stored in a vacuum desiccator over calcium chloride. The pyrone melted at 51° (reported 51.5°).

A weighed amount of the pyrone was placed in a 50-ml. flask equipped with a stirrer and a cork stopper. The flask was placed in a water-bath with a thermometer and stirrer. Weighed quantities of freshly distilled water were added from a weighing bottle, the water-bath heated above the upper consolute temperature, and the bath allowed to cool slowly, during which time the upper and lower solution temperatures were recorded; then more water was added and the procedure repeated. These temperatures were reproducible within ±0.5°. Readings were recorded at the temperature at which there was a definite separation into two phases. The data are plotted in Fig. 1.

Acknowledgment.—The authors wish to thank the Research Corporation for a grant made in support of a program under which this work was done.

DEPARTMENT OF CHEMISTRY
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RECEIVED NOVEMBER 6, 1950

COMMUNICATIONS TO THE EDITOR

NEOMYCINS B AND C, AND SOME OF THEIR DEGRADATION PRODUCTS

Sir:

In the course of purification of the antibiotic complex termed neomycin by Waksman and Lechevalier,[1] we have obtained by chromatography of the hydrochloric acid salt[2] in 80% methanol over alumina two homogeneous biologically active fractions. One of these closely resembles the neomycin B described by Regna and Murphy,[3] while the properties of the other, named by us neomycin C, sharply differentiate it from neomycin A[4] (see Table I).

TABLE I

COMPARISON OF PROPERTIES OF NEOMYCINS

	Neomycin component B, amorphous hydrochloride	Neomycin component C, amorphous hydrochloride	Neomycin A[4] amorphous hydrochloride	Neomycin B[4] amorphous sulfate
Biopotency in[a] nutrient broth *vs. Klebsiella pn.*	265 units/mg.	180 units/mg.	20 units/mg.[b]	260
Biopotency on nutrient Agar *vs. B. subtilis*	86 units/mg.	121 units/mg.	1700 units/mg.[b] 710 units/mg.[b]	255 units/mg. .....
$[\alpha]_D$	+54°	+80°	+83°	+58°

[a] The units are based on a comparison with a Waksman standard preparation. [b] These values determined with a sample of neomycin A hydrochloride kindly supplied by Dr. Karl Folkers.

Crystalline reineckates, picrolonates and *p*-(*p*'-hydroxyphenylazo)-benzenesulfonates have been prepared from both entities. In addition, antibacterially inactive, crystalline N-acetates have been obtained (*Neomycin B N-acetate*, needles from aqueous acetone; m.p. 200–205° with decomposition, after darkening and softening 186–190°; $[\alpha]^{22}D$ +62°, 0.4% in water. *Anal.* Found:[5] C, 50.71; H, 6.95; N, 9.45; acetyl, 32.4. *Neomycin C N-acetate*, needles from aqueous acetone; m.p. similar to that of neomycin B N-acetate, but showing a depression of 10° in mixed melting point determination; $[\alpha]^{22}D$ +90°, 0.4% in water. *Anal.* Found: C, 51.09; H, 7.47; N, 9.24; acetyl, 32.7). These data suggest that the two bases may be isomeric. All the nitrogen atoms are present as primary amino groups (Van Slyke).

Methanolysis of the hydrochlorides afforded the following two fragments:

(1) An amorphous hydrochloride, identical from both neomycin B and C, which was devoid of reducing power and yielded all its nitrogen as amino nitrogen in the Van Slyke determination. The empirical formula, $C_9H_{19}O_5N_3 \cdot 3HCl$, may be derived from the analyses of the following crystalline derivatives. *N-acetate*, needles from methanol; decomposes *ca.* 300° without melting, after sintering at 260°; $[\alpha]^{22}D$ +88°, 0.3% in water. *Anal.* Calcd. for $C_9H_{16}O_5N_3(COCH_3)_3$: C, 48.00; H, 6.71; N, 11.20; acetyl, 34.4. Found: C, 47.76, 47.94; H, 6.95, 7.76; N, 11.46; acetyl, 36.5. *N-Benzoate*, needles from aqueous methanol, decomposes to black tar at 299–300° after darkening at 270°; $[\alpha]^{22}D$ +70°,0.5 % in methanol. *Anal.* Calcd. for $C_9H_{16}O_5N_3(COC_6H_5)_3$. C, 64.16; H, 5.57; N, 7.48. Found: C, 64.54; H, 6.02; N, 7.43. *Heptaacetate*, prisms from acetone, dried sample softens at 165°, undergoes transition 190–215°, liquifies at 260–262°; the cooled and solidified material (needles) then melts (without transition) at 262–265°; $[\alpha]^{22}D$ +49°, 0.5% in methanol. *Anal.* Calcd. for $C_9H_{12}O_5N_3(COCH_3)_7$ (mol. wt., 5.44): C, 50.83; H, 6.12; N, 7.73; total acetyl,

(1) S. A. Waksman and H. A. Lechevalier, *Science*, **109**, 305 (1949).

(2) The crude neomycin preparations used were produced by the Divisions of Microbiological and Chemical Development, E. R. Squibb & Sons, New Brunswick, N. J.

(3) P. P. Regna and F. X. Murphy, THIS JOURNAL, **72**, 1045 (1950).

(4) R. Peck, C. E. Hoffhine, P. Gale and K. Folkers, *ibid.*, **71**, 2590 (1949).

(5) All analytical determinations were carried out by Mr. J. F. Alicino, Microanalytical Laboratory, E. R. Squibb & Sons, New Brunswick, N. J.

55.4; O-acetyl, 31.7. Found: C, 49.45, 49.71; H, 6.51, 6.61; N, 7.65; total acetyl, 53.4; O-acetyl, 30.2; mol. wt. (Rast, in camphor), 543, 512.

(2) Non-identical methyl glycosidic moieties, which for reasons given below have been provisionally termed methyl neobiosaminides B and C ($[\alpha]_D$ of the amorphous hydrochlorides, 1.0% in water, +22° and +78°, respectively). The analysis of a chromatographically purified amorphous *polyacetate of methyl neobiosaminide B* was best compatible with the composition $C_{11}H_{16}O_6N_2(OCH_3)(COCH_3)_5$: $[\alpha]^{22}D$ +2°, 0.5% in ethanol. *Anal.* Calcd.: C, 50.96; H, 6.61; N, 5.40; CH_3O, 5.98; 5 acetyl, 41.5. Found: C, 49.62; H, 6.70; N, 5.41; CH_3O, 5.16; acetyl, 41.9.

On vigorous hydrolysis with hydrochloric acid, methyl neobiosaminide C yielded the dihydrochloride of a reducing diamine, $C_6H_{14}O_3N_2.2HCl$, platelets from methanol–ether; m.p. 182–185° with decomposition after shrinking and darkening 155–175°, $[\alpha]^{22}D$ +69°, equilibrium value, 0.4% in water. *Anal.* Calcd.: C, 30.65; H, 6.86; N, 11.92; Cl, 30.16. Found: C, 30.79; H, 7.18; N, 12.52; Cl, 30.51. The reducing diamine from methyl neobiosaminide B has not yet been obtained in crystalline form but mutarotation and paper chromatographic data indicate that it is not identical with that from neomycin C. The remainder of the neobiosaminide fragment seems to be accounted for by a pentose, as evidenced by the formation, on acid hydrolysis, of furfural from both neomycin B and C and the corresponding methyl neobiosaminides. This property has been utilized for the chemical assay of the two antibiotics.

The average weight ratio, in several methanolyses, of non-glycosidic fraction to methyl biosaminide fraction was 1.85; on the other hand, the ratio of the molecular weight of the C_9 base hydrochloride (358) to that of the methyl biosaminide hydrochloride (381) is 0.94. Hence it appears that actually two moles of the C_9 base are liberated, conceivably from acetalic linkage, by the methanolysis. On this basis the neomycins B and C would appear to have the composition $C_{29}H_{58}O_{16}N_8$.

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RECEIVED JANUARY 25, 1951

A TERMINAL AMINO ACID RESIDUE OF LYSOZYME AS DETERMINED WITH 2,4-DINITROFLUOROBENZENE

Sir:

Studies on lysozyme to determine which amino acid residues occupy terminal positions at the amino ends of the polypeptide chains have shown that one of the lysine residues occupies such a position in the protein. This conclusion is drawn from the fact that α,ϵ-di-DNP-lysine was the only DNP-amino acid isolated chromatographically from the ether extract of an acidic hydrolysate of DNP-lysozyme. No attempt has yet been made to determine whether the hydrolysate contains basic DNP-amino acids other than ϵ-DNP-lysine which are not extracted with ether.

DNP-Lysozyme was prepared from lysozyme (Armour and Company Lot 805L1) in a manner similar to that described by Sanger[1] for insulin. A sample of the DNP-lysozyme was refluxed with 6 *N* hydrochloric acid for 8 hours and the ether extract of the hydrolysate was examined chromatographically according to a scheme which has recently been developed in these Laboratories.[2] This scheme for the separation of 16 ether-soluble DNP-amino acids by adsorption chromatography on 2:1 silicic acid–Celite is considerably faster than that described by Sanger,[1] Porter and Sanger,[3] and Blackburn,[4] and gives satisfactory qualitative results on a large number of samples of silicic acid. The ether extract of the hydrolysate gave two yellow zones on a silicic acid column. The chromatographic characteristics of the lower zone were identical with those of α,ϵ-di-DNP-lysine with three different solvent systems as developers, even after a second hydrolysis. The upper zone, which did not behave like any known DNP-amino acid, yielded α,ϵ-di-DNP-lysine on further hydrolysis and, therefore, contained a DNP-lysyl peptide (or peptides), highly resistant to hydrolysis, which is now being investigated. The changes in development behavior of α,ϵ-di-DNP-lysine from one developer to another are greater than those of other similarly adsorbed DNP-amino acids so that its chromatographic behavior is an excellent criterion for its identity.

In preliminary quantitative studies it has been found that the ether extract of an 8-hour hydrolysate contained α,ϵ-di-DNP-lysine equivalent to about 0.35 terminal amino groups per molecule (mol. wt. 13,900[5]), and in addition a considerable amount of DNP-lysyl peptide(s). After hydrolysis for 24 hours, α,ϵ-di-DNP-lysine equivalent to 0.75 terminal amino groups per molecule was isolated. This value, which includes corrections for 7% loss during chromatographic operations and for 15% destruction of the lysine derivative during hydrolysis, is considerably less than one terminal group per molecule and further improvements in the analytical method are now being sought. There was still evidence of a small amount of DNP-lysyl peptide(s) after 24 hours hydrolysis but probably not enough to account for the low yield of lysine derivative. The absence of free lysine when the amino acid content of the hydrolysate was determined by starch chromatographic methods indicates the completeness of the reaction between the protein and dinitrofluorobenzene. From the results obtained thus far it seems unlikely that more than one lysine residue occupies an amino terminal position in lysozyme.

CONTRIBUTION NO. 1512
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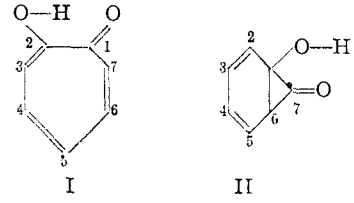
RECEIVED JANUARY 11, 1951

(1) F. Sanger, *Biochem. J.*, **39**, 507 (1945).
(2) F. C. Green and L. M. Kay, to be published.
(3) R. R. Porter and F. Sanger, *Biochem. J.*, **42**, 287 (1948).
(4) S. Blackburn, *ibid.*, **45**, 579 (1949).
(5) K. J. Palmer, M. Ballantyne and J. A. Galvin, THIS JOURNAL, **70**, 906 (1948).

MOLECULAR STRUCTURE OF TROPOLONE

Sir:

Doering and Knox[1] have recently reported the synthesis of the interesting and important compound tropolone, I, which had been previously conceived by Dewar[2] as a structural unit having special resonance stability. The chemical and spectroscopic evidence obtained by Doering and Knox,



I II

they remark, is consistent with I but does not exclude the alternative structure II. We wish to report the preliminary results of an electron diffraction investigation of tropolone which (a) exclude II and (b) constitute direct evidence in support of I.

The sample, kindly supplied by Dr. Doering, was vaporized in a boiler and photographs were prepared in the usual way[3] with an electron wave length of about 0.06 Å. and a camera distance of about 11 cm. The radial distribution curve calculated[4] from our visual interpretation of the photographs is shown in Fig. 1. The following interpretations of the peaks

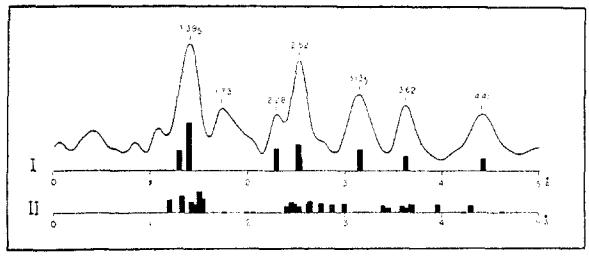


Fig. 1.—Radial distribution function for tropolone: vertical bars indicate distances calculated for models of structural types I and II; heights of bars are proportional to weights of distances.

of the radial distribution curve may be made in terms of I, if, just for purposes of orientation, the molecule is assumed to be approximately coplanar, and to have approximately all bonded C–C distances alike, bonded C–O distances alike, C–C–C angles alike, and corresponding O–C–C angles alike. The peak at 1.39(5) Å. is due to bonded C–C and bonded C–O interactions, which are too close together to be resolved. The peak at 2.28 Å. may be assigned to the distances of the type $O_1\cdots C_2$ and $O_1\cdots C_7$, that at 2.52 Å. to $C_1\cdots C_3$ and $O_1\cdots O_2$ (unresolved), and the remaining peaks at 3.13_5, 3.62, and 4.41 Å. to $C_1\cdots C_4$, $O_1\cdots C_3$ and $O_1\cdots C_6$, and $O_1\cdots C_4$ and $O_1\cdots C_5$, respectively. The peak at 1.73 Å. does not correspond to any possible distance in I or in any other reasonable model of tropolone, and must be considered as due to error in interpretation of the photographs. The interatomic distances for the completely symmetrical version of I defined in Table I correspond closely with the observed peak positions as may be seen in the figure. On the other hand, the distances for a reasonable model of II (C_1–C_2 = 1.54 Å., C_2–C_3 = 1.33 Å., C_3–C_4 = 1.46 Å., C_1–C_7 = C_1–C_6 = 1.52 Å., C_1–O_1 = 1.43 Å., C=O = 1.21 Å., $\angle C_1C_3C_2$ = 125°, $\angle OC_1C_2$ = $\angle OC_1C_6$ = 109.5°, and plane of 3-membered ring bisecting $\angle OC_1C_2$) do not correspond at all well. Indeed, examination of the general situation indicates that it is extremely unlikely that a fit to the radial distribution curve can be obtained with models of II even on assumption of unreasonable values for the structural parameters.

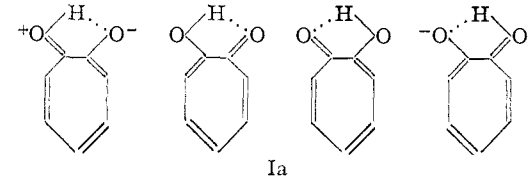
TABLE I

TROPOLONE. COMPARISON OF OBSERVED AND CALCULATED DISTANCES

The calculated distances are for a coplanar model of symmetry C_{2v}, with regular seven-membered ring, C–C = 1.40 Å., C–O = 1.30 Å., and $\angle C_1C_2O_2$ = 115¾°.

Identification	Rel. weight	Distances Obsd.	Calcd.
C–C	100	1.39(5)	1.40 (assumed)
C–O	41		1.30 (assumed)
$O_1\cdots C_2$	23	2.28	2.28
$O_1\cdots C_7$	23		2.28
$C_1\cdots C_3$	56	2.52	2.52
$O_1\cdots O_2$	28		2.53
$C_1\cdots C_4$	44	3.13(5)	3.15
$O_1\cdots C_3$	15	3.62	3.62
$O_1\cdots C_6$	15		3.62
$O_1\cdots C_4$	12	4.41	4.42
$O_1\cdots C_5$	12		4.42

Our discussion of the data in terms of a symmetrical model for I might seem to imply that the tropolone molecule actually is symmetrical with the enolic hydrogen atom occupying a position midway between the two oxygen atoms as would correspond to symmetrical contributions from all the resonance structures including the typical ones shown in Ia. Presumably such a symmetrical



Ia

structure is attained by the somewhat analogous azulene molecule. It must be emphasized, however, that notwithstanding the excellent agreement shown in Table I, the radial distribution curve for tropolone does *not* exclude an unsymmetrical structure with unequal C–C bond distances and unequal C–O bond distances corresponding to unsymmetrical contributions of the resonance structures (Dewar[2] at first discussed only the symmetrical possibility but later favored an unsymmetrical structure) provided that in each group the distances do not differ by more than about 0.1 Å. Further, although the radial distribution curve definitely excludes large deviations of the heavy atom structure from coplanarity, small

(1) W. von E. Doering and L. H. Knox, THIS JOURNAL, **72**, 2305 (1950).
(2) M. J. S. Dewar, *Nature*, **155**, 50, 141, 479 (1945).
(3) L. O. Brockway, *Rev. Modern Phys.*, **8**, 231 (1936).
(4) R. Spurr and V. Schomaker, THIS JOURNAL, **64**, 2693 (1942).

deviations are not ruled out. Such small deviations cannot be regarded as likely, however.

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CONTRIBUTION NO. 1477 KENNETH HEDBERG

RECEIVED OCTOBER 16, 1950

ISOLATION OF CRYSTALLINE PYROPHOSPHATASE FROM BAKER'S YEAST

Sir:

The presence in yeast of an enzyme capable of catalyzing the hydrolysis of inorganic pyrophosphate to orthophosphate has been established by Bauer[1] in 1936. The enzyme was named "pyrophosphatase." Several attempts have been made by various workers to purify the enzyme. The most notable advance in the purification of the enzyme was made in 1944 by Bailey and Webb.[2] They have been unsuccessful, however, in their attempt to obtain the enzyme in crystalline form. The enzyme has now been crystallized from Fleischmann's baker's yeast, in the form of fine needles and thin rectangular prisms.

The method of isolation consists essentially of the following steps: 1. Plasmolysis of compressed yeast with toluene at 38–40°, followed by extraction with water, at 5°. 2. Concentration and fractionation between 0.5 and 0.7 saturation of ammonium sulfate. 3. Removal of inert components by autolysis at 5°, accompanied by precipitation of the enzyme with ammonium sulfate. 4. Further removal of impurities by adsorption on $Ca_3(PO_4)_2$ gel, followed by precipitation of the enzyme with ammonium sulfate. 5. Removal of electrolytes by dialysis against distilled water at 5°. 6. Crystallization in dilute ethyl alcohol solution at −8°.

Crystalline pyrophosphatase is a soluble, colorless protein of the albumin type, free of phosphorus (C, 54.5; H, 7.4; N, 16.2; S, 0.14; ash, 0.36).

Details of the method of isolation, also a description of some of the physico-chemical and catalytic properties of the newly isolated crystalline enzyme, are to be submitted for publication in the *Journal of General Physiology*.

(1) E. Bauer, *J. Physiol. Chem.*, **239**, 195 (1936).
(2) K. Bailey and E. C. Webb, *Biochem. J.*, **38**, 394 (1944).

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RECEIVED JANUARY 25, 1951

PAPER CHROMATOGRAPHY OF HYDROXY AND KETOACIDS[1]

Sir:

Paper chromatography has been applied in the last few years to the detection of small amounts of various types of organic acids. Various procedures have been reported for the saturated aliphatic acids,[2–6] and Lugg and Overell[7] have developed an excellent method for the paper chromatography of polycarboxylic and other non-volatile acids. They employed butanol–water or other solvents, in combination with a volatile organic acid, such as formic or acetic, in order to decrease the ionization of the test acids and thus prevent streaking or "tailing."

In connection with an investigation of fatty acid metabolism in this laboratory, it was necessary to develop a technique for the separation and identification of small quantities of certain hydroxy and ketoacids of intermediate chain length. In attempting to apply a paper chromatographic method, it was found that the solvent system of Lugg and Overell was not suitable for most of these acids: they exhibited very high R_f values and poor resolution; furthermore, their moderate degree of volatility limited the length of time permissible to carry out the procedure.

It has been found, however, that a solvent system composed of toluene–acetic acid–water provides an excellent method for the analysis of many hydroxy and ketoacids. A mixture of 100 cc. of toluene and 5 cc. of acetic acid is equilibrated with 60 cc. of distilled water; after separation of the layers, an additional 4 cc. of acetic acid is added to the toluene layer. Whatman No. 1 filter paper is used without prior washing or other treatment. The papers are run in the descending manner for several hours, depending on the particular acids being chromatographed. After the removal of the paper from the chamber, it is dried several hours in a current of air. The test acids are located in a novel manner: the dried papers are exposed a few minutes to ammonia vapor in a closed chamber, the excess ammonia is removed by allowing the paper to stand 30 minutes, and the spots of ammonium salts are then located by dipping the paper in Nessler solution. Small, intensely orange spots against a light background result.

Since the mobile solvent is allowed to overrun the paper during the chromatographing, R_f values do not apply, but the distances the acids move from the starting point are equally characteristic. The excellent resolution obtained is indicated by the following data from a 6-hour chromatogram: α-hydroxyvaleric acid and α-hydroxycaproic acid move 5.3 cm. and 14.5 cm. from the starting point, respectively; β-hydroxycaproic acid moves 10.5 cm.; and α-ketovaleric acid moves 8.6 cm.

Preliminary experiments indicate the method of color development described above may be used for quantitative estimation of the test acids: After exposing to ammonia, the material can be eluted with water, Nesslerized, and the intensity of color determined in a photoelectric colorimeter or spectrophotometer.

(1) This work was supported in part by funds granted by the National Dairy Council on behalf of the American Dairy Association, and by the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) K. Fink and R. M. Fink, *Proc. Soc. Exp. Biol. Med.*, **70**, 654 (1949).
(3) E. R. Hiscox and N. J. Berridge, *Nature*, **166**, 522 (1950).
(4) F. Brown and L. P. Hall, *ibid.*, **166**, 66 (1950).
(5) F. Brown, *Biochem. J.*, **47**, 598 (1950).
(6) L. A. Liberman, A. Zaffaroni and E. Stotz, in press.
(7) J. W. H. Lugg and B. T. Overell, *Australian J. Scientific Res., Ser. A*, **1**, 98 (1948).

Further details of the method and some biological applications will be published elsewhere.

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SCHOOL OF MEDICINE AND DENTISTRY L. A. LIBERMAN
UNIVERSITY OF ROCHESTER ALEJANDRO ZAFFARONI
ROCHESTER 20, N. Y. ELMER STOTZ

RECEIVED JANUARY 30, 1951

PHASE BOUNDARY POTENTIALS OF NICKEL IN FOREIGN ION SOLUTIONS[1]

Sir:

An investigation was started early in 1947 concerning the phase boundary potentials of inert metals in contact with solutions initially free from the common metal ions. The study of such systems, which remain relatively free from common ions, might contribute to a better understanding of the initial processes which induce corrosion of the metal. Our approach aims at very careful control of all experimental factors, since most metals show a greater or lesser tendency to interact with electrolyte solutions.

In a recent publication by El Wakkad and Salem,[2] the behavior of the potentials of mercury in buffer solutions initially free from mercury ions is discussed. An earlier article by Tourky and El Wakkad[3] dealt with an analogous investigation of the potentials of copper in foreign ion solutions.

Since in these laboratories work already has been done on several inert metals,[4] we believe that a preliminary account of our measurements on nickel may be of interest. Stable potentials could be obtained within 5 to 15 hours in a series of potassium hydroxide solutions, and in a series of phosphate buffer solutions covering the entire *p*H range. The stationary potentials, calculated against the standard hydrogen electrode, are plotted as a function of the *p*H of the solutions (Fig. 1).

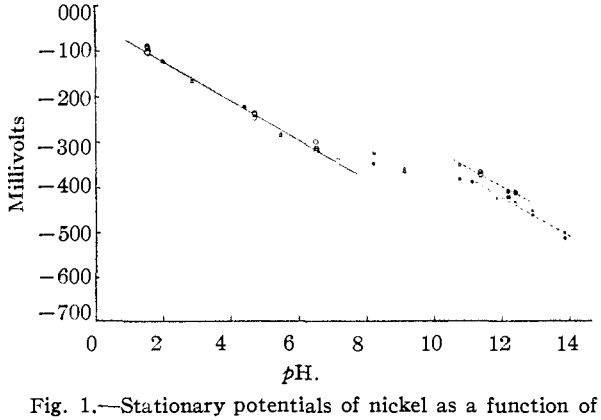

Fig. 1.—Stationary potentials of nickel as a function of *p*H in potassium hydroxide (× runs 4 and 5) and in 0.100 *M* phosphate solutions (O run XI, □ run XII, △ run XIV).

(1) This paper was presented before the General Meeting of the Division of Physical and Inorganic Chemistry of the American Chemical Society, Chicago, Ill., September, 1950.

(2) S. E. S. El Wakkad and T. M. Salem, *J. Phys. and Coll. Chem.*, **54**, 1371 (1950).

(3) A. R. Tourky and S. E. S. El Wakkad, *J. Chem. Soc.*, 740, 749 (1948).

(4) J. J. Singer, Jr., Ph.D. Dissertation, Clark University, 1949; J. H. Rosenbaum, Ph.D. Dissertation, Clark University, 1950.

Since aeration greatly accelerates the corrosion of nickel in acid media, oxygen was excluded from the half cells. Lengths of nickel wire of high purity were thoroughly cleaned and pretreated. The samples were freed from oxides and gases by induction heating first in hydrogen and then in a high vacuum. The solutions were freed from oxygen before making contact with the nickel samples. More details of the apparatus and the procedure will be given in a more extensive article. After each run the solutions were tested for nickel which might have gone into solution. The results were always negative, even in the acid phosphate solutions, unless oxygen had been admitted. The potentials were measured with a Leeds and Northrup K-2 potentiometer, using a Coleman electrometer as null point indicator.

Our graph of the nickel potentials in the phosphate buffers shows analogy with the curves obtained for copper[3] and mercury[2] in contact with a set of buffer solutions. In a more complete article we hope to discuss the interesting aspects both of an experimental and a theoretical nature.

Acknowledgment.—This work was supported by the Office of Naval Research to whom the authors express their appreciation.

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RECEIVED JANUARY 29, 1951

AN INTERMEDIATE IN THE CONVERSION OF FIBRINOGEN TO FIBRIN[1]

Sir:

When bovine fibrinogen and thrombin react in the presence of 0.4 *M* hexamethylene glycol (at *p*H 6.3, ionic strength 0.45), no clot is formed. However, the fibrinogen, whose sedimentation constant is about 9 *S*, appears to be gradually replaced by a new molecular species with a sedimentation constant of 25 *S*, which is evidently an intermediate polymerization product.[2] (All sedimentation constants given here are extrapolated to zero protein concentration.) We have now found very similar behavior with urea instead of glycol as the inhibitor. At *p*H 6.3 in 1.0 *M* urea, or at *p*H 7.5 in 2.35 *M* urea (ionic strength 0.15), sedimentation diagrams of a fibrinogen–thrombin system show, after 24 hours, two peaks; the sedimentation constant of one corresponds to that of unaltered fibrinogen, and the other is about 25 *S*.

Urea, unlike hexamethylene glycol, can in concentrated solution dissolve fibrin clots prepared in the absence of calcium and an unidentified serum factor.[3] A solution of fibrin in 3.5 *M* urea at *p*H 7.5, ionic strength 0.15, shows a single component in the ultracentrifuge with a sedimentation constant of 8 to 9 *S*; and its intrinsic viscosity is the same as that of fibrinogen, so that the fragments appear to

(1) This is paper 4 of a series on "The Formation of Fibrin and the Coagulation of Blood" from the University of Wisconsin, supported in part by research grants from the National Institutes of Health, Public Health Service. Grateful acknowledgment is made also of a grant from Eli Lilly and Company.

(2) S. Shulman and J. D. Ferry, *J. Phys. Coll. Chem.*, **55**, 135 (1951).

(3) E. Mihályi, *Acta Chem. Scand.*, **4**, 344 (1950); L. Lóránd, *Nature*, **166**, 694 (1950).

be very similar to fibrinogen molecules in size and shape. But when the urea concentration is reduced to 2.35 *M*, two peaks appear in the sedimentation diagram, with constants of 9 and 25 *S*.

The presence of the fast peak, both in partly polymerized fibrinogen inhibited by glycol or urea, and in depolymerized fibrin in urea, is always accompanied by a high viscosity which depends markedly on the rate of shear. The reduced specific viscosity falls rapidly with dilution of the protein, however, approaching that characteristic of the original fibrinogen, indicating dissociation of long linear aggregates.

The behavior of the intermediate represented by the fast peak is thus the same whether it is formed from fibrinogen by the action of thrombin or from urea-depolymerized fibrin by decreasing the concentration of urea.[4]

We are much indebted to Professor J. W. Williams for use of the Svedberg oil turbine ultracentrifuge.

(4) In general agreement with the experience of Mihályi,[5] there was no evidence, under the conditions of our experiments, of the denaturation which is observed[5] at somewhat higher urea concentrations or temperatures (or lower *p*H). Fibrinogen in 2.35 *M* urea had the same intrinsic viscosity as in the absence of urea; and the solubility of fibrinogen was not impaired by contact for 18 hours with 3.5 *M* urea, at *p*H 7.5, room temperature, and subsequent removal of the urea by dialysis. These criteria are of course not applicable to fibrin, but the susceptibilities of fibrinogen and fibrin to denaturation should be similar. Also, the viscosity of a fibrin solution in 3.5 *M* urea at *p*H 6.3 showed no change with time for two days, indicating that no progressive changes were taking place.

(5) E. Mihályi, *Acta Chem. Scand.*, **4**, 317 (1950).

DEPARTMENT OF CHEMISTRY
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RECEIVED FEBRUARY 12, 1951

DIETHYL CHLOROARSENITE AS A REAGENT FOR THE PREPARATION OF PEPTIDES

Sir:

In the course of an investigation in these Laboratories of methods of peptide synthesis, new reagents for forming the peptide linkage at either the amino or carboxylic function of an aminoacid or peptide chain have been found. The use of diethyl chlorophosphite has been reported recently.[1] Similarly, diethyl chloroarsenite[2] reacts readily with aminoacid esters and with N-substituted aminoacids to give highly reactive amides and anhydrides, respectively. The new reagent has advantages over the phosphite analog in being stable and readily prepared. Comparable yields are obtained with either reagent.

Both the amides, $(C_2H_5O)_2AsNHCH(R)COOR'$, and the anhydrides, $R'NHCH(R)COOAs(OC_2H_5)_2$, are non-distillable oils which are conveniently prepared and reacted without isolation. The reactions are accomplished in an inert solvent in the presence of an equivalent of triethylamine as the acid acceptor. After removal of the precipitated triethylamine hydrochloride, the solution of the intermediate diethylarsenite amide or anhydride is refluxed one hour with an equivalent of a second N-substituted aminoacid or aminoacid ester, respectively. The by-product, presumably diethyl arsenite in both cases, is precipitated quantitatively as arsenic trioxide by addition of water.

The N-substituted peptide ester prepared by either of these procedures is obtained crystalline by first extracting the reaction solution successively with dilute sodium bicarbonate and dilute hydrochloric acid and then concentrating in an air stream. One crystallization from ethanol–water or ethyl acetate–petroleum ether generally has given pure products.

Prepared by the intermediate amide method were carbobenzoxyglycine anilide[3] (79%), m.p. 144–145°; carbobenzoxyglycine morpholide[4] (70%), m.p. 144–145°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (59%), m.p. 91–92°; ethyl phthalyl-DL-alanyl-DL-valinate[4] (71%), m.p. 121–123°; and ethyl carbobenzoxyglycyl-L-tyrosinate (74%), m.p. 125–126° (a mixed m.p. with an authentic sample[6] was not depressed).

Prepared by the anhydride method were carbobenzoxyglycine anilide[3] (63%), m.p. 146–147°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (52%), m.p. 92–93°; ethyl carbobenzoxy-DL-alanyl-DL-phenylalanate[4] (60%), m.p. 104–106°; ethyl carbobenzoxy-L-leucyl-DL-phenylalanate[4] (74%), m.p. *ca.* 90° $[\alpha]^{24}D$ −9.2° (*c* = 5, 95% ethanol) and ethyl carbobenzoxyglycylglycyl-DL-phenylalanate monohydrate[4] (30%), m.p. 80–82° (from ethyl phenylalanate and the diethyl arsenite anhydride from carbobenzoxyglycylglycine).

(1) Anderson, Welcher and Young, THIS JOURNAL, **73**, 501 (1951).
(2) McKenzie and Wood, *J. Chem. Soc.*, **117**, 406 (1920).
(3) Wieland and Sehring, *Ann.*, **569**, 122 (1950).
(4) Carbon, hydrogen and nitrogen analyses were satisfactory.
(5) Neurath, *et al.*, *J. Biol. Chem.*, **170**, 222 (1947).
(6) Bergmann and Fruton, *ibid.*, **118**, 412 (1937).

CHEMOTHERAPY DIVISION
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RECEIVED FEBRUARY 7, 1951

CRYSTALLINE XYLOBIOSE AND XYLOTRIOSE

Sir:

Charcoal chromatography of partially hydrolyzed xylan permits the separation and isolation of a considerable amount of crystalline xylobiose and xylotriose. This is the first isolation of crystalline di- and trisaccharides composed only of pentose sugar units.

In one instance a 2% solution of xylan in 42% hydrochloric acid was hydrolyzed at 0° until the reaction was 66% complete as indicated by reducing value and by optical rotation. The hydrolyzate was neutralized with sodium bicarbonate and chromatographically separated on charcoal columns following the method of Whistler and Durso.[1] After washing the column with water, xylobiose was removed with 5% ethanol. The sirupy concentrate from this extraction was dissolved in a small amount of warm water and hot methanol added. On cooling, crystallization occurred. The yield was 4.8% of the xylan used, m.p. 186–187°; $[\alpha]D^{25}$ −32.0→−25.5 (1 hour) (*c*, 1 in water).

(1) Roy L. Whistler and Donald F. Durso, THIS JOURNAL, **72**, 677 (1950).

Anal. Calcd. for $C_{10}H_{18}O_9$: C, 42.53; H, 6.42. Found: C, 42.5; H, 6.4.

Iodimetric titration gave the expected equivalent value for a disaccharide and on hydrolysis the final reducing value and specific rotation were those calculated for D-xylose. On acetylation of the disaccharide, there was produced the same crystalline hexaacetyl xylobiose as reported by Bachrach and Whistler.[2] Thus, the structure of the disaccharide is 4-(β-D-xylopyranosyl)-β-D-xylopyranose.

In another instance a 2% xylan solution in 42% hydrochloric acid solution was hydrolyzed to 50% of completion. It was neutralized and chromatographically separated as before. After washing the column with water and 5% ethanol, a trisaccharide fraction was removed with 9% ethanol. The concentrated sirup was dissolved in a small amount of warm water and hot absolute ethanol was added to produce a solution of 80–85% alcohol concentration. Upon cooling, crystallization occurred. The yield was 8.0% of the original xylan; m.p. 205–206°; $[\alpha]_D^{25}$ 46.96 (1.06% in water).

Anal. Calcd. for $C_{15}H_{26}O_{13}$: C, 43.48; H, 6.33. Found: C, 43.4; H, 6.4.

Iodimetric titration gave the expected value for a trisaccharide and on hydrolysis the reducing value and specific rotation were those calculated for D-xylose.

(2) J. Bachrach and R. L. Whistler, paper presented before the Division of Sugar Chemistry, 116th meeting of American Chemical Society, Atlantic City, 1949.

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RECEIVED FEBRUARY 3, 1951

ADRENAL CONVERSION OF C^{14} LABELED CHOLESTEROL AND ACETATE TO ADRENAL CORTICAL HORMONES[1]

Sir:

It previously has been demonstrated that beef adrenals, perfused with blood containing added ACTH, synthesize and release into the perfusion medium a mixture of corticosteroids, the principal components of which are 17-hydroxycorticosterone (I) and corticosterone (II).[2,3] We wish to report that when similar experiments are carried out in the presence of either C^{14} labeled acetate or cholesterol, the I and II isolated from adrenal perfusates are radioactive, and have approximately the same number of counts per mg. per min. (c.m.m.) as determined under identical conditions.

Groups of 5 glands were perfused in parallel from a manifold with 1 liter of homologous citrated blood containing 25 mg. of ACTH (Armour) for four hours, the perfusate being recycled through the glands. The corticosteroids were extracted from perfusates with activated carbon (Darco G-60),[4] and fractionated by paper partition chromatography. The compounds were characterized by the method of mixed chromatograms,[5] both of the free steroids and of their esters, and by measuring the chromogen produced by H_2SO_4.[6] All counts were made using a thin-window Geiger counter with 0.1–0.7 mg. samples (diluted with non-isotopic compound when necessary) plated as a thin film. Ten milligrams of carboxyl labeled sodium acetate having radioactivity of 5.8×10^6 c.m.m. was added to the medium at the initiation of the perfusion. I and II were isolated in **3.0** and **1.0** mg. amounts, and had activity of 319 and 305 c.m.m. respectively, in one experiment; in a second similar experiment, the c.m.m. for each were 219 and 208 and the total amounts isolated were **4.5** and **1.5** mg. for I and II, respectively. The counts were made with 0.09 to 0.13 mg. samples; with our technique the c.m.m. of I or II remains constant in the range 0.09 to 0.7 mg.

A similar perfusion of cholesterol labeled in position 3 with C^{14} prepared from radio-cholestenone[7] by Drs. Schwenk, Gut and Belisle[8] was conducted in which 90 mg. of radiocholesterol (300 c.m.m.) was used. I and II were isolated in **1.0** and **0.4** mg. amounts and had activities of 25 and 18 c.m.m., respectively.

The data of Table I indicate that the radioactivity is a property of the compounds isolated since (a) rechromatography on paper and (b) the preparation of two derivatives led to no significant alteration of the specific activity. It is recognized that the method of mixed chromatograms of the free compounds and their esters plus determination of the H_2SO_4 chromogen does not constitute a classical characterization of I and II. In our experience, however, no substance proved to be homogeneous by this method, has failed to meet classical criteria of purity and composition.

TABLE I

The specific activities of cortical hormones and their derivatives isolated from an adrenal perfusion experiment with $CH_3C^{14}OONa$.

		mg.	c.m.m.
1	Free 17-hydroxycorticosterone (I)[a]	0.125	319
2	I after rechromatography	.115	340
3	I acetate[b]	.130	362
4	I propionate[b]	.098	332
1	Free corticosterone (II)[a]	.130	305
2	II after rechromatography[a]	.090	326
3	II acetate[b]	.123	294
4	II propionate[b]	.110	342

[a] Isolated from paper following partition chromatography using the propylene glycol–toluene system. [b] Isolated from paper following partition chromatography using the formamide–benzene system. The samples were then rechromatographed on paper.

These data indicate that both acetate and cholesterol can be transformed by the isolated adrenal

(1) Aided by United States Public Health Service Grant GG-2742 and G. D. Searle and Company.

(2) Hechter, Zaffaroni, Jacobsen, Levy, Jeanloz, Schenker and Pincus, *Recent Progress in Hormone Research*, in press.

(3) Pincus, Hechter and Zaffaroni, *2nd Clin. ACTH Conf.*, The Blakiston Co., Philadelphia, Pa., 1951, in press.

(4) Hechter, Jacobsen, Jeanloz, Levy, Marshall, Pincus and Schenker, *Arch. Biochem.*, **25**, 477 (1950).

(5) Zaffaroni, Burton and Keutman, *Science*, **111**, 6 (1950).

(6) Zaffaroni, THIS JOURNAL, **72**, 3828 (1950).

(7) Turner, THIS JOURNAL, **69**, 726 (1947).

(8) Schwenk, Gut and Belisle, in press.

gland into adrenocortical steroids. While cholesterol appears to be a more efficient precursor than acetate, further experiments are necessary to accurately define the percentage conversion with both precursors. Since in adrenal slices cholesterol has been shown to arise from acetate condensation[9] it is not inconceivable that cholesterol may be an intermediary in the reactions leading to corticosteroid synthesis from acetate. We hope in further studies to determine whether cholesterol is an obligatory intermediate in steroidogenesis.

(9) Srere, Chaikoff and Dauben, *J. Biol. Chem.*, **176**, 829 (1948).

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RECEIVED JANUARY 29, 1951

BOOK REVIEWS

Principles of Ionic Organic Reactions. By ELLIOT R. ALEXANDER, Assistant Professor of Chemistry in the University of Illinois. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1950. viii + 318 pp. 15.5 × 23.5 cm. Price, $5.50.

This book applies ionic principles in presenting the mechanisms of those organic reactions where the ionic concept has been established or appears very likely. The treatment is entirely from the point of view of the organic chemist and actual kinetic data and mathematics are omitted. The author has not merely presented reaction explanation but has also presented the important data leading to the validity of the ionic mechanisms. The documentation is adequate but not exhaustive.

The book will serve admirably as a primary textbook for an advanced course in organic reaction mechanisms to follow a more orthodox advanced chemistry course, or as a supplementary book for a comprehensive advanced organic course. The research organic chemist whose formal schooling was completed say ten years ago will find this an indispensable means of mastering the newer concepts.

The style and treatment are, for the most part, quite clear although more explanation of the symbolism employed might be suggested. More detail would be helpful in explaining the actual physical nature of carbonium ions and the sequence of events leading up to their transitory existence. Conspicuous omissions include ionic oxidation reactions and the application of the transition state concept to ionic processes.

The complete objectivity of the book impresses this reviewer. Although Dr. Alexander is a firm believer in ions he accepts them, applies them, and submits the evidence without any apparent attempt to crusade for them. This is a healthy attitude in a book of this type; one regrets that the same approach has not yet been used in free radical books. The book is excellent and highly recommended.

DEPARTMENT OF CHEMISTRY
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Technique of Organic Chemistry. Volume I, Part II. **Physical Methods of Organic Chemistry.** Edited by ARNOLD WEISSBERGER. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N. Y. 1949. xi + 1024 pp. 15.5 × 23 cm. Price, $12.50.

The chapter headings, and the authors, are as follows: X-Ray Diffraction, by I. Fankuchen; Electron Diffraction, by L. O. Brockway; Refractometry, by N. Bauer and K. Fajans; Spectroscopy and Spectrophotometry by W. West; Colorimetry, Photometric Analysis, Fluorimetry and Turbidimetry, by W. West; Polarimetry, by W. Heller; Determination of Dipole Moments, by C. P. Smyth; Conductometry, by T. Shedlovsky; Electrophoresis, by D. H. Moore; Potentiometry, by L. Michaelis; Polarography, by O. H. Müller; Determination of Magnetic Susceptibility, by L. Michaelis; Determination of Radioactivity, by W. F. Bale and J. F. Bonner, Jr.; Mass Spectrometry, by D. W. Stewart.

This book is necessarily of encyclopedic proportions and character, and like other encyclopedias it must suffer from obsolescence setting in before the ink is dry. Indeed, if a book of this nature is to be any good, its publication must accelerate its own obsolescence, for if the authors are conscientious in pointing out the pitfalls and the weaknesses of existing procedures and instruments this must in turn stimulate new advances. By this criterion the book is not a very good one. On the whole it shows little dissatisfaction with present-day instrumentation.

The authors have been faced with the necessity of compromising between theory and experimental techniques; for the most part they have elected to present an adequate treatment of fundamental principles at the expense of detailed experimental procedures. The book is thus in no sense a working manual but it is a comprehensive and authoritative reference source of material not otherwise readily available to the analyst.

If any one chapter excels in excellence and thoroughness, the reviewer would choose the discussion of polarimetry by W. Heller. Granted the advantage of dealing with a quiescent, maturely developed field, this presentation is worthy of special comment for the abundant illustrations, the preciseness of its formulation, and for the wealth of information it contains.

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CHICAGO, ILLINOIS

Fortschritte der Alkaloidchemie Seit 1933. Scientia Chimica, Band 2. By HANS-G. BOIT, Chemisches Institute der Universität, Berlin. Akademie-Verlag G. m. b. H., Presseabteilung, Schiffbauerdamm 19, Berlin NW 7, Germany. 1950. xxxii + 425 pp. 18 × 25 cm. Price, paper, 49 DM, bound, 53 DM.

A progress report is one of the devices which a busy worker in one field can use to keep abreast with advances in another field. The present monograph serves this purpose excellently. It does not present the extensive background in alkaloid chemistry which preceded 1933, the year chosen for the start of the report. It does attempt to supplement some of the excellent reviews available at that time, such as Winterstein-Trier, with a broad account of the work which has been completed in the intervening years. In spite of this, the book is self-contained. The author follows the practice of giving sufficient information in each discussion to pick up the thread of the argument.

The major portion of the book is devoted to a discussion of the chemistry of alkaloids whose structures are either completely elucidated or tentatively assigned. These are