COMMUNICATIONS TO THE EDITOR

RELATIONSHIP OF THE FOLINIC ACID GROUP AND THE LEUCONOSTOC CITROVORUM FACTORS

Sir:

The reported nutritional requirement of *Leuconostoc citrovorum* 8081 for thymidine[1] or a factor(s) in refined liver extracts[2] suggested the possibility that the organism required an antipernicious anemia principle. In the isolation of thymidine,[3] this factor and erythrotin,[4] a member of the vitamin B_{12} group and probably identical with vitamin B_{12}, were separated quantitatively. While neither of these fractions possess the activity of the original liver extract for *Leuconostoc citrovorum* in a previously described medium[5] supplemented with pyridoxine, the two fractions on recombination possessed the full activity. The thymidine containing fraction could be replaced by 0.03–0.1 γ per cc. of crystalline thymidine while the erythrotin-containing fraction could not be replaced by crystalline erythrotin. However, concentrates of the folinic acid[6] were found to be highly effective in replacing the erythrotin fraction, and the relative potencies of these fractions determined by *Lactobacillus casei* test and this modified test parallelled closely.

A concentrate of folinic acid 200,000 times as active as an enzymatic digest of liver in the *Lactobacillus casei* test, elicited a half-maximal response in the *Leuconostoc citrovorum* test at 0.0001–0.0002 γ per cc. In the absence of thymidine, 0.001 γ per cc. of this factor was required for the same response. Thymidine at a concentration of 10–20 γ per cc. can also replace the folinic acid group; consequently, the synergistic action of the folinic acid group and thymidine in stimulating the growth of the organism resulted in the high activity of purified liver extracts which we have previously found to contain large amounts of thymidine.[3]

Other factors associated with the folinic acid appear to be effective for this organism, but folic acid is essentially inactive under our testing conditions. Very mild acid hydrolysis destroys folinic acid but forms a compound with biological activities corresponding to folic acid.

This synergistic action and interchangeability of thymidine and the folinic acid group indicate the functioning of this group in the biosynthesis of thymidine as well as further involvement of thymidine concerning the biosynthesis of the active coenzyme form of folinic acid for this organism.

THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH AUSTIN, TEXAS

THOMAS J. BARDOS
THOMAS J. BOND
JEAN HUMPHREYS
WILLIAM SHIVE

RECEIVED SEPTEMBER 23, 1949

(1) Snell, *et al.*, *J. Biol. Chem.*, **175**, 473 (1948).
(2) Sauberlich and Baumann, *ibid.*, **176**, 165 (1948).
(3) Shive, *et al.*, THIS JOURNAL, **70**, 2299 (1948).
(4) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. Science, Feb., 1949.
(5) Snell, *et al.*, *J. Biol. Chem.*, **143**, 519 (1942).
(6) Bond, *et al.*, THIS JOURNAL, **71**, 3852 (1949).

THE FOLINIC ACID GROUP, A SERIES OF NEW VITAMINS RELATED TO FOLIC ACID

Sir:

By application of *inhibition analysis* to a study of factors functionally related to *p*-aminobenzoic and folic acids, testing procedures for a wide variety of new factors occurring in refined liver extracts have been developed.[1,2] One of these methods developed about two years ago, involved the prevention of the toxicity of methylfolic acid for *Lactobacillus casei* in a previously described medium[3] supplemented with thymine, purines, folic acid (0.001 γ per cc.) and methylfolic acid (1 γ per cc.). Under these conditions, only the folic acid group was known to prevent competitively the toxicity of methylfolic acid. However, liver extracts, both crude and refined, which prevent the toxicity in a competitive manner, are approximately 15 times as active as can be accounted for on the basis of their folic acid content determined by assay with *Lactobacillus casei* in the absence of the inhibitor. A similar technique has been employed with *Streptococcus faecalis* R in demonstrating an unusual activity for formylfolic acid.[4]

With the aid of this assay based on this differential in activity, one of the active principles has been concentrated more than 200,000 fold from enzymatic digests of hog liver. A half-maximal response of *Lactobacillus casei* is obtained in the presence of 0.002 γ per cc. of the concentrate which is somewhat more active than folic acid under these testing conditions. Depending upon the time of incubation and response at which the comparison is made, the concentrate is from 10 to 100 times as active as folic acid in preventing the toxicity of methylfolic acid (1 γ per cc.) for *Streptococcus faecalis* R. On the basis of structure and functional relationship to folic acid, this active principle has been termed folinic acid. On the basis of estimated purity of the concentrate, folinic acid does not appear to be less active than folic acid in promoting the growth of either organism in the absence of the inhibitor.

(1) Shive, *et al.*, THIS JOURNAL, **70**, 2299 (1948).
(2) Shive, *Ann. New York Acad. Science*, in press, presented before the New York Acad. of Science, Feb., 1949.
(3) Rogers and Shive, *J. Biol. Chem.*, **172**, 100, 751 (1947).
(4) Gordon, *et al.*, THIS JOURNAL, **70**, 878 (1948).

While folinic acid accounts for the major portion of the activity of extracts of hog liver, another factor with similar physical and biological properties occurs in these extracts. At least two other substances possessing activity in the assay have been detected by means of paper chromatography. Consequently, it appears that a group of compounds, the folinic acid group, possess activity similar to that of folinic acid.

Since the folinic acid group is utilized more effectively than folic acid for several organisms, the possibility exists that it may be more active than folic acid in the treatment of sprue, nutritional and pernicious anemia, and other nutritional deficiencies related to the folic acid and vitamin B_{12} groups.

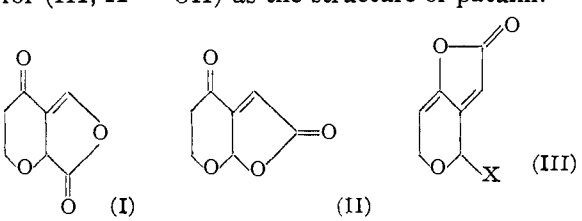
THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH, AUSTIN, TEXAS
THOMAS J. BOND
THOMAS J. BARDOS
MARGARET SIBLEY
WILLIAM SHIVE

RECEIVED SEPTEMBER 23, 1949

THE STRUCTURE OF PATULIN

Sir:

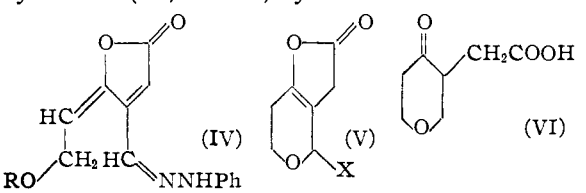
Recent evidence has required revision of the accepted structure (I)[1] of the antibiotic mold metabolite, patulin, and two new formulations, (II)[2] and (III, X = OH),[3] have been advanced. The following data now afford additional strong support for (III, X = OH) as the structure of patulin.



(I) (II) (III)

Structure (III, X = OH) possesses three structural characteristics: free hydroxyl group, lactal ring and doubly-unsaturated lactone system. Presence of a free O–H band (2.73μ), in the infrared spectrum of patulin and its absence in patulyl acetate (III, X = OAc) and in patulyl chloride (III, X = Cl), retention of the characteristic double bond ultraviolet and infrared spectra of patulin in these derivatives (Patulin: u.v., 275 mμ, log ϵ 4.22; ir., 5.58μ, 5.94μ, 6.11μ. Acetate: u.v., 277 mμ, log ϵ 4.24; ir., 5.58μ, 5.93μ, 6.11μ. Chloride: u.v., 277 mμ, log ϵ 4.18; ir., 5.61μ, 5.94μ, 6.13μ), and conversion of each in high yield to patulin phenylhydrazone by aqueous phenylhydrazine indicate the presence of a non-enolic hydroxyl group and exclude occurrence of enolization or isomerization during their preparation.

Patulin shows reactions (negative Schiff, positive Tollens, positive Fehling)[1] characteristic of a lactal ring. Lactal ring opening by phenylhydrazone formation usually unmasks a hydroxyl group and the conversion of patulin phenylhydrazone (IV, R = H) by treatment with sodium



(IV) (V) (VI)

acetate–acetic anhydride to patulin phenylhydrazone acetate (IV, R = Ac), m. p. 143° (calcd. for $C_{15}H_{14}O_4N_2$: C, 63.00; H, 4.93. Found: C, 63.40; H, 5.27) fits this interpretation. Infrared spectra of patulin phenylhydrazone (5.86μ, 6.04μ, 6.23μ) and its acetate (5.84μ, 6.00μ, 6.22μ) indicate retention of the doubly-unsaturated lactone system in these derivatives. Demonstration of a lactone ring in the phenylhydrazone and its acetate is shown by consumption of 1.05 and 1.92 equivalents, respectively, of sodium hydroxide. Dihydropatulin (V, X = OH) phenylhydrazone[4] contains only a singly-unsaturated lactone system (u.v., 380 mμ, log ϵ 4.55; 1.07 equivalents sodium hydroxide).

Treatment of patulin with warm excess thionyl chloride followed by sublimation furnishes unstable patulyl chloride (III, X = Cl) in 78% yield, m. p. 92–94° (calcd. for $C_7H_5O_3Cl$: C, 48.70; H, 2.92; Cl, 20.55. Found: C, 48.94; H, 2.63; Cl, 20.43); structural evidence given above. Patulyl chloride in anhydrous dioxane with palladium–barium sulfate catalyst absorbs 2.0 of moles hydrogen in two hours to give a neutral fraction which furnishes on distillation oily dihydrodesoxypatulin (V, X = H) in 34% yield, b. p. 90–95° (0.5 mm.). (Calcd. for $C_7H_8O_3$: sapon. equiv. 140.1. Found: 141.2); immediate Legal test; u.v., at 212 mμ, log ϵ 3.93; ir., 5.57μ, 6.01μ. Accordingly, dihydrodesoxypatulin contains a β,γ-unsaturated-γ-lactone system and its exact structure is established by hydrolysis in aqueous alcoholic sodium hydroxide to dihydrodesoxypatulinic acid (VI), identified by its well-known derivatives[2,3,4,5]: 2,4-dinitrophenylhydrazone, m. p. 193–195°; methyl ester 2,4-dinitrophenylhydrazone, m. p. and m. m. p. 149–150° (calcd. for $C_{14}H_{16}O_7N_4$: C, 47.70; H, 4.58. Found: C, 47.50; H, 5.02); *p*-phenylphenacyl ester, m. p. 124–127°.

DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING
UNIVERSITY OF WASHINGTON
SEATTLE 5, WASHINGTON
HYP J. DAUBEN, JR.
FRANK L. WEISENBORN

RECEIVED SEPTEMBER 9, 1949

(1) Birkinshaw, Bracken, Michael and Raistrick, *Lancet*, **245**, 625 (1943); *cf. Quart. Revs. Chem. Soc.*, **2**, 53 (1948).

(2) Engel, Brzecki and Plattner, *Helv. Chim. Acta*, **32**, 1166, 1752 (1949).

(3) Woodward and Singh, THIS JOURNAL, **71**, 758 (1949).

(4) Bergel, Morrison, Moss and Rinderknecht, *J. Chem. Soc.*, 415 (1944).

(5) Acknowledgments are made gratefully to Professor Raistrick and the Therapeutic Research Corporation of Great Britain for the supply of patulin, to Professor Woodward and Dr. Singh for helpful discussions, spectral determinations on the phenylhydrazone derivatives and an authentic sample of the methyl ester dinitrophenylhydrazone, and to E. I. du Pont de Nemours and Co. for the Fellowship granted to one of us (F. L. W.).

POLARIZED INFRARED SPECTRA FOR SILKWORM-GUT AND OTHER FIBROUS PROTEINS

Sir:

Infrared spectra observed with anisotropic specimens in polarized light depend upon their orientations relative to both direction of incidence and plane of polarization of the light. Spectra for different orientations of the same specimen can be correlated with structure and arrangement of its molecules. We have been employing this technique to study fibrous proteins. A recent account[1] of results obtained in this way by others encourages us to report some of our own findings at this time.

Polarized spectra for silkworm-gut, a material giving[2] the X-ray diffraction pattern characteristic of fibroin, are shown in Fig. 1. Each spectrum was traced from original instrumental records of *per cent.* transmission through two different samples, one about twenty, another about five microns thick, at left and right, respectively.

These spectra indicate an arrangement that is highly ordered in some respects. The degree of polarization exhibited by several bands approaches the best observed here for single crystals of favorably arranged, simple molecules, exceeding that for the other fibrous proteins we have examined.

The *rachis* of seagull feather exhibited many features appearing in Fig. 1 while quill from pigeon feather was polarized perceptibly at the lower frequencies only. Several natural collagens gave spectra similar to ones described above, with polarizations varying vicariously like ones for feather keratins. Films of rabbit myosin, oriented[3] so as to produce the α-keratin structure, and porcupine quill, showed only small effects of different sort.

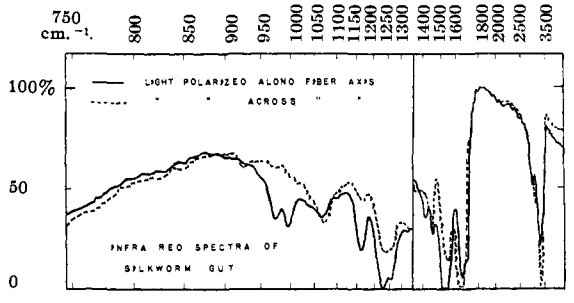


Fig. 1.

Our findings are confirmed in part by those of Ambrose, Elliott and Temple,[1] who worked just in the limited spectral range between 2800 and 3500 cm.$^{-1}$. There they found only slight differences for myosin (91% *vs.* 87% absorption, recalculated from their optical densities) and even smaller ones for porcupine quill and tropomyosin. They report larger effects, however, with feather keratin, as do we.

A full report will be submitted when our work has been completed.

DEPARTMENT OF CHEMISTRY
COLUMBIA UNIVERSITY MARTIN GOLDSTEIN
NEW YORK, N. Y. RALPH S. HALFORD

RECEIVED JULY 25, 1949

(1) E. J. Ambrose, A. Elliott, and R. B. Temple, *Nature*, **163**, 859 (1949).

(2) R. S. Bear, THIS JOURNAL, **66**, 2043 (1944).

(3) W. T. Astbury and S. Dickinson, *Proc. Roy. Soc.* (*London*), **B129**, 307 (1940).

USE OF *p*H INDICATORS WITH ION EXCHANGE RESINS

Sir:

I wish to report the use of *p*H indicators as a means of detecting acids adsorbed on a strong base type resin, Amberlite I.R.A 400.[1]

When the basic form of this resin is treated with phenolphthalein the indicator is quantitatively removed from solution and the resin becomes the characteristic deep pink. Washing has no effect and even strong acid or base will not elute the indicator. Acid decolorizes the resin but base restores the color. If the resin is left for a few days in neutral solution, however, the color is destroyed and base will not restore it. When methyl orange is adsorbed the characteristic color is given with hydrogen ion. Washing removes the color. Again neither strong acid or base will elute the indicator.

The following experiment on the determination of I.R.A 400 capacity for aspartic acid will serve to illustrate the use of a column indicator: Aspartic acid solution was exchanged on the basic form of the resin. The column was washed and 50–100 drops of 1% phenolphthalein passed through. The top of the column remained colorless and a pink zone formed at the junction of the aspartic acid and unreacted resins. The columns were micro burets containing 5–6 g. of 20–30 mesh resin. The acid was 4 mg./ml. and a flow rate of 0.2 ml./min. was used. The value obtained was: 1.5 cc. wet R:NOH resin = 100 mg. aspartic acid.

The method gave a visual illustration of resin efficiency. Not one colored particle was located in the aspartic resin. The pink zone was perfectly even. There was no measurable displacement of aspartic by phenolphthalein.

(1) Rohm & Haas Co.

1922 STEPHENS ST., NO. 5
VANCOUVER, B. C., CANADA DAVID R. IDLER

RECEIVED SEPTEMBER 19, 1949

THE HYDROLYSIS OF NICOTINYL-L-TYROSYL-HYDRAZIDE BY CHYMOTRYPSIN

Sir:

The recent report that benzoyl-L-tyrosylhydrazide is ineffective as an inhibitor in the chymotrypsin catalyzed hydrolysis of benzoyl-L-tyrosylamide or ethyl ester[1] would lead one to infer

(1) S. Kaufman, H. Neurath and G. W. Schwert, *J. Biol. Chem.*, **177**, 793 (1949).

that the hydrazide analogs of the specific chymotrypsin amide or ester type substrates[2-5] are not hydrolyzed by this enzyme. We therefore wish to point out that at least one of the acylated α-amino acid hydrazides possessing the structural characteristics required of ester or amide type specific chymotrypsin substrates,[2-5] *i. e.*, nicotinyl-L-tyrosylhydrazide is hydrolyzed by this enzyme (Table I).

TABLE I

HYDROLYSIS OF NICOTINYL-L-TYROSYLHYDRAZIDE BY CHYMOTRYPSIN

t, min.	Hydrolyis, %	$\frac{1}{t}\log\frac{s_0}{s}$
1.2	1.2	0.064
6.6	7.0	.064
10.6	10.0	.061
20.3	21.8	.067
61.0	49.4	.064
91.0	63.0	.063

Nicotinyl-L-tyrosylhydrazide, m.p. 242–243° (cor.)(*Anal.* Calcd. for $C_{15}H_{16}O_3N_4$; C, 60.0; H, 5.4; N, 18.7. Found; C, 59.9; H, 5.3; N, 18.6) was prepared from nicotinyl-L-tyrosine ethyl ester, m.p. 147–149° (cor.) obtained by the acylation of L-tyrosine ethyl ester with nicotinyl azide.[6] The enzymatic hydrolysis was conducted at 25° and *p*H 7.9 (0.02 *F* ethylenediamine–hydrochloric acid buffer) with an initial substrate concentration, s_0 of 5.0 micromoles per ml. reaction mixture and an initial enzyme concentration, E_0, of 0.075 mg. protein nitrogen per ml. reaction mixture. A formol titration was used to determine the extent of hydrolysis.

(2) M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 405 (1937).
(3) J. S. Fruton and M. Bergmann, *ibid.*, **145**, 253 (1942).
(4) J. E. Snoke and H. Neurath, *Arch. Biochem.*, **21**, 351 (1949).
(5) S. Kaufman and H. Neurath, *ibid.*, **21**, 437 (1949).
(6) T. Curtius and E. Mohr, *Ber.*, **31**, 2493 (1898).

CONTRIBUTION NO. 1335 R. V. MACALLISTER
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA CARL NIEMANN

RECEIVED SEPTEMBER 29, 1949

SEPARATION OF COLUMBIUM AND TANTALUM WITH ANION EXCHANGE RESINS[1]

Sir:

In a previous communication[2] an experiment was described indicating a partial separation of zirconium and hafnium on an anion exchange column in HCl–HF mixtures. While this separation was unusually difficult, the separation of the adjacent elements columbium and tantalum by the same method, under similar conditions, was very efficient.

The experiments were carried out with a 12.5-cm. column (0.0226 sq. cm. cross-section) of the anion exchange resin Dowex-1 using columbium[95] (β-emitter $T_{1/2}$ = 35 days[3]) and tantalum[182] (β-emitter $T_{1/2}$ = 117 days[3]). The columbium was carrier-free fission product and the tantalum was prepared by a neutron bombardment of tantalum metal. In a typical experiment, the tracers were added to the column in a mixture of 9 *M* HCl and 0.05 *M* HF and elution carried out in the same medium at an average flow rate of *ca.* 0.3 ml./sq.cm./min.

The results are shown in Fig. 1 which represents a transcribed continuous record of the activity of the eluent. The bands were identified by standard radiochemical procedures.

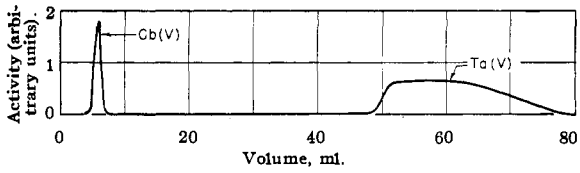


Fig. 1.—Separation of columbium (V) and tantalum (V) by anion exchange: dowex-1 column 12.5 cm. length 0.0226 sq. cm. cross-section, average flow rate; 0.3 ml./sq. cm./min.

Columbium eluted relatively rapidly in a sharp, well-shaped band and the tantalum very much more slowly in a somewhat diffuse band with a sharp front edge. The separation appears to be complete and could probably be achieved with better than 99% purity of the fractions using columns of considerably shorter length.

The experiments prove that both columbium and tantalum can form negatively charged complexes in this medium with probable negative charge minus two or greater. From the slower elution rate of the tantalum one can conclude that the average negative charge on this element is greater than that on columbium.

The very large difference in elution behavior of these two elements is somewhat surprising since both elements, in some complexes at least, show practically the same size. Thus Hoard[4] found no significant difference in the lattice constants of the complex fluorides K_2CbF_7 and K_2TaF_7. The rather large difference in the behavior of these elements on anion-exchangers may thus be due to comparatively large differences in their polarizability, causing considerable differences in the chloride complex constants, or to small differences in the value of each stability constant with a resulting large difference in the negatively charged series due to the fact that for these the product of a considerable number of such constants is involved.

OAK RIDGE NATIONAL LABORATORY KURT A. KRAUS
OAK RIDGE, TENNESSEE GEORGE E. MOORE

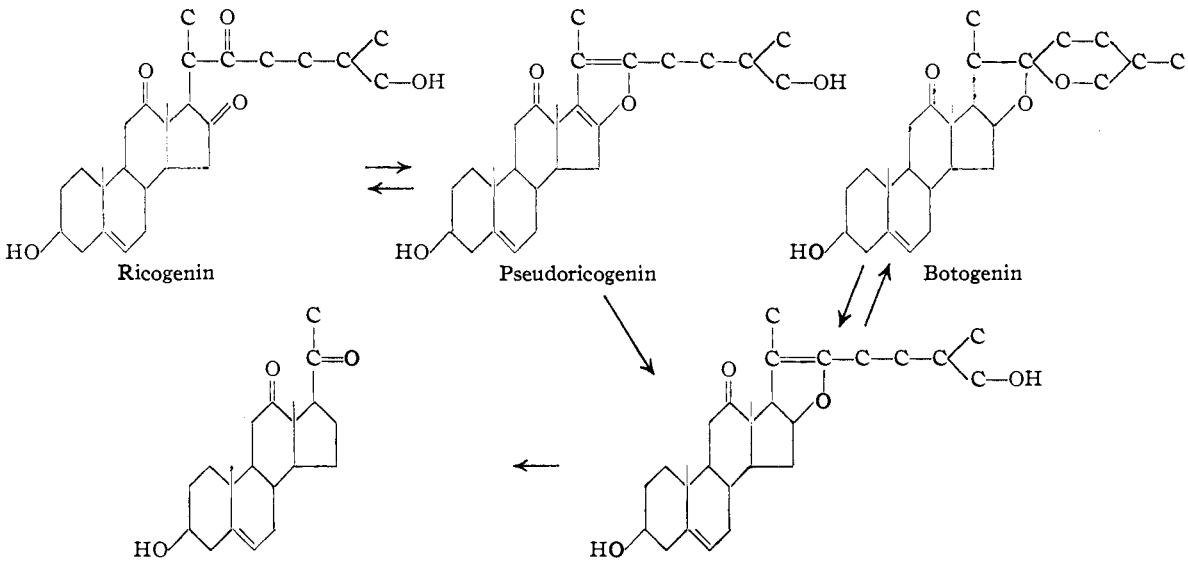
RECEIVED AUGUST 20, 1949

(1) This document is based on work performed under Contract Number W-7405 eng 26 for the Atomic Energy Project at Oak Ridge National Laboratory.
(2) K. A. Kraus and G. E. Moore, THIS JOURNAL, **71**, 3263 (1949).
(3) Information from G. T. Seaborg and I. Perlman, "Table of Isotopes," *Rev. Mod. Phys.*, **20**, 585 (1946).
(4) J. L. Hoard, THIS JOURNAL, **61**, 1252 (1939).

STEROIDAL SAPOGENINS, 173. 16-DEHYDROPREGNEN-5-OL-3-DIONE-12,20 FROM RICOGENIN, A NEW STEROIDAL SAPOGENIN

Sir:

In an extensive search for naturally occurring steroidal sapogenins having substituents in ring C which may be utilized for the synthesis of cortisone, the anti-arthritic hormone, a new saponide, riconin, m.p. 285–289° dec., was isolated from the mixture of glycosides occurring in *Dioscorea Macrostachya*.



Hydrolysis of riconin with alcoholic hydrochloric acid gave ricogenin, m.p. 225–227°. *Anal.* Calcd. for $C_{27}H_{40}O_5$: C, 72,9; H, 9.1. Found: C, 72.9; H, 9.1.

Ricogenin formed a diacetate, m.p. 195–197°, and contains three ketonic groups having the same side-chain structure as kryptogenin. *Anal.* Calcd. for $C_{31}H_{44}O_7$: C, 70.4; H, 8.4. Found: C, 70.2; H, 8.2.

Treatment of ricogenin with acetic anhydride at 195° for eight hours followed by hydrolysis gave pseudoricogenin, m.p. 220–222°. *Anal.* Calcd. for $C_{27}H_{38}O_4$: C, 76.0; H, 9.0. Found: C, 76.2; H, 9.0.

When heated with alcoholic hydrochloric acid for fifteen minutes, pseudoricogenin was converted into ricogenin, m.p. and mixed m.p. 225–227°. Catalytic reduction of the diacetate of pseudoricogenin, using palladium-on-barium sulfate as catalyst, saturated only the conjugated double bond in ring D, giving the diacetate of pseudobotogenin. This product upon alkaline hydrolysis followed by isomerization with alcoholic hydrochloric acid gave botogenin, m.p. and mixed m.p. 260–262°. *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.7; H, 9.4. Found: C, 75.5; H, 9.4.

Acetylation of this product gave botogenin acetate, m.p. and mixed m.p. 246–248°. *Anal.* Calcd. for $C_{29}H_{42}O_5$: C, 74.0; H, 9.0. Found: C, 74.1; H, 9.3.

The pseudobotogenin diacetate produced by the catalytic reduction of the diacetate of pseudoricogenin was oxidized with chromic anhydride in acetic acid, followed by hydrolysis,[1] giving 16-dehydropregnen-5-ol-3-dione-12,20 acetate, m.p. and mixed m.p. with the product prepared from naturally occurring botogenin, 225–227°. *Anal.* Calcd. for $C_{29}H_{38}O_4$: C, 74.8; H, 8.2. Found: C, 74.6; H, 81.

(1) Marker, THIS JOURNAL, 71, 2656 (1949).

BOTANICA-MEX, S. A.
PLAZA DE SAN PABLO NO. 6 RUSSELL E. MARKER
TEXCOCO, MEXICO HOTEL GENEVE, MEXICO CITY

RECEIVED OCTOBER 4, 1949

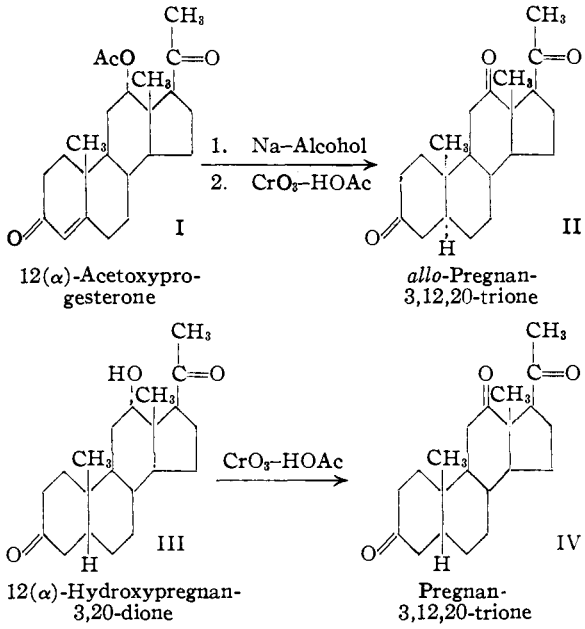
ALLO-PREGNAN-3,12,20-TRIONE

Sir:

The current interest in Kendall's substance E for rheumatoid arthritis has stimulated great interest in the search for starting materials for its synthesis. Recently, Marker reported the possibility of utilizing a steroidal sapogenin, botogenin, isolated from *Dioscorea Mexicana*.[1] Its degradation product was 5-pregnen-3(β)-ol-12,20-dione, characterized by conversion to *allo*-pregnan-3,12,-20-trione, m.p. 264°. The latter was identical with *allo*-pregnan-3,12,20-trione[2] from the degradation of hecogenin.

We have prepared an authentic sample of *allo*-pregnan-3,12,20-trione by an entirely different route and have found it completely different from the trione from hecogenin. Desoxycholic acid has been degraded to 12(α)-acetoxyprogesterone (I), m.p. 181°, $[\alpha]^{26}D$ +215°, $[\alpha]^{25}_{5461}$ +259° (chloroform), absorption maximum at 240mμ(log ε 4.14 in ethanol).[3] This compound (I) upon sodium–alcohol reduction followed by chromic acid oxidation furnished *allo*-pregnan-3,12,20-trione (II), m.p. 206–208°, $[\alpha]^{23}D$ +184°, $[\alpha]^{23}_{5461}$ +224° (chloroform), no maximum at 240mμ. *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.0; H, 8.9. The reduction was also accomplished with hydrogen and Adams catalyst in acetic acid; subsequent hydrolysis and oxidation gave the same product (II). The course of these methods of reduction has been shown previously[4]

(1) Marker, THIS JOURNAL, 71, 2656 (1949).
(2) Marker, Wagner and co-workers, *ibid.*, 69, 2167 (1947).
(3) Shoppee and Reichstein, *Helv. Chim. Acta*, 24, 351 (1941).
(4) Marker and Wittle, THIS JOURNAL, 59, 2704 (1937); Butenandt and Fleischer, *Ber.*, 68, 2094 (1935).



12(α)-Acetoxyprogesterone — *allo*-Pregnan-3,12,20-trione

12(α)-Hydroxypregnan-3,20-dione — Pregnan-3,12,20-trione

to lead to the *allo*-configuration at C-5. Nevertheless, we have prepared the corresponding isomer, pregnan-3,12,20-trione (IV), by the mild oxidation of an authentic sample of 12(α)-hydroxypregnan-3,20-dione (III). It had the following properties: m.p. 204–206°, $[\alpha]^{26}D$ +181°, $[\alpha]^{26}_{5461}$ +225° (chloroform). *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.3; H, 9.2. Found: C, 76.0; H, 9.1. Reichstein and von Arx[5] report for pregnan-3,12,20-trione: m.p. 201–202°; $[\alpha]^{17}D$ +182 ± 7, $[\alpha]^{17}_{5461}$ +219 ± 8 (ethanol). A mixture of IV with II showed a melting point depression of 36°. The melting point of each of these compounds was depressed 10–20° by the trione from hecogenin.

Since the properties of *allo*-pregnan-3,12,20-trione (II) are different from those of the samples derived from hecogenin and botogenin, some doubt must be entertained as to the structures of the degradation products from both of these sapogenins.

We thank Parke, Davis and Company for their help.

(5) Reichstein and von Arx, *Helv. Chim. Acta*, **23**, 747 (1940).

THE WHITMORE LABORATORIES
SCHOOL OF CHEMISTRY AND PHYSICS — R. B. WAGNER
THE PENNSYLVANIA STATE COLLEGE — JAMES A. MOORE
STATE COLLEGE, PENNSYLVANIA — ROBERT F. FORKER

RECEIVED OCTOBER 10, 1949

SYNTHESES IN THE DIRECTION OF MORPHINE. I. 7-METHOXY- AND 7,8-DIMETHOXY-2-TETRALONE.

Sir:

We wish to report the synthesis of 7,8-dimethoxy-2-tetralone, which may serve as a useful intermediate for elaboration in the direction of morphine and certain of its degradation products,[1] and may open a way for the preparation of physiologically active substances oxygenated at points corresponding to the 3 and 4 positions in morphine. 7-Methoxy-2-tetralone may serve in the syntheses of substances similarly substituted in the 3 position; and is of particular interest in view of the recent report that 3-hydroxymorphinane is a powerful analgesic surpassing morphine in clinical tests[2].

1,2,7-Trimethoxynaphthalene,[3] m.p. 38.5–39.5°, b.p. 133° at 1 mm. (picrate[3], m.p. 113°), gave by reduction[4] with sodium and alcohol, the crystalline ketone, m.p. 76° (*anal.* calcd. for $C_{10}H_8O(OCH_3)_2$: OCH_3, 30.1. Found: OCH_3, 29.5, 29.3), characterized as the semicarbazone, m.p. 191–191.5°, and the 2,4-dinitrophenylhydrazone, m.p. 167° dec. (*anal.* calcd. for $C_{18}H_{18}O_8N_4$: C, 56.0; H, 4.7; N, 14.5. Found: C, 55.7; H, 4.6; N, 15.0, 14.8). The structure of the ketone was shown by oxidation, with alkaline permanganate, to hemipinic acid, identified by its m.p.[5] (177–179°) and by the m.p.[6] (166–167°) and characteristic fluorescence[6] of the pure anhydride.

2,7-Dimethoxynaphthalene similarly[4] gave on reduction 7-methoxy-2-tetralone, m.p. 27–28°, b.p. 124–126° (1.5 mm.); semicarbazone, m.p. 174–176° (*anal.* calcd. for $C_{12}H_{15}O_2N_3$: C, 61.8; H, 6.5. Found: C, 62.1, 62.1; H, 6.4, 6.4); 2,4-dinitrophenylhydrazone m.p. 177–181° (*anal.* calcd. for $C_{17}H_{16}N_4O_5$: C, 57.3; H, 4.5. Found: C, 57.2, 57.5; H, 4.4, 4.6).

(2) Schnider and Grussner, *Helv. Chim. Acta*, **32**, 821 (1939).
(3) Chakravarti and Pasupati, *J. Chem. Soc.*, 1859 (1937).
(4) Cornforth, Cornforth and Robinson, *ibid.*, 689 (1942).
(5) Perkin, *ibid.*, **109**, 922 (1916).
(6) Dobbie and Lauder, *ibid.*, **67**, 19 (1895).

DEPARTMENT OF CHEMISTRY — MILTON D. SOFFER
SMITH COLLEGE — J. CHARLES CAVAGNOL
NORTHAMPTON, MASS. — HILDA E. GELLERSON

RECEIVED OCTOBER 19, 1949

DEGRADATION OF GLYCOGEN TO ISOMALTOSE

Sir:

Methylation studies[1] have indicated that the glycogen molecule has a highly ramified structure composed of α-D-glucopyranosyl units joined 1,4 with branching at C6 on one out of twelve units. As additional evidence in support of this structure we report the isolation of crystalline 6-α-D-glucopyranosyl-β-D-glucopyranose octaacetate (β-D-isomaltose octaacetate)[2] from an acetylated acid hydrolysate of glycogen.

Animal (rabbit liver) glycogen (5.00 g., $[\alpha]^{25}D$ +200°, *c* 0.92, water) in 2% concentration was hydrolyzed at 100° in 0.05 *N* sulfuric acid for nine hours (degree of hydrolysis *ca.* 75%). After acid neutralization with barium carbonate and ion removal with exchange resins (Amberlite IR-100 and IR-4), the amorphous solid obtained on solvent removal was acetylated with hot acetic anhydride and sodium acetate. The resultant sugar acetate mixture (6.08 g.) was chromatographed[2] on Magnesol–Celite under such developmental conditions that monosaccharides were removed from the column. β-D-Glucose pentaacetate was identified,

(1) Fieser and Holmes, THIS JOURNAL, **60**, 2548 (1938); **58**, 2319 (1936); Cahn, *J. Chem. Soc.*, 2565 (1926).

(1) W. N. Haworth and E. G. W. Percival, *J. Chem. Soc.*, 2277 (1931); W. N. Haworth, E. L. Hirst and F. Smith, *ibid.*, 1914 (1939).

(2) M. L. Wolfrom, L. W. Georges and I. L. Miller, THIS JOURNAL, **69**, 473 (1947); **71**, 125 (1949).

by melting point and rotation, in the effluent. The material from the lowest zone consisted of β-D-maltose octaacetate (m. p. 158–160°, unchanged on admixture with an authentic specimen; $[\alpha]^{25}D$ +62.5°, *c* 1.1, chloroform). The material from the next higher zone was rechromatographed in the same manner, and the eluent from the lower zone which crystallized from ethanol was identified as β-D-isomaltose octaacetate (m. p. 144–145°, unchanged on admixture with an authentic specimen; $[\alpha]^{26}D$ +98°, *c* 1.0, chloroform); yield 92 mg.

DEPARTMENT OF CHEMISTRY
THE OHIO STATE UNIVERSITY M. L. WOLFROM
COLUMBUS 10, OHIO A. N. O'NEILL[3]

RECEIVED OCTOBER 8, 1949

(3) Corn Industries Research Foundation Fellow in the Department of Chemistry.

ERYTHEIN AND APOERYTHEIN AND THEIR RELATION TO THE ANTIPERNICIOUS ANEMIA PRINCIPLE

Sir:

Normal gastric juice has been found to contain a non-dialyzable, heat labile substance which combines, apparently stoichiometrically, with erythrotin,[1] (vitamin B_{12})[2] to form a complex (erythein) which is non-dialyzable and not dissociated by dialysis. Erythrotin in this combination is not available to microörganisms (*Escherichia coli*, *Lactobacillus lactis Dorner*, *Lactobacillus leichmannii*), but is released by heat, much as biotin is released from avidin, whereupon it is again microbiologically active. Heated gastric juice contains no principle capable of combining with erythrotin.

Quantitative determination of heat labile principle (apoerythein) is readily performed by measuring in an erythrotin assay (*Escherichia coli*)[1] the inhibition of growth resulting when aliquots of the juice are added (unheated) to cultures containing just sufficient erythrotin to elicit a maximum response. The erythrotin combining capacities (millimicrograms of erythrotin per ml. of secretion) of samples of gastric juice from normal and anemic subjects were found to be respectively, 20, 60[3], 60, 15; and 5, <5,[3] <1, <1 >15.

Commercial preparations of hog gastric mucosa made for therapeutic use have been found to be rich in a principle which appears on the basis of chemical and biological properties to be analogous to the apoerythein in gastric juice. Other biological materials tested, including commercial pepsins, contain very little or none of the active substance. Less than 2000 parts by weight of a concentrate prepared from hog gastric mucosa completely counteracted consistently the microbiological growth stimulation of one part of erythrotin.

For preparative purposes hog gastric mucosa has been used, and the principle can be precipitated from an aqueous extract by alcohol, acetone or ammonium sulfate (80% saturation). The principle is highly selective in its action and inactivates erythrotin but does not diminish the biological action of the end-products of erythrotin-catalyzed processes which can substitute for this vitamin in microbiological assays—methionine (*Escherichia coli* test)[1] and desoxyribosides (*Lactobacilli* tests).[4]

The complex formed when erythrotin combines with apoerythein decomposes upon heating (120° fifteen minutes) into erythrotin (or a compound which cannot be distinguished from it biologically or chromatographically), and a residue no longer possessing the ability to bind erythrotin. In combined form erythrotin is not as susceptible to destruction by alkaline or oxidative treatments which inactivate the unbound vitamin, since heat liberation following such treatment of the complex yields the original erythrotin activity.

These experiments point to the probability that apoerythein is the intrinsic factor of Castle or an important component thereof. Clinical trials are now in progress to test this conclusion.

We are deeply indebted to Dr. William Shive for generous supplies of erythrotin before vitamin B_{12} was available and for prepublication disclosures concerning erythrotin tests, and to Dr. Edward Campbell, Eli Lilly and Company, who furnished biological preparations and gastric samples.

THE BIOLOGICAL INSTITUTE AND
THE DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF TEXAS, AND
THE CLAYTON FOUNDATION FOR JESSIE L. TERNBERG
RESEARCH, AUSTIN, TEXAS ROBERT E. EAKIN

RECEIVED SEPTEMBER 20, 1949

(1) W. Shive, *Ann. N. Y. Acad. Sci.*, in press.
(2) E. L. Rickes, *et al.*, *Science*, **107**, 396 (1948).
(3) Pooled samples from at least three subjects.
(4) W. Shive, J. M. Macow and R. E. Eakin, THIS JOURNAL, **70**, 2614 (1948).