(and working up with barium hydroxide to remove ammonia in the usual manner), but the crude amino acid melted at 151–164°. When recrystallized from water to constant melting point, 166–168°, the yield was 10.4 g. of amino acid from one mole of acetamidocyanoacetic ester. This is essentially the same over-all yield as reported above. No attempt was made to isolate the second DL-stereoisomer which is presumably present.

5-Carboxy-3-methyl-2-pyrrolidone.—The pyrrolidone was obtained in an attempt to prepare the hydantoin. A solution of 10.4 g. of 4-methylglutamic acid in 100 ml. of water was brought to *p*H 7 with sodium hydroxide and heated with 7.1 g. of potassium cyanate on the steam-bath for one hour. It was then acidified with hydrochloric acid and heated for three hours more. The solvent was removed *in vacuo* and the residue extracted with hot alcohol. The product, recrystallized from water, melted at 173°.

Anal. Calcd. for $C_6H_9NO_3$: C, 50.43; H, 6.34; N, 9.79. Found: C, 49.92; H, 6.23; N, 9.53.

STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, N. Y. JEANNE L. FILLMAN
NOEL F. ALBERTSON

RECEIVED APRIL 29, 1952

COMMUNICATIONS TO THE EDITOR

A NEW PROCEDURE FOR THE DETERMINATION OF THE FINE STRUCTURE OF POLYSACCHARIDES

Sir:

In a previous communication[1] it was reported that the dialdehydes obtained from simple glycosides by periodate oxidation could readily be reduced to the corresponding alcohols in almost quantitative yield.

We wish to report here that periodate oxidation followed by reduction with either hydrogen and a Raney nickel catalyst between 60 and 100° under pressure or with sodium borohydride in aqueous solution represents a general procedure which can also be applied to polysaccharides. Now, whereas the periodate-oxidized polysaccharides or "polyaldehydes" usually undergo profound decomposition when hydrolyzed even in the cold, the corresponding new "polyalcohols" can be subjected to hydrolysis with boiling dilute mineral acid with little or no decomposition to give cleavage products which can be separated by partition chromatography and determined quantitatively. Results obtained in this manner provide information concerning the nature and amount of glycosidic linkages in a polysaccharide.

Glucose residues linked so that free OH groups are present at C_2 and C_3 will give rise to erythritol and glycolic aldehyde when subjected to periodate oxidation followed by reduction and hydrolysis; this applies to residues linked through positions 1 and 4 or 1, 4, and 6. Glucose residues with free hydroxyl groups at C_3 and C_4 such as those in terminal positions and those joined through positions 1 and 6 or 1, 2 and 6 will provide glycerol instead of erythritol. However, any glucose residue linked so that no pair of adjacent hydroxyl groups is present will not be affected by periodate oxidation and will therefore appear as free glucose after the final hydrolysis step. Similar considerations, which are clearly not restricted to polyglucosans, will also apply to polysaccharides composed wholly or in part of furanose residues.

The deductions that can be made from the results of an examination of the polyalcohol produced from a given polysaccharide do not necessarily permit a clear cut solution to a structural problem but taken in conjunction with other experimental results such as, for example, those of methylation it is feasible to restrict greatly the number of structural possibilities.

The few typical examples given below will serve to illustrate the usefulness of the proposed new procedure. In the case of the branched chain polysaccharides amylopectin and glycogen which are composed of glucopyranose residues joined by 1,4 bonds and have branches at certain C_6 positions, the non-reducing terminal unit will give rise to glycerol while the glucose units of the main chain joined through positions 1 and 4 and those at which branching occurs with linkages at positions 1, 4 and 6 will all give erythritol. Hence the molecular ratio of glycerol to erythritol, as determined by the chromotropic acid procedure,[2] should equal the molecular ratio of terminal to non-terminal glucose residues. For glycogen a ratio of 1:10 has been found for the glycerol/erythritol ratio. This is in good agreement with the value of 1:11 for the molecular ratio of tetramethyl- to the sum of the trimethyl- and dimethyl-glucose components derived from methylation studies. The result is also in good agreement with the figure of 1:11 for the ratio of terminal to non-terminal residues as determined from the amount of formic acid liberated by periodate oxidation of glycogen itself.[3] Similar correlations have been obtained with amylopectin.

In a polysaccharide composed of hexopyranose residues joined by 1,6 and 1,4 linkages the ratio of the number of these two types of linkages should correspond to the mole ratio of the glycerol to the erythritol obtained from the corresponding polyalcohol by hydrolysis. By application of the new procedure reported herein to one type of dextran,[4] produced by *Leuconostoc mesenteroides* NRRL-B-512, followed by chromatographic separation and determination of glycerol and erythritol,[2] the ratio of 1,6 to 1,4 linkages has been found to be approximately 45:1.

Paper partition chromatographic investigation of

(1) Bertha Lewis, R. Montgomery, F. Smith and J. Van Cleve, 121st A.C.S. Meeting, Milwaukee, Wisconsin, April, 1952.

(2) Marguerite Lambert and A. C. Neish, *Can. J. Res.*, **B28**, 83 (1950).

(3) M. Abdel-Akher and F. Smith, THIS JOURNAL, **73**, 994 (1951).

(4) The authors thank Dr. Allene Jeanes of the Northern Regional Research Laboratory, Peoria, for the sample of dextran.

the hydrolysis fragments of the above dextran polyalcohol revealed also 2–2.5% glucose as determined by the phenol–sulfuric acid method.[5] This is believed to be derived from glucose residues which are immune to periodate oxidation as a consequence of being linked through positions 1 and 3, or 1, 2, and 4.

In an analogous manner it has been shown that glycogen and amylopectin contain about 1.0 and 0.5% glucose, respectively, which is immune to prolonged treatment with sodium periodate. This glucose, which still remains intact even when the derived polyalcohols themselves are treated with sodium periodate, could arise because of incomplete oxidation or because of fixed *trans* OH groups arising from stereochemical strain[6] but the present evidence suggests that it arises from glucose residues in the polysaccharides linked by 1, 3 bonds. While it is probable that these same glucose residues correspond to those which give rise to the 2,6-dimethyl-D-glucose fragment produced from the methylated polysaccharides by hydrolysis, the possibility exists that they correspond to glucose residues joined through positions 2 and 4.

In similar experiments on amylose and cellulose the indications are that these two polysaccharides contain approximately 0.2 to 0.5 and 0.1 to 0.2% glucose, respectively, which is immune to periodate oxidation. It is believed, therefore, that the possibility of a hitherto unrecognized linkage in these polysaccharides is worthy of some consideration. The details and constitutional significance of these and similar experiments on other polysaccharides such as fructosans, hemicelluloses, fungus glucosans, plant gums and degraded plant gums will be published later.

(5) M. Dubois, K. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Nature*, **168** 167 (1951).

(6) B. H. Alexander, R. J. Dimler and C. L. Mehltretter, THIS JOURNAL, **73**, 4658 (1951).

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RECEIVED AUGUST 4, 1952

X-RAY EXAMINATION OF IRON BISCYCLOPENTADIENYL

Sirs:

Crystals of iron biscyclopentadienyl, prepared by Dr. E. O. Brimm of Linde Air Products Co., and forwarded to us by Professor W. C. Fernelius, were examined by X-ray methods at the suggestion of the latter. Rotation and Weissenberg photographs, using MoKα radiation, revealed a monoclinic cell, space group $P2_1/n$, with $a = 9.00$ Å; $b = 7.52$ Å., $c = 5.94$ Å., $\beta = 92.5°$. The measured density of 1.516 g./cc. showed 2 molecules per cell, and thus required the two iron atoms to be at the cell corner and body center. The cell symmetry requires that the molecule is centrosymmetric, with the iron atom at its center.

The molecular structure was determined automatically on X-RAC, the electronic computer for X-ray analysis,[1] by use of the non-negativity criterion, as previously utilized by us on the structure of fructose[2] and in several other analyses.[3] The iron atoms contribute positive phases to $(h,k,0)$ terms with $h + k = 2n$. All such structure factors were inserted into X-RAC with positive phases, and the strongest term with $h + k$ odd was also entered as positive. The effects of phase permutation of the remaining odd terms were examined consecutively and in order of decreasing amplitude, and phases were assigned so as to minimize negative excursions of the density function. A projection on the (x,y) plane concomitant with the "sandwich" structures proposed by Wilkinson, Rosenblum, Whiting and Woodward[4] and Woodward, Rosenblum and Whiting[5] immediately appeared. The density projection did not indicate that the cyclopentadiene groups were rotating, and the center of symmetry then demanded the anti-prismatic structure of Wilkinson, *et al.*[4]

A correct form factor for iron as it occurs here is not known, and thus a refinement of carbon positions has not yet been possible. Using an empirical Fe^{++} curve with an approximate temperature factor, an R-factor of 0.17 was found for a planar carbon ring with C–C distances of 1.41 Å. and Fe–C distance of 2.0 Å. A three-dimensional analysis is in progress, to establish the nature of the bonding and the electronic configuration of the iron atom.

We are grateful to Dr. Brimm and Prof. Fernelius for suggesting the problem and supplying the crystalline material.

(1) R. Pepinsky, *J. Appl. Phys.*, **18**, 601 (1947).

(2) P. F. Eiland and R. Pepinsky, *Acta Cryst.*, **3**, 160 (1950).

(3) X-RAC Computations supported by Office of Naval Research.

(4) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2126 (1952).

(5) R. B. Woodward, M. Rosenblum and M. C. Whiting, *ibid.*, **74**, 3458 (1952).

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PHILIP FRANK EILAND
RAY PEPINSKY

RECEIVED AUGUST 12, 1952

CHROMATOGRAPHIC SEPARATION OF THE ADRENOCORTICOTROPIC HORMONE ON PARTITION COLUMNS[1]

Sir:

Fractionation of pig and sheep pituitary extracts on oxycellulose columns[2] has yielded materials with ascorbic acid depleting activities up to 100 u./mg.[3] After *peptic* digestion of such fractions of porcine origin, Brink, *et al.*,[4] applied the countercurrent distribution technique in the isolation of an apparently homogeneous material with an activity of 300 u./mg.

(1) This work was aided in part by grants to Professor C. H. Li from the National Institutes of Health, the United States Public Health Service, the Armour Laboratories, Merck and Company, Inc., and the Eli Lilly Laboratories.

(2) (a) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951); (b) C. H. Li, *ibid.*, **74**, 2124 (1952).

(3) Assays reported here were performed by the adrenal ascorbic acid depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948). Results are expressed in U. S. P. units per milligram.

(4) N. G. Brink, F. A. Kuehl, J. W. Richter, A. W. Bazemore, M. A. P. Meisinger, D. E. Ayer and K. Folkers, THIS JOURNAL, **74**, 2120 (1952).

We wish to report the use of partition columns[5] in the isolation of sheep hormone preparations of high biological activities (200 to 400 u./mg.) *without prior peptic digestion.*

Three to seven mg. of material purified by the oxycellulose method[2b] and containing 30 to 40 u./mg. were placed on a column 1.2 cm. in diameter containing 12 g. of kieselguhr[6] and 10 ml. of 0.2 *N* HCl saturated with isobutyric acid[7] as the stationary phase. The moving phase was composed of isobutyric acid saturated with 0.2 *N* HCl. The column was run at a constant temperature of 24° and at a flow rate not greater than 6 ml. per hour. After the fraction containing the biological activity had emerged, the remainder of the material was eluted with 6 *N* HCl.

The results of a typical experiment in which material containing 0.820 mg. of nitrogen was placed on the column are shown in Fig. 1 (open circles). The amount of substance in each tube was determined by the method of Lowry, *et al.*[8] As judged by this colorimetric procedure, the starting material was recovered completely from the column. Fraction I (11% of the nitrogen and 5% of the activity) started to emerge from the column after the first 17 ml. and was contained in the following 14 ml. The material in the next 17 ml., Fraction II, contained 10% of the nitrogen and 95% of the activity. The material eluted by 6 *N* HCl, Fraction III, contained 74% of the nitrogen and no activity.

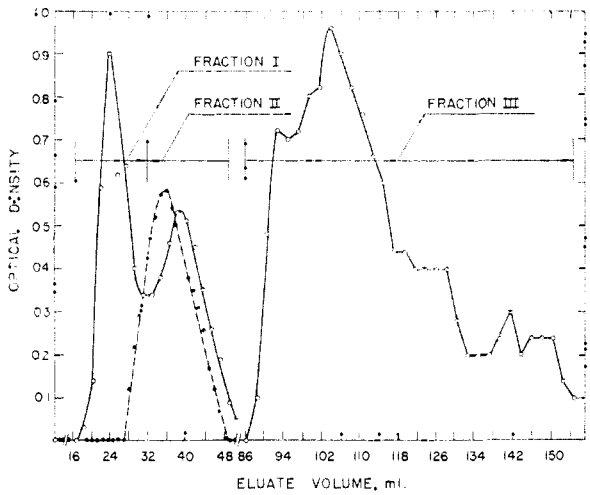

Fig. 1.—Chromatogram of oxycellulose purified sheep ACTH preparation (open circles); rechromatogram of Fraction II (closed circles).

In many experiments on different preparations of starting material similar results were obtained. The material in Fraction II had approximately the same *R* value[5a] (0.60 to 0.70) from experiment to experiment and represented on a nitrogen basis an 8- to 12-fold purification of the starting material. This purification was also reflected in the biological assays in which starting materials with assay values of 30 to 40 u./mg. gave Fractions II with 200 to 400μ./mg.[9] When 1.1 mg. of the solids of Fraction II were rechromatogramed, Fig. 1 (solid circles), a symmetrical peak containing virtually all of the material (96%) emerged close to the expected position (*R* value of 0.72). Thus the material comprising Fraction II showed no gross inhomogeneity by the criterion of partition chromatography which was applied.

The authors are greatly indebted to Professor C. H. Li for the starting materials and for furnishing the facilities for the biological assays reported in this paper.

(9) Based on weight as determined by the colorimetric procedure.[8]

DEPARTMENT OF BIOCHEMISTRY
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RECEIVED SEPTEMBER 2, 1952

INCORPORATION OF LABELED CARBON DIOXIDE INTO PYRUVATE AND α-KETOGLUTARATE[1]

Sir:

The pyruvic oxidase of pigeon breast muscle[2] catalyzes the incorporation of $C^{14}O_2$ into pyruvate (Table I). No addition of cofactors is required for this reaction but with added cocarboxylase the rate of incorporation is increased 3 to 4 fold. However, without added Mg^{++} or cocarboxylase there is no detectable decarboxylation of pyruvate to acetoin. Similarly the enzymatic oxidation of pyruvate by ferricyanide requires Mg^{++} and cocarboxylase.[3] Thus the only activity exhibited by pyruvic oxidase without any additions is the incorporation of CO_2, presumably by an exchange reaction as follows

$$\text{Pyruvate} \rightleftharpoons (\text{acetaldehyde-enzyme}) + CO_2$$

The only known cofactor present in pyruvic oxidase is protogen or thioctic acid[3] which according to Gunsalus, *et al.*,[4] is part of a more complex coenzyme tentatively identified by Reed, *et al.*,[5] as lipoicyl-thiamine pyrophosphate.

TABLE I

INCORPORATION OF LABELED CARBON DIOXIDE INTO PYRUVATE

The components of the system were pyruvate (35 μmoles), $KHC^{14}O_3$ (2.1×10^5 cts./min.) and pyruvic oxidase (250 units) in a total volume of 1.3 ml. at *p*H 7.0; incubated in nitrogen at 37°.

Time of incubation, minutes	Cts./min./μ mole
0	0
30	20
60	63
120	150

A similar equilibration of $C^{14}O_2$ with α-ketoglutarate is catalyzed by the α-ketoglutaric oxidase

(1) Supported by a grant from the American Heart Association.

(2) V. Jagannathan and R. S. Schweet, *J. Biol. Chem.*, **196**, 551 (1952).

(3) R. S. Schweet and K. Cheslock, *ibid.*, in press.

(4) I. C. Gunsalus, L. Stuglia and D. J. O'Kane, *ibid.*, **194**, 859 (1952).

(5) L. J. Reed and B. G. DeBusk, THIS JOURNAL, **74**, 3964 (1952).

(5) (a) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941); (b) A. J. P. Martin and R. R. Porter, *ibid.*, **49**, 215 (1952).

(6) Hyflo Super-cel, Johns-Manville Company.

(7) F. H. Carpenter, G. P. Hess and C. H. Li, *J. Biol. Chem.*, **197**, 7 (1952).

(8) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. S. Randall, *ibid.*, **193**, 265 (1951).

of pig heart[6] (Table II). Cysteine used in this experiment was later found to be unnecessary. This enzyme contains bound protogen and cocarboxylase. Addition of cocarboxylase, or of diphosphopyridine nucleotide (DPN), coenzyme A (CoA) and cysteine did not change the rate of incorporation. C^{14} labeled formate and succinate were not incorporated with or without CoA and DPN.

TABLE II

INCORPORATION OF LABELED CARBON DIOXIDE INTO α-KETOGLUTARATE

The components of the system were α-ketoglutarate (10 μmoles), cysteine (10 μmoles), $NaHC^{14}O_3$ (1.8×10^5 cts./min.) and α-ketoglutaric oxidase in a total volume of 0.5 ml. at *p*H 7.0; incubated at 37° for 15 min.

Oxidase units	Cts./min./μ mole
5	15
13	40
25	86
50	198
75	256
75 (boiled)	0

The keto acids were purified for counting by (a) partition chromatography of the 2,4-dinitrophenylhydrazones on silica gel column[7] and (b) recrystallization of the 2,4-dinitrophenylhydrazones (with carrier) to constant specific activity. Both methods showed the same specific activity.

(6) D. R. Sanadi and J. W. Littlefield, XIIth International Congress of Pure and Applied Chemistry, New York, Sept., 1951; *J. Biol. Chem.*, in press.

(7) D. O. Brummond, "The oxidation of organic acids by mitochondria from plants," M.S. Thesis, University of Wisconsin, 1952.

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RECEIVED AUGUST 7, 1952

THE CONFIGURATION OF UNDISSOCIATED SULFAMIC ACID

Sir:

In a recent paper on the ionization constant of sulfamic acid,[1] the statement is made that "Although the acid occurs in its crystals as a dipolar ion, $^+NH_3SO_3^-$, the un-ionized acid in aqueous solution is largely in the form of the neutral molecule, NH_2SO_3H." As a basis for this statement, reference is made to a paper by Baumgarten.[2] This same concept of the configuration of undissociated sulfamic acid in aqueous solution is expressed by Kanda and King,[3] apparently on the same basis.

It is the purpose of this communication to point out (1) that Baumgarten's paper gives no evidence for this configuration (in fact, the statement made by Baumgarten which has been interpreted[4] as claiming the normal configuration for the undissociated molecules probably was not meant to imply this at all) and further, (2) that there appears to be no compelling reason to expect the undissociated molecules in aqueous solution to be other than the dipolar ion form, which clearly is the configuration in the crystals,[3] although it is not at present possible to determine unambiguously the configuration in solution.

In order to determine, in the absence of direct molecular structure evidence, which of the two configurations is correct, one can only attempt to decide which would be the weaker acid. Then, since the ion produced by either configuration is the same, the addition of hydrogen ions to this sulfamate ion to form the undissociated molecules must necessarily form this more weakly acidic configuration.

Unfortunately, it does not seem possible to calculate expected acid strengths for either configuration with sufficient accuracy to permit an unequivocal answer. Using the semi-empirical method of Branch and Calvin,[5] which is known to be not too satisfactory for acids of this strength, one may estimate for the dipolar ion form $pK(^+NH_3SO_3^-) = pK(NH_4^+) + \log 4/3 + \{pK(HOSO_3^-) - pK(HOH) + \log 2 \times 4\} = 9.3 + 0.1 + \{1.7 - 16.0 + 0.09\} = -4.0$, or from the inductive constants given by Branch and Calvin, $pK(^+NH_3SO_3^-) = pK(NH_4) - I_s - 2I_+ - (3/2.8)I_0 - (3/2.8)I_- + \log 4/3 = 9.3 - 3.4 - 2(12.3) - (3/2.8)(4) - (3/2.8)(-12.3) + 0.1 = -9.7$. Similarly for the neutral molecule form $pK(NH_2SO_3H) = pK(H_2O) - I_s - 2I_+ - (2/2.8)I_0 - (2/2.8)I_- - (1/2.8)I_N + \log 3 = 16.0 - 3.4 - 2(12.3) - (2/2.8)(4) - (2/2.8)(-12.3) - (1/2.8)(1.3) + 0.5 = -6.2$ or $pK(NH_2SO_3H) = pK(HSO_4^-) + \log 3/4 + (1/2.8)\cdot I_0 + (1/2.8)I_- - (1/2.8)I_N = 1.7 - 0.1 + (1/2.8)(4) + (1/2.8)(-12.3) - (1/2.8)(1.3) = -1.9$. While these values appear to be slightly in favor of the neutral molecule form, it is obvious that the difference is not sufficient to make any real decision possible. The fact that the S–N bond distance in the sulfamate ion is 1.60 Å.,[6] indicating considerable double bond character, is in the direction to hinder the attachment of the proton to the nitrogen atom, again does not compel the assumption of the neutral molecule form for the acid.

Thus, since there seems to be no evidence indicating that the dipolar ion form is any less likely than the neutral form, and since it clearly exists in this form in the solid, it appears most reasonable to assume that the dipolar ion form is the configuration in aqueous solution, until more definite evidence is forthcoming. It should be pointed out that it is, of course, quite possible that both forms are present in equilibrium.

If this is the case, then the equilibrium with the intermediate, X, postulated by Maron and Berens[7] in their discussion of the kinetics of the hydrolysis of sulfamic acid, is merely the dissociation equilibrium. It is interesting to note that the values of ΔH and ΔS given by King and King[1] for the dissociation equilibrium, when extrapolated to 90°, are of the correct sign required for Maron and Berens' explanation of their data. This is certainly not any argument in favor of the dipolar ion form over the neutral molecule form, but does show that there is no difficulty in this interpreta-

(1) E. J. King and G. W. King, THIS JOURNAL, **74**, 1212 (1952).
(2) P. Baumgarten, *Ber.*, **62B**, 820 (1929).
(3) F. A. Kanda and A. J. King, THIS JOURNAL, **73**, 2315 (1951).
(4) *Cf. C. A.*, **23**, 5159 (1929).
(5) G. E. K. Branch and M. Calvin, "Theory of Organic Chemistry," Prentice-Hall, New York, N. Y., 1941, p. 201.
(6) G. A. Jeffrey and H. P. Stadler, *J. Chem. Soc.*, 1467 (1951).
(7) S. H. Maron and A. R. Berens, THIS JOURNAL, **72**, 3571 (1950).

tion. It should be further pointed out that Baumgarten[2] suggested that the hydrolysis probably proceeds by way of the dipolar ion.

DEPARTMENT OF CHEMISTRY E. C. LINGAFELTER
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RECEIVED APRIL 7, 1952

EVIDENCE OF NEW LINKAGES IN DEXTRANS

Sir:

We have been able to demonstrate that a significant fraction of the anhydroglucose units in a certain dextran apparently is not attacked by sodium metaperiodate at 25°. Previous investigations[1] of several dextrans showed that substantially all the units were attacked. Methylation studies[2] on dextrans so far investigated indicate that the principal glucosidic linkage is 1,6′, and that, in some cases, 1,4′-linkages are also present. Units at branch points carry linkages on both the 4- and 6-positions. Our results strongly suggest that this dextran contains units linked in the 3-positions, or both the 2- and 4-positions (branch points), or a combination of these possibilities.

This dextran, produced by *Leuconostoc mesenteroides* NRRL B-742, and purified by precipitation between 41 per cent. and 90 per cent. ethyl alcohol, consumed 1.43 moles of periodate and produced 0.64 mole of formic acid per anhydroglucose unit when oxidized at 25°[1] for 250 hours, at which time the consumption of oxidant and production of acid had ceased. Sixty-four per cent. of the glucopyranosyl units are therefore substituted only on the 6-position. Two moles of periodate are consumed by each unit so linked. The percentage of anhydroglucopyranose units consuming only one mole of periodate is then 15% [1.43 − (2 × 0.64)]. These are probably linked on the 4- and 6-positions. According to these calculations, the remaining 21 per cent. of the anhydroglucose units are not oxidized.

To confirm the presence of unoxidized units, a method developed by Smith[3] and his associates at the University of Minnesota has been applied. After removal of salts, the oxidized polymer was catalytically reduced and then hydrolyzed in 2 *N* sulfuric acid on the steam-bath. The only optically active products expected from a polyanhydroglucopyranose treated as above are D-glyceraldehyde, from 2- or 2- and 6-linked units, and D-glucose, from unoxidized units. The optical activity of the hydrolysate, if assumed to be due entirely to glucose, corresponded to 11.7% of unoxidized anhydroglucose units in the original dextran. Catalytic reduction of the neutralized hydrolysate yielded a solution having a small negative optical rotation in good agreement with that expected from the conversion of glucose to sorbitol. Sorbitol was isolated as the pyridine complex[4] and characterized as the hexaacetate, m.p. and mixed m.p., 98–99°; $[\alpha]^{25}D + 10.0°$ (*c*, 3.8; $CHCl_3$). The yield of the hexaacetate corresponded to 5.8% unoxidized anhydroglucopyranose in the original dextran.

The simplest explanation for the lack of oxidation by periodate is the presence of 1,3′-glucosidic linkages. Linkage in the 3-position, regardless of other linkages on the same anhydroglucopyranosyl unit, would prevent oxidation. Oxidation wuld be prevented also by the presence of units at branch points linked in both the 2- and 4-positions. However, the fact that the optical activity of the reduced hydrolysate indicated conversion of D-glucose to sorbitol, rather than of D-glyceraldehyde to glycerol, seems to rule out the presence of 1,2′-glucosidic linkages. Hence, if any 2-linked units are present, they probably occur only at branch points.

Dextran from *L. mesenteroides* NRRL B-742 has been found by Dr. Hellman at this Laboratory to consist of at least two discrete fractions.[5] Periodate analysis of the less soluble fraction, *i.e.*, that portion *precipitated* by 41% ethyl alcohol, does not indicate the presence of unoxidized anhydroglucose units. The fractions have been found by other workers here to differ also in specific rotation, viscosity, and infrared absorption.

Periodate oxidation data on dextrans produced by several other organisms have exhibited similar indications of unoxidized anhydroglucose units. In those cases where calculations indicate the presence of such units, unusual infrared absorption[6] is also found.

Methylation studies are in progress at this Laboratory to establish the positions involved in glycosidic linkage.

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PEORIA, ILLINOIS ROLLAND LOHMAR

RECEIVED AUGUST 4, 1952

(1) Allene Jeanes and C. A. Wilham, THIS JOURNAL, **72**, 2655 (1950).

(2) M. Stacey and C. R. Ricketts, *Fortschr. Chem. Org. Naturstoffe*, **8**, 28 (1951).

(3) F. Smith, personal communication.

(4) H. H. Strain, THIS JOURNAL, **56**, 1756 (1934).

(5) N. N. Hellman, in "Report of Working Conference on Dextran," National Research Council, Subcommittee on Shock, and Northern Regional Research Laboratory, Peoria, Illinois, Oct. 29, 1951, p. 36.

(6) S. C. Burket and E. H. Melvin, *Science*, **115**, 516 (1952).

(7) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

STEREOSPECIFIC TOTAL SYNTHESIS OF CORTISONE

Sir:

We should like to report a stereospecific[1] total synthesis of 11-ketoprogesterone, dehydrocorticosterone and cortisone in both the natural and *dl* modifications. *dl*-4b-Methyl-7-ethylenedioxy-1, 2,-3, 4. 4aα, 4b, 5, 6, 7, 8, 10, 10aβ-dodecahydrophenanthrene-4β-ol-1-one[2] (I) with methyl iodide and potassium *t*-butoxide gave the 2-methyl derivative. m.p. 189–192°. *Anal.* Found: C, 70.58; H, 8.42. The latter was alkylated in turn with methallyl iodide to give 2β,4b-dimethyl-2-meth-

(1) "Stereospecific" is taken to mean that in each reaction producing a fixed asymmetric center, the ratio of isomer having the same configuration as the end product to all other isomers is greater than unity. In point of fact, each of such ratios in the present synthesis is 8:1 or greater.

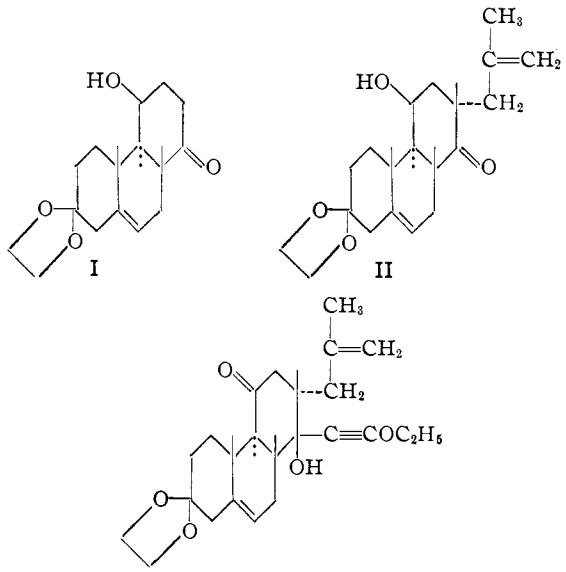
(2) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, in press.

allyl - 7 - ethylenedioxy - 1,2,3,4,4aα,4b,5,6,7,8,10,-10aβ-dodecahydrophenanthrene-4β-ol-1-one (II), m.p. 166–168°. *Anal.* Found: C, 73.15; H, 8.96. Oxidation of II to the corresponding 1,4-diketone, m.p. 139° (*anal.* Found: C, 73.97; H, 8.42) with the chromium trioxide–pyridine complex[2] followed by condensation with ethoxyacetylene magnesium bromide[3] yielded 2β,4b-dimethyl-2-methallyl-1-ethoxyethinyl-7-ethylenedioxy-1,2,3,4,-4aα,4b,5,6,7,8,10,10aβ - dodecahydrophenanthrene-1ζ-ol-4-one (III), m.p. 131–132°. *Anal.* Found: C, 72.88; H, 8.36. Treatment of III with dilute sulfuric acid afforded 2β,4b-dimethyl-2-methallyl-1-carbethoxymethylene - 7 - ethylenedioxy - 1,2,3,4,-4aα,4b,5,6,7,8,10,10aβ - dodecahydrophenanthrene-4-one, m.p. 94–96°. *Anal.* Found: C, 72.65; H, 8.25. Free acid, m.p. 203–205°. *Anal.* Found: C, 71.75; H, 7.78. Reduction of the keto acid with sodium borohydride to the 4α-hydroxy acid followed by reduction of the conjugated double bond with potassium–ammonia–isopropyl alcohol[4] gave 2β,4b-dimethyl-1β-carboxymethyl-2-methallyl-7-ethylenedioxy-1,2,3,4,4aα,4b,5,6,7,8,10,10aβ-dodecahydrophenanthrene - 4α - ol, m.p. 255–257°. *Anal.* Found: C, 71.36; H, 8.73. The 1β-(β-hydroxyethyl) derivative, m.p. 199–201°; 210–211° (*anal.* Found: C, 73.56; H, 9.55) was obtained by reduction with lithium aluminum hydride; 1β-(β-*p*-toluenesulfonate), m.p. 157–158°. *Anal.* Found: C, 68.10; H, 8.17. Successive oxidations of the monotosylate with the chromium trioxide–pyridine complex,[2] with osmium tetroxide, and with periodic acid gave 2β,4b-dimethyl-1β-(β-*p*-toluenesulfonyloxyethyl) - 2 - acetonyl - 7 - ethylenedioxy - 1,2,3,4,4aα,4b,5,6,7,8,10,10aβ - dodecahydrophenanthrene-4-one (IV), m.p. 105–108°. *Anal.* Found: C, 66.15; H, 7.19. The initial reaction product of IV with sodium methoxide was *dl*-3-ethylenedioxy-Δ^5-17α-pregnene-11,20-dione, m.p. 212–214° (*anal.* Found: C, 74.26; H, 8.58) which on equilibration with alkali gave the 3-ethylenedioxy derivative of *dl*-ketoprogesterone (V), m.p. 181–182.5°. *Anal.* Found: C, 74.34; H, 8.36. (Acid hydrolysis of V gave *dl*-11-ketoprogesterone,[5] m.p. 175–176°; *anal.* Found: C, 76.72; H, 8.65). Resolution of V via the strychnine salt (dec. 212–214°; *anal.* Found: N, 3.21) of the *dl*-21-oxalyl acid (VI) dec. 174–177°; *anal.* Found: C, 67.36; H, 7.08) followed by hydrolysis of the oxalyl acid group gave 3-ethylenedioxy-Δ^5-pregnene-11,20-dione,[5,6] m.p. and mixed m.p. 175–176.5°, $[\alpha]^{25}D$ + 52 ±2° ($CHCl_3$). (*Anal.* Found: C, 74.37; H, 8.45). Acid hydrolysis of the ethylenedioxy derivative gave 11-ketoprogesterone,[5] m.p. and mixed m.p. 178°, $[\alpha]^{23}D$ + 231 ± 4° (acetone).

Iodination and acetoxylation[7] of the 21-oxalyl acid of 3-ethylenedioxy-Δ^5-pregnene-11,20-dione, m.p. 183–185°, $[\alpha]^{25}D$ + 61 ± 2° (THF) (*anal.* Found: C, 67.71; H, 7.17), obtained in the above resolution, yielded successively crystalline 3-ethylenedioxy - 21 - iodo - Δ^5 - pregnene - 11,20 - dione and 3-ethylenedioxy-Δ^5-pregnene-21-ol-11,20-dione acetate[5] (VII), m.p. and mixed m.p. 193.5–194°; $[\alpha]^{25}D$ + 52 ± 2° ($CHCl_3$). *Anal.* Found: C, 70.02; H, 7.65.

The 20-cyanhydrin of VII, dec. 220–224° (*anal.* Found: C, 68.17; H, 7.63) was dehydrated to the $\Delta^{5,17}$-20-cyanopregnadiene, m.p. 203°. Oxidation with potassium permanganate[8] gave 3-ethylenedioxy - Δ^5- pregnene - 17α,21 - diol - 11,20 - dione acetate, dec. 262–267°. *Anal.* Found: C, 67.50; H, 7.49. Acid hydrolysis of the latter yielded cortisone acetate[5] VIII, m.p. and mixed m.p. 239–244°, $[\alpha]^{25}D$ + 210° ($CHCl_3$).

Alkaline iodination of the *dl*-21-oxalyl acid VI gave crystalline *dl*-3-ethylenedioxy-21-iodo-Δ^5-pregnene-11,20-dione which with potassium acetate yielded *dl*-3-ethylenedioxy-Δ^5-pregnene-21-ol-11,-



(3) *Cf.* D. A. Van Dorp and J. F. Arens, *Nature*, **160**, 189 (1947).

(4) *Cf.* A. J. Birch, *J. Chem. Soc.*, 430 (1944), and succeeding papers.

(5) Identity confirmed by infrared comparison.

(6) A sample prepared from 11-ketoprogesterone had $[\alpha]^{25}D$ + 52.5 ± 2°, m.p. 175–176°.

(7) *Cf.* C. R. Addinall, FIAT Final Report, 996, Jan. 29 (1947).

(8) Unpublished procedure of R. Tull, R. E. Jones and Huang-Minlon. See also von J. Heer and K. Miescher, *Helv. Chim. Acta*, **34**, 859 (1951).

20-dione acetate VII, m.p. 190–191°. *Anal.* Found: C, 69.87; H, 7.68. Acid hydrolysis of VII gave *dl*-11-dehydrocorticosterone 21-acetate, m.p. 154°; 166–168°; free *dl*-11-dehydrocorticosterone,[5] m.p. 173–179°. *Anal.* Found: C, 72.97; H, 8.11.

dl-Cortisone acetate,[5] m.p. 240–245° (*anal.* Found: C, 68.89; H, 7.47) was prepared from *dl*-VII by the same route; *dl*-20-cyanhydrin, dec. 220–225°; *dl*-unsaturated nitrile, m.p. 181–183°; *dl*-3-ethylenedioxy-Δ^5-pregnene-17α,21-diol-11,20-dione acetate, dec. 247–252°.

Acknowledgment.—The authors wish to express their indebtedness to Dr. J. van de Kamp, Mr. W. Paleveda, and Mr. R. Gasser, for the preparation of materials.

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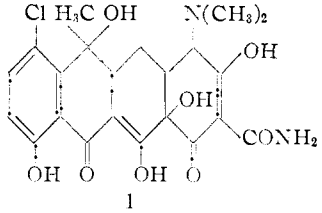
RECEIVED SEPTEMBER 20, 1952

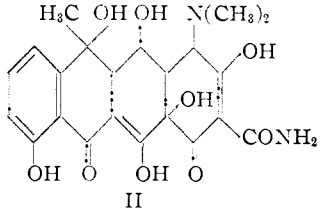
TERRAMYCIN. VIII. STRUCTURE OF AUREOMYCIN AND TERRAMYCIN

Sir:

Published physical data[1,2] on aureomycin and Terramycin and the results of our studies on the structure of Terramycin[3] require a relationship between these compounds which is expressed by structures I and II, respectively.



I



II

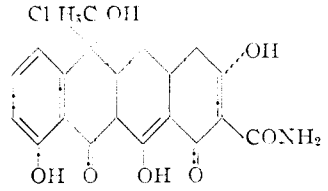
The structure I is in agreement with our analytical data which indicate that the molecular formula of aureomycin is $C_{22}H_{23}N_2O_8Cl$: *Anal.* Calcd. for $C_{22}H_{23}N_2O_8Cl$: C, 55.17; H, 4.84; N, 5.85; Cl, 7.40. Found: C, 55.10; H, 4.90; N, 5.72; Cl, 7.27. Calcd. for $C_{22}H_{23}N_2O_8Cl \cdot HCl$: C, 51.27; H, 4.69; N, 5.43; Cl, 13.76. Found: C, 51.24; H, 4.66; N, 5.40; Cl, 13.80.

(1) (a) R. Broschard, A. Dornbush, S. Gordon, B. Hutchings, A. Kohler, G. Krupka, S. Kushner, D. Lefemine and C. Pidacks, *Science*, **109**, 199 (1949); (b) B. M. Duggar, U. S. Patent 2,482,055 (1949); (c) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, **73**, 4211 (1951).

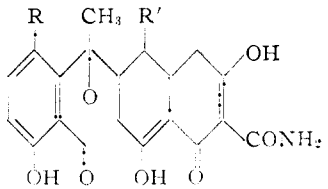
(2) (a) D. J. Hiscox, *J. Am. Pharm. Assoc.*, **40**, 237 (1951); (b) J. Dunitz and J. Robertson, THIS JOURNAL, **74**, 1108 (1952); (c) R. Pepinsky and T. Watanabe, *Science*, **115**, 541 (1952).

(3) F. A. Hochstein, C. R. Stephens, L. H. Conover, P. P. Regna, R. Pasternack, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **74**, 3708 (1952).

The naphthacene skeleton in aureomycin is demonstrated, as in the case of Terramycin, by reduction to desdimethylaminodesoxyaureomycin (III) (*Anal.* Calcd. for $C_{20}H_{18}NO_7Cl$: C, 57.21; H, 4.31; N, 3.33. Found: C, 57.09; H, 4.64; N, 3.38) and the acid dehydration of this product to a red compound (*Anal.* Calcd. for $C_{20}H_{16}NO_6Cl$: C, 59.78; H, 4.00; N, 3.48; Cl, 8.83. Found: C, 60.13; H, 4.14; N, 3.57; Cl, 8.90) from which naphthacene has been obtained by zinc dust distillation.



III



IV, R = Cl, R' = H
V, R = H, R' = OH

The ultraviolet absorption spectrum of aureomycin and its acidity constants (for the hydrochloride, pK_a's 3.4, 7.4, 9.2) are very similar to those of Terramycin (pK_a's 3.5, 7.6, 9.2). Thus, the polycarbonyl system of Terramycin is common to both compounds. The slightly longer wave length absorption of aureomycin is attributable to the effect of the aromatic chlorine atom, the position of which has been shown by the isolation of 5-chlorosalicylic acid[4] and 5-chloro-7-hydroxy phthalides[5] from aureomycin.

The desdimethylaminodesoxy compounds (*e.g.*, III) from both antibiotics have very similar absorption spectra, which exhibit marked shifts from the parent compounds. This shift is a consequence of the removal of the C_6 hydroxyl group since desdimethylaminoterramycin (*Anal.* Calcd. for $C_{20}H_{19}NO_9$: C, 57.55; H, 4.59; N, 3.36. Found: C, 57.42; H, 4.62; N, 3.34) and desdimethylaminoaureomycin (*Anal.* Calcd. for $C_{20}H_{18}NO_8Cl \cdot CH_3OH$: C, 53.91; H, 4.72; N, 2.99; Cl, 7.57; OCH_3, 6.62. Found: C, 54.08; H, 4.95; N, 3.12; Cl, 7.59; OCH_3, 6.24) possess absorption characteristics essentially identical with those of the respective antibiotics.

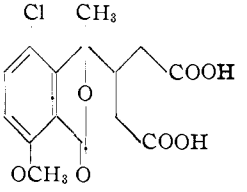
The presence of a C_{14}-hydroxyl in aureomycin is apparent from the alkali-induced rearrangement of desdimethylaminodesoxyaureomycin (III) to the substituted phthalide (IV) (*Anal.* Calcd. for $C_{20}H_{18}NO_7Cl$: C, 57.21; H, 4.31; N, 3.33. Found: C, 56.87; H, 4.50; N, 3.40). Similarly in the Terramycin series desdimethylaminodesoxyterramycin[3] yields an analogous compound (V) (*Anal.* Calcd. for $C_{20}H_{19}NO_8$: C, 59.85; H, 4.71; N, 3.49. Found: C, 59.82; H, 5.06; N, 3.55). Pyrolysis

(4) R. Kuhn and K. Dury, *Ber.*, **84**, 563 (1951).

(5) B. Hutchings, C. Waller, S. Gordon, R. Broschard, C. Wolf, A. Goldman and J. Williams, THIS JOURNAL, **74**, 3710 (1952).

of IV yields 4-chloro-7-hydroxy-3-methylphthalide (VI), m.p. 101–103°, while pyrolysis of V yields 7-hydroxy-3-methylphthalide (VII).[6] Further evidence for the similarity of the two terminal ring systems in aureomycin and in Terramycin is provided by the virtual identity of the difference curves obtained by the subtraction of the ultraviolet absorption of VI from that of IV, and VII from V.

All eight oxygens in aureomycin have now been placed and, therefore, in addition to the substitution of a chlorine atom at C_{16}, aureomycin differs from Terramycin by the absence of a hydroxyl group at C_{12}.[7] These deductions are supported by the recently described isolation of the acid (VIII)

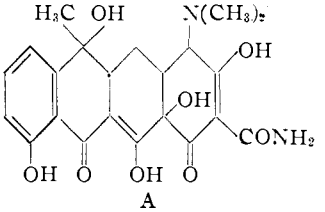


VIII

from aureomycin by methylation, followed by permanganate oxidation.[5]

(6) F. Hochstein and R. Pasternack, THIS JOURNAL, **73** 5008 (1951).

(7) Common to both Terramycin and aureomycin is the structure A for which we propose the name tetracycline. Terramycin has, therefore, been assigned the generic name oxytetracycline.



A

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R. B. WOODWARD

RECEIVED AUGUST 8, 1952

HYDROLYSIS OF CONDENSED PHOSPHATES

Sir:

The ever-increasing importance of condensed phosphates and other polyelectrolytes to science and industry combined with the inadequacies and misconceptions of the published data on phosphate hydrolyses necessitated the initiation of a fundamental research program in this field. Some of the findings in the program are presented below. These results will be discussed more fully in a forthcoming paper.

Condensed phosphates hydrolyze in aqueous solutions to yield less condensed phosphates and ultimately pure orthophosphate. The rate of hydrolysis is dependent upon the temperature, *p*H, concentration of phosphate,[1–5] and ionic environment. The ionic environment may affect the rate by complexing with the phosphate and by forming an ionic atmosphere about the phosphate.[6,7]

Condensed phosphates are believed not to form complexes with tetramethylammonium ions. We have hydrolyzed tetramethylammonium tripoly- and pyrophosphates in solutions of ten per cent. tetramethylammonium bromide and in water.[7] Sodium tripoly- and sodium pyrophosphates have also been hydrolyzed in sodium bromide solutions of the same ionic strength as the tetramethylammonium bromide solutions. The *p*H of these solutions was continuously controlled to ±0.1 *p*H unit at *p*H 1, 4, 7, 10, or 13, whereas the temperatures were held at 30, 60, 90 or 125°. In every case the concentration of the solution was adjusted to give one per cent. of orthophosphate ion on complete hydrolysis.

The degradations from tripoly to pyro and from pyro to ortho were found to follow a first-order law. Although Watzel,[3] in agreement with other authors, finds a minimum rate for the hydrolysis of sodium tripolyphosphate at *p*H 10, our results show that the rate of hydrolysis of tetramethylammonium phosphate in 10% tetramethylammonium bromide solution continuously decreases with increase in *p*H from 1 to 13.

The temperature dependence of the first-order rate constant, k, in hr.$^{-1}$ for the conversion from tripoly- to pyrophosphate can be given by the equation: $k = Ae^{-E/RT}$ where A is the frequency factor and E is the activation energy. The variation of these quantities with *p*H is given in Figs. 1 and 2.

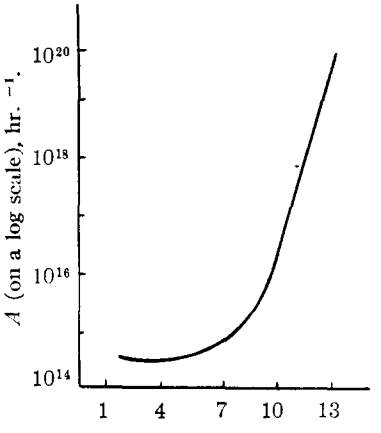

Fig. 1.—Frequency factor for hydrolysis of 1% tetramethylammonium tripolyphosphate in 10% tetramethylammonium bromide solution as a function of *p*H.

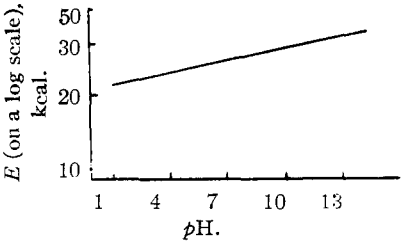

Fig. 2.—Activation energy for hydrolysis of 1% tetramethylammonium tripolyphosphate in 10% tetramethylammonium bromide solution as a function of *p*H.

(1) J. Muus, *Z. physik. Chem.*, **159A**, 268 (1932).
(2) S. J. Kiehl and E. Claussen, THIS JOURNAL, **57**, 2284 (1935).
(3) R. Watzel, *Die Chemie*, **55**, 356 (1942).
(4) R. N. Bell, *Ind. Eng. Chem.*, **39**, 136 (1947).
(5) L. M. Postnikov, *Ser. Fiz.-Mat. Estest. Nauk*, **3**, 63 (1950).
(6) J. Green, *Ind. Eng. Chem.*, **42**, 1542 (1950).
(7) J. R. Van Wazer, THIS JOURNAL, **72**, 639 (1950).

An ionic atmosphere of tetramethylammonium bromide decreases the rate of hydrolysis in both acidic and basic solutions. Thus, at 90° the tripoly-to-pyro rate constant in hr.$^{-1}$ decreases from 0.46 to 0.37 at *p*H 4 and from 0.0172 to 0.0158 at *p*H 10 on adding ten per cent. of tetramethylammonium bromide to the tetramethylammonium tripolyphosphate solution. This is added proof that the hydrolyses of pyro- and tripolyphosphates are not catalyzed by hydroxyl ions.

As would be expected from complex formation, it was found that substitution of tetramethylammonium ion by sodium ion increases the rate of hydrolysis, and this increase is intensified by the presence of excess sodium. For example, at 90° and *p*H 7 the tripoly-to-pyro constants in hr.$^{-1}$ are, for sodium ion, 0.192 and 0.152. and, for tetramethylammonium ion, 0.108 and 0.147, with the first number in each group corresponding to the presence of 0.6 *N* bromide of the respective cation and the second to a pure solution without swamping electrolyte.

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DAYTON, OHIO J. F. MCCULLOUGH

RECEIVED JULY 31, 1952

DEGRADATION OF AUREOMYCIN. III. 3,4-DIHYDROXY-2,5-DIOXOCYCLOPENTANE-1-CARBOXAMIDE

Sir:

The isolation and identification of dimethylamine and β-(4-chloro-7-hydroxy-3-methylphthalide-3)-glutaric acid as degradation products of aureomycin have been outlined.[1] Reported herein is the isolation and characterization of a $C_6H_7NO_5$ compound, I.

These degradation products thus account for the carbon. chlorine and nitrogen of the original molecule.

When aureomycin is treated with 5 *N* sodium hydroxide, desdimethylaminoaureomycinic acid, m.p. 210–212°, *anal.* Calcd. for $C_{20}H_{18}NClO_9$: C, 53.26; H, 4.04; N, 3.15; Cl, 7.86. Found: C, 52.83; H, 4.32; N, 2.99; Cl, 7.59, and dimethylamine are formed. The former compound is an optically active ($[\alpha]^{25}D$ + 100° (in methanol)), tribasic, monocarboxylic acid with *pKa*'s of 6.4, 7.8 and 10.2.

On air (or oxygen) oxidation in *N* sodium hydroxide desdimethylaminoaureomycinic acid cleaves to yield β-(4-chloro-7-hydroxy-3-methylphthalide-3)-glutaric acid and a $C_6H_7NO_5$ monobasic acid, I, (*pKa* 2.65) m.p. 198–200° (dec.), *anal.* Calcd. for $C_6H_7NO_5$: C, 41.63; H, 4.05; N, 8.09. Found: C. 41.78; H, 4.19; N, 8.26. The bulk of the C_6 acid is isolated as a *dl* compound but the residual crops have a specific rotation of −65°. The product forms ketonic derivatives, crystalline basic salts, a triacetate (isolated as a pyridine or sodium salt), but no carboxylic acid derivatives. The compound exhibits an ultraviolet absorption spectra characteristic of a cyclic β-diketone with maxima at 252 mμ (*E* 22,500) in 0.1 *N* sodium hydroxide and at 247 mμ (*E* 17,200) in 0.1 *N* hydrochloric acid.

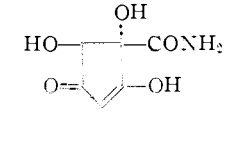
On refluxing I with hydriodic acid and red phosphorus a mole of ammonia and carbon dioxide is evolved, and a $C_5H_6O_2$ monobasic acid, II (*pKa* 4.5), m.p. 151–152° is isolated, *anal.* Calcd. for $C_5H_6O_2$: C, 61.22; H, 6.12. Found: C, 61.33; H, 6.56. This latter product was identified as 1,3-cyclopentanedione by oxidation to succinic acid and by a positive iodoform reaction. The compound has a characteristic ultraviolet absorption spectrum with maxima at 257 mμ (*E* 29,400) in 0.1 *N* sodium hydroxide and 242 mμ (*E* 20,700) in 0.1 *N* hydrochloric acid.

When I is heated in 48% hydrobromic acid, a mole of ammonia and carbon dioxide is evolved and a $C_5H_4O_3$ monobasic acid, III (*pKa* 3.0), m.p. 172.5–173° (dec.) is formed, *anal.* Calcd. for $C_5H_4O_3$: C, 53.6; H, 3.57. Found: C, 53.56, H. 3.90. The ultraviolet absorption spectra are characterized by maxima at 310 mμ (*E* 13,450) in 0.1 *N* sodium hydroxide and at 267 mμ (*E* 10,850) in 0.1 *N* hydrochloric acid. The compound was identified as 1,2,4-cyclopentanetrione by a positive iodoform reaction and by formation of an *o*-phenylenediamine derivative. Reduction of III with zinc and hydrochloric acid gives 4-hydroxy-1,3-cyclopentanedione, IV. This product and III can be converted to 1,3-cyclopentanedione by treatment with hydriodic acid and phosphorus. The synthesis[2] of II and III unequivocally proved their assigned structures.

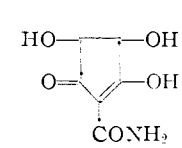
When the pyridine salt of the triacetate of I is refluxed with acetic anhydride, a descarboxamido triacetate, $C_5H_3O(OCOCH_3)_3$, V, is formed. The acetyl groups are removed by dilute acid hydrolysis to yield a monobasic acid, $C_5H_6O_4$, VI, m.p. 153–154°, *anal.* Calcd. for $C_5H_6O_4$: C, 46.2; H, 4.62. Found: C, 46.77; H, 4.84, positive iodoform reaction. This acid is also obtained from barium hydroxide hydrolysates of I. On refluxing V or VI with hydrobromic acid or hydriodic acid and phosphorus III and II are formed, respectively.

The ultraviolet and infrared spectra and the chemical characteristics of the $C_5H_6O_4$ compound (VI) are consistent with its formulation as 4,5-dihydroxy-1,3-cyclopentanedione.

Structures VII or VIII are therefore possible for the $C_6H_7NO_5$ compound.



VII



VIII

The inability to condense I with aldehydes and the marked stability of I to alkaline hydrolysis[3] es-

(1) B. L. Hutchings, C. W. Waller, S. Gordon, R. W. Broschard, C. F. Wolf, A. A. Goldman, and J. H. Williams, THIS JOURNAL, **74**, 3710 (1952).

(2) J. H. Boothe, R. G. Wilkinson, S. Kushner and J. H. Williams, to be published.

(3) The stability of I is analogous to the stability of C-acetyl dimedone to alkaline cleavage, A. J. Birch, *J. Chem. Soc.* 3026 (1951).

tablishes the structure of I as 3,4-dihydroxy-2,5-dioxocyclopentane-1-carboxamide (VIII).

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RECEIVED SEPTEMBER 15, 1952

DEGRADATION OF AUREOMYCIN. IV. DESDIMETHYLAMINOAUREOMYCINIC ACID

Sir:

The formation of β-(4-chloro-7-hydroxy-3-methylphthalide-3)-glutaric acid, I, and 3,4-dihydroxy-2,5-dioxocyclopentane-1-carboxamide, II, from desdimethylaminoaureomycinic acid, III, has been described.[1] In this "Communication" sufficient additional chemical data are presented for the structural formulation of III.

Desdimethylaminoaureomycinic acid, III, contains a phthalide nucleus as shown by the lactone band in the infrared spectra at 5.7 μ and by its ultraviolet absorption spectra before and after methylation. The presence of a free carboxyl group is apparent from the formation of the half ester of I on methylation and oxidation of III. A carboxamide grouping is shown by the formation of ammonia and carbon dioxide on hydrolysis of III with 1 *N* sodium hydroxide in ethylene glycol.[2] Furthermore, the ready elimination of carbon dioxide indicates this position to be activated.

The *pKa*'s of 6.4, 7.8 and 10.2 of III allows for the assignment of the carboxylic acid and the 7-hydroxyl of the phthalide to the *pKa*'s of 6.4 and 7.8, respectively, while the 10.2 value might be a polyhydroxylated benzene ring. The acidity of II (*pKa* 2.65) definitely excludes this structure in III.

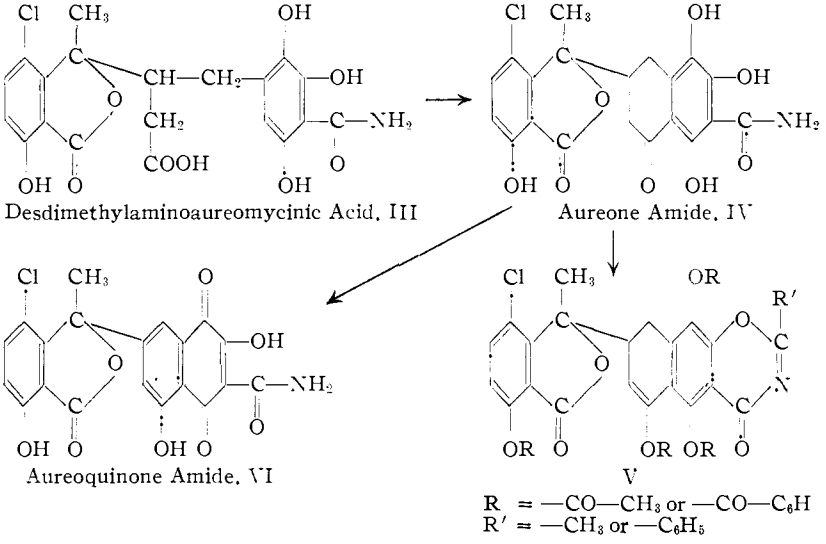
The subtraction of the ultraviolet absorption spectra of I from the spectra of III gives a remaining chromophore comparable to that of a 2,6-dihydroxybenzoic acid (dihydrocitrinin). Thus, the structure of I must contain a 2,6-dihydroxybenzamide further substituted with a hydroxyl group and with the γ-(β-[4-chloro-7-hydroxy-3-methylphthalide-3])-butyric acid radical.

When III is dehydrated with heat or sulfuric acid, aureone amide, IV, m.p. 295–305° (dec.), $[\alpha]^{25}D$ +24.6° (methyl cellosolve), *anal.* Calcd. for $C_{20}H_{16}NClO_8$: C, 55.35; H, 3.71; N, 3.25; Cl, 8.17; C—CH_3, 3.43. Found: C, 55.31; H, 4.11; N, 3.18; Cl, 7.97; C—CH_3, 3.41. is obtained. Acetylation or benzoylation of IV results in penta acylation with the loss of the elements of water. This acylation allows for the presence of three hydroxyl groups, an enolizable ketone and oxazine formation between one hydroxyl and the carboxamide group (Structure V). The presence of the ketonic group is also established by the formation of a 2,4-dinitrophenylhydrazone. Furthermore, the spectra of IV show this ketone to be conjugated with an existing chromophore.

Methylation of IV yields a methyl ether in the 7 position of the phthalide. This methylated compound (and IV) forms a stable crystalline diborate complex indicating the presence of two pairs of adjacent hydroxyl groups (or *peri* positions of a naphthalene type) in the non-phthalide portion of the molecule.

On air oxidation in 5*N* sodium hydroxide aureone amide is aromatized to aureoquinone amide, VI, m.p. 142–148°, *anal.* Calcd. for $C_{20}H_{12}NClO_8$: C, 55.81; H, 2.79; N, 3.26; Cl, 8.25. Found: C, 55.31; H, 3.15; N, 3.02; Cl, 8.15. The ultraviolet absorption spectra and *pKa* values of VI identify the compound as a 2-hydroxy-1,4-naphthoquinone.

Aureone amide on hydrolysis[2] yields aureone, VII, m.p. 296–300 (dec.), $[\alpha]^{25}D$ +19° (in ethanol), *anal.* Calcd. for $C_{19}H_{15}ClO_7$: C, 58.39; H 3.84; Cl, 9.09. Found: C, 58.16; H, 4.08; Cl. 9.04. Spectra studies and the formation of a mono 2,4-dinitrophenylhydrazone of aureone establish the presence of a ketonic group. Reduction of this ketone gives a product which has the same ultraviolet absorption spectra as a composite sample of I and 1,2,4-trihydroxybenzene.



The data allow the exact assignment of structure to III, IV and VI. The arrangement of the hydroxyl groups in the terminal benzene ring are in the 1,2,4-positions as shown by the spectra of reduced aureone and by the formation of a 2-hydroxy-1,4-naphthoquinone. The identification of II and the spectral characteristics of III and VI places the carboxamide at the 3 position. The cyclization of III to IV and the formation of a diborate complex of the ether of IV requires the arrangement in the dihydronaphthalene system of IV to be a 1,2,4,5-tetrahydroxy-7,8-dihydronaphthalene-3-carboxamide.

(1) C. W. Waller, B. L. Hutchings, C. F. Wolf, R. W. Broschard, A. A. Goldman, and J. H. Williams, THIS JOURNAL, **74**, 4978 (1952).
(2) S. Olesen, *Die Chemie*, **56**, 202 (1943).

The data further require that the benzamide nucleus of III have the three hydroxyl groups at the 2, 3 and 6 positions with the γ-(β-[4-chloro-7-hydroxy-3-methylphthalide-3])-butyric acid radical at position 4. Position 5 is free for ring closure in the formation of IV.

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RECEIVED SEPTEMBER 15, 1952

DEGRADATION OF AUREOMYCIN. V. AUREOMYCINIC ACID

Sir:

When aureomycin is treated with 5 *N* sodium hydroxide containing a reducing agent, α- or β-aureomycinic acid, I, is formed. With sodium hydrosulfite and a reaction time of 2.5 hours at room temperature α-aureomycinic acid, m.p. 225–230° for the hydrochloride, $[\alpha]^{25}D$ +54° (dilute hydrochloric acid), *anal.* Calcd. for $C_{22}H_{25}N_2ClO_9 \cdot HCl$: C, 49.53; H, 4.88; N, 5.25; Cl, 13.32; C-CH_3, 2.82. Found: C, 49.38; H, 5.20; N, 5.34; Cl, 13.58; C-CH_3, 2.54, is obtained. If the reaction time is increased to four days, β-aureomycinic acid, m.p. 174–185° (dec.) for the hydrochloride, $[\alpha]^{25}D$ −10.2° (dilute hydrochloric acid), *anal.* Calcd. as for the α-isomer. Found: C, 49.60; H, 5.62; N, 5.23; Cl, 13.35, is isolated. The β-isomer also results if zinc dust is used in lieu of hydrosulfite and the reaction mixture is heated for two hours on the steam-bath.

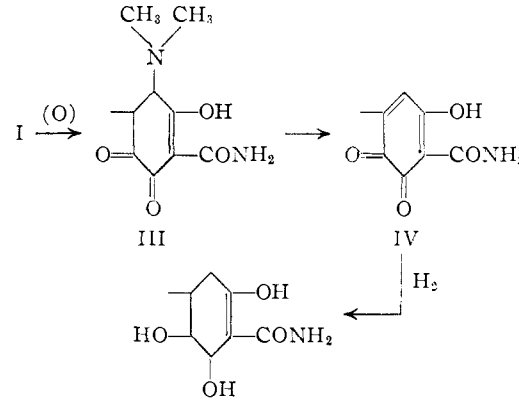
A free carboxyl group in I is indicated by the facile formation of a monoester, *anal.* Calcd. for $C_{21}H_{24}N_2ClO_7COOCH_3 \cdot HCl$: OCH_3, 5.66. Found: OCH_3, 5.11, with methanolic hydrogen chloride. The preparation of the monomethyl ester monomethyl ether, II, of I with diazomethane or methsulfate and sodium carbonate and the subsequent oxidation of II to the half ester of β-(4-chloro-7-methoxy-3-methylphthalide-3)-glutaric acid confirms the presence of a carboxyl group in I.

The lactone band at 5.7 μ in the infrared spectra of I establishes the presence of the phthalide nucleus. Similarly, the ultraviolet absorption spectra of I and II clearly show the presence of a phthalide moiety.

The titration curve of I, in addition to showing the acid functions due to the carboxyl and 7-hydroxyphthalide, demonstrates the presence of an acid function of *pKa* 7.2.

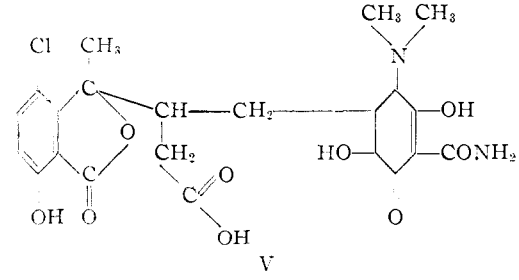
Subtraction of the ultraviolet absorption spectra of β-(4-chloro-7-methoxy-3-methylphthalide-3)-glutaric acid from those of I, gives spectra with absorption maxima at 282 mμ (*E* 15,500) in 0.1 *N* sodium hydroxide and at 267 mμ (*E* 15,400) in 0.1 *N* hydrochloric acid. The spectra of this added chromophore compares favorably with those of dimedone which has maxima at 282 mμ (*E* 23,700) in 0.1 *N* sodium hydroxide and at 260 mμ (*E* 14,000) in 0.1 *N* hydrochloric acid, except the extinction coefficient of dimedone in alkali is greater. The molecular extinction coefficient in alkaline solution is decreased when a carboxamide group is located on the central carbon of a β-diketone system.[1] The presence of this added chromophore and the acidic function at *pKa* 7.2 suggests that an isolated cyclic β-diketone is present in I. The infrared bands in the 6 to 7 μ region substantiate this conclusion.

When aureomycinic acid, I, is further treated with 5 *N* sodium hydroxide (in the absence of reducing agents), dimethylamine and desdimethylaureomycinic acid is formed. This elimination of dimethylamine with the introduction of a double bond readily explains the formation of the aromatic group, 2,3,6-trihydroxybenzamide, of desdimethylaminoaureomycinic acid.[2] The placing of dimethylamine in the 5 position of the cyclohexanedione ring makes possible the β-elimination of this group when a trace of oxygen forms the α-diketone, III, from I.



The final step in the reaction shows the *o*-quinone, IV, acting as a hydrogen acceptor for the oxidation of another molecule of I. If more than a trace of oxygen is present, further changes are initiated.

The formulation of the structure of aureomycinic acid as V is consistent with the chemical and physical data.



Aureomycinic Acid

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RECEIVED SEPTEMBER 5, 1952

(1) For comparison of 1,3-cyclopentanedione with that of 3,4-dihydroxy-2,5-dioxocyclopentane-1-carboxamide see C. W. Waller, B. L. Hutchings, C. F. Wolf, R. W. Broschard, A. A. Goldman and J. H. Williams, THIS JOURNAL, **74**, 4978 (1952).

(2) C. W. Waller, B. L. Hutchings, A. A. Goldman, C. F. Wolf, R. W. Broschard and J. H. Williams, *ibid.*, **74**, 4979 (1952).

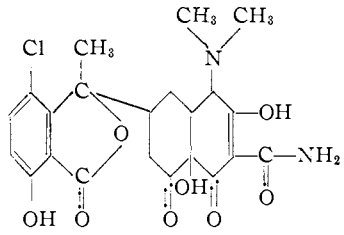
DEGRADATION OF AUREOMYCIN. VI. ISOAUREOMYCIN AND AUREOMYCIN

Sir:

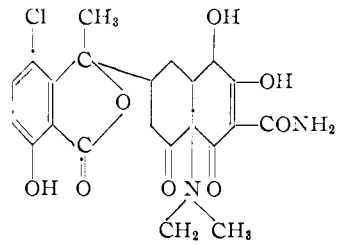
When aureomycin is dissolved in dilute alkali and allowed to stand for twenty-four hours at room temperature, isoaureomycin, I, m.p. 195–197°, and 248–250° as the hydrochloride,[1] $[\alpha]^{25}D$ −94° (dilute hydrochloric acid), *anal.* Calcd. for $C_{22}H_{23}NClO_8 \cdot HCl$: C, 51.26; H, 4.66; N, 5.44; Cl, 13.79. Found: C, 51.29; H, 4.91; N, 5.29; Cl, 13.88, is formed.

The infrared and ultraviolet absorption spectra of I establishes the presence of the phthalide nucleus. Alkaline hydrolysis[2] indicates the presence of the carboxamide grouping. The two acid functions of I have *pKa* values of 6.8 and 8.1, respectively. The latter value is due to the 7-hydroxyphthalide. When the ultraviolet absorption spectra of the phthalide nucleus are subtracted from the spectra of I, a chromophore similar to that in aureomycinic acid[3] is found to be present. The diketone bands in the 6–7 μ region of the infrared spectra again substantiates the conclusions from the *pKa* and ultraviolet data that a cyclic β-diketone structure exists in I. In addition, an absorption band at 5.80–5.85 μ is present. The absorption in this region is typical of a non-conjugated ketone of a cyclohexanone.[4]

When isoaureomycin subsequently reacts with 5 *N* sodium hydroxide in the presence of sodium hydrosulfite, α-aureomycinic acid is formed. This reaction involves a ketonic hydrolysis and causes the formation of a carboxyl group from the non-conjugated β-diketone of I. Since the central carbon atom of the hydrolyzed β-diketone is completely substituted, isoaureomycin has structures II or III.



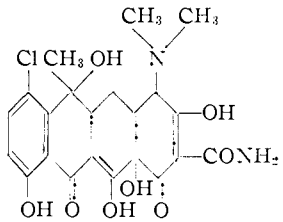
II



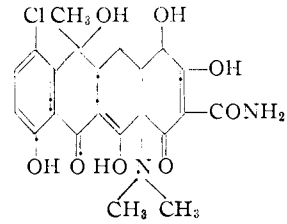
III

The infrared absorption spectrum of aureomycin[5,6] showed no absorption bands between 5 and 6 μ which not only eliminates the presence of a phthalide structure but also excludes the presence of a non-conjugated ketonic group. The formation of isoaureomycin involves the alkaline cleavage of a carbon to carbon bond of an enolizable β-diketone to form a carboxyl group which subsequently lactonizes to give a phthalide. The remaining ketonic group of the original β-diketone is now not capable of forming a conjugated system.

Since there are only two possible structures for isoaureomycin, aureomycin must have structure IV or V.



IV



V

(1) The compound reported by A. C. Dornbush, J. J. Oleson, A. L. Whitehill and B. L. Hutchings, *Proc. Soc. Exptl. Biol. Med.*, **76**, 676 (1951), when dried over boiling toluene lost water of hydration.

(2) S. Olesen, *Die Chemie*, **56**, 202 (1943).

(3) B. L. Hutchings, C. W. Waller, R. W. Broschard, C. F. Wolf, P. W. Fryth and J. H. Williams, THIS JOURNAL, **74**, 4980 (1952).

(4) Cyclopentanones absorb from 5.70–5.75 μ.

(5) B. M. Duggar, U. S. Patent 2,482,055 (1949).

(6) Analytical data obtained subsequent to the preliminary values reported by R. W. Broschard, *et al.*, *Science*, **109**, 2826 (1949), are: *Anal.* Calcd. for $C_{22}H_{23}N_2ClO_8 \cdot HCl$: C, 51.26; H, 4.66; N, 5.44; Cl, 13.79. Found: C, 51.12; H, 4.75; N, 5.39; Cl, 13.75.

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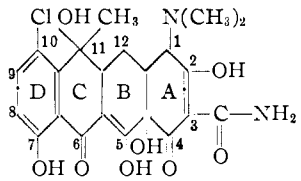
RECEIVED SEPTEMBER 15, 1952

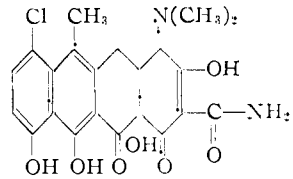
DEGRADATION OF AUREOMYCIN. VII.[1] AUREOMYCIN AND ANHYDROAUREOMYCIN

Sir:

The existence of a naphthacene nucleus in Aureomycin I has been postulated.[2]



Aureomycin I



Anhydroaureomycin II

Substituents and the nature of rings D and A have been rigorously established with the one exception the 1-dimethylamino and the 4a-hydroxyl groups may be reversed (the acidity of I would not allow the dimethylamino group to be on carbon 2 or 4).

Ring C of aureomycin is further established by dehydration and aromatization. In concentrated hydrochloric acid at 60° for thirty minutes I is converted in excellent yields to anhydroaureo-

(1) The data in this series of papers was presented at the Medicinal Section of the Gordon Research Conferences, New London, N. H., on August 20, 1952.

(2) (VI) C. W. Waller, B. L. Hutchings, C. F. Wolf, A. A. Goldman, R. W. Broschard, and J. H. Williams, THIS JOURNAL, **74**, 4981 (1952).

mycin II, m.p. 220–235° (dec.), *pKa* 5.5 and 8.5, $[\alpha]^{25}D$ + 16° (in cellosolve), *anal.* Calcd. for $C_{22}H_{21}N_2ClO_7$: C, 57.32; H, 4.56; N, 6.08; Cl, 7.70. Found: C, 57.30; H, 4.62; N, 5.81; Cl, 8.02.

The infrared absorption spectrum of II shows no bands from 5 to 6 μ (amide carbonyl at 6.0 to 6.1 μ) thus eliminating the presence of a phthalide, other lactones, and any non-conjugated ketonic groups. The ultraviolet absorption spectra in 0.1 *N* sodium hydroxide exhibits maxima at 230 (*E* 25,500), 272 (*E* 37,200), 345 (*E* 6,440) and 445 mμ (*E* 11,000) and in 0.1 *N* hydrochloric acid at 227 (*E* 26,500), 277 (*E* 44,000) and 445 mμ (*E* 8,250).

The aromatization of ring C by the loss of the water is not only shown by spectral changes but also by the failure of the compound to form 5-chlorosalicylic acid on alkali fusion (all previously discussed C_{20} and C_{22} compounds do give 5-chlorosalicylic acid) yet ring D is found to be unaffected since on alkaline peroxide oxidation 5-chloro-6-acetylsalicylic acid is obtained.

Dimethylamine and ammonia (with loss of carbon dioxide) are eliminated from II as from other C_{22} compounds.

It is unlikely that rings B and A are affected since optical activity persists after the acid treatment.

When 48% hydriodic acid is used in lieu of hydrochloric acid for the elimination of water from I there is produced deschloroanhydroaureomycin (III), m.p. 225–226° (dec.), *pKa*'s 6.0 and 8.6 $[\alpha]^{25}D$ +24° (in cellosolve), *anal.* Calcd. for $C_{22}H_{22}N_2O_7$: C, 62.00; H, 5.15; N, 6.57. Found: C, 61.88; H, 5.36; N, 6.02. Anhydroaureomycin, II, also forms III on heating with hydriodic acid.

The absorption spectra and chemical properties of II and III are very similar.

Since the removal of the chlorine atom from II changed only its first *pKa* (5.5 to 6.0), this acid function is represented by the 1,8-dihydroxynaphthalene portion of the molecule.

Not only is ring C established as a six-membered ring but also the steric relationship of the hydroxyl group at carbon 11 and the hydrogen at carbon 11a is indicated to be *trans*.

Ring B of aureomycin cannot be seven membered. Each carbon of ring A in aureomycinic acid carries at least one substituent. The γ-butyric acid group must be *para* to the carboxamide and the closing of ring B to form a seven-membered ring would then involve one of the ketonic carbons. Such an involvement would destroy the acidity of the β-diketones of ring A.

The infrared spectra of iso-aureomycin shows a band at 5.80 to 5.85 μ which demands a six rather than a five membered ring for B.

The difficulty of eliminating dimethylamine or water from the A and B rings suggests that the dimethylamino group at 1 (or 4a) the hydroxyl group at carbon 4a (or 1) and the hydrogen at carbon 12a may be a *cis,cis*-configuration. The relationship of the configuration at carbons 11 and 11a to those at carbons 1, 4a and 12a cannot be stated at this time.

Further work is in progress to establish unequivocally the position of the dimethylamino group in aureomycin.

The independent and quite dissimilar methods of proof of structure for aureomycin and terramycin[3] tend to substantiate the structures of these two compounds.

(3) F. A. Hochstein, *et al.*, THIS JOURNAL, **74**, 3708 (1952).

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RECEIVED SEPTEMBER 15, 1952

BOOK REVIEWS

The Enzymes. Chemistry and Mechanism of Action. Volume II, Part 1 and Part 2. By JAMES B. SUMNER, Laboratory of Enzyme Chemistry, Cornell University Ithaca, New York, and KARL MYRBACK, Institute for Organic Chemistry and Biochemistry, University of Stockholm, Sweden (Editors). Academic Press, Inc., 125 E. 23rd Street, New York 10, N. Y. Part 1—1951. Pages xi + 790. 16.5 × 23.5 cm. Price, $14.80. Part 2—1952. Pages xi + 791–1440. 16.5 × 23.5 cm. Price, $14.00.

Reviews of Volume I, Parts 1 and 2 of this encyclopedic presentation of the important aspects of the chemistry and mechanism of action of the enzymes appeared recently in THIS JOURNAL [**74**, 284 (1952)]. Volume II, Parts 1 and 2, composed of thirty-five chapters, has now appeared. This comprehensive survey of the present knowledge in this field is now complete in two volumes, four parts, seventy-eight chapters, 1743 pages. Each chapter is on a well defined specific topic and is written by one of the seventy-five authorities who contributed to the work.

Volume II continues the same high quality of organization and presentation as shown previously. Although the reviews naturally reflect to some extent the individuality of the respective writers, there is a remarkable degree of uniformity and a minimum of unessential repetition for a work shared by so many individuals. The editors have unquestionably fulfilled their aim expressed in the introduction to the first volume "to gather and sift available knowledge and present it in an orderly fashion," for the use of those interested in advancing the field of enzymology. Time was ripe for undertaking this tremendous task. The available information in this field has become too extensive for even an expert to obtain it from the original literature; the number interested in enzymes has expanded far beyond biochemistry into all the allied fields in chemistry and biology, and the subject, while still changing rapidly, has reached a stage sufficiently definitive to be summarized on a broad basis.

These volumes, as was pointed out by a previous reviewer, will be valuable not only for reference but as a source of in-