From molecular weight determinations using benzene solutions, Hill[15] concluded that at higher concentrations, two or even three ion pairs of silver perchlorate associated into clusters. Estimations of the size of the solute particle by Luder, *et al.*,[21] also indicate dimerization. It may prove possible to determine the solute particle geometry by an X-ray diffraction technique, using concentrated solutions of silver perchlorate or fluoborate in toluene, such as was carried out by Vaughan, *et al.*,[22] with complex ions of certain heavy metals.

(21) W. L. Luder, P. B. Kraus, C. A. Kraus and R. M. Fuoss, THIS JOURNAL, **58**, 255 (1936).

(22) P. A. Vaughan, J. H. Sturdivant and L. Pauling, *ibid.*, **72**, 5477 (1950).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SOUTHERN CALIFORNIA
LOS ANGELES 7, CALIFORNIA

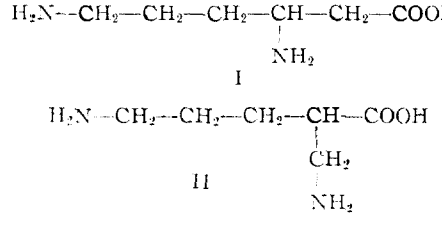
COMMUNICATIONS TO THE EDITOR

STRUCTURE OF THE DIAMINOHEXANOIC ACID FROM STREPTOTHRICIN[1]

Sir:

Previously we have reported that streptothricin on hydrolysis yields three ninhydrin-positive products, one of which, the fastest-moving on paper-grams, was characterized as a diaminohexanoic acid.[2,3] The same amino acid has been isolated from viomycin[3,4,5] and appears to be present also in streptolin.[3,6]

Possible structures for this substance were limited to isomers I and II on the basis of the following data[2,3]: negative Kuhn–Roth, negative



periodate, and negative α-amino acid tests; degradation *via* the Curtius reaction to a triaminopentane which reacted with periodate (one mole) yielding ammonia and formaldehyde.

Since further degradation studies were unproductive, the synthesis of isomers I and II was undertaken. I was obtained from α-N-phthalyl-δ-N-benzoyl-L-ornithine by application of the Arndt-Eistert reaction in a manner similar to that described by Balenović, *et al.*,[7] for derivatives of tyrosine. The resulting β,ϵ-diamino-*n*-caproic acid was optically active ($[\alpha]^{28}D$ +24°, *c* 1.1 in 1 *N* hydrochloric acid), and yielded the following derivatives: *p*-hydroxyazobenzene-*p'*-sulfonate, m.p. 246–249°; N,N-dibenzoyl acid, m.p. 113–116° (found: C, 67.94; H, 6.30; N, 7.73); N,N-dibenzoyl methyl ester, m.p. 147–150° (found: C, 68.47; H, 6.42; N, 7.77). The corresponding derivatives of the natural substance ($[\alpha]^{28}D$ +25°, *c* 1.1 in 1 *N* hydrochloric acid) melted at 224–246° (found: C, 51.52; H, 4.44; N, 12.23; S, 9.30), 113–116° (found: C, 68.02; H, 5.73; N, 7.93), and 148–150° (found: C, 68.63; H, 6.22; N, 7.86), respectively. The infrared spectra of the N,N-dibenzoyl acids and esters of the synthetic and natural substances were respectively identical. Moreover, crystallization from ethanol of the crude benzoylation products of both the synthetic and natural substances yielded neutral dibenzoyl derivatives (synthetic, m.p. 143–146°, found: C, 68.95; H, 6.77; N, 7.43; natural, m.p. 146–148°, found: C, 68.71; H, 6.97; N, 7.89) having superimposable infrared spectra.

Isomer II was prepared in racemic form by reduction of ethyl α,γ-dicyanobutyrate in a glacial acetic–sulfuric acid mixture with platinum oxide as catalyst. The racemic N,N-dibenzoyl acid (m.p. 156–158°, found: C, 68.13; H, 5.92; N, 7.66) and N,N-dibenzoyl methyl ester (m.p. 119–122°) of II gave infrared spectra differing significantly from those of the corresponding derivatives of the natural substance.

These data establish conclusively that the diaminohexanoic acid from streptothricin and viomycin has structure I (β,ϵ-diamino-*n*-caproic acid). It is suggested that the trivial name β-lysine be assigned to this compound.

Structural studies upon streptothricin and its hydrolytic products will be reported later in more detail.

HERBERT E. CARTER
WALTER R. HEARN
DIVISION OF BIOCHEMISTRY — EDWIN M. LANSFORD, JR.
NOYES LABORATORY OF CHEMISTRY — A. C. PAGE, JR.
UNIVERSITY OF ILLINOIS — NORMAN P. SALZMAN
URBANA, ILLINOIS — DAVID SHAPIRO[8]
W. R. TAYLOR

RECEIVED JUNE 2, 1952

(1) Supported by a grant from the Abbott Laboratories, Eli Lilly and Company, and the Upjohn Company.

(2) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers," 119th Meeting, American Chemical Society, Cleveland, Ohio, April, 1951, p. 25A.

(3) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers" 120th Meeting, American Chemical Society, New York, N. Y., September, 1951, p. 3L.

(4) T. H. Haskell, S. A. Fusari, R. P. Frohardt and Q. R. Bartz, THIS JOURNAL, **74**, 599 (1952).

(5) We are indebted to Parke, Davis and Company for a generous supply of viomycin.

(6) E. E. Smissman, R. W. Sharpe and E. E. van Tamelen, "Abstracts of Papers" 121st Meeting, American Chemical Society, Milwaukee, Wisconsin, April, 1952, p. 8O.

(7) von K. Balenović, W. Thaller and I. Filipović, *Helv. Chim. Acta*, **34**, 744 (1951).

(8) On leave from the Weizman Institute of Science, Rehovot, Israel.

UTILIZATION OF PTEROYLGLUTAMIC ACID CONJUGATES IN THE *IN VITRO* SYNTHESIS OF *L. CITROVORUM* ACTIVITY[1]

Sir:

The demonstration that liver slices convert synthetic pteroylglutamic acid (I) to a substance(s) possessing microbiological activity for *L. citrovorum*[2] led us to study the ability of chick liver tissue to convert pteroyldiglutamic acid (II) and pteroyltriglutamic acid (III) to a substance(s) possessing *L. citrovorum* activity. Using the procedure of Nichol and Welch[2] and employing the modifications reported previously[3] it was observed (Table I) that liver slices from chicks deficient in I synthesized *L. citrovorum* activity, under the experimental conditions, at the same rate regardless of whether the substrate was I, II or III. The values presented were obtained using synthetic 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid (leucovorin) as a standard. Ascorbic acid was added to the flasks (10 mg./flask) to augment the synthesis of *L. citrovorum* activity.[2]

TABLE I

L. Citrovorum ACTIVITY SYNTHESIZED BY PTEROYLGLUTAMIC ACID DEFICIENT CHICK LIVER TISSUE[a]

	Substrate		
	I	II	III
None	10γ/flask	≅ 10γI/flask	≅ 10γI/flask
0.12 ± 0.06	3.78 ± 0.51	3.50 ± 0.25	3.60 ± 0.34

[a] Values are the average of six experiments and are expressed as γ of leucovorin per g. liver slices, fresh wt., present after incubation. This amount of I is far in excess of that needed to saturate the system in respect to I. The standard error is included.

Bioautographic analyses of the total *L. citrovorum* activity synthesized under these conditions were carried out. The modified procedure of Winsten and Eigen[4] reported previously[5] was employed. Under the experimental conditions two zones of growth were consistently observed. The faster moving spot had the same R_f value and could not be separated chromatographically from leucovorin. Both zones of growth were obtained regardless of whether the substrate was I, II or III.

These observations indicate that I, II and III apparently have a common metabolic pathway in the synthesis of *L. citrovorum* activity. It is furthermore indicated that more than one substrate possessing activity for *L. citrovorum* is synthesized by chick liver tissue.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN

L. S. DIETRICH
W. J. MONSON
C. A. ELVEHJEM

RECEIVED MAY 16, 1952

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by funds supplied by Swift and Co., Chicago, Ill., and by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. We are indebted to Merck and Co., Rahway, N. J., for some of the crystalline vitamins, to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic pteroylglutamic acid, synthetic pteroyldiglutamic acid and synthetic pteroyltriglutamic acid.

(2) C. A. Nichol and A. D. Welch, *Proc. Soc. Expt. Biol. Med.*, **74**, 52 (1950).

(3) L. S. Dietrich, W. J. Monson and C. A. Elvehjem, *ibid.*, **77**, 93 (1951).

(4) W. A. Winsten and E. Eigen, *J. Biol. Chem.*, **184**, 155 (1950).

(5) L. S. Dietrich, W. J. Monson, H. Gwoh and C. A. Elvehjem, *ibid.*, **194**, 549 (1952).

EVIDENCE FOR THE EXCHANGE OF HYDROXYL RADICAL WITH WATER[1]

Sir:

Other work has shown that the reaction of H_2O_2 and O_3 produces a powerful $1e^-$-oxidizing agent which reacts with O_3, H_2O_2 as well as with less reactive substances such as Cl^-, Br^-, HOAc, etc.[2] It has also been shown that the same species is generated by the reaction of HCOOH with O_3.[3] This species has been described as the hydroxyl radical, and the decomposition of O_3 which it catalyzes has been formulated[2,4] as taking place by the steps

$$HO + O_3 \longrightarrow HO_2 + O_2$$
$$HO_2 + O_3 \longrightarrow HO + 2O_2$$

This reaction scheme suggests a simple tracer experiment for testing exchange of HO and H_2O, since the hydroxyl oxygen is converted to O_2, which is known not to exchange readily with H_2O. Hydrogen peroxide chemistry offers no similar convenient means of studying the exchange, since the hydroxyl oxygen in reaction with H_2O_2 is presumably converted to water.

$$HO^* + H_2O_2 = H_2O^* + HO_2$$

This communication presents some results on the exchange of O_3 with H_2O induced by the reaction with H_2O_2. In all experiments ozonized oxygen of normal isotopic composition (N = mole fraction of $O^{18} = 2.000 \times 10^{-3}$; all isotopic compositions quoted have been normalized to this value for N°) was left in contact with a liquid phase containing water enriched in O^{18} ($N = 14.6 \times 10^{-3}$). The ratio of gas volume to liquid was approximately 5. After a time, the gas was removed, dried, any residual O_3 was decomposed and the isotopic composition determined. In an experiment on the direct exchange of O_3 with water, ozonized oxygen at atmospheric pressure, 7% O_3, was left in contact with enriched water, 0.04 M in $HClO_4$ for 5 days. The isotopic composition of the gas remained unchanged[5] at 2.000×10^{-3}. In a typical experiment with H_2O_2 present, all conditions were the same except the liquid contained 9×10^{-4} M H_2O_2 (normal isotopic composition, $N = 1.997 \times 10^{-3}$). After 3 days, during which time 40% of the O_3 disappeared and 30% of the H_2O_2, the isotopic composition of the gas was found to be 2.050×10^{-3}.

The results quoted correspond to the exchange of about one-tenth of the oxygen contained in the O_3 which has decomposed. By the mechanism for decomposition which has been suggested, a maximum exchange of one-sixth of the O_3 oxygen can be expected.

It should be stressed that neither the earlier data nor the present data prove that the intermediate in question is HO. The present exchange data provide additional strong evidence however. H_2O_2, O_2 and O_3 do not exchange at all rapidly with water.

(1) This research is supported by Office of Naval Research under contract N6-ori-02026. The funds for the purchase of the mass spectrometer were supplied by the Atomic Energy commission under contract At(11-1)-92.

(2) H. Taube and W. C. Bray, THIS JOURNAL, **62**, 3357 (1940).

(3) H. Taube, *ibid.*, **63**, 2453 (1941).

(4) J. Weiss, *Trans. Faraday Soc.*, **31**, 1547 (1934).

(5) In alkaline solution extensive exchange of ozone and water does take place.

Of the radicals HO, HO_2 and HO_3, rapid exchange seems possible only for HO.

More complete experimental results will be presented in a later report, containing also data on related systems.

JONES HERBERT JONES LABORATORY
UNIVERSITY OF CHICAGO OTTO L. FORCHHEIMER
CHICAGO 37, ILLINOIS HENRY TAUBE

RECEIVED JUNE 7, 1952

TRACER STUDIES ON SOME REACTIONS OF THIOSULFATE AND TETRATHIONATE

Sir:

The oxidation of thiosulfate to tetrathionate with iodine, the reduction of tetrathionate to thiosulfate with sulfide, and the decomposition of both thiosulfate and tetrathionate with mercuric chloride in presence of bisulfite and excess formaldehyde have been studied with the aid of S^{35}.

In order to separate the products of the reactions with mercuric chloride, three successive precipitations were made: $HgCl_2 \cdot 2HgS$ (from thiosulfate) and $HgCl_2 \cdot 2HgS + S$ (from tetrathionate) were precipitated in the cold by means of a large excess of concentrated buffered mercuric chloride and addition of some ammonia after one hour (I), sulfate was precipitated, also in the cold, with acetic acid and barium chloride (II), finally the protected bisulfite was oxidized with potassium hypobromite and precipitated as barium sulfate (III). Radioactive contamination of III was excluded by an intermediate scavenging operation which consisted of the addition of inactive thiosulfate or tetrathionate, mercuric chloride and barium chloride. These intermediate precipitates were checked to be practically inactive.

If thiosulfate labeled at the central S-atom was treated in this way the activity distribution was: 1% in I, 95% in II, none in III.

If the same thiosulfate was titrated to tetrathionate with iodine, and analyzed in the same manner, again 1% was found in I, and 95% in II, but this time 2–3% entered into III.

Save for the infrequent side-reaction which caused the formation of radioactive sulfite during the decomposition of tetrathionate, the reactions may be assumed to proceed according to

$$2SS^*O_3^- + 3HgCl_2 + 2H_2O \longrightarrow HgCl_2 \cdot 2HgS + 2S^*O_4^- + 4Cl^- + 4H^+$$

$$2SS^*O_3^- \longrightarrow O_3S^*SSS^*O_3^- + 2e^-$$

$$2O_3S^*SSS^*O_3^- + 3HgCl_2 + 4H_2O \longrightarrow HgCl_2 \cdot 2HgS + 4S^*O_4^- + 4Cl^- + 8H^+ + 2S$$

If tetrathionate, obtained by titration of the same thiosulfate with iodine, was reduced immediately with inactive sulfide (in presence of inactive bisulfite and excess formaldehyde), the sulfur formed was inactive. The filtrates from this reaction were analyzed with mercuric chloride both directly, and after they had been titrated back to tetrathionate. In the first case the activity distribution was found to be: 1% in I, 96% in II, 1–2% in III; in the second case: 1% in I, 95% in II and 3% in III.

The activities found in all fractions I may well be introduced into the thio-S of the thiosulfate by side-reactions during its formation.

If tetrathionate was prepared from thiosulfate labeled at the thio-S, 2–3% of the total activity was always found in the solid sulfur; if inactive tetrathionate was reduced with active sulfide, 97% was found in the sulfur.

The results indicate that, save for a minor side-reaction, the reduction of tetrathionate with sulfide proceeds according to

$$O_3S^*S\dagger S\dagger S^*O_3^- + S^- \longrightarrow 2S\dagger S^*O_3^- + S$$

This investigation represents part of the research program of the Foundation for Fundamental Research of Matter (F. O. M.). It was performed with the financial aid of the Netherlands Organization for pure Research (Z. W. O.).

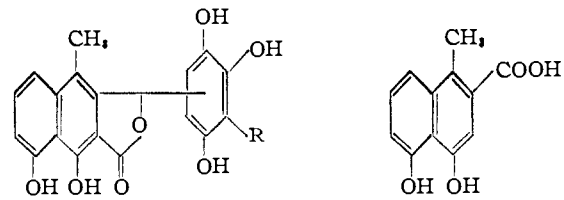
INSTITUUT VOOR KERNPHYSISCH ONDERZOEK
18 OOSTERRINGDIJK, AMSTERDAM HERMAN B. V. D. HEIJDE
THE NETHERLANDS A. H. W. ATEN, JR.

RECEIVED JUNE 9, 1952

TERRAMYCIN. V. STRUCTURE OF TERRINOLIDE. AN ACID DEGRADATION PRODUCT OF TERRAMYCIN

Sir:

Among the products formed by the degradation of terramycin[1] in dilute hydrochloric acid at elevated temperatures is terrinolide (I), pK_{a_1} = 4.6, pK_{a_2} = 7.5 (dimethylformamide–water); $[\alpha]_D$ −16.0° (*c* 1% in 1:1 methanol–0.1 *N* hydrochloric acid). *Anal.* Calcd. for $C_{20}H_{15}NO_8$: C, 60.45; H, 3.81; N, 3.53. Found: C, 60.46; H, 4.10; N, 3.52. On hydrolysis in hot 12 *N* sulfuric acid, terrinolide loses ammonia and carbon dioxide to yield a nitrogen-free, optically-inactive compound, decarboxamidoterrinolide (II),[2] pK_{a_1} = 4.7, pK_{a_2} = 10.2 (dimethylformamide–water). *Anal.* Calcd. for $C_{19}H_{14}O_7$: C, 64.41; H, 3.98; C-methyl, 4.25. Found: C, 64.10; H, 4.41; C-methyl, 3.82. Pentamethyldecarboxamidoterrinolide: m.p. 152–153°, *Anal.* Calcd. for $C_{24}H_{24}O_7$: C, 67.91; H, 5.70; CH_3O, 36.55. Found: C, 67.85; H, 5.74; CH_3O, 35.95. Terrinolide and decarboxamidoterrinolide have been assigned structures I and II, respectively.



I, R = $CONH_2$
II, R = H
III

Alkali fusion of II yields 1,8-dihydroxy-4-methyl-3-naphthoic acid (III).[3] I, II and III enhance the

(1) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, 73, 4211 (1951).

(2) Terrinolide and decarboxamidoterrinolide were originally assigned the formulas $C_{18}H_{17}NO_8$ and $C_{18}H_{12}O_6$, respectively, in our first Communication (R. Pasternack, P. Regna, R. Wagner, A. Bavley, F. Hochstein, P. Gordon and K. Brunings, THIS JOURNAL, 73, 2400 (1951)). The formation of stable solvates and a tendency of these compounds to decompose under conditions of molecular weight determination complicated the assignment of the molecular formulas.

(3) F. A. Hochstein, *et al.*, to be published.

acidity of boric acid to the marked degree characteristic of 1,8-naphthalenediols.[4]

Lithium aluminum hydride reduction of pentamethyldecarboxamidoterrinolide consumes 0.5 mole of hydride and yields no hydrogen; the reaction product is a dialcohol (IV), m.p. 114–115°, *anal.* Calcd. for $C_{24}H_{28}O_7$: C, 67.27; H, 6.59. Found: C, 67.09; H, 6.69, which is readily dehydrated in dilute mineral acid to an ether, m.p. 141–142°. *Anal.* Calcd. for $C_{24}H_{26}O_6$: C, 70.23; H, 6.38. Found: C, 70.06; H, 6.06. That the lactone structure shown by this reduction is a phthalide is indicated by the extreme resistance of decarboxamidoterrinolide to hydrolysis. This assignment is in agreement with the carbonyl band at 5.73 μ (dioxane) in the infrared absorption spectra of I and II. The marked similarity of the ultraviolet spectra of I, II and 1,8-dihydroxynaphthalene-2-carboxylic acid[3] determines the orientation of the phthalide ring on the naphthalenediol system. Further, the acidity of terrinolide, $pK_{a_1} = 4.5$, is in good agreement with the acidity of 1,8-dihydroxy-2-naphthaldehyde, $pK_a = 4.5$ (dimethylformamide–water).

The presence of five phenolic hydroxyl groups in I and II is shown by the formation of pentamethyl and pentaacetyl derivatives. The stability of II in strong acid, and the marked susceptibility of I and II to air oxidation suggest that the $C_6H_5O_3$ moiety not accounted for by the dihydroxybenzophthalide system is a trihydroxybenzene. Comparison of the ultraviolet spectrum of the dialcohol (IV) to the composite curves derived from 3-hydroxymethyl-4-methyl-1,8-naphthalenediol[3] and the three isomeric trihydroxybenzenes indicates that the $C_6H_5O_3$ group is hydroxyhydroquinone.

The presence in terrinolide of a carboxamide group which is lost by hydrolysis in sulfuric acid is supported by the infrared absorption spectra of I, II and their derivatives. The second acid constant of I, $pK_{a_2} = 7.5$ (compare $pK_{a_1} = 10.2$ for II) requires that the carboxamide group in terrinolide (I) be attached to the hydroxyhydroquinone ring between two phenolic groups.

(4) J. Böeseken, J. de Bruin and W. van Rijswijk de Jong, *Rec. trav. chim.*, **58**, 3 (1939).

RESEARCH LABORATORIES — F. A. HOCHSTEIN
CHAS. PFIZER AND CO., INC. — P. P. REGNA
BROOKLYN 6, N. Y. — K. J. BRUNINGS
CONVERSE LABORATORY
HARVARD UNIVERSITY — R. B. WOODWARD
CAMBRIDGE, MASSACHUSETTS

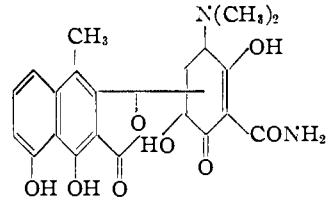
RECEIVED JUNE 25, 1952

TERRAMYCIN. VI. THE STRUCTURE OF α- AND β-APOTERRAMYCIN, ACID REARRANGEMENT PRODUCTS OF TERRAMYCIN

Sir:

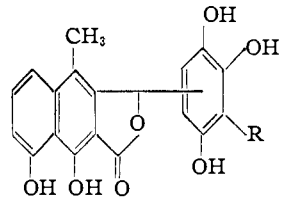
In 1.5 *N* aqueous hydrochloric acid at 60°, terramycin[1] loses a molecule of water and rearranges to form two closely related optically active compounds: α-Apoterramycin hydrochloride (I), $[\alpha]^{25}D$ +123° (*c* 1% in ethanol), $pK_{a_1} = 4.0$, $pK_{a_2} = 5.1$, $pK_{a_3} = 8.4$ (dimethylformamide–water). *Anal.* Calcd. for $C_{22}H_{22}N_2O_8 \cdot HCl$: C, 55.17; H, 4.84; N, 5.85; Cl, 7.40. Found: C, 54.85; H, 5.13; N, 5.97; Cl, 7.19. β-Apoterramycin hydrochloride (I)[2], $[\alpha]^{25}D$ −28° (*c*, 1% in ethanol), $pK_{a_1} = 3.6$, $pK_{a_2} = 5.2$, $pK_{a_3} = 7.8$ (dimethylformamide–water). *Anal.* Calcd. for $C_{22}H_{22}N_2O_8 \cdot HCl \cdot H_2O$: C, 53.17; H, 5.07; N, 5.64; Cl, 7.14. Found: C, 52.90; H, 4.76; N, 5.65; Cl, 6.90.

We consider α- and β-apoterramycin to be stereoisomers of structure I. The two compounds are interconvertible in acid and alkaline solution, and their ultraviolet spectra are virtually identical.



I

Alkali fusion of the apoterramycins yields 1,8-dihydroxy-4-methylnaphthalene-3-carboxylic acid[3] and 2,5-dihydroxybenzoquinone. Both isomers lose carbon dioxide, ammonia and dimethylamine in hot concentrated hydrochloric acid, and are converted to decarboxamidoterrinolide (II).[4] Pentamethylterrinolide is formed by treatment of α-apoterramycin with methyl iodide and potassium carbonate in acetone. The presence of the dihydroxybenzophthalide system in the apoterramycins and terrinolide (III) is evident since the absorption spectra of these compounds are practically superimposable in the 330–420 mμ region of the ultraviolet spectrum, and all three compounds show the strong enhancement of the acidity of boric acid characteristic of 1,8-naphthalenediols.[5] Thus, the apoterramycins differ from terrinolide (III) only in the structure of the isolated six-membered ring.



II, R = H
III, R = $CONH_2$

The isolation of 2,5-dihydroxybenzoquinone from α-apoterramycin suggests the relative positions of two carbonyl groups, a hydroxyl and a dimethylamine group in the isolated ring. The stability of the apoterramycins under the conditions of their formation from terramycin excludes α- or γ-diketone structures within the carbocyclic ring since

(1) P. P. Regna, I. A. Solomons, K. Murai, A. B. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, **73**, 4211 (1951).

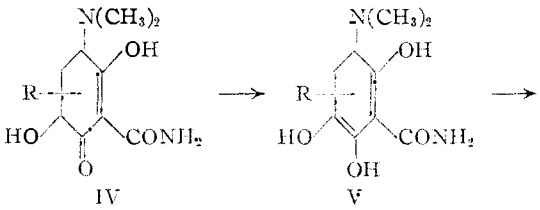
(2) This compound was described by R. Pasternack, P. P. Regna, R. L. Wagner, A. Bavley, F. A. Hochstein, P. N. Gordon and K. J. Brunings, *ibid.*, **73**, 2400 (1951), as $C_{22}H_{24}N_2O_8 \cdot HCl$. It has since been found that Karl Fischer reagent shows the presence of one molecule of water, and that recrystallization from methanol displaces the water with a molecule of methanol.

(3) F. A. Hochstein, *et al.*, to be published.

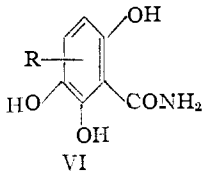
(4) F. A. Hochstein, P. P. Regna, K. J. Brunings and R. B. Woodward, THIS JOURNAL, **74**, 3706 (1952).

(5) J. Böeseken, J. de Bruin and W. van Rijswijk de Jong, *Rec. trav. chim.*, **58**, 3 (1939).

such systems necessitate the attachment of either a hydroxylic or a dimethylamino function β to the carbonyl group. The β-diketone structure (I) expresses the stability of the hydroaromatic ring except under conditions which bring about enolization of both carbonyl functions, thus permitting ready elimination of the dimethylamino group and the formation of a hydroxyhydroquinone ring, as, for example in IV → V → VI.



IV V



VI

RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
BROOKLYN 6, N. Y.

F. A. HOCHSTEIN
C. R. STEPHENS
P. N. GORDON
P. P. REGNA
F. J. PILGRIM
K. J. BRUNINGS

CONVERSE LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

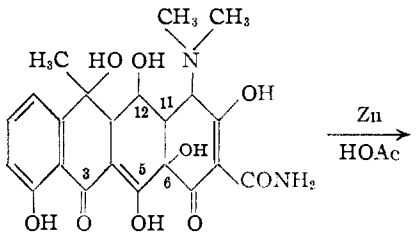
R. B. WOODWARD

RECEIVED JUNE 25, 1952

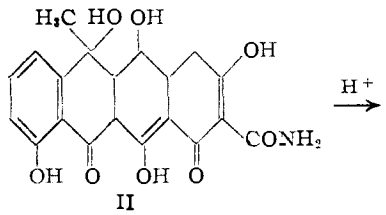
TERRAMYCIN. VII. THE STRUCTURE OF TERRAMYCIN

Sir:

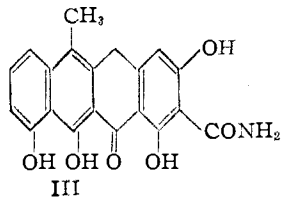
Terramycin[1] has been assigned the structure I.



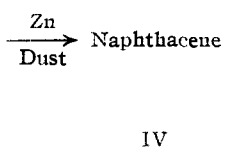
I



II



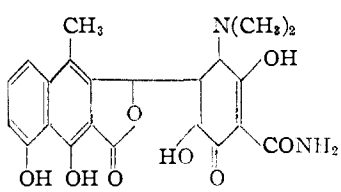
III



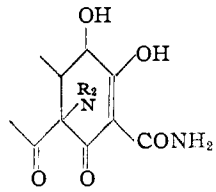
IV

The naphthacene carbon skeleton is demonstrated by the reaction sequence I → IV. Terramycin is reduced with zinc and acetic acid at room temperature to form desoxydesdimethylaminoterramycin (II)[2]: *Anal.* Calcd. for $C_{20}H_{19}NO_8 \cdot {}^1/_2CH_3COCH_3$: C, 59.99; H, 5.15; N, 3.26. Found: C, 59.99; H, 5.17; N, 3.29. In methanolic hydrochloric acid, II is readily converted to the orange-red crystalline compound (III) or a tautomer: *Anal.* Calcd. for $C_{20}H_{15}NO_6$: C, 65.75; H, 4.14; N, 3.83. Found: C, 65.74; H, 4.29; N, 4.15. Zinc dust distillation of III yields naphthacene.

Structure I is consistent with the dehydration and rearrangement of terramycin in acid media to form the dihydroxybenzophthalide structure known to be present in α- and β-apoterramycin,[3] to which we now assign structure V.



V



VI

The phthalide carbonyl in the apoterramycins must be derived from a highly conjugated or enolized carbonyl group, since the infrared absorption spectrum of terramycin shows no absorption between 5 and 6 μ. In order to permit ready cleavage to form the apoterramycins, this carbonyl must be incorporated in an actual or potential β-dicarbonyl system. With this limitation, only two formulas (I and VI) can be written for terramycin. The alternative VI is excluded, *inter alia*, by the acidity relationships of terramycin and its transformation products. For example, the *p*Ka (8.0 in dimethylformamide–water) of the dimethylamino group in terramycin does not change markedly in the conversion to the apoterramycins.

Structure I is also consistent with the formation of terracinoic acid,[4] 7-hydroxy-3-methylindanone-2-acetic acid,[5] 7-hydroxy-3-methylphthalide,[6] 6-acetylsalicylic acid,[7] and 3-hydroxymethyl-4-methyl-1,8-naphthalenediol,[5] in the alkaline degradation of terramycin, which involves cleavages at the 3–4, 5–6, and 11–12 positions. The indanone ring of terracinoic acid is presumed to form through the intermediate VII by aldehyde condensation in the position para to the phenolic group, while 7-hydroxy-3-methylindanone-2-acetic acid is formed through condensation in the ortho position with loss of the carboxyl group.

(1) P. P. Regna, I. A. Solomons, K. Murai, A. E. Timreck, K. J. Brunings and W. A. Lazier, THIS JOURNAL, **73**, 4211 (1951).

(2) R. Pasternack, P. P. Regna, R. L. Wagner, A. Bavley, F. A. Hochstein, P. N. Gordon and K. J. Brunings, *ibid.*, **73**, 2400 (1951). In this paper II was assigned the formula $C_{28}H_{21}NO_8$.

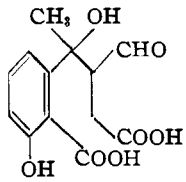
(3) F. A. Hochstein, C. R. Stephens, P. N. Gordon, P. P. Regna, F. J. Pilgrim, K. J. Brunings and R. B. Woodward, *ibid.*, **74**, 3707 (1952).

(4) R. Pasternack, L. H. Conover, A. Bavley, F. A. Hochstein, G. B. Hess and K. J. Brunings, *ibid.*, **74**, 1928 (1952).

(5) F. A. Hochstein, to be published.

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

(7) R. Kuhn and K. Dury, *Ber.*, **84**, 848 (1951).



VII

RESEARCH LABORATORIES
CHAS. PFIZER AND CO., INC.
BROOKLYN 6, N. Y.

F. A. HOCHSTEIN
C. R. STEPHENS
L. H. CONOVER
P. P. REGNA
R. PASTERNACK
K. J. BRUNINGS

CONVERSE LABORATORY
HARVARD UNIVERSITY
CAMBRIDGE, MASS.

R. B. WOODWARD

RECEIVED JUNE 25, 1952

THE EXCHANGE OF VANADIUM(II) AND VANADIUM(III) IONS IN PERCHLORIC AND SULFURIC ACID SOLUTIONS

Sir:

The successful rate measurement[1] of the rapid electron-transfer exchange reaction between iron(II) and iron(III) ions in perchloric acid solutions with the aid of a separation based on formation of a stable complex between α,α'-dipyridyl (dipy) and iron(II) ions has led us to examine exchange in the similar vanadium(II)–vanadium(III) system with this same separating reagent and also with a cation-exchange-resin separation. Vanadium(II) and vanadium(III) ions in 1 *f* perchloric acid appear to exist predominantly as the hydrated V^{++} and V^{+++} ions, respectively. The hydrolysis constant[2] of the latter is such that only *ca.* 0.1% of the vanadium(III) should be in hydrolyzed forms, principally as hydrated VOH^{++} ion. Consequently, this system appears satisfactory for examination with respect to a possible electron-transfer exchange reaction.

Solutions of vanadium(II) perchlorate (or sulfate) and labeled vanadium(III) perchlorate (or sulfate) were mixed in the appropriate acid, aliquots removed at definite time intervals, the two oxidation states separated, and the specific activities of both fractions determined by counting the solutions in a reproducible geometry with a dipping Geiger–Mueller tube and determining total vanadium by oxidation to the +5 state, followed by reduction to the +4 state with sulfite ion and titration of the vanadium(IV) with standardized potassium permanganate. The chloride-ion concentration was less than 1×10^{-5} *f* in most of the runs; in one run it was as high as 0.02 *f*. Because vanadium(II) and vanadium(III) in aqueous solution are both easily oxidized by air, all experiments were carried out in a reaction vessel under nitrogen freed from oxygen by passage through a chromium(II) chloride solution, and all preparations and transfers were handled similarly. Oxidation of the vanadium species during an exchange run was found to be entirely negligible. All but two runs were made at 2° in the absence of light.

The vanadium(II) solutions were made in some cases by electrolytic reduction of vanadium(V) oxide suspended in perchloric (or sulfuric) acid and in others by reduction of vanadium(IV) perchlorate (or sulfate) solutions at 0.5° with a Jones reductor; a stoichiometric concentration of zinc ions was present during the exchange runs in the latter case. The vanadium(III) solutions were prepared by mixing equivalent amounts of standardized vanadium(IV) and vanadium(II) stock solutions, which react rapidly and completely to produce vanadium(III). The tracer used was 16-day V^{48} produced on the U.C.L.A. cyclotron by Ti(p, xn) reactions and radiochemically purified; the purity was checked by half-life measurements.

Separation with dipy was achieved by running the exchange mixture into dipy in 50% ethanol, forming the green tris(dipy)vanadium(II) ion, then precipitating the vanadium(III), which apparently remains uncomplexed, by addition of ammonium hydroxide or ammonium fluoride solutions. Separation with a cation-exchange resin (250–500-mesh Ion-X) was effected by eluting the vanadium(II) from the resin column with 2 *f* perchloric acid. Vanadium(III) was subsequently eluted with 5 *f* perchloric acid. Both separation methods were quantitative.

TABLE I

V(II)-V(III) EXCHANGE, 1.0–1.2 *f* $HClO_4$, 2°, DARK RUNS

Sepn. method	$[V(ClO_4)_2]$, *f*	$[V(ClO_4)_3]$, *f*	Exch. time, min.	% exch.
Dipy	0.072	0.144	1	100 ± 7
Ion exch.	.072	.144	3	99 ± 7
	.072	.144	5	104 ± 7[a]
	.071	.039	4	120 ± 15[a]
	.063[b]	.126[b]	3	112 ± 7

[a] At 25°, in laboratory lighting. [b] Sulfates, in 0.86 *f* H_2SO_4.

From the average results of duplicate runs given in Table I one sees that the exchange appears to be complete within the one- to five-minute interval required to get the exchange mixture into the dipy reagent or onto the resin column (the over-all time for each separation was five to ten minutes).

In order to check the possibility that the rapid apparent exchange with the dipy separation is a result of exchange between tris-(dipy)-vanadium(II) and vanadium(III) ions during the separation, exchange between these species was investigated at 25° in ordinary laboratory lighting, using the perchlorates with excess dipy present. Separation was brought about by the addition of ammonium hydroxide to precipitate the vanadium(III), followed by centrifugation, all within ten minutes total time. The results are shown in Table II.

TABLE II

TRIS-(DIPY)–V(II)-V(III) EXCHANGE, 0.5 *f* $HClO_4$, 25°

$[V(dipy)_3^{++}]$, *f*	$[V(ClO_4)_3]$, *f*	Exch. time, min.	% exch.
0.017	0.034	0.4	44 ± 4
.017	.034	1	15 ± 1
.017	.034	10	43 ± 2
.025[a]	.050[a]	2	38 ± 2

[a] Exchange mixture 0.75 *f* in $HClO_4$.

The exchange reported in Table II appears to be a zero-time exchange, and is sufficiently incomplete to suggest that the exchange observed with the hydrated ions in the case of the dipy separation is not primarily between tris-(dipy)-vanadium(II) and

(1) R. W. Dodson, THIS JOURNAL, 73, 3315 (1950).
(2) S. C. Furman and J. T. Denison, private communication.

vanadium(III) ions. The possibility of rapid separation-induced exchange brought about when the reaction mixture is added to alcoholic α,α'-dipyridyl is not excluded by these observations. Dodson's work with the iron(II)–iron(III) exchange showed exchange half-times of the order of 15–50 seconds with the reactant species at *ca.* 0.001 *f* each. Reduction of the concentrations of the vanadium reactants may slow the observed rapid exchange rate to a point where kinetic studies could be undertaken, but the techniques used so far have not permitted this because of difficulties with oxidation of both vanadium species in solutions at high dilution by traces of oxygen present.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA WILLIAM R. KING, JR.
LOS ANGELES 24, CALIFORNIA CLIFFORD S. GARNER

RECEIVED JUNE 6, 1952

SYNTHESIS OF DEGRADATION PRODUCTS OF AUREOMYCIN

Sir:

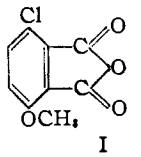
The synthesis of several degradation products[1] of Aureomycin by unequivocal methods has been accomplished. In each case these synthetic products were compared with the degradation products by means of m.p., mixed m.p., ultraviolet and infrared absorption spectra, and other properties to prove their identity.

TABLE I

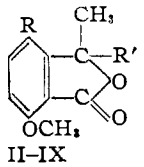
No.	R	R'	M.p., °C.	Theory C	Theory H	Theory OCH₃	Theory Cl	Found C	Found H	Found OCH₃	Found Cl
II	H	—H	73–75	67.4	5.6			66.9	6.0		
III	Cl	—H	113–14	56.5	4.2		16.7	57.0	4.6		16.3
IV	H	—OH	164–65	61.8	5.2	16.0		62.1	5.9	16.4	
V	Cl	—OH	204–206	52.4	3.9		15.5	52.3	3.9		15.4
VI	H	—COOH	168–70	59.5	4.5			59.8	5.2		
VII	Cl	—COOH	199–200	51.5	3.5		13.8	51.7	3.9		13.8
VIII	H	—CH_2COOH / —CH_2COOH	207–209.5	57.1	4.8	10.5		57.3	5.2	9.7	
IX	Cl	CH_2COOH / CH_2COOH	209–210.5	51.1	4.0		10.8	51.4	4.4		10.9

The first of these products is 6-chloro-3-methoxyphthalic anhydride (I) which definitely places the position of the methoxyl and chloro groups in relation to the other 2 substituents on the benzene ring. This compound was prepared from 3-methoxy-6-chloroanthranilic acid[2] by replacement of the amino group by a cyano group through a Sandmeyer reaction and hydrolysis to the phthalic acid derivative, m.p. 187–188°. *Anal.* Calcd. for $C_9H_5O_4Cl$: C, 50.8; H, 2.4; Cl, 16.7. Found: C, 51.5; H, 2.7; Cl, 16.7.

The remaining compounds are phthalides of the following general formula in which R = chlorine and R′ = groups as shown in Table I. These phthalides can be degraded to phthalic or benzoic acid derivatives to prove the position of the chlorine atom as indicated in the previous paper.[1]



I



II–IX

4-Chloro-7-methoxy-3-methyl-phthalide (III) was prepared from 2-amino-3-methoxyacetophenone[3] by reducing the ketone to the alcohol and replacing the amino by a cyano group. On hydrolysis, II was formed which was chlorinated to III with chlorine in acetic acid or with sodium hypochlorite and hydrochloric acid.

4-Chloro-3-hydroxy-7-methoxy-3-methylphthalide (V) was prepared by replacement of the amino by cyano in 2-amino-3-methoxyacetophenone, hydrolysis to IV and chlorination to V. Both IV and V probably exist in equilibrium with the corresponding *o*-carboxyacetophenone structure and both will form "normal" esters with diazomethane and "pseudo" esters with acid–methanol or acid chloride–methanol procedures.

4-Chloro-7-methoxy-3-methyl-3-phthalidecarboxylic acid (VII) was prepared by adding hydrogen cyanide to 2-cyano-3-methoxyacetophenone, hydrolysis of the product to VI and chlorination to VII. The compound was resolved by crystallizing the brucine salt from water; $[\alpha]^{28}D$ +25° (1.2% in ethanol).

3-(4-Chloro-7-methoxy-3-methylphthalidyl)-succinic acid (IX) was prepared by treating IV with phosphorus pentachloride to form the "pseudo" acid chloride which reacted with sodio diethyl carbethoxysuccinate. Hydrolysis and decarboxylation yielded VIII which was chlorinated to IX. Two racemates resulted and the higher melting one, m.p. 228–229° with gas, was resolved by crystallizing the brucine salt from ethanol; m.p. 209–210.5° with gas; $[\alpha]^{25}D$ −20.4° (5% in alcohol).

(1) B. L. Hutchings, *et al.*, THIS JOURNAL, **74**, 3710 (1952).
(2) B. R. Baker, *et al.*, *J. Org. Chem.*, **17**, 160 (1952).
(3) J. C. E. Simpson, *et al.*, *J. Chem. Soc.*, 646 (1945).

S. KUSHNER
CONTRIBUTION FROM THE JAMES H. BOOTHE
LEDERLE LABORATORIES DIVISION JOHN MORTON II
AMERICAN CYANAMID COMPANY JOSEPH PETISI
PEARL RIVER, NEW YORK J. H. WILLIAMS

RECEIVED JUNE 23, 1952

DEGRADATION OF AUREOMYCIN

Sir:

In a preliminary report[1] certain of the physical and chemical properties of aureomycin were out-

(1) R. W. Broschard, A. C. Dornbush, S. Gordon, B. L. Hutchings, A. R. Kohler, G. Krupka, S. Kushner, D. V. Lefemine and C. Pidacks, *Science*, **109**, 199 (1949).

lined. From the analytical data presented an empirical formula of $C_{22}H_{27}N_2ClO_8$ could be calculated. In the present communication a number of degradation products of aureomycin will be described.

From alkaline fusion of aureomycin 5-chlorosalicylic acid, dimethylamine and ammonia were obtained.[2]

The methylation and subsequent permanganate oxidation of aureomycin resulted in the formation of a number of *p*-chloromethoxybenzene derivatives which were separated by fractional extraction with various buffers and by selective crystallization.

The simplest oxidation product was identified as 6-chloro-3-methoxyphthalic acid, m.p. 186–187°, *anal.* Calcd. for $C_9H_7ClO_5$: C, 46.91; H, 3.06; Cl, 15.37. Found: C, 47.04; H, 3.57; Cl, 15.13. The anhydride of this compound melted at 187–188°.

Further fractionation yielded a monobasic acid, m.p. 199–200° (dec.) $[\alpha]^{25}D$ +25° (methanol), *anal.* Calcd. for $C_{11}H_9O_5Cl$: C, 51.5; H, 3.54; Cl, 13.8; OCH_3, 12.1; C-CH_3, 5.35. Found: C, 51.5; H, 4.02; Cl, 13.8; OCH_3, 11.9; C-CH_3, 5.18, which readily formed a monomethyl ester, m.p. 96–100°. Infrared absorption spectra showed the presence of carboxyl, lactone, carbonyl, aromatic unsaturation, terminal methyl and aromatic ether absorption. Decarboxylation resulted in the formation of carbon dioxide (one mole) and a neutral compound, m.p. 112–113°. The latter was identified as 4-chloro-7-methoxy-3-methylphthalide, *anal.* Calcd. for $C_{10}H_9O_3Cl$: C, 56.5; H, 4.24; Cl, 16.7. Found: C, 56.6; H, 4.5; Cl, 16.75. Thus, the monobasic acid was 4-chloro-7-methoxy-3-methylphthalide-3-carboxylic acid.

When 4-chloro-7-methoxy-3-methylphthalide was oxidized with alkaline permanganate, 6-chloro-3-methyloxyphthalonic acid or 6-chloro-3-methoxyphthalic acid was obtained, depending on whether the manganese dioxide was filtered off before or after acidification. The former compound melted at 224–227° (dec.), *anal.* Calcd. for $C_{10}H_7O_6Cl$: C, 46.4; H, 2.71; Cl, 13.7. Found: C, 46.8; H, 3.12; Cl, 13.9; C-CH_3, 0.0. If the oxidation was carried out in neutral solution, 3-hydroxy-3-methyl-4-chloro-7-methoxyphthalide, m.p. 198–293°, *anal.* Calcd. for $C_{10}H_9ClO_4$: C, 52.5; H, 3.94; Cl, 15.54; OCH_3, 13.6; C-CH_3, 6.6. Found: C, 52.65; H, 4.47; Cl, 15.26; OCH_3, 10.36; C-CH_3, 6.13, was formed. Methylation of this compound yielded a normal ester, m.p. 69–70°, and a pseudo ester, m.p. 188–190°.

The oxidation residues further yielded a dibasic acid, m.p. 211–212°, $[\alpha]^{25}D$ −20.2 (ethanol), *anal.* Calcd. for $C_{14}H_{13}O_7Cl$: C, 51.1; H, 3.96; Cl, 10.8; OCH_3, 9.45; C-CH_3, 4.56. Found: C, 51.1; H, 5.54; Cl, 10.7; OCH_3, 9.24; C-CH_3, 4.53. An anhydride, m.p. 209–210°, was readily formed when the dibasic acid was heated in acetic anhydride. Ultraviolet and infrared absorption spectra indicated the presence of the phthalide nucleus in both the acid and the anhydride. The typical absorption bands of the latter compound at 5.3 and 5.6 microns further suggested the presence of a succinic acid moiety. The dibasic acid was postulated to be 4-chloro-7-methoxy-3-methylphthalide-3-succinic acid.

The synthesis[3] of the above compounds unequivocally prove the assigned structures.

Finally, a second dibasic acid, m.p. 203–204°, *anal.* Calcd. for $C_{15}H_{15}O_7Cl$: C, 52.6; H, 4.38; Cl, 10.4; C-CH_3, 4.40; OCH_3, 9.58. Found: C, 52.3; H, 4.81; Cl, 10.5; C-CH_3, 4.89; OCH_3, 9.60, was isolated from the oxidation mixture. The formation of the dimethyl ester, m.p. 108–109.5°, and the anhydride, m.p. 200–201°, established the presence of two carboxylic acid groups. The infrared absorption spectra of the anhydride showed typical bands for glutaric anhydride, in contrast to the bands for succinic anhydride in the previous compound. The ultraviolet absorption spectra of the two dibasic acids were almost identical. The unknown dibasic acid was demethylated with hydrobromic acid to the phenolic acid, m.p. 172.5–175°, and then oxidized with acid permanganate to yield tricarballylic acid. The dibasic acid was, therefore, postulated to be β-(4-chloro-7-methoxy-3-methylphthalide-3)-glutaric acid.

Alkaline fusion of the phthalide derivatives, with the exception of 4-chloro-7-methoxy-3-methylphthalide, gave 5-chloro-2-methoxybenzoic acid.

(2) R. Kuhn and K. Dury, *Chem. Ber.*, **84**, 563 (1951), reported the finding of 5-chlorosalicylic acid and dimethylamine but no ammonia in a similar experiment on aureomycin.

(3) S. Kushner, J. H. Boothe, J. Morton, J. Petisi and J. H. Williams, THIS JOURNAL, **74**, 3710 (1952).

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

B. L. HUTCHINGS
C. W. WALLER
S. GORDON
R. W. BROSCHARD
C. F. WOLF
A. A. GOLDMAN
J. H. WILLIAMS

RECEIVED JUNE 23, 1952

STEROIDS. XXXVII.[1] A TEN STEP CONVERSION OF PROGESTERONE TO CORTISONE

Sir:

Taking advantage of our recently described[2] methods for the introduction of the 11α-hydroxy group into ring C unsubstituted steroids we have started a program directed at the chemical synthesis of the 11α-hydroxy analogs of the various natural hormones; their application to the case of 11α-hydroxyprogesterone (I) has already been reported.[3] The physical constants of the synthetic product proved to be in excellent agreement with those reported by Peterson and Murray[4] for a substance obtained in 10% yield by the microbiological oxidation of progesterone with the mold *Rhizopus arrhizus* (their strain RH 176) and assigned the 11α-hydroxyprogesterone structure.

(1) Paper XXXVI, J. Romo, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, in press.

(2) G. Stork, J. Romo, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **73**, 3546 (1951); C. Djerassi, O. Mancera, G. Stork and G. Rosenkranz, *ibid.*, **73**, 4496 (1951); C. Djerassi, E. Batres, M. Velasco and G. Rosenkranz, *ibid.*, **74**, 1712 (1952); F. Sondheimer, R. Yashin, G. Rosenkranz and C. Djerassi, *ibid.*, **74**, 2696 (1952).

(3) O. Mancera, J. Romo, F. Sondheimer, G. Rosenkranz and C. Djerassi, *J. Org. Chem.*, **17**, in press (1952).

(4) D. H. Peterson and H. C. Murray, THIS JOURNAL, **74**, 1871 (1952).

We now wish to report that by the use of an as yet unidentified fungus of the *Rhizopus* family (our strain SY 152) isolated[5] from a Mexican soil sample (Molino de Bezares, D.F., Mexico) it has been possible to achieve the oxidation of progesterone to its 11α-hydroxy analog I in 45% yield. In striking contrast to the catalytic hydrogenation of 11-keto[6] and 11β-hydroxy[7] steroids which yields predominantly the 5α(*allo*) dihydro derivative, it was observed that catalytic hydrogenation of I with palladized charcoal catalyst in ethanol solution preferably in the presence of potassium hydroxide for 30 minutes yields only small amounts of the *allo* isomer,[3] the main product being the *normal* derivative, pregnane-3,20-dione-11α-ol (II) [m.p. 116–118°, $[\alpha]^{20}D$ +91° (all rotations in chloroform), $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free –OH; found: C, 75.97; H, 9.92; *acetate*, m.p. 148–149°. $[\alpha]^{20}D$ +65°, $\lambda_{max}^{CHCl_3}$ 1736, 1720 and 1700 cm.$^{-1}$]. This reversal of the stereochemical course of the catalytic hydrogenation of 11-oxygenated Δ^4-3-ketosteroids in the case of the 11α-epimer thus permits the conversion of ring C unsubstituted precursors (progesterone, diosgenin, stigmasterol) to cortisone by way of the desirable 5β(normal) series.

Chromium trioxide oxidation of II furnished pregnane-3,11,20-trione (m.p. 158–160°, [ ]^{20}D +128°, $\lambda_{max}^{CHCl_3}$ 1702 cm.$^{-1}$, identified by comparison with an authentic specimen,[8] m.p. 160–162°, $[\alpha]^{20}D$ +126°) and reduction of the latter with sodium borohydride in *pyridine solution*[9] smoothly yielded the known[8] pregnane-11,20-dione-3α-ol (m.p. 169–171°, $[\alpha]^{20}D$ + 105°, $\lambda_{max}^{CHCl_3}$ 1700 cm.$^{-1}$ and free –OH, identified by comparison with an authentic sample, m.p. 168–171°, $[\alpha]^{20}D$ +103°) and upon acetylation pregnane-11,20-dione-3α-ol acetate m.p. 134–135°, $[\alpha]^{20}D$ +135°). Experimental details of the further transformations of this substance to cortisone have already been recorded.[10] The consistently high yields, the ready availability of the starting materials and the paucity of steps (ten from progesterone or fourteen from diosgenin) appear to make this combined microbiological-chemical route the best yet described synthesis of cortisone.

RESEARCH LABORATORIES
SYNTEX, S. A.
LAGUNA MAYRAN 413
MEXICO CITY 17, D.F.

O. MANCERA
ALEJANDRO ZAFFARONI
B. A. RUBIN
FRANZ SONDHEIMER
G. ROSENKRANZ
CARL DJERASSI[11]

RECEIVED JUNE 18, 1952

(5) This work was aided by the use of a screening technique based on a color reaction specific for 11α-hydroxyprogesterone (A. Zaffaroni, *et al.*, to be published).

(6) C. Djerassi, G. Rosenkranz, J. Pataki, and St. Kaufmann, *J. Biol. Chem.*, **194**, 115 (1952).

(7) J. Pataki, G. Rosenkranz and C. Djerassi, *ibid.*, **195**, 751 (1952), and references cited therein.

(8) J. von Euw, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **27**, 821 (1944).

(9) In ethanol solution, the product was chiefly the known pregnane-3α,20β-diol-11-one (*cf.* L. H. Sarett, THIS JOURNAL, **70**, 1690 (1948).

(10) T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *ibid.*, **74**, 483 (1952).

(11) Department of Chemistry, Wayne University, Detroit, Michigan.

ON A PHOSPHO-TRI-ANHYDRIDE FORMULA FOR THE NUCLEIC ACIDS

Sir:

Last year Dr. Edward Ronwin[1] suggested a phospho-tri-anhydride formula for the nucleic acids, with as its core a polymer chain of phosphorus atoms held together by oxygen atoms, each phosphorus atom having five oxygen atoms attached to it, of which three bind it to adjacent phosphorus atoms, one is in a hydroxyl group, and one is in a sugar ester group. We then stated[2] that in formulating a hypothetical structure for a substance one must take care that the structural elements of which use is made are reasonable ones or one must show that there is an overwhelming necessity for a radical proposal, that there is no precedent for a structure in which phosphorus is bonded to five oxygen atoms, that in every one of the scores of quinquepositive phosphorus compounds that have been subjected to complete structural investigation the phosphorus atom is surrounded by four oxygen atoms, and that the ligation of five oxygen atoms about each phosphorus atom is such an unlikely structural feature that the proposed phospho-tri-anhydride formula for the nucleic acids deserves no serious consideration.

Dr. Ronwin has now kindly informed us that he has become aware of earlier references in the literature to compounds to which structures have been attributed involving quinquepositive phosphorus bonded to five oxygen atoms or to a total of five oxygen atoms and similar atoms. Anschütz[3] prepared four compounds to which he assigned structures involving ligation of five oxygen atoms to a phosphorus atom. The synthesis of several compounds described as having one oxygen atom and four NHR groups bonded to a phosphorus atom has been reported by Lemoult,[4] and Autenrieth and Meyer[5] have reported similar compounds with two oxygen atoms, two NHR groups, and one SH group presumed to be bonded to a phosphorus atom.

Although there may be some question about the correctness of the structures attributed to some of these compounds, and although no complete structure determination has been made for any of them, the compounds reported by Anschütz may indeed have the structures suggested by him, involving five oxygen atoms ligated to a quinquepositive phosphorus atom. His suggested formulas for the four substances are $P(OC_6H_5)_5$, $PO_2C_6H_4(OC_6H_5)_3$, $P(OC_6H_5)(O_2C_6H_4)_2$, and $P_2(O_2C_6H_4)_5$, in which $O_2C_6H_4$ is the *o*-phenylene group. Our statement that there is no precedent for a structure in which a phosphorus atom is bonded to five oxygen atoms must accordingly be withdrawn.

It is pertinent to the proposed phospho-tri-anhydride formula for the nucleic acids that the four compounds reported by Anschütz are described by him as being extremely sensitive to moisture, so sensitive as to make it impossible to

(1) E. Ronwin, THIS JOURNAL, **73**, 5141 (1951).

(2) L. Pauling and V. Schomaker, *ibid.*, **74**, 1111 (1952).

(3) L. Anschütz, *Ann.*, **454**, 71 (1927).

(4) P. Lemoult, *Compt. rend.*, **141**, 1241 (1905).

(5) W. Autenrieth and W. Meyer, *Ber.*, **58**, 840 (1925).

determine, except roughly, their melting points and other physical properties.

GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY LINUS PAULING
PASADENA 4, CALIFORNIA VERNER SCHOMAKER

RECEIVED JUNE 7, 1952

PARTIAL PURIFICATION AND AMINO ACID CONTENT OF VASOPRESSIN FROM HOG POSTERIOR PITUITARY GLANDS

Sir:

A highly purified vasopressin preparation (400–500 pressor units per mg.) from beef posterior pituitary glands has recently been prepared[1] by countercurrent distribution of concentrates between *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. Analysis of hydrolysates by chromatography on starch columns[2] showed phenylalanine, tyrosine, proline, glutamic acid, aspartic acid, glycine, arginine and cystine in approximately equimolar amounts, plus three moles of ammonia per mole of any one amino acid. The preparation and the amino acid analysis have been verified on several batches of posterior pituitary material of bovine origin.

We wish to report here an unexpected result encountered when pressor concentrates from hog posterior pituitary lobes were used as starting material for vasopressin preparation. A mixture of pressor fractions obtained by a solvent fractionation procedure (fractions "e" and "f" of Kamm, *et al.*[3]) were subjected to a twenty-transfer countercurrent distribution at room temperature in an all-glass machine[4] in the system *s*-butyl alcohol and 0.1% acetic acid. The material from tubes 1–4 inclusive was then submitted to a fifty-transfer countercurrent distribution at 5–10° in the system *n*-butyl alcohol and 0.09 *M* *p*-toluenesulfonic acid. The peak of pressor activity seemed to be in the vicinity of tube 20, which indicated a distribution constant of 0.66. The vasopressin of bovine origin had a distribution constant of 1.25 in this solvent system.[1]

Material from tubes 10–24 inclusive was subjected to a 150-transfer distribution in the same solvent system. Analysis of the distribution pattern by quantitative ninhydrin reaction[5] on aliquots of the lower phase showed a peak at tube 59 (distribution constant 0.65) which corresponded to the peak of pressor activity. The combined material from tubes 54–65 inclusive had a potency of approximately 175 pressor units per mg. This potency probably does not represent the highest obtainable for this principle. We have reason to believe that some inactivation has occurred in the process of working up the material.

Analysis of a hydrolysate of this material by starch column chromatography showed a pattern similar to that of vasopressin of bovine origin except that arginine was absent and a peak occupying the position of lysine was present.[6] If calculated as lysine this peak represented approximately one mole per mole of each of the other amino acids. One-half mg. of this preparation gave a negative Sakaguchi test both before and after acid hydrolysis. The same amount of a purified beef vasopressin preparation or an equimolar amount of arginine gave a strong positive test. Two-dimensional paper chromatograms of a hydrolysate with arginine or lysine added showed clearly that the basic amino acid present was not arginine, and was inseparable from lysine under these conditions. Microbiological assay of a hydrolysate for L-lysine[7] gave a value in reasonable agreement with the value from the starch column analysis.

Efforts are being continued toward further purification of lysine-vasopressin. Additional efforts are being made to ascertain whether lysine-vasopressin can be found in beef glands and arginine-vasopressin in hog glands, or whether this interesting and unexpected result represents a qualitative species difference.

The authors wish to thank Dr. Dorothy S. Genghof for the microbiological determination and Miss Sachi Fujii and Miss Naida Das for their capable assistance.

DEPARTMENT OF BIOCHEMISTRY EDWIN A. POPENOE[8]
CORNELL UNIVERSITY MEDICAL COLLEGE H. CLAIRE LAWLER
NEW YORK, N. Y. VINCENT DU VIGNEAUD[9]

RECEIVED JUNE 16, 1952

(1) R. A. Turner, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **191**, 21 (1951).

(2) S. Moore and W. H. Stein, *ibid.*, **178**, 53 (1949).

(3) O. Kamm, T. B. Aldrich, I. W. Grote, L. W. Rowe and E. P. Bugbee, THIS JOURNAL, **50**, 573 (1928).

(4) L. C. Craig, *Anal. Chem.*, **22**, 1346 (1950).

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(6) It is of interest that *oxytocin* preparations from beef and hog sources had shown no difference in amino acid composition (Pierce, Gordon and du Vigneaud, manuscript in preparation).

(7) L. M. Henderson and E. E. Snell, *J. Biol. Chem.*, **172**, 15 (1948).

(8) Public Health Service Postdoctorate Research Fellow of the National Institutes of Health.

(9) Appreciation is expressed to Lederle Laboratories, American Cyanamid Company for a grant-in-aid and to Parke, Davis and Company and Armour and Company for gifts of material.

STREPTOLIN. THE STRUCTURE AND SYNTHESIS OF ISOLYSINE

Sir:

Hydrochloric–formic acid hydrolysis of the antibiotic streptolin[1] followed by chromatographic separation on Dowex-50 has given five major fractions; the last to be eluted possesses the empirical formula $C_6H_{14}O_2N_2$ for the free base and is designated as "*iso*-lysine." This substance, which is also a hydrolysis product of viomycin[2] and streptothricin,[3] we have characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate)[4] (I), dec. 243.5–244°, $[\alpha]^{25}D$ +6.5 ± 1 (alc.) (found: C, 50.91; H, 5.08; N, 12.00) and the dipicrate,[4] m.p. 200–201° (found: C, 35.38; H, 3.63; N, 18.06).

Isolysine gave a positive hydroxamic acid test[5]

(1) R. W. Rivett and W. H. Peterson, THIS JOURNAL, **69**, 3006 (1947).

(2) T. H. Haskell, S. A. Fusari, R. P. Frohardt and Q. R. Bartz, *ibid.*, **74**, 599 (1952).

(3) H. E. Carter, W. R. Hearn and W. R. Taylor, "Abstracts of Papers" 119th Meeting, American Chemical Society, Cleveland, Ohio, April 1951, p. 25A.

(4) These derivatives have been previously reported by Haskell *et al.* (ref. 2) and Carter, *et al.* (ref. 3).

(5) F. Feigl, "Qualitative Analysis by Spot Tests," Elsevier Publishing Co., Inc., New York, N. Y., 1946, p. 369.

but no cobalt(II) complex and no C-methyl in the Kuhn–Roth determination. The substance did not consume periodate; it yielded succinic acid upon permanganate oxidation. These observations, coupled with the absence of N-alkyl functions in streptolin itself, allow three structural possibilities: (i) β,ϵ-diaminocaproic acid, (ii) γ,ϵ-diaminocaproic acid, and (iii) α-aminomethyl-δ-aminovaleric acid.[6] Through synthesis we have confirmed the first possibility. L-Di-(N-phthaloyl)-ornithine, m.p. 187–188.5°, $[\alpha]^{25}D$ −31.5 ± 0.5° (alc.) (found: C, 63.95; H, 4.09) was homologated *via* the Arndt–Eistert sequence[7] to methyl β,ϵ-diphthalimido caproate (II), m.p. 156–157°, $[\alpha]^{25}D$ −13.5 ± 0.5° (chf.) (found: C, 65.33; H, 4.64). The melting point of II was not changed on admixture with the corresponding derivative (m.p. 155.5–156.5°) from iso-lysine; the infrared spectra of the two substances were identical. Hydrazinolysis[8] and subsequent acid hydrolysis of II afforded β,ϵ-diaminocaproic acid, which was purified and characterized as the di-(*p*-hydroxyazobenzene-*p'*-sulfonate) (III), dec. 242.2–243°, $[\alpha]^{25}D$ +6 ± 1° (alc.) (found: C, 51.31; H, 5.09). The infrared spectra of I and III were indistinguishable, as were the paperstrip chromatograms (R_f = 0.65; developed with phenol–water–formic acid).

Details of the present work as well as syntheses of (ii) and (iii) and the demonstration of their nonidentity with iso-lysine will be published shortly.

This research was supported by grants from The Parke-Davis Co. and The National Institute of Health.

(6) H. E. Carter and associates have independently arrived at similar conclusions (presented at the 120th meeting, American Chemical Society, New York, N. Y., September, 1951).

(7) K. Balenović and D. Fleš, *J. Org. Chem.*, **17**, 347 (1952).

(8) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **71**, 1856 (1949).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN EUGENE E. VAN TAMELEN
MADISON 6, WISCONSIN EDWARD E. SMISSMAN

RECEIVED MAY 22, 1952

BOOK REVIEWS

The Theory of Isotope Separation as Applied to the Large-scale Production of U^{235}. By KARL COHEN, Director, Atomic Energy Division, The H. K. Ferguson Company; formerly Director, Theoretical Division, SAM Laboratories. Edited by George M. Murphy, Washington Square College, New York University; formerly at SAM Laboratories, Columbia University. McGraw-Hill Book Co., Inc., 330 West 42nd Street, New York 18, N. Y. 1951. xviii + 165 pp. 16 × 23.5 cm. Price, $2.00.

This book gives a presentation of the theory of isotope separation by methods in which elementary processes are multiplied to reach large end results. The first five chapters develop the theory of cascades. Applications to centrifuges, two-phase separation, thermal diffusion, and the concentration of deuterium are included in the latter two chapters. The theory has been presented in clear and logical fashion and the numerous tables and very clearly drawn graphs present numerical data in usable form. A wide variety of problems in the design of efficient cascades has been treated. These include necessary deviations from ideality. A variety of types of operation is discussed.

It is probable that the book has suffered because of security restrictions in that examples of the use to which theoretical results can be put are virtually absent. The author has not attempted to compensate for this by elaborating the physical significance of the mathematical relations. It is not until one reaches Chapter 6 on centrifuges that any of the discussions relate to the characteristics of equipment itself. It may be of interest to note that the terms "barrier," "barrier diffusion," and "mass spectrograph" do not appear in the index.

In many respects the derivation of the theory of cascade processes is analogous to the study of thermodynamics since the theory does not depend on the nature of the element. It is based on a fundamental axiom—the conservation of matter—and the consequences are derived through mathematical treatment without reference to physical phenomena. The significance of the results is unfortunately not elaborated.

This book is useful for the engineer who needs specific numerical solutions as well as for the theorist who is interested in the mathematical formulations which have been developed. For the reader having the empirical approach, the book will bear fruit in proportion to the background on cascade processes which he brings to it.

W. R. KANNE

Starch Chemistry. By JIRO NIKUNI (Editor), Osaka University. Asakura Publishing Co., 1–10 Nishiki-Cho, Kanda, Chiyoda Ku, Tokyo, Japan. 1951. 540 pp. Price, regular 1000 Yen ($2.80); special 880 Yen.

The book is edited by Professor Jiro Nikuni of Osaka University and is divided into seven chapters which are contributed by ten men including the editor. The following are the titles of the various chapters: I. General Discussion on Starch Chemistry; II. Metabolism of D-Glucose in the Organism; III. X-Ray Diffraction of Starch; IV. Enzymatic Studies of Starch; V. Fundamental Experimental Methods; VI. Experimental Enzymatic Methods; and VII. Industrial Preparation.

As it stated in the preface, a part of the aim of this publication is to bring up to date the recent advances in starch chemistry which the Japanese missed during the war years. For this reason the material for the most part is probably familiar to Western students. The book is intended primarily for students and research men in Japan. However, the volume undoubtedly will be an excellent source of information and reference to all students in the field of starch chemistry.

THE NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND YOSHIO SATO