolonged contact with silica gel, as in attempted column chromatography, led to decomposition, with the production of benzaldehyde. Aqueous acetic acid at 70° caused complete hydrolysis of **6** in 10 min, and a non-reducing, optically active, unsaturated product was formed in high yield. The latter was obtained as an oil which crystallized below room temperature, and it was formulated as 2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**). The assigned structure is supported by the microanalytical data, and by the fact that the compound gives a crystalline dibenzoate and di-*p*-nitrobenzoate, both having the proper elemental analyses. Proof of the structure of **7** was provided by periodate oxidation; the substance consumed one mole of periodate per mole, with the production of one mole of formaldehyde per mole. The 2-furaldehyde produced by cleavage of **7** was characterized as its crystalline (2,4-dinitrophenyl)hydrazone.

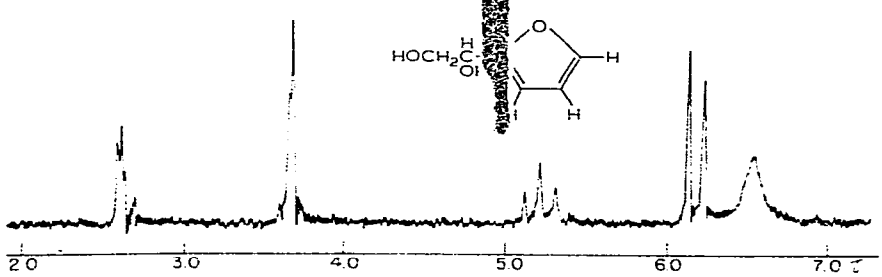


Fig. 1. The n.m.r. spectrum of 2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**) in deuteriochloroform at 60 Mc.p.s.

The n.m.r. spectrum of **7** (Figure 1) provided independent proof of the assigned structure. The aromatic hydrogen atoms of the 2-substituted furan moiety give a

recognizable[23] pattern of signals, a one-proton narrow multiplet at τ 2.60 (H-5) and a two-proton, narrow multiplet at τ 3.68 (H-3 and H-4). The apparent simplicity of this portion of the spectrum may be ascribed to the near-equivalence of the H-3 and H-4 protons. The two-proton, broad singlet at τ 6.53 was observed at lower field with higher concentrations of sample, and it disappeared when the sample was deuterated; the signal was, therefore, assigned to the hydroxyl protons. Exchange of these protons is evidently rapid under the conditions of observation, and the signals of the methylene and methine protons of the side-chain were observed as an apparent A_2X system[24]. The two-proton doublet at τ 6.18 was assigned to the methylene group, and the one-proton triplet at τ 5.22 was assigned to the methine group.

Details of the n.m.r. spectra, including first-order analyses and assignments, are recorded in the Experimental section, for the thionocarbonate **9**, the alkene **6**, and the dibenzoate of the furan derivative **7**. The optical rotatory dispersion spectrum of **7** showed a positive Cotton effect[25].

The 2,3-disulfonic ester derivatives **2**, **3**, **4**, and **5** showed strong bands in their infrared spectra at 8.4–8.5 μ and at 7.3–7.4 μ, characteristic of the symmetrical and antisymmetrical stretching modes of the $-SO_2-$ group[26,27]. In each case, a strong band at 11.8–11.9 μ and a weak one at 11.2–11.4 μ were present. It has been proposed[27] that bands of the latter type are characteristic of the C–O–S vibration mode of an equatorial sulfonic ester group on a pyranoid ring.

The order of reactivity, in the conversion of the sulfonic esters of **1** into the alkene **6** by potassium ethylxanthate in butyl alcohol at reflux (see Table I), was di-*p*-bromobenzenesulfonate **5** $\approx$ di-*p*-nitrobenzenesulfonate **4** $>$ di-*p*-toluenesulfonate **2** $>$ dimethanesulfonate **3**. The epoxide **8** and the episulfide **9** underwent rapid conversion into **6** under similar conditions. The product **6** did not suffer destruction, by the reagent, at a significant rate. The data suggest that 2,3-epoxides or 2,3-episulfides are possible intermediates in the conversion of **2**, **3**, **4**, and **5** into **6**. The conversion of **2** into **8** by alkoxide ion is well known[4,13]; episulfides (and trithiocarbonates) can be formed by the action of alkylxanthate ion on epoxides[20,28,29]; and episulfides have been converted into alkenes by the action of nucleophiles[30,31]. Conversion of certain steroidal[31] and inositol[32] epoxides into alkenes by alkylxanthate ion has been noted. Treatment of **8** with thiourea, under forcing conditions[12], gives the D-*manno* analog of **9** (*cf.* Ref. 29), with **6** as a side product.

A number of unidentified side-products were formed in the preparation of **6** by the potassium ethylxanthate method. Characterization of these products should throw further light on the detailed mechanism of these transformations. Continuing studies in this laboratory are concerned with this aspect, and with the kinetics of acid hydrolysis of **6** and related derivatives.

It is noteworthy that, in the preparation of **6** by the Tipson–Cohen method (Table II), the product was destroyed by the reagent at prolonged times of reaction, but this decomposition could be markedly retarded by the inclusion of 10 moles of water per mole of starting material.

EXPERIMENTAL

General methods

Melting points were determined with a Thomas–Hoover melting-point apparatus (Arthur H. Thomas Co., Philadelphia, Pennsylvania) and are uncorrected. Specific rotations were determined in a 2-dm polarimeter tube. Infrared spectra were measured with a Perkin–Elmer Model 137 "Infracord" infrared spectrophotometer. Ultraviolet spectra were measured with a Bausch and Lomb "Spectronic 505", or a Carey Model 14, recording spectrophotometer. Nuclear magnetic resonance spectra were measured at 60 Mc.p.s. with a Varian A-60 n.m.r. spectrometer. Chemical shifts are given on the τ-scale, and refer to spectra measured at *ca.* 30° with solutions (*ca.* 10%) in deuteriochloroform, with tetramethylsilane (τ 10.00) as the internal standard. Deuteration was effected by codistilling the sample with a few drops of deuterium oxide. Microanalyses were determined by W.N. Rond. X-Ray powder diffraction data give interplanar spacings, Å, for CuKα radiation. Relative intensities were estimated visually; s, strong; m, moderate; w, weak; v, very. The strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities. Thin-layer chromatography was performed with Desaga equipment, with Silica Gel G (E. Merck, Darmstadt, Germany) activated at 110° (denoted S), or with alumina prepared as described below (denoted A), as the adsorbent. Indication was effected with sulfuric acid.

Deactivated alumina for chromatography

Basic alumina (Woelm, 20 g) was ground in a mortar with water (12 ml), and more water (12 ml) was gradually added, with continued grinding. The slurry was poured into a large dish (for use in column chromatography) or applied to glass plates (for t.l.c.), and the dish (or plates) were heated for 2 h at 150°.

If the grinding step was omitted, the adsorbent failed to adhere to the plates. Untreated alumina (Woelm) proved to be too strong an adsorbent for the following experiments; no products could be desorbed with carbon tetrachloride, benzene, or chloroform.

*Preparation of methyl 4,6-O-benzylidene-α-D-glucopyranoside 2,3-di-*p*-toluenesulfonate* (**2**) *and 2,3-dimethanesulfonate* (**3**)

Substance **2** was prepared from **1** by the method of Richtmyer[4]; m.p. 154–155°; $[\alpha]_D^{22}$ +13.0 ±0.2° (*c* 5.5, chloroform) [lit.[4] m.p. 154—155°; $[\alpha]_D^{22}$ +12° (chloroform)]; R_F 0.85 (S, 1:1 chloroform–ether); λ_{max}^{KBr} 6.24, 6.72, 6.90 (aryl), 7.37, 8.48 (SO_2), 11.35 w, 11.89 s (C–O–S)[27], 12.25 μ (substituted phenyl).

Substance **3** was prepared from **1** by the method of Honeyman and Morgan[6]; m.p. 186–188°; $[\alpha]_D^{22}$ +51.5 ±0.4° (*c* 1.6, chloroform) [lit.[6] m.p. 188–189° ; $[\alpha]_D^{10}$ +49° (chloroform)]; R_F 0.60 (S, 1:1 chloroform–ether); λ_{max}^{KBr} 7.35, 8.47 (SO_2), 11.36 w, 11.86 s μ (C–O–S)[27].

Methyl 4,6-O-benzylidene-2,3-di-O-(p-nitrophenylsulfonyl)-α-D-glucopyranoside (**4**)

To a solution of **1** (2.0 g) in dry pyridine (14 ml) was added *p*-nitrobenzenesulfonyl chloride (4.72 g, 3 molar equivalents), and the mixture was kept for 24 h at room temperature. Water (0.5 ml) was added, and, after 1 h, the solution was poured into ice and water (250 g). The precipitated solid was filtered off, washed thoroughly with water, and dried; yield 4.61 g (100%). The crude product was dissolved in chloroform (40 ml), the solution was filtered, ether (15 ml) was added to the filtrate, and the solution was kept 1 day at 0° to give the pure product as yellow needles, yield 3.70 g (80%); m.p. 184–186°; $[\alpha]_D^{22}$ +62.1 ±0.5° (*c* 1, chloroform); R_F 0.82 (S 1:1 chloroform–ether); λ_{max}^{KBr} 6.21, 6.74, 6.88 (aryl), 6.53 (NO_2), 7.25, 7.41 (NO_2, SO_2), 8.42 (SO_2), 11.35 w, 11.90 s μ (C–O–S)[27]; X-ray powder diffraction data: 11.23 m, 10.08 m, 7.14 w, 6.46 m, 5.73 vw, 5.24 s (3), 4.73 s (1), 4.43 w, 4.09 w, 3.82 s (2), 3.55 m, 3.22 m.

Anal. Calc. for $C_{26}H_{24}N_2O_{14}S_2$: C, 47.85; H, 3.71; N, 4.29; S, 9.83. Found: C, 47.55; H, 3.91; N, 4.42; S, 9.54.

Methyl 2,3-di-O-(p-bromophenylsulfonyl)-4,6-O-benzylidene-α-D-glucopyranoside (**5**)

Treatment of **1** (4.0 g) with *p*-bromobenzenesulfonyl chloride (10.8 g, 3 molar equivalents) in pyridine, by the procedure used for the preparation of **4**, gave crude **5** as a gum, which was washed with water by decantation, and then dissolved in 1,2-dichloroethane (100 ml). The solution was washed successively at 0° with N hydrochloric acid, water, saturated sodium hydrogen carbonate, and water (25-ml portions), and the dried (magnesium sulfate) extract was evaporated to 40 ml. Addition of Skellysolve C* caused deposition of an oil which solidified to give the crystalline product after 1 day at room temperature; yield 6.90 g (68%); m.p. 113–116°; $[\alpha]_D^{22}$ +26.8 ±0.5° (*c* 1.2, chloroform); R_F 0.90 (S, 1:1 chloroform–ether); λ_{max}^{KBr} 6.33, 6.80, 6.87 (aryl), 7.32, 8.42 (SO_2), 11.34 w, 11.89 s μ (C–O–S)[27]; X-ray powder diffraction data: 9.12 vw, 8.70 m, 7.95 w, 6.97 s (3), 6.30 w, 5.78 m, 5.40 m, 4.81 s (2,2), 4.36 m, 3.94 s (1), 3.72 w, 3.52 s (2,2).

Anal. Calc. for $C_{26}H_{24}Br_2O_{10}S_2$: C, 43.35; H, 3.36; Br, 22.19; S, 8.91. Found: C, 43.49; H, 3.52; Br, 22.28; S, 8.62.

The X-ray diffraction pattern indicated that the product was definitely crystalline, but no solvent mixture could be found that would give **5** as well-formed crystals.

Methyl 4,6-O-benzylidene-α-D-mannopyranoside 2,3-thionocarbonate (**10**)

To a solution of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[16] (**8**, 4.00 g) in acetone (150 ml) was added bis(imidazol-1-yl)thione[2,17] (1.96 g; 1.5 molar equivalents) and the mixture was refluxed for 1 h under nitrogen. The solution was evaporated to a syrup which was treated with cold methanol (15 ml). The white solid which separated was filtered off and dried; yield 2.01 g, m.p. 143–144°. From the mother

*Petroleum ether, b.p. 90–100°, Skelly Oil Co., Kansas City, Missouri.

liquors, 0.72 g of the starting material (**10**) was recovered, giving a total conversion yield of 53%. Recrystallization of the product from chloroform–petroleum ether gave pure **9**; m.p. 144–145°; $[\alpha]_D^{23}$ −80 ±1° (*c* 1, chloroform); R_F 0.30 (S, 1:1 benzene–dichloromethane); λ_{max}^{KBr} 6.75, 6.85 (aryl), 8.40 (C=S)[33], 13.3, 14.3 μ (phenyl); λ_{max}^{EtOH} 238 mμ (ε 12,000), 211 mμ (ε 5280); n.m.r. data: τ 6.63 (3-proton singlet, OMe), τ 6.12 (3-proton multiplet, H-6,6′,5), τ 5.68 (1-proton multiplet, H-4), τ 4.95-5.37 (2-proton multiplet, H-2,3), τ 4.93 (1-proton singlet[34], $J_{1,2}$ 0 c.p.s. H-1) τ. 4.45 (1-proton singlet, benzylic H), τ 2.60 (5-proton multiplet, Ph); X-ray powder diffraction data: 10.03 s (2) 7.04 m, 6.05 m, 4.58 vs (1) 4.35 m, 4.08 s (3), 3.92 m, 3.57 m, 3.38 w, 3.04 w, 2.90 w.

Anal. Calc. for $C_{15}H_{16}O_6S$: C, 55.54; H, 4.97; S, 9.88. Found: C, 55.19; H, 4.85; S, 9.91.

Recrystallization of **10** from carbon disulfide gave an isomorphous form, m.p. 150-151°, X-ray powder diffraction data: 13,17 w, 11.24 m, 9.31 m, 8.50 w, 7.63 s (2,2), 6.81 vw, 5.99 w, 5.51 w, 4.99 s (2,2), 4.56 vw, 4.13 s (1), 3.90 m, 3.74 m.

Methyl 4,6-O-benzylidene-α-D-erythro-hex-2-enopyranoside (**6**)

(*a*) *From methyl 4,6-O-benzylidene-2,3-di-O-(p-tolylsulfonyl)-α-D-glucopyranoside* (**2**) *and potassium ethylxanthate.* A mixture of the di-*p*-toluenesulfonate **2** (1.28 g) and potassium ethylxanthate (Fisher Scientific Co., Fair Lawn, New Jersey) (3.5 g) in butyl alcohol (18 ml) was refluxed for 3 h, the mixture was evaporated at 85–90°, and the residue was extracted with three 50-ml portions of boiling Skellysolve C. The cooled solution (from which a small amount of solid separated) was passed through a column (1.2 × 1.8 cm) of deactivated alumina (to remove most of the colored impurities), and the solution was evaporated to a reddish syrup which crystallized on cooling; yield 550 mg. This product was dissolved in a few ml of benzene, and chromatographed on a column (2 × 10 cm) of deactivated alumina, with benzene as the eluant. A yellow substance, R_F 0.35 (minor), 0.45 (major) (A, benzene), was eluted in the 20–30 ml portion of the effluent, and the unsaturated sugar **6**, R_F 0.25 (A, benzene) was eluted in the 30–55 ml portion of the effluent. Evaporation of the latter fraction gave **6** as an almost white solid, yield 210 mg (39%); m.p. 117–119°. Recrystallization from Skellysolve C gave pure **6**, m.p. 119–120°; $[\alpha]_D^{22}$ +130 ±2° (*c* 0.54, chloroform) [lit.[10] m.p. 117–119°, $[\alpha]_D^{20}$ +126° (*c* 1, chloroform)]; R_F 0.15 (S, 1:1 chloroform–benzene); λ_{max}^{KBr} 13.3, 14.4 μ (phenyl); λ_{max}^{EtOH} 210 (ε 7,200), 250 (220), 256 (270), 260 (230), 262 (230), 266 mμ (160); $\lambda_{max}^{CHCl_3}$ 210 (ε 7,200), 250 (240), 256 (310), 260 (280), 262 (280), 266 mμ (170); n.m.r. data: τ 6.53 (3-proton singlet, OMe), τ 5.57–6.26 (4-proton multiplet, H-4,5,6,6′) τ 5.10 (1-proton unresolved multiplet, width at half-height 4.5 c.p.s., H-1), τ 4.40 (1-proton singlet, benzylic H), τ 3.84, 4.25 (broadened doublets, 2 protons, $J_{2,3}$ ~10 c.p.s. H-2, H-3), τ 2.58 (5-proton multiplet, phenyl); X-ray powder diffraction data: 12.03 s (3), 6.03 s (2), 5.68 w, 5.29 w, 4.12 vs (1,1), 3.93 vs (1,1), 3.77 w, 3.63 w, 3.39 m, 2.96 m, 2.78 w, 2.61 w, 2.48 vw.

Anal. Calc. for $C_{14}H_{16}O_4$: C, 67.72; H, 6.47. Found: C, 67.57; H, 6.62.

For this substance, Richards[8] reported λ_{max} 212 (ε 11.6), 220 (17.2), 235 (17.9), 245 (17.3), 260 mμ (18.1) (solvent not stated), and Christensen and Goodman[10] reported τ 3.94, 4.31 (2 protons, vinylic H).

The yellow fraction of the effluent gave a distillable (1 mm Hg) yellow oil, yield 120 mg, which contained sulfur.

Further elution of the column, with isopropyl alcohol, gave a mixture of minor products (50 mg) having t.l.c. mobilities less than that of **6**. No carbohydrate material could be detected in the original Skellysolve C-extracted residue.

Comparable yields of **6** were obtained when the reaction was conducted on a larger scale. The use of column chromatography in this preparation provided the best method for obtaining **6** free from impurities. Direct isolation[1] gave a higher yield of crude product, but removal of all impurities by direct crystallization was difficult.

(*b*) *From various disulfonic esters of methyl 4,6-O-benzylidene-α-D-glucopyranoside with potassium ethylxanthate.* A series of experiments was performed wherein a mixture of the disulfonic ester **2**, **3**, **4**, or **5** (1.0 mmole) and potassium ethylxanthate (10 mmoles) in butyl alcohol (10 ml) was refluxed, the solution was evaporated, the residue was extracted with three 20-ml portions of Skellysolve C, the extract was filtered after 1 h at room temperature, and the filtrate was evaporated to dryness. The weighed residues were dissolved in a measured volume of benzene, and the approximate yield (±5%) of the unsaturated sugar **6** was determined by t.l.c. (S, 1:1 chloroform–benzene) of measured aliquots of this solution, in comparison with measured volumes of a standard solution of **6** in benzene. The results are given in Table I.

TABLE I

YIELD OF METHYL 4,6-*O*-BENZYLIDENE-α-D-*erythro*-HEX-2-ENOPYRANOSIDE (**6**) BY THE ETHYLXANTHATE PROCEDURE[a]

Compound	*Yield, %* (±5%)				
	5 min	15 min	30 min	1 h	3 h
2	40	40	45	45	45
3	10	20	25	45	45
4	30	30	30	30	30
5	35	45	45	45	45
8	45				
9	50				50

[a]See Experimental for details

(*c*) *From methyl 2,3-anhydro-4,6-O-benzylidene-α-D-allopyranoside*[4,13] (**8**) *and methyl 4,6-O-benzylidene-2,3-dideoxy-2,3-epithio-α-D-allopyranoside*[10] (**9**) *with potassium ethylxanthate.* A mixture of **8** (589 mg) and potassium ethylxanthate (3.57 g) in butyl alcohol (20 ml) was refluxed for 7 min, the solution was evaporated, and the residue was processed, essentially by the method used in (*a*) above, to give crystal-

line **6**, yield 220 mg (40%), which, after recrystallization from hexane, had m.p. 119-120°. The substance was identical with the product produced by method (*a*), in regard to t.l.c., mixed melting point, and infrared spectrum. Under similar conditions, the episulfide[10] **9** gave **6** in comparable yield. Data for these transformations at different times of reaction are recorded in Table I.

(*d*) *From methyl 4,6-O-benzylidene-2,3-di-O-(p-tolylsulfonyl)-α-D-glucopyranoside* (**2**), *sodium iodide, and zinc dust.* A mixture of **2** (1.18 g, 2 mmole), sodium iodide (dried at 250°, 15 g, 100 mmole), zinc dust (7 g), and *N,N*-dimethylformamide (25 ml, 0.1% water content) was refluxed for 2 h, evaporated, and the residue was extracted with three 70-ml portions of Skellysolve C. The combined extracts were filtered, and evaporated, and the residue was dissolved in ether (6 ml) and transferred to a column of alumina (Woelm, not deactivated, 3.5 g). The column was eluted with ether, and the first 20 ml of effluent was evaporated, to give crystalline **6**, yield 260 mg (52%). Recrystallization from hexane gave pure **6**, yield 205 mg; m.p. 119–120°. The product was identical, by mixed m.p., i.r. spectrum, and t.l.c., with the product obtained by method (*a*).

(*e*) *From various disulfonic esters of methyl 4,6-O-benzylidene-α-D-glucopyranoside, with sodium iodide and zinc dust.* A series of experiments was performed wherein **2**, **3**, **4**, or **5** (1 mmole), dried sodium iodide (7.5 g, 50 mmole), zinc dust (3.5 g), and dry *N,N*-dimethylformamide (12.5 ml) was refluxed, with or without addition of water (0.2 ml), and the resulting mixture was evaporated. The residue was extracted with three 35-ml portions of hot Skellysolve C, and the approximate yield of **6** was determined by the method described under (*b*) above. The results are given in Table II.

TABLE II

YIELD OF METHYL 4,6-*O*-BENZYLIDENE-α-D-*erythro*-HEX-2-ENOPYRANOSIDE (**6**) BY THE SODIUM IODIDE–ZINC DUST PROCEDURE[a]

Compound	*Yield, %* (±5%)				
	30 min	1 h	2 h	4 h	8 h
2	45	55	50	45	7
2[b]	50	55	55	45	45
3	30	40	40	25	6
3[b]	25	30	45	40	25
4[b]		15			
5	45	45	35	20	3
5[b]	50	50	50	40	40

[a]See Experimental for details. [b]With water added.

When the procedure was applied to the di-*p*-nitrobenzenesulfonate (**4**), the mixture became dark before it was heated.

(*f*) *From methyl 4,6-O-benzylidene-α-D-mannopyranoside 2,3-thionocarbonate* (**11**). A solution of **11** (500 mg) in trimethyl phosphite (15 ml) was refluxed for 60 h under nitrogen. To the cooled mixture was added carbon tetrachloride (100 ml), and then

N sodium hydroxide solution was added with vigorous stirring until the aqueous phase remained basic. The organic layer was separated, the aqueous layer was re-extracted with carbon tetrachloride (50 ml), and the combined extracts were dried (magnesium sulfate) and evaporated to a syrup. Crystallization from Skellysolve C gave **6** as white rosettes, yield 151 mg (40%); m.p. 115–117°. The product was identical, by mixed m.p., i.r. spectrum, and X-ray powder diffraction pattern, with **6** prepared by method (*a*).

2-(D-glycero-1,2-Dihydroxyethyl)furan (7)

A solution of **6** (1.00 g) in 1:3 water–acetic acid (20 ml) was heated for 10 min at 70°, evaporated at 40°, and the residue was coevaporated twice with toluene to remove most of the benzaldehyde liberated. The resultant syrup was dissolved in ether (3 ml) and chromatographed on a column (2 × 11 cm) containing silica gel (grade 950, 60–200 mesh, Davison Division of W. R. Grace Co., Baltimore, Maryland) (20 g), with ether as eluant, and the fractions from the column were examined by t.l.c. (S, ether). The first 50 ml gave benzaldehyde and a small amount of yellow material. Fractions eluted in 60–180 ml of column effluent contained a single component, R_F 0.3 (S, ether), and evaporation of these fractions gave the product 7 as a chromatographically homogeneous, pale-yellow syrup; yield 449 mg (87%); $[\alpha]_D^{22}$ +36.7 ±0.4° (*c* 1.6, chloroform); λ_{max}^{film} 2.95–3.15 (OH), 6.66, 9.85, 11.38 μ (furan); λ_{max}^{EtOH} 218 mμ (ε 8,000); n.m.r. data (see Fig. 1): τ 6.53 (2-proton broad singlet, shifts with change of concentration, disappears on deuteration, OH), τ 6.18 (2-proton doublet, $J_{1',2'}$ 5.7 c.p.s., H-2'), τ 5.22 (1-proton triplet, $J_{1',2'}$ 5.7 c.p.s., H-1), τ 3.68 (2-proton multiplet, H-3,4), τ 2.60 (1-proton multiplet, H-5).

Anal. Calc. for $C_6H_8O_3$: C, 56.33; H, 6.29. Found: C, 56.68; H, 6.62.

A sample of the syrup crystallized upon refrigeration, but the crystals melted when brought to room temperature.

2-(D-glycero-1,2-Dihydroxyethyl)furan di-p-nitrobenzoate

To a solution of 7 (60 mg) in dry pyridine (3 ml) was added *p*-nitrobenzoyl chloride (340 mg), and the mixture was kept for 24 h at room temperature. Water (0.1 ml) was added, the mixture was diluted with ether (80 ml), and the ethereal solution was washed successively with aqueous sodium hydrogen carbonate (twice), cold N hydrochloric acid (twice), cold water, aqueous sodium hydrogen carbonate, and again with water. The dried (magnesium sulfate) extract was evaporated, to give a yellow solid, yield 167 mg (84%), which was recrystallized from benzene–hexane to give the pure di-*p*-nitrobenzoate of 7, m.p. 95–97°; $[\alpha]_D^{22}$ +82.6 ±1.0° (*c* 0.7, chloroform); λ_{max}^{KBr} 5.76, 7.89 (ArCO–O), 6.19, 6.69 (aryl), 6.53, 7.40 (NO_2), 9.85, 11.38 μ (furan).

Anal. Calc. for $C_{20}H_{14}N_2O_9$: C, 56.35; H, 3.31; N, 6.57. Found: C, 56.57; H, 3.49; N, 6.33.

2-(D-glycero-1,2-Dihydroxyethyl)furan dibenzoate

To a solution of 7 (53.3 mg) in dry pyridine (2 ml) was added benzoyl chloride

(0.2 ml), and the solution was treated as described for the di-*p*-nitrobenzoate analog; yield 112 mg (80%). Recrystallization from hexane (5 ml) gave the product as very pale-yellow plates; yield 82 mg; m.p. 75° (after solidification, the product remelted at 79.5–80.5°); $[\alpha]_D^{21}$ +72.0 ±0.7° (*c* 1.9, chloroform); λ_{max}^{KBr} 5.69, 5.72 (OBz), 6.24, 6.69, 6.90 (aryl), 9.78, 11.35 μ (furan); n.m.r. data: τ 5.13 (2-proton doublet, $J_{1',2'}$ 5.7 c.p.s., H-2'), τ 3.51 (3-proton multiplet, H-3,4, H-1'), τ 1.95, 2.49 (multiplets, phenyl, H-5); X-ray powder diffraction data (low-m.p. form): 8.72 s (3), 7.04 vw, 6.14 w, 5.58 w, 5.26 s (2), 4.76 vs (1), 4.52 m, 4.37 w, 4.21 w, 4.11 w, 3.97 w, 3.73 s (2).

Anal. Calc. for $C_{20}H_{16}O_5$: C, 71.42; H, 4.80. Found: C, 71.44; H, 5.00.

Periodate oxidation of 2-(D-glycero-*1,2-dihydroxyethyl)furan* (7)

(*a*) *Analytical.* To a solution of 7 (12.8 mg, 0.1 mmole) in acetate buffer (0.05 M in acetic acid and 0.05 M in sodium acetate) (10 ml) was added 0.03 M sodium metaperiodate in acetate buffer (12 ml), and the mixture was made up to 25 ml. Periodate consumption, determined by the arsenite method[35], was found to be 1.08 moles per mole for an aliquot taken immediately after mixing, and an aliquot taken after 15 h at room temperature gave a value of 1.08 moles per mole also.

To a suspension of 7 (12.8 mg, 0.1 mmole) in water (2 ml) was added periodic acid (34.2 mg, 0.15 mmole), together with sodium hydrogen carbonate (42 mg), and the mixture, turbid initially, was agitated for 1 h at room temperature. Formaldehyde production, determined by the chromotropic acid method[36] on the resultant clear solution, was 0.91 mole per mole.

(*b*) *Preparative.* To a suspension of 7 (94 mg) in water (10 ml) was added periodic acid (324 mg). The mixture became clear after 20 min at room temperature, and t.l.c. (S) of aliquot samples revealed conversion of the starting material, R_F 0.3 (S, ether) into a product R_F 0.75 (S, ether) chromatographically indistinguishable from 2-furaldehyde. The solution was neutralized with hot 10% strontium hydroxide solution, and the precipitated salts were filtered, and washed with water. A small amount of strontium carbonate was added to the combined filtrates, and the mixture was distilled under diminished pressure. Water (20 ml) was added to the residue, and the mixture was distilled. To the combined distillate (60 ml) was added a solution (20 ml) containing 2.5% of (2,4-dinitrophenyl)hydrazine in 30% perchloric acid[37]. After 1 h at room temperature, the resultant red precipitate was filtered, washed thoroughly with water, and dried, yield 267 mg. This product was boiled with absolute ethanol (20 ml) whereupon formaldehyde (2,4-dinitrophenyl)hydrazone and part of the 2-furaldehyde (2,4-dinitrophenyl)hydrazone were dissolved. The mixture was filtered while hot, and the filtered mass was washed with hot ethanol to give a deep-red solid, yield 94 mg, m.p. 190–195°. Recrystallization from ethyl acetate gave pure 2-furaldehyde (2,4-dinitrophenyl)hydrazone, m.p. 201–203° (lit.[38] m.p. 202°), indistinguishable from an authentic sample by mixed m.p., infrared spectrum, and X-ray powder diffraction pattern; X-ray powder diffraction data: 12.29 m, 10.41 m, 9.39 w, 8.26 w, 7.02 w, 6.57 m, 5.85 s (3), 5.50 vw, 5.24 w, 4.89 vw, 4.65 m, 4.30 s (2), 3.92 w, 3.75 m, 3.54 m, 3.25 s (1), 3.12 m.

The starting material **7** gave an intense black spot on t.l.c. as soon as the plate was sprayed with sulfuric acid at room temperature. A sample of furfuryl alcohol (R_F 0.7, S, ether) behaved similarly. 2-Furaldehyde did not give a black spot on t.l.c. until the sprayed plate was heated.

*Treatment of 1,2-O-isopropylidene-5,6-di-O-(p-tolylsulfonyl)-α-D-glucofuranose with sodium iodide and zinc dust**

A mixture of 1,2-*O*-isopropylidene-5,6-di-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose[20] (20g), sodium iodide (45g), and zinc dust (35 g) in acetone (375ml) was refluxed for 21 h under nitrogen. The solution was filtered, the filtrate was evaporated, and the residue was dissolved in dichloromethane (100 ml), filtered from sodium iodide, and evaporated to a syrup, yield 7 g. Extraction of the syrup with hot Skellysolve C (500 ml), followed by refrigeration of the extract, gave 1,2-*O*-isopropylidene-α-D-*xylo*-hex-5-enofuranose, yield 3.5 g (50%), m.p. 57–58°, which after purification by sublimation was identical with an authentic sample[1,2] by mixed m.p. and i.r. spectrum.

Extraction of the crude syrup with a further two 500-ml portions of hot Skellysolve C gave, on refrigeration, 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose; yield 3.0 g (22%), m.p. 128°; identical with an authentic sample[21] by mixed m.p., and i.r. and n.m.r. spectra[39].

A second experiment, employing exhaustively dried materials, gave the crude product as a syrup (~7 g). Addition of methanol (30 ml) caused the anhydro sugar derivative to separate, and it was filtered off and washed with methanol; yield 0.52 g (4%), m.p. 128°. The filtrate, which contained exclusively the alkene derivative (t.l.c.) was evaporated, and the syrup was crystallized from Skellysolve C; yield 5.2 g (74%); m.p. 59–60°.

ACKNOWLEDGMENTS

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SUMMARY

Procedures for the introduction of the alkene type of unsaturation into cyclic sugar derivatives, from *trans*- and from *cis*-diol precursors, have been evaluated. The *trans*-diol system in methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) was converted

*This experiment was performed by C.G. Tindall.

into the corresponding alkene, methyl 4,6-*O*-benzylidene-α-D-*erythro*-hex-2-enopyranoside (**6**), by treatment of various disulfonic esters (**2**, **3**, **4**, or **5**) of **1** with potassium ethylxanthate. The latter reagent also converted the epoxide **8** and the episulfide **9** into **6**. The esters **2**, **3**, **4**, and **5** could also be converted into **6** by the Tipson–Cohen procedure, namely, the action of sodium iodide–zinc dust. The *cis*-diol analog of **1**, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**11**), was converted into the alkene **6** by way of the 2,3-thionocarbonate **10**. The alkene **6** is extremely acid-labile, and, under very mild hydrolytic conditions, it is converted into 2-(D-*glycero*-1,2-dihydroxyethyl)furan (**7**). Treatment of 1,2-*O*-isopropylidene-5,6-di-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose with sodium iodide–zinc dust in acetone gave the 5,6-alkene, together with 3,6-anhydro-1,2-*O*-isopropylidene-5-*O*-(*p*-tolylsulfonyl)-α-D-glucofuranose.

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ISOLATION OF HYALURONIC ACID BY GEL FILTRATION ON AGAROSE

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INTRODUCTION

Samples of hyaluronic acid from normal and pathological synovial fluids have been shown[1] to contain the same repeating unit, namely →4)-β-D-GpA-(1→3)-β-D-GNAc-(1→4)-. That subtle differences exist between normal and pathological hyaluronic acids has been suggested by work on the protein part of the acid complex by Sandson and Hamerman[2], and from the comparison of the mucopolysaccharides by Barker *et al.*[3]. A method is now reported which enables the undegraded protein–polysaccharide complex to be isolated rapidly for analysis and, at the same time, indicates its molecular dispersity.

METHODS

Materials and assays

Agar was obtained from Davis Gelatine Ltd., Warwick, England, and agarose from Seravac Laboratories (Pty) Ltd., Maidenhead, England. Emulphor El was a gift from Kodak Ltd., Kirkby, Lancashire, England.

The synovial fluids were obtained by aseptic aspiration from patients having rheumatoid arthritis.

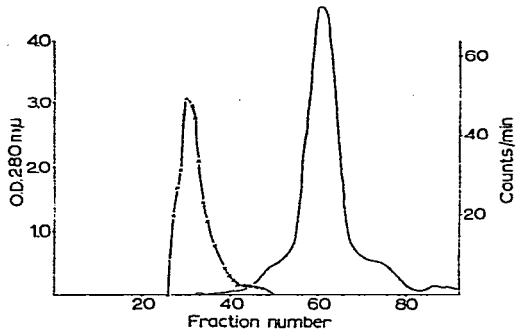
Ultraviolet absorption was measured at 280 mμ in 1-cm silica cells, using a Unicam SP 500 spectrophotometer. Protein concentrations were measured by a modification[4] of the Folin method[5]. Reagents were prepared in the same manner, but 1 ml of alkaline copper solution was added to each 3 ml of sample. After 10 min, 0.1 ml of Folin–Ciocalteu reagent was added. The absorbance at 750 mμ was read after 0.5 h. The standard used was a solution (50 γ/ml) of human serum albumin.

Uronic acid was determined by the Gregory[6] modification of the Dische[7] carbazole reaction.

Separations on 5% agar gel

The gel was prepared as described by Andrews[8]. A column (bed volume, 2.5 × 34 cm) packed with 5% (w/v) gel of 100/200 mesh, and having a 2.5-cm head, had a flowrate of 8 ml/h of phosphate buffer (pH 7.0, $I = 0.1$)[9]. Human serum (2 ml) containing 0.005% of sodium azide was run through the column. Fractions were

collected at 20-min intervals and assayed for u.v. absorbance. The column was then thoroughly washed with buffer, and a solution of ^{14}C-labelled hyaluronic acid[8] (2 ml) was similarly eluted. Fractions were assayed for radioactivity by drying a sample (0.05 ml) on a planchet and counting, for 15 min, with an end-window counter. The results are superimposed in Fig. 1.



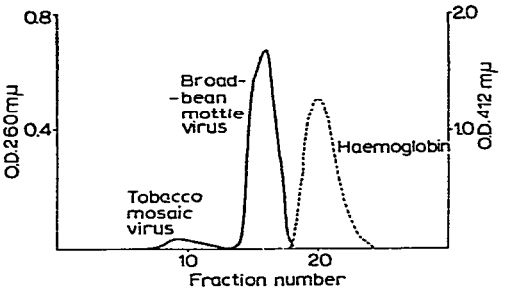


Fig. 1. Separations on 5% agar gel. ————, optical density (O.D.) at 280 mμ (protein); x—x radioactive hyaluronic acid.

Fig. 2. Virus separation on 3% "block" agarose. ————, O.D. at 260 mμ (nucleic acid); – – –, O.D. at 412 mμ (haemoglobin).

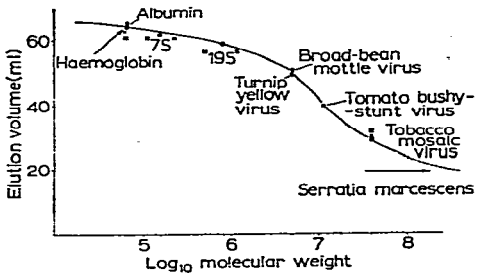
Separations on agarose gel

(a) Gels prepared by the disintegration of blocks of agarose gel. Agarose gels (1, 2, and 3%, w/v) were prepared by the method used for 5% agar[8]. Columns (1.4 × 50 cm) of these gels were equilibrated with phosphate buffer (pH 7.0, $I = 0.1$)[9]. A mixture of tobacco mosaic virus, broad-bean mottle virus, and haemoglobin (1-ml portions) was run through each gel, at flowrates of 3.6, 2.7, and 3.6 ml/h for the 1, 2, and 3% gels, respectively. Fractions were taken at hourly intervals, and the u.v. absorbances at 260 mμ and 280 mμ were measured (Fig. 2).

Calibration of the 3% gel (Fig. 3) was continued with whole serum, turnip yellow virus, and broad-bean mottle virus by collecting 1-ml fractions. To obtain the void volume of the 3% column, a culture of *Serratia marcescens* was separated, and the collected fractions were tested by growing on plates of nutrient media.

(b) Gels prepared as beads. Agarose gels (1 and 2%) were prepared, according to the method of Hjertén[11], using the emulsifying agent, Emulphor El. Samples for protein investigation were prepared on a column (34 × 2 cm) of 2% bead agarose (100/200 mesh) equilibrated in M sodium chloride–0.01M phosphate (pH 7.0). Synovial fluids were diluted four times with this buffer and centrifuged at 34.000 × g in an MSE 17,000 centrifuge for 10 min at 4°. Portions of the supernatant solution (4–6 ml, depending on hyaluronic acid concentration) were applied to the column and eluted with the buffer, using a 5-cm head of liquid. Fractions (4 ml) were assayed for uronic acid content and u.v. absorbance at 280 mμ.

A 0.001% solution (3 ml) of Blue Dextran 2000 (Pharmacia Ltd.) was similarly passed through this column. The fractions were assayed for u.v. absorbance



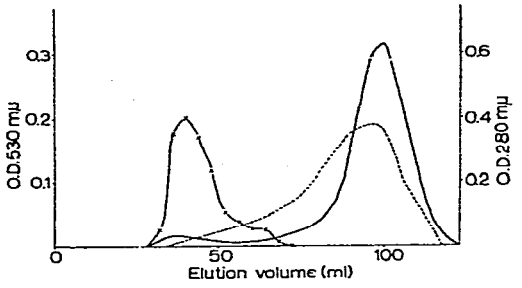


Fig. 3. Calibration of "block" agarose. o, 3% gel; x, 2% gel; □, 1% gel.

Fig. 4. Short-column runs of Blue Dextran and synovial fluid. ————, O.D. at 280 mμ (protein); x—x, O.D. at 530 mμ (hyaluronic acid); — — —, O.D. at 280 mμ (on left-hand scale) (Blue Dextran 2000).

at 280 mμ. This separation is shown superimposed on a synovial fluid separation in Fig. 4.

To investigate the dependence of intrinsic viscosity on elution volume, columns (50 × 2 cm) were prepared in the buffer described by Blumberg and Ogston[12]. With a 5-cm head, the flowrate of a 1% column (100/200 mesh) was 13 ml/h and that of a 2% column (60/100) was 25 ml/h. The synovial fluid was diluted and centrifuged as above, and a sample (6 ml, 1.5 ml of synovial fluid) was passed through each column (Fig. 5). Fractions (4 ml and 6.3 ml) from the 1% and 2% runs, respectively, were assayed for uronic acid content and u.v. absorbance. Intrinsic viscosities were measured, using four concentrations in a suspended-level dilution viscometer (buffer flowtime, 188.80 sec), for the following fractions:

Fraction (1% run)	*Intrinsic Viscosity dl/g*	*Fraction (2% run)*	*Intrinsic Viscosity dl/g*
16 + 17	28.6	6	28.4
20 + 21	25.0	8	21.4
24 + 25	20.9	10	17.7
28 + 29	18.8	12	13.8
32 + 33	14.7		

Stability of the protein–polysaccharide complex

(*a*) A solution (5 ml) of hyaluronate–protein complex (0.1 mg/ml), prepared by chromatography on agarose gel, was rechromatographed on a column (34 × 2 cm) of 2% agarose equilibrated in 3M guanidine hydrochloride, adjusted to pH 7.0 with M Tris buffer[9] (4.4 ml/l). Fractions (4.5 ml) were assayed for uronic acid content and u.v. absorbance at 280 mμ.

(*b*) A solution (50 ml) of the complex (0.05 mg/ml) in 0.05M sodium chloride–0.0005M phosphate buffer (pH 7.0) was applied to a column (10 × 1.2 cm) of DEAE Sephadex A50, equilibrated in the same buffer. After a 50-ml wash, the column was

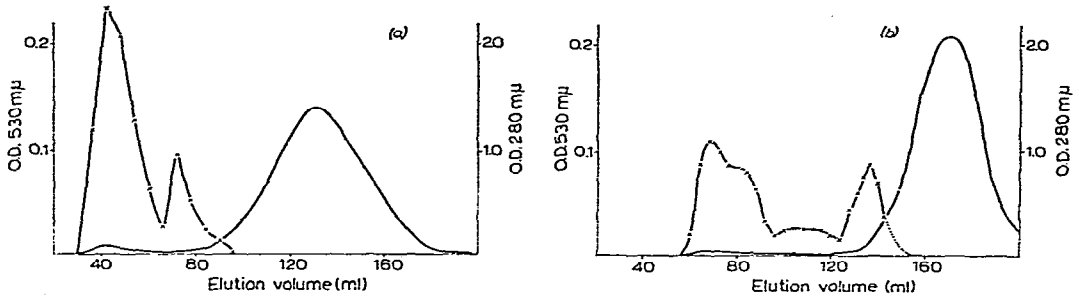


Fig. 5. Agarose-bead runs: (*a*) 2% gel; (*b*) 1% gel. ————, O.D. at 280 mμ (protein); x—x, O.D. at 530 mμ (hyaluronic acid).

developed with a linear gradient formed from 100 ml each of 0.05M and M sodium chloride buffer. Finally, 2M sodium chloride (25 ml) was passed through the column. Fractions (5.5 ml) were assayed for protein and uronic acid. The complex was also assayed and found to contain 11% (w/w) of protein before and after separation from fractions eluted at salt concentrations between 0.3M and 0.4M.

(*c*) A solution of hyaluronate–protein complex (25 ml, *ca.* 0.150 mg/ml) was dialysed against phosphate buffer (pH 7.4, $I = 0.1$)[9]. The solution was centrifuged at 34000 × g for 10 min at 4°, divided into portions (see below), and fractionated on a column (36 × 2 cm) of 2% agarose (100/200 mesh, equilibrated with the 0.05M sodium chloride buffer described above).

A sample (10 ml) was treated, with stirring, for 30 min at room temperature with succinic anhydride (1 mg). During the reaction, the pH fell from 7.4 to 7.3. A portion (9.5 ml) was then separated on agarose. A further sample (5 ml) was added to a solution of perfluoro-octanoic acid (0.05 g in 4 ml of 0.05M sodium chloride buffer adjusted to pH 7.0). The mixture was allowed to stand for 24 h at 4° before being centrifuged and passed through the agarose column. A final sample (8 ml) was run on agarose without any treatment. Fractions (4 ml) were assayed for uronic acid and protein content, and for u.v. absorbance at 280 mμ.

RESULTS AND DISCUSSION

The proteins of synovial fluid are derived from serum[13]. Using 5% agar gel for fractionation (chosen after examination of Andrews' data[8]), it was found that hyaluronic acid emerged before serum proteins (Fig. 1) and hence would be expected to emerge before the synovial fluid proteins. However, it was not clear to what extent this might be due to a repulsion of the hyaluronate anion by the sulphate groups of the agaropectin component of agar[14], or to true molecular sieving. Agarose, the sulphate-free, neutral component of agar, became commercially available at this time, and studies were begun to find a suitable concentration of gel by elution of viruses of known molecular size (Fig. 2). Hjertén[15] has published some calibration data.

The first gels were prepared by the method used[8] for agar gel, that is, by disintegration of a gel block. These gels were peculiar in that their sieving properties seemed not to vary with the concentration of the gel (Fig. 3), perhaps because of changes in concentration (through condensation or "bumping" of the solutions) while the materials were being autoclaved. It was decided from the results with the viruses that the required concentration was 3%, or less.

Publication of a method for preparing agarose gels in bead form[11] suggested that this would allow better control of concentration and give gels of higher flow rates, without loss of resolution. Consequently, 1% and 2% gels were prepared according to this method[11], using Emulphor El as the emulsifying agent. These concentrations were chosen because the agarose could then be dissolved by refluxing, whereas higher concentrations required repeated autoclaving. Further, the more-open gels would, it was hoped, separate the hyaluronic acid, not only from protein, but also into fractions having various molecular sizes.

Samples of synovial fluid were always diluted before gel filtration. With columns of 50 × 2 cm, samples (1.5 ml) of synovial fluid, diluted (to 6 ml) with the appropriate buffer, could be clearly separated into hyaluronic acid and protein components. The flow rate of the column falls as the hyaluronic acid is eluted and then rises to the initial value. Agarose columns, unlike dextran and polyacrylamide molecular sieves, contract slightly (2–4%) when elution is begun and, therefore, samples were applied to columns that had been eluted for at least one hour and had settled to a steady length. Column measurements reported herein are for flowing columns. Samples are best applied by layering them above the gel (no added solute being required since the diluted fluid is more dense than the buffer). With larger columns (4 × 50 cm), up to 5 ml of synovial fluid could be used, but the flow rate must then be restricted.

Fig. 5 shows results (using 1% and 2% gels) for 1.5-ml samples of a synovial fluid from a case of rheumatoid arthritis. This synovial fluid was unique, amongst those studied, in having two polysaccharide peaks in its elution pattern, both of high molecular weight. This feature permitted an investigation of the intrinsic viscosity of fractions in the centre of the gels' sieving range (other fluids gave insufficient hyaluronic acid at this position). Elution of Blue Dextran 2000 (Pharmacia Ltd.) from 2% agarose gave a distribution rising to a peak before the peak for normal protein (Fig. 4), and the second polysaccharide peak was eluted before that of the Blue Dextran. The increased pore size of the 1% gel leads to a spread of the hyaluronic acid over 80 ml of eluate, compared to 40 ml for the 2% gel. It also is completely permeable to serum proteins, whilst the 2% gel is slightly less permeable.

Measurements of intrinsic viscosity were made on fractions from each column, and are shown in Fig. 6. The results fit a logarithmic relationship better than a linear relationship for intrinsic viscosity and elution volume. Fractions having intrinsic viscosities up to 34 dl/g have been obtained with other synovial fluids. The elution position is therefore related to intrinsic viscosity and hence to molecular size. Granath[16] has built up molecular weight distributions from the fractionation of dextran on dextran gels, but, for a comparison of materials, the elution profile is sufficient.

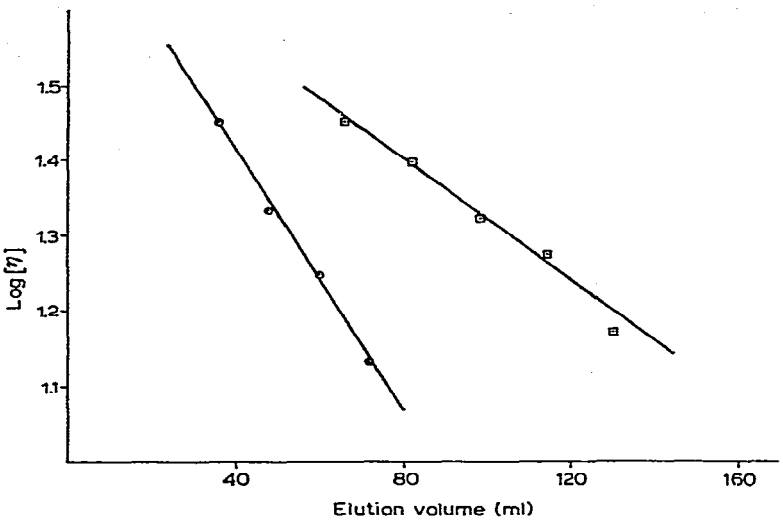


Fig. 6. Intrinsic viscosity as a function of elution volume □, 1% gel; o, 2% gel.

However, since a solution of hyaluronic acid itself acts as a molecular sieve[17], the elution profile will be influenced by the concentration and size distribution of the hyaluronic acid (a molecule would tend to be eluted more slowly in the presence of hyaluronic acid). If possible, comparative experiments should, therefore, be performed at the same concentration of hyaluronic acid (0.75 mg is a suitable quantity).

From the point of view of preparing the protein–polysaccharide complex, the 2% gel gives the better separation of hyaluronic acid from protein, whilst the 1% gel shows better resolution of the hyaluronic acid into molecular sizes. However, since the second peak is unusual, the 1% gel will normally be suitable for both purposes. Oberg *et al.*[18] have shown the 1% gel to be superior to 2% gel for separating deoxyribo- from ribo-nucleic acid. When another rheumatoid fluid was separated, the protein peak was examined for hyaluronic acid of low molecular weight. The protein peak was separated on DEAE Sephadex as described by Barker *et al.*[19]. This showed that hyaluronic acid of low molecular weight accounted for not more than 5% of the total hyaluronic acid.

There is, under both the 1% and 2% hyaluronic acid peaks, a small peak of u.v.-absorbing material. This is lower and more diffuse in the 1% elution pattern, indicating that it may be associated with the hyaluronic acid. U.v. spectra showed that this absorbance arose from protein rather than u.v. scatter from the polysaccharide of high molecular weight. Protein has been found in hyaluronic acid prepared by other means[2,20], but it is not yet certain whether this is a covalent or ionic association. When it was found that re-passage of the hyaluronic acid through 2% agarose did not produce any new u.v. peak near the protein region, the hyaluronic acid was treated under various conditions which would have led to the dissociation of an ionic complex, or to precipitation of an impurity having a high molecular

weight (insufficient material was available for ultracentrifugal and electrophoretic examination). The first method was to elute the agarose column with a buffer of greater dissociating power[21], 3M guanidine hydrochloride. This failed to produce a new u.v. peak when hyaluronic acid was re-passed through the agarose column. Next, DEAE Sephadex, which has been shown to break otherwise inseparable complexes[20,22] was used. The uronic acid coincided again with the protein, the amount (11%) of which was unchanged.

Another possible approach with ionic complexes is to alter the charges on the basic amino acids by treatment with succinic anhydride[23]. When hyaluronic acid, which had been treated in this way, was compared with a blank, the protein percentage was only slightly changed (10.4 to 9.4%). A similar result was obtained when the hyaluronic acid was treated with perfluoro-octanoic acid, which has been found to precipitate protein from nucleic acid complexes[24]. The change is probably not significant at the very low levels of protein being assayed here.

A complex containing *ca.* 10% of protein, from pathological fluid, has been reported by Sandson and Hamerman[2] They also reported that normal synovial fluid contained a complex having a lower content of protein. We have not yet examined normal synovial fluid, but, in preliminary amino-acid analyses of the protein in synovial hyaluronic acid isolated from rheumatoid fluids, the most abundant amino acid was found to be serine, which has been shown to form, with D-xylose, the protein to polysaccharide linkage in other mucopolysaccharides[25,26].

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SUMMARY

A method is described for preparing an undegraded, hyaluronic acid–protein complex from synovial fluid, and which gives, at the same time, a picture of molecular weight heterogeneity in the hyaluronic acid. The protein is shown to be firmly bound to the polysaccharide.

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SUBSTITUTED CEREBROSIDES

PART II. SYNTHETIC DIHYDROSULFATIDES

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INTRODUCTION

Sulfated glycolipids (sulfatides), which were first obtained from nervous tissue[1,2], were characterized as cerebroside sulfate esters more than thirty years ago[3]. Since then, they have been isolated from many animal tissues[4]. In metachromatic leucodystrophy (a sphingolipidosis), there is an accumulation of sulfatides in various organs, and the biochemistry of the disorder is under active investigation at present[5,6].

For about a decade, the accepted structure for these compounds was galactosylceramide 6-sulfate. The point of attachment of the sulfate ester to the sugar moiety was established on the basis of the lack of reactivity of the molecule with chlorotriphenylmethane[7] and the isolation of 2,3,4-tri-*O*-methyl-D-galactopyranose from the hydrolyzed permethylated material[8]. However, it was shown that in cerebrosides (with an unsubstituted C-6 hydroxyl group) reaction with chlorotriphenylmethane does not take place, even under forcing conditions[8]. Furthermore, the identification of the methylated galactose was performed by comparison of its rate of migration on paper chromatograms with that of an authentic specimen. Unfortunately, this method was not conclusive since the R_F values of 2,3,4-tri-*O*-methyl-D-galactopyranose and of its 2,4,6-tri-*O*-methyl isomer are almost identical. More careful work involving the separation of the methyl ethers by gas–liquid chromatography, and also the preparation of crystalline derivatives[9–11], showed that the earlier conclusions were invalid. The specimens of sulfatides investigated were shown to be galactose 3-sulfate esters. The possibility of the occurrence of a 3,6-cyclic sulfate[12,13], however, still exists.

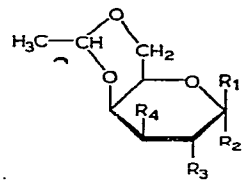
Chemical sulfation of natural cerebrosides has been achieved [14,15], and a product was isolated which was shown to be different from the sulfatide[15]. It was concluded that a derivative of galactose 6-sulfate had been obtained.

Apart from the possibility of different sites of linkage of the sulfate group, the sulfatides are, in fact, isolated as mixtures of the general structure (galactosyl sulfate)-*N*-acylsphingosine, where the amino group of the sphingosine moiety is acylated by substituents derived from a variety of straight-chain fatty acids. The latter may be divided into two main groups, namely non-hydroxylated and hydroxylated straight-chain fatty acids. The naturally occurring compounds thus correspond to the sulfate esters of the cerebrosides cerasine (non-hydroxylated fatty acids) and

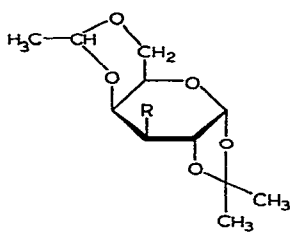
phrenosine (hydroxylated fatty acids). On t.l.c. they migrate as two spots, which have been assigned to the two different types of sulfatides present [16,17].

It was considered of value to synthesize these substances unequivocally for comparison with the natural products. The synthesis would make available pure sulfatides bearing only one defined fatty acid moiety which could be varied at will. The method might also give a possibility of preparing ^{35}S-labelled compounds whose metabolism could be followed.

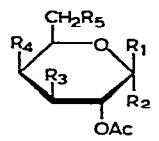
The simplest type of compound in this series is the one derived from *N*-acyl-DL-dihydrosphingosine*, where the acyl group is non-hydroxylated. For an unambiguous synthesis, suitably substituted cerebrosides are required. Protecting groups must be such that their removal does not lead to undesirable side-reactions at other



	R_1	R_2	R_3	R_4
1	H	OH	OH	OH
8	H	Br	OAc	OTs
9	OMe	H	OAc	OTs



2 R = OTs
3 R = $OCH_2C_6H_5$



	R_1	R_2	R_3	R_4	R_5
5	H	Br	OH	OAc	OAc
7	OMe	H	OTs	OAc	OAc
10	OMe	H	OTs	OH	OH

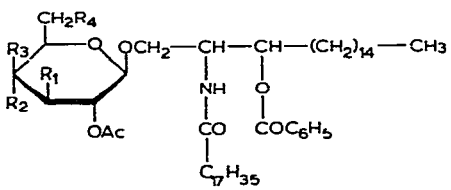
parts of the molecule. Dihydrocerebroside derivatives of the required structure for the preparation of 6-sulfate esters have recently been described[18], but the corresponding starting-material for the 3-sulfate is not yet available.

Discussion

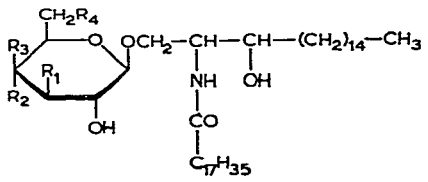
4,6-*O*-Ethylidene-1,2-*O*-isopropylidene-α-D-galactose[19] (a sirup) was converted into the crystalline benzyl ether (**3**). Mild acid hydrolysis removed the acetal groups. Acetylation of the product with acetic anhydride in pyridine solution gave 1,2,4,6-tetra-*O*-acetyl-3-*O*-benzyl-D-galactopyranose which was purified by chromatography on silica gel, but did not crystallize. Hydrogenolysis of the anomeric mixture removed the benzyl ether, as shown by i.r. and n.m.r. spectroscopy, and gave 1,2,4,6-tetra-*O*-acetyl-D-galactopyranose (**4**). This was purified by chromatography on silica gel, and a colorless sirup was isolated which was converted into the unstable 2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) by boiling the solution in chloroform with titanium bromide. Reaction of compound (**5**) with *N*-octadecanoyl-3-*O*-benzoyl-DL-dihydrosphingosine gave 1-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine (**6**).

* In this paper, the name "DL-dihydrosphingosine" indicates DL-*erythro*-2-amino-1,3-octadecanediol.

The structure of (**5**) was confirmed by conversion, in high yield, into methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**7**). The pure, crystalline, compound was identical with an authentic specimen, prepared by the following method: The known 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranose (**2**)[19] was treated with a solution of hydrogen bromide in acetic acid with the expectation of removing the acetal groups and producing, directly, 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranosyl bromide. However, to facilitate solution of compound (**2**) in the acid mixture, dilution with anhydrous chloroform was necessary. After treatment under these mild conditions, a crystalline bromide was isolated, the elementary analysis of which indicated the structure 2-*O*-acetyl-4,6-*O*-ethylidene-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranosyl bromide (**8**). Treatment of this bromide with methanol in the presence of mercuric cyanide gave methyl 2-*O*-acetyl-4,6-*O*-ethylidene-3-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**9**) which was converted into methyl 2-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**10**) and methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**7**). This series of reactions was followed by n.m.r. spectroscopy, the presence of the ethylidene group in compound (**9**) being clearly shown by a doublet at 8.65 τ which was absent in the spectra of the products (**10**) and (**7**). The number of acetyl groups (n.m.r.) in compounds **7**, **9**, and **10** also agreed with the proposed structures, as did their elementary analyses. Compound (**7**) is different from the derivative described by Wolfrom *et al.*[20] as a methyl tri-*O*-acetyl-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside with the tolylsulfonyl group being located at C-2,3, or 4. The present work thus eliminates one of these three possibilities.



	R_1	R_2	R_3	R_4
6	OH	H	OAc	OAc
11	OAc	H	OAc	OH
12	OAc	OAc	H	OH
13	OSO_3H	H	OAc	OAc
14	OAc	H	OAc	OSO_3H
15	OAc	OAc	H	OSO_3H



	R_1	R_2	R_3	R_4
16	OSO_3H	H	OH	OH
17	OH	H	OH	OSO_3H
18	OH	OH	H	OSO_3H

The dihydrocerebroside derivatives (**6**, **11**, and **12**) reacted with pyridine–sulfur trioxide[21] in pyridine to give the sulfated esters (**13**, **14**, and **15**) in good yield. In two cases (**13** and **14**), crystallization from methanol sufficed to render the products analytically pure.

Catalytic transesterification gave the dihydrosulfatides (**16**, **17**, and **18**) in

reasonable yield. After treatment of a solution in chloroform–methanol (1:1) with dilute acid to remove inorganic salts, compounds **16** and **17** were purified by crystallization from chloroform–methanol (2:1). A more impure specimen (**18**) required prior chromatography on silicic acid. The phenomenon, previously described[18], of preliminary sintering with formation of a translucent glass which cleared sharply at the temperature indicated as the m.p., was also encountered with the dihydrosulfatides. The galactose 3- and 6-sulfates (**16** and **17**) were distinguishable on t.l.c., in which the 6-sufate migrated significantly faster. Their i.r. spectra were similar, showing strong absorptions at 820 and 1250 cm^{-1} due to the equatorial sulfate group, and were almost identical with that of a specimen of natural sulfatide[22].

EXPERIMENTAL

Melting points were taken between glass slides on a Fisher–Johns apparatus and are corrected. Rotations were determined in semimicro tubes using a Perkin–Elmer No. 141 polarimeter. N.m.r. spectra were recorded with a Varian A-60 n.m.r. spectrometer using tetramethylsilane as internal standard and deuteriochloroform as solvent. "Silica gel" refers to silica gel Davison, grade 950, 60–200 mesh, used without pretreatment; the flowing method was used and elution was stepwise, in order of increasing polarity of the eluants. The proportion of weight of substance added to the column to weight of adsorbent was 1 to 50–100. The fractions eluted were 2 ml/g. of the column. "Silicic acid" refers to Bio-Rad silicic acid, used after a preliminary heating for 60 min. at 120°, and the chromatographic columns were prepared using chloroform–methanol (19:1). Thin layer chromatograms were run on Kieselgel G. (E. Merck, Darmstadt). Analyses were carried out in the Institute's Microanalytical Laboratory under the direction of Mr. R. Heller.

4,6-O-Ethylidene-1,2-O-isopropylidene-3-O-p-tolylsulfonyl-α-D-galactopyranose (**3**)

The method of preparation described in the literature[19] was modified slightly[23]. A mixture of dry D-galactose (60 g), freshly distilled paraldehyde (220 ml), and sulfuric acid (1.8 ml) was shaken vigorously for 24 h. Removal of paraldehyde by decantation left a sticky solid which dissolved almost completely in hot, sodium-dried dioxane. The insoluble material was removed by filtration, and the filtrate was cooled to room temperature and brought to pH 6 by the addition of sodium hydrogen carbonate (20 g) while stirring. After removal of the inorganic salts by filtration, the clear solution was concentrated *in vacuo* to a sirup. Dioxane (50 ml) was added and the solution seeded. A crystalline solid (20 g, m.p. 182–184°) precipitated slowly. Recrystallization from alcohol raised the m.p. to 183–184°.

The thoroughly dried product (4,6-*O*-ethylidene-α-D-galactopyranose, **1** was converted into compound **2** as described[19].

3-O-Benzyl-4,6-O-ethylidene-1,2-O-isopropylidene-α-D-galactopyranose (**3**)

Treatment of the pure *p*-toluenesulfonate (**2**) with lithium aluminum hydride

gave sirupy 4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactose[19]. A mixture of the sirup (1.2 g), toluene (1.5 ml), benzyl chloride (1.5 ml), and powdered potassium hydroxide (1.7 g) was stirred for 5 h at 100–105°. The reaction mixture was cooled, and extracted with toluene (20 ml). The toluene solution was washed with water until the washings were free of alkali, dried, and concentrated *in vacuo* to an oil which was purified by chromatography on silica gel. Benzene–ether (9:1) eluted fractions which crystallized on evaporation of the solvent. Recrystallization from ether–pentane gave prisms (0.5 g, 31%, m.p. 115–117°). An analytical sample, obtained by a second crystallization from ether–pentane, had m.p. 116–118°, $[\alpha]_D^{25}$ +19.0° (*c* 1.54, chloroform).

Anal. Calc. for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.58; H, 7.01.

In later preparations, compound (**3**) was obtained directly from the unpurified oil resulting from the reaction of (**1**) with acetone and zinc chloride. In a typical example, 10 g of compound **1** gave 6 g of the crude oil, from which was produced 2.4 g (15% yield) of the pure benzyl ether (**3**), m.p. 116–118°.

1,2,4,6-Tetra-O-*acetyl*-D-*galactopyranose* (**4**)

A solution of the benzyl ether (**3**, 1.98 g) in 60% acetic acid (15 ml) was kept for 3 h at 100°. The sirup obtained on concentration *in vacuo* showed no n.m.r. peaks at 8.5–8.65 τ, indicating complete removal of the acetal groups. Acetylation, in the usual fashion, with acetic anhydride in pyridine gave a sirup (2.4 g), $[\alpha]_D^{20}$ +63.9° (*c* 1.00, chloroform). N.m.r. aromatic (**1**), acetate at 7.88–8.0 τ (**4**), no peak at 8.5–8.65 τ.

Anal. Calc. for $C_{21}H_{26}O_{10}$: C, 57.53; H, 5.98. Found: C, 57.70; H, 5.57.

A solution in distilled alcohol (100 ml) was hydrogenolyzed at 50 p.s.i., at room temperature, for 2 days, in presence of 10% palladium on charcoal as catalyst. After removal of the catalyst and the solvent, a sirup was obtained which was purified by chromatography on silica gel. Benzene–ether (3:1) eluted nonhydrogenolyzed material (190 mg), and benzene–ether (1:1) eluted fractions (1.60 g, 78% from **3**) from which the benzyl ether had been removed, as shown by the i.r. and n.m.r. spectra, and which consisted principally of the desired tetraacetate (**4**).

2,4,6-Tri-O-*acetyl*-α-D-*galactopyranosyl bromide* (**5**)

A solution of **4** (1.50 g) in alcohol-free chloroform (15 ml), containing titanium tetrabromide (3.8 g), was boiled under reflux, with careful exclusion of moisture, for 30 min. The solution was washed with a cold, saturated solution of sodium hydrogen carbonate and with water, dried, and concentrated *in vacuo* to a pale-yellow sirup (700 mg, 39%), $[\alpha]_D^{20}$ +146° (*c* 1.60, chloroform). The product was unstable and darkened after a few days, even when preserved in a desiccator over sodium hydroxide.

1-O-(*2,4,6-Tri*-O-*acetyl*-β-D-*galactopyranosyl*)-*3*-O-*benzoyl*-N-*octadecanoyl*-DL-*dihydrosphingosine* (**6**)

A solution of **5** (650 mg) in anhydrous benzene (5 ml) was added dropwise

over a period of 60 min. to a stirred mixture of *N*-octadecanoyl-3-*O*-benzoyl-DL-dihydrosphingosine (1.1 g) and mercuric cyanide (400 mg), in nitromethane (30 ml) and benzene (10 ml), at 70°. The reaction was allowed to continue overnight at 70°. Purification by chromatography[18], followed by crystallization from methanol, gave a product which was not completely pure (600 mg, 38%, m.p. 42–44°), $[\alpha]_D^{25}$ −0.3° (*c* 1.10, chloroform). T.l.c. in benzene–ether (2:1) revealed a spot with R_F 0.2 contaminated with traces of slower-moving material (presumably oligosaccharide derivatives).

Anal. Calc. for $C_{55}H_{93}NO_{12}$: C, 68.79; H, 9.76. Found: C, 69.68; H, 9.88.

1-O-(*β*-D-*Galactopyranosyl 3-sulfate*)-N-*octadecanoyl*-DL-*dihydrosphingosine* (**16**)

Pyridine–sulfur trioxide[21] (45 mg) was added to a solution of **6** (250 mg) in pyridine (1 ml). The mixture was stirred for 2 days at room temperature with exclusion of moisture. On addition of cold water, a gel-like precipitate was obtained. This was separated by centrifugation at low speed and washed thoroughly with water. Crystallization from cold methanol gave a product (**13**) weighing 200 mg (71% yield), m.p. 52–54°, $[\alpha]_D^{25}$ +2.3° (*c* 0.20, chloroform). T.l.c. in benzene–ether (2:1), R_F 0.0, and in benzene–methanol (9:1), R_F 0.10.

Anal. Calc. for $C_{55}H_{93}NO_{15}S$: C, 63.49; H, 9.01; S, 3.07. Found: C, 63.65; H, 8.89; S, 2.85.

A portion of this product (150 mg) was dissolved in anhydrous methanol containing a catalytic amount of sodium methoxide, and the solution was kept at room temperature overnight. The addition of a little ice precipitated an amorphous solid which was separated by filtration, and dissolved in chloroform–methanol (1:1). The solution was washed, first with cold dilute hydrochloric acid and then with water, dried, and concentrated *in vacuo*. The residue (80 mg, 70%) crystallized from chloroform–methanol (2:1); m.p. 183–185°, $[\alpha]_D^{25}$ +2.6 (*c* 0.83, pyridine).

Anal. Calc. for $C_{42}H_{83}NO_{10}S \cdot H_2O$: C, 62.11; H, 10.55; S, 3.95. Found: C, 62.17; H, 10.26; S, 3.78.

1-O-(*β*-D-*Galactopyranosyl 6-sulfate*)-N-*octadecanoyl*-DL-*dihydrosphingosine* (**17**)

Sulfation of 1-*O*-(2,3,4-tri-*O*-acetyl-*β*-D-galactopyranosyl)-*N*-octadecanoyl-3-*O*-benzoyl-DL-dihydrosphingosine[18] (**11**, 360 mg) in pyridine with pyridine–sulfur trioxide (65 mg), and crystallization of the product (**14**) from methanol gave 200 mg (50%) of m.p. 60–62°, $[\alpha]_D^{27}$ −1.3° (*c* 0.82, chloroform). T.l.c.: R_F 0.05 in benzene–methanol (9:1).

Anal. Calc. for $C_{55}H_{93}NO_{15}S$: C, 63.49; H, 9.01; S, 3.07. Found: C, 63.25; H, 8.79; S, 2.88.

Catalytic transesterification, followed by acidification of a solution in chloroform–methanol as described for **16**, and evaporation of the solvent, left a residue weighing 90 mg (80%); crystallization from methanol gave a product melting at 183–185° (63 mg), $[\alpha]_D^{25}$ +1.0° (*c* 0.76, pyridine).

Anal. Calc. for $C_{42}H_{83}NO_{10}S \cdot H_2O$: C, 62.11; H, 10.55; S, 3.95. Found: C, 62.17; H, 10.42; S, 3.89.

1-O-(*β*-D-*Glucopyranosyl 6-sulfate*)-N-*octadecanoyl*-DL-*dihydrosphingosine* (**18**)

Sulfation of 1-*O*-(2,3,4-tri-*O*-acetyl-*β*-D-glucopyranosyl)-*N*-octadecanoyl-3-*O*-benzoyl-DL-dihydrosphingosine[18] (**12**, 280 mg) and isolation as described for **13** gave a product (**15**) having m.p. 58–60° (200 mg, 63%), $[\alpha]_D^{27}$ −9.4° (*c* 0.85, chloroform) which was not completely free of unsulfated material, as shown by t.l.c.

Anal. Calc. for $C_{55}H_{93}NO_{15}S$: C, 63.49; H, 9.01; S, 3.07. Found: C, 64.24; H, 9.61; S, 3.27.

A portion (190 mg) was transesterified catalytically, and the crude product was purified by chromatography on silicic acid. Chloroform–methanol (19:1) eluted fractions which weighed 30 mg and crystallized from methanol, which gave a product m.p. 181–182°, $[\alpha]_D^{25}$ −2.0° (*c* 1.00, pyridine), identical with 1-*O*-*β*-D-glucopyranosyl-*N*-octadecanoyl-DL-dihydrosphingosine[18] (mixed m.p. and t.l.c.). Chloroform–methanol (9:1) eluted fractions weighing 120 mg (80%). This material was dissolved in chloroform–methanol (1:1) and washed succesively with hydrochloric acid and with water, as described for **16**. The solvent was removed by evaporation *in vacuo* and the residue crystallized from methanol; m.p. 185–187°, $[\alpha]_D^{21}$ −7.4° (*c* 0.68, pyridine).

Anal. Calc. for $C_{42}H_{83}NO_{10}S \cdot H_2O$: C, 62.11; H, 10.55; S, 3.95. Found: C, 62.21; H, 10.21; S, 3.98.

2-O-*Acetyl-4,6*-O-*ethylidene-3*-O-p-*tolylsulfonyl*-*α*-D-*galactopyranosyl bromide* (**8**)

To a solution of **2** (1.0 g) in dry chloroform (5 ml) was added a solution of hydrogen bromide in glacial acetic acid (45%, w/v, 3 ml), and the clear solution was kept for 3 h at room temperature. Additional chloroform and ice were added, and the organic extract was washed with a saturated solution of sodium hydrogen carbonate until the washings were no longer acid, and then with water, and dried with calcium chloride. Concentration *in vacuo* left an oily residue which crystallized on addition of dry ether. The product (0.7 g, 64%, m.p. 165° dec.) was recrystallized from chloroform–ether to give needles having m.p. 167° dec., $[\alpha]_D^{25}$ +240° (*c* 0.80, chloroform).

Anal. Calc. for $C_{17}H_{21}O_8SBr$: C, 43.88; H, 4.55; S, 6.89; Br, 17.17. Found: C, 43.80; H, 4.37; S, 6.69; Br, 17.14.

Methyl 2-O-*acetyl-4,6*-O-*ethylidene-3*-O-p-*tolylsulfonyl*-*β*-D-*galactopyranoside* (**9**)

Treatment of **8** (200 mg) with dry methanol (0.02 ml), and mercuric cyanide (150 mg) in nitromethane–benzene (2:1) (20 ml) for 18 h at 50–60°, and concentration of the solution *in vacuo*, left a solid residue which was crystallized from methanol. The product (100 mg, 56%, m.p. 155–157°) was recrystallized from methanol; m.p. 157–158°, $[\alpha]_D^{25}$ +39.9° *c* 1.00, chloroform). T.l.c.: in benzene-methanol (9:1), R_F 0.60.

N.m.r.: A doublet at 8.65 *τ*, *J* 5 c.p.s., was attributed to CH_3 of the ethylidene group, split by H; peaks at 8.12 *τ* (OAc), 7.55 *τ* (CH_3 attached to an aromatic ring), 6.50 *τ* (OCH_3), 2.7 *τ* and 2.2 *τ* (doublets, aromatic sulfonate).

Anal. Calc. for $C_{18}H_{24}O_9S$: C, 51.92; H, 5.81; S, 7.69. Found: C, 52.01; H, 5.58; S, 7.40.

Methyl 2-O-acetyl-3-O-p-tolylsulfonyl-β-D-galactopyranoside (**10**)

A solution of **9** (120 mg) in 60% acetic acid (1 ml) was kept at 100° for 2 h. The solvent was removed by evaporation, and the residue (m.p. 157–159°) was crystallized from acetone–ether: yield 75 mg (66%) of rosettes, m.p. 160–162°, R_F 0.20 in benzene–methanol (9:1) (t.l.c.), $[\alpha]_D^{25}$ +20.0° (*c* 1.03, acetone).

Anal. Calc. for $C_{16}H_{22}O_9S$: C, 49.22; H, 5.68; S, 8.21. Found: C, 49.25; H, 5.65; S, 7.98.

Methyl 2,4,6-tri-O-acetyl-3-O-p-tolylsulfonyl-β-D-galactopyranoside (**7**)

(*i*) Acetylation of compound **10** (60 mg) with acetic anhydride in pyridine, in the usual fashion, and removal of the solvents by evaporation, gave 65 mg (89%) of rhombic prisms, m.p. 176–177°. Recrystallization from acetone–ether did not affect the m.p., $[\alpha]_D^{23}$ +14.4° (*c* 1.00, chloroform).

N.m.r.: The doublet at 8.65 τ had disappeared; peaks at 8.00 τ and 7.92 τ (OAc, 3) 7.55 τ (CH_3 attached to an aromatic ring), 6.50 τ (OCH_3), 2.7 τ, and 2.2 τ.

Anal. Calc. for $C_{20}H_{26}O_{11}S$: C, 50.62; H, 5.52; S, 6.76. Found: C, 50.76; H, 5.36; S, 6.70.

(*ii*) Compound **5** (150 mg) was added to a stirred solution of mercuric cyanide (150 mg) in nitromethane–benzene (2:1) (20 ml) containing dry methanol (0.03 ml), and the mixture was kept at 50–60° for 18 h. More benzene was added, and the solution was washed with a saturated solution of sodium hydrogen carbonate and with water, dried, and concentrated *in vacuo*. The residue (130 mg) was dried and dissolved in pyridine (1 ml). *p*-Toluenesulfonyl chloride (65 mg) was added, and the solution was kept overnight at room temperature and then for 20 min at 60°. The sirup remaining after concentration *in vacuo* crystallized, on addition of ether, in prisms weighing 90 mg (47% from **5**), m.p. 175–177°. Recrystallization from acetone–ether gave a product of m.p. 176–177°, $[\alpha]_D^{23}$ +14.8° (*c* 1.20, chloroform). T.l.c.: R_F 0.80 in benzene–methanol (9:1), indistinguishable from the R_F of an authentic specimen (**7**).

ACKNOWLEDGMENT

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SUMMARY

The preparation of the unstable, sirupy 2,4,6-tri-*O*-acetyl-α-D-galactopyranosyl bromide (**5**) from crystalline 3-*O*-benzyl-4,6-*O*-ethylidene-1,2-*O*-isopropylidene-α-D-galactopyranose (**3**) is described. Condensation of the bromide (**5**) with 3-*O*-benzoyl-*N*-octadecanoyl-DL-dihydrosphingosine afforded the dihydrocerebroside derivative 1-*O*-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-benzoyl-*N*-octadecanoyl-DL-di-

hydrosphingosine (**6**). Treatment of the bromide (**5**) with methanol, followed by *p*-toluenesulfonation, gave a good yield of methyl 2,4,6-tri-*O*-acetyl-3-*O*-*p*-tolylsulfonyl-β-D-galactopyranoside (**7**).

Sulfation of the dihydrocerebroside esters (**6**, **11**, and **12**) with pyridine–sulfur trioxide, followed by catalytic transesterification, gave sulfated products from which the dihydrosulfatides **16**, **17**, and **18** could be isolated.

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OPTICAL ROTATORY DISPERSION OF SUGARS*

III. RELATIONSHIP TO STRUCTURES OF OLIGOSACCHARIDES

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INTRODUCTION

Optical rotations at the sodium D-line have been reported for numerous oligosaccharides[1,2], and Hudson extended his rules of isorotation derived for monosaccharides to several of these compound sugars[3–7]. By using the molar rotations of methyl glycosides, these rules have been modified to obtain a more accurate representation of the rotatory contribution of the glycosidic linkage. Although accurate quantitative agreement has not generally been obtained, there have been many useful applications of these treatments; in particular, deductions have been made concerning the configuration of the glycosidic linkage in some oligosaccharides[3–11].

Recently, optical rotatory dispersion measurements have been employed for studying simple monosaccharides[12,13]. General rules have been proposed relating the direction of rotation in the far-ultraviolet spectral region to the configuration and conformation of aldo- and keto-furanosides and -pyranosides[13,14]. In these cases, Cotton effects with expected first peaks at wavelengths below 185 mμ were predicted to have a considerable effect on the rotations in the far-ultraviolet region. Cotton effects at higher wavelengths in the ultraviolet region have been reported for certain sugars containing optically active chromophores[15–18].

Based on an examination of a select group of di-, tri-, and tetra-saccharides, correlations have been made between the optical rotatory dispersion properties and the structures of these oligosaccharides in solution. The data in the visible and near-ultraviolet spectral regions are in agreement with the previously presented interpretations[3–7,19] of the rotations at 589 mμ. In the far-ultraviolet region, the rotatory dispersion curves exhibit characteristic features of shape, including changes in direction of rotation, which have been related to the curves obtained for the constituent monosaccharides[13,14].

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RESULTS AND DISCUSSION

Cyclic sugars generally exhibit plain optical rotatory dispersion curves in the visible and near-ultraviolet spectral regions[20,21]. In many instances, however, an inversion in the direction of rotation occurs[12–14] near 210 mμ. The optically active chromophore having the greatest influence on the rotations in the 200-mμ spectral region was predicted to be the ring oxygen atom, whose absorption band is[13] below 185 mμ. The configurations at the carbon atoms adjacent to the ring oxygen atom affect these rotations to a larger extent than do the configurations at carbon atoms farther removed from the ring oxygen atom. Therefore, information concerning the stereochemistry about the ring oxygen atom can be deduced from the direction of the rotatory dispersion curve, below 200 mμ, as the first peak (or trough) of an expected Cotton effect is approached[13,14].

The neighboring stereochemistry has been shown to have a pronounced influence on the rotatory contribution associated with a given position on the ring. Thus, the configuration at C-4 of an aldopyranoside affects the rotations in the far-ultraviolet region, mainly because of its influence on the configuration of the hydroxymethyl group[13] at C-5. It is, therefore, difficult to resolve the rotational contribution of any isolated configuration, because of the effect of its neighboring groups. For oligosaccharides, it is to be expected that interaction between monomeric residues is less than the interaction of neighboring positions on a single ring. Consequently, the treatment of the optical rotatory dispersion data proposed for monosaccharides should apply to oligosaccharides.

The oligosaccharides. Although slight conformational variations of the individual monomeric rings have been reported for some oligosaccharides[22–24], it will be assumed, in discussing the optical rotatory dispersion data, that the favored conformation predicted for a monosaccharide residue[25] is retained upon its incorporation into an oligosaccharide. In addition, the relative proportion of each anomeric form of the reducing sugar residue of the oligosaccharide is assumed to be nearly the same as that of the corresponding monosaccharide.

The optical rotatory dispersion curves for a select group of disaccharides and for some tri- and tetra-saccharides in the spectral region between 600 and 185 mμ are shown in Figs. 1–4. The distinct shape of each of these curves, especially in the far-ultraviolet region, may be closely approximated from the sum of the curves of the constituent monosaccharide residues. The dispersion curves for disaccharides composed of only D-glucopyranose residues are shown in Fig. 1. As was to be expected, sugars having α-D-glycosidic links have much greater positive rotations than have the β-D-glycosides. Small differences are observed between the (1→4)-linked sugars and the corresponding (1→6)-linked sugars, even though the constituent monosaccharide residues are the same. Evidently, the site of the glycosidic linkage on the reducing residue has some effect on the rotational magnitude. Rotatory dispersion curves of disaccharides containing D-glucose residues and D-fructose residues are shown in Fig. 2, and those containing D-galactose residues, in Figs. 3 and 4.

The shape and, in some cases, the approximate magnitude of the curves shown in Figs. 1 to 4 may be estimated from the sum of the rotations of the component monomeric residues. The rotatory contribution of the glycosyl moiety may be esti-

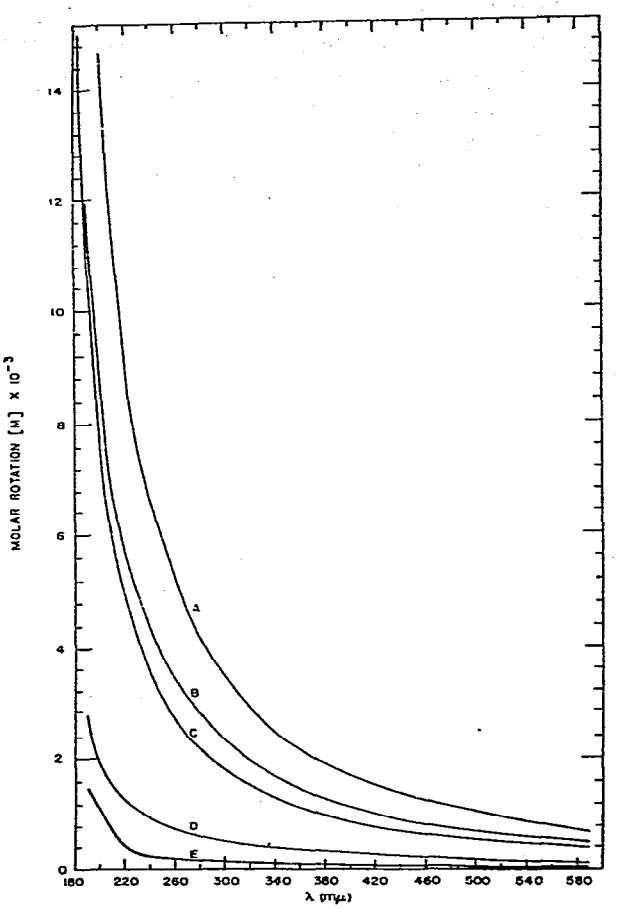

Fig. 1. Optical rotatory dispersion curves for disaccharides containing D-glucopyranose residues. Curve A, α-D-glucopyranosyl α-D-glucopyranoside (α,α-trehalose); Curve B, 4-*O*-α-D-glucopyranosyl-D-glucose (maltose); Curve C, 6-*O*-α-D-glucopyranosyl-D-glucose (isomaltose); Curve D, 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose); Curve E, 6-*O*-β-D-glucopyranosyl-D-glucose (gentiobiose)

mated from the rotation of the corresponding methyl glycoside[8]. Some sample calculations at several wavelengths are shown in Table I. Exact numerical agreement between the calculated and experimental rotational values for many of the oligosaccharides studied is not always achieved; this may be a result of the minor conformational differences in the monomeric residues of particular oligosaccharides or the changes in the environment of the asymmetric chromophore at the glycosidic linkage, or both. However, reasonably close agreement is achieved in most cases.

As previously shown, the direction of the far-ultraviolet rotatory contribution of the hydroxymethyl group at C-5 of an aldohexopyranoside is inverted by replace-

ment of an axial substituent with an equatorial substituent[13] at C-4. Thus, the rotatory contribution near 200 mμ of an equatorial C-5 hydroxymethyl group of a D-aldohexopyranoside in the *C1* conformation is in the positive direction if the C-4 hydroxyl

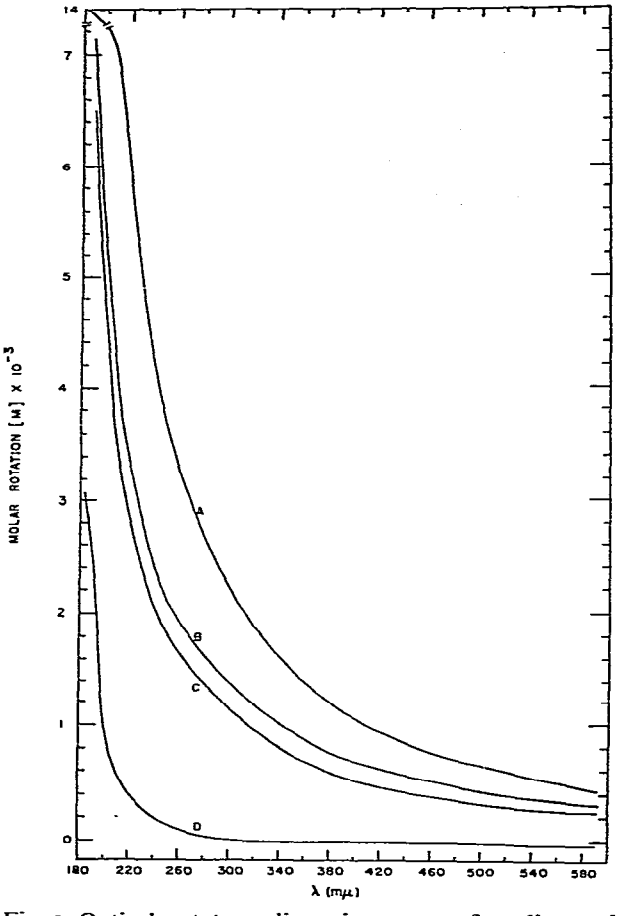

Fig. 2. Optical rotatory dispersion curves for oligosaccharides containing D-glucopyranosyl and D-fructosyl units. Curve A, *O*-α-D-glucopyranosyl-(1→3)-*O*-β-D-fructofuranosyl α-D-glucopyranoside (melezitose); Curve B, 3-*O*-α-D-glucopyranosyl-D-fructose (turanose); Curve C, β-D-fructofuranosyl α-D-glucopyranoside (sucrose); Curve D, 5-*O*-α-D-glucopyranosyl-D-fructose (leucrose).

group is equatorial, and in the negative direction if the hydroxyl group is axial. Because of this "C-4 effect", oligosaccharides containing D-galactose residues exhibit optical rotatory dispersion curves having slopes which are less positive in the far-ultraviolet region than those of the corresponding D-glucose-containing oligosaccharides. Thus, the observed (Fig. 1) and calculated (Table 1) rotations for 4-*O*-α-D-glucopyranosyl-D-glucose (maltose) and 4-*O*-β-D-glucopyranosyl-D-glucose (cellobiose) continue in the positive direction below 210 mμ. On the other hand, the curve for 4-*O*-β-D-galactopyranosyl-D-glucose (lactose) (see Fig. 3) is characterized by positive

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TABLE I

MOLAR ROTATIONS FOR SELECTED OLIGOSACCHARIDES, COMPARED TO THE SUM OF THE MOLAR ROTATIONS OF THE CONSTITUENT SUGARS

			Molar rotation, degrees × 10^{-2}				
		Wavelength mμ	*500*	*400*	*300*	*250*	*200*
1	Calculated for	Methyl α-D-glucopyranoside + methyl β-D-fructofuranoside	290	527	1053	1780	4100
	Observed for	β-D-Fructofuranosyl α-D-glucopyranoside (Sucrose)	328	547	1160	1850	4580
2	Calc.	Methyl α-D-galactopyranoside + methyl β-D-fructofuranoside	417	750	1480	2440	4950
	Obs.	β-D-Fructofuranosyl α-D-galactopyranoside ("Galsucrose")[a]	390	673	1330	2155	4510
3	Calc.	Methyl α-D-glucopyranoside + D-glucopyranose	597	967	1960	3310	7620
	Obs.	6-*O*-α-D-Glucopyranosyl-D-glucose (Isomaltose)	568	906	1810	3150	7700
	Obs.	4-*O*-α-D-Glucopyranosyl-D-glucose (Maltose)	677	1130	2380	3890	8640
4	Calc.	Methyl β-D-glucopyranoside + D-glucopyranose	66	86	186	333	1420
	Obs.	6-*O*-β-D-Glucopyranosyl-D-glucose (Gentibiose)	27	48	123	232	1190
	Obs.	3-*O*-β-D-Glucopyranosyl-D-glucose (Laminaribiose)[a]	96	130	305	472	923
5	Calc.	Methyl α-D-galactopyranoside + D-glucopyranose	720	1190	2390	3970	8490
	Obs.	6-*O*-α-D-Galactopyranosyl-D-glucose (Melibiose)	690	1210	2490	4080	9260
6	Calc.	Methyl β-D-galactopyranoside + D-glucopyranose	151	230	457	662	285
	Obs.	4-*O*-β-D-Galactopyranosyl-D-glucose (Lactose)	295	432	828	1220	936
7	Calc.	2 × Methyl α-D-glucopyranoside	892	1474	2940	4960	10,500
	Obs.	α-D-Glucopyranosyl α-D-glucopyranoside (α,α-Trehalose)	1040	1700	3550	6010	14,700
8	Calc.	Sucrose + methyl α-D-glucopyranoside	774	1280	2630	4330	9820
	Obs.	*O*-α-D-Glucopyranosyl-(1→3)-*O*-β-D-fructofuranosyl α-D-glucopyranoside (Melezitose)	656	1080	2260	3660	9040
9	Calc.	Sucrose + methyl α-D-galactopyranoside	900	1510	3060	4990	10,690
	Obs.	*O*-α-D-Galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside (Raffinose)	950	1530	3030	5050	10,200
	Obs.	*O*-α-D-Galactopyranosyl-(1→6)-*O*-β-D-fructofuranosyl α-D-glucopyranoside (Planteose)	1020	1660	3530	5640	12,300
10	Calc.	Sucrose + 2 × methyl α-D-galactopyranoside	1472	2470	4960	8030	16,800
	Obs.	*O*-α-D-Galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside (Stachyose)	1210	2100	4400	7200	18,000

[a] Data for these components are not shown in the Figures.

rotations above 210 mμ, followed by a sharp transition to the negative direction at lower wavelengths. The sum of the rotations of the two component monomeric residues of lactose (methyl β-D-galactopyranoside + D-glucopyranose) also exhibit

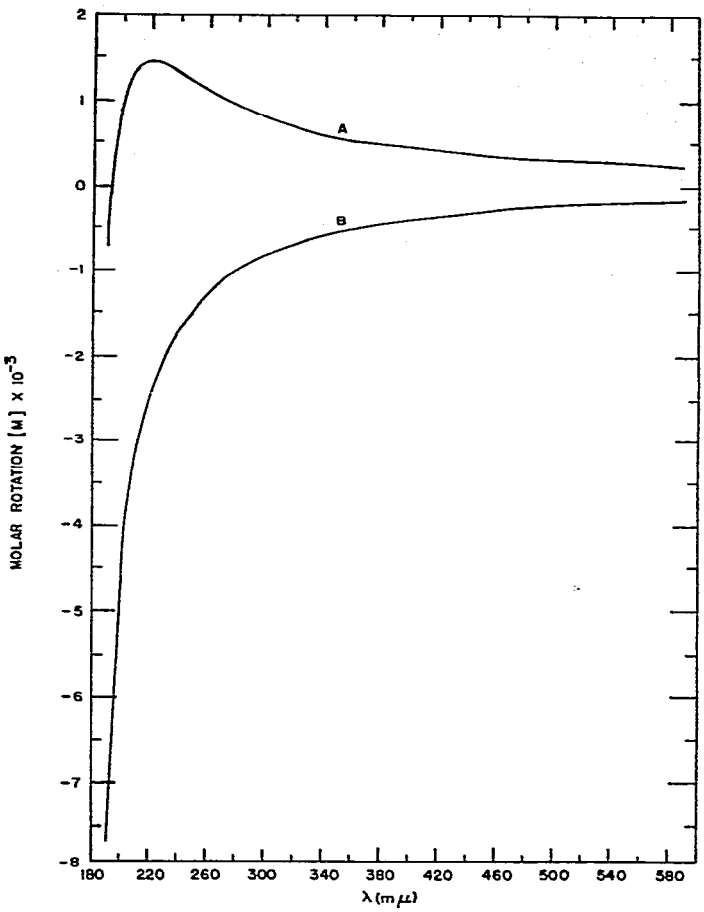

Fig. 3. Optical rotatory dispersion curves: Curve A, 4-*O*-β-D-galactopyranosyl-D-glucose (lactose); Curve B, 4-*O*-β-D-galactofuranosyl-D-fructose (lactulose).

this change in rotational direction in the far-ultraviolet region (see Table I). The curve for 2-*O*-β-D-xylopyranosyl-L-arabinose (not shown), as well as the calculated sum of its component monosaccharide rotations, also exhibited a transition from the positive to the negative direction near 210 mμ.

The effect of the configuration at C-4 may also be seen by examining the optical rotatory dispersion difference curves shown in Fig. 5. The rotations of disaccharides containing nonreducing D-glucopyranoside groups are subtracted from those containing nonreducing D-galactopyranoside residues. A sharp decline toward negative rotations near 210 mμ is evident in each curve. The curves for 6-*O*-α-D-galactopyranosyl-D-glucose (melibiose) minus 6-*O*-α-D-glucopyranosyl-D-glucose

(isomaltose) (see Fig. 5) and for α-D-galactopyranosyl β-D-fructofuranoside ("galsucrose") minus α-D-glucopyranosyl β-D-fructofuranoside (sucrose) (not shown in Fig. 5) are very similar to the curve previously obtained for methyl α-D-galacto-

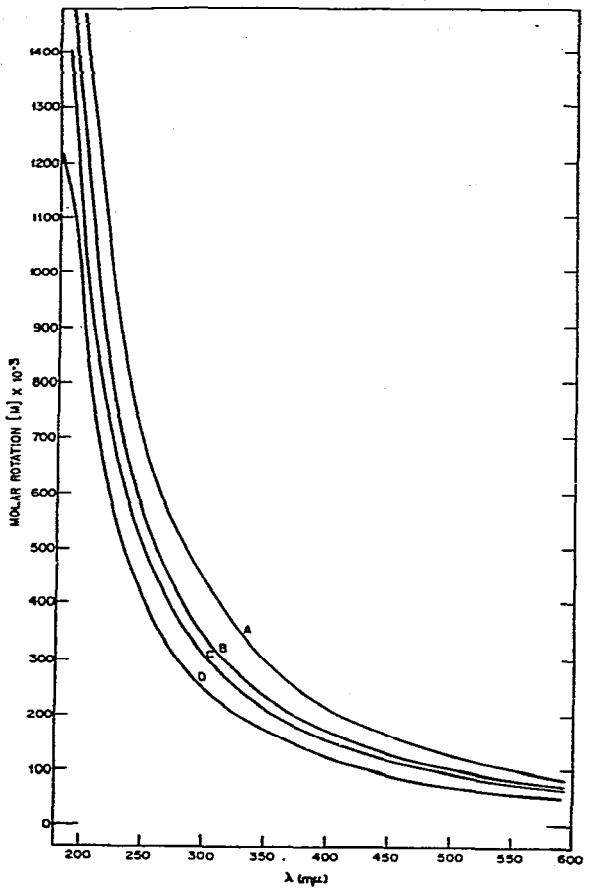

Fig. 4. Optical rotatory dispersion curves for oligosaccharides containing D-glucopyranose, D-galactopyranose, and D-fructose residues. Curve A, *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside (Stachyose); Curve B, *O*-α-D-galactopyranosyl-(1→6)-*O*-β-D-fructofuranosyl α-D-glucopyranoside (Planteose); Curve C, *O*-α-D-galactopyranosyl-(1→6)-*O*-α-D-glucopyranosyl β-D-fructofuranoside (Raffinose); Curve D, 6-*O*-α-D-galactopyranosyl-D-glucose (Melibiose).

pyranoside minus methyl α-D-glucopyranoside[13]. Likewise, there is great similarity between the difference curves obtained for the β-D-glycosidically linked disaccharides (see Fig. 5) and the curve for methyl β-D-galactopyranoside minus methyl β-D-glucopyranoside[13].

Difference values between anomeric forms (Hudson's 2A values)[6,26,27] for D-glucopyranosides having various substituents at C-1, and for methyl α-D-galactopyranoside minus methyl β-D-galactopyranoside, are given in Table II. It is evident

TABLE II

HUDSON'S 2A VALUES FOR SOME MONO- AND DI-SACCHARIDES

	2A Value							
Wavelength (mμ)	*590*	*500*	*400*	*300*	*250*	*210*	*200*	*190*
Methyl α-D-galactopyranoside minus methyl β-D-galactopyranoside	380	560	960	1930	3300	6440	8200	11,500
Methyl α-D-glucopyranoside minus methyl β-D-glucopyranoside	364	531	881	1770	2975	5040	6200	7,600
4-*O*-α-D-Glucopyranosyl-D-glucose minus 4-*O*-β-D-glucopyranosyl-D-glucose (maltose — cellobiose)	336	475	836	1780	2980	5800	6660	9,200
6-*O*-α-D-Glucopyranosyl-D-glucose minus 6-*O*-β-D-glucopyranosyl-D-glucose (isomaltose — gentiobiose)	350	540	858	1690	2920	5410	6510	10,100

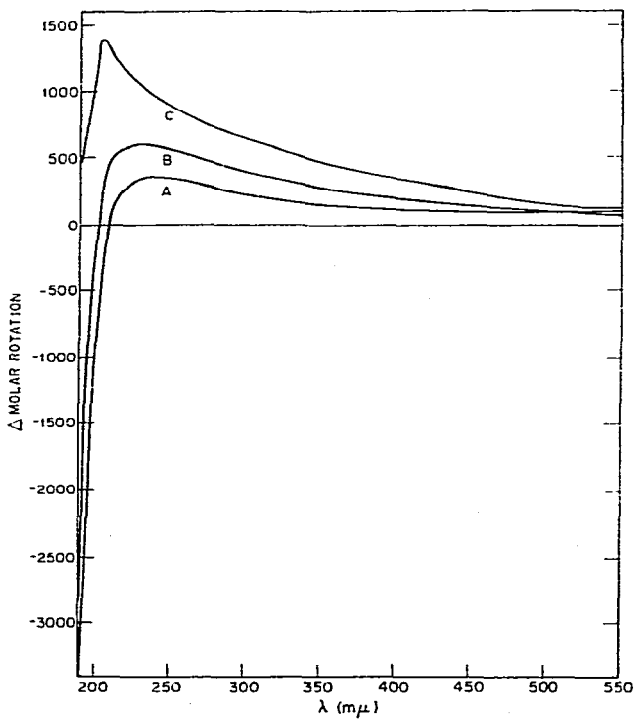

Fig. 5. Molar rotation difference curves. Each curve represents the difference in rotation between disaccharides containing D-galactopyranosyl and D-glucopyranosyl groups. Curve A, 4-*O*-β-D-galactopyranosyl-D-glucose minus 4-*O*-β-D-glucopyranosyl-D-glucose; Curve B, 4-*O*-β-D-galactopyranosyl-D-glucitol (Lactitol) minus 4-*O*-β-D-glucopyranosyl-D-glucitol (Cellobiitol); Curve C, 6-*O*-α-D-galactopyranosyl-D-glucose minus 6-*O*-α-D-glucopyranosyl-D-glucose.

that, at wavelengths above 250 mμ, these 2A values agree very well for each of the compounds shown in Table II. Between 250 and 200 mμ, the values calculated from the D-glucopyranoside-containing derivatives are in close agreement with each other, but diverge significantly from the value calculated from the pair of D-galactopyranoside derivatives. At 190 mμ, significant deviations occur among all of the calculated 2A values. It is evident that the nature of the aglycon or other residue attached to C-1 does not significantly affect the magnitude of the rotatory contribution of the anomeric carbon atom. Configurational variations within the glycosyl group, however, have a large effect on the calculated value of the C-1 rotatory contribution of this ring; this effect becomes more pronounced in the far-ultraviolet region as the anomalous portion of the curve is approached.

CONCLUSIONS

The optical rotatory dispersion curves of the oligosaccharides examined in the present study reflect the rotatory properties of the constituent monosaccharide residues. Therefore, the same general methods used in the interpretation of the optical rotatory data of monosaccharides[13] may be applied to the study of oligosaccharides. In addition, interactions between the rings in the series of oligosaccharides studied appear to have much smaller effects on the rotations than asymmetric interactions between positions on a single ring. Indeed, Beychok and Kabat[29] have reported no unusual rotatory dispersion characteristics of oligomers (dimer through heptamer) of maltose and isomaltose. Thus, it has been possible for us to estimate approximate optical rotatory dispersion curves of oligosaccharides from the sum of the curves of the constituent monomeric residues (*cf.* Table I). The accuracy of these estimates for the visible and near-ultraviolet rotations is generally in agreement with those made for the sodium D-line rotations[3–7]. Of greater importance, however, is the fact that the shapes of the curves, including reversals in the direction of rotations near 200 mμ, agreed with the previously proposed rules relating the structure of monosaccharides to the direction of rotation in the far-ultraviolet region[13,14].

Difference curves between galactopyranosides and the corresponding glucopyranosides, for both mono- and di-saccharides, have been compared. The shape of these curves is influenced significantly by the configuration of the anomeric carbon atom (C-1), but is relatively unaffected by the nature of the substituent at C-1. Thus, the difference curves obtained from sugars containing either a methyl (α-D) or a 6-*O*-D-glucose (α-D) substituent at C-1 are the same, but are different from the difference curves obtained from sugars with either a methyl (β-D), 4-*O*-D-glucose (β-D), or 4-*O*-D-glucitol (β-D) substituent at C-1. In addition, values for the rotatory contribution of the anomeric carbon atom (Hudson's 2A value) are consistent over the 200- to 600-mμ spectral region for a series of D-glucopyranoside derivatives, irrespective of the substituent at the anomeric carbon atom. At wavelengths above 250 mμ, the 2A values calculated from a pair of D-galactopyranosides also agree with those obtained from the D-glucopyranoside series.

An approach to the analysis of optical rotatory dispersion data of sugars has been summarized here and in the preceding papers[13,14] of this series. It is evident that other treatments are possible and that additional potential information is inherent in the rotatory data. It may also be suggested that the high rotations in the far-ultraviolet region have analytical usefulness for quantitating and distinguishing between various sugars. Spectropolarimetric techniques for the enzymic assay of various sugar interconversions at low concentrations of substrate may become feasible. Elucidation of the configuration and conformation of sugars of unknown structure may also be possible by application of the rules presented in these discussions.

EXPERIMENTAL

Optical rotatory dispersion measurements were performed on a Cary Model 60 spectropolarimeter. A cell of 0.6-ml capacity and 1-cm path-length was used for most of the measurements, as previously described[13]. The slit width was programmed for a resolution of better than ± 0.75 mμ throughout the entire spectral region. Rotation values were not reported when the solutions exceeded an absorption of 2.0. Concentrations of 0.1 percent were used for most sugars throughout the entire spectral range (185–600 mμ) examined. The solutions of the mutarotating sugars were kept in the cell until constant specific rotation values were obtained.

Isomaltose, gentiobiose, leucrose, and melibiose were samples generously supplied by Dr. E. Hehre, and laminaribiose was obtained from Dr. W. J. Whelan. All other sugars were commercial samples having the highest possible purity.

SUMMARY

Optical rotatory dispersion curves have been obtained for a series of oligosaccharides. The rotatory data for the oligosaccharides, over the spectral region 600–185 mμ, were in general agreement with the sum of the rotations of the constituent monomeric residues. Distinct features of shape of the rotatory dispersion curves, including changes in the directional trends of rotation in the far-ultraviolet region, reflected the specific configurational features of the monomeric residues. Based on the previously proposed treatment of optical rotatory dispersion data for monosaccharides, a method has been outlined for relating the rotations in the far-ultraviolet region to the structure of oligosaccharides in solution.

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INTERACTION BETWEEN SERUM ALBUMIN AND ACIDIC POLYSACCHARIDES*

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INTRODUCTION

Steric interactions between proteins and polysaccharides have been investigated recently in one of our laboratories. Proteins transported through polysaccharide media by sedimentation and diffusion are retarded by a sieve mechanism[1-3]. The observation that the activity coefficients of proteins are increased in the presence of polysaccharides can be interpreted as an indication that a polysaccharide mechanically excludes a protein from part of the solution. This exclusion hypothesis has been tested by a number of methods[4]. The steric interaction phenomena were studied at high ionic strengths and pH values where electrostatic interactions were expected to be negligible.

However, it is important to determine the conditions where charge interactions do occur, in order to separate the two types of interaction phenomena. This information is also important in discussion of the physiological role of polysaccharide–protein interactions.

We have studied the charge interactions between serum albumin and polygalacturonate (a polycarboxylic polysaccharide) or chondroitin 4-sulfate, which contains both carboxyl and sulfate groups. Albumin was used in many of the earlier experiments. The methods used are chromatography, sedimentation, and free electrophoresis.

Polyanions and proteins are known to form insoluble complexes under conditions where electrostatic attraction between the substances occurs. A detailed description of this and related phenomena has been given by Bungenberg de Jong and his co-workers[5]. Precipitation reactions involving connective tissue polysaccharides have been described by a number of authors[6,7]. The theoretical aspects of interacting protein systems have been reviewed recently by Nichols *et al.*[8], who discuss, in detail, such transport systems as sedimentation, electrophoresis, and chromatography. Electrophoretic studies of the interaction between polysaccharides and proteins have been reported by several authors[7,9-16].

*This paper is No. 10 in the series "Interaction between polysaccharides and other macromolecules" from the Department of Medical Chemistry, University of Uppsala, Sweden.

MATERIALS

Human serum albumin (HSA) was obtained through the courtesy of Mr. H. Björling, AB KABI, Stockholm, Sweden. At a concentration of 10^{-2} g/ml, it moved as a single component in the ultracentrifuge ($s^{\circ}_{20,w} = 4.3 \times 10^{-13}$ sec) and in free electrophoresis (salt concentration 0.15M; pH between 4.65 and 7.05). The albumin to be used for chromatographic experiments was first subjected to gel filtration on Sephadex G-200, in order to remove a small amount of material of high-molecular weight (probably dimer) and a u.v.-absorbing contaminant of low-molecular weight.

Polygalacturonate (PG) was obtained as the free acid from Mann Research Laboratories, New York, N. Y. (Lot No. L 2266). Titration data indicated a purity of 93–95% for the dry material which contained 1.6% of ash. The sodium salt of PG showed a single boundary upon sedimentation in 0.05M sodium acetate ($s^{\circ}_{20,w} = 1.6 \times 10^{-13}$ sec). The molecular weight, determined by light-scattering, was 27,000 and the limiting-viscosity number at 25° in 0.15M sodium chloride was 120 ml/g.

Chondroitin 4-sulfate (CS). Two preparations of the sodium salt of CS were used. One of them (I) was a gift from Dr Lennart Rodén, La Rabida-University of Chicago Institute, Chicago, Ill. This was a commercial preparation (Wilson Laboratories), purified by precipitation using cetylpyridinium chloride[17]. It contained 34% of uronic acid[18] and 31% of hexosamine[19], calculated on a dry-weight basis. The ratio, hexuronic acid : hexosamine : nitrogen[20] : sulfate[21] was 1.00:0.96:1.33:1.02, and $s^{\circ}_{20,w}$ in 0.05M sodium acetate was 1.2×10^{-13} sec. The second preparation (II) had been used in a previous investigation, and its analytical values have been reported[22]. Glucosamine represented about 5% of the total hexosamine in both preparations[19].

Polysaccharide gels. Gels were prepared from pectin (N.F. pectin, Mann Laboratories) and from preparation I of CS by cross-linking them with epichlorohydrin[23]. Pectin (the methyl ester of PG) was used as starting material instead of PG, since the latter would not form gels under the conditions used. The gels were prepared as follows: CS (10 g) and sodium hydroxide (2.85 g) were dissolved in water (10 ml). Pectin (10 g) and sodium hydroxide (5 g) were dissolved in water (20 ml). Sodium borohydride (0.2 g) was added to each solution. The solutions were then mixed with epichlorohydrin (6.0 and 8.9 g), respectively, and the mixtures were kept for 18 h at 50°. The higher concentration of sodium hydroxide used for cross-linking of pectin was required to neutralize the carboxyl groups which are released. The ester linkages are hydrolysed completely in the alkaline medium.

The gels were disintegrated in a Waring Blendor and washed repeatedly with distilled water. Very fine particles of gel, which did not settle during the washing procedure, were decanted. After removal of portions for analysis, the gel suspensions were transferred into 0.2M sodium chloride. An analysis of the CS-gel gave an S:N ratio of 0.63, as compared to 0.76 for the starting material. Apparently, the polysaccharide had been partly desulfated during the cross-linking procedure. The GP- and CS-gels were also converted into the hydrogen-form by treatment with

M hydrochloric acid, followed by exhaustive dialysis against distilled water and lyophilization. This material was titrated with sodium hydroxide to determine the content of acidic groups. PG contained 3.5 meq/g and CS contained 3.35 meq/g. As the degree of substitution with epichlorohydrin is unknown, it is not possible to compare these data with theoretical values. In unsubstituted polysaccharides, they would correspond to 55 and 77% of the expected values.

METHODS

Chromatography. Two columns were packed with the CS-gel and two with the PG-gel. The columns were 7–14 cm long and had volumes of 25–50 ml. The amount of dry gel used for each column was 1–2 g. The void volumes and total volumes of the columns were determined in runs with dextran of high molecular weight (Pharmacia, FDR 922) and tritiated water, respectively. The columns were eluted with upward flow (6 ml/h) by means of a pump. The chromatographic runs were performed at 4°.

Before each run, the columns were washed with 5–10 column volumes of the appropriate buffer. Samples (13 mg) of HSA were applied to the columns in a volume of 1 ml. The effluents were collected in 3-ml fractions, and the pH and absorbance at 280 mμ of each fraction were determined.

After each run, the columns were regenerated with 10-ml portions of 0.1N sodium hydroxide and re-equilibrated with buffer.

Ultracentrifugation. Sedimentation runs were made at 20° in a Spinco Model E analytical ultracentrifuge in ordinary 12-mm, 4°, single-sector alumina cells at 59,780 r.p.m. The sedimentation coefficients were calculated from plots of ln x against time, where x is the distance between the boundary and the axis of rotation. The areas under the schlieren peaks were determined from enlargements by planimetry.

Electrophoresis. Free-electrophoresis experiments were carried out in a Portable Aminco Electrophoresis Apparatus equipped with schlieren optics. Currents of 10–25 mA were used, depending on the ionic strength of the media. The duration of a run was 1.5–3 h. In each run, an average of 6 pictures was taken on both the ascending and descending arms, and mobilities were calculated by averaging the displacement measurements. Conductivity measurements was performed with a Philips PR bridge which was calibrated with standard solutions of potassium chloride. All solutions to be used for electrophoresis or for measurement of refractive increment were first dialysed against appropriate buffers for at least 24 h. To determine whether concentration changes had occurred during dialysis, HSA and polysaccharide were determined by the biuret[24] and carbazole[18] methods, respectively.

Relative concentrations in the boundaries were determined by planimetric measurements on enlargements. The absolute concentrations of free HSA were calculated[25] from its refractive increment of 0.186 ml/g. The absolute concentrations of the polysaccharides could not be calculated because the moving boundaries left behind large, stationary salt peaks (*cf.* ref. 16).

Measurements of pH were made with a Radiometer pH-meter Type PHM 22r

equipped with a Type G 200B glass electrode. The meter was calibrated with commercial phosphate and biphthalate standard buffers.

Dry weights of samples were determined after drying for 12 h at 100°/0.03 mm over phosphoric anhydride. The vials containing the samples were closed at 100° *in vacuo* before weighing.

RESULTS

Chromatography

The chromatographic behavior of HSA on the polysaccharide gels was studied in three series of experiments. Each series of runs was made in 0.05M sodium acetate buffer at pH values between 3.4 and 7.6. In two series, 0.1M and 0.5M sodium chloride was included in the buffer.

In each series of experiments, the results can be subdivided into three regions. At high pH, all of the HSA emerged in a peak at the void volume. (Sometimes the recovery from the PG columns was not quantitative). At low pH, all of the HSA was adsorbed to the columns and could not be eluted with 10–15 column volumes of buffer. In a narrow pH-range between these two regions, some of the HSA was eluted at the void volume while the rest remained adsorbed to the columns. The column length did not influence the results significantly.

Some results are shown in Fig. 1. In 0.05M acetate, PG adsorbs HSA at higher pH than CS does, but, at higher ionic strengths, there seems to be no difference between the two types of gels.

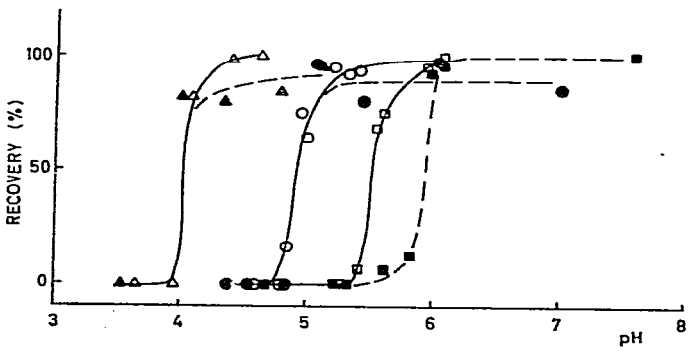

Fig. 1

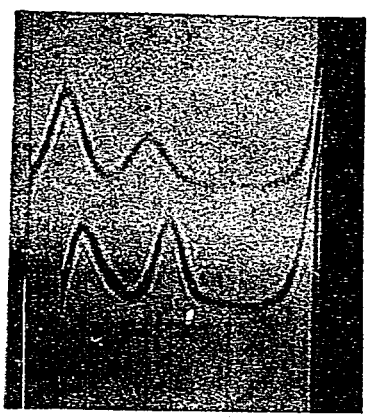

Fig. 2

Fig. 1. Recoveries of serum albumin from chromatography on CS and PG gels as a function of the pH of the eluant. Open symbols: CS; filled symbols: PG; (□, ■) 0.05M acetate; (○, ●) 0.05M acetate + 0.1M NaCl; (△, ▲) 0.05M acetate + 0.5M NaCl.

Fig. 2. Ultracentrifuge patterns from runs with mixtures of 4.6×10^{-3} g/ml of HSA and 8×10^{-3} g/ml of CSA in 0.05M acetate buffers of pH 7.6 (top) and 5.1 (bottom). Speed: 59,780 r.p.m. Picture taken 171 min after start. Bar angle: 60°.

Ultracentrifugation

Sedimentation rates. Sedimentation studies were performed on several mixtures of HSA and PG, or HSA and CS (I), at various salt concentrations and pH values where chromatography and electrophoresis indicated various degrees of interaction between the protein and the polyacids. The sedimentation rates of the polysaccharide boundaries (slow boundaries) did not vary appreciably. Some representative values for the sedimentation rates of the "HSA" boundaries (fast boundary) are given in Table I. In the presence of the polysaccharides, the sedimentation rate is decreased even at neutral pH in 0.15M salt, presumably because of a sieving effect of the polymers[1–3]. A further decrease is observed in the region where other methods indicate strong charge interaction, *e.g.*, at pH 5.1 in 0.05M acetate buffer. However, the latter effect is small and, at maximum, is of the same magnitude as the sieve effect.

TABLE I

SEDIMENTATION RATE (SEC $\times$ 10^{13}) OF THE FAST BOUNDARY IN RUNS ON MIXTURES OF PG OR CS AND HSA. Data obtained at a specified polysaccharide concentration and at five to six different HSA concentrations between 10^{-3} and 9×10^{-3} ml/g have been extrapolated to zero HSA concentration.

Concentration of polysaccharide (*g/ml*) $\times$ 10^3	0	PG(4.4)	CS(4.0)	CS(8.0)	CS(16.0)
Medium					
0.05M acetate buffer pH~7.5 + 0.1M sodium chloride[a]	4.3	3.6	4.0	3.8	2.6
0.05M acetate buffer pH~5 + 0.1M sodium chloride[b]	4.3	3.4	3.8	3.3	2.3
0.05M acetate buffer pH 5.1.	—	3.4	—	3.0	2.4

[a] pH values in the five experiments were 7.5, 7.85, 7.45, 7.45, and 7.45, respectively.
[b] pH values in the five experiments were 4.8, 5.1, 4.8, 4.8, and 4.65, respectively.

Concentrations of components. Fig. 2 shows patterns obtained with mixtures of HSA and CS in 0.05M acetate at pH 7.6 and 5.1. The following features are apparent: (*a*) the fast-moving boundary is larger at pH 5.1 than it is at pH 7.6; (*b*) the slow boundary is larger at pH 7.6 than it is at pH 5.1; (*c*) there is a greater accumulation of material at the bottom of the cell at the lower pH.

The areas under the peaks in Fig. 2 were followed during the runs and were corrected for radial dilution[26]. The areas of the slow peaks remained constant throughout the runs, but the fast peaks decreased in area, especially at pH 5.1 where the decrease was approximately 40%. This decrease was accompanied by an accumulation of material at the bottom of the cell. When the areas of the peaks were extrapolated to zero time, the area of the fast peak at pH 5.1 was 1.37 times that at pH 7.6; the area of the slow peak at pH 5.1 was 0.85 times that at pH 7.6.

Patterns similar to those shown in Fig. 2 were obtained in runs with mixtures of HSA and PG. In this case, however, the area of the faster boundary did not change during the run at pH 5.1. Instead, material accumulated at the bottom of the cell

from the beginning, and both the fast and slow peaks were smaller than expected from the original concentrations of PG and HSA.

Electrophoresis

The electrophoretic experiments were carried out with the individual components and with mixtures containing 4.6×10^{-3} g/ml of HSA and 4.0×10^{-3} g/ml of CS (II) or 4.6×10^{-3} g/ml of HSA and 4.4×10^{-3} g/ml of PG. Two series of experiments were made: one in 0.05M acetate buffer (in Figs. marked A) and the other in 0.05M acetate buffer containing 0.1M sodium chloride (B). The pH was varied within each series.

The electrophoretic patterns obtained with mixtures of HSA and CS at pH 7 and pH 5.15 in 0.05M sodium acetate are shown in Fig. 3. Each pattern shows two

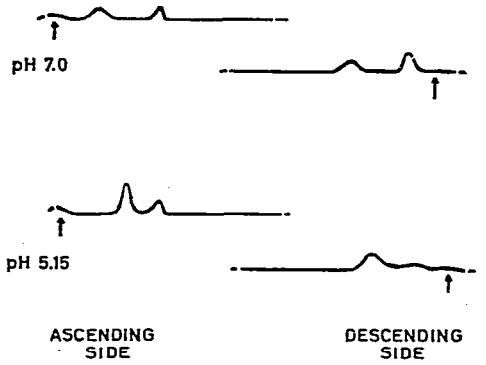

Fig. 3. Electrophoretic runs of a mixture of 4.6×10^{-3} g/ml of HSA and 4×10^{-3} g/ml of CS in 0.05M acetate buffer pH 7.0 and 5.15. Pictures were taken 35 and 28 min after start, respectively. Current: 10 mA. Bar angle: 30°. Arrows indicate place of boundaries at start.

components and a salt boundary. Both the mobilities and the concentrations of the components differ in the two runs.

Mobilities. The mobilities of the fast-moving boundaries on the ascending side did not vary significantly with pH and corresponded to those of free CS (16×10^{-5} and 13×10^{-5} $cm^2\ V^{-1}\ sec^{-1}$) and free PG (14×10^{-5} and 11×10^{-5} $cm^2\ V^{-1}\ sec^{-1}$ in 0.05M and 0.15M sodium chloride, respectively. The mobility of the fast-moving boundaries on the descending side did not differ significantly from those of CS or PG, but the values tended to be somewhat lower than those of the free polysaccharides. The mobilities were approximately 13×10^{-5} and 12×10^{-5} $cm^2\ V^{-1}\ sec^{-1}$ for CS and 12×10^{-5} and 9.5×10^{-5} $cm^2\ V^{-1}\ sec^{-1}$ for PG, at the salt concentrations given above.

The largest variation in mobility was found for the slow-moving boundary on the ascending side. This is shown in Figs. 4 and 5 for CS-HSA and PG-HSA mixtures, respectively. The mobility of free albumin is given for comparison. Deviations from the behaviour of free albumin are observed below pH 7 in 0.05M acetate and below pH 6 in the presence of 0.15M salt. In mixtures of HSA and PG, the

slow-ascending boundary tended to split into three peaks at approximately neutral pH in 0.05M salt. This effect is illustrated in Fig. 5(A).

The mobility of the slow-moving peak on the descending side adheres very closely to that of free albumin. The only deviation from this behaviour was observed in 0.05M acetate at the lowest pH (<6.0), where the boundary broadened (see Fig. 3), occasionally split into two, and showed a higher mobility than free albumin.

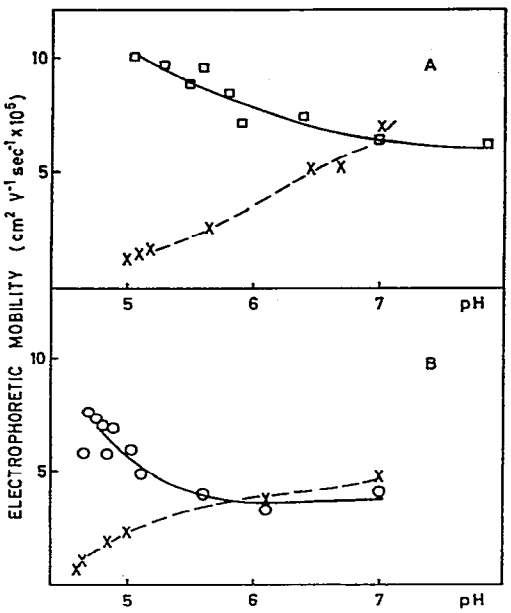

Fig. 4

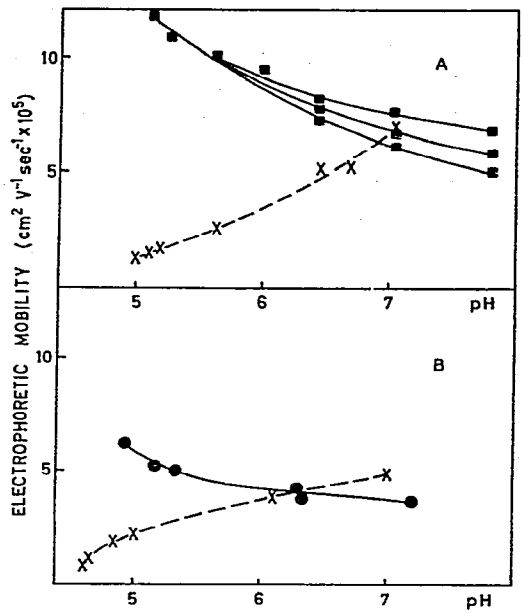

Fig. 5

Fig. 4. Electrophoretic mobility of the slow-moving boundary (□, ○) in the ascending arm for mixtures of CS and HSA as a function of pH. A: in 0.05M acetate buffer; B: in 0.05M acetate buffer + 0.1M NaCl; (x— — —x): the mobility of free albumin.

Fig. 5. The same as in Fig. 4 for mixtures of PG and HSA.

Concentration of components. When the pH is decreased, the fast-ascending and slow-descending boundaries decrease and the slow-ascending and fast-descending boundaries increase in size. The sizes of the peaks usually remained constant during any particular run. The only exception is the slow-descending peak in the system PG-HSA: this peak diminished slightly during the first 40 min, but remained constant thereafter.

It is not possible to calculate the concentrations of components in the slow-ascending and fast-descending boundaries because the refractive increments of the polysaccharide–protein complexes are not known. However, if one assumes that the fast-ascending peak represents free polysaccharide and the slow-descending peak represents free albumin, as indicated by the mobilities, their relative concentrations can be calculated by planimetry. Figs. 6 and 7 show the variation of material in

these peaks as a function of pH at the two different concentrations of salt. The material in the slow-descending arm in Fig. 6 (A and B) and Fig. 7 (B) corresponds, at pH 7, to the total amount of HSA in the system, as calculated from the known refractive increment of the protein.

DISCUSSION

The experiments described above were performed in order to determine the conditions where electrostatic interactions might occur between the acid mucopolysaccharides of connective tissue and proteins. Human serum albumin (HSA) is the only protein which we have studied so far. Chondroitin 4-sulfate (CS) was used as a representative of the class of polysaccharides which carries both sulfate and carboxyl groups. Hyaluronic acid would have been chosen to represent the carboxylated polysaccharides if the investigation had not required such large quantities of material. Instead, we used polygalacturonate (PG), which can be obtained reasonably pure

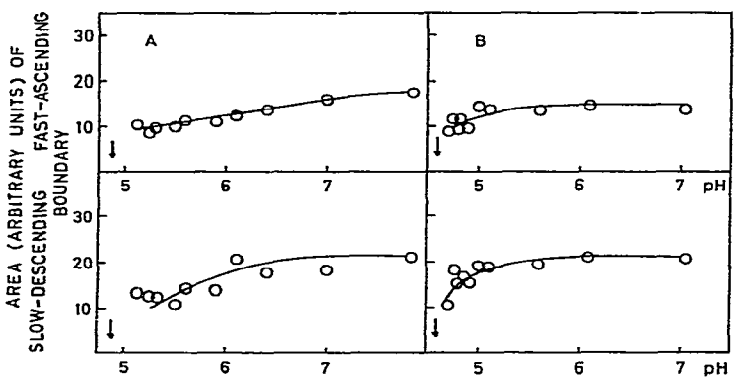

Fig. 6. Relative amount of material in the fast boundary in the ascending arm and in the slow boundary in the descending arm during runs of CS-HSA mixtures at various pH values. A: in 0.05M acetate; B: in 0.05M acetate + 0.1M NaCl; Arrows indicate pH values at which the constituents precipitate.

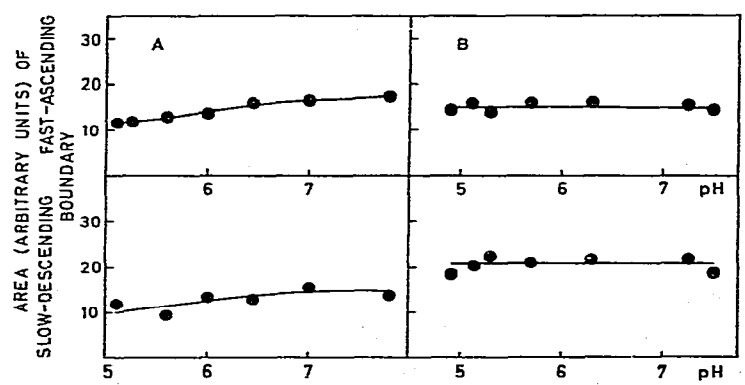

Fig. 7. The same as Fig. 6 for PG-HSA mixtures.

in large quantities. PG has the same charge density as CS. From the many possible methods for studying the interaction, chromatography and sedimentation were selected because they had been used in earlier studies of the sterical interaction between polysaccharides and proteins[1–4], and electrophoresis was chosen because it has been used extensively in studies with other interacting systems in protein chemistry[8]. Several conclusions can be drawn on the basis of our experiments.

The pH-range of interaction. The pH-range in which charge interaction occurs between the polysaccharides and HSA is a function of the ionic strength, as shown by chromatographic and electrophoretic experiments. In 0.05M acetate buffer, HSA is adsorbed to the columns below pH 6 (Fig. 1), whereas electrophoretic experiments indicate that interaction begins just below pH 7 (Figs. 4 and 5). Similarly in 0.05M acetate containing 0.1M sodium chloride, chromatographic experiments demonstrate interaction below pH 5, whereas electrophoretic experiments indicate interaction already below pH 6. In 0.55M salt, adsorption to the columns does not occur above pH 4. This pH- and ionic strength-dependence indicates strongly that the interaction is electrostatic.

The electrophoretic experiments show that soluble complexes are formed in a pH-range above the isoelectric point of HSA (pH 4.7[27]), where both the polysaccharides and HSA are negatively charged. The fact that interaction occurs might indicate an uneven charge distribution on the albumin surface. Insoluble complexes are formed at pH 4.9 in 0.05M acetate and at pH 4.65 in 0.15M salt at the concentrations of CS and HSA used in the electrophoretic experiments. The adsorption of the protein to the chromatographic columns seems to be correlated with formation of insoluble rather than soluble complexes. That adsorption occurs at pH-values slightly above the precipitation point can be ascribed to the fact that the polysaccharide concentrations in the gel grains are much higher than those used in free solution.

Fig. 1 illustrates the interesting fact that in 0.05M acetate buffer, but not at higher ionic strengths, PG has a stronger affinity for albumin than has CS. This might reflect different association of carboxyl and sulfate groups to albumin. Noguchi[12] found no difference between the affinities of albumin for sulfate groups and carboxyl groups, but he did not state the ionic strength of the medium. Electrophoretic experiments also indicate a difference between the interactions of HSA with the two polysaccharides in 0.05M acetate. The slow-ascending peak in HSA–PG mixtures seems to separate into several components at pH>6 (Fig. 5). Furthermore, the concentrations of free HSA and PG in 0.05M acetate at neutral pH deviate from the total concentrations of HSA and PG in the system (Compare Fig. 7A and B). This more complicated interaction between PG and HSA does not occur in 0.15M salt.

In a study of the interaction between albumin and dextran sulfate, which had a charge density 2.3 times greater than that of the polysaccharides used here, Thompson and McKernan[14] observed complex formation even at pH 7.4 at an ionic strength of 0.1. They noted no complex formation at pH 8.5.

The electrophoretic picture. In most runs, only two boundaries were observed in the ascending and descending arms (Fig. 3). This differs from the results obtained

in many previous studies of the interaction between polysaccharides and proteins[7,9–13,15]. However, our result is very similar to that obtained by Tsang and Thompson[16] with the system, dextran sulfate–carbomonoxyhemoglobin. A thorough review of the electrophoretic behaviour of interacting systems has been written by Nichol *et al.*[8], who also discuss the model systems of Gilbert and Jenkins[28]. The interactions involved in our study seem to belong to their Class IV: the rate constant(s) for the formation and dissociation of the soluble complex(es) is (are) large and similar in magnitude. Our system seems too complicated for theoretical treatment at this stage, and we have not attempted to calculate rate constants for the formation of the complexes. The data in Figs. 6 and 7 indicate the complexity of the interactions. If we assume that the differences between the total concentrations of the constituents in the system and the amounts of free constituents measured in the fast-ascending and slow-descending boundaries (Figs. 6 and 7) represent the amounts of constituents bound in the complexes, we can calculate the ratio of HSA and the polysaccharides in the complexes as a function of pH and ionic strength. Such calculations indicate that the composition of the complexes varies strongly with pH and ionic strength.

The sedimentation picture. The sedimentation runs also show only two boundaries. The slower one sediments like the free polysaccharide, whereas the faster one has a sedimentation rate intermediate between those of the polysaccharide and free HSA. Apparently, this decrease in the sedimentation rate is largely due to frictional effects, as discussed earlier[1–3], since it occurs even when chromatography and electrophoresis show no signs of charge interaction. Complexes are formed at low pH, as indicated by the transfer of material from the slow boundary to the fast one in the CS-HSA system, and by the disappearance of material from both boundaries in the PG-HSA system. An additional decrease in the sedimentation rate of the fast boundary occurs when soluble complexes are present, despite the fact that the complexes have higher molecular weights than the original components. Therefore, the complexes must encounter a high frictional resistance indicating that they retain the open-chain conformation of the polysaccharides. Furthermore, the complexes must vary widely in size, since some material seems to disappear from the boundaries and collect at the bottom of the cell. In order to explain the disappearance of material from the boundary containing the complexes, one must also assume that the large complexes dissociate more slowly than the small ones. However, the electrophoretic mobilities of these large aggregates must be very similar to those of the smaller complexes in order to account for the electrophoretic picture.

Physiological considerations. No electrostatic interaction between HSA and the polysaccharides was observed under "physiological" conditions. This substantiates the view that the experiments designed to study steric interactions[1–4] have not been disturbed by charge effects. However, it is possible that proteins with isoelectric points closer to neutral pH might form transient complexes with polyanions. Such soluble complexes occur at pH values considerably above the precipitation point[6] and are best studied by electrophoresis.

Our findings indicate that the interaction between hyaluronic acid and serum

albumin, observed by Pigman and co-workers[15] at pH 8.6 and ionic strength 0.1, is not electrostatic and is probably accountable to frictional effects[29].

ACKNOWLEDGMENTS

This investigation was supported by a grant (HD-01249) of the National Institute of Child Health and Human Development, U.S. Public Health Service (to F.A.B.) and grants from the Swedish Medical Research Council (13x-4-02A) and the Swedish Cancer Society (to T.C.L.).

SUMMARY

The electrostatic interaction between human serum albumin and chondroitin 4-sulfate or polygalacturonate has been studied as a function of pH and ionic strength. The protein is adsorbed on columns of the polysaccharides at pH values slightly above the range where insoluble complexes between albumin and these polysaccharides form in free solution ($pH < 5.5$–6 in 0.05M salt; $pH < 5$ in 0.15M salt). However, free electrophoresis shows that soluble protein–polysaccharide complexes exist at pH-values ($pH < 7$ in 0.05M salt; $pH < 6$ in 0.15M salt) well above the range where precipitation occurs. In most cases, only two boundaries are detectable, but reversible complex formation is indicated by changes in boundary sizes and mobilities. The complexes seem to be heterogenous as regards composition, size, and, probably, rate of formation and dissociation. Differences between the two polysaccharides in their interaction with albumin were observed only at low ionic strength. Apparently, no electrostatic interaction between albumin and the polysaccharides occurs under physiological conditions, which supports our view that the main interaction *in vivo* is of steric nature.

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STUDIES ON URONIC ACID MATERIALS

PART XVI[1]. INTER-NODULE VARIATION AND THE ACIDIC COMPONENTS IN *Acacia nilotica* GUM

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INTRODUCTION

In a preliminary study of the gum exudates from several *Acacia* species, *Acacia nilotica* (L.) Willd. ex Del. was found[2] to differ in a number of interesting respects from those species studied prior to 1963. Thus, *A. nilotica* gum gave a high, positive specific rotation (+106°), a high methoxyl content (1.05%), and contained only traces of rhamnose that could not be estimated satisfactorily by paper chromatography.

In addition, *A. nilotica* gum gave solutions of low viscosity, and its unusually low nitrogen content (0.08%) was decreased to 0.02% on electrodialysis (corresponding decreases are not shown by other *Acacia* gums[2,3]). *A. nilotica* could, therefore, constitute a useful limiting case in investigations into the extent of the dependence of the physico-chemical properties of *Acacia* gum solutions on their natural nitrogenous content, and it was therefore selected for study in preference to other available species[2].

In view of the inter-nodule variation observed in *A. seyal* gum[3], a preliminary analytical survey of ten nodules of *A. nilotica* gum was undertaken.

EXPERIMENTAL

Origin of Specimens. Ten large nodules of gum from *Acacia nilotica* were collected by (the late) M.P. Vidal-Hall, formerly Gum Research Officer to the Republic of the Sudan, at Hawata, Kassala Province, Eastern Sudan, on February 3rd, 1963. All of the nodules originated by natural exudation; nodules **(1)**, **(2)**, **(3)**, and **(4)** were taken from individual small trees, nodules **5–10** from individual large trees, all growing in close proximity.

Analytical Methods. The general methods have been described[2], except that paper chromatography was carried out with the following solvent systems (v/v): (*A*) butan-1-ol–ethanol–water (4:1:5, top layer); (*B*) butan-1-ol–pyridine–water–benzene (5:3:3:1, top layer); (*C*) ethyl acetate–pyridine–water (10:4:3); (*D*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4); (*E*) ethyl acetate–acetic acid–water (9:2:2); (*F*) butan-1-ol–acetic acid–water (4:1:5, top layer).

TABLE I

ANALYTICAL DATA [a] FOR PURIFIED NODULAR SPECIMENS OF *Acacia nilotica* GUM

	Nodules										*Average*	
	1	2	3	4	5	6	7	8	9	10	*All nodules*	*omitting* 5
Ash, %	0.02	—	—	—	0.04	—	—	—	—	—	—	—
Nitrogen, %	0.02	0.02	0.02	0.03	0.02	0.01	0.03	0.01	0.01	0.01	0.02	0.02
Methoxyl, %	0.96	0.75	1.25	1.18	1.44	1.30	1.25	1.03	1.13	1.28	1.16	1.12
Reducing power[b]	0.07	0.05	0.13	0.05	0.16	0.13	0.07	0.35	0.20	0.10	0.13	0.13
Flow time[c], sec	304	312	305	307	333	305	307	306	304	309	309	307
Limiting flow time number[c]	9.5	—	—	—	10.4	—	—	—	—	—	—	—
$[\alpha]_D^{20}$ (*c* 3.0, water)	+108°	+109°	+108°	+108°	+54°	+107°	+108°	+106°	+106°	+106°	102°	107°
Neutralisation equivalent	1890	1890	1860	1930	1645	1960	1860	1880	1860	1850	1860	1890
Hence, uronic anhydride, %[d]	9.3	9.3	9.5	9.1	10.7	9.0	9.5	9.4	9.5	9.5	9.5	9.3
Uronic anhydride (decarbox.)	9.2	9.2	9.4	9.3	10.9	9.3	9.2	9.3	9.1	9.3	9.4	9.3
D-Galactose	44	43	41	43	33	40	43	42	43	39	41	42
L-Arabinose	46	47	46	47	55	46	45	47	46	49	47	46

[a] All results corrected to a dry-weight basis. [b] Expressed as apparent % of free pentose. [c] In 4% saline at 25.0°; water = 200 sec. [d] If all acidity arises from uronic acid residues.

RESULTS

Studies on the crude gum. The results of analyses of nodule (**1**) have been published[2] under the heading "*A. nilotica*" in Table II therein.

Studies on gum samples purified by electrodialysis. Each of the ten nodules of gum was purified separately by electrodialysis[4], and the free gum acids were isolated by freeze-drying; analytical data are given in Table I. Particular care was taken with duplicate analyses when it was observed that the results for nodule (**5**) differed significantly from the others; the results quoted are average values for satisfactory replicates.

Partial hydrolysis of the gum acid, and separation of acidic sugars. Electrodialysed specimen (**1**) (16 g) was hydrolysed with N sulphuric acid (300 ml) for 12 h at 100°. After neutralisation (barium carbonate), de-ionisation [Amberlite IR-120 (H^+ form)], and concentration, paper chromatography (solvents *B* and *D*) revealed the presence of galactose, arabinose, glucuronic acid, and 4-*O*-methylglucuronic acid. Two acidic disaccharides were also indicated (these were, in fact, two mixtures of acidic disaccharides).

The neutral and acidic components were separated by passage through Duolite A4 (formate form). Elution with water gave a neutral syrup (10.7 g) which contained galactose and arabinose; this fraction was not examined further. The acidic components were eluted with dilute formic acid to give a syrup (4.4 g) after the necessary isolation stages.

Fractionation of the acidic sugars. The acidic syrup (4.4 g) was added to a cellulose column (80×4 cm) and eluted with solvent *E*. Fractions (10 ml) were collected; the contents of every third tube were examined in solvents *D* and *E*. Five main fractions were obtained: fraction *a* (tubes 24–28), 216 mg; fraction *b* (30–61), 1498 mg; fraction *c* (62–100), 931 mg; fraction *d* (134–307), 941 mg; fraction *e* (492–600), 309 mg. The total recovery was therefore 88%.

Examination of the acidic fractions. (*a*) Fraction *a* had R_{Gal} 3.0 (solvent *E*) and crystallised readily. Recrystallisation from water gave D-glucopyranurono-6→3-lactone, m.p. and mixed m.p. 177°, $[\alpha]_D^{20}$ +19° (*c* 1.0, water).

(*b*) Fraction *b* gave a single chromatographic spot having R_{Rha} 0.91 (solvent D) and R_{Gal} 0.39 (solvent *B*). It had $[\alpha]_D^{20}$ +37° (*c* 1.5, water) (Found: OCH_3, 14.6. Calc. for a monomethylhexuronic acid: OCH_3, 14.9%).

The pale syrup (0.4 g) was converted into the methyl ester methyl glycoside (0.34 g) with dry, 2% methanolic hydrogen chloride, reduced with potassium borohydride, and hydrolysed. The product (0.24 g) had R_{Rha} 0.94 in solvent *A* and gave a single spot, having R_{Gal} 1.94 and 2.1, respectively, in solvents *B* and *D*. This behaviour was identical with that of 4-*O*-methylglucose. After purification on Whatman No. 3MM

paper in solvent *A*, the product had OCH_3, 15.0% (calc. for $C_7H_{14}O_6$: OCH_3, 15.6%), and the crystalline phenylosazone had m.p. 156° (lit., 157–160°), after two recrystallisations from hot water. Fraction *b* was thus 4-*O*-methyl-D-glucuronic acid; the amide of methyl 4-*O*-methyl-α-D-glucopyranuronoside was obtained as large, colourless plates, m.p. 231° (lit., 232–236°), $[\alpha]_D^{20}$ +140° (*c* 0.5, water).

(*c*) Fraction *c* gave a single spot corresponding to D-glucuronic acid in solvent *D*: examination in solvent *B* showed the presence of a trace of galactose. In view of the identity of fraction (*a*), this fraction was not examined further.

(*d*) Fraction *d* had $[\alpha]_D^{20}$ +55° (*c* 1.0, water), and partially crystallised. Examination in solvents *D* and *E* gave spots (brown with aniline oxalate spray) having R_{Gal} 0.69 and 0.66, respectively. Hydrolysis (2N sulphuric acid, 6 h) and subsequent examination (solvent *D*) showed the presence of galactose and 4-*O*-methylglucuronic acid in equal proportions (visual examination). A small portion of the fraction (80 mg) was treated with methanolic hydrogen chloride, reduced with potassium borohydride, hydrolysed, and fractionated on 3MM paper in solvent *A*, to give 4-*O*-methyl-D-glucose [28 mg; phenylosazone, m.p. 158° (lit., 159°)], $[\alpha]_D^{20}$ +58° (*c* 0.1, water); and D-galactose (31 mg; m.p. and mixed m.p. 156°).

Fraction *d* (500 mg) was methylated with dimethyl sulphate and sodium hydroxide, followed by methyl iodide and silver oxide, to give a product (286 mg; OCH_3, 52.1%) which was reduced with lithium aluminium hydride. The methylated, neutral product (213 mg) was hydrolysed (N hydrochloric acid, 4 h); the hydrolysate gave 3 spots (R_G 0.84, 0.72, 0.69) in solvent *A*. The three components were separated on a cellulose column (2×38 cm) using butan-1-ol–light petroleum (7:3), saturated with water, as eluant. The first component (68 mg) had R_G 0.85 (solvent *A*), was identical with 2,3,4-tri-*O*-methyl-D-glucose in solvents *A*, *B*, and *F*, and gave an anilide, m.p. 152° (lit., 145–150°). The second component (18 mg) had R_G 0.71 (solvent *A*), was identical with 2,3,6-tri-*O*-methyl-D-galactose in solvents *A* and *F*, and was oxidised with bromine to 2,3,6-tri-*O*-methyl-D-galactonolactone, m.p. 96–97° (lit., 98°). The third component (17 mg) was identical with 2,3,4-tri-*O*-methyl-D-galactose in solvents *A*, *B*, and *F*, and gave an anilide, m.p. and mixed m.p. 166° (lit., 167–170°).

Separation of the aldobiouronic acids in Fraction d

Fraction *d* was suspected to be a mixture of monomethylaldobiouronic acids when the rotation for the corresponding fraction from the other specimens of *A. nilotica* was observed to vary significantly from +48 to +71°. Separation of fraction *d* into two aldobiouronic acids was achieved on strips (4″ wide) of 3MM paper, in solvent *D* for 96 h. The chromatograms were freed from acidic solvent by air-drying for 48 h, followed by heating for 5 min at 150°. The located zones were eluted with cold water to give aldobiouronic acids *A* (101 mg) and *B* (95 mg).

Examination of aldobiouronic acids A *and* B

Acid *A* (101 mg) had R_{Gal} 0.79 (solvent *D*) and $[\alpha]_D^{20}$ +93° (*c* 1.0, water).

A sample (40 mg) was converted into the methyl ester methyl glycoside; one half-portion of the product was reduced with potassium borohydride and hydrolysed. The products were chromatographically identified (solvent *A*) as galactose and 4-*O*-methylglucose. The second half-portion reduced 1.8 mol. of sodium periodate. In duplicate experiments, the aldobiouronic acid gave, on periodate oxidation, 1.01 and 1.04 mol. of formaldehyde, indicating that C-6 of the D-galactose residue was not involved in a linkage. These experiments, considered in conjunction with the methylation evidence reported above for acidic fraction (*d*), led to identification of acid *A* as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose.

Acid *B* (yield 95 mg) had R_{Gal} 0.68 (solvent *D*) and $[\alpha]_D^{20}$ +6° (*c* 0.95, water). Methanolysis, followed by potassium borohydride reduction and subsequent hydrolysis, gave only galactose and 4-*O*-methylglucose (solvent *D*). Periodate oxidation of the aldobiouronic acid at pH 8 gave no formaldehyde. The methyl ester methyl glycoside, on oxidation with sodium periodate in darkness for 2 days at room temperature consumed 3.1 mol. of periodate. These experiments, considered in conjunction with the methylation evidence reported above for acidic fraction (*d*), led to the identification of acid *B* as 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose.

(*e*) Fraction *e* (309 mg) had $[\alpha]_D^{20}$ +31° (*c* 1.2, water) and gave, after hydrolysis, only galactose and glucuronic acid (solvents *B* and *D*). Methanolysis, followed by potassium borohydride reduction and hydrolysis, gave only galactose and glucose. Chromatographic separation in solvent *D* for 120 h was required to reveal the presence of two components having R_{Gal} 0.21 (major component) and 0.28. Fraction *e* was then fractionated on 3MM paper (4″ wide) in solvent *D* for 160 h to give aldobiouronic acids *C* and *D*.

Examination of aldobiouronic acids C *and* D

Acid *C* (103 mg) had R_{Gal} 0.21 (solvent *D*) and gave $[\alpha]_D^{20}$ −5° (*c* 0.4, water). It was chromatographically homogeneous and identical in solvents *D*, *E*, and *F* with 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose. Reduction of the methyl ester methyl glycosides with potassium borohydride, followed by hydrolysis, gave only galactose and glucose. Methylation of a portion (80 mg) by the Haworth and the Purdie methods, followed by reduction (lithium aluminium hydride), gave a product (38 mg) which, after hydrolysis, was fractionated on 3MM paper in solvent *A* to give 2,3,4-tri-*O*-methyl-D-glucose (14 mg) [R_{Gal} 0.85 (solvent *A*); anilide, m.p. +148° (lit., 145–150°)] and 2,3,4-tri-*O*-methyl-D-galactose (11 mg) [R_{Gal} 0.67 (solvent *A*), chromatographically identical with an authentic specimen in solvents *A*, *D*, and *F*; attempted preparation of the anilide did not yield a crystalline product].

Acid *D* (18 mg) had R_{Gal} 0.28 and 0.32 on Whatman No. 1 and 3MM papers, respectively, in solvent *D*, and had $[\alpha]_D^{21}$ +107° (*c* 0.1, water). Hydrolysis gave only galactose and glucuronic acid; periodate oxidation at pH 8 for 24 h gave 0.93 mol. of formaldehyde. This acid has subsequently been obtained from other *Acacia* species, and has been more rigorously characterised[5] as 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose.

DISCUSSION

The analytical results in Table I supplement the limited data of this type extant[3,6]; the additional work involved in such an approach is justified for the following reasons.

For such complex polysaccharides, it is reasonable to expect that some variation in composition and properties may exist between gum specimens exuded by different trees of a particular species (it is becoming apparent that the inter-nodule variation for one species may be greater than that for another). This investigation has shown that a knowledge of the inter-nodule variation is useful whenever an aspect of heterogeneity, or the possibility of fractionation, is involved.

In this study of *A. nilotica*, the natural exudates, collected on the same day, from ten different trees growing in close proximity, have been examined. This approach can be extended to examine (*i*) the seasonal variation for a species, (*ii*) the variation between specimens from geographical locations differing in climate and type of soil, and (*iii*) the variation between exudations resulting from different stimuli, *e.g.*, from tapping, from natural processes, from diseased trees, and from trees attacked by ants or borer beetles. Such studies could provide useful evidence regarding the mode of biosynthesis of gum and the nature of the carbohydrate systems serving as gum precursors in the tree.

The available analytical data substantiate the view[3] that a single, gum nodule is itself complex and offers the simplest system available for structural investigation. Recent developments in analysis make such an approach possible if the collection of reasonably large nodules can be arranged. A preliminary analytical survey of several nodules is, nevertheless, required, to select the most representative nodule of the species for structural study, and to establish the extent to which it varies from other specimens. The value of this approach is seen in Table I, from which specimen (5) must be regarded as atypical (in some respects) of the *A. nilotica* species. On the basis of present knowledge, there is, however, no evidence that specimen (5) did not originate from *A. nilotica*. The ten specimens studied were collected by an accepted authority on the Sudanese *Acacias*, whose undertaking was, in the research collaboration between the Sudanese Department of Forests and this laboratory, to collect, personally, only specimens which could be authenticated beyond doubt. *Acacia nilotica* is, moreover, distinctly characteristic to a trained fieldsman; the species resembling it most closely is *A. arabica*. Inspection of the available analytical data for *Acacia* species[2] indicates that although *A. pycnantha*, *A. arabica*, and *A. fistula* each have *some* feature in common with specimen (5) in Table I, its analytical characteristics, *taken as a whole*, do not suggest that it would be more correctly assigned to any other species for which information exists. Although this view may require alteration in the future, the present reserve associated with this specimen further justifies a limited, preliminary, analytical survey of the nodules of any gum species under structural investigation.

Determinations of the traces of rhamnose in *A. nilotica* could only be achieved

by a spectroscopic method developed[7] for the purpose. Several *Acacia* species are now known to contain <1% of rhamnose, and this cannot now be considered a major constituent in all *Acacia* gums (*cf.* ref. 8) Furthermore, polysaccharides containing both D-glucuronic acid and its 4-*O*-methyl ether are no longer unusual (*cf.* ref. 8); these acids occur conjointly in species of the *Albizia*[4,8], *Khaya*[10] and, now, of the *Acacia* genera.

Methoxyl groups were recently reported[9] to occur in *Acacia* species; the relatively high methoxyl content in *A. nilotica* has facilitated the present confirmation that the methoxyl groups are structurally significant[9]. Prior to this investigation, only *A. karroo*[11] and *A. senegal*[1] had been reported to contain two aldobiouronic acids; on re-examination, those species previously reported to contain only 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose may be found to be more complex.

The optical rotation data presented for acid fraction (*d*) and its components give an indication of the extent to which heteropolymolecularity[1] is displayed by *A. nilotica* gum. Calculation shows that the proportions of the monomethylaldobiouronic acids *A* and *B* vary, in the nodules examined, from approximately 1:1 to 3:1. Furthermore, with the recovery of these monomethyl acids accounting for the observed methoxyl content, and with no evidence of alternative locations for the methoxyl groups in this or other *Acacia* species[1,5], the inter-nodule variations in methoxyl content suggest that the heteropolymolecularity extends to differences in the proportions of the monomethyl acids (*A* + *B*) and the non-methylated acids (*C* + *D*).

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SUMMARY

Inter-nodule variations in the composition and properties of *Acacia nilotica* gum have been investigated. The results confirm the value of this type of analytical survey of a species, prior to structural studies on a single, representative nodule.

The acidic components of the gum have been examined in detail. For the first time in the genus *Acacia*, four aldobiouronic acids, two of which contain 4-*O*-methyl-D-glucuronic acid, were present and were identified as 4-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-galactose (*A*), 6-*O*-(4-*O*-methyl-β-D-glucopyranosyluronic acid)-D-galactose (*B*), 6-*O*-(β-D-glucopyranosyluronic acid)-D-galactose (*C*), and 4-*O*-(α-D-glucopyranosyluronic acid)-D-galactose (*D*).

The analytical data indicate that there is an inter-nodule variation in the proportions of acids *A* and *B*, and, further, in the proportions of the monomethyl acids (*A* + *B*) and the unsubstituted acids (*C* + *D*).

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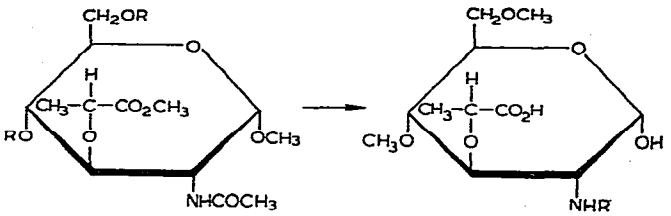
Notes

Synthesis of the 4,6-dimethyl ether of muramic acid: 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-4,6-di-*O*-methyl-α-D-glucose*

Investigation, by the methylation procedure, of the chemical structure of the carbohydrate backbone of bacterial cell wall, and of the tetrasaccharide obtained after degradation with lysozyme[1,2] requires the preparation of various methylated derivatives of 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid) as reference compounds. The present work reports the synthesis of the 4,6-dimethyl ether.

Methylation of methyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methyl carboxylate) ethyl]-α-D-glucopyranoside[3] (I) in *N*,*N*-dimethylformamide solution[4] gave the crystalline 4,6-dimethyl ether (II). This compound was hydrolyzed to give crystalline 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-4,6-di-*O*-methyl-α-D-glucose (III). Crystalline 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-4,6-di-*O*-methyl-α-D-glucose (IV) was obtained by *N*-acetylation of compound (III).



I R=H
II R=CH3

III R=H
IV R=COCH3

EXPERIMENTAL**

*Methyl 2-acetamido-4,6-di-*O*-methyl-2-deoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-D-glucopyranoside* (II)

A mixture of methyl 2-acetamido-2-deoxy-3-*O*-[D-1-(methyl carboxylate) ethyl]-α-D-glucopyranoside[3] (I, 500 mg), methyl iodide (4 ml), *N*,*N*-dimethylformamide (10 ml), and freshly prepared silver oxide (3.0 g) was stirred overnight at room

*Amino Sugars XLVI. This is publication No. 409 of the Robert W. Lovett Memorial Group for the Study of Crippling Diseases, Harvard Medical School at the Massachusetts General Hospital, Boston, Massachusetts. Preliminary communication: *Abstracts Papers Am. Chem. Soc. Meeting*, 145 (1963) 18D.

**For conditions, see previous publication[5].

temperature. The insoluble material was separated by filtration, and washed thoroughly with chloroform. The combined filtrate and washings were evaporated, and the residue (520 mg) was dissolved in benzene and purified by chromatography on silica gel. A 1:1 mixture of ether and ethyl acetate eluted a fraction (400 mg), which crystallized upon evaporation. Recrystallization from ether and pentane gave needles (280 mg, 52%), m.p. 113–115°. After recrystallization from the same solvent mixture, the product had m.p. 114–116° and $[\alpha]_D^{25}$ +137° (*c* 1.10, chloroform).

Anal. Calc. for $C_{15}H_{27}NO_8$: C, 51.56; H, 7.79; OCH_3, 35.53. Found: 51.34; H, 7.71; OCH_3, 35.44.

2-Amino-3-O-(D-1-carboxyethyl)-2-deoxy-4,6-di-O-methyl-α-D-glucose (III)

A solution of II (80 mg) in 2N hydrochloric acid (1 ml) was heated in a sealed tube for 8 h at 100°. The clear solution was passed through a column of Dowex-50 (H^+, 200–400 mesh, 480 × 10 mm) which had been equilibrated with 0.3N hydrochloric acid and washed with water (5 ml). The column was eluted with 0.3N hydrochloric acid and 6 ml fractions were collected, and tested with ninhydrin. Fractions 62–89 showed a positive ninhydrin reaction and were lyophilized. The residue was dissolved in water (25 ml), and shaken with small portions of Dowex-1 (X-8, OH^-) until the solution had reached pH 6. After filtration and evaporation, the residue (30 mg, 50%) was crystallized from ethanol to give needles (18 mg, 30%), m.p. 183–185°. Recrystallization from ethanol raised the m.p. to 187–188°. The compound showed mutarotation: $[\alpha]_D^{22}$ +136° (9 min) → +119° (7 h) (*c* 0.59, water); $R_{2\text{-amino-2-deoxy-D-glucose}}$ 2.75 on Whatman No. 1 paper, descending, in the solvent system 4:1:5 1-butanol–acetic acid–water (upper phase); infrared maxima: 660, 695, 775, 850, 870, 915, 925, 980, 1015, 1050, 1075, 1095, 1120, 1150, 1165, 1200, 1225, 1240, 1275, 1290, 1305, 1320, 1370, 1410, 1450, 1515, 1535, 1565, 1625, 2910, 2930, 2960, 2990, 3010, 3030, and 3340 cm^{-1}.

Anal. Calc. for $C_{11}H_{21}NO_7$: C, 47.30; H, 7.58; OCH_3, 22.22. Found: C, 47.52; H, 7.65; OCH_3, 22.41.

2-Acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-4,6-di-O-methyl-α-D-glucose (IV)

Compound II (100 mg) was hydrolyzed, and the hydrolyzate was chromatographed and neutralized, as previously described. The residue was dissolved in water (2 ml), and acetylated with acetic anhydride (0.3 ml) in the presence of saturated sodium hydrogen carbonate solution (0.6 ml). After 1 h at room temperature, the solution was passed through a column of Dowex-50 (H^+), to remove the sodium ions, and was lyophilized. The residue (40 mg, 44%) was crystallized from methanol–ethyl acetate to give long needles (23 mg, 24%), m.p. 184–186°. The compound showed mutarotation: $[\alpha]_D^{23}$ +69° (15 min) → +65.5° (2 h) (*c* 0.56, water); infrared maxima: 730, 790, 855, 955, 980, 1055, 1095, 1135, 1200, 1260, 1300, 1325, 1385, 1450, 1545, 1620, 1655, 1720, 1770, 2920, 3000, 3080, 3280, 3330, and 3385 cm^{-1}.

Anal. Calc. for $C_{13}H_{23}NO_8$: C, 48.59; H, 7.21; N, 4.36. Found: C, 48.66; H, 7.23; N, 4.31.

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Synthesis of oligosaccharides containing galactose and xylose

Studies by Rodén and coworkers[1–4] have indicated that chondroitin 4-sulfate and heparin are bound to protein by a glycosidic linkage between D-xylose and the hydroxyl group of L-serine. Two galactose residues also seem to be present in the carbohydrate–protein linkage region, a tentative structure of which is outlined in Fig. 1. In order to facilitate and confirm the identification of oligosaccharides obtained in low yields from the linkage region of the two polysaccharides by partial acid hydrolysis, it was desirable to synthesize authentic compounds with structures possibly present in the linkage area. The synthesis of *O*-β-D-xylopyranosyl-L-serine has been previously described[5] and the synthetic compound seems to be identical with the compound isolated from the polysaccharide–protein complexes.

In the present paper, the syntheses of 3-*O*-β-D-galactopyranosyl-D-xylose (**1**), 4-*O*-β-D-galactopyranosyl-D-xylose (**2**), and *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-xylose (**3**) are reported.

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In the present paper, the syntheses of 3-*O*-β-D-galactopyranosyl-D-xylose (**1**), 4-*O*-β-D-galactopyranosyl-D-xylose (**2**), and *O*-β-D-galactopyranosyl-(1→3)-*O*-β-D-galactopyranosyl-(1→4)-D-xylose (**3**) are reported.

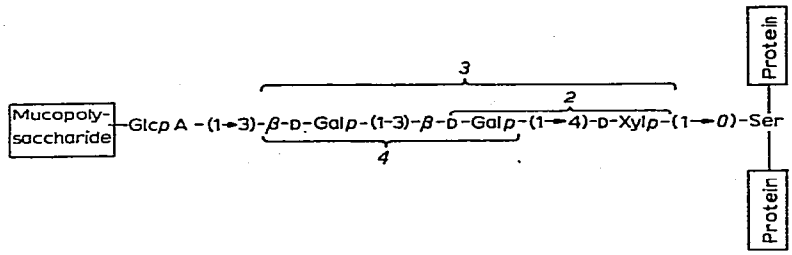

Fig. 1. Tentative structure of the carbohydrate–protein linkage region in the protein complexes of heparin and chondroitin 4-sulfate.

The syntheses followed conventional lines using the Koenigs–Knorr reaction. Compound **1** was prepared by condensation of tetra-*O*-acetyl-α-D-galactopyranosyl bromide with 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose followed by removal of the protective groups. The yield of the crystalline disaccharide, m.p. 194–197°, $[\alpha]_{5780}^{20}$ +12°, was 1.3%. The same disaccharide has recently been prepared enzymically by Gorin *et al.*[6], and the two substances proved to be indistinguishable.

Disaccharide **2** was prepared from tetra-*O*-acetyl-α-D-galactopyranosyl bromide and benzyl 2,3-anhydro-β-D-ribopyranoside[7]. Condensation of the two compounds was followed by deacetylation, alkaline opening of the epoxide, and catalytic hydrogenation yielded **2**, in analogy with the synthesis of 4-*O*-β-D-xylopyranosyl-D-xylose, reported by Aspinall and Ross[8]. The yield of the amorphous product, $[\alpha]_{5780}^{20}$ +15°, was 36%. This disaccharide, m.p. 210–211°, $[\alpha]_{D}^{20}$ +15°, has previously been isolated from a partial acid hydrolyzate of corn-hull hemicellulose[9].

3-*O*-β-D-Galactopyranosyl-D-galactose (**4**) was prepared by partial acid hydrolysis of larch wood arabinogalactan. The trisaccharide **3** was prepared from the *O*-acetylglycosyl bromide of **4** and benzyl 2,3-anhydro-β-D-ribopyranoside, as described above for the synthesis of **2**. The yield of amorphous **3**, $[\alpha]_{D}^{20}$ +18°, was 26%. The structure, evident from the mode of synthesis, was further confirmed by partial acid hydrolysis to give a mixture containing **2** and **4**, and by total acid hydrolysis to give D-galactose and D-xylose. Quantitative analysis showed that D-galactose and D-xylose were present in a molar ratio of 2:1. The amount of trisaccharide obtained was insufficient for the preparation of crystalline derivatives.

TABLE I

PAPER-CHROMATOGRAPHIC AND PAPER-ELECTROPHORETIC MOBILITIES OF THE OLIGOSACCHARIDES.

Oligosaccharide	M_G^a	R_{Gal}^b
β-D-Galp-(1→3)-D-Xyl (1)	1.9	0.83
β-D-Galp-(1→4)-D-Xyl (2)	0.7	0.55
β-D-Galp-(1→3)-D-Gal (4)	1.6	0.43
β-D-Galp-(1→3)-β-D-Galp-(1→4)-D-Xyl (3)	0.6	0.27

[a]Paper electrophoretic mobility, relative to that of D-glucose, in germanate buffer[10] at pH 10.7. [b]Paper chromatographic mobility, relative to D-galactose, in solvent A.

The paper electrophoretic mobilities of the synthetic oligosaccharides in germanate buffer[10] were of the expected magnitudes. These values, together with some R_F values, are given in Table I.

The disaccharides described in this paper have been used for the investigation of the structure of the galactose-xylose linkage in the chondroitin 4-sulfate–protein complex[4]. Though a (1→3) linkage had first been suggested on the basis of periodate oxidation data[3], a comparison with the authentic compounds demonstrated unequivocally that the linkage is (1→4)[4]. Compound **3** appears to be identical with a trisaccharide isolated from the chondroitin 4-sulfate–protein complex, and the characterization of the latter oligosaccharide will be reported in detail elsewhere.

EXPERIMENTAL

Concentrations were carried out under reduced pressure, at 40°. Melting points are corrected. The solvent mixtures used for paper chromatography were : (A), ethyl acetate–acetic acid–water (3:1:1) and (B), ethyl acetate–pyridine–water (10:4:3).

Quantitative analyses for galactose and xylose were carried out by the anthrone[11] and orcinol[12] methods, respectively.

3-O-β-D-Galactopyranosyl-D-xylose (**1**)

A mixture of 5-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-xylofuranose[13] (5.0 g), Drierite (17 g), and freshly prepared silver oxide (4.4 g) in anhydrous, ethanol-free chloroform (20 ml) was stirred in the dark for 1 h. A solution of iodine (0.9 g) and tetra-*O*-acetyl-α-D-galactopyranosyl bromide (14.1 g) in chloroform (50 ml) was added during 3 h, and stirring was continued for 20 h. The reaction mixture was then filtered through a layer of Celite, and the salts were washed with chloroform. The combined filtrate and washings were washed with aqueous sodium thiosulfate, dried over calcium chloride, and concentrated to a sirup (19 g).

The sirup was dissolved in 80% aqueous methanol (250 ml) and stirred with Dowex 1 (OH^-) (250 ml) overnight. The mixture was filtered, the resin was washed with water, and filtrate and washings were concentrated to a sirup. This sirup (3.5 g) was dissolved in 0.05 M hydrochloric acid (175 ml) and kept at 100° for 1.5 h. The hydrolyzate was neutralized with 2 M sodium hydroxide, concentrated, and applied to a carbon–Celite column (31 × 4 cm). The column was washed with water (2 l) and then eluted with aqueous ethanol (4 l), the concentration of which was increased from 0 to 20%, following a linear gradient. Fractions were collected, and examined by paper chromatography in solvent A, and those containing pure **1** were combined and concentrated. The resulting sirup (70 mg) was crystallized from methanol, m.p. 194–197°, $[\alpha]_D^{20}$ +12° (equil.) (*c* 0.7 water).

4-O-β-D-Galactopyranosyl-D-xylose (**2**)

Benzyl 2,3-anhydro-β-D-ribopyranoside[7] (8.9 g) was condensed with tetra-*O*-acetyl-α-D-galactopyranosyl bromide (16.5 g), in a Koenigs–Knorr reaction, as

described above for the synthesis of **1**. The resulting sirupy reaction product (25 g) was heated in 2 M aqueous sodium hydroxide (500 ml) at 100° for 16 h. The cooled solution was deionized by passage through columns of Dowex 50 (H^+) and Dowex 3 (free base), and concentrated to a sirup (8.2 g).

The sirup was dissolved in 50% aqueous ethanol (250 ml) and stirred, in an atmosphere of hydrogen, with 10% palladium–charcoal (5 g). After 5 h, when the uptake of hydrogen (about 360 ml) had ceased, the catalyst was removed by filtration, and the filtrate was concentrated to a sirup (4.5 g). As revealed by paper chromatography in solvent A, this sirup consisted essentially of disaccharide **2**, contaminated by xylose, galactose, and some unknown compounds. Part of the sirup was purified by cellulose column chromatography in solvent A, yielding chromatographically pure **2**. The disaccharide, which has not crystallized, showed $[\alpha]_{5780}^{22}$ +15° (*c* 0.5, water).

O-*β*-D-*Galactopyranosyl*-(*1*→*3*)-O-*β*-D-*galactopyranosyl*-(*1*→*4*)-D-*xylose* (**3**)

3-*O*-*β*-D-Galactopyranosyl-D-galactose (**4**) was obtained by graded hydrolysis of larch (*Larix occidentalis*) arabinogalactan[14,15]. The polysaccharide (50 g) was subjected to mild hydrolytic treatments, first with 0.02 M hydrochloric acid (2 l) at 100° for 4.5 h, to remove arabinose, then three times with 0.2 M hydrochloric acid (1 l) at 100° for 1 h. After each hydrolysis, the high-molecular-weight material was recovered by neutralization with ion-exchange resin, concentration to 200 ml, and precipitation with ethanol (1800 ml). The supernatant fluids from the last three ethanol precipitations (containing mono- and oligo-saccharides) were combined, concentrated to a sirup (32 g), and fractionated by chromatography on a carbon–Celite column (70 × 10 cm), using a gradient elution with aqueous ethanol. The effluent was analyzed by paper chromatography in solvent B, and the fractions containing pure **4** were combined, concentrated, and crystallized from aqueous ethanol, to yield the pure substance (0.75 g), m.p. 159–162°, $[\alpha]_D^{22}$ +64° (*c* 1.0, water).

Compound **4** (0.50 g) was acetylated with acetic anhydride (5 ml) and sodium acetate (0.5 g) in the usual manner to yield the amorphous disaccharide acetate (1.0 g). This acetate was dissolved in a mixture of acetic acid (1 ml) and acetyl bromide (2 ml). The solution was kept at room temperature for 2 h, diluted with chloroform (5 ml), and poured into ice-water (10 ml). The chloroform phase was washed with water and aqueous sodium hydrogen carbonate, dried with calcium chloride, and concentrated to a sirup (0.8 g).

The sirupy acetylated glycosyl bromide of **4** (0.8 g) was condensed with benzyl 2,3-anhydro-*β*-D-ribopyranoside (0.45 g), and the reaction product was subjected to hot alkaline treatment, deionization, and catalytical hydrogenation, as described above for the analogous synthesis of **2**. The product (0.18 g), obtained after these treatments was fractionated by paper chromatography on Whatman 3MM filter papers to yield the chromatographically pure, amorphous trisaccharide (0.13 g), $[\alpha]_D^{22}$ +18° (*c* 1.0, water).

Total hydrolysis of **3** (1 N hydrochloric acid, 3 h, 100°) yielded only galactose and xylose. The presence of the disaccharides **2** and **4** in a partial hydrolyzate (0.2 N

hydrochloric acid, 1 h, 100°) of **3** was demonstrated by paper chromatography in solvent A. Quantitative analyses showed that **3** contained 68.7% of galactose and 23.4% of xylose (molar ratio 2.01: 1).

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Preparation of phenyl and *p*-nitrophenyl α-D-glucopyranosides for use in the assay of yeast maltase

Phenyl α-D-glucopyranoside and the corresponding *p*-nitrophenyl derivative have both been recommended as suitable substrates for the assay of yeast maltase (α-D-glucoside glucohydrolase, EC 3.2.1.20)[1]. Several descriptions[2] of the preparation of these substances have been published, but the experimental detail given is often scant, and the recommendations of different authors are not always in agreement. After numerous trials, the techniques described below were selected as being fairly simple and reproducible. With their aid, the phenyl glycoside is readily prepared, though the preparation of the *p*-nitrophenyl glycoside requires rather more care.

EXPERIMENTAL

Phenyl tetra-O-acetyl-α-D-glucopyranoside

Regulation of temperature (oil bath, thermostatically controlled at 120°) was important in the Helferich reaction[2], since, above 140°, a great deal of tar was formed. As the reaction was initially exothermic, the reaction mixture, which was viscous and difficult to stir, was shaken mechanically.

Phenol (96 g) and penta-*O*-acetyl-α-D-glucopyranose[3] (100 g) were melted together, and powdered zinc chloride (25 g) was added. The mixture was shaken for 2 h at 120° in a slow stream of nitrogen, and then extracted with benzene (700 ml) at *ca.* 60°. After being cooled to room temperature, the benzene solution was decanted, and the residue was extracted with more benzene (300 ml). The combined extracts were washed successively with ice-cold water, chilled 5% aqueous sodium hydroxide (4×200 ml), water, enough N acetic acid to bring the pH of the aqueous layer to 4, and water. The extract was dried ($CaCl_2$), filtered, and concentrated under diminished pressure. A solution of the partly crystalline syrup in warm ethanol (500 ml) was left at room temperature; on shaking (and if necessary seeding), the acetyl derivative crystallised. The mixture was then chilled overnight, and the product was collected, washed with ethanol (100 ml), and air-dried. A second crop was obtained by concentrating the filtrates (to 100 ml).

Though the yield was low (28%), the product was mainly the α-D form (97%). After one recrystallisation from ethanol (3 ml/g), phenyl tetra-*O*-acetyl-α-D-glucopyranoside (28 g), $[\alpha]_D^{20}$ +168° (*c* 2.0, chloroform), was obtained.

When the reaction was carried out at 20 mm, as described by Montgomery *et al.*[4], the yield of crude product (72 g) was much greater, but this contained only 47 g of the α-D form. The ratio of the solubilities in ethanol of the tetra-*O*-acetyl-α-D and -β-D derivatives was found to be 0.64, and by recrystallisation only 31 g of the α-D form could be recovered. In view of the ready availability[3] of penta-*O*-acetyl-α-D-glucopyranose, the low yield of the first method was more than counterbalanced by its simplicity.

Phenyl α-D-glucopyranoside

Phenyl tetra-*O*-acetyl-α-D-glucopyranoside was dissolved in boiling methanol (10 ml/g), 0.5M methanolic sodium methoxide (1 ml/g) was added, and the solution was boiled for 5 min and then left overnight at room temperature. An equal volume of water was added, and the solution was deionised with Amberlite Monobed MB-1 resin (1.5 g/g of tetra-*O*-acetyl derivative). Elution with 50% aqueous methanol gave the glucoside (97%), and slow crystallisation from isopropyl alcohol (solubility, 0.7 g per 100 ml at room temperature), gave the title compound, m. p. 173° (lit.[2], 173–174°) and $[\alpha]_D^{20}$ +182° (*c* 1, water) (lit., +181°). From moist ethyl acetate[5], the monohydrate separated; the water of crystallisation was retained after air-drying, but was lost over desiccants.

p-Nitrophenyl α-D-glucopyranoside

With solid zinc chloride as catalyst, the Helferich reaction at atmospheric pressure gave very poor yields of the tetra-*O*-acetyl derivative. Consequently, a solution of zinc chloride (25 g) in a mixture of acetic acid (75 ml) and acetic anhydride (5 ml) was used[4]. The reaction between *p*-nitrophenol (144 g) and penta-*O*-acetyl-α-D-glucopyranose (90 g) was allowed to proceed for 45 min at 125°/20 mm.

The *p*-nitrophenyl compounds were extremely unstable to alkali[6]. The benzene extract was therefore washed successively with water, 2.5% (to avoid the separation of sodium *p*-nitrophenate) and 5% aqueous sodium carbonate, water, dilute acetic acid, and water. A hot solution in ethanol (500 ml) of the syrup obtained by evaporation of the benzene was treated with activated carbon, filtered, and left to cool *very slowly* overnight to room temperature, after which it was chilled to 4° for several days. Rapid cooling caused the separation of a glass, which could be induced to crystallise only with difficulty. The product (25 g, 23%) contained 80% of the α-D form.

Published statements[4,7] on the relative solubility of the α-D and β-D forms in ethanol are not consistent. We completely failed to resolve the anomers by recrystallisation from ethanol, isopropyl alcohol, or benzene–light petroleum (1:2, v/v). Fortunately, the *p*-nitrophenyl glucosides themselves could be readily separated.

The crude *p*-nitrophenyl tetra-*O*-acetyl-α- and -β-D-glucopyranosides were dissolved in hot ethanol (2.5 ml/g), and sodium methoxide (0.15 mmole/g) was added to the warm solution, which was allowed to cool and then chilled overnight. The addition of a little glacial acetic acid removed the yellow colouration of the crystals, which were collected and dissolved under reflux in methanol (7.5 ml/g of tetra-*O*-acetyl derivative). Charcoal was added, and the hot solution was filtered, allowed to cool, and chilled overnight. The product had m.p. 214°, $[\alpha]_{20}^{D}$ +219° (*c* 1, water); the pure α-D form has m.p. 216°, $[\alpha]_D^{20}$ +215° (water)[2]. The yield was 44% in the deacylation step; another 13% could be recovered from the mother liquors.

The lower yields of the *p*-nitrophenyl glycoside appear to reflect the greater instability of this substance, but no difficulty should be experienced in making enough for several hundred enzyme assays.

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Announcement

In view of the exceptionally good response to *Carbohydrate Research* by authors, we are now in a position to expedite publication of papers by initiating a monthly publication schedule ahead of the dates previously proposed. It has now been decided that no further bi-monthly issues will appear. Therefore Vol. 3 will commence with issue No. 1 in November 1966. Three volumes of 500 pages each will be published between November 1966 and December 1967.

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2,4:3,5-DI-*O*-BENZYLIDENE-D-GLUCITOL

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INTRODUCTION

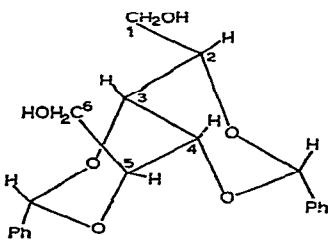
The diacetal obtained by condensing 1,6-di-*O*-benzoyl-D-glucitol with benzaldehyde, in the presence of zinc chloride, was assumed to have a 2,4:3,5-arrangement of acetal rings[1]. Recently, this assignment of acetal groups was confirmed by n.m.r. spectroscopy[2]. The chemical evidence below provides an independent proof of the structure.

RESULTS AND DISCUSSION

The parent diacetal (**1**), obtained by saponification of the dibenzoate, yielded a diacetate and a dimethyl ether, both crystalline. Proof that the two free hydroxyl groups of the diacetal were at the 1- and 6-positions was obtained as follows. Its dimethyl ether, on mild, acid hydrolysis, yielded a syrupy D-glucitol dimethyl ether (**2**) which, in turn, afforded a crystalline tetra-acetate. The tetra-acetate, after deacetylation, consumed 2.8 mol. of periodate, liberating 2.0 mol. of formic acid and methoxyacetaldehyde, characterised as its known *p*-nitrophenylhydrazone (1.5 mol.). The dimethyl ether (**2**) migrated on molybdate ionophoresis as expected[3] for a 1,6-disubstituted glucitol.

The positions and sizes of the acetal rings in the diacetal (**1**) were not determined by partial, acid hydrolysis, because this reaction was shown to lead to acetal migration[4]. Instead, partial hydrogenolysis was used; this reaction was shown to avoid migration. Chromatography on alumina of the reaction mixture yielded a glassy solid and the known 2,4-*O*-benzylidene-D-glucitol (**3**). The 2,4-acetal (**3**) was shown to be identical with an authentic sample prepared, in good yield, by directly condensing D-glucitol and benzaldehyde (*cf.* preparation of 2,4-*O*-furfurylidene-D-glucitol[5]). Acetylation of the glassy solid gave a compound which gave correct elemental analyses for a tetra-*O*-acetyl-mono-*O*-benzylidenehexitol. Acid hydrolysis of the saponified tetra-acetate showed the hexitol to be D-glucitol (isolated as its hexa-acetate); benzaldehyde was obtained as its dimedone (5,5-dimethylcyclohexane-1,3-dione) derivative. That the benzylidene group was in fact spanning the 3,5-positions (**4**) was shown by periodate oxidation (1.02 mol. of oxidant being consumed and 0.94 mol. of formaldehyde being liberated). Mild, acid hydrolysis of the main oxidation fission product yielded D-arabinose, which was characterised as its bisphenylboronate.

Borohydride reduction of 2,4-*O*-benzylidene-*aldehydo*-D-arabinose from the oxidation yielded 2,4-*O*-benzylidene-D-arabinitol, isolated as its crystalline triacetate. Saponification, followed by mild, acid hydrolysis, gave material which moved as arabinitol on paper chromatography.



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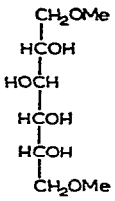

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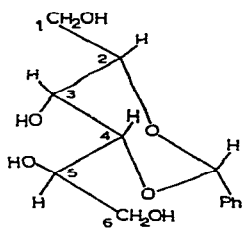

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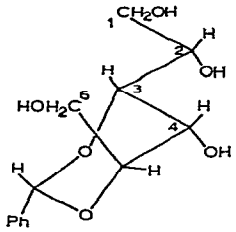

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A 3,5-acetal of D-glucitol (**4**) and 2,4-acetals of D-arabinose and D-arabinitol are less thermodynamically stable than other monoacetals which can be formed by the parent unsubstituted compounds (*cf.* Mills[6]). In particular, the D-arabinose acetal must exist with the sugar moiety in the open-chain form.

Hydrogenolysis thus provides a synthetic route to thermodynamically unstable benzylidene acetals not readily accessible by acid hydrolysis or synthesis. Di- and tri-acetals are liable to isomerise during treatment with acid and, in cases of this sort, evidence from hydrogenolysis is likely to be a more reliable indication of structure.

EXPERIMENTAL

Whatman No. 1 paper was used for paper chromatography. Solvent (*A*) was butan-1-ol–ethanol–water (40:11:19, v/v). Potassium periodate–silver nitrate–sodium hydroxide or 2,4-dinitrophenylhydrazine[7] were used for detection. Quantitative periodate oxidations[8], and formaldehyde[9,10] and formic acid determinations[11] were performed by standard procedures. 2,4:3,5-Di-*O*-benzylidene-D-glucitol (m.p. 205–207°) was prepared by the method[12] of Haworth *et al.*, who recorded m.p. 208°.

1,6-Di-O-acetyl-2,4:3,5-di-O-benzylidene-D-glucitol

The diacetal (0.50 g) gave needles of the diacetate (0.41 g, 66%), m.p. 188.5–190° (from 10 parts of benzene), $[\alpha]_D^{25}$ −10.9° (*c* 1.8, chloroform). (Found: C, 65.1; H, 5.8; Ac, 19.15. $C_{24}H_{26}O_8$ calc.: C, 65.15; H, 5.9; Ac, 19.5%).

2,4:3,5-Di-O-benzylidene-1,6-di-O-methyl-D-glucitol

The diacetal (3.0 g), dry methyl iodide (4.7 ml), silver oxide (3 g), and dry *N*,*N*-dimethylformamide (9 ml) were shaken for 27 h at room temperature. The silver salts and volatile material were removed in the usual way, and the product was purified on alumina (75 g), using benzene as eluent. The material (0.6 g, m.p. 104–109°) in fraction 1 (50 ml) could not be purified by crystallisation. The remaining material from the column gave the dimethyl ether (0.98 g, 30%), m.p. 119–120°,

$[\alpha]_D^{22}$ +0.5° (*c* 2.1, chloroform), as needles from 8 parts of ethanol (Found: C, 68.2; H, 6.8; OMe, 16.3. $C_{22}H_{26}O_6$ calc.: C, 68.35; H, 6.8; OMe, 16.1%).

*2,3,4,5-Tetra-*O*-acetyl-1,6-di-*O*-methyl-*D*-glucitol*

2,4:3,5-Di-*O*-benzylidene-1,6-di-*O*-methyl-D-glucitol (0.30 g) was refluxed for 15 min with a mixture of ethanol (1 ml) and 0.1N hydrochloric acid (1 ml). The mixture was concentrated. The acid treatment and concentration were repeated, and then sodium hydrogen carbonate (0.02 g) was added and the whole evaporated to dryness. 1,6-Di-*O*-methyl-D-glucitol had R_F 0.51 in solvent (*A*) and M_S (rate relative to glucitol) 0.94 on molybdate[3] ionophoresis. Acetylation of the residue yielded, after crystallisation from a mixture of light petroleum (3 ml) and ethanol (0.3 ml), the 2,3,4,5-tetra-acetate (0.20 g, 68%), m.p. 72–74°, $[\alpha]_D^{13.5}$ +24.0° (*c* 1.5, chloroform) (Found: C, 50.7; H, 6.7; OMe, 16.5; Ac, 45.8. $C_{16}H_{26}O_{10}$ calc.: C, 50.8; H, 6.9; OMe, 16.4; Ac, 45.55%).

*Periodate oxidation of 1,6-di-*O*-methyl-*D*-glucitol*

Tetra-*O*-acetyl-1,6-di-*O*-methyl-D-glucitol (1 mol.) was deacetylated with methanolic sodium methylate. The methanol was removed, and the residue consumed 2.7, 2.8, and 2.8 mol. of periodate (7.55 mol. initially present) (theor., 3.0) after 0.5, 2.0, and 7.3 h, respectively, and gave 2.0 mol. of formic acid (corrected for methoxide) (theor., 2.0).

The tetra-acetate (0.036 g) was deacetylated as above, and the solvent-free residue was treated with sodium periodate (0.08 g) in water (2 ml). After 0.5 h, the solution was concentrated, more water (1.5 ml) was added, and the solution was concentrated. The total distillate was treated with a warm solution of *p*-nitrophenylhydrazine[13]. Crystallisation of the precipitate from aqueous ethanol gave methoxyacetaldehyde *p*-nitrophenylhydrazone (0.030 g, 1.5 mol.), m.p. and mixed m.p. 113–116°.

*Partial hydrogenolysis of 2,4:3,5-di-*O*-benzylidene-*D*-glucitol*

The diacetal (4 g) in methanol (170 ml) was added to pre-hydrogenated palladium black (3–4 g) in methanol. The suspension was allowed to stand, with occasional shaking, until *ca.* 0.5 l of hydrogen was consumed (1–6 days). The catalyst was removed, and the solution was concentrated. The residue, dissolved in absolute ethanol (200 ml), was passed through alumina (100 g). Fraction 1, eluted with absolute ethanol (0.6 l), gave unchanged diacetal (1.3 g), R_F in solvent (*A*) 0.91; using dimethyl sulphoxide–benzene (1:50, v/v) on dimethyl sulphoxide[14], R_F 0.38 (1,3:2,4-di-*O*-benzylidene-D-glucitol[15], R_F 0.43, was absent). Increasing amounts of water (up to 5%, v/v) in the eluent gave 3,5-*O*-benzylidene-D-glucitol (2,4-*O*-benzylidene-L-gulitol), R_F 0.71 in solvent (*A*). Industrial methylated spirit–water (98:2, v/v) gave 2,4-*O*-benzylidene-D-glucitol (*ca.* 0.2 g), m.p. and mixed m.p. with the sample prepared below, 172–174.5°, R_F 0.74 in solvent (*A*). The 3,5-benzylidene acetal yielded a tetra-acetate (0.4–0.6 g), m.p. 98–99° (from ethanol), $[\alpha]_D^{27}$ −13.8° (*c* 1.55,

chloroform), (Found: C, 57.25; H, 5.9; Ac, 39.3. $C_{21}H_{26}O_{10}$ calc.: C, 57.5; H, 6.0; Ac, 39.3%).

2,4-O-Benzylidene-D-glucitol

The acetal was prepared by using a modification of the preparation[5] of 2,4-*O*-furfurylidene-D-glucitol. D-Glucitol (9 g), dissolved in 3N sulphuric acid (2.5 ml), was treated with benzaldehyde (5 ml) and warmed to 70°. After cooling and standing overnight, the mixture was crystallised from water (50 ml) containing sodium hydrogen carbonate (0.8 g), any insoluble material being removed by filtration. The product (5.6 g, 42%) had m.p. 173–175° (lit.[16], 176–177°).

Hydrolysis of 3,5-O-benzylidene-D-glucitol

Tetra-*O*-acetyl-3,5-*O*-benzylidene-D-glucitol (0.0281 g) was saponified, and the residue was hydrolysed, as described[17] for 4,6-*O*-butylidene-D-glucitol, but using 0.1N acid (3 ml). The benzaldehyde bisdimedone derivative (65%) had m.p. and mixed m.p. 194–196°. The D-glucitol hexa-acetate (78%) had m.p. and mixed m.p. 97–100°.

Periodate oxidation of 3,5-O-benzylidene-D-glucitol

Tetra-*O*-acetyl-3,5-*O*-benzylidene-D-glucitol (1 mol.) was deacetylated with methanolic sodium methylate. The methanol was removed, and the residue consumed 0.80, 0.87, 0.95, and 1.02 mol. of periodate (3.4 mol. initially present) (theor., 1.0) after 0.5, 5.5, 12.5, and 23 h, respectively, and gave 0.94 mol. of formaldehyde (theor., 1.0) after 25 h. Under similar conditions, tetra-*O*-acetyl-2,4-*O*-benzylidene-D-glucitol, after deacetylation, consumed 0.99 mol. of periodate (theor., 1.0).

Tetra-*O*-acetyl-3,5-*O*-benzylidene-D-glucitol (0.58 g) was treated with 0.2N methanolic sodium methylate (0.8 ml). The methanol was removed, and a solution of sodium periodate (0.4 g) in water was added. After 3 h, the suspension was freeze-dried, and the residue was extracted with boiling ethyl acetate. Part (0.08 g) of the extracted material [*ca.* 0.28 g, R_F 0.87 (single spot) in solvent (*A*)] was hydrolysed as described above for the acid hydrolysis of 3,5-*O*-benzylidene-D-glucitol. The dry, neutralised residue, which moved as arabinose, but not xylose, in solvent (*A*), was treated with phenylboronic anhydride (0.08 g) in boiling methanol. The methanol was evaporated, and the residue was extracted with boiling light petroleum. Four crystallisations from light petroleum gave D-arabinose bisphenylboronate (0.012 g, 11%), m.p. 157–159°, mixed m.p. with authentic D-arabinose bisphenylboronate (see below, m.p. 159–161.5°), 157–158°; the infrared spectra (KBr discs) were identical.

D-*Arabinose bisphenylboronate*

This compound, m.p. 159–161.5°, $[\alpha]_D^{18}$ −8.4° (*c* 1.8, dry benzene). (Found: C, 63.7; H, 5.1. $C_{17}H_{16}B_2O_5$ calc.: C, 63.4; H, 5.0%), was prepared from D-arabinose (0.3 g), in 75% yield, as described above. L-Arabinose bisphenylboronate[18] has m.p. 166°, $[\alpha]_D^{25}$ +8.5° in benzene.

1,3,5-Tri-O-acetyl-2,4-O-benzylidene-D-arabinitol.

2,4-*O*-Benzylidene-*aldehydo*-D-arabinose (0.1 g, from the oxidation) was reduced by borohydride, as described[17] for the reduction of 4,6-*O*-butylidene-D-glucose. The product was acetylated and yielded, from ethanol, 1,3,5-tri-*O*-acetyl-2,4-*O*-benzylidene-D-arabinitol (0.05 g), m.p. 79–80° (Found: C, 58.9; H, 5.9. $C_{18}H_{22}O_8$ calc.: C, 59.0; H, 6.0%).

Hydrolysis of 2,4-O-benzylidene-D-arabinitol

The triacetate was deacetylated, and the residue was hydrolysed with 0.1N hydrochloric acid in the usual way. The hydrolysate moved as arabinitol (R_F 0.27), and not xylitol (R_F 0.25), in solvent (*A*).

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SUMMARY

Chemical proof is given that the acetal groups in the known 2,3,4,5-di-*O*-benzylidene-D-glucitol span the 2,4- and 3,5-positions.

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ALKALINE HYPOCHLORITE OXIDATION OF METHYL β-CELLOBIOSIDE

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INTRODUCTION

Methyl β-cellobioside has been used as a model compound in studying the reactions occurring during the bleaching of cellulose. Wolfrom and Lucke[1] reported kinetic studies on the oxidation of methyl β-cellobioside with sodium hypochlorite at pH 9 and 50.5°. The purpose of the present work was the isolation, characterization, and quantitative determination of the major products formed in this reaction. Simple methyl glycosides were used by Dyfverman, Lindberg, and Wood[2–4] as model compounds for polysaccharides. They found that methyl glycosides were oxidized by chlorine water, under acidic conditions, to give mainly the corresponding aldonic acids. Henderson[5] identified D-glucose, D-arabinose, carbon dioxide, and oxalic acid as the major products formed on oxidation of methyl β-D-glucopyranoside with sodium hypochlorite at pH 4.5. Whistler, Linke, and Kazeniac[6] studied the oxidation at 25° of methyl 4-*O*-methyl-β-D-glucopyranoside with 10 equivalents of sodium hypochlorite buffered at pH 9. They found that, under these conditions, there was a preferential attack between C-2 and C-3 of the glycoside, as indicated by the isolation of glyoxylic acid and glyoxal from the hydrolyzate of the oxidation mixture. Theander[7,8] oxidized methyl β-D-glucopyranoside with hypochlorite at different pH values and found that, under acidic conditions, a maximum yield of D-gluconic acid was obtained with only traces of glyoxylic acid and D-erythronic acid. He found a maximum formation of glyoxylic and D-erythronic acids near neutrality. Selective attack between C-2 and C-3 of the D-glucose units in glucans has also been reported when they were oxidized by sodium hypochlorite under neutral or alkaline conditions. Whistler and co-workers[6,9] oxidized amylose and amylopectin and found the C-2–C-3 cleavage reaction to be very specific between pH 7 and 9, whereas at pH 3 only traces of glyoxylic acid and D-erythronic acids were formed. Similar results were reported by McKillican and Purves[10], who, on oxidation of starch with hypochlorous acid at pH 4, found an 80–90% attack at the primary alcohol group and none between C-2 and C-3. On the other hand, Eisenbraun and Purves[11] reported that, on the oxidation of starch with calcium hypochlorite at pH 12, oxalic acid, isolated directly from the oxidation mixture, and D-erythronic acid, from the hydrolyzate, were the major products. The influence of the hydrogen-ion concentration on the hypochlorite oxidation of cellulose was studied by Birtwell, Clibbens, and Ridge[12], and by Kaverz-

neva, Ivanov, and Solova[13]. Both groups of investigators reached the conclusion that, under slightly acidic or neutral conditions, reducing-type oxidized celluloses were obtained, whereas under alkaline conditions, particularly between pH 9 and 11, a maximum of carboxyl groups and a minimum of ketonic groups were produced.

A model compound, methyl β-cellobioside, was used, in our present investigation, to study the preferential location of the carboxyl groups when the oxidation was effected at 50° with a sodium hypochloride solution buffered at pH 9. A large excess of oxidant was employed to increase the yield of the final products.

EXPERIMENTAL

Materials

Methyl β-cellobioside was prepared from α-cellobiose octaacetate by the procedure of Wolfrom and Haq[14,15]; m.p. 193°, $[\alpha]_D^{20}$ −19° (water). Reference D-erythronolactone was prepared by the oxidation of D-arabinoascorbic acid according to Weidenhagen and co-workers[16]. Sodium hypochlorite was standardized from commercial Clorox (Procter and Gamble).

X-Ray powder diffraction data

X-Ray powder diffraction data refer to interplanar spacings in Å with Cu K_α radiation. Relative intensities were estimated visually: s, strong; m, moderate; w, weak; v, very. The first three strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities.

Chromatographic methods

Thin-layer chromatography was performed on microcrystalline cellulose[17] (Avicel-Technical Grade, formerly known as Avirin; Avicel Sales Division of American Viscose Division, FMC Corp., Marcus Hook, Pa.) and on Silica Gel G (E. Merck, Darmstadt, Germany); paper chromatography was done on Whatman No. 1 paper. Developers used were: *A*, butanone–acetone–water–formic acid (40 : 2 : 6 : 1 v/v) and *B*, ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4 v/v). Spray indicator reagents used were: (*a*), silver nitrate–sodium hydroxide[18]; (*b*), aniline hydrogen phthalate[19]; (*c*), sulfanilamide–β-naphthol–sodium nitrite[20]; (*d*) hydroxylamine–ferric chloride[21]; (*e*) sodium periodate–potassium permanganate[22].

Oxidation of methyl β-cellobioside

Methyl β-cellobioside (4.88 g, 0.013 mole) was dissolved in 700 ml of 0.4M sodium hypochlorite solution buffered with sodium bicarbonate–sodium carbonate to pH 9. Approximately 20 moles of hypochlorite per mole of glycoside were used. The solution was maintained in a thermostatted bath for 5 h at 50°. It was then acidified to pH 3–4 by stirring with Amberlite IR-120 (H^+) resin and the excess of chlorine was eliminated with a stream of air until a negative reaction was obtained with iodide–starch paper.

Identification and assay of oxalic acid and formaldehyde

The chlorine-free oxidation solution was neutralized with calcium hydroxide; the precipitate obtained gave a positive reaction (red color) for oxalic acid[23], by reduction to glyoxylic acid and subsequent reaction with phenylhydrazine and hydrogen peroxide in the presence of hydrochloric acid. Oxalic acid was isolated by treating the barium salt from another experiment with the calculated amount of sulfuric acid; the filtrate was taken to dryness under diminished pressure and the acid obtained was purified by sublimation. Identification was made by comparison with an authentic sample of sublimed oxalic acid, by X-ray powder diffraction data: 5.90 m (3), 4.73 m (2), 3.43 m, 3.01 s, (1), 2.90 w, 2.81 m, 2.57 w, 2.51 w, 2.41 m, 2.35 w, 2.32 w.

Oxalic acid was further identified as phenylhydrazine oxalate, m.p. and mixed m.p. 180°, as reported by Henle and Schupp[24], X-ray powder diffraction data: 16.10 s, (3), 5.52 s, 4.45 vs (1), 4.12 m, 3.93 s, 3.71 w, 3.54 s (2), 3.10 m, 3.03 w, 2.86 m, 2.79 w, 2.56 s.

The amount of oxalic acid was determined by permanganate oxidation[25] (Table I).

TABLE I

OXIDATION PRODUCTS OF METHYL β-CELLOBIOSIDE WITH SODIUM HYPOCHLORITE AT pH 9 AND 50°

Product	*Mole of product per mole of glycoside*	*Product*	*Mole of product per mole of glycoside*
D-Erythronic acid[a]	0.50	D-Glucose[a,b]	0.14
D-Glyceric acid[a]	0.45	D-Arabinose[a,b]	0.03
Glyoxylic acid[a]	0.44	Total aldehyde[a]	0.68
Formic acid[a]	0.33	Glycolic acid[a]	Trace
Oxalic acid[b]	0.04	Formaldehyde[b]	0.005

[a]Identified after hydrolysis of the oxidation mixtures. [b]Identified in the oxidation mixture before hydrolysis.

Formaldehyde

An aliquot of the filtrate from the calcium oxalate precipitation was distilled in a Kjeldahl apparatus; the distillate gave a positive reaction for formaldehyde with chromotropic acid, and this was assayed[26] (Table I).

Hydrolysis of the oxidized mixture

After filtration of the calcium oxalate, the solution was made N in acid by the addition of dilute sulfuric acid, and the chlorate ion was destroyed with sulfur dioxide. The solution was again adjusted to N in acid, and hydrolyzed by heating in a water bath for 14 h at 90°. Barium carbonate was added to remove sulfate, followed by a few drops of dilute hydrochloric acid to make the solution weakly

acid (pH 3–4) before filtration. This is the hydrolyzate referred to in the remainder of the paper.

Formic acid

An aliquot of the acid hydrolyzate, freed from formaldehyde as described above, was concentrated under diminished pressure and the distillate was collected in an ice-cooled flask. Formic acid was characterized qualitatively in the distillate, after reduction to formaldehyde with magnesium, by the reaction with chromotropic acid[27], and also by precipitating the dimedone derivative, m.p. 187–189°. Formic acid was also determined in the distillate by heating with mercuric chloride in an acetate-buffered solution and weighing the mercurous chloride precipitate[28] (Table I).

Chromatographic examination of the hydrolyzate

An aliquot of the acid hydrolyzate was decationized by passing through a column of Amberlite IR-120 (H^+) ion-exchange resin. The effluent was concentrated under diminished pressure, the hydrochloric acid was removed by treatment with silver carbonate, and the excess of silver ion was removed by stirring with Amberlite IR-120 (H^+). Paper chromatography, using double development with solvent *A*, (5 h for each development, with 1 h of air-drying between), showed, with indicator (*a*), compounds having R_G (G = glucose) values 1, 1.52, 3.31, 4.46, 5.32, and 6.44, corresponding, respectively, to glucose, arabinose, erythronic acid, glyoxylic acid, glyceric acid, and erythronolactone. Other faint spots that could not be identified, had R_G values of 0.52, 8.06, and 8.68. With the spray reagent (*d*), which is specific for lactones only, the compound having R_G 6.44, corresponding to erythronolactone, appeared as a purple spot. With spray reagent (*c*) the acids having $R_{Glycerlc}$ 0.60, 1.00, and 1.40, corresponding, respectively, to erythronic, glyceric, and glycolic acids, were shown as orange spots, glyoxylic acid ($R_{Glycerlc}$ 0.81) appeared as a yellow spot; not identified were faint spots having $R_{Glycerlc}$ values of 0.2, 1.60 (yellow), and 1.67. When aniline hydrogen phthalate[19] was used as spray reagent, the component having mobility corresponding to that of glucose showed as a brown spot, the one corresponding to D-arabinose showed as a reddish-brown spot, and glyoxylic acid appeared as a light yellow spot. The same components were shown by developing with solvent *B* on paper or on thin-layers of microcrystalline cellulose[17], and using indicator (*e*). A compilation of all the identified mobility values obtained is shown in Table II.

Glyoxylic acid, isolation and identification; isolation of the barium salt of an oxidation product

Five g of methyl β-cellobioside was oxidized under the conditions described above. After precipitation of the calcium oxalate, the solution was concentrated under diminished pressure to 200 ml, acidified by stirring with Amberlite IR-120 (H^+), filtered, and neutralized with barium hydroxide; 0.160 g of a barium salt of an organic acid was obtained. (Found: Ba, 39.28).

This salt was fairly insoluble, even in hot water, and gave a deep blue color when a small sample was heated in a water bath with 2,3,4-trihydroxybenzoic acid in con-

centrated sulfuric acid, a very specific color test for glyoxylic acid[29]. A sample of 0.100 g of the salt was treated with 5 ml of N sulfuric acid; after 30 min in a water bath at 70°, the suspension was filtered, and the solution was heated in a water bath for 5 h at 90°. The solution was neutralized with barium carbonate, slightly

TABLE II

CHROMATOGRAPHIC PROPERTIES OF THE OXIDATION PRODUCTS

	Paper[a]		*Thin-layer*[a]
	Developer A[b] R_G	*Developer B*[b] R_G	*Developer B* R_F
Methyl β-cellobioside	0.70		
Glucose	1.00	1.00	0.04
Arabinose	1.52	1.50	0.16
Erythronic acid	3.31	1.86	0.24
Glyoxylic acid	4.46	2.29	0.34
Glyceric acid	5.32	2.55	0.50
Erythronolactone	6.44	3.04	0.55
Glycolic acid	7.48		0.62
Oxalic acid			0.65
Arabinonic acid[c]			0.18
Erythraric acid[c]			0.30
Gluconic acid[c]			0.15

[a] See section on chromatographic methods. [b] Chromatogram developed for 5 h, dried in the air for 1 h, and again developed for 5 h. [c] Not identified in the oxidation mixture.

acidified with hydrogen chloride, and filtered. The solution was concentrated under diminished pressure and chromatographed on paper with developer *B*. Spray reagent (*a*) showed compounds having the same mobilities as glyoxylic acid, erythronolactone, and glyceric acid. The syrup was treated with 0.030 g of dimedone dissolved in 2 ml of 40% ethanol. A crystalline precipitate was obtained after 18 h at room temperature. Thin-layer chromatography on Silica Gel G with ethyl ether–hexane–acetic acid (5 : 5 : 0.2 v/v) as developer, and alkaline potassium permanganate[30] as spray indicator, showed glyoxylic acid dimedone, R_F 0.37, in the largest amount, and traces of free dimedone, R_F 0.09. The crude dimedone was purified by fractional sublimation and was recrystallized from 50% aqueous acetone. Glyoxylic acid dimedone, m.p. 236–238° (lit.[31] m.p. 239°), was obtained. The product was further identified by comparison with an authentic sample, prepared from pure glyoxylic acid, by mixed m.p. and X-ray powder diffraction data: 11.88 s (1), 8.71 m, 8.10 m (3), 6.60 m, 5.56 m, 5.29 w, 4.99 w, 4.70 s (2), 4.25 m. The glyoxylic acid could also be identified in an aliquot of the acid hydrolyzate of the oxidized mixture, by continuous extraction with ether for 48 h and characterization as the dimedone derivative.

Quantitative determination of glyoxylic acid

The color test first described by Eegriwe[32] was adapted for the quantitative

determination of glyoxylic acid. A solution of 50 mg of 2,3,4-trihydroxybenzoic acid in 100 ml of concentrated sulfuric acid was used as a colorimetric reagent. The calibration curve was prepared from crystalline glyoxylic acid dissolved in 2N sulfuric acid to make an approximately 0.2% solution. The solution was standardized, after neutralization, by titration with alkaline hypoiodite according to Hirst, Hough, and Jones[33]. In 15-ml test tubes, 0.2 ml of the glyoxylic acid solution containing between 4 and 30 μg was introduced with a micropipet. Then, while the tubes were kept in a cold water bath, 5 ml of the reagent solution was added from a microburet, the first two drops slowly with shaking, and then the rest of the solution more rapidly. The contents were homogenized in a Vortex mixer. The tubes, covered with aluminum foil, were kept in a thermostatted bath for 1 h at 58°. After the solutions had been cooled to room temperature, transmittance curves for the different concentrations of glyoxylic acid were taken with a Bausch and Lomb Spectronic 505 recording spectrophotometer. The data obtained showed that a transmittance minimum was present at 602 mμ, the position of which was independent of the concentration of glyoxylic acid used. The standard curve was obtained by reading the transmittance at 602 mμ with a Beckman DU Model 2400 spectrophotometer against a reagent blank prepared by substituting 2N sulfuric acid for the glyoxylic acid solution. The glyoxylic acid concentration in an aliquot of the acid hydrolyzate, conveniently diluted with 2N sulfuric acid, was determined by reference to the standard curve. Under the same conditions, erythronolactone, glyceric acid, glucose, and glycolic acid did not give any color, even with concentrations tenfold higher than the maximum used for glyoxylic acid.

Total aldehyde assay

Total aldehyde (Table I) in the hydrolyzate was determined by the method of Hirst, Hough, and Jones[33].

Preparative thin-layer chromatography on microcrystalline cellulose

An aliquot of the hydrolyzate was concentrated under diminished pressure and, after removal of inorganic salts by filtration, two fractions of about 0.100 g each of the syrup were respectively separated on two plates (8 in × 8 in) of microcrystalline cellulose. Double development with solvent mixture *B*, to a distance 15 cm from the starting line, was used. The plates were dried overnight, and the zones in one were located by spraying one edge with the reagent for acids (*c*). The main zones corresponded to glyoxylic and glyceric acids (Table II). Faint spots corresponding to erythronic and glycolic acids were obtained. From the glyceric acid zone the acid was eluted with water and characterized as the brucine salt, which gave identical X-ray powder diffraction data as did an authentic sample of the brucine salt of D-glyceric acid. To prepare this, the calcium salt of DL-glyceric acid (0.280 g) was decationized by stirring an aqueous solution with an excess of Amberlite IR-120 (H^+) ion-exchange resin. The filtered solution was refluxed with 0.400 g of brucine for 1 h. After cooling, the filtered solution was taken to dryness and the

brucine salt of D-glyceric acid was crystallized from absolute ethanol and was recrystallized from the same solvent, white prisms, m.p. 220–222°, $[\alpha]_D^{24}$ −23.7° (*c* 1.2, water), lit.[34] m.p. 218–219°, $[\alpha]_D^{20}$ −23.0° (water), X-ray powder diffraction data: 9.10 s, (1) 7.69 m, 6.01 s (2), 5.74 w, 4.88 vw, 4.32 s (3), 4.12 m, 3.79 m, 3.46 m.

The eluate from the zone having a mobility corresponding to glycolic acid gave a characteristic color reaction for this compound. When heated with 2,7-dihydroxynaphthalene in sulfuric acid a red-violet color was formed[35]. Derivatization could not be effected because of the small amount available.

Erythronolactone was located on the other plate by spraying with the reagent for lactones (*d*); it was eluted with water, and characterized as the brucine salt of D-erythronic acid, identical with an authentic sample prepared from pure D-erythronolactone, m.p. 208–210° (dec.), $[\alpha]_D^{25}$ −23.5° (*c* 1.8, water), lit.[36] m.p. 211°, $[\alpha]_D^{20}$ −22.6 (water), X-ray powder diffraction data: 9.40 s (1), 7.75 m, 6.22 s (2), 5.56 m, 4.99 w, 4.32 s (3), 4.11 w, 3.83 m, 3.68 vw, 3.56 vw.

A small cellulose plate (2 in × 8 in), of the same thickness as the large ones, was run at the same time and sprayed with the aniline hydrogen phthalate reagent. After the plate had been heated at 110°, two zones corresponding to glucose and arabinose were shown. By comparison, the zones corresponding to the sugars in the large plates were cut, and then eluted with water. Glucose was characterized as its phenylosazone, m.p. 207–209°, X-ray powder diffraction pattern identical with that of an authentic sample.

Quantitative determination of glucose and arabinose

A determination of glucose and arabinose in an aliquot of the salt-free hydrolyzate was made by the quantitative paper chromatographic method described by Wilson[19] (Table I).

Quantitative determination of glyceric acid

Glyceric acid was determined on a paper chromatogram by direct photometry. Two appropriate dilutions of the hydrolyzate, free from inorganic salts, and four standards containing from 100 to 200 μg of pure DL-glyceric acid were chromatographed on Whatman No. 1 paper with solvent mixture *A* (double development). After being dried overnight at room temperature, the paper was sprayed evenly with the reagent for acids (*c*). The densities of the spots were then determined densitometrically (Photometer Model 501A with transmission Density Unit Model 52C, Photovolt Corp., New York, N. Y.). The standard curve was obtained by plotting the logarithm of the concentrations against the densities. The concentrations in the solutions to be analyzed were determined by reference to the standard curve (Table I).

Quantitative determination of erythronolactone

An aliquot of the hydrolyzate was taken to dryness and extracted with methanol. Paper chromatography of the methanolic solution showed almost complete lacton-

ization of the erythronic acid since only a faint spot for the latter appeared with the reagent for acids. A determination of the erythronolactone was made by densitometry on paper, using double development with solvent mixture *A*. The paper was dried overnight and sprayed with the reagent (*d*) for lactones. Standards of pure D-erythronolactone in methanolic solution were applied in 80, 160, 200, and 240 μg quantities. From the standard curve the amount of D-erythronolactone in the hydrolyzate was determined (Table I).

Oxidation of methanol by hypochlorite

Methanol (1 ml, 0.792 g) was added to 300 ml of 5% sodium hypochlorite buffered to pH 9 with sodium bicarbonate–sodium carbonate. The solution was kept for 5 h at 50°. After this time it was acidified to pH 3–4 by stirring with Amberlite IR-120 (H^+) resin, while excess chlorine was removed with a stream of air. A determination of formic acid with mercuric chloride gave a yield of 4%, which is 0.028 mole of formic acid per mole of methanol. Formaldehyde was detected qualitatively in the oxidation solution.

Periodate oxidation of methyl β-cellobioside

To 92 ml of 0.29M periodic acid, 1.775 g (5 mmoles) of methyl β-cellobioside was added. The solution was diluted to 100 ml and maintained at room temperature. After 24 h, an aliquot was analyzed for periodic acid by the arsenite method. The consumption of periodic acid corresponded to 3.0 mole per mole of glycoside. The rotation was $[\alpha]_D$ −84°, calculated on the basis of the aldehyde. Hamilton, Huffman, and Smith[37] reported $[\alpha]_D$ −93.9°, constant after 8 h. The solution was then neutralized to phenolphthalein with hot strontium hydroxide solution, any excess of which was destroyed with solid carbon dioxide, and the inorganic precipitate was removed by filtration and washed with water. The general procedure of Jackson and Hudson[38] was followed for the isolation of the tetra-aldehyde as a syrup and its further oxidation with bromine to the tetra-acid (Fig. 1).

The distrontium salt trihydrate was obtained as a microcrystalline solid by adding half a volume of warm methanol to its warm aqueous solution. Purified twice in the same manner and dried to constant weight at 110° under diminished pressure, the anhydrous salt showed $[\alpha]_D^{20}$ +11.7° (*c* 0.9, water). X-ray powder diffraction data for the trihydrate were: 14.15 m, 7.25 m (1), 4.46 w, 4.19 w, 3.91 m (2), 3.60 m (3), 3.38 w, 3.24 w, 3.05 m.

Anal. Calc. for $C_{12}H_{14}O_{14}Sr_2.3H_2O$: H_2O, 8.85. Found: H_2O, 8.58. Calc. for $C_{12}H_{14}O_{14}Sr_2$: C, 25.85; H, 2.53; Sr, 31.44. Found: C, 25.68; H, 3.12; Sr, 31.41.

A sample of 12 mg of the distrontium salt in 10 ml of N sulfuric acid was hydrolyzed by heating for 7 h in a water bath at 85–90°. A determination of glyoxylic acid by the spectrophotometric method described above gave 95% of the calculated value. The barium salt was obtained from the strontium salt by liberating the acid with the calculated amount of sulfuric acid and neutralizing to phenolphthalein with barium hydroxide. By adding methanol to the aqueous solution, the barium salt

was precipitated as an amorphous solid. This salt was readily soluble in water and was different from the water-insoluble barium salt obtained from the oxidation mixture as described above.

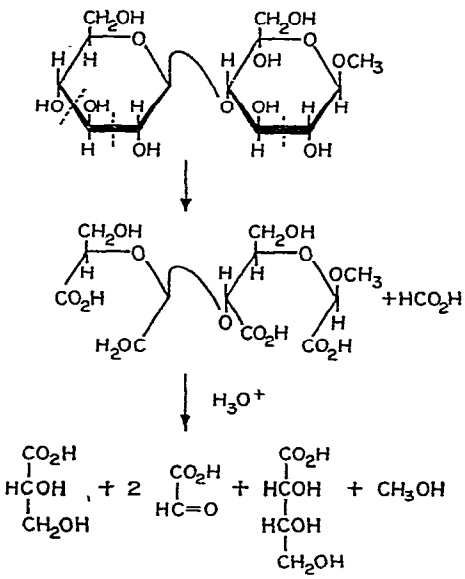

Fig. 1. Postulated main course of the hypochlorite oxidation of methyl β-cellobioside.

RESULTS AND DISCUSSION

Oxidation of methyl β-cellobioside with 20 moles of sodium hypochlorite per mole of sugar at pH 9 and 50° appears to be a fairly specific reaction. As seen in Table I, D-erythronic, D-glyceric, glyoxylic, and formic acids are the major products identified and quantitized in the acid hydrolyzate of the oxidation mixture. The first three acids are also produced by hydrolysis of the tetrabasic acid (Fig. 1) obtained, together with formic acid, by oxidation of methyl β-cellobioside with periodic acid and further oxidation with bromine water. This tetrabasic acid could also be the main product in the oxidation of methyl β-cellobioside with sodium hypochlorite. In one experiment, a barium salt was isolated, in low yield, from an aliquot of the hypochlorite-oxidized mixture. This salt gave a color test which is specific for glyoxylic acid[29]. The acid of this salt was hydrolyzed, and examination of the hydrolyzate by paper chromatographic methods showed compounds with the same mobilities as glyoxylic acid, D-glyceric acid, and D-erythronolactone. Glyoxylic acid could be isolated from the mixture as its dimedone derivative. This barium salt, as well as the strontium salt, was almost insoluble, even in hot water. On the other hand, the strontium and barium salts of the tetrabasic acid (Fig. 1), prepared for comparative purposes by periodate oxidation of methyl β-cellobioside, were very soluble in cold water. Further studies are in progress on the determination of the structure of the

acid isolated from the hypochlorite oxidation mixture, as well as are studies aimed at the isolation, from the oxidation mixture, of the tetrabasic acid shown in Fig. 1. That such an acid is a probable product is shown by the experiments of Jackson and Hudson[38] on the oxidation of methyl α-D-mannopyranoside with barium hypobromite. These workers obtained, by elimination of C-3 as formic acid and attack at C-2 and C-4, a dibasic acid isolated, in 25% yield, as the strontium salt dihydrate, which proved to be the same as the strontium salt prepared by oxidation of methyl α-D-mannopyranoside with periodic acid followed by oxidation of the dialdehyde with bromine water.

A simple, specific, quantitative micromethod for glyoxylic acid, based on the color test mentioned above, has been developed and used for the determination of this acid in the hydrolyzate of the oxidation mixture. None of the other major products formed interfere with the test. A determination of glyoxylic acid in the hydrolyzate of the distrontium salt of the tetrabasic acid (Fig. 1), prepared by the periodate method, gave 95% of the theoretical value, thus confirming the validity of the spectrophotometric method.

Preparative thin-layer chromatography on microcrystalline cellulose, following the general technique given by Wolfrom, Patin, and Lederkremer[17], proved to be useful for the separation of D-glyceric acid and D-erythronolactone. D-Glyceric and D-erythronic acids were characterized as their brucine salts, which had physical properties in agreement with literature values. Free use was made of comparative X-ray powder diffraction data for identification purposes. A quantitative determination of these two acids was performed on paper chromatograms by direct photometry (Table I).

The formation of glyoxylic acid and D-erythronic acid in these large proportions agrees with the results found by Whistler, Linke, and Kazeniac[6], who isolated these two acids as the main products in the oxidation of methyl 4-*O*-methyl-β-D-glucopyranoside with sodium hypochlorite at pH 9 and 25°. They suggested that the first step was an oxidation of either C-2 or C-3, or both, to carbonyl; under the alkaline conditions of the reaction, enolization would take place, followed by further oxidation to carboxylic acids. This is supported by Theander[7], who isolated 2-keto and 3-keto methyl β-D-"gluco"-pyranosides and also a small proportion of "glucosone" as neutral products on oxidation of methyl β-D-glucopyranoside with chlorine water at pH 2, 4, and 7. Only traces of glyoxylic acid and D-erythronic acid were formed under these conditions. The opposite was true for the oxidation at pH 9 and 10.

In our investigation no keto compounds were detected by using the resorcinol–hydrochloric acid spray reagent[2]. Glyoxylic acid and D-erythronic acid would be formed by preferential cleavage between C-2 and C-3 in either one of the rings; however, the isolation of D-glyceric and formic acids in high yields would indicate that D-erythronic acid originates mainly from the ring that carries the methyl aglycon and D-glyceric acid would be formed from carbon atoms 4′, 5′, and 6′, with removal of C-3′ as formic acid, as is the case in the periodate oxidation. Formic acid was identified among the products of the oxidation and was determined by the mercuric

chloride gravimetric method. The actual amount formed (Table I) could be even larger, since oxidation of formic acid by sodium hypochlorite has been reported[39]. The presence of D-glucose and D-arabinose, detected by chromatography in the oxidation mixture even before hydrolysis, and also the isolation of oxalic acid as a direct product of oxidation, indicate some cleavage of the acetal linkage under alkaline conditions when normal hydrolysis would not take place. Dyfverman[4] found that hydrolysis of methyl β-cellobioside is negligible, even in 2N hydrochloric acid for 16 days at room temperature. He also reported that, on oxidation of methyl β-cellobioside by chlorine water, cellobionic acid was formed in the early stages and was later degraded to D-gluconic acid, indicating that the methyl glycoside bond was more labile than the disaccharide linkage. If the degradation takes place according to a scheme proposed by Theander[8,40] some methanol could be set free during the oxidation. When methanol was oxidized under the same conditions used for the oxidation of the methyl cellobioside, 5% of formic acid was obtained (formaldehyde was also detected), that is, if even all the methyl aglycon would be free for attack by the hypochlorite, this would account for only 0.028 mole of formic acid. These results confirm that formic acid arises mainly from degradation of the pyranose ring in the nonreducing cellobiose entity. The finding that formaldehyde can be detected in the hypochlorite oxidation mixture from methanol would explain the traces of formaldehyde detected in the oxidation mixture before hydrolysis. Structures postulated by Theander[8] would also explain the formation of traces of glycolic acid. This acid was tentatively identified in the hydrolyzate of the oxidation mixture by paper and thin-layer chromatography on cellulose with two different developers. A very small amount, separated by the latter technique, gave the characteristic color reaction for glycolic acid[35]. Oxalic acid was isolated from the oxidation mixture as the calcium salt and assayed by titration with potassium permanganate. Henderson[5] detected oxalic acid on oxidation of methyl β-D-glucopyranoside with aqueous chlorine at pH 4.5, and Eisenbraum and Purves[11] found 0.21 mole of oxalic acid per hexose unit in the oxidation of starch with calcium hypochlorite at pH 12. Oxalic and glycolic acids would add to the glyoxylic acid to account for the cleavage between carbons 2 and 3 and also 2′ and 3′.

Even with the large excess of oxidant used, some unattacked sugar remained. D-Glucose was isolated by preparative thin-layer chromatography on microcrystalline cellulose, identified by its phenylosazone, and analyzed by quantitative paper chromatography, using Wilson's[19] method.

Arabinose was detected in the oxidation mixture by chromatography; it appeared as a reddish-brown spot on spraying with aniline hydrogen phthalate reagent and was assayed by the same method as was used for D-glucose. D-Arabinose was found by Theander[7] as a minor product on hypochlorite oxidation of methyl β-D-glucopyranoside over the pH range of 2 to 10, and Henderson[5] isolated the pentose on similar oxidation of cellulose and methyl β-D-glucopyranoside at pH 4.5. This sugar could be formed by cleavage between C-1 and C-2 of either unit.

The nature of the acidic products formed is critically dependent on the pH

at which hypochlorite oxidations are conducted. Gluconic acid was not detected in our studies, nor in those of Whistler and co-workers[6] when they oxidized methyl 4-*O*-methyl-D-glucopyranosides with sodium hypochlorite at pH 9. In contrast, D-gluconic acid was the main product formed on oxidation of methyl β-D-glucopyranoside[2] and methyl β-cellobioside[4] by chlorine water.

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SUMMARY

Methyl β-cellobioside was oxidized with 20 molar equivalents of sodium hypochlorite per mole of glycoside at pH 9 and 50°. D-Erythronic, glyoxylic, D-glyceric, and formic acids were the major products isolated from the hydrolyzate of the oxidized mixture. This indicates preferential attack on C-2 and C-3 of one of the rings and C-2′, C-3′, and C-4′ of the other, with removal of C-3′ as formic acid. Oxalic acid, a minor product of the oxidation, was isolated as the calcium salt before hydrolysis. Glucose and arabinose were detected in the oxidation mixture, before hydrolysis, indicating oxidative cleavage of the acetal linkage. The proportions present were small. Very small proportions of formaldehyde were also present and were shown to arise probably from methanol. Some glucose was found in the hydrolysis mixture, indicating incomplete oxidation, even with the large excess of oxidant used. All of these components were determined quantitatively (Table I). A specific micromethod for the determination of glyoxylic acid is described. A postulated intermediate tetrabasic acid was synthesized. Another acid, probably of related structure, was isolated in small amount.

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LABELING OF ACID MUCOPOLYSACCHARIDES WITH TRITIUM
I. LABELING OF HEPARIN WITH TRITIUM AND SULFUR-35

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INTRODUCTION

Purified preparations of ^{35}S-labeled heparin (labeled biologically or chemically) have been widely used for metabolic studies[1,2]. It has been found that, after intravenous injection, the radioactive heparin leaves the circulation very rapidly, its disappearance being parallel to that of the anticoagulant and lipolytic activities[3,4]. By the time the circulating radioactivity has declined below a measurable level, the radioactivity in the urine accounts for only a fraction of the dose injected[2,3], indicating a considerable retention of heparin in the organism. Radiochemical or autoradiographic analyses of different organs, obtained at different times after injection, have revealed a widespread distribution of the label throughout the organism, with a possible predilection for the liver, kidneys, and spleen[1,3]. These data, which have been repeatedly interpreted as an indication of the distribution and excretion of heparin, should be evaluated with a great deal of caution in view of the demonstrated ability of the whole organism to cleave some sulfate ester groups of acid mucopolysaccharides and sulfamido groups of heparin[5,6,7]. Therefore, the demonstration of ^{35}S radioactivity in particular sites may reflect not only the presence of ^{35}S-heparin, but also that of $^{35}SO_4$ — either free or bound to compounds of various chemical structures.

Labeling of heparin, or other acid mucopolysaccharides, on the pyranose ring of their monosaccharide components not only would obviate this inconvenience, but would also allow a more detailed investigation of the metabolism of the polymer. In fact, if the compound injected underwent depolymerization, the oligosaccharides, still radioactive, could be traced and easily recognized as to their derivation, even in the event of loss of sulfate ester or sulfamido groups or of disappearance of biological or physicochemical properties. Moreover, detection of radioactivity in the products of complete oxidation or in carbohydrates different from the compound injected would indicate a depolymerization to monosaccharides, followed by their oxidation or partial reutilization.

Theoretically, isotopes of carbon would be a better label for the pyranose ring

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than isotopes of hydrogen. In practice, however, the use of uniformly labeled D-glucose-^{14}C as a precursor in the biosynthesis of acid mucopolysaccharides is limited by its cost, by the small amounts of purified polysaccharide which can be obtained, and by the low specific activities attained. These same considerations recommend tritium as a more practical label, provided that the destruction of the material being labeled could be limited and the stability of the tritium label proved. Three different techniques have been used in our attempts to label acid mucopolysaccharides with tritium. We have chosen heparin as a model compound, because it is readily available in a fairly pure state, it is labile under a variety of conditions, and it may be labeled with ^{35}S using chemical methods. Moreover, its biological activities may be used as additional parameters to indicate structural changes, which may not be detected with physicochemical analyses.

The purpose of this paper is to evaluate the merits of the methods employed by comparing the properties of the labeled products obtained.

EXPERIMENTAL

(*a*) *Tritium-labeling of heparin by catalytic exchange*

Into a heavy-wall pyrex test tube (15 × 80 mm), connected to a three-way stopcock (No. AT-5168, 4 mm bore, Eck & Krebs, Inc., Long Island City, N.Y.) by a 28/15 ball joint, palladium black (4 mg) and tritiated water (0.5 ml, 500 mc) were added to potassium heparinate (100 mg) (A), dissolved in 0.5 ml of a buffered saline solution of pH 7.20. The saline solution was made by mixing 0.9% sodium chloride (10 ml), 0.03 M potassium dihydrogen phosphate (2.39 ml), and 0.03 M disodium phosphate (7.60 ml).

The potassium heparinate (A) was prepared as follows: Barium heparinate (a gift of Dr. D. D. Dziewiatkowski, Rockefeller University, New York) was passed through a column of Dowex 50 (*X*-8,200-400 mesh, H^+ form) and the water eluate was precipitated with 10% potassium acetate in 95% ethanol. Potassium heparinate was adsorbed on ECTEOLA modified cellulose and eluted with a stepwise sodium chloride gradient. The material eluted with concentrations of sodium chloride between 1 and 2 M was collected, desalted by gel filtration, and further purified by precipitation with cetylpyridinium chloride, according to Laurent[13]. The fraction that was precipitated by the quaternary ammonium compound at a magnesium chloride concentration of 1 M was collected, desalted by gel filtration, and dried with ethanol and ether. This material (A) had $[\alpha]_D^{21}+40.0°$ (*c* 3, water).

The 28/15 ball joint was greased with silicone lubricant and closed with a metal clamp. The tube, evacuated and filled with nitrogen several times was eventually filled with this gas, closed, and heated at 110° for 20 h in a heating block. Continuous agitation of the contents of the tube was insured by mounting the heating block on a shaker. After cooling the tube to 4°, the stopcock was opened and the contents of the tube, frozen to −40°, were dried from the frozen state and redissolved in water three times. The final aqueous solution was centrifuged, and the dark-brown super-

natant was precipitated with 3 vol. of 10% potassium acetate in 95% ethanol. The precipitate was washed repeatedly with 95% and absolute ethanol, then with ether, and dried to give 87.7 mg of heparin B. Two aliquots of heparin B (19 mg each) were dissolved in water and precipitated with a 2% solution of cetyltrimethylammonium bromide in water (2 ml). Each precipitate was suspended in distilled water, transferred to a cellophane tube, and dialyzed at 4° against running distilled water. One dialysis tubing was removed after three days; the heparin–quaternary ammonium salt complex was collected by centrifugation, washed with 10% potassium acetate in 95% ethanol, and dried with ether to give 13 mg of heparin C. Heparin C (11 mg) was dissolved in water (1 ml) and the solution was applied to a column of Dowex 50 (*X*-4, 200–400 mesh, H^+ form, 30 × 1 cm) which was then eluted with water. The volume of the hexuronic acid-containing eluate was reduced in a flash evaporator (60°), and the heparin was precipitated with 10% potassium acetate in 95% ethanol, and washed with absolute ethanol and ether to give 10 mg of heparin D.

The second dialysis tubing was removed after 6 days of dialysis and the heparin recovered was applied to a column of Sephadex G 25 (1.9 × 140 cm, Pharmacia, Uppsala, Sweden) which was eluted with distilled water. The hexuronic acid-containing material appeared with the void volume of the column as a sharp peak, followed by a slowly declining tail. The eluate corresponding to the sharp peak and that corresponding to the slowly declining part were reduced in volume, precipitated with potassium acetate in ethanol, and dried with ether. Heparin S1 (15 mg) was recovered from the first, and heparin S2 (2.5 mg) from the second, eluate.

TABLE I

SPECIFIC ACTIVITY OF HEPARIN LABELED BY CATALYTIC EXCHANGE, AT DIFFERENT STAGES OF PURIFICATION

Preparation	*Specific Activity ^3H cpm/μg heparin*
Heparin A	0
Heparin B	321
Heparin D	279
Heparin S 1	260
Heparin S 2	362
Heparin SS 1	280

The radioactivity was measured in a scintillation counter, using 50 μl of aqueous solution of the compound to be counted, 5 ml of 2-methoxyethanol, and 10 ml of toluene containing 15 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene per liter. The efficiency of the instrument for ^3H was calculated by use of a known amount of tritiated water as a standard. The degree of quenching was assessed, and when necessary corrected, by use of tritiated water as internal standard for each counted sample. The concentration of heparin in each solution was measured with a borate modification of the Dische test[8].

Milligram amounts of heparin A, B, D, S 1, and S 2 were dissolved in water and aliquots were used for determination of specific activity and prothrombin time. The results obtained are given in Table I and Fig. 1.

Heparin S 1 (3.7 mg was again passed through the Sephadex G-25 column and 3.2 mg was eluted in a symmetrical peak (Heparin SS 1). Aliquots from each tube composing the peak were used for measuring the specific activity of heparin. The results are given in Fig. 2.

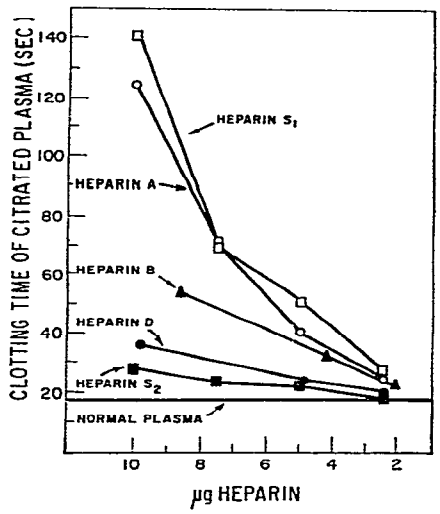

Fig. 1. Biological activity (prothrombin time) of heparin, labeled by catalytic exchange, at different stages of purification. The prothrombin time was measured by addition of heparin (1 to 10 μg) in 0.9% sodium chloride (0.1 ml) to citrated human plasma (0.1 ml) and simplastin (0.2 ml) at 37°. The low biological activity of heparin D is presumably due to cleavage of *N*-sulfate groups during concentration at 60° of the strongly acidic material eluted from a column of Dowex 50 (X-4, H^+ form).

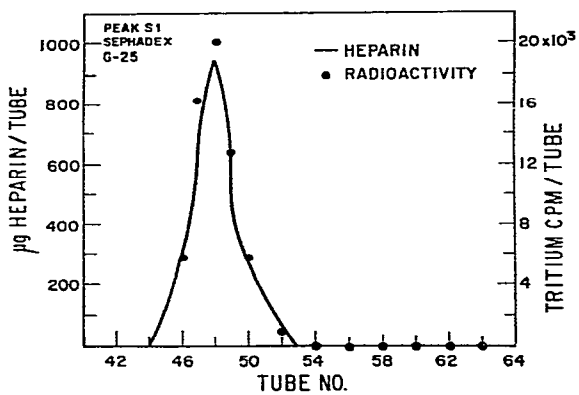

Fig. 2. Second passage through Sephadex G 25 (1.9 × 140 cm column) of Heparin S 1, labeled with tritium by catalytic exchange.

Heparin S 1 (8.7 mg) was hydrolyzed in a sealed tube (4N hydrochloric acid at 110° for 4 h.). Analysis of amino sugars, performed on the hydrolyzate according to Gardell's technique[9], indicated that glucosamine was the only amino sugar

present. Known amounts of glucosamine isolated from the hydrolyzate and known amounts of heparin S 1 were dissolved in 50 μl of water and used for radioactivity measurements. It was found that 280 μg of isolated glucosamine account for 45.5% of the radioactivity present in 1 mg of heparin S 1.

(*b*) *Tritium-labeling of heparin by Wilzbach's method*[10]

Potassium heparinate (A) (500 mg) was exposed to 5 curies of tritium gas for a period of 2 weeks in a conventional Wilzbach apparatus. After removal of the gas, the heparin was dissolved in water, precipitated, and washed with ethanol and ether, and dried, as described for the catalytic-exchange technique. The heparin (340 mg) recovered was dissolved in water and precipitated with cetylpyridinium chloride, the final yield being 104 mg (heparin E). Part of this preparation was dissolved in water, and aliquots were used for measurement of biological activity (prothrombin time) and radioactivity. The material was found to have no biological activity left, and a specific activity of 28,600 cpm/μg. It is unlikely that this material was radiochemically pure, but it was not purified further because of lack of biological activity.

(*c*) *Tritium-labeling of heparin by exposure to tritium gas under electric discharge*

Potassium heparinate (A) (500 mg) was placed in the metal cup of the cell shown in Fig. 3. The surface of the joint was greased, and the cell was connected into a vacuum system for the introduction of tritium gas. The cell electrodes were connected to the secondary winding of a neon-light transformer whose input was regulated with a Variac. The cell was evacuated for at least 30 min, and degassing was considered to be sufficient, when the intensity of the discharge produced by 800 volts across the electrodes did not decrease, the cell being isolated from the vacuum line. Tritium gas (1 curie; 0.4 cm^3) was introduced into the cell to a pressure of 5–8 mm of mercury and the input voltage of the cell was started at approximately 1000 volts. During the following 20–30 min, the voltage had to be gradually increased to 2000 volts in order to maintain the intensity of the discharge. After 1 h, the glow of the discharge turned from pink to blue, indicating that the tritium gas had been depleted considerably. At this point, the discharge was discontinued, the cell was evacuated, and replenished with another curie of tritium gas, and the whole operation was repeated. Upon completion of the second exposure, the electric input was discontinued, and the cell was evacuated with a diffusion pump, for at least 1 h, in order to remove residual tritium gas. Thereafter, air was slowly let into the apparatus. The cell was removed from the vacuum system, its upper part was carefully lifted, and replaced with a 125-ml Erlenmeyer flask provided with the male part of a 34/45 joint shortened to fit the bottom part of the cell. The assembly was rapidly inverted and gently tapped on the bottom until all the solid material had been transferred to the flask. After removal of the bottom part of the cell, the heparin was dissolved in water, precipitated with 10% potassium acetate in 95% ethanol, and dried with ether. The material (360 mg) recovered was redissolved in water and precipitated with cetylpyridinium

chloride in a quantitative yield (heparin F). A few mg of this preparation was dissolved in water and tested for biological activity (prothrombin time) and radioactivity. The material was found to have full biological activity and a specific activity of 11,500 cpm/μg. Repeated gel filtration of this preparation and several precipitations with ethanol and cetylpyridinium chloride failed to change its specific activity or biological activity.

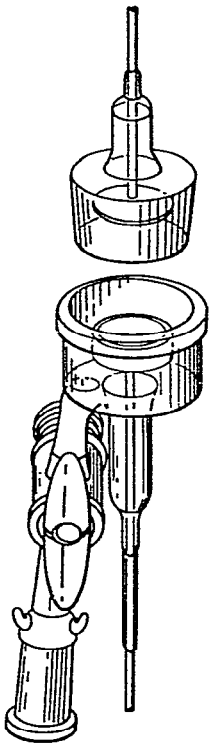

Fig. 3. The discharge cell consists of a 34/45 joint, cut off to provide a joint surface 1.5 cm high. The stainless steel cup and plate are connected to Kovar (Carborundum Corp., Niagara Falls, N.Y.) electrodes. When the cell is closed, the distance between the plate and the rim of the cup is 1 cm. The cell is connected to a vacuum line with an appropriate joint, provided with a stopcock.

Table II summarizes some chemical analyses and molecular-weight determinations of the original potassium heparinate and of the tritiated preparations. Infrared spectra of the original material and of the labeled preparations did not show any qualitative difference.

(*d*) *Labeling of tritiated heparin with* ^{35}S

Tritium-labeled heparins B and F, which had retained full biological activity, were labeled with ^{35}S using the technique described by Levy and Petraceck for partial *N*-desulfation and resulfation[2], with minor modifications. A solution of tritiated heparin (300 mg) in 0.04N hydrochloric acid (3 ml) in a sealed Pyrex tube was im-

mersed for 4 h in a boiling methanol–acetone azeotrope (12.1% of methanol, 87.9% of acetone w/w, b.p. 55.7°). The hydrolyzate was neutralized and an aliquot was removed for analysis. The remainder was transferred to a 40-ml Pyrex centrifuge tube and stirred at room temperature for 2 h with equal weights of sodium carbonate and pyridine-$^{35}SO_3$ complex, the latter being added gradually during the 2-h period. Care was taken to maintain the pH of the solution between 9 and 10. At the end of the

TABLE II

ANALYSIS OF ORIGINAL HEPARIN AND OF HEPARIN LABELED WITH TRITIUM

Heparins	*Molecular weight*[a]	*C %*	*N %*	*S %*	*Ash %*	*^3H dpm/μg*	*Biological activity*
A	11,000 ± 1,000	21.5	1.7	9.84	46.5	0	100
E	7,500 ± 600	18.9	1.7	10.64	45.8	28,600	0
F	10,500 ± 700					11,500	100
S 1	10,500 ± 500	21.1	1.6	9.86	48.38	314	100

[a]Sedimentation equilibrium[11].
[b]The biological activity of the original preparation was arbitrarily taken as 100.

reaction period, the heparin was precipitated with cetylpyridinium chloride, and the precipitate was washed and dried by centrifugation with 10% potassium acetate in 95% ethanol, and with ether. The heparin thus recovered was dissolved in water and passed through a column of Amberlite CG 120 (200–400 mesh, 15×1 cm, H^+ form) and through at least three columns of Dowex 1 (*X*8, 200–400 mesh, 5×1 cm, OH^- form). The complete removal of inorganic $^{35}SO_4$ was assessed by measuring the ratio ^3H cpm/^{35}S cpm of consecutive aliquots of each eluate. When a constant ratio was obtained, the solution was neutralized, and the heparin precipitated with cetylpyridinium chloride, washed several times with potassium acetate in ethanol and with ether, and dried. Analyses of the original preparation, of the aliquots removed after partial *N*-desulfation, and of the final products indicated that the partly *N*-desulfated material had very little anticoagulant activity left, whereas the final, doubly-labeled products had an anticoagulant activity consistently higher than that of the original preparation.

DISCUSSION

The analytical data for the tritiated heparin preparations indicate that the two of them obtained by methods (*a*) (heparin B) and method (*c*) (heparin C) had undergone little or no degradation. As indicated by the first gel filtration of heparin B, approximately 13% of this product (heparin S 2), retarded in its elution from the column, has a higher specific activity and a lower biological activity than the material eluted with the void volume. In all probability, the retarded aliquot represents material which has become more labeled but has also been degraded to a larger extent. Because of its lower biological activity, this aliquot is not suitable for metabolic experiments. Its removal by gel filtration, however, produces a good yield of heparin

(heparin S1) which is equally labeled in the glucosamine and hexuronic acid moieties, has properties similar to those of the original preparation, and therefore seems to be suitable for metabolic experiments.

In both labeling techniques, the mechanism for exchange of hydrogen with tritium probably consists of two steps: at first, activation of the substrate by hydrogen, atom abstraction with formation of a free radical; then activation of tritium gas or tritiated water with addition of tritium atoms to the free radicals. In the technique of catalytic exchange, however, the activation of the substrate depends on the formation of a proper complex with the catalyst surface[12]. A functional complex is usually achieved by chemisorption through delocalization of π electrons of the substrate into the empty *d*-shells of the catalyst. In the case of heparin, which lacks π electrons, a weak complex may be formed between σ electrons and the most active, but less abundant, sites of the catalyst surface. Hence, a small number of free radicals are formed. Moreover, the abundance of sulfur in heparin may greatly reduce the efficiency of the catalyst, limiting to a further extent the degree of labeling of the substrate. Despite the low specific activities obtained (amenable, however, to measurement with modern scintillation counters), the technique of labeling heparin by catalytic exchange is worth description, and possibly further refinements because it may be performed under technical conditions which are simpler and less hazardous than those required for labeling under electric discharge. The loss of biological activity of the heparin labeled with Wilzbach's method is probably due to cleavage of glycosidic bonds rather than to loss of *O*- or *N*-sulfate groups. This is not an unexpected result, since it is known that radiation-induced labeling proceeds simultaneously with radiolytic degradation[12]. An attempt to fractionate this heparin preparation into fractions of increasing molecular weight[13] has indicated that the larger ones still have a discrete biological activity, but a lower specific activity, than the small, biologically inactive fractions. Thus, it is probable that the material more directly exposed to the effects of radiation (surface material) has become more labeled, but has also been degraded to a larger extent.

The usefulness of doubly-labeled heparin in metabolic studies is demonstrated by the results obtained by perfusing it through isolated, surviving, rat livers and by injecting it intravenously into rabbits[14]. Analysis of blood and bile samples collected during 5 h of liver perfusion have indicated a constant $^{3}H/^{35}S$ ratio for the heparin isolated from blood, and absence of tritium-labeled material from the bile.

Analyses of blood and urine samples collected from rabbits have confirmed the rapid disappearance of heparin from the circulation, and its slow urinary excretion. However, analyses of the heparin isolated from blood and urine samples have demonstrated a gradual removal of *N*-sulfate groups, as indicated by a ratio $^{3}H/^{35}S$ progressively higher than the ratio of the injected heparin or of the heparin extracted from blood immediately after injection.

Barring improbable species differences, it appears that the mammalian organism is capable of severing the *N*-sulfate linkages of heparin. The liver, however, does not seem to be involved in this reaction, or in the excretion of heparin through the bile.

The reported presence of ^{35}S radioactivity in the bile of dogs injected with *N*-(^{35}S-resulfated) heparin[2] may be interpreted as excretion of $^{35}SO_4$ (either free or conjugated in ester form) which was cleaved from heparin in sites other than the liver.

SUMMARY

Heparin has been labeled with tritium by exposing it to tritium gas under an electric discharge or to tritiated water in presence of palladium black. The tritium label is stable under a variety of conditions, and, at least in the heparin labeled by catalytic exchange, is equally distributed between hexosamine and hexuronic acid moieties. Tritium-labeled heparin has also been labeled with ^{35}S by replacing some *N*-sulfate groups with similar groups labeled with ^{35}S. The final products retain the original physicochemical and biological properties, and appear to be suitable for metabolic experiments.

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DEGRADATION OF CARBOHYDRATES

PART VIII*. FORMATION OF A 3(2*H*)-FURANONE FROM HEX-2-ENOFURANOSES

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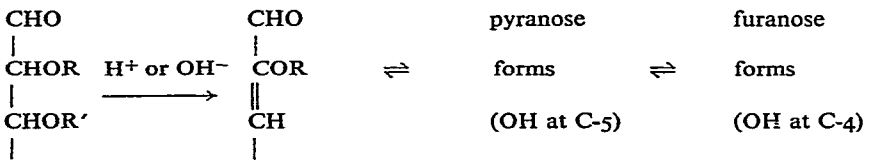
INTRODUCTION

Many sugars and their derivatives are degraded by acid to 2-furaldehydes; the first reactions are probably 1,2-enolizations which are followed by β-elimination of the C-3 substituent to give unsaturated 3-deoxyaldoses (which may then be dehydrated to 2-furaldehydes[1]). In the case of 2-*O*-methylaldoses, these unsaturated sugars can conveniently be obtained by a mild alkaline treatment, the reactions proceeding no further in alkali[1,2].



Pyranose forms of these unsaturated sugars, obtained by the alkaline treatment of 2,4-di-*O*-methylaldoses, have been shown to be dehydrated under mild acid treatment, to yield 3,4-dideoxy-3-enopyranosuloses and then 2-furaldehydes[1,3]. The preparation and study of the chemistry of the corresponding furanose forms** is of special interest because sugars lacking a potential hydroxyl group at C-5 do not appear to yield 2-furaldehydes on acid treatment[4,5].

This paper reports the preparation of such an unsaturated furanose sugar, 3-deoxy-2,5,6-tri-*O*-methyl-D-*erythro*-hex-2-enofuranose (**4**) and, its degradation by acid to give, not a 2-furaldehyde, but a 3(2*H*)-furanone **5*****.

RESULTS AND DISCUSSION

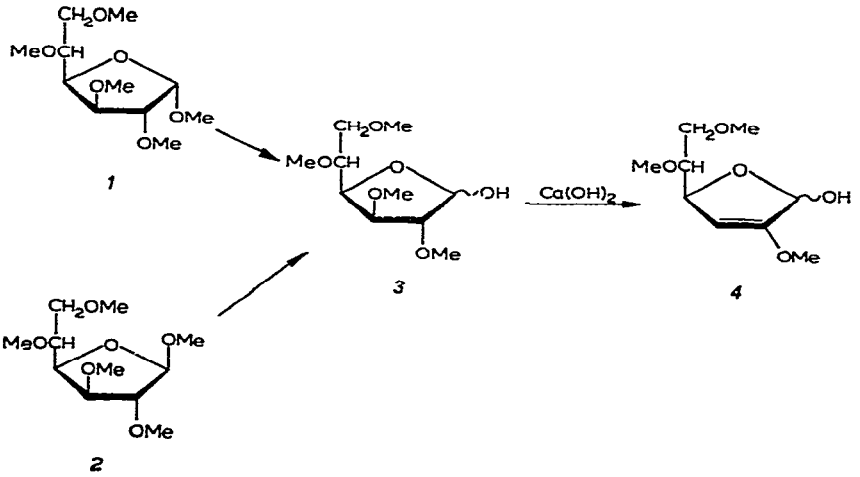
The glycenofuranose **4** was obtained from 2,3,5,6-tetra-*O*-methyl-D-glucose (**3**) by the action of lime-water, as reported for the glycenopyranoses[2]. The crystalline

*For Part VII, see Ref. 7. A preliminary account of some of this work has been given[4].

**The author[2] has previously obtained a crystalline furanose form, 3-deoxy-2-*O*-methyl-β-D-*erythro*-hex-2-enose. However, as (in solution) it gave an equilibrium mixture of the furanose and pyranose forms, the chemical reactions could not be studied independently.

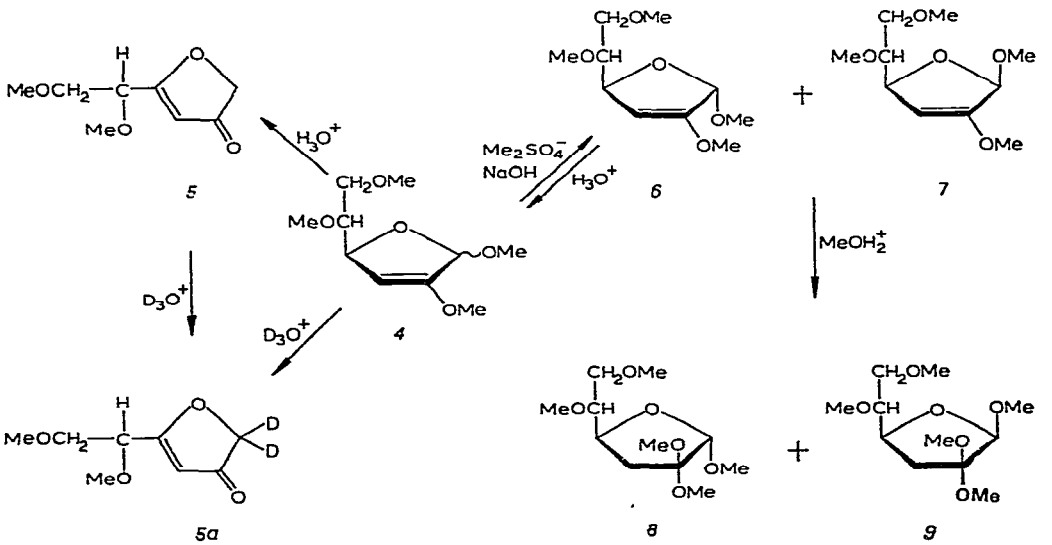
***Carbohydrate numbering is used for compounds **1**-**4** and **6**-**9**, and heterocyclic numbering for the furanone **5**.

methyl glycosides **1** and **2**, used for the preparation of **3**, were synthesized essentially by the method of Levene and Meyer[6], namely, by methylation of 1,2-*O*-isopropylidene-α-D-glucofuranose, methanolysis–glycosidation to the methyl 3,5,6-tri-*O*-methyl-glucofuranosides, separation of the α-D and β-D anomers by distillation, and, finally, methylation of the C-2 hydroxyl groups to give the glycosides **1** and **2**. Although



Levene and Meyer did not obtain these glycosides in a pure state; the only modification of their method that was required was that the intermediates (all liquids) be carefully purified. The purity of all these compounds and of the final glycosides was checked by gas–liquid chromatography (g.l.c.).

Methylation of the glycenofuranose **4** with methyl sulfate and alkali yielded the methyl furanosides, the α-D-anomer **6** being isolated by preparative g.l.c., and the β-D anomer **7** by crystallisation.



The unsaturated methyl furanosides **6** and **7** were found to be very labile in acid media. In contrast to the unsaturated pyranosides[7], anomerization takes place rapidly in dilute acid solution at room temperature. In acidic carbon tetrachloride solution, these furanosides are rapidly polymerized to an insoluble, black resin. In methanolic hydrogen chloride, much insoluble, black polymer is also formed, but addition of methanol occurs at the double bond, to yield the methyl glycosidulose dimethyl acetals **8** and **9**.

By contrast, in *aqueous* acids, even at 100°, little darkening of compounds **4**–**9** takes place. The furanosides **6** and **7** are reversibly hydrolyzed to the glycenose **4**. Under slightly more vigorous conditions, **4**, **6**, and **7** all give the furanone **5** in about 70% yield. Formation of the furanone from the methyl glycosidulose dimethyl acetals **8** and **9** also occurs, but more slowly and in poor yield.

The furanone **5** is stable for some hours at 100° in aqueous 0.1N hydrochloric acid. There was no spectral indication of the formation of 2-furaldehydes, either in this instance, or when **4**–**9** were treated with acid.

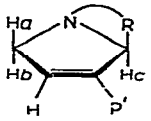
Structures of compounds **4**–**9**

(*a*) *Unsaturated sugars* **4**, **6**, *and* **7**

The presence of a double bond in each compound, **4**, **6**, and **7**, was indicated by their infrared absorption at 1670 cm^{-1} (pyranose analogs 1668–1678 cm^{-1})[2,7], and by the maxima in the ultraviolet absorption at 194 mμ (pyranose analogs 198–200 mμ)[2,7]. The optical rotations (higher positive value for **6** than **7**) suggested that **6** and **7** were α-D and β-D anomers, respectively. However, assignation of the structures rests mainly on the evidence from the n.m.r. spectra.

The signals from H-1, H-3, and H-4 in the n.m.r. spectrum of each of the furanosides **6** and **7** were well resolved and approximately first-order (see Figs. 1 and 2). The assignments followed from the chemical shifts and the splitting patterns. The significant differences between the two spectra were in the values of $J_{1,4}$: 0.9 c.p.s. for **6**, and 4.0 c.p.s. for **7**.

The spatial disposition of H-1 and H-4 differed in the two anomers, H-1 and H-4 being *trans* for **6** and *cis* for **7**. The very large value of $J_{1,4}$ for **7** is most unusual for either a 1,3 coupling across an oxygen atom or for a homoallylic coupling[8]. An analogous structure occurs in the pyrrolizidine alkaloids, where the hetero ring-atom is a nitrogen instead of an oxygen atom. These alkaloids give $J_{b,c}$ values



of *ca.* 5 c.p.s. (*cis* protons) and lower values for $J_{a,c}$ (*trans* protons)[9]. Culvenor *et al.*[9] explained the large coupling in the *cis* compounds as being due to the addition of a long-range coupling, *via* the rear residual lobes of their C-H σ-orbitals, to the π-orbital, homoallylic interaction. Whatever the mechanism, it is clear that the *cis*

compounds showed the large coupling[9]; by analogy, the furanoside **7** (having the large $J_{1,4}$) has the *cis* configuration and is, therefore, the β-D anomer.

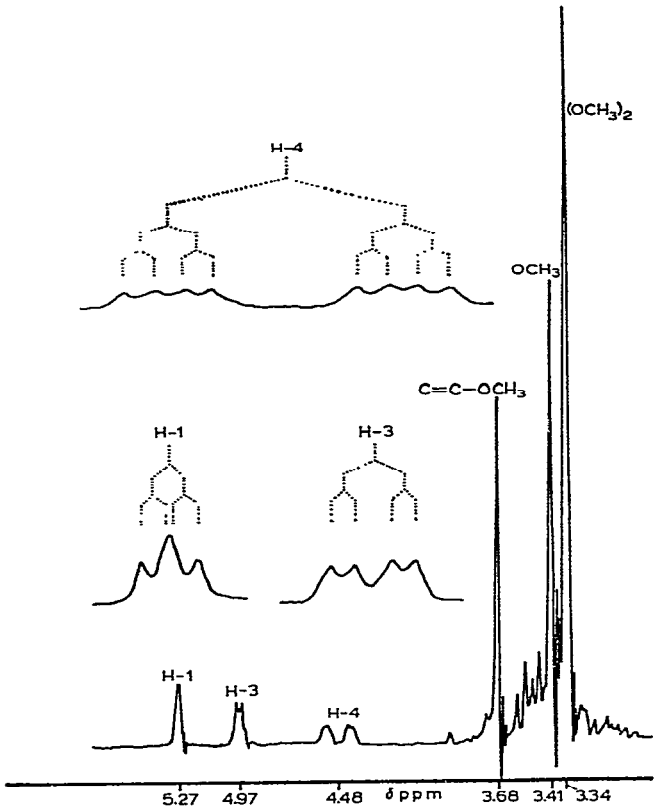


Fig. 1. N.m.r. spectrum of methyl 3-deoxy-2,5,6-tri-*O*-methyl-α-D-*erythro*-hex-2-enofuranoside (**6**) in carbon tetrachloride solution with internal tetramethylsilane (δ 0.00 p.p.m.) at 60 Mc.p.s. The spectrum amplitude of inserts was 50 c.p.s.

The n.m.r. spectrum of the glycenose **4** was complicated, since it was the sum of the spectra of its α-D and β-D forms. Furthermore, the signals of H-3 and H-4 of at least one anomer overlapped each other. However, the H-1 signals were well downfield of the others; with pyridine as solvent, the doublet of the β-D anomer and the singlet of the α-D anomer were clearly resolved, and it was concluded from the intensities of the peaks that they were present in approximately equal proportions. The n.m.r. spectra of deuterium oxide solutions of **4** and of (**6** + **7**) were similar, except that the signals of H-1 and, to a lesser extent, of H-4 were shifted downfield, owing to the presence of a hydroxyl group at C-1 in **4**, instead of the glycosidic methyl group in **6** and **7**.

(*b*) *Furanone* **5**

In some of the 3(2*H*)-furanones synthesized by Rosenkranz *et al.*[10], the furanone

ring is not conjugated to substituents, and the spectra of these furanones can, therefore, be usefully compared with **5**. For example, 2,5-dimethyl-3(2*H*)-furanone shows an absorption peak, λ_{max} at 260 mμ (ε 12,200), but, in alkaline solution, this gradually changes to 296 mμ; under similar conditions, the peak given by **5** at 262 mμ drifts to 296 mμ. (These authors[10] ascribed the change in spectrum to the formation of

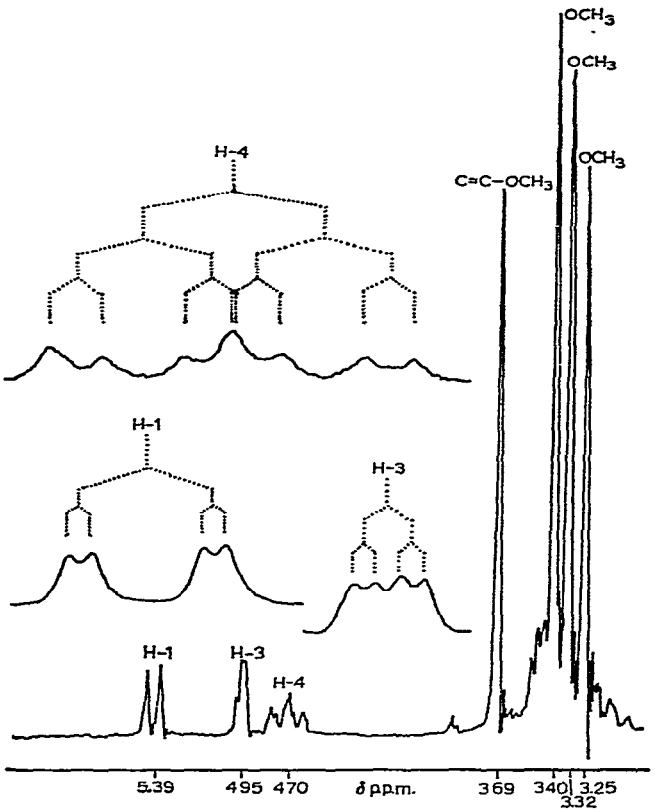


Fig. 2. N.m.r. spectrum of methyl 3-deoxy-2,5,6-tri-*O*-methyl-β-D-*erythro*-hex-2-enofuranoside (**7**) in carbon tetrachloride solution with internal tetramethylsilane (δ 0.00 p.p.m.) at 60 Mc.p.s. The spectrum amplitude of inserts was 50 c.p.s.

an enolic form of 2,4-pentanedione derivative.) Also, in carbon tetrachloride, the infrared spectrum of their furanone shows bands at 1712 and 1610 cm^{-1}, whereas **5** shows bands at 1700 and 1602 cm^{-1}. The same authors[10] also reported the n.m.r. spectrum of 5-(dimethylaminomethyl)-2-methyl-3(2*H*)-furanone. The chemical shifts (in carbon tetrachloride solution) of the ring protons H-2 (δ 4.40) and H-4 (singlet δ 5.48) were comparable to those of the furanone **5** (δ 4.48 and 5.62, respectively) (*cf.* Fig. 3).

Since the 2,2-dideuterofuranone **5a** was readily formed in deuterium oxide, the furanone and the 3-hydroxyfuran forms were in equilibrium. The equilibrium

must, however, lie on the side of the furanone, because the furan form was not detected by the n.m.r. spectra.

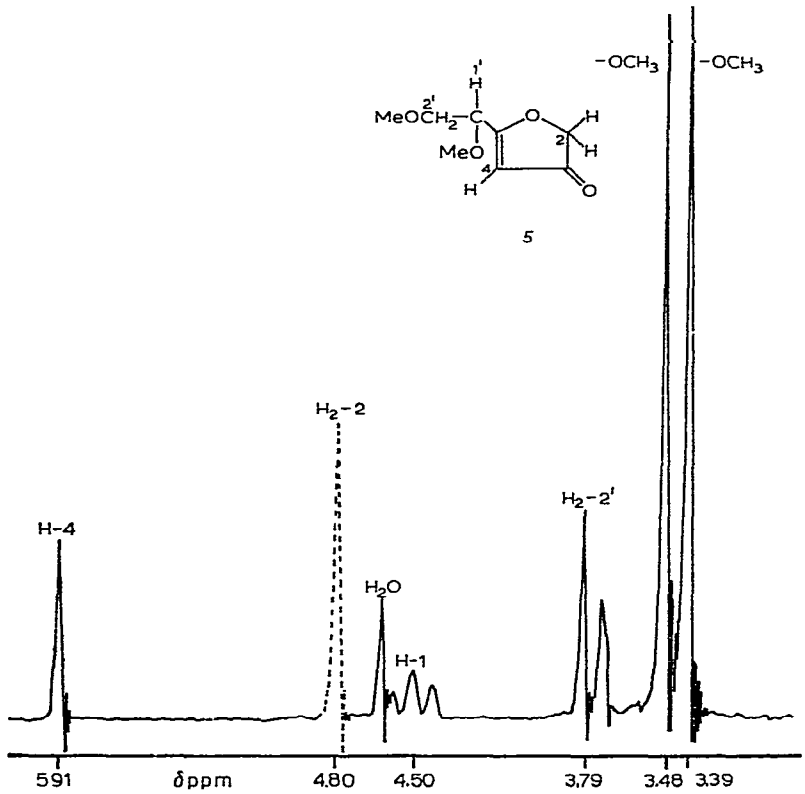


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Fig. 3. N.m.r. spectrum of 5-(D-*glycero*-1,2-dimethoxyethyl)-3(2*H*)-furanone (**5**) in deuterium oxide from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (δ 0.00 p.p.m.) at 60 Mc.p.s. The signal from H-2 (dotted peak) was absent from the spectrum when the furanone was treated with acid in deuterium oxide or was formed in deuterium oxide (2,2 dideutero form **5a**).

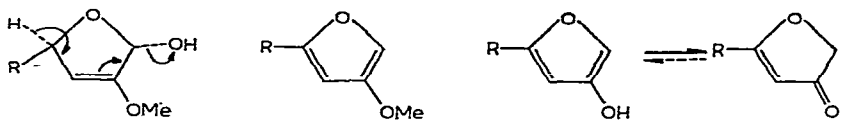
(*c*) *Glycosidulose acetals* **8** *and* **9**

The structure of these acetals **8** and **9** was largely determined from spectral evidence. The infrared and ultraviolet spectra showed the absence of both C=C and C=O bonds, and of hydroxyl groups. The n.m.r. spectra showed that each acetal contains five methoxyl groups, an isolated glycosidic proton (singlet downfield), and a methylene group (C-3) bonded to two carbon atoms, one with no hydrogen atoms and the other with one (two quartets at high field, with the expected large geminal coupling, $J_{3a,b}$ 13.1 and 13.4 c.p.s.). The anomeric configurations of the glycosidic groups, assigned from the optical rotations, should be reliable, since there are no interfering features.

Mechanism for the formation of the furanone **5**

The transformation of the glycenose **4** to the furanone **5** involves the loss of

a molecule of water and the hydrolysis of the vinylic methoxyl group. The reaction could take the path **10**→**13**. For this scheme to be possible, the hydrolysis of **11** to **12** must proceed readily under mild conditions, because furans did not accumulate during the formation of the furanone (when the reaction was followed by n.m.r.



10 *11* *12* *13*

spectroscopy, no definite peaks were seen between δ 6 and 8). Not much is known about the rate of hydrolysis of 3-methoxyfurans; in the case of 4(3)-methoxyfuran-5(2)-carboxylic acid, treatment with 0.2N hydrochloric acid for 2 h at 100° was required[5]. Therefore, if 3-methoxyfurans are intermediates, they must be hydrolyzed at an appreciably faster rate–a likely possibility, since the furan **11** is not stabilized by conjugation to a carboxyl group.

In the alternative mechanism, the vinylic methoxyl group is hydrolyzed first, to give the 3-deoxyglycosulose (R–CHOH–CH_2–CO–CHO). This compound is, probably, an intermediate in the formation of the furanone **5** from the methyl glycofuranosidulose acetals **8** and **9**, but, in the latter instance, the furanone is formed slowly and in poor yield. The low rate of formation of the furanone could be due to the slowness of the hydrolysis of the glycoside or acetal groups of **8** and **9**.

EXPERIMENTAL

General

The microanalyses were performed by the Australian Microanalytical Service, Melbourne, and the nuclear magnetic resonance spectra were obtained on a Varian model A60 spectrometer by Mr. P.J. Collins of the Division of Coal Research, C.S.I.R.O. The chemical shifts were measured against internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate when the solvent was deuterium oxide, and, with other solvents, against internal tetramethylsilane as standard (δ 0.00 p.p.m.). The chemical shifts were corrected against chloroform in carbon tetrachloride solution containing tetramethylsilane (δ 7.27). Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter, the infrared spectra on a Perkin–Elmer Model 221 spectrometer, and the ultraviolet spectra with a Beckman Model DK-2A spectrometer.

Gas–liquid chromatography was performed on a Wilkens Aerograph Model 200 gas chromatograph fitted with a flame ionization detector. For analytical separations, two columns of 3-mm stainless steel were used: Column A, 2 m long, was packed with 5% methylsilicone gum on Chromosorb* W, 60–80 mesh, and was

*Trademark of the Johns–Manville Product Corporation.

operated at 115°; and column B, 2.5 m long, was packed with 5% Carbowax* 20M on Chromosorb G, 60–72 mesh, and was operated at 145°. Preparative separations were achieved by repeatedly injecting 10-μl samples on 9 mm × 3 m columns, splitting the effluent streams with a Wilkens variable splitter, and passing one part to the detector and 10–15 parts to the collector. Preparative column C was of glass, packed with 5% methylsilicone gum on Chromosorb G, 45–60 mesh, and operated at 120°; and column D was of stainless steel, packed with 5% Carbowax 20M on Chromosorb G, 45–60 mesh, and was used at 140°. The retention times are all given relative to methyl 2,3,5,6-tetra-*O*-methyl-α-D-glucofuranoside (**1**) (*R* 1.00).

Vacuum distillations were performed in stills (designed by Dr. K. E. Murray) characterized by having a short vapor-path of large cross-section (see Fig. 4). The temperatures given are bath temperatures.

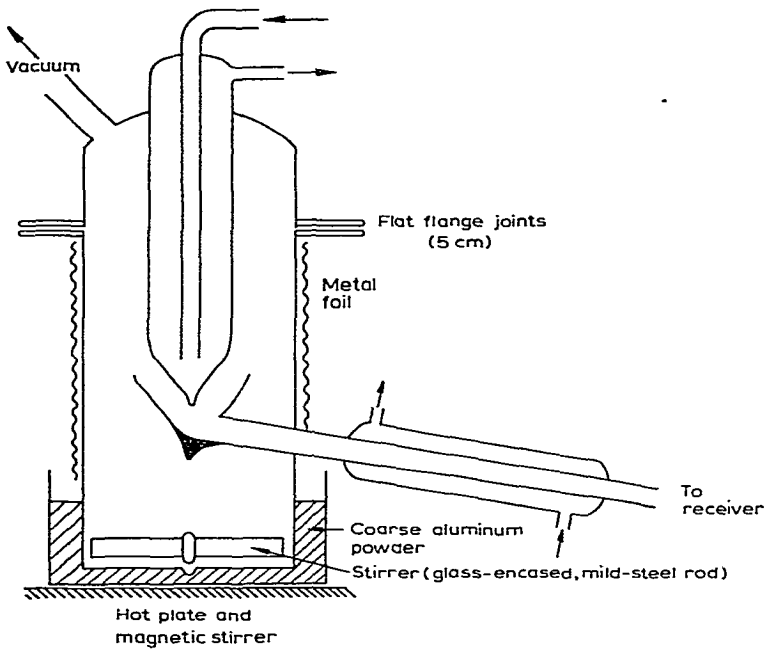


Fig. 4. Vacuum distillation apparatus designed by Dr. K. E. Murray.

*Methyl 2,3,5,6-tetra-*O*-methyl-*D*-glucofuranosides* (**1–2**)

The α- and β-D-glucosides were prepared according to the method of Levene and Meyer[6], starting from pure 1,2-*O*-isopropylidene-α-D-glucofuranose. The success of the preparation depended on purifying each intermediate; in particular, the methyl 3,5,6-tri-*O*-methyl-α- and -β-D-glucofuranosides had to be as pure as possibly obtainable. The purity of these two liquids was checked by g.l.c. on columns A and B, and the completeness of the methylations was checked by infrared spectroscopy.

*Trademark of the Union Carbide Corporation.

(*a*) α-D *Anomer* (**1**). The distilled product crystallized readily, and was recrystallized twice from pentane to give large needles, m.p. 16–17°, $[\alpha]_D^{25}$ +113°, +122°, and +117° (*c* 1; water, methanol, and chloroform, respectively) (Haworth *et al.*[11] gave m.p. 11°, $[\alpha]_D^{18}$ +106.5° (*c* 2, methanol).

Anal. Calc. for $C_{11}H_{22}O_6$: C, 52.8; H 8.9. Found: C, 52.9, H, 9.0.

The glucoside gave a single peak on columns A (*R* 1.00) and B (*R* 1.00).

(*b*) β-D *Anomer* (**2**). The distilled product crystallized rapidly when seeded, and was obtained as stout rods from pentane, m.p. 23–23.5°, $[\alpha]_D^{25}$ −88°, −94°, and −95° (*c* 1; water, methanol, and chloroform, respectively). [Hess and Heumann[12] gave $[\alpha]_D$ −72.7° (methanol) for a noncrystalline product prepared by a different method].

Anal. Calc. for $C_{11}H_{22}O_6$: C, 52.8; H 8.9. Found: C, 52.7; H, 8.9.

The glucoside gave a single peak on columns A (*R* 0.90) and B (*R* 0.85).

*2,3,5,6-Tetra-*O*-methyl-*D*-glucofuranose* (**3**)

The methyl D-glucosides above were hydrolyzed with 0.1N sulfuric acid at 100°; the α-D anomer (**1**) required 30 min, and the β-D anomer (**2**), 60 min. The cooled solutions were neutralized with barium carbonate, and filtered. The filtrates were deionized with ion-exchange resins, and concentrated to sirups which were distilled at 80°/0.001 mm to give, in both instances, the methylated glucofuranose (**3**), $[\alpha]_D^{25}$ −20.3°, and −34.7° (*c* 1; water and methanol, respectively), (lit.[11,12] $[\alpha]_D$ −7.6° and −21°.)

Anal. Calc. for $C_{10}H_{20}O_6$: C, 50.8; H, 8.5. Found: C, 51.0; H, 8.6.

The α,β-D mixture was only partially resolved on column A (*R* 1.25).

*3-Deoxy-2,5,6-tri-*O*-methyl-*D*-*erythro*-hex-2-enofuranose* (**4**)

A solution of 2,3,5,6-tetra-*O*-methyl-D-glucose (**3**) (35 g) in 3,500 ml of 0.04N calcium hydroxide (O_2-free) was kept at 50° for 16 h, cooled, neutralised with carbon dioxide, and filtered. The filtrate was concentrated to a sirup which was extracted with chloroform. The extracts were concentrated, and the residue distilled at 85°/0.002 mm to give **4**, a colorless liquid, $[\alpha]_D^{25}$ +35 +9.6° (30 min) (*c* 1, water), $\lambda_{max}^{H_2O}$ 194 mμ (ε 7,100); it showed a strong band at 1668 cm^{-1}.

Anal. Calc. for $C_9H_{16}O_5$: C, 52.6; H, 7.9. Found: C, 52.6; H, 7.9.

The n.m.r. spectrum of **4** was taken for a deuterium oxide solution, and, after the removal of the deuterium oxide, for a pyridine solution. In pyridine solution, approximately equimolar amounts of the two anomers were present, as judged from the intensities of the H-1 signals (a doublet for the β-D anomer, δ 6.20, $J_{1,4}$ 4.0 c.p.s., and a singlet for the α-D-anomer, δ 6.11); also, one of the three methoxyl bands (δ 3.31, ~3.49, and 3.62) consisted of two peaks of equal intensity (δ 3.48 and 3.50), each due to one anomer. In deuterium oxide solution, the differences in chemical shifts of H-1 of the two anomers were not as great, so that the signals appeared as two peaks, the one at δ 5.72 being due to the α-D anomer and the high-field part of the doublet of the β-D anomer; the second peak at δ 5.77, was of about one-third the

intensity, and was due to the low-field part of the β-D anomer signal. The behavior in these two solvents was thus similar to that shown by 3-deoxy-2-*O*-methyl-α, β-D-*erythro*-hex-2-enofuranose[2].

In acid solution (0.1N at 70° for 30 min), **4** was converted into the furanone **5** in 70% yield, as indicated by the ultraviolet absorption.

5-(D-glycero-1,2-Dimethoxyethyl)-3(2H)-furanone (**5**)

A solution of **4** (12 g) in 0.1N hydrochloric acid (150 ml) was heated at 100° for 6 min, cooled, neutralized to pH 7 with sodium bicarbonate, and extracted four times with chloroform. The extracts were dried (Na_2SO_4) and evaporated, and the residue was distilled at 90°/0.001 mm to give the furanone **5**, which was recrystallized from ether as colorless needles, m.p. 27–27.5°, $[\alpha]_D^{25}$ +107.6° (*c* 1, water), intense bands at 1700 cm^{-1} (C=O) and at 1602 cm^{-1} (C=C–C=O), $\lambda_{max}^{H_2O}$ 187 and 262 mμ (ε 2,400 and 12,800), changing after 30 min in 0.01N sodium hydroxide to λ_{max} 296 mμ (ε 17,200).

Anal. Calc. for $C_8H_{12}O_4$: C, 55.8; H, 7.0. Found: C, 55.7, H, 7.1.

The furanone **5** gave a single peak on g.l.c. on column A (*R* 0.38) and B (*R* 1.17), showing its strong polar character, which was confirmed by its insolubility in pentane. The n.m.r. spectrum recorded for its deuterium oxide solution is shown in Fig. 3. In carbon tetrachloride, small chemical shifts took place (H-4 at δ 5.62, H_2-2 at 4.48, H-1′ at 4.13, H_2-2′ at 3.62, OCH_3 at 3.45, and OCH_3 at 3.57).

When deuterium oxide replaced water as the solvent in the formation of the furanone **5** from **4**, or when the furanone was subjected to the same acid treatment in deuterium oxide, the product was the 2,2-dideuterofuranone **5a**, as indicated by the absence of the H_2-2 absorption band in the n.m.r. spectra.

In aqueous 0.1N hydrochloric acid, the furanone **5** was stable, very little change in the ultraviolet spectrum occurring even after 1 h at 100°. In acid, nonaqueous solvents, the furanone rapidly darkened, to form an insoluble, black polymer. In a sample of carbon tetrachloride which contained a trace of acid, the furanone was completely polymerized at room temperature in less than one hour.

*Methyl 3-deoxy-2,5,6-tri-*O*-methyl-α- and -β-*D-erythro-*hex-2-enofuranoside* (**6** *and* **7**)

The glycenofuranose **4** (25 g) in ether (15 ml) was methylated at room temperature by the addition during 10 min of a solution of sodium hydroxide (35 g in 90 ml) and methyl sulfate (30 ml). The reaction mixture was vigorously stirred during the addition and for a further 2 h. The furanosides were extracted into ether, and the extracts concentrated to a sirup which was distilled at 75°/0.01 mm. The product consisted of equal amounts of **6** and **7**.

(*a*) α-D *Anomer* (**6**). The α-D anomer was separated by g.l.c. on column D; $[\alpha]_D^{25}$ +33° (*c* 0.4, water), $\lambda_{max}^{H_2O}$ 194 mμ (ε 7,330), strong band at 1669 cm^{-1}.

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.0; H, 8.3. Found: C, 54.7; H, 8.1.

It gave single peaks on g.l.c. on columns A (*R* 0.62) and B (*R* 0.78). The n.m.r. spectrum is shown in Fig. 1. ($J_{1,3}$ 0.7, $J_{1,4}$ 0.9, $J_{3,4}$ 1.8, and $J_{4,5}$ 7.2 c.p.s.).

(*b*) β-D *Anomer* (**7**) The β-D anomer crystallized from the anomeric mixture at −20°. It was recrystallized twice from ether at −40 to −50°; m.p. 30°, $[\alpha]_D^{25}$ +13° (*c* 0.5, water), $\lambda_{max}^{H_2O}$ 194 mμ (ε 8,500), strong band at 1670 cm^{-1}.

Anal. Calc. for $C_{10}H_{18}O_5$: C, 55.0; H, 8.3. Found: C, 54.8; H, 8.2.

It gave single peaks on g.l.c. on columns A (*R* 0.66) and B (*R* 0.93). The n.m.r. spectrum is shown in Fig. 2 ($J_{1,3}$ 0.7, $J_{1,4}$ 4.0, $J_{3,4}$ 1.5, and $J_{4,5}$ 5.4 c.p.s.). In deuterium oxide, the n.m.r. signals of H-1 from the two anomers were well separated (singlet at δ 5.42 for **6**, and a doublet at δ 5.53 for **7**), but those of H-3 and H-4 formed a complex band.

(*c*) *Action of acid on* **6** *and* **7**. When **6** or **7** was treated with 0.1M methanolic hydrogen chloride for 40 min at 25°, or for 3 min at 50°, the reaction mixture turned black, and, after it had been neutralized with sodium hydroxide, g.l.c. and n.m.r. spectroscopy showed that, in each case, the good yield of recovered product was an almost equimolar mixture of **6** and **7**, with a trace of **8**. Longer reaction times yielded **8** and **9**.

In 0.1N hydrochloric acid in water or deuterium oxide, **6** and **7** were rapidly hydrolyzed at room temperature to an equilibrium mixture consisting, on a molar basis of, about eight parts each of the free sugar **4** (α,β) and methanol and one part each of the furanosides **6** and **7**. This result was obtained by an examination of n.m.r. signals of H-1 (**4**, two peaks at δ 5.72, 5.77 in the ratio of 3:1; **6**, a singlet at δ 5.42; and **7**, a doublet at δ 5.53) and from the methoxyl signals (methanol gave a peak at a slightly higher field). When the reaction mixture was warmed, the furanone **5** was formed, and **4**, **6**, and **7** disappeared at the same rate. The ultraviolet absorption indicated a yield of furanone of about 70%.

*Methyl 3-deoxy-5,6-di-*O*-methyl-α- and -β-*D-erythro-*hexofuranosidulose dimethyl acetal* (**8** *and* **9**)

The unsaturated furanoside **7** (1 g) was heated at 50° for 25 min in 0.1M anhydrous, methanolic hydrogen chloride (20 ml); during this treatment, a relatively large amount of insoluble black material formed. The reaction mixture was cooled, and made slightly alkaline with N sodium hydroxide; most of the black material dissolved when the alkali was added. The methanol was evaporated, and the residue was extracted with pentane. The extract was evaporated to a clear sirup (530 mg), which was subjected to preparative g.l.c. on column C to give three fractions. The first fraction was an unresolved double peak, and the second and third fractions came from two slightly overlapping peaks, but enough of the overlap was rejected to ensure that fraction 2 was free from fraction 3. Each fraction was analyzed on columns A and B. The first fraction consisted mainly of the unsaturated furanosides **6** and **7**, with slightly more of the β-D anomer; the second was the pure β-D-furanosidulose acetal **8**; and the third was the α-D anomer **9** contaminated with about 3% of **8**. In the original preparation, fractions 1, 2, and 3 were present in the ratio of 0.6:2:1, as estimated by g.l.c.

(*a*) α-D *Anomer* (**8**). This anomer was obtained pure, as judged by g.l.c. on

columns A (R 0.90) and B (R 0.72); $[\alpha]_D^{25}$ +92.5° (c 2, chloroform), no λ_{max} from 185 to 400 mμ, no infrared OH stretch bands or bands in the double-bond region (1500–2000 cm^{-1}).

Anal. Calc. for $C_{11}H_{22}O_6$: C, 52.8; H, 8.9. Found: C, 52.9; H, 8.8.

The n.m.r. spectrum in carbon tetrachloride showed: two quartets (2 protons, H-3*a* δ 1.83, and H-3*b* δ 2.11), a group of three strong, sharp bands above weaker ones with a total of 18 protons (split up into: 6 protons due to two OMe at δ 3.17, 7 protons due to two OMe at δ 3.28 and H_2-6, and 5 protons due to one OMe at δ 3.38 and H-5 and H-6), a multiplet (1 proton, H-4 at $\delta\sim$4.0), and a singlet (1 proton, H-1 at δ 4.46) ($J_{3a,b}$ 13.4, $J_{3a,4}$ 5.0 and $J_{3b,4}$ 8.6 c.p.s.).

(*b*) *β*-D *Anomer* (**9**). The sample of this anomer still contained about 3% of the α-D anomer, as indicated by g.l.c. on columns A, (R 1.0) and B (R 0.93); $[\alpha]_D^{25}$ −68° (c 1.6, chloroform), the ultraviolet spectrum was as for **8**, and it showed no infrared OH stretch bands or double-bond bands, the n.m.r. spectrum was similar to that of the α-D anomer, except for the following differences: H-3*a* at δ 1.70, H-4 at $\delta\sim$3.75, and H-1 at δ 4.40; also, $J_{3a,b}$ 13.1, $J_{3a,4}$ 6.4 and $J_{3b,4}$ 9.4 c.p.s.

(*c*) *Formation of furanone from* (**8**). In 0.03N hydrochloric acid at 100°, the yield of the furanone **5** was only 20–25% after about 2 h. The furanone was identified by n.m.r. and u.v. spectroscopy, and by g.l.c.

ACKNOWLEDGMENTS

The author is indebted to Mr. D. Gallimore for technical assistance, and to Dr. K.E. Murray for the design of the still shown in Fig. 4.

SUMMARY

3-Deoxy-2,5,6-tri-*O*-methyl-D-*erythro*-hex-2-enofuranose (**4**) was prepared by the action of lime-water on 2,3,5,6-tetra-*O*-methyl-D-glucofuranose (**3**). The α-D (**6**) and β-D (**7**) anomers of the methyl glycosides of **4** were obtained by methylation, and each was converted by methanolic acid into their anomeric mixture, and then into methyl 3-deoxy-5,6-di-*O*-methyl-α- and -β-D-*erythro*-hexofuranosidulose dimethyl acetal (**8** and **9**). The 5-(D-*glycero*-1,2-dimethoxyethyl)-3(2*H*)-furanone (**5**) was formed by the action of aqueous acid on **4** and **6**–**9**.

Preparative methods for **3** and for its methyl glycosides were improved.

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D-GLUCOSE DIPHENYL DITHIOACETAL, PHENYL 1-THIO-α-D-GLUCOPYRANOSIDE, PHENYL 1-THIO-α-D-GLUCOFURANOSIDE, AND SOME RELATED COMPOUNDS*,**

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Emil Fischer[3] stated in 1894 that aldoses do not condense with benzenethiol in the presence of acids to form dithioacetals, and he repeated that statement[4] in 1908. Fifty years later El-Hewehi[5], using a mixture of concentrated hydrochloric acid and fused zinc chloride as a combined condensing and hydrolytic agent, succeeded in preparing crystalline D-galactose diphenyl dithioacetal from lactose and benzenethiol; he did not report a yield. El-Hewehi[5] described tetra-*O*-acetyl-D-ribose diphenyl dithioacetal as a yellowish oil distillable in a high vacuum. More recently, Horton and Wander[6] have prepared the crystalline diphenyl dithioacetals of D-ribose and L-arabinose.

In this laboratory we have prepared crystalline D-glucose diphenyl dithioacetal (**1**) in a 71% yield simply by shaking D-glucose, benzenethiol, and concentrated hydrochloric acid for 11 days at room temperature. Phenyl 1-thio-α-D-glucopyranoside (**2**) could be isolated in low yield from the mother liquor of **1**, or it could be obtained in a 31% yield by the direct action of concentrated hydrochloric acid upon **1**. The pyranoid ring structure of **2** was proved by reductive desulfurization with Raney nickel to 1,5-anhydro-D-glucitol (**3**). Both **2** and its tetraacetate were quite dextrorotatory, having $[\alpha]_D^{20}$ values of +258° and +234° in pyridine, respectively. When the phenyl 1-thio-α-D-glucopyranoside tetraacetate was treated with hydrogen peroxide in glacial acetic acid at room temperature it was converted into the corresponding sulfone, which was then deacetylated to yield α-D-glucopyranosyl phenyl sulfone (**4**).

When D-glucose diphenyl dithioacetal (**1**) was heated briefly with a mixture of mercuric chloride and mercuric oxide in ethyl alcohol, a 61% yield of phenyl 1-thio-α-D-glucofuranoside (**5**; $[\alpha]_D^{20}$ +216° in pyridine) was obtained. Its furanoid ring structure was proved by reductive desulfurization to 1,4-anhydro-D-glucitol (**6**).

*For the preceding paper from this laboratory on aryl thioglycopyranosides and aryl glycopyranosyl sulfones, see Ref. 1.

**For a recent review on developments in the chemistry of thio sugars, including many references to the earlier work in this field, see Ref. 2.

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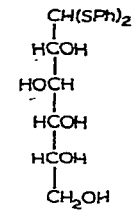
Acetylation of **5**, followed by oxidation with hydrogen peroxide and deacetylation, yielded α-D-glucofuranosyl phenyl sulfone (**7**).

Although sulfoxides have been described in the sugar series[7], we reported in the preceding paper[1] that we had been unable to isolate one. Now we have succeeded in obtaining, in very low yield by the brief action of hydrogen peroxide in glacial acetic acid on phenyl 1-thio-α-D-glucofuranoside (**5**), what we believe is a crystalline α-D-glucofuranosyl phenyl sulfoxide (**8**). It seems probable that the use of peroxybenzoic acid in chloroform at −15°, as described recently by Wagner and Wagler for a series of phenyl tetra-*O*-acetyl-1-thio-β-D-glycopyranosides[7], would have been more successful. Some data on the infrared spectrum of our sulfoxide, together with similar data for some related compounds described in this paper, are given in the Experimental. Some reactions that had been successful with the aryl 1-thio-β-D



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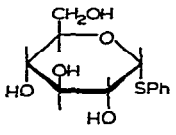

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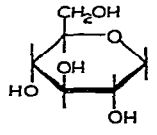

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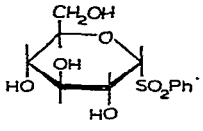

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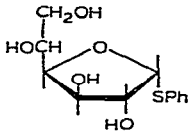

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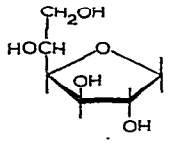

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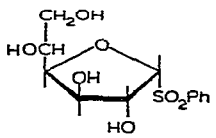

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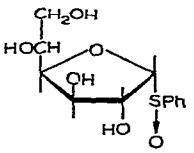

8

glucopyranosides and the aryl β-D-glucopyranosyl sulfones described in the preceding paper[1] were unsuccessful when applied to the α-D compounds of this paper. Brief mention is made of these reactions in the Experimental, and the diphenyl dithioacetals of D-galactose and D-mannose are also described there.

EXPERIMENTAL

Paper chromatography was performed on Whatman No. 1 filter paper by the descending method at room temperature with butyl alcohol–pyridine–water (6:4:3) unless otherwise noted. The locations of the spots were revealed by dipping the

papers in silver nitrate in acetone followed by sodium hydroxide in 95% ethyl alcohol[8].

D-*Glucose diphenyl dithioacetal* (**1**)

The best yields of the title compound were obtained by shaking a solution of 18 g of anhydrous D-glucose in 20 ml of concentrated hydrochloric acid with 35 ml of benzenethiol for 11 days at 20–25°. At the end of that time the mixture contained a heavy, sludge-like precipitate that was isolated by cooling the mixture in an ice bath, diluting with ice and ice water, and filtering. The product was washed well with cold water and then ethyl ether. The air-dried D-glucose diphenyl dithioacetal weighed 27.2 g (71%). It was recrystallized from 16 parts of ethyl alcohol, yielding 24.9 g of prismatic needles, m.p. 155–157° and $[\alpha]_D^{20}$ +1.5 ±1° (*c* 1, pyridine).

Anal. Calc. for $C_{18}H_{22}O_5S_2$: C, 56.52; H, 5.80; S, 16.77. Found: C, 56.49; H, 5.59; S, 16.45.

The mother liquors from three such preparations were combined, neutralized with solid sodium hydrogen carbonate, and concentrated *in vacuo* to a solid residue. Extraction several times with hot ethyl acetate followed by evaporation of that solvent left 7 g of crystalline residue that appeared, from paper chromatography, to be principally phenyl 1-thio-α-D-glucopyranoside (**2**). Recrystallization from a small amount of hot water yielded 3.8 g of crystals of **2**, identified by comparison with the same compound prepared as described below.

Phenyl 1-thio-α-D-glucopyranoside (**2**)

A suspension of 10 g of **1** in 100 ml of concentrated hydrochloric acid was stirred for 2 h at room temperature, during which time the color of the reaction mixture turned from a light yellow to an olive green. The mixture was chilled, diluted with an equal volume of cold water, and the undissolved dithioacetal (7.0 g) filtered and washed with a small amount of water. The filtrate was neutralized with solid sodium hydrogen carbonate, and concentrated *in vacuo* to a solid residue that was extracted twice with 250-ml portions of hot ethyl acetate and then with 100 ml of hot ethyl alcohol. Evaporation of the ethyl acetate extracts yielded 0.9 g of crystalline material that paper chromatography showed to be nearly pure phenyl 1-thio-α-D-glucopyranoside (**2**). The residue (0.5 g) from the ethyl alcohol extract contained some **2** and at least 4 other substances that, from their low mobility on paper chromatograms, appeared not to be glycosides. When the 7.0 g of recovered D-glucose diphenyl dithioacetal was washed with ethyl alcohol, the olive-green color was removed. The remaining 6.7 g of colorless **1** was stirred again with concentrated hydrochloric acid. An additional 1.3 g of **2** was thus obtained and 3.0 g of **1** recovered. The 2.2 g of phenyl 1-thio-α-D-glucopyranoside was recrystallized from ethyl alcohol to give 1.55 g of needles having m.p. 156–157° and $[\alpha]_D^{20}$+258° (*c* 0.6, pyridine). This represents a 31% yield based upon the 7 g of **1** consumed.

Anal. Calc. for $C_{12}H_{16}O_5S$: C, 52.93; H, 5.92; S, 11.77. Found: C, 52.92; H, 5.96; S, 11.81.

Reductive desulfurization of phenyl 1-thio-α-D-glucopyranoside (**2**) *to 1,5-anhydro-D-glucitol* (**3**)

A stirred mixture of 0.9 g of **2**, 7 g of Raney nickel, and 75 ml of 70% aqueous ethyl alcohol was refluxed for 3 h. The solution was decanted, and the Raney nickel was washed several times with a total of 200 ml of 30% aqueous ethyl alcohol. Upon evaporation of most of the solvent, the syrupy residue crystallized. Filtered and washed with ethyl alcohol, it weighed 169 mg (31%). This product was recrystallized from ethyl alcohol and identified as 1,5-anhydro-D-glucitol (polygalitol, **3**) through its m.p. 141–142° and $[\alpha]_D^{20}$ +42.7° (*c* 1, water); a mixed m.p. with authentic material[9] having m.p. 141–142° and $[\alpha]_D^{20}$ +42.5° (*c* 2, water) was not depressed, and infrared spectra of the two substances were identical. Paper-chromatographic examination of the mother liquor from the 169 mg showed the presence of three compounds in addition to **3**, but none of them corresponded to 1,4-anhydro-D-glucitol.

Phenyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-glucopyranoside

Acetylation of 200 mg of **2** with acetic anhydride and pyridine in the usual manner afforded 295 mg (91%) of the crystalline tetraacetate. The product was recrystallized from ethyl alcohol–hexane and chloroform–pentane: needles, having m.p. 91–92° and $[\alpha]_D^{20}$ +234° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{24}O_9S$: C, 54.53; H, 5.49; S, 7.28; CH_3CO, 39.1. Found: C, 54.81; H, 5.32; S, 7.47; CH_3CO, 38.8.

Tetra-O-acetyl-α-D-glucopyranosyl phenyl sulfone

A solution of 3.5 g of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside in 40 ml of glacial acetic acid and 7.4 ml of 30% hydrogen peroxide was kept for 9 days at room temperature and then diluted with 80 ml of cold water. The mixture was kept for a few hours in a refrigerator and then the precipitated sulfone was filtered and washed with water: yield 3.1 g (83%). It was recrystallized from ethyl alcohol and from chloroform–pentane; the clusters of elongated prisms melted at 161–162° and showed $[\alpha]_D^{20}$ +149° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{24}O_{11}S$: C, 50.84; H, 5.12; S, 6.79; CH_3CO, 36.4. Found: C, 50.82; H, 5.09; S, 6.69; CH_3CO, 36.9.

α-D-Glucopyranosyl phenyl sulfone (**4**)

The catalytic deacetylation of 1.5 g of tetra-*O*-acetyl-α-D-glucopyranosyl phenyl sulfone with methanolic sodium methoxide resulted in a practically quantitative yield of the free sulfone. It was recrystallized from ethyl acetate and then from ethyl alcohol. The prismatic needles melted at 125–126° (dec.) and showed $[\alpha]_D^{20}$ +150° (*c* 1.1, water).

Anal. Calc. for $C_{12}H_{16}O_7S$: C, 47.36; H, 5.30; S, 10.54. Found: C, 47.58; H, 5.37; S, 10.48.

Phenyl 1-thio-α-D-glucofuranoside (**5**)

A solution was prepared by dissolving 19 g (0.05 mole) of D-glucose diphenyl

dithioacetal (**1**) in 350 ml of boiling ethyl alcohol in a 750-ml flask. The flask was placed on the preheated hot plate of a magnetic stirrer, and to it was added 5.4 g (0.025 mole) of powdered mercuric oxide followed by a hot solution of 6.8 g (0.025 mole) of mercuric chloride in 50 ml of ethyl alcohol. The hot solution was stirred for 15 min and then filtered by suction to remove most of the PhSHgCl (*ca.* 15 g), and the latter was washed with a small amount of ethyl alcohol. As the solution cooled and became concentrated during the filtration, an additional amount of PhSHgCl separated. After a second filtration, 10 ml of pyridine was added, to form an insoluble complex with any unchanged mercuric chloride, and the solution was kept overnight in a refrigerator. After filtration, the solution was concentrated *in vacuo* to a crystalline residue that was mixed well with ethyl ether, and then filtered and washed with ethyl ether. The 11.8 g of material was stirred with 250 ml of water for 1 h, and filtered to remove 1.4 g of unreacted dithioacetal. The aqueous filtrate was concentrated *in vacuo* to a crystalline residue that was filtered and washed with ethyl ether; wt. 10.3 g. Recrystallization from ethyl alcohol–pentane gave 7.7 g (61%, based on the 17.6 g of **1** consumed) of needles of phenyl 1-thio-α-D-glucofuranoside, m.p. 115–119°. Additional recrystallizations from ethyl acetate raised the m.p. to 119–121°; $[\alpha]_D^{20}$ +216° (*c* 0.9, pyridine).

Anal. Calc. for $C_{12}H_{16}O_5S$: C, 52.93; H, 5.92; S, 11.77. Found: C, 52.96; H, 6.16; S, 11.70.

Reductive desulfurization of phenyl 1-thio-α-D-glucofuranoside (**5**) *to 1,4-anhydro-D-glucitol* (**6**)

Reductive desulfurization of 0.9 g of the furanoside (**5**) with Raney nickel as described for the pyranoside (**2**) yielded 0.5 g of a syrup. Paper chromatograms showed that one of the two major spots had the mobility of 1,4-anhydro-D-glucitol; there was no evidence for any 1,5-anhydro-D-glucitol. The syrup was digested with warm isopropyl alcohol, and the extract was decanted and concentrated to a syrup that crystallized in part overnight. The crystals, filtered and washed free of syrup with ethyl alcohol, weighed 115 mg (19%). This product was recrystallized from ethyl alcohol–pentane and identified as 1,4-anhydro-D-glucitol through its m.p. 111–114° and $[\alpha]_D^{20}$ −22.0° (*c* 1.6, water) as compared with the published values[10] of m.p. 115–116° and $[\alpha]_D^{27}$ −21.9° (*c* 2.5, water). A mixed melting point with authentic material was not depressed, and infrared spectra of the two specimens were identical.

Tetra-O-acetyl-α-D-glucofuranosyl phenyl sulfone

Acetylation of 0.4 g of the furanoside (**5**) with acetic anhydride and pyridine yielded a syrupy acetate. This was oxidized with hydrogen peroxide as described above for the phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-glucopyranoside. Crystals of the sulfone tetraacetate (0.55 g, 79% overall from **5**) were obtained without difficulty; upon recrystallization from ethyl alcohol or from chloroform–pentane the compound separated as chunky prisms having m.p. 128–129° and $[\alpha]_D^{20}$ +106° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{24}O_{11}S$: C, 50.84; H, 5.12; S, 6.79; CH_3CO, 36.4. Found: C, 50.71; H, 5.30; S, 6.73, 6.92; CH_3CO, 36.6.

α-D-Glucofuranosyl phenyl sulfone (7)

Catalytic deacetylation of 2.0 g of the sulfone acetate gave a 97% yield of the free sulfone. The product was recrystallized once from ethyl alcohol–pentane and once from ethyl alcohol, forming diamond-shaped prisms having m.p. 127–128° (dec.) and $[\alpha]_D^{20}$ +49.8° (*c* 1.1, water).

Anal. Calc. for $C_{12}H_{16}O_7S$: C, 47.36; H, 5.30; S, 10.54. Found: C, 47.24; H, 5.05; S, 10.31.

α-D-Glucofuranosyl phenyl sulfoxide (8)

A 3-g sample of phenyl 1-thio-α-D-glucofuranoside (5) was dissolved in a mixture of 25 ml of glacial acetic acid and 6 ml of 30% hydrogen peroxide. After 1 h at room temperature, the clear, colorless solution was diluted with 300 ml of ethyl ether. The resulting precipitate, filtered and washed with ethyl ether, weighed 2.4 g and appeared, from a paper-chromatographic examination, to contain at least four components. Trituration of the 2.4 g of product with ethyl alcohol and filtration left 1.4 g of a mixture that was not identified. The filtrate was concentrated to a crystalline residue that was broken up and filtered with a small amount of ethyl alcohol. The 0.4 g that was then left undissolved appeared to be mainly one compound, which was purified by two recrystallizations from methanol–ethyl ether. The 150 mg of shiny needles thus obtained melted about 150–155° with some discoloration and this melting point varied with the rate of heating. The rotation $[\alpha]_D^{20}$ was +4° (*c* 1.1, water) and the mobility on paper chromatograms developed with butyl alcohol–ethyl alcohol–water (40:11:19) was about the same as that of the corresponding sulfone (7). An attempt to obtain a crystalline acetate was unsuccessful.

Anal. Calc. for $C_{12}H_{16}O_6S$: C, 49.99; H, 5.59; S, 11.1. Found: (two different preparations): C, 50.20, 50.43; H, 5.67, 5.70; S, 11.1, 10.6.

Results of some miscellaneous experiments

Treatment of 50 mg of α-D-glucopyranosyl phenyl sulfone (4) with boiling aqueous potassium hydroxide for 19 h gave a light-yellow, levorotatory solution from which 1.5 mg of crude 1,6-anhydro-β-D-glucopyranose could be isolated; identification was made by paper chromatography and mixed melting-point. Similar treatment of 200 mg of α-D-glucofuranosyl phenyl sulfone (7) resulted in a very dark red solution having no detectable rotation (after clarification) and from which no crystalline material could be isolated.

The action of 30% hydrogen peroxide in glacial acetic acid upon *p*-tolyl 1-thio-β-D-glucopyranoside for a week at room temperature had earlier given an excellent yield of the 6-acetate of the corresponding sulfone[1]. The similar treatment of phenyl 1-thio-α-D-glucopyranoside (2) and phenyl 1-thio-α-D-glucofuranoside (5) failed to yield crystalline products.

Raney-nickel desulfurization of a 250-mg sample of α-D-glucofuranosyl phenyl

sulfone (7) gave a mixture of at least five substances according to its paper chromatogram. Desulfurizations, with nickel chloride and sodium borohydride, according to Truce and Roberts[11], of the dithioacetal (1) and the tetraacetate of the sulfone 7 were likewise unproductive of any crystalline material.

D-Galactose diphenyl dithioacetal

When 18 g of D-galactose was dissolved in 50 ml of ice-cold, concentrated hydrochloric acid and 25 g of benzenethiol, and the mixture stirred for 90 min at 25° before being worked up as described for the D-glucose analog (1), the yield of recrystallized D-galactose diphenyl dithioacetal was only 6.25 g (16%). In a second experiment, 9 g of D-galactose, 25 ml of hydrochloric acid, and 25 g of benzenethiol were stirred for 19 h, and the yield of recrystallized dithioacetal was 13.3.g (69.5%). Upon a second recrystallization from ethyl alcohol, the D-galactose diphenyl dithioacetal separated as small prismatic needles having m.p. 173–174° and $[\alpha]_D^{20}$ −31.5° (*c* 4.4, pyridine). El-Hewehi[5] reported m.p. 178° but no rotation.

Anal. Calc. for $C_{18}H_{22}O_5S_2$: C, 56.52; H, 5.80; S, 16.77. Found: C, 56.80; H, 5.76; S, 17.02.

Reductive desulfurization of D-galactose diphenyl dithioacetal to 1-deoxy-D-galactitol (L-fucitol)

A mixture of 2.5 g of D-galactose diphenyl dithioacetal, 25 g of Raney nickel, and 75 ml of ethyl alcohol was refluxed for 20 h, filtered, and the Raney nickel washed with hot water. The combined filtrates were concentrated to a syrup that crystallized readily. The product was recrystallized from ethyl alcohol to give 0.18 g of 1-deoxy-D-galactitol having m.p. 154–155°, alone and also when mixed with authentic L-fucitol.

D-Mannose diphenyl dithioacetal

Unlike D-glucose and D-galactose, when 18 g of D-mannose was dissolved in 50 ml of cold, concentrated hydrochloric acid and the mixture stirred for 90 min at 25°, the mixture appeared to thicken at first and then became a clear, pink, homogeneous solution. It was kept overnight at 5° and then diluted with chipped ice to give a fine, white precipitate. The product was filtered and recrystallized, first from aqueous ethyl alcohol and then from ethyl alcohol, to give about 6 g of fine needles of D-mannose diphenyl dithioacetal having m.p. 138–139° and $[\alpha]_D^{20}$ −30.0° (*c* 2.6, pyridine).

Anal. Calc. for $C_{18}H_{22}O_5S_2$: C, 56.52; H, 5.80; S, 16.77. Found: C, 56.71; H, 5.97; S, 16.72.

Infrared data

The infrared spectra were obtained with a Perkin–Elmer recording infrared spectrophotometer Model 21. Nujol mulls were used. Since infrared data on glycosyl sulfones are rare[1] and such data on glycosyl sulfoxides are practically nonexistent[7], we have listed the principal absorption bands of some of our compounds to supple-

ment the data reported in our earlier paper[1]; the most intense bands are shown in italic type, and sh denotes shoulder. It will be noted that all sulfones have one or more absorption bands near 1325 cm^{-1} (the mean absorption frequency attributed to asymmetric SO_2 stretching vibrations in C–SO_2–C compounds[12]) but the nearest band in our sulfoxide is a relatively weak one at 1306 cm^{-1}. Both of the new sulfones and their tetraacetates show strong absorption between 1155 and 1150 cm^{-1}, a value that is of a frequency slightly higher than the mean value of 1140 cm^{-1} attributed to symmetric SO_2 stretching in C–SO_2–C compounds[12], but still within the range of 1160–1140 cm^{-1} assigned to sulfones[12]. Our sulfoxide shows a strong absorption band at 1052 cm^{-1}, which is very close to the mean value of 1050 cm^{-1} cited by Bellamy[12] for sulfoxides. Like Wagner and Wagler[7], however, we do not place any significance on this value as being especially characteristic of the sulfoxide group, because most of our thioglucosides and sulfones also have strong absorption bands in the region 1060–1040 cm^{-1}.

Phenyl 1-thio-α-D-glucofuranoside (**5**): 688, *737*, 774, 785, 823, 864, 882, 907, 925, 951, *1005*, *1046*, *1072*, 1095, 1144, 1218, 1266, 1587, 1723, *3250*, *3380* cm^{-1}.

Phenyl 1-thio-α-D-glucopyranoside (**2**): 691, *740*, 848, 863, 970, *1017*, 1034, *1048*, *1058*, *1077*, 1088, 1103, 1117, 1134, 3220, *3370*, cm^{-1}.

Phenyl 1-thio-β-D-glucopyranoside[13] (for comparison with **2**): 685, 697, *736*, 819, 876, 1003, 1015, *1033*, *1057*, *1068*, 1087, *1117*, 1270, *3150*, *3330* cm^{-1}.

Phenyl 1-thio-α-D-glucopyranoside tetraacetate: 688, *742*, 907, 920, 976, 983, *1037*, 1065, 1085, 1093, 1113, 1139, 1155, 1205 (sh), *1225* (sh), *1234*, *1258* (sh), 1283 (sh), 1303 (sh), 1317 (sh), 1326, 1340, *1742* cm^{-1}.

α-D-Glucofuranosyl phenyl sulfone (**7**): 687, *722*, 766, 779, 808, 892, 977, *1012*, 1035, *1055*, *1068*, *1094*, 1115, *1155*, 1234, 1283, *1303*, *1318*, *3150*, 3420, 3530 cm^{-1}.

α-D-Glucopyranosyl phenyl sulfone (**4**): 689, 737, 760, 775, 859, 918, 967, 999, *1032*, 1054, *1075*, *1088*, *1098* (sh), *1127*, *1153*, 1225, 1238, 1255, *1289*, 1303, 1323, *1334*, 3300, 3530 cm^{-1}.

α-D-Glucofuranosyl phenyl sulfone tetraacetate: 686, 738, 763, 846, 908, 955, 978, 999, 1009, 1027, *1073*, 1097, 1112, 1131, *1150*, 1182, *1231*, 1250 (sh), *1267*, 1288, *1323*, *1755* cm^{-1}.

α-D-Glucopyranosyl phenyl sulfone tetraacetate: 692, 737, 759, 886, 914, *1040*, 1074, 1084, 1104, *1117*, 1143, *1154*, 1165, *1204*, *1224*, *1238*, *1250* (sh), 1282, 1309, 1326, 1350, *1745*, *1757*, 1776 cm^{-1}.

α-D-Glucofuranosyl phenyl sulfoxide (**8**): 688, 697, 723 (sh), 734–752 (broad), 808, 853, 886, 907, 964 (sh), *984*, *1001*, *1052*, *1072*, 1115, 1133, 1205, 1252, *1288*, 1306, 2720, *3275*, 3560, 4300 cm^{-1}.

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SUMMARY

D-Glucose diphenyl dithioacetal has been prepared for the first time, in a 71% yield, by the action of benzenethiol and concentrated hydrochloric acid on D-glucose. It has been converted by acid into phenyl 1-thio-α-D-glucopyranoside and by mercuric chloride–mercuric oxide into phenyl 1-thio-α-D-glucofuranoside; the ring structures of these thioglucosides were established by reductive desulfurization. The thioglucoside acetates, upon oxidation with hydrogen peroxide in glacial acetic acid, yielded the corresponding α-D-glucopyranosyl and α-D-glucofuranosyl phenyl sulfones. An α-D-glucofuranosyl phenyl sulfoxide could be obtained only in very small yield. Some infrared data on these compounds are included.

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DERIVATE DER ZUCKER-THIOACETALE

TEIL XXXVIII[1]. CYCLOHEXYLIDENVERBINDUNGEN DER D-ARABINOSE-THIOACETALE

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(Eingegangen den 24. März, 1966)

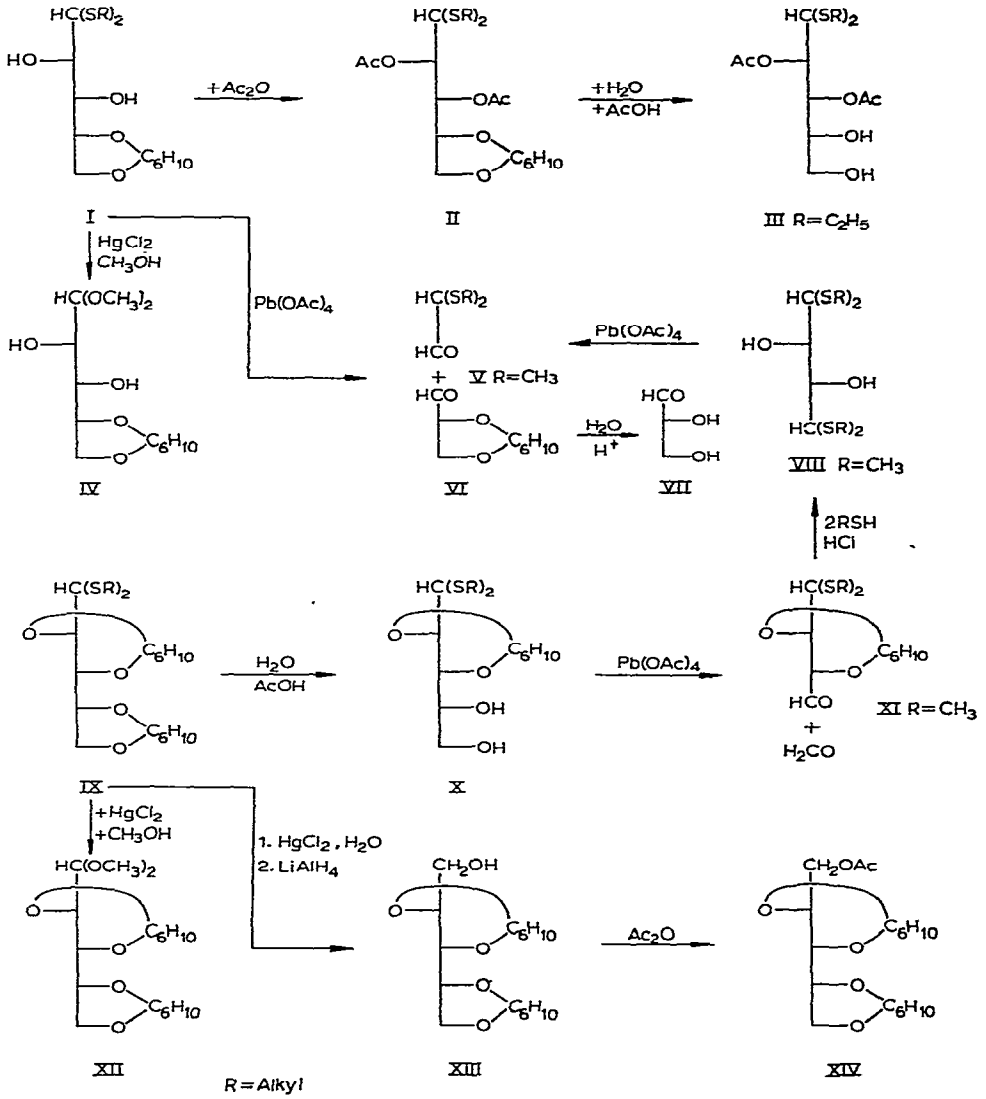
Die D-Arabinose-thioacetale[2] bilden beim Schütteln mit Cyclohexanon und Zinkchlorid 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetale (I). Nach dem Aufarbeiten gewinnt man die reinen, kristallisierten Verbindungen mit Ausbeuten von 57 bis 76%. Wir versuchten, aus ihnen durch Abspalten der Thioacetal-Gruppierungen mit Quecksilber(II)-chlorid und Quecksilberoxyd in wäßrigem Aceton die 4,5-*O*-Cyclohexyliden-*aldehydo*-D-arabinose darzustellen. Die amorphe Verbindung war jedoch nicht rein; sie konnte auch nicht in ein reines, kristallisiertes Derivat übergeführt werden. Erfolgreich verlief die Umsetzung der Cyclohexyliden-D-arabinose-thioacetale mit Methanol und Quecksilber(II)-chlorid bei Gegenwart von Quecksilberoxyd; man gewinnt auf diesem Wege das sirupöse, aber reine 4,5-*O*-Cyclohexyliden-D-arabinose-dimethylacetal (IV). Da alle Cyclohexyliden-D-arabinose-thioacetale (siehe Versuchsteil) das gleiche Dimethylacetal IV liefern, muß sich die Cyclohexyliden-Gruppierung bei allen Vertretern in der gleichen Stellung befinden.

Die 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetale besitzen zwei freie Hydroxyl-Gruppen, die sich mit Acetanhydrid und Pyridin acetylieren lassen. Die so entstandenen 2,3-Di-*O*-acetyl-4,5-*O*-cyclohexyliden-D-arabinose-thioacetale (II) sind Öle, die sich durch Destillation im Hochvakuum reinigen lassen. Aus dem Diäthylthioacetal II spalteten wir den Cyclohexyliden-Rest durch Hydrolyse mit Essigsäure ab. Die dabei gewonnene Verbindung (III) ist mit dem bekannten 2,3-Di-*O*-acetyl-D-arabinose-diäthylthioacetal[3] identisch. Diese Reaktionsfolge ist ein Beweis dafür, daß sich die Cyclohexyliden-Gruppe im Diäthylthioacetal II und damit auch in den Cyclohexyliden-D-arabinose-thioacetalen (I) in 4,5-Stellung befinden muß.

Ein weiterer Strukturbeweis für die 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetale kann durch Oxydation der Verbindungen mit Bleitetraacetat und anschließende Papierchromatographie der Spaltprodukte erbracht werden. Die Verbindungen müßten für die Glykolspaltung an den C-Atomen 2 und 3 theoretisch 1.0 Mol Bleitetraacetat verbrauchen. Nach 4 Stunden tritt jedoch ein Verbrauch von etwa 1.5 Mol auf. Es findet also neben der Glykolspaltung zusätzlich eine Oxydation an der Thioacetal-Gruppierung statt. Als Spaltprodukte sind Glyoxal-1-thioacetal (V) und *O*-Cyclohexyliden-D-glycerinaldehyd (VI) zu erwarten, wobei ersteres wegen der auftretenden „Überoxydation" teilweise in oxydierter Form vorliegen wird.

Für den papierchromatographischen Nachweis des *O*-Cyclohexyliden-D-glycerinaldehyds wurde die Verbindung zunächst mit verdünnter Schwefelsäure zum

D-Glycerinaldehyd (VII) hydrolysiert, der dann zusammen mit einem D-Glycerinaldehyd chromatographiert wurde, den wir aus *O*-Isopropyliden-D-glycerinaldehyd nach bekannter Vorschrift[4] dargestellt hatten. Beide zeigten gleiche Wanderungsgeschwindigkeit.



Zur Identifizierung des oben genannten Glyoxal-1-thioacetals (Dimethylthioacetal) stellten wir zunächst ein Vergleichspräparat durch Oxydation von D-*threo*-Dihydroxy-succindialdehyd-1,4-bis-dimethylthioacetal[5] (VIII) mit Bleitetraacetat dar. Das zu identifizierende und das als Vergleichssubstanz hergestellte Glyoxal-1-dimethylthioacetal wurden dann auf acetyliertem Papier chromatographiert. Erwartungsgemäß war die Wanderungsgeschwindigkeit beider Präparate gleich.

Während sich beim Schütteln der D-Arabinose-thioacetale mit Cyclohexanon unter dem Einfluß von Zinkchlorid fast nur 4,5-*O*-Cyclohexyliden-Verbindungen (siehe oben) bilden, erhält man bei Verwendung von Chlorwasserstoff oder Schwefelsäure als Kondensationsmittel ein Gemisch von 4,5-*O*-Cyclohexyliden- und 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetalen (IX) sowie 1-Cyclohexylidencyclohexanon-(2)[6], das durch Selbstkondensation des Cyclohexanons ensteht. Die Ausbeute an 2,3:4,5-Di-*O*-cyclohexyliden-Verbindungen kann dadurch vergrößert werden, daß man das Gemisch unter Verwendung von Kupfersulfat nachkondensiert. Am einfachsten gestaltet sich jedoch die Darstellung von IX, wenn man das Thioacetal und Cyclohexanon von Anfang an mit Schwefelsäure und Kupfersulfat schüttelt, das Reaktionsgemisch anschließend mit wäßrigem Ammoniak neutralisiert, die organische Phase vom festen Kupferkomplex abtrennt, das im Überschuß zugesetzte Cyclohexanon abdampft und den Rückstand bei 10^{-4} Torr fraktioniert destilliert. Das 1-Cyclohexyliden-cyclohexanon-(2) geht bei einer Badtemperatur von 60–70° über, die 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetale (IX) werden dann bei einer Badtemperatur von 150–160° destilliert.

In allen Vertretern von IX befinden sich die beiden Cyclohexyliden-Reste in der gleichen Stellung, denn beim Behandeln der Verbindungen mit Quecksilber(II)-chlorid und Methanol bei Gegenwart von Quecksilberoxyd erhält man immer das 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-dimethylacetal (XII). Die 2,3:4,5-Stellung der Cyclohexyliden-Reste wird unten noch bewiesen.

Zu einem analogen Ergebnis führt die Abspaltung der Alkylthio-Gruppierungen aus den Verbindungen IX durch Behandeln mit Quecksilber(II)-chlorid und Quecksilberoxyd in wäßrigem Aceton. Alle Vertreter von IX liefern hierbei die sirupöse 2,3:4,5-Di-*O*-cyclohexyliden-*aldehydo*-D-arabinose, die wir jedoch nicht ganz rein gewinnen konnten. Die Verbindung liefert aber nach Reduktion mit Lithiumaluminiumhydrid und Destillation im Hochvakuum den reinen 2,3:4,5-Di-*O*-cyclohexyliden-D-arabit (XIII). Die gleiche Verbindung erhält man aus D-Arabit durch Kondensation mit Cyclohexanon bei Gegenwart von Schwefelsäure und Kupfersulfat. Das auf diesem Wege erhaltene Präparat ist aber weniger rein. Zur weiteren Charakterisierung führten wir XIII in den 1-*O*-Acetyl-2,3:4,5-di-*O*-cyclohexyliden-D-arabit (XIV) über.

Die Struktur der 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetale (IX) kann durch partielle Hydrolyse zu den 2,3-*O*-Cyclohexyliden-D-arabinose-thioacetalen (X) und deren Oxydation mit Bleitetraacetat erbracht werden. Die partielle Hydrolyse, die wir mit dem Dimethyl-, Diisopropyl-, und dem Di-*n*-butylthioacetal IX durchführten, ist schwierig. Die besten Ergebnisse erhielten wir durch 2.5-stündiges Erwärmen von IX mit 80-proz. Essigsäure auf 65°. Aber auch hier enthält das Hydrolysegemisch neben dem gewünschten 2,3-*O*-Cyclohexyliden-D-arabinose-thioacetal (X) nicht hydrolysiertes Di-*O*-cyclohexyliden-thioacetal (IX) und D-Arabinose-thioacetal, das durch vollständige Hydrolyse entsteht. Aus dem Gemisch der drei Verbindungen läßt sich zunächst das D-Arabinose-thioacetal durch Kristallisation weitgehend abtrennen. Die Trennung der Mono-*O*-cyclohexyliden- (X) von der Di-*O*-cyclohexyliden-Verbindung (IX) gelingt durch Chromatographie an Aluminiumoxyd. Auf diese

Weise konnten wir das Diisopropyl- und das Di-*n*-butylthioacetal der 2,3-*O*-Cyclohexyliden-D-arabinose als reine Öle gewinnen; das Dimethylthioacetal war nicht ganz rein darstellbar.

Die Struktur der 2,3-*O*-Cyclohexyliden-D-arabinose-thioacetale (X) läßt sich durch eine übliche analytische Oxydation mit Bleitetraacetat in Benzol beweisen. Es findet eine Glykolspaltung zwischen den C-Atomen 4 und 5 statt. Der dabei gebildete Formaldehyd kann als Dimedon-Verbindung mit einer Ausbeute von 55–65% nachgewiesen werden. Der Cyclohexyliden-Rest muß nach diesem Ergebnis die 2,3-Stellung einnehmen.

Am Beispiel des Dimethylthioacetals X ist es uns gelungen, auch das zweite bei der Oxydation gebildete Bruchstück, das 2,3-*O*-Cyclohexyliden-D-*threo*-dihydroxy-succindialdehyd-1-dimethylthioacetal (XI), zu gewinnen. Die sirupöse, nicht ganz reine Verbindung wurde mit Methylmercaptan und Chlorwasserstoff in Dioxan behandelt. Dabei reagiert die noch freie Aldehydgruppe in XI unter Thioacetal-Bildung, gleichzeitig wird der Cyclohexyliden-Rest abgespalten; es entsteht das bekannte, kristallisierte D-*threo*-Dihydroxy-succindialdehyd-1,4-bis-dimethylthioacetal[5] (VIII).

EXPERIMENTELLER TEIL

Darstellung der 4,5-O-Cyclohexyliden-D-arabinose-thioacetale (I)

Man löst 0.01 Mol eines D-Arabinose-thioacetals[2] unter schwachem Erwärmen in 30 ml Cyclohexanon, läßt auf 20° abkühlen, fügt 3.0 g wasserfreies Zinkchlorid hinzu, schüttelt 2.5 Stunden bei 20°, verdünnt dann mit 50 ml Äther, wäscht die ätherische Phase zweimal mit je 30 ml Wasser, einmal mit 20 ml Natriumhydrogencarbonat-Lösung und nochmals mit 30 ml Wasser, destilliert den Äther ab und vertreibt das Cyclohexanon durch Wasserdampfdestillation im Vakuum. Das zurückbleibende Rohprodukt kristallisiert nach kurzer Zeit. Das Cyclohexyliden-D-arabinose-äthylenthioacetal wird aus Äthanol–Wasser, alle übrigen, folgend aufgeführten Verbindungen werden aus *n*-Hexan umkristallisiert.

Dimethylthioacetal: Ausb. 2.32 g (75%), Schmp. 91°, $[\alpha]_D^{20}$ −55.3° (*c* 1.44, Chloroform) (Gef.: C, 50.62; H, 7.46. $C_{13}H_{24}O_4S_2$ Ber.: C, 50.61, H, 7.84%).

Diäthylthioacetal: Ausb. 2.22 g (66%), Schmp. 72°, $[\alpha]_D^{20}$ −54.3° (*c* 2.65, Chloroform) (Gef.: C, 53.40; H, 8.37. $C_{15}H_{28}O_4S_2$ Ber.: C, 53.54; H, 8.39%).

Di-n-propylthioacetal: Ausb. 2.08 g (57%), Schmp. 67°, $[\alpha]_D^{20}$ −65.7° (*c* 1.98, Chloroform) (Gef.: C, 55.77; H, 9.13. $C_{17}H_{32}O_4S_2$ Ber.: C, 56.00; H, 8.85%).

Diisopropylthioacetal: Ausb. 2.37 g (65%), Schmp. 79°, $[\alpha]_D^{20}$ −42.1° (*c* 3.61, Chloroform) (Gef.: C, 55.93; H, 8.67. $C_{17}H_{32}O_4S_2$ Ber.: C, 56.00; H,8.85%).

Di-n-butylthioacetal: Ausb. 2.51 g (64%), Schmp. 76°, $[\alpha]_D^{20}$ −61.9° (*c* 3.00, Chloroform) (Gef.: C, 58.30; H, 9.34. $C_{19}H_{36}O_4S_2$ Ber.: C, 58.12; H, 9.24%).

Diisobutylthioacetal: Ausb. 2.27 g (58%), Schmp. 73°, $[\alpha]_D^{20}$ −66.9° (*c* 3.23, Chloroform) (Gef.: C, 57.96; H, 9.19. $C_{19}H_{36}O_4S_2$ Ber.: C, 58.12; H, 9.24%).

Dibenzylthioacetal: Ausb. 2.72 g (59%), Schmp. 96°, $[\alpha]_D^{20}$ −45.6° (*c* 2.66, Chloroform) (Gef.: C, 65.07; H, 7.23. $C_{25}H_{32}O_4S_2$ Ber.: C, 65.17; H, 7.00%).

Äthylenthioacetal: Ausb. 2.33 g (76%), Schmp. 107°, $[\alpha]_D^{20}$ −14.3° (*c* 2.53, Chloroform) (Gef.: C, 51.15; H, 7.30. $C_{13}H_{22}O_4S_2$ Ber.: C, 50.96; H, 7.24%).

4,5-O-Cyclohexyliden-D-arabinose-dimethylacetal (IV)

Man rührt 0.01 Mol eines 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetals mit 60 ml Methanol, 6.0 g Quecksilber(II)-chlorid und 6.0 g gelbem Quecksilberoxyd 5 Stunden bei 40°, filtriert, wäscht den Rückstand mit 30 ml Methanol, dampft die vereinigten Filtrate bei Gegenwart von 1.0 g Quecksilberoxyd ein, extrahiert den Rückstand dreimal mit je 30 ml heißem Chloroform, wäscht die vereinigten Extrakte mit je 30 ml N KJ sowie mit 10 ml Wasser, trocknet mit Natriumsulfat, dampft das Chloroform ab und destilliert den Rückstand bei 10^{-4} Torr und 120–130° Badtemperatur. Ausb. 1.73 g (63%), farbloser Sirup, $[\alpha]_D^{19}$ −5.5° (*c* 3.83, Chloroform), n_D^{19} 1.4812 (Gef.: C, 56.25; H, 8.83. $C_{13}H_{24}O_6$ Ber.: C, 56.51; H, 8.76%).

Darstellung der 2,3-Di-O-acetyl-4,5-O-cyclohexyliden-D-arabinose-thioacetale (II)

0.01 Mol eines 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetals wird in 10 ml Pyridin gelöst und mit 3.0 ml Acetanhydrid versetzt. Man läßt die Lösung 16 Stunden bei 20° stehen, gießt dann in 150 ml Wasser, läßt einige Stunden stehen, nimmt den ausgefallenen Sirup in 30 ml Chloroform auf, wäscht den Chloroform-Extrakt je zweimal mit 30 ml Kaliumhydrogensulfat- und gesättigter Natriumhydrogencarbonat-Lösung sowie mit 20 ml Wasser, trocknet mit Natriumsulfat, destilliert das Chloroform ab und destilliert das zurückbleibende Rohprodukt bei 10^{-4} Torr, wobei man die folgend aufgeführten Verbindungen als farblose Öle erhält. Das rohe Äthylenthioacetal kristallisiert nach dem Abdampfen des Chloroforms aus; es wird aus *n*-Hexan umkristallisiert.

Diäthylthioacetal: Ausb. 3.49 g (83%), Badtemperatur der Destillation 135–145°, $[\alpha]_D^{18}$ +8.4° (*c* 2.38, Chloroform), n_D^{20} 1.5037 (Gef.: C, 54.33; H, 7.77. $C_{19}H_{32}O_6S_2$ Ber.: C,54.25; H, 7.67%).

Di-n-propylthioacetal: Ausb. 2.87 g (64%), Badtemperatur der Destillation 135–145°, $[\alpha]_D^{18}$ +10.7° (*c* 2.46, Chloroform), n_D^{20} 1.5006 (Gef.: C, 56.46; H, 8.26. $C_{21}H_{36}O_6S_2$ Ber.: C, 56.21; H, 8.09%).

Diisopropylthioacetal: Ausb. 2.60 g (58%), Badtemperatur der Destillation 140–145°, $[\alpha]_D^{18}$ +13.7° (*c* 1.58, Chloroform), n_D^{20} 1.4985 (Gef.: C, 55.92; H, 8.32. $C_{21}H_{36}O_6S_2$ Ber.: C, 56.21; H, 8.09%).

Di-n-butylthioacetal: Ausb. 2 96 g (62%), Badtemperatur der Destillation 145–150°, $[\alpha]_D^{18}$ +10.5° (*c* 2.52, Chloroform), n_D^{20} 1.4968 (Gef.: C, 57.62; H, 8.56 $C_{23}H_{40}O_6S_2$ Ber.: C, 57.95; H, 8.46%).

Äthylenthioacetal: Ausb. 2.81 g (72%), Schmp. 75°, $[\alpha]_D^{18}$ +16.4° (*c* 1.47, Chloroform) (Gef.: C, 52.47; H, 6.84. $C_{17}H_{26}O_6S_2$ Ber.: C, 52.28; H, 6.71%).

2,3-Di-O-acetyl-D-arabinose-diäthylthioacetal (III)

2,3-Di-*O*-acetyl-4,5-*O*-cyclohexyliden-D-arabinose-diäthylthioacetal (4.21 g, 0.01 Mol) wird mit 25 ml 80-proz. Essigsäure 90 Minuten im siedenden Wasserbad erhitzt. Dann engt man im Vakuum zu einem Sirup ein, nimmt diesen in 100 ml Chloroform auf, filtriert, wäscht das Filtrat mit 30 ml Natriumhydrogencarbonat-Lösung sowie mit 30 ml Wasser, trocknet mit Natriumsulfat, engt zu einem Sirup ein und destilliert diesen bei 10^{-4} Torr und 135–140° Badtemperatur. Ausb. 2.29 g (67%), farbloser Sirup, $[\alpha]_D^{19}$ −27.0° (*c* 2.52, Chloroform), n_D^{20} 1.5063. Lit.[3]: $[\alpha]_D^{17}$ −27.3° (*c* 1.23, Chloroform), n_D^{20} 1.5050.

Oxydation des 4,5-O-Cyclohexyliden-D-arabinose-dimethylthioacetals (I) mit Bleitetraacetat

Man löst 0.31 g (0.001 Mol) I in 35 ml Benzol, fügt 0.6 g Bleitetraacetat hinzu, schüttelt eine Stunde bei 20°, läßt anschließend 3 Stunden stehen, schüttelt die Lösung dreimal mit je 3.0 ml gesättigter Natriumhydrogencarbonat-Lösung sowie mit 2.0 ml Wasser, trocknet mit Natriumsulfat und dampft im Vakuum ein. Der Rückstand wird in 1.0 ml Methanol aufgenommen und filtriert. Die so gewonnene Lösung dient zur unten angegebenen Chromatographie.

Chromatographischer Nachweis von Glyoxal-1-dimethylthioacetal (V)

Auf einem Keilstreifen[7] wird eine Probe vorstehend genannter Lösung aufgetragen; auf einem zweiten Keilstreifen trägt man von einer Lösung auf, die man durch Oxydation von 0.28 g (0.001 Mol) D-*threo*-Dihydroxy-succindialdehyd-1,4-bis-dimethylthioacetal[5] und Aufarbeiten, wie zuvor beschrieben, erhält. Zur Chromatographie werden das Lösungsmittelgemisch Butylacetat–Pyridin–Wasser (1:5:10) und das Papier „Schleicher & Schüll 2043 bmgl acetyliert" benutzt. Zum Sichtbarmachen von V auf den Chromatogrammen dient Anilinphthalat[8]. Die beiden Proben zeigen auf den parallel behandelten Chromatogrammen gleiche Wanderungsgeschwindigkeit. Der R_F-Wert beträgt 0.48.

Chromatographischer Nachweis von D-Glycerinaldehyd (VII)

Die methylalkoholische Lösung (0.5 ml), die nach der Oxydation von I erhalten wurde (siehe oben), wird mit 0.5 ml N H_2SO_4 30 Minuten auf 80°erwärmt; anschließend neutralisiert man durch Schütteln mit einem Anionenaustauscher (Wofatit L) und filtriert. Parallel werden 0.13 g (0.001 Mol) *O*-Isopropyliden-D-glycerinaldehyd[4] durch Erwärmen mit 2.0 ml N H_2SO_4 hydrolysiert, wie zuvor angegeben. Proben beider Lösungen werden nach der Keilstreifenmethode[7] auf dem Papier „Schleicher & Schüll 2043 bmgl" mit dem Lösungsmittelgemisch *n*-Butanol–Äthanol–Wasser (5:1:4) bei 20° chromatographiert. Zum Nachweis des D-Glycerinaldehyds auf dem Chromatogramm dient Anilinphthalat[8]. Die beiden chromatographierten Proben zeigen einen R_F-Wert von 0.65.

Darstellung der 2,3:4,5-Di-O-cyclohexyliden-D-arabinose-thioacetale (IX)

Man schlämmt 0.01 Mol eines D-Arabinose-thioacetals[2] und 5.0 g wasserfreies Kupfersulfat in 25 ml Cyclohexanon auf, fügt eine unter Kühlung hergestellte Mischung von 0.5 ml konz. Schwefelsäure und 25 ml Cyclohexanon hinzu, schüttelt 20 Stunden bei 20°, neutralisiert mit konz. Ammoniak-Lösung, filtriert vom festen Kupferkomplex ab, entfernt aus dem Filtrat das Cyclohexanon durch Wasserdampfdestillation im Vakuum und destilliert den Rückstand bei 10^{-4} Torr. Das 1-Cyclohexylidencyclohexanon-(2)[6] geht bei einer Badtemperatur von 60–70° über; anschließend destilliert man die Di-*O*-cyclohexyliden-thioacetale bei einer Badtemperatur von 150–155°; nur die Destillation des Di-*O*-cyclohexyliden-di-*n*-butylthioacetals benötigt eine Temperatur von 150–160°. Die rein dargestellten Vertreter werden folgend aufgeführt.

Dimethylthioacetal: Ausb. 2.06 g (53%), $[\alpha]_D^{20}$ +63.9° (*c* 2.39, Chloroform), n_D^{20} 1.5210 (Gef.: C, 58.97; H, 8.65. $C_{19}H_{32}O_4S_2$ Ber.: C, 58.72; H, 8.30%).

Diäthylthioacetal: Ausb. 2.75 g (66%), $[\alpha]_D^{20}$ +69.5° (*c* 1.72, Chloroform), n_D^{20} 1.5200 (Gef.: C, 60.62; H, 8.93. $C_{21}H_{36}O_4S_2$ Ber.: C, 60.52; H, 8.71%).

Di-n-propylthioacetal: Ausb. 3.02 g (68%), $[\alpha]_D^{20}$ +63.9° (*c* 1.82, Chloroform), n_D^{20} 1.5164 (Gef.: C, 62.08; H, 8.83. $C_{23}H_{40}O_4S_2$ Ber.: C, 62.11; H, 9.07%).

Diisopropylthioacetal: Ausb. 2.76 g (62%), $[\alpha]_D^{20}$ +78.5° (*c* 1.76, Chloroform), n_D^{20} 1.5109 (Gef.: C, 62.17; H, 8.96. $C_{23}H_{40}O_4S_2$ Ber.: C, 62.11; H, 9.07%).

Di-n-butylthioacetal: Ausb. 3.35 g (71%), $[\alpha]_D^{20}$ +74.3° (*c* 3.33, Chloroform), n_D^{20} 1.5092 (Gef.: C, 63.68; H, 9.57. $C_{25}H_{44}O_4S_2$ Ber.: C, 63.51; H, 9.38%).

Diisobutylthioacetal: Ausb. 2.69 g (57%), $[\alpha]_D^{20}$ +68.3° (*c* 3.25, Chloroform), n_D^{20} 1.5090 (Gef.: C, 63.72; H, 9.56. $C_{25}H_{44}O_4S_2$ Ber.: C, 63.51; H, 9.38%).

2,3:4,5-Di-O-cyclohexyliden-D-arabinose-dimethylacetal (XII)

2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetal (0.01 Mol) wird behandelt, wie oben zur Darstellung von IV angegeben. Nach einer Destillation bei 10^{-4} Torr und 110–120° Badtemperatur erhält man 2.54 g (71%) eines farblosen Sirups, $[\alpha]_D^{17}$ +10.3° (*c* 2.67, Chloroform), n_D^{17} 1.4810 (Gef.: C, 64.20; H, 9.01. $C_{19}H_{32}O_6$ Ber.: C, 64.01; H, 9.05%).

2,3:4,5-Di-O-cyclohexyliden-D-arabit (XIII)

2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetal (0.01 Mol) wird mit 60 ml Aceton, 6.0 ml Wasser, 6.0 g Quecksilber(II)-chlorid und 6.0 g Quecksilberoxyd 5 Stunden unter Rühren auf 40° erwärmt. Dann filtriert man, wäscht den Rückstand mit 30 ml Aceton, dampft das vereinigte Filtrat bei Gegenwart von 1.0 g Quecksilberoxyd ein, extrahiert den Rückstand dreimal mit je 30 ml heißem Chloroform, schüttelt die Lösung mit 30 ml N KJ sowie mit 10 ml Wasser, trocknet über Natriumsulfat, dampft im Vakuum ein und destilliert den Rückstand bei 10^{-3} Torr und 120–130° Badtemperatur. Die so gewonnene 2,3:4,5-Di-*O*-cyclohexyliden-*aldehydo*-D-arabinose (1.92 g) zeigt eine spezif. Drehung von $[\alpha]_D^{20}$ +0.4° (*c* 6.45, Chloroform), sie ist nicht analysenrein. Sie wird in 80 ml Äther mit 0.5 g $LiAlH_4$

5 Stunden unter Rückfluß erwärmt. Dann zerstört man das überschüssige $LiAlH_4$ unter Kühlen mit 2.0 ml Wasser, saugt den Hydroxydniederschlag ab, dampft das Filtrat ein und destilliert den zurückbleibenden Sirup bei 10^{-4} Torr und 140–145° Badtemperatur. Man gewinnt 1.55 g (50%) eines farblosen Sirups, $[\alpha]_D^{20}$ +7.9° (*c* 3.49, Chloroform), n_D^{18} 1.4978 (Gef.: C, 65.52; H, 9.23. $C_{17}H_{28}O_5$ Ber.: C, 65.36; H, 9.04%).

1-O-Acetyl-2,3:4,5-di-O-cyclohexyliden-D-arabit (XIV)

XIII (3.12 g, 0.01 Mol) wird mit 1.5 ml Acetanhydrid und 10 ml Pyridin 16 Stunden bei 20° acetyliert. Man arbeitet auf, wie oben zur Darstellung von II angegeben. Nach einer Destillation bei 10^{-4} Torr und 120–125° Badtemperatur erhält man 2.83 g (80%) eines farblosen Sirups mit $[\alpha]_D^{20}$ +9.9° (*c* 2.67, Chloroform) und n_D^{19} 1.4906 (Gef.: C, 64.57; H, 8.60. $C_{19}H_{30}O_6$ Ber.: C, 64.37; H, 8.53%).

Darstellung der 2,3-O-Cyclohexyliden-D-arabinose-thioacetale (X)

Man erwärmt 0.01 Mol eines 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetals unter Rühren mit 25 ml 80-proz. Essigsäure 2.5 Stunden auf 65°, dampft anschließend im Vakuum bei 40–50° Badtemperatur zu einem Sirup ein, löst diesen mit 30 ml Chloroform, filtriert, wäscht das Filtrat mit 20 ml gesättigter Natriumhydrogencarbonat-Lösung sowie mit 20 ml Wasser, trocknet mit Natriumsulfat und dampft zu einem Sirup ein. Dieser wird in 3.0 ml Chloroform gelöst, die Lösung mit 30 ml Benzin (Sdp. 100–110°) versetzt und 16 Stdn. bei −15° stehengelassen. Dann filtriert man das auskristallisierte D-Arabinose-thioacetal ab, dampft wieder im Vakuum ein, löst den zurückbleibenden Sirup in 30 ml Benzol und filtriert die Lösung durch eine Säule (Durchmesser 2.0 cm), die auf 20 cm Länge mit Aluminiumoxyd der Aktivitätsstufe II[9] gefüllt ist, wäscht zunächst mit 80 ml Benzol und dann mit 80 ml 96-proz. Äthanol nach. Die ersten 100 ml der durchlaufenden Flüssigkeit enthalten die gesamte Menge an nicht hydrolysierter Di-*O*-cyclohexyliden-Verbindung IX, in den nächsten 80 ml befindet sich die 2,3-*O*-Cyclohexyliden-Verbindung X; man dampft die Lösung im Vakuum ein und destilliert den zurückbleibenden Sirup bei 10^{-4} Torr und 155–165° Badtemperatur. Die dargestellten sirupösen 2,3-*O*-Cyclohexyliden-D-arabinose-thioacetale werden folgend aufgeführt.

Dimethylthioacetal: Ausb. 0.50 g (16%), $[\alpha]_D^{20}$ +40.1° (*c* 2.24, Chloroform), n_D^{20} 1.5323; die Verbindung ist nicht ganz rein.

Diisopropylthioacetal: Ausb. 1.05 g (29%), $[\alpha]_D^{20}$ +58.5° (*c* 1.96, Chloroform), $n_D^{18.5}$ 1.5196 (Gef.: C, 56.03; H, 9.15. $C_{17}H_{32}O_4S_2$ Ber.: C, 56.00; H, 8.85%).

Di-n-butylthioacetal: Ausb. 1.15 g (29%), $[\alpha]_D^{20}$ +68.0° (*c* 1.55, Chloroform), n_D^{21} 1.5138 (Gef.: C, 57.97; H, 9.53. $C_{19}H_{36}O_4S_2$ Ber.: C, 58.12; H, 9.24.%).

D-threo-*Dihydroxy-succindialdehyd-1,4-bis-dimethylthioacetal (VIII)*

Man löst 3.08 g (0.01 Mol) 2,3-*O*-Cyclohexyliden-D-arabinose-dimethylthioacetal in 100 ml Benzol, fügt 5.0 g gepulvertes Bleitetraacetat hinzu, rührt 20 Minuten bei 20°, filtriert das ausgefallene Bleisalz ab, wäscht das Filtrat dreimal mit je 30 ml gesättigter Natriumhydrogencarbonat-Lösung sowie einmal mit 20 ml Wasser,

trocknet mit Natriumsulfat und engt zu einem Sirup ein, der aus rohem *O*-Cyclohexyliden-D-*threo*-dihydroxy-succindialdehyd-1-dimethylthioacetal (XI) besteht. Den Sirup löst man in 30 ml einer 10-proz. Lösung von Chlorwasserstoff in Dioxan, kühlt die Lösung auf 0° ab, gibt 3.0 ml Methylmercaptan hinzu, läßt 3 Stunden in einem gut verschlossenen Kolben bei 20° stehen, dampft im Vakuum ein, löst die Kristallmasse in 25 ml Benzol, dampft erneut im Vakuum ein und kristallisiert den Rückstand aus *n*-Hexan um. Ausb. 1.68 g (61%), Nadeln, Schmp. 116°, $[\alpha]_D^{22}$ +5.3° (*c* 2.13, Methanol). Lit.[5]: Schmp. 116°, $[\alpha]_D^{22}$ +5.5° (Methanol).

ZUSAMMENFASSUNG

D-Arabinose-thioacetale werden mit Cyclohexanon bei Gegenwart von Zinkchlorid zu 4,5-*O*-Cyclohexyliden-D-arabinose-thioacetalen kondensiert. Deren Struktur wird einmal durch Überführen in das bekannte 2,3-Di-*O*-acetyl-D-arabinose-diäthylthioacetal und weiterhin durch Oxydation mit Bleitetraacetat und chromatographischen Nachweis des als Bruchstück enstandenen Glyoxal-1-thioacetals und *O*-Cyclohexyliden-D-glycerinaldehyds bewiesen. Bei Verwendung von Schwefelsäure und Kupfersulfat als Kondensationsmittel erhält man aus den D-Arabinose-thioacetalen und Cyclohexanon als Hauptprodukte 2,3:4,5-Di-*O*-cyclohexyliden-D-arabinose-thioacetale, die sich durch Erwärmen mit Essigsäure zu 2,3-*O*-Cyclohexyliden-D-arabinose-thioacetalen hydrolysieren lassen. Die Strukturaufklärung dieser Verbindungen erfolgt am Beispiel des Dimethylthioacetals durch Bleitetraacetatoxydation in Benzol; als Bruchstücke werden Formaldehyd und 2,3-*O*-Cyclohexyliden-D-*threo*-dihydroxy-succindialdehyd-1-dimethylthioacetal nachgewiesen. Letzteres wird in das bekannte D-*threo*-Dihydroxy-succindialdehyd-1,4-bis-dimethylthioacetal übergeführt.

SUMMARY

Condensation of D-arabinose dialkyl dithioacetals with cyclohexanone in the presence of zinc chloride gives 4,5-*O*-cyclohexylidene derivatives for which structures have been established by conversion of the diethyl dithioacetal into the known 2,3-di-*O*-acetyl-D-arabinose diethyl dithioacetal, and by chromatographic identification of the fragments resulting from oxidation with lead tetra-acetate. By using sulphuric acid–copper sulphate as condensing agent, the principal product is the 2,3:4,5-di-*O*-cyclohexylidene-D-arabinose dialkyl dithioacetal, which, with warm acetic acid, yields the 2,3-*O*-cyclohexylidene-D-arabinose dialkyl dithioacetal. Structural assignments for these compounds follow from the oxidation of the dimethyl dithioacetal with lead tetra-acetate in benzene which gives formaldehyde and 2,3-*O*-cyclohexylidene-D-*threo*-dihydroxysuccindialdehyde 1-(dimethyl dithioacetal). The latter compound was converted into the known bis(dimethyl dithioacetal) of D-*threo*-dihydroxysuccindialdehyde.

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TRANSFORMATION OF DISACCHARIDES DURING BORATE ION-EXCHANGE CHROMATOGRAPHY. ISOMERIZATION OF LACTOSE INTO LACTULOSE

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INTRODUCTION

In our studies on the biosynthesis of lactose and "neuramin-lactose"– α-*N*-acetylneuraminyllactose or *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-nonulopyranosid)onic acid-(2→3)-*O*-β-D-galactopyranosyl-(1→4)-D-glucose – by rat mammary glands[1,2], ion-exchange chromatography of neutral sugars as borate complexes[3] was selected for the isolation and purification of radioactive lactose. When the chromatography was performed in a cold room at 4°, a single peak of lactose was eluted from the column, whereas experiments at room temperature with similar rat mammary-gland extracts gave two peaks positive to sugar reagents, one corresponding to lactose, and the other to an unidentified sugar.

This report describes experiments performed with several disaccharides which were found to undergo chemical changes, when subjected to borate ion-exchange chromatography at room temperature. Lactose was studied in some detail, and the sugar formed during its chromatography was identified as lactulose (4-*O*-β-D-galactopyranosyl-D-fructofuranose).

MATERIALS AND METHODS

Columns (1 × 14.5 cm) of Dowex 1-*X*8 (200–400 mesh) were utilized initially, but later on, the 50–100 mesh of the same resin was found more satisfactory, because it gave the same elution patterns and permitted easier regulation of the flow rate of the eluting buffers. The resin was washed with hot 4N hydrochloric acid, as described by Svennerholm[4], prior to its conversion into the borate form.

The chromatographic separations were performed as described by Khym and Zill[3]. The disaccharide (50 mg) was dissolved in 0.005M potassium tetraborate (10 ml), and allowed to equilibrate for 2 h prior to introduction into the column. The elution of materials containing carbohydrate was followed by analysis of 1-ml aliquots of the effluent by the anthrone reaction in a final volume of 4 ml, using a method previously described[5]. The fractions corresponding to the individual peaks were pooled, and the potassium tetraborate was removed by passage through a

column of Dowex 50-W (H^+), followed by evaporation and repeated distillation with methanol[6].

The presence of ketoses was qualitatively established by the Seliwanoff reaction[7].

Hydrolysis of disaccharides was performed in 0.5 N sulfuric acid at 100° for 2 h. The hydrolyzates were cooled, and neutralized with saturated barium hydroxide, and the precipitated barium sulfate was removed by centrifugation.

Ionophoresis experiments were performed either on Schleicher and Schuell no. 598 or on Whatman No. 3 MM filter papers in 0.05 M borax buffer[8], at pH 9.2. The separations were done at 375 volts, for 4 h, in a water-cooled instrument of the horizontal type (Research Equipment Corporation, Model E-800-2).

Paper chromatography was performed on Whatman no. 1 filter paper with the following solvents: 3-methyl-1-butanol–pyridine–0.1 N hydrochloric acid (4:4:2)[9]; ethyl acetate–pyridine–water (100:55:65). The chromatograms were developed by descending irrigation for 23 h, and the front of the solvent was allowed to flow out of the serrated end of the paper. The dried chromatograms were treated either with an anthrone[10] or with a urea[11] spray reagent (both of which are specific for ketoses), the spots were outlined with pencil, and the paper was then sprayed with the benzidine[12] reagent to locate all reducing sugars.

An authentic sample of lactulose was prepared by alkaline isomerization of lactose with lime water[13].

RESULTS

The elution patterns of the experiments with maltose and cellobiose are shown in Fig. 1 and Fig. 2. The electrophoretic mobility of the sugar presents in each of the two main peaks was studied; the results are shown in Table I. The third peak was heterogeneous in both cases, and the electrophoretograms showed two faint spots, one with the mobility of glucose and another migrating half-way between the sugar from peak II and standard glucose.

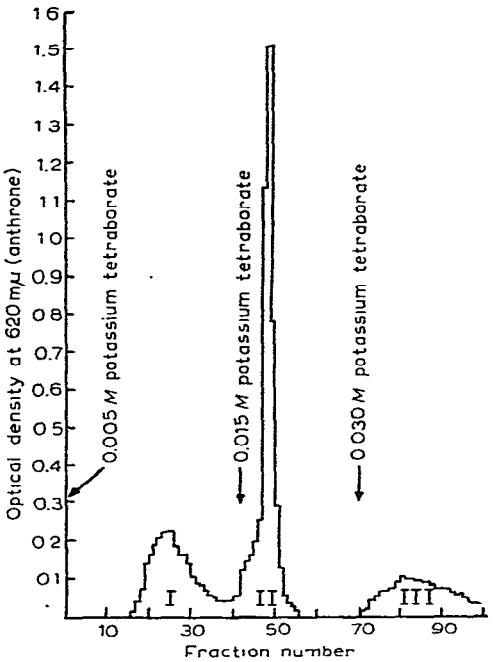
TABLE I

IONOPHORETIC MOBILITIES OF THE SUGARS OBTAINED FROM MALTOSE AND CELLOBIOSE BY BORATE ION-EXCHANGE CHROMATOGRAPHY

Substance	$M_{glucose}$[a]
Maltose column (Fig. 1)	
Peak I	0.28
Peak II	0.55
Standard Maltose	0.28
Cellobiose column (Fig. 2)	
Peak I	0.27
Peak II	0.54
Standard Cellobiose	0.27

[a] Uncorrected experimental values obtained with 0.05 M borate buffer, pH 9.2.

The elution patterns obtained from the experiments with lactose at room temperature and in the cold (4°) are shown in Fig. 3 and Fig. 4, respectively. Elutions carried out in the cold yielded a single peak of lactose, whereas those carried out



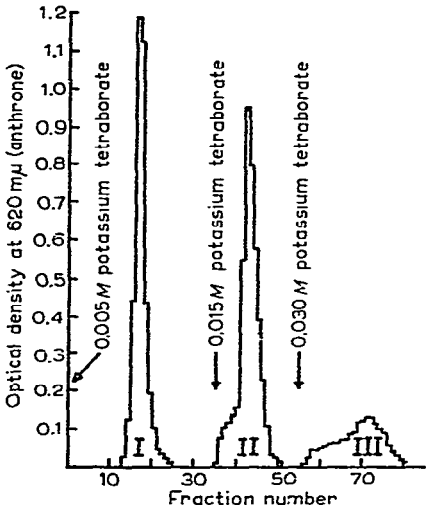


Fig. 1. Elution pattern of a 50-mg sample of maltose, subjected to ion-exchange chromatography on a column (1 × 14.5 cm) of Dowex 1 (borate form, 50–100 mesh), at room temperature. The vol. of the fractions was 50–60 ml.

Fig. 2. Elution pattern of a 50-mg sample of cellobiose subjected to ion-exchange chromatography on a column (1 × 14.5 cm) of Dowex 1 (borate form, 50–100 mesh), at room temperature. The vol. of the fractions was 50–60 ml.

at room temperature yielded two peaks. Material from Peak I corresponds to lactose, whereas that from Peak II was identified as lactulose, on the basis of the following experimental findings: positive Seliwanoff test, chromatographic identification of

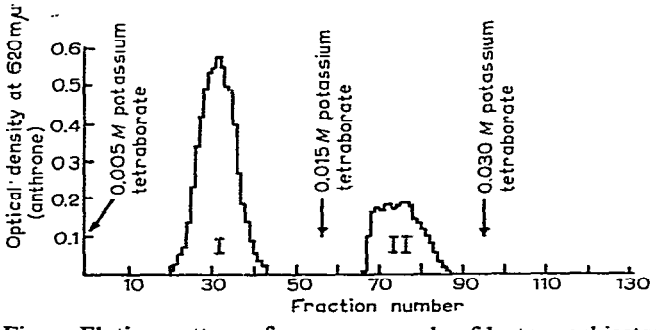


Fig. 3. Elution pattern of a 50-mg sample of lactose subjected to ion-exchange chromatography on a column (1 × 14.5 cm) of Dowex 1 (borate form, 50—100 mesh), at room temperature. The vol. of the fractions was 50—60 ml.

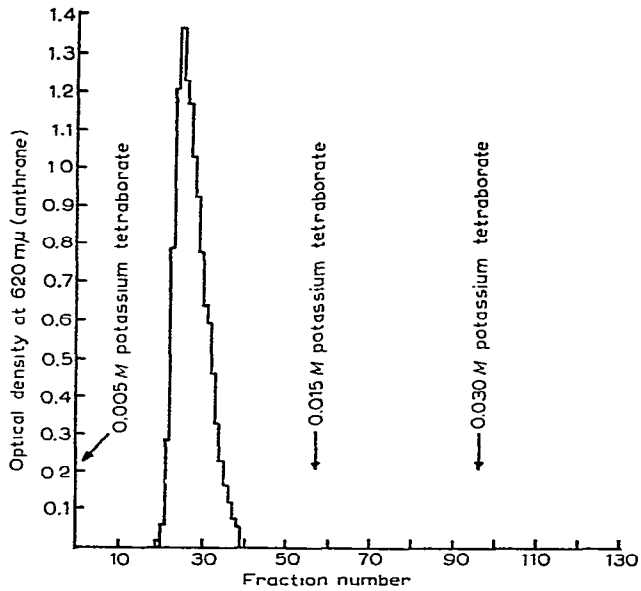


Fig. 4. Elution pattern of a 50-mg sample of lactose subjected to ion-exchange chromatography on a column (1 × 14.5 cm) of Dowex 1 (borate form, 50–100 mesh), at 4°. The vol. of the fractions was 50–60 ml.

galactose and fructose in the products of acid hydrolysis, and chromatographic mobility and ionophoretic migration in borate buffer identical with those of authentic lactulose. Furthermore, the ketoses gave typical positive reactions with specific spray reagents. Table II shows the results of these experiments.

DISCUSSION

The formation of Böeseken complexes[14] between neutral sugars and borate ions has been utilized advantageously for the separation of sugars by paper chromatography[15], paper ionophoresis[8], and ion-exchange chromatography[3]. The column-chromatographic technique[3] is especially suited for preparative purposes and it is widely used in carbohydrate chemistry. The chromatography of lactose gives satisfactory results, provided that the columns are maintained at low temperature. At room temperature, however, a considerable proportion of the initial material undergoes chemical changes that result in the elution of two sugar-containing peaks in the chromatography of lactose and three peaks in the case of either maltose or cellobiose. Experiments with lactose, carried out at room temperature, showed that a considerable (and variable) amount of lactose was isomerized to lactulose.

Present methods of preparation of lactulose[16] are, in general, minor modifications of the original method of Montgomery and Hudson[17], based on the alkaline isomerization of lactose by the action of calcium hydroxide. In alkaline medium, the D-glucose moiety of lactose undergoes a Lobry de Bruyn–Alberda van Ekenstein rearrangement[18]

TABLE II

CHROMATOGRAPHIC AND IONOPHORETIC MOBILITIES OF THE SUGARS OBTAINED FROM LACTOSE BY BORATE ION-EXCHANGE CHROMATOGRAPHY

Substance	*R_G by paper chromatography in*		*M_G[a] by ionophoresis in 0.05 M borate buffer, pH 9.2*
	3 Methyl-1-butanol–pyridine–0.1 N HCl (4:4:2)	*Ethyl acetate–pyridine–water (100:55:65)*	
Standard Sugars			
Lactose	0.63	0.72	0.42
Lactulose	0.73	0.78	0.72
Galactose	0.88	0.94	0.92
Fructose	1.06	1.06	0.89
Lactose Column (Fig. 3)			
Peak I	0.63	0.72	0.41
Peak II	0.73	0.79	0.72
Hydrolyzate[b] of peak II	0.88; 1.06	0.93; 1.07	0.90

[a] Uncorrected experimental values.

[b] Paper chromatograms of the hydrolyzate showed two spots positive to sugar reagents. The $R_{glucose}$ is given for each spot in both solvent systems. The ionophoretic experiment gave a single spot, because mixtures of galactose and fructose cannot be resolved, as shown by the $M_{glucose}$ of the standard sugars.

to form a terminal D-fructose. The yield of lactulose is low because of the behavior of β-alkoxycarbonyl compounds at alkaline pH; as a result of β-elimination, D-galactose and isosaccharinic acids are formed[19].

The protective and catalytic action of borate ions in the alkali-catalyzed isomerization of sugars was studied by Mendicino[20], who reported the formation of a D-fructose-containing disaccharide (presumably lactulose) from lactose, under rather drastic conditions of pH and temperature. The fructose moiety of lactulose is known to occur in the furanose form[21] which, as stressed by Böeseken[14], is the most favorable structure for the formation of sugar–borate complexes. The fact that the borate complex of lactulose is more negatively charged than that of lactose is evident from their ionophoretic and ion-exchange behavior.

It is likely that a similar transformation might occur in the experiments with maltose and cellobiose (see Figs. 1 and 2) and that the small amounts of glucose eluted with 0.030 M potassium tetraborate would be derived from the corresponding keto-disaccharides by β-elimination. In the case of lactulose, β-elimination results in liberation of D-galactose rather than D-glucose. Since galactose is eluted by 0.0015 M potassium tetraborate[3], trace amounts of free galactose, if present, could be eluted together with lactulose, and hence only two peaks positive to sugar reagents would appear in the elution pattern.

The equilibrium of the various possible forms of the borate complexes of a given sugar is known to be influenced by pH, sugar concentration, and the ratio of borate to sugar[22]. These factors, as well as the temperature effect reported here and

the ratio of sugar to resin, should be taken into account in a detailed study of the mechanism of isomerization of disaccharides. The use of these observations in the development of rapid methods for the preparation of lactulose and other disaccharides is being further investigated.

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The author expresses his sincere appreciation to Mr. W. N. White for technical assistance and to Mr. H. E. May for help in the preparation of this manuscript. This investigation was supported in part by a grant (AI-04954) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.

SUMMARY

Three reducing disaccharides, lactose, maltose, and cellobiose, were found to undergo chemical changes when subjected to ion-exchange chromatography on columns of Dowex 1 (borate) at room temperature. The new product formed during the chromatography of lactose was found to be lactulose. This isomerization of lactose was found to be temperature-dependent, since a single peak of lactose was eluted from columns operated at 4°.

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REAKTIONEN ENOLISCHER ZUCKERDERIVATE

TEIL II*. DIE RADIKALISCHE ADDITION VON BENZYLMERKAPTAN AN ENOLISCHE ZUCKERDERIVATE**

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(Eingegangen den 28. März, 1966)

EINFÜHRUNG

Die Einführung einer C–S-Bindung anstelle einer C–O-Bindung in ein Zuckermolekül wurde bisher durch nucleophilen Angriff geeigneter Thioverbindungen auf reaktive Zuckerderivate erreicht. Die Vielzahl der dabei möglichen Reaktionen wurde von Horton und Hutson[1] zusammenfassend dargestellt. Beiläufig werden auch Arbeiten erwähnt, in denen auf die Möglichkeit hingewiesen wird, Schwefelverbindungen[2–4] an ungesättigte Zuckerderivate zu addieren. Daß sich enolische Zuckerverbindungen für radikalische Additionsreaktionen, z.B. von Bisulfit, in hervorragendem Maße eignen, wurde bereits gezeigt[5]. Im Folgenden soll an einigen Beispielen die Reaktion von Benzylmerkaptan mit enolischen Zuckerderivaten unter Bestrahlung mit ultraviolettem Licht beschrieben werden. Von besonderem Interesse ist dabei die Stereoselektivität der radikalischen Addition. Gegenüber der Reaktion ungesättigter cyclischer Kohlenwasserstoffe, die in diesem Zusammenhang fast ausschließlich untersucht wurden, sind in der genannten Verbindungsklasse Unterschiede zu erwarten, die durch die Wirkung des Enolsauerstoffs als Elektronendonator und die stabilisierende Wirkung freier oder substituierter Hydroxylgruppen auf die Konformation von Ringverbindungen zu erklären sind.

DISKUSSION

Die radikalische Reaktion von Thiolen mit Olefinen ist seit langer Zeit bekannt[6] und in ihrem Mechanismus vor allem von Kharasch *et al.*[7] und Back *et al.*[8] geklärt. Die sterischen Verhältnisse dabei wurden an Reaktionen von Thiolen mit Cyclohexen- und Cyclopenten-derivaten untersucht. Im Falle des 1-Chlorcyclohexens[9], des 1-Methylcyclopentens und des 1-Methylcyclohexens[4] erfolgt vorwiegend *Trans*-addition. Weiterhin wurde gezeigt, daß die Bevorzugung der *Trans*- gegenüber der *Cis*-addition von Thiophenol über Schwefelwasserstoff nach Thioessigsäure abnimmt[9]. Außergewöhnliche Verhältnisse existieren bei der Addition von Thiolen an 1-Chlorbicycloalkene, wo fast nur *Cis*-addition beobachtet wurde[10].

* Teil I: J. LEHMANN, *Carbohydrate Res.*, 2 (1966) 1.
** Herrn Prof. Dr. A. Lüttringhaus zum 60. Geburtstag gewidmet.

Die Lebensdauer des Intermediärradikals, das nach dem Angriff des Thiylradikals ensteht, hängt von der Art des eingesetzten Olefins ab, außerdem von der Geschwindigkeit, mit der im zweiten Schritt der Additionsreaktion ein Wasserstoffatom aus einem weiteren Thiolmolekül abstrahiert werden kann. Bei genügend grosser Lebensdauer ist im Intermediärradikal die Einstellung einer energetisch günstigen Konformation möglich, wie schon von Bordwell *et al.*[4] angedeutet wurde.

Als Thiol-Acceptoren wurden Methyl-6-desoxy-α-D-*xylo*-hex-5-enopyranosid (I)[11], 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*erythro*-hex-3-enose (II)[12–14], und D-Glucal (III)[15,16] gewählt. Durch die großen strukturellen Unterschiede kann mit diesen Verbindungen ein recht klares Bild der radikalischen Addition von Benzylmerkaptan an enolische Zuckerderivate gegeben werden. Als Thiol wurde Benzylmerkaptan eingesetzt. Die glatt verlaufende Hydrogenolyse der Thiobenzyläther durch Natrium in flüssigem Ammoniak eröffnet einen Weg zur Darstellung von Thiozuckern[17–19]. Die reduktive Entschwefelung mit Raney-Nickel[20] führt zu Desoxyzuckern. Neben diesen präparativen Möglichkeiten war für die Wahl von Benzylmerkaptan die weit größere Stabilität der S–H-Bindung gegenüber der in Thioessigsäure maßgebend. Nach den schon erläuterten Gesichspunkten ist dadurch die Additionsreaktion sehr verlangsamt. Die parallel laufende Erhöhung der Lebensdauer des Intermediärradikals ermöglicht diesem, sich in die stabilste Konformation einzustellen.

Im Gegensatz zu Benzylmerkaptan reagiert Thioessigsäure auch ohne u.v.-Bestrahlung in wenigen Minuten quantitativ mit I unter Bildung schlecht isolierbarer Reaktionsprodukte, von denen zwei in größerer Menge entstehen. Die weitere Untersuchung dieser Reaktion soll Gegenstand einer späteren Arbeit sein. Das Tri-*O*-acetyl-Derivat von I reagiert unter analogen Bedingungen nicht. Ebensowenig konnten Maki *et al.*[21] ein Additionsprodukt von Thioessigsäure an Tri-*O*-acetyl-D-glucal nachweisen. Sie erhielten statt dessen ein Di-*O*-acetyl-1-thio-D-pseudoglucal.

Die Reaktion von I mit Benzylmerkaptan ergibt nach zweistündiger Bestrahlung mit einer Quecksilber-Hochdrucklampe ausschließlich ein Produkt. Der zeitliche Ablauf der Reaktion wurde papierchromatographisch verfolgt. Bereits nach 10 Minuten ist Reaktionsprodukt nachzuweisen, nach 30 Minuten ist der Umsatz 80%ig und nach zwei Stunden quantitativ. Es entsteht dabei Methyl-6-*S*-benzyl-6-thio-α-D-glucopyranosid (IV), dessen Konstitution durch Entschwefelung mit Raney-Nickel unter Bildung von Methyl-6-desoxy-α-D-glucopyranosid (V) bewiesen wurde. IV reagiert in siedendem Methanol nicht mit Quecksilberchlorid, es kann also kein Thioacetal durch Addition in 5-Stellung von I entstanden sein. Auf gleiche Weise konnte bei allen folgenden Reaktionen eine Markownikoff-Addition ausgeschlossen werden. Dieser Befund entspricht den Erwartungen, da bei radikalischen Additionen von Thiolen an Olefine bisher nur anti-Markownikoff-Verlauf beobachtet wurde. IV ist eine wachsartige amorphe Substanz, die nach Acetylierung ein kristallines Acetylderivat VI liefert. Theoretisch wären bei der Addition von Benzylmerkaptan zwei Isomere zu erwarten, nämlich das bereits beschriebene D-Glucose- sowie ein L-Idose-Derivat. Bei der Addition von Diboran an Derivate von I ist dies auch der Fall[22]. Zwei Isomere werden ebenfalls bei der Addition von Thioessigsäure an β-Pinen

gefunden[4], das wie I ein sechsgliedriges Ringsystem mit exocyclischer Doppelbindung ist. Die beobachtete ausschließliche Bildung von IV kann so verstanden werden, daß das intermediäre Radikal Ia induktiv durch den benachbarten Ringsauerstoff stabilisiert wird. Die so erreichte höhere Lebensdauer ermöglicht die Einstellung des Gleichgewichts zugunsten von Ia (Abb. 1) mit einer äquatorialen $-CH_2-S-CH_2-C_6H_5$-Gruppierung. Das Wasserstoffatom aus einem zweiten Molekül Benzylmerkaptan kann dann nur in axialer Position eintreten. Die Frage nach der sterischen Anordnung des trigonalen Kohlenstoffradikals[4,9] beim Eintritt des Wasserstoffatoms, planare oder tetraedrische Form, ist damit in diesem speziellen Fall zugunsten der tetraedrischen

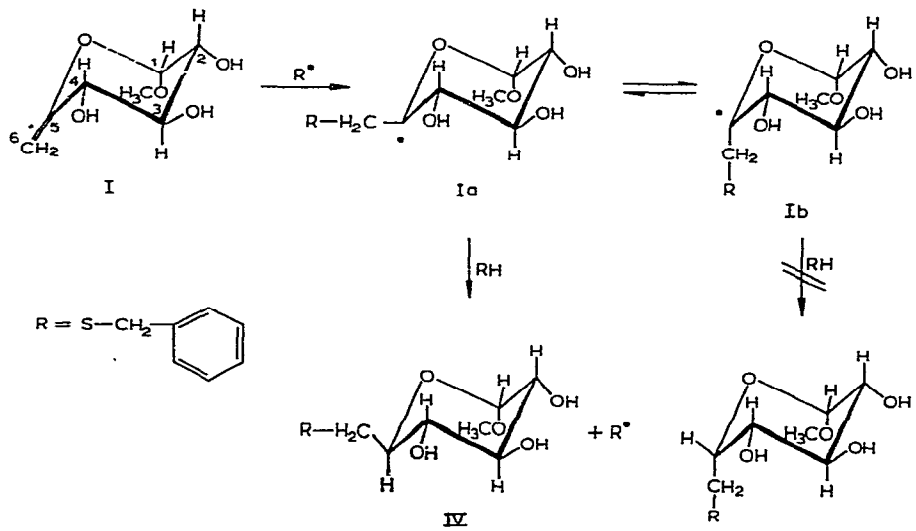

Abb. 1

Form beantwortet. Bei der Entbenzylierung von VI in flüssigem Ammoniak mit überschüssigem Natrium wird auch ein Teil der Acetylgruppen angegriffen. Wahrscheinlich ist es günstiger, derartige Debenzylierungen mit den nicht acetylierten Verbindungen durchzuführen[19]. Reacetylierung des Reaktionsproduktes mit Acetanhydrid in Pyridin ergibt eine im Hochvakuum destillierbare einheitliche Substanz von leichter Gelbfärbung. Nach Entacetylierung ensteht amorphes Methyl-6-thio-α-D-glucopyranosid (VII) mit einer S–H-Absorption im Infrarotspektrum bei 2500 cm^{-1}. Das Acetat von VII wurde auf anderem Wege von Machell und Richards[23] erhalten. Bei der Behandlung von VII mit Raney-Nickel in siedendem Äthanol wurde in fast quantitativer Ausbeute kristallines Methyl-6-desoxy-α-D-glucopyranosid (V) erhalten.

II setzt sich mit Benzylmerkaptan nach dreistündiger u.v.-Bestrahlung in Stickstoffatmosphäre quantitativ um. Die Reaktion wurde dünnschicht- und gaschromatographisch verfolgt. Es entstehen in genau gleichen Mengen zwei Additionsprodukte VIII und IX, die ohne Trenneffekt im Hochvakuum destilliert, aber sowohl dünnschicht- wie auch gaschromatographisch unterschieden werden können. Eine präparative Trennung soll mit Hilfe von Tritiummarkierung durch Papier-

chromatographie an Acetylcellulose noch erfolgen. Daß es sich bei VIII und IX um 3-*S*-Benzyl-1,2:5,6-di-*O*-isopropyliden-3-thio-α-D-glucofuranose bzw. 3-*S*-Benzyl-1,2:5,6-di-*O*-isopropyliden-3-thio-α-D-galaktofuranose handelt, erklärt sich folgendermaßen. Nach der Entschwefelung mit Raney-Nickel liefert das redestillierte sirupöse Isomerengemisch wiederum zwei Substanzen (X und XI), die im Vakuum als Öl gemeinsam überdestillieren. Eine Trennung kann durch Dünnschicht- oder Gaschromatographie erfolgen. Die eine der beiden Substanzen, XI, ist gaschromatographisch nicht von 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*xylo*-hexofuranose zu unterscheiden. Die Vergleichssubstanz wurde durch katalytische Hydrierung[14] von II erhalten. Nach Animpfen des Isomerengemisches mit 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*xylo*-hexofuranose und Aufbewahren über Nacht bei 0° enstand ein dicker Kristallbrei, der auf Tonscherben abgepreßt, eine kristalline Verbindung, XI, lieferte. Aufgrund von Mischschmelzpunkt und i.r.-Spektrum konnte sie als 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*xylo*-hexofuranose identifiziert werden.

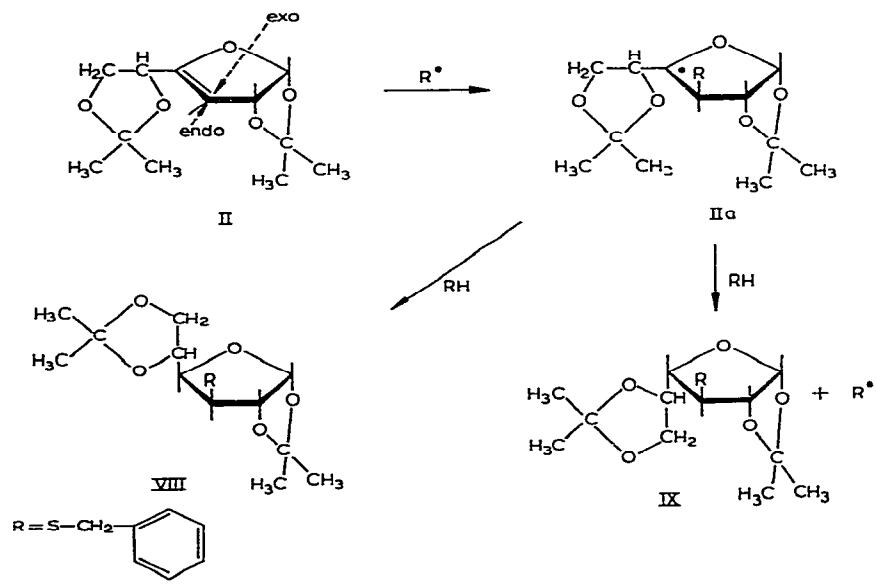

Abb. 2

In verschiedenen Arbeiten wurde gezeigt [22,24], daß die *endo*-Seite von II durch die 1,2-*O*-Isopropylidengruppe sterisch stark gehindert ist. Der Angriff eines Thiylradikals in 3-Stellung ist demnach nur von der *exo*-Seite möglich und das resultierende Intermediärradikal nimmt Konfiguration IIa an. Eine bevorzugte Konfiguration zu IIa ist aufgrund des vorliegenden Furanoseringes nicht zu erwarten, im Gegensatz zum Pyranosering, d.h., die Aufenthaltswahrscheinlichkeit des ungepaarten Elektrons ist auf beiden Seiten des Furanoseringes gleich groß. Das Wasserstoffatom kann also in Übereinstimmung mit den experimentellen Ergebnissen gleichberechtigt unter Bildung des D-Glucose- (VIII), bzw. des D-Galaktose-derivates (IX) eintreten. Am

Molekülmodell ist zu erkennen, daß die Benzylthiogruppe eine *Cis*-addition des Wasserstoffs nicht behindert (Abb. 2).

Bei der Addition von Benzylmerkaptan an III besteht ein grundlegender Unterschied zu den gleichartigen Reaktionen von I und II in der stark herabgesetzten Reaktionsgeschwindigkeit. Nach 5 Stunden findet man einen nur etwa 50%igen Umsatz. Verlängerung der Reaktionszeit führt zu Nebenprodukten und ein starker Geruch nach Schwefelwasserstoff läßt es nicht ratsam erscheinen, den Umsatz auf diesem Wege erhöhen zu wollen. Wahrscheinlich ist die unterschiedliche Reaktionsgeschwindigkeit von I und II gegenüber III dem Vorhandensein eines quartären Kohlenstoffatoms in I und II (5- bzw. 4-Position) zuzuschreiben. Dagegen ist in III das dem Ringsauerstoff benachbarte Kohlenstoffatom (1-Position) tertiär.

Die Trennung des Reaktionsgemisches erfolgte papier- und gaschromatographisch. Im Gaschromatogramm erkennt man neben nicht umgesetztem III zwei Produkte (XII, XIII) im Mengenverhältnis 1:1. Wegen der unterschiedlichen Löslichkeit gelang es, XII und XIII in reiner, kristalliner Form zu erhalten. Daraus wurden die Acetate XIV und XV ebenfalls in kristalliner Form hergestellt. Sowohl XII als auch XIII ergeben nach Entschwefelung mit Raney-Nickel Dihydro-D-glucal (1,5-Anhydro-2-desoxy-D-*arabino*-hexit), das in seinen Eigenschaften (Gaschromatogramm, Schmelzpunkt, i.r.-Spektrum) mit dem aus D-Glucal durch katalytische Hydrierung gewonnenen Dihydro-D-glucal[15] identisch war.

In der Umsetzung von Thiolen mit 1-substituierten Cyclohexenen findet die Addition von Benzylmerkaptan an III eine Parallele. Allerdings besteht ein Unterschied

TABELLE I

BILDUNG VON BENZYLTHIOÄTHERN DURCH RADIKALADDITION VON BENZYLMERKAPTAN AN ENOLISCHE ZUCKERDERIVATE

Enol	*Additionsprodukte*	*Mengenverhältnis der Reaktionsprodukte*[a]
Methyl 6-Desoxy-α-D-*xylo*-hex-5-enopyranosid(I)	Methyl 6-*S*-Benzyl-6-thio-α-D-glucopyranosid (IV)	
3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*erythro*-hex-3-enose(II)	3-*S*-Benzyl-1,2:5,6-di-*O*-isopropyliden-3-thio-α-D-galaktose (IX)	1:1
	3-*S*-Benzyl-1,2:5,6-di-*O*-isopropyliden-3-thio-α-D-glucose (VIII)[b]	
D-Glucal (III)[c]	1,5-Anhydro-2-*S*-benzyl-2-thio-D-mannit (XII)	1:1
	1,5-Anhydro-2-*S*-benzyl-2-thio-D-glucit (XIII)	

[a]Die Mengenverhältnisse wurden durch quantitative Auswertung der Gaschromatogramme ermittelt.
[b]Die Struktur konnte noch nicht eindeutig geklärt werden.
[c]III ist strenggenommen kein enolisches Zuckerderivat, sondern ein enolischer 1,5-Anhydro-zuckeralkohol.

darin, daß in III drei äquatoriale Substituenten (zwei Hydroxylgruppen, eine Hydroxymethylgruppe) den ungesättigten 6-Ring in der vorgegebenen Konformation fixieren (Abb. 3). Die beschriebenen Versuche sprechen gegen das Postulat von Goering *et al.*[9]. Danach soll der Angriff des Thiylradikals in axialer Stellung erfolgen. Je nach Lebensdauer des gebildeten Intermediärradikals findet eine Isomerisierung zur äquatorialen Konformation statt. Daraus wird die Bevorzugung der *Trans*- gegenüber

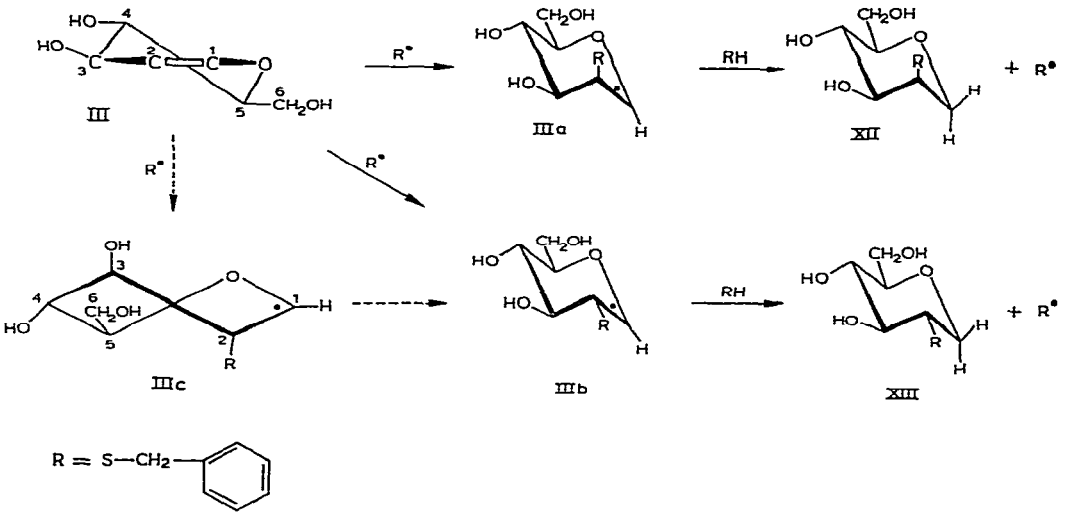

Abb. 3

der *Cis*-addition geschlossen. Wahrscheinlicher ist, daß bereits im Intermediärradikal (IIIa, IIIb) die axiale oder äquatoriale Stellung fixiert ist. Modellbetrachtungen zeigen, daß ein Angriff am 2-Kohlenstoffatom von III sowohl von „oben" als auch von „unten" möglich ist, demnach axiale oder äquatoriale Stellung der eintretenden Thiylgruppe resultiert. Sterische Hinderung ist in keinem Fall zu erwarten, d.h., daß das *Cis*-*Trans*-Verhältnis bereits im ersten Reaktionsschritt bestimmt wird. Huyser *et al.*[25] untersuchten die Reaktion von 4-*t*-Butylcyclohexen mit Methylmerkaptan und fanden, daß die Additionsprodukte mit axialer Thiylgruppe gegenüber denen mit äquatorialer überwiegen. Mit Recht wird immer vorausgesetzt, daß eine äquatoriale Fixierung des *t*-Butylrestes als Ankergruppe in 4-Stellung vorliegt. Die Bevorzugung axialer gegenüber äquatorialen Thiylgruppen scheint den beschriebenen Umsetzungen von III zu widersprechen. Für das Produkt mit äquatorialer Thiylgruppe wird jedoch von Huyser als erstes Intermediärradikal eine Verbindung mit „twisted boat"-Konformation postuliert. Bei der Reaktion von III ist ein ähnlicher Verlauf kaum zu erwarten, da eine vergleichbare „twisted boat"-Konformation (IIIc) die 3- und 4- Hydroxylgruppen in energetisch ungünstiger axialer Stellung verlangen würde. Vielmehr ist anzunehmen, daß sich das Intermediärradikal, auch wegen der längeren Lebensdauer, in die für IIIb angegebene Konformation einstellt. Die beschriebenen Ergebnisse sind demnach mit denen von Huyser nicht ohne weiteres vergleichbar.

Die Isomeren XII und XIII sind nicht nur gaschromatographisch eindeutig zu unterscheiden, sondern auch nach ihren Schmelzpunkten, i.r.-Spektren und vor allem auch durch ihre verschiedenen Drehwerte. Es liegt nahe, eine strukturelle Zuordnung mit Hilfe der optischen Drehung vorzunehmen. Entsprechend den gleichsinnigen Drehwerten gegenüber 1,5-Anhydro-D-glucit (XVII, positiv) und 1,5-Anhydro-D-mannit (XVI, negativ)[26], könnten XII und XIII als 1,5-Anhydro-2-*S*-benzyl-2-thio-D-glucit bzw. 1,5-Anhydro-2-*S*-benzyl-2-thio-D-mannit aufgefaßt werden; das hieße, XII (positiver Drehwert) besitzt D-*Gluco*-, XIII (negativer Drehwert) D-*Manno*-Konfiguration. Eine derartige Zuordnung ist jedoch nicht überzeugend, da die Acetate von XII wie auch von XIII (XIV bzw. XV) positiv, die Acetate von XVI und XVII dagegen im gleichen Sinn wie die nicht acetylierten Produkte drehen[26]. Daneben sprechen auch die längere Retentionszeit im Gaschromatogramm und der tiefere Schmelzpunkt von XIII gegenüber XII nicht für eine Entscheidung aufgrund der optischen Drehung. Erfahrungsgemäß wäre zu erwarten, daß das kompaktere Molekül, das mit einer axialen Benzylthiogruppe, eine kürzere Retentionszeit und einen höheren Schmelzpunkt hat. In der Tat konnte inzwischen durch Kernresonanz-Untersuchungen bewiesen werden, daß XIII trotz des negativen Drehwertes die 1,5-Anhydro-D-glucit-Konfiguration und XII die Konfiguration des 1,5-Anhydro-D-mannits besitzt[27].

Der seltene Fall, daß ein 1,5-Anhydrohexit-, 1,5-Anhydropentit- oder auch ein Hexopyranosid-Derivat ohne Veränderung der Konfiguration durch Acetylierung in Pyridin bei Zimmertemperatur einen entgegengesetzten Drehwert annimmt, ist experimentell zu verfolgen. XIII wird in einer Küvette acetyliert. Wie Abbildung 4 zeigt, findet momentan eine Drehwertänderung in positiver Richtung statt, die nach 80 Minuten einen konstanten Endwert erreicht. Aus dem Reaktionsgemisch kann XV in fast quantitativer Ausbeute isoliert werden. Nimmt man umgekehrt das so gewonnene XV in absolutem Methanol auf und verfolgt nach Zusatz einer katalytischen Menge

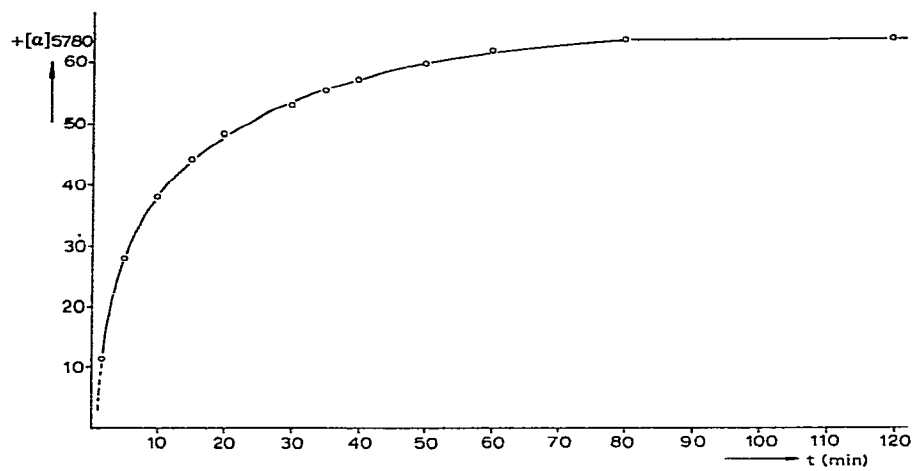

Abb. 4. Abhängigkeit des spezifischen Drehwertes von der Zeit bei der Acetylierung von XIII in Pyridin–Acetanhydrid (2:1) (bezogen auf nicht acetyliertes Produkt).

Natriummethylat den Drehwert in Abhängigkeit von der Zeit, so sinkt die anfangs positive Drehung rasch ab und erreicht bereits nach 8 Minuten den Endwert (Abb. 5). Aus der Lösung kann XIII mit guter Ausbeute in reiner kristalliner Form zurückgewonnen werden.

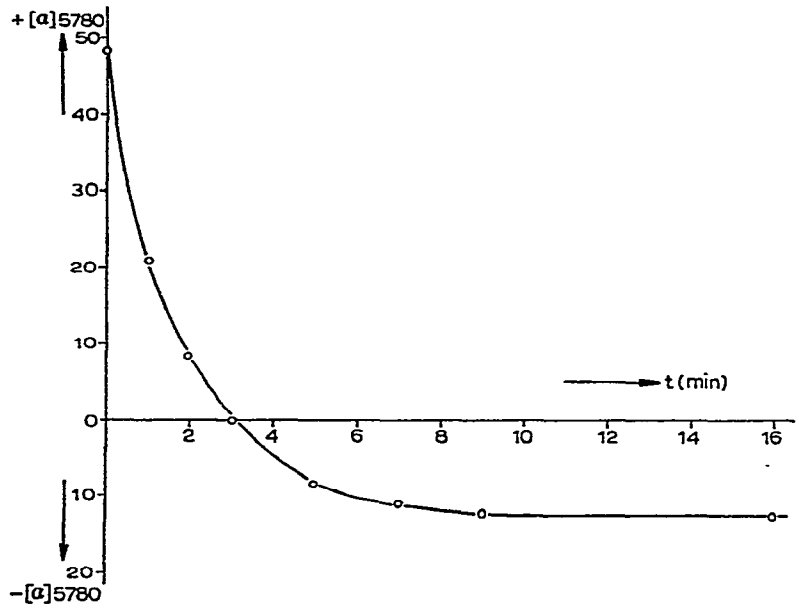

Abb. 5. Abhängigkeit des spezifischen Drehwertes von der Zeit bei der Entacetylierung von XV in absolutem Methanol mit einer katalytischen Menge Natriummethylat (bezogen auf nicht acetyliertes Produkt).

Das Phänomen der Drehsinnumkehrung kann also nicht auf Konfigurationsänderung beruhen, sondern ist wahrscheinlich einer Konformationsänderung in XV zuzuschreiben, die auf einer gewissen sterischen Hinderung der Benzylthiogruppe (äquatorial) durch die eintretende Acetylgruppe (äquatorial) in 3-Stellung beruhen könnte.

In diesem besonderen Fall erlaubt also die Messung der Drehwerte eine bequeme Kontrolle des Reaktionsablaufes. Im allgemeinen werden bei Acetylierungen und Entacetylierungen von Zuckerderivaten die Reaktionslösungen selbst bei homogenen Gemischen mehrere Stunden, manchmal sogar Tage, aufbewahrt, um eine vollständige Umsetzung zu erreichen. Wie schon häufig geäußert wurde, konnte auch hier wieder deutlich gezeigt werden, daß kürzere Reaktionszeiten ausreichend sind. Das bedeutet neben dem Zeitgewinn bei manchen instabilen Verbindungen Schonung der Substanz und damit bessere Ausbeuten.

EXPERIMENTELLER TEIL

Allgemeine Methoden

Papierchromatographie. Papierchromatogramme wurden absteigend auf What-

man Nr. 1—Chromatographiepapier in 1-Butanol–Pyridin–Wasser (6:4:3, v/v/v) angefertigt. Die Methode diente zur Untersuchung des zeitlichen Verlaufs der Additionsreaktionen von I und III mit Benzylmerkaptan, da sich die Enole von den Additionsprodukten sehr gut trennen lassen. Die Differenz der R_F-Werte beträgt in beiden Fällen etwa 0.2. Jedoch sollen individuelle R_F-Werte wegen ihrer relativ schlechten Reproduzierbarkeit nicht angegeben werden. Der stark lipophile Charakter der Benzylthioäther IV, XII, und XIII schließt eine ausreichende Trennbarkeit der Additionsprodukte nach der hier angeführten Methode sowieso aus. Als Entwicklungsreagenz wurde Natriumperjodat–Fuchsinschweflige Säure verwendet[28].

Gaschromatographie. Gaschromatographische Trennungen wurden wie schon an anderer Stelle beschrieben ausgeführt[22].

Bestrahlung der Reaktionsgemische. Die Proben wurden in Reagenzgläsern aus klarem Quarzglas bestrahlt. Als Lichtquelle diente eine wassergekühlte Quecksilberhochdrucklampe. Die Reagenzgläser wurden nahe an der Lichtquelle befestigt und von außen mit Aluminiumfolie abgeschirmt, um eine möglichst hohe Lichtausbeute zu erzielen.

Entschwefelung mit Raney-Nickel. Sämtliche Reaktionen wurden mit der zehnfachen Gewichtsmenge Raney-Nickel in der 40-fachen Volumenmenge Alkohol, bezogen auf eingesetzte Schwefelverbindung, durchgeführt. Die Suspension ließ man in allen Fällen 5 Stunden am Rückfluß kochen.

Methyl 6-S-Benzyl-6-thio-α-D-glucopyranosid (IV)

Methyl 6-Desoxy-α-D-*xylo*-hex-5-enopyranosid (1) (3 g) wird in Äthanol (15 ml) gelöst und mit frisch destilliertem Benzylmerkaptan (6 ml) versetzt. Die klare Lösung wird zwei Stunden in Quarzgläsern, die mit Korkstopfen verschlossen sind, bestrahlt. Die Gläser sollten fast vollständig gefüllt sein. Vor der Bestrahlung wird kurze Zeit Stickstoff durchgeleitet. Um eine möglichst gleichmässige Belichtung des Reaktionsgemisches zu erreichen, schüttelt man die Proben von Zeit zu Zeit um. In einem Parallelversuch mit kleineren Mengen wird der Reaktionsverlauf papierchromatographisch verfolgt. Nach 1.5–2 Stunden ist I nicht mehr nachzuweisen. Dafür tritt in gleichem Maße wie I verschwindet ein neuer Fleck mit größerer Wanderungsgeschwindigkeit auf. Die Reaktionsmischung wird in einer offenen Schale in einem kräftigen Abzug aufgestellt und das Lösungsmittel und überschüssiges Benzylmerkaptan mit einem Stickstoffstrom verjagt. Es bildet sich bald eine feste Masse. Durch gelegentliches Umrühren wird die Entfernung des Benzylmerkaptans erleichtert. Dieser Prozeß dauert etwa 15 Stunden. Dem wachsartigen Rückstand haftet noch starker Thiolgeruch an. Kristallisationsversuche in verschiedenen Lösungsmitteln schlugen fehl. Durch Verreiben mit einer geringen Menge Äther–Petroläther (60–70°), 1:1, v/v, und scharfes Absaugen der Suspension erhält man IV als weißes amorphes Pulver, das ohne weitere Reinigung acetyliert wird.

Methyl 2,3,4-Tri-O-acetyl-6-S-benzyl-6-thio-α-D-glucopyranosid (VI)

Die gesamte im zuvor beschriebenen Versuch gewonnene Substanz (IV) wird

in trockenem Pyridin (30 ml) gelöst und mit Acetanhydrid (25 ml) versetzt. Man läßt das Reaktionsgemisch über Nacht stehen und gießt dann unter Rühren in Eiswasser. Das ausgefallene Öl kristallisiert nach 3–4 Stunden. Umkristallisation kann aus Äthanol oder Äther–Petroläther (60–70°) erfolgen. Ausbeute: 6.5 g = 87% d.Th., bezogen auf eingesetztes I; Fp 105°; $[\alpha]_{5780}^{22}$ +135° (*c* 1.5, Chloroform). (Gef.: C, 56.02; H, 6.25; S, 7.38. $C_{20}H_{26}O_8S$ Ber.: C, 56.32; H, 6.15; S, 7.52%).

Methyl 6-Desoxy-α-D-glucopyranosid (V) aus IV

IV (2 g) werden mit Raney-Nickel wie im Abschnitt „Allgemeine Methoden" beschrieben behandelt. Nach Filtration und Einengen der alkoholischen Lösung im Vakuum erstarrt der Rückstand kristallin. Er wird aus Essigester umkristallisiert. Fp 98° (Lit.[30], 98–99°); $[\alpha]_{5780}^{22}$ +159 (*c* 1, Wasser). Das i.r.-Spektrum von V sowie der Schmelzpunkt und die optische Drehung waren mit einer aus Methyl-6-desoxy-6-jod-α-D-glucopyranosid[29] durch Behandlung mit Raney-Nickel gewonnenen Probe identisch. Der Mischschmelzpunkt zeigte keine Depression. Auch die Konstanten für acetyliertes V stimmen mit den Literaturwerten[30] überein: Fp 75°, $[\alpha]_D^{22}$ +159.2°.

3-S-Benzyl-1,2:5,6-di-O-isopropyliden-3-thio-α-D-galaktose (IX) und 3-S-Benzyl-1,2:5,6-di-O-isopropyliden-3-thio-α-D-glucose (VIII).

II (1.4 g) wird in frisch destilliertem Benzylmerkaptan (3 ml) gelöst und in einem offenen Quarzreagenzglas belichtet. Während der Reaktion wird Stickstoff mit einer Geschwindigkeit von 20–40 Blasen pro Minute aus einem spitz zulaufenden Glasrohr durchgeleitet. Man erreicht dadurch eine ständige Durchmischung. Nach 3 Stunden hat sich II, wie dünnschichtchromatographisch festgestellt wurde, quantitativ umgesetzt. Überschüssiges Benzylmerkaptan wird im Hochvakuum unter Zwischenschaltung einer mit flüssigem Stickstoff gekühlten Kühlfalle bei 60–70° abdestilliert. Der ölige, schwach gelb gefärbte Rückstand (2.1 g) besteht nach gaschromatographischer Analyse zu etwa 90% aus zwei Substanzen mit wenig unterschiedlichen Retentionszeiten im genau gleichen Mengenverhältnis. Die Mischung wird im Klebevakuum destilliert. Der etwas trübe Vorlauf (90–150°) wird verworfen. Die Hauptfraktion geht als viskoses gelbliches Öl bei 160–162° über (1.3 g). Im Gaschromatogramm dieses Produktes erscheinen nur noch die beiden bereits erwähnten Komponenten (VIII und IX), wobei die Komponente mit kleinerer Retentionszeit (IX) in etwas geringerer Menge als im Rohprodukt vorliegt. Es ist anzunehmen, daß ein Teil mit dem Vorlauf überdestilliert ist. Aufgrund des Gaschromatogramms kann geschlossen werden, daß das kompaktere Molekül IX die Galaktokonfiguration hat. (Gef.: C, 62.10; H, 7.07; S, 9.33. $C_{19}H_{26}O_5S$ Ber: C, 62.27; H, 7.15; S, 8.75%).

3-Desoxy-1,2:5,6-di-O-isopropyliden-α-D-xylo-hexose (XI)

Die destillierte Mischung von VIII und IX (1 g) wird mit Raney-Nickel wie im Abschnitt „Allgemeine Methoden" behandelt. Nach Filtration und Eindampfen im Vakuum wird der farblose Rückstand bei 0.2 Torr destilliert. Zwischen 90 und 105° gehen 0.5 g eines klaren farblosen Öls über, das nach dem Gaschromatogramm

zu etwa 95% aus zwei Hauptkomponenten besteht. Die Komponente mit der kürzeren Retentionszeit ist gaschromatographisch von authentischer 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*xylo*-hexose nicht zu unterscheiden. Nach Animpfen des Öls mit 3-Desoxy-1,2:5,6-Di-*O*-isopropyliden-α-D-*xylo*-hexose kristallisiert über Nacht bei 0° ein Teil aus. Der Kristallbrei wird auf Tonscherben abgepreßt. Die hinterbleibenden feinen Nadeln (XI, 0.1 g) kristallisiert man aus Petroläther (60–70°) um. Nach dem Gaschromatogramm ist die Substanz rein und mit 3-Desoxy-1,2:5,6-Di-*O*-isopropyliden-α-D-*xylo*-hexose identisch. Fp 79–80° [Lit., 81° (ref. 14), 81.5° (ref. 13)]. Der Mischschmelzpunkt mit authentischem XI zeigt keine Depression. Auch die i.r.-Spektren sind identisch.

Die Identifizierung der zweiten Komponente (X) steht noch bevor.

*1,5-Anhydro-2-S-benzyl-2-thio-*D*-mannit (XII) und 1,5-Anhydro-2-S-benzyl-2-thio-*D*-glucit (XIII)*

Sirupöses D-Glucal (III, 5 g) werden in Äthanol (15 ml) gelöst, mit frisch destilliertem Benzylmerkaptan (7 ml) vermischt und wie für I beschrieben bestrahlt. Der Reaktionsverlauf wird papierchromatographisch verfolgt. Nach 4 Stunden tritt Geruch nach Schwefelwasserstoff auf, der sich laufend verstärkt, sodaß die Reaktion, obwohl ein großer Teil des D-Glucals noch nicht umgesetzt ist, nach 5 Stunden abgebrochen wird. Lösungsmittel und Benzylmerkaptan werden wie bei der Umsetzung von I beschrieben entfernt. Der gewonnene Kristallbrei wird mit wenig trockenem Äther verrieben und dieser vom Ungelösten abgegossen. Diesen Prozeß wiederholt man 2–3 mal. Die gaschromatographische Analyse zeigt neben nicht umgesetztem III (50%) zwei Komponenten (XII und XIII) in gleichen Mengen. Nach Zugabe von Wasser zu dem Rückstand erhält man eine Kristallsuspension, die nach zweistündigem Stehen im Eisschrank scharf abgesaugt wird. Das kristalline Produkt (XII) wird zweimal aus Äthanol umkristallisiert und ist dann gaschromatographisch einheitlich. Ausbeute: 1.7 g = 19.5% d.Th.; Fp 167–168°; $[\alpha]_{5780}^{22}$ +30.5° (*c* 0.5, Methanol). (Gef.: C, 57.69; H, 6.78; S, 12.16. $C_{13}H_{18}O_4S$ Ber.: C, 57.76; H, 6.71; S, 11.86%).

Die vereinigten Mutterlaugen (wässrige und alkoholische) werden mit Wasser auf 100 ml aufgefüllt und kontinuierlich 48 Stunden mit warmem Chloroform (200 ml) extrahiert. Die Chloroformlösung wird mit geglühtem Natriumsulfat getrocknet und im Vakuum eingedampft. Der Rückstand ist kristallin. Durch Umkristallisieren aus Aceton–Äther erhält man die Substanz (XIII) gaschromatographisch einheitlich. Ausbeute: 1.2 g = 13% d.Th.; Fp 151–151.5°; $[\alpha]_{5780}^{22}$ −12.8° (*c* 1, Methanol). (Gef.: C, 57.80; H, 6.80; S, 11.72. $C_{13}H_{18}O_4S$ Ber.: C, 57.76; H, 6.71; S, 11.86%).

*3,4,6-Tri-O-acetyl-1,5-anhydro-2-S-benzyl-2-thio-*D*-mannit (XIV)*

XII (1 g) läßt man in trockenem Pyridin (10 ml) mit Acetanhydrid (5 ml) 6 Stunden stehen und gießt dann unter Rühren in Eiswasser. Das ausgefallene Öl kristallisiert nach Anreiben mit einem Glasstab. Die trocken gesaugte kristalline Masse wird aus Petroläther (60–70°) umkristallisiert. Ausbeute: 1.2 g = 82% d.Th.; Fp 104–105°; $[\alpha]_{5780}^{22}$ +28° (*c* 0.5, Chloroform). (Gef.: C, 57.67; H, 6.29; S, 8.31. $C_{19}H_{24}O_7S$ Ber.: C, 57.55; H, 6.10; S, 8.09%).

3,4,6-Tri-O-acetyl-1,5-anhydro-2-S-benzyl-2-thio-D-glucit (XV)

XIII (1 g) wird wie im vorhergehenden Versuch beschrieben acetyliert und aufgearbeitet. Das erhaltene Produkt kristallisiert man aus Petroläther um. Ausbeute 1.3 g = 89% d.Th.; Fp 79–80°; $[\alpha]_{5780}^{22}$ +14.5° (*c* 1.5, Chloroform); $[\alpha]_{5780}^{22}$ +34° (*c* 1.2, Methanol). (Gef.: C, 57.56; H, 6.35; S, 7.88. $C_{19}H_{24}O_7S$ Ber.: C, 57.55; H, 6.10; S, 8.09%).

Dihydro-D-glucal aus XII und XIII

XII und XIII wurden getrennt mit Raney-Nickel behandelt und das Reaktionsprodukt nach Aufarbeitung und Destillation im Hochvakuum jeweils als klares farbloses Harz erhalten. Nach Animpfen mit authentischem kristallinen Dihydro-D-glucal, das durch katalytische Hydrierung aus D-Glucal gewonnen wurde[15], erstarrten beide Proben nach einigen Tagen kristallin. Beide durch Entschwefelung erhaltenen Proben waren außer einer geringen Verunreinigung (etwa 5%) mit niedrigerer Retentionszeit gaschromatographisch identisch mit authentischem Dihydro-D-glucal. Nach Umkristallisieren aus absolutem Äthanol–Petroläther stimmten die Schmelzpunkte der Präparate überein; Fp 84–86° (Lit.[15], 86–87°). Der Mischschmelzpunkt zeigte keine Depression. Die i.r.-Spektren waren untereinander identisch.

Geschwindigkeit der Acetylierung von XIII und der Entacetylierung von XV

XIII (15.8 mg) werden in einer 1 dm-Küvette in Pyridin (2 ml) gelöst, mit Acetanhydrid (1 ml) vermischt und sofort kräftig umgeschüttelt. Die Zeit wird vom Moment des Umschüttelns an gemessen. Die erste Messung des Drehwertes erfolgte nach 2 Minuten. Um vergleichbare Werte zu erhalten, wird die spezifische Drehung aufgrund von eingesetztem XIII berechnet. Nach 2 Minuten beträgt $[\alpha]_{5780}^{22}$ +11.4°. Die Drehung zur Zeit O liegt nach dem Kurvenverlauf (Abb. 4) bei negativen Werten. Der Endwert ist mit $[\alpha]_{5780}^{22}$ +63.5° nach 80 Minuten erreicht. Die Aufarbeitung der Lösung ergibt 20 mg XV = 87% d.Th. Der Mischschmelzpunkt mit authentischem XV zeigt keine Depression. In Methanol gemessen ergibt sich wieder die bereits gefundene spezifische Drehung für XV mit $[\alpha]_{5780}^{22}$ +34°.

XV (63.8 mg) werden in absolutem Methanol (5 ml) gelöst und davon 3 ml in einer 1 dm-Küvette bei 578 mμ gemessen. Bezogen auf die entsprechende Menge entacetylierter Substanz XIII (45 mg) ergibt sich ein spezifischer Drehwert von $[\alpha]_{5780}^{22}$ +48.5°. Dieser Wert wird für die Zeit O eingesetzt. In die Küvette pipettiert man dann 0.025 ml einer 1.5N Natriummethylatlösung und beginnt sofort mit der Messung der Drehwerte. Die durch Zugabe der Methylatlösung entstandene Konzentrationsänderung ist zu vernachlässigen. Der Anfangswert sinkt rasch ab und ist bereits nach 9 Minuten annähernd konstant ($[\alpha]_{5780}^{22}$ −12.2°). Nach 16 Minuten wird der Endwert mit $[\alpha]_{5780}^{22}$ −12.7° abgelesen, der dem einer reinen Probe XIII sehr gut entspricht ($[\alpha]_{5780}^{22}$ −12.8°). Die eingedampfte Lösung ergibt nach dem Umkristallisieren des Rückstandes aus Aceton–Äther 30 mg reines XIII = 67% d.Th. Der Mischschmelzpunkt mit einer authentischen Probe zeigt keine Depression.

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ZUSAMMENFASSUNG

Die radikalische Addition von Benzylmerkaptan an enolische Zuckerderivate wird beschrieben. Die Stereoselektivität dieser Reaktion wird an Hand der Umsetzung von Methyl-6-Desoxy-α-D-*xylo*-hex-5-enopyranosid, 3-Desoxy-1,2:5,6-di-*O*-isopropyliden-α-D-*erythro*-hex-3-enose, und D-Glucal untersucht und mit entsprechenden Additionen von Thiolen an cyclische Alkene verglichen.

Die Geschwindigkeit der Acetylierung und Entacetylierung in homogener Lösung wurde durch Messung der Drehwertsänderungen von 1,5-Anhydro-2-*S*-benzyl-2-thio-D-glucit bzw. dessen Tri-*O*-acetylderivat als geeigneten Untersuchungsobjekten verfolgt.

SUMMARY

The free-radical addition of toluene-α-thiol to enolic sugar derivatives is described. The stereoselectivity of this reaction was investigated by studying the addition to methyl 6-deoxy-α-D-*xylo*-hex-5-enopyranoside, 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*erythro*-hex-3-enose, and D-glucal, and by comparison with analogous additions of thiols to cyclic alkenes.

The velocity of acetylation and deacetylation of sugar derivatives in homogeneous solutions was measured by means of changes in optical rotation. 1,5-Anhydro-2-*S*-benzyl-2-thio-D-glucitol and its triacetate were studied in this manner.

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Notes

Reaktionen enolischer Zuckerderivate

Teil III. Strukturaufklärung der Additionsprodukte von Benzylmerkaptan an D-Glucal

Bei der radikalischen Addition von Benzylmerkaptan an D-Glucal in äthanolischer Lösung unter Stickstoff und Bestrahlung mit ultraviolettem Licht entstehen[1] zwei kristalline Substanzen (I und II) im Verhältnis 1:1". Der Angriff des Thiylradikals in 2-Stellung des D-Glucals wurde durch Entschwefelung mit Raney-Nickel unter Bildung von Dihydro-D-glucal sowohl aus I als auch aus II bewiesen, sowie durch die Resistenz beider Verbindungen gegenüber Quecksilberchlorid in siedendem Methanol[1].

Es liegt nahe, I ($\alpha+$) und II ($\alpha-$) strukturell mit Polygalit (1,5-Anhydro-D-glucit) bzw. Styracit (1,5-Anhydro-D-mannit) zu vergleichen[2]. Die Messung der optischen Drehung der Reaktionsprodukte I ($[\alpha]_{5780}^{22}$ +30.5°, *c* 0.5, Methanol) und II ($[\alpha]_{5780}^{22}$ −12.8°, *c* 1, Methanol) würde somit schon eine strukturelle Zuordnung möglich erscheinen lassen. Das hieße, daß I, mit positiver Drehung, die Konfiguration von 1,5-Anhydro-D-glucit, II, mit negativer Drehung die von 1,5-Anhydro-D-mannit hätte. Die lange Zeit umstrittene strukturelle Zuordnung der Naturprodukte Styracit und Polygalit führte zu widersprechenden Ergebnissen, unter anderem aufgrund der optischen Drehung. Die endgültige Struktur der Acetate von I und II (Ia und IIa) konnte jetzt mit Hilfe der Protonenspektroskopie aufgeklärt werden.

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Notes

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TABELLE I

τ-WERTE IN p.p.m. VON Ia UND IIa GEGEN TMS ALS INNERER STANDARD

Protonen	*Ia*	*IIa*
1a	6.32	6.78
1e	6.08	6.15
2a		~7.25
2e	6.80	
3a	4.97	~5.20
4a	4.77	~5.05
5a	~6.50	~6.55
6	5.85	5.88
		6.55
CH_2S-	6.25	6.36
Phenyl-	2.80	2.80
CH_3CO-	~8.00	~8.00

TABELLE II

KOPPLUNGSKONSTANTEN VON Ia UND IIa IN Hz

	Ia	*IIa*
$J_{1a,1e}$	12	11.5
$J_{1a,2a}$		11.5
$J_{1a,2e}$	3	
$J_{1e,2a}$		4
$J_{1e,2e}$	3	
$J_{2a,3a}$		8–9
$J_{2e,3a}$	4	
$J_{3a,4a}$	9	8–9
$J_{4a,5a}$	8.5–9	8–9
$J_{5a,6'}$		5
$J_{5a,6''}$		2

Wie die Analyse des PR-Spektrums von Ia zeigt, steht das Proton an C-2 äquatorial, der Thiobenzylrest also axial. Für diese Position sprechen eindeutig die Kopplungskonstanten $J_{1e,2e}$ und $J_{1a,2e}$ 2.5 Hz. Stände das Proton an C-2 axial, müßte eine weit größere, *ca.* 7–10 Hz große Kopplungskonstante ($J_{1a,2a}$) auftreten. Dies ist nun bei IIa der Fall. Die Kopplungskonstanten $J_{1a,2a} = 11.5$ Hz und $J_{1e,2a}$ 4 Hz zeigen eindeutig die axiale Stellung des Protons an C-2, das heißt der Thiobenzylrest steht äquatorial.

τ-Werte und Kopplungskonstanten der übrigen Protonen in Ia und IIa sind den Tabellen I und II sowie den Ausschnitten der PR-Spektren (Abb. 1 und Abb. 2) zu entnehmen.

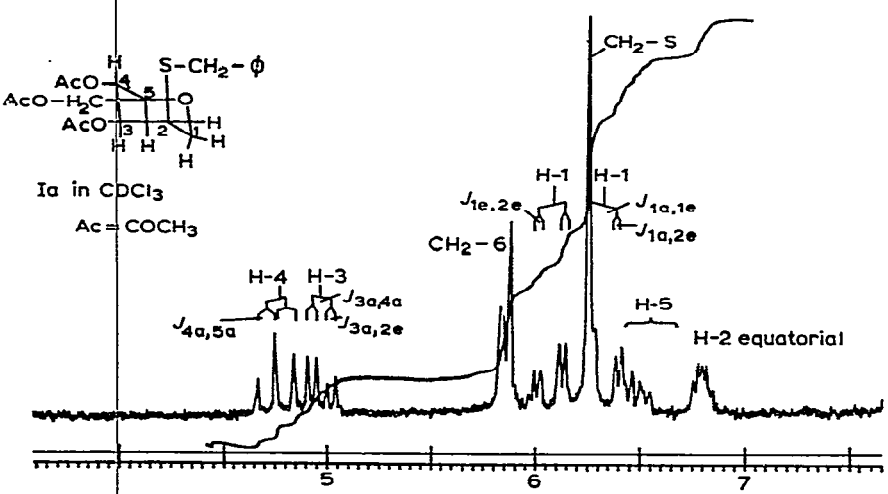

Abb. 1 Ausschnitt des PR-Spektrums von Ia, aufgenommen mit einem Varian-Spektrometer HA-100 bei 100 MHz.

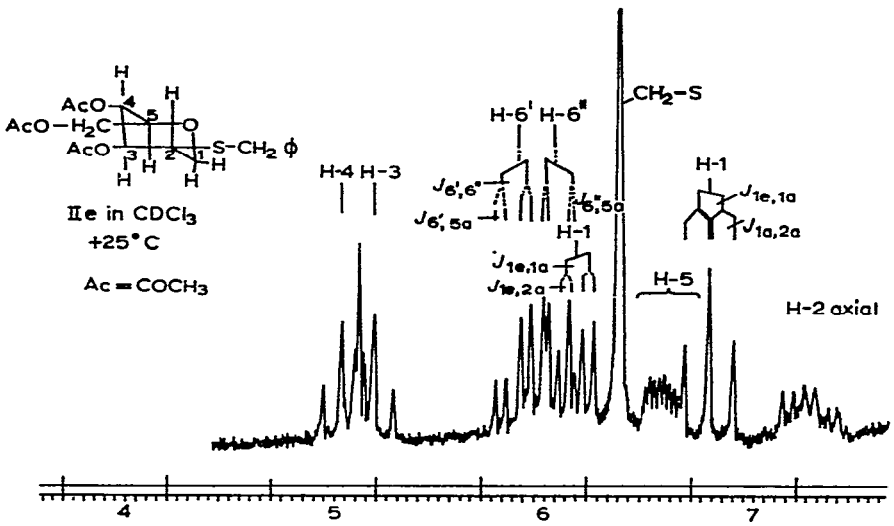

Abb. 2 Ausschnitt des PR-Spektrums von IIa, aufgenommen mit einem Varian-Spektrometer HA-100 bei 100 MHz.

Merkwürdig ist, daß das Triacetat von II (IIa) im Gegensatz zur Ausgangssubstanz einen positiven Drehsinn aufweist[1]. Das Phänomen der Drehsinnumkehrung unter gleichen Meßbedingungen durch Acetylierung wurde bei Pyranosiden oder 1,5-Anhydro-zuckeralkoholen, soweit uns bekannt ist, bisher nicht beobachtet. Eine Erklärung läßt sich durch Vergleich der PR-Spektren von II und IIa (II in Dimethylsulfoxid, Trifluoressigsäure, und Deuterochloroform gemessen) nicht geben, da die Spektren von II für eine einwandfreie Deutung zu komplex sind.

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Enzymic assay of the average length of the unit-chain of amylopectin

Currently, the estimation of the average length of unit-chain of amylopectin samples is most often carried out by periodate oxidation[1]. This method has disadavantages. In particular, it is difficult to apply adequate corrections for over-oxidation and for contaminating, amylose-like material. These problems are very pertinent in the study of amylomaize starch and its sub-fractions[2].

Recently, Whelan *et al.*[3,4] have described an enzymic method of chain-length assay. The glucan is degraded by the concurrent action of β-amylase and the debranching enzyme pullulanase. Pullulanase has been shown to specifically remove the α-(1 → 6)-linkages in amylopectin[5], and hence the whole polysaccharide molecule is rendered susceptible to the action of β-amylase. At high concentrations, the β-amylase will convert (*1*) chains having an even number of D-glucose residues into maltose, and (*2*) chains having an odd number of units into maltose and one D-glucose molecule (arising from the maltotriose) which can be specifically estimated by glucose oxidase[6]. The average length of the unit-chain of the amylopectin is then calculated on the assumption that the polysaccharide has a random distribution (*i.e.*, equal numbers) of even and odd lengths of chain.

Through the courtesy of Professor Whelan, we have been able to make further tests of the application of this technique to amylopectin.

Table I shows, for a variety of amylopectin samples, the results obtained for the enzymic assay method, compared to those from periodate oxidation[7]. It can be seen that the agreement is good, except for the amylopectin samples having high iodine-affinity. However, the enzymic assay may tend to give lower, average chain-lengths.

The method can also be used to estimate long chain-lengths, as shown by the results for the amylose-type samples (amylomaize 5 and 6) in Table I.

Our experience with the enzymic assay suggests that it gives much more reproduceable results than periodate oxidation. Its inherent specificity is an added advantage. We have found that the production of D-glucose reaches a constant value which is (*1*) independent of the incubation time, and (*2*) unchanged on the further addition of enzyme mixture. We now intend to use this method routinely.

EXPERIMENTAL

The following enzymes were used: pullulanase (by courtesy of Professor

TABLE I

COMPARISON OF AVERAGE CHAIN-LENGTH OF AMYLOPECTIN SAMPLES BY ENZYMIC AND PERIODATE ASSAY

Sample[a]	*Iodine affinity*	*Chain length*		*Sample*[b]	*Iodine affinity*	*Chain length*	
		Enzymic	*Periodate*			*Enzymic*	*Periodate*
Barley	0.60	20	20	Amylomaize 1	2.30	34	38
Maize	0.10	24	27	Amylomaize 2	4.20	45	37
Oats	0.50	19	18	Amylomaize 3	0.12	27	n.d.[c]
Potato	0.02	22	26	Amylomaize 4	0.19	27	28
Waxy maize	0.04	17	23	Amylomaize 5	20.8	240	n.d.
Wheat	0.50	21	20	Amylomaize 6	20.2	250	n.d.

[a]Amylopectin prepared from laboratory-extracted starches by removal of the thymol–amylose complex from aqueous dispersions.
[b]Fractions of amylomaize starches[2].
[c]n.d. = not determined.

Whelan); crystalline β-amylase (Worthington Biochemical Corporation); glucose oxidase (Boehringer Corporation); horseradish peroxidase (Boehringer Corporation).

The digest conditions were those discussed by Whelan *et al.*[5], except that the amylomaize samples were dispersed in dimethyl sulphoxide[2]. Assay of D-glucose was carried out as described by Fleming and Pegler[6].

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