out by the fact that a simple visual examination of iodine solutions of equal concentration in benzene, toluene, *o*-xylene, mesitylene and α-methylnaphthalene shows that the color shifts stepwise in that order ending with a brown solution. Preliminary measurements of the absorption spectra of these iodine solutions show an absorption band in the ultraviolet region similar to that of benzene.

This work is being continued and a complete report of the results will be given in a paper soon to be submitted for publication.

DEPARTMENT OF CHEMISTRY
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RECEIVED JULY 15, 1948

β-PELTATIN, A NEW COMPONENT OF PODOPHYLLIN

Sir:

The fractionation of the drug podophyllin by chromatographic adsorption on alumina has yielded, beside podophyllotoxin and α-peltatin,[1] a new crystalline substance in about 4% yield for which the name β-peltatin is proposed. The new compound possesses about the same high necrotizing activity[2] for mouse sarcoma 37 as α-peltatin.

β-Peltatin crystallizes from alcohol in colorless, transparent prisms, m. p. 231.1–238.0° (shrinks at 225.5°) cor.; $[\alpha]^{20}D$ −115° (*c*, 1.009, absolute alcohol). *Anal.*[3] Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 64.0; H, 5.6. Calcd. for three methoxyl groups: 22.5; found, 22.2. Molecular weight values (Rast) for derivatives of both α- and β-peltatin agree with the formula $C_{22}H_{22}O_8$ and indicate that the peltatins are thus isomeric with podophyllotoxin.[4] α-Peltatin has one less methoxyl group than β-peltatin and podophyllotoxin.

Beside the methoxyl content, α- and β-peltatin differ in their color reactions with sulfuric acid and in the properties of their derivatives. With concentrated sulfuric acid, both peltatins give an immediate yellow color, rapidly turning reddish brown with α-peltatin and green with β-peltatin; the final color with both peltatins is red. A series of derivatives of the peltatins has been prepared and will be reported at a later date.

Structural and biological studies with β-peltatin are in progress.

NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTE OF HEALTH
U. S. PUBLIC HEALTH SERVICE JONATHAN L. HARTWELL
BETHESDA, MARYLAND WENDELL E. DETTY

RECEIVED JULY 22, 1948

(1) J. L. Hartwell, THIS JOURNAL, **69**, 2918 (1947).

(2) Unpublished results of Joseph Leiter and Faith Jouvenal.

(3) By Mrs. M. M. Ledyard and Mrs. Evelyn Peake National Institute of Health.

(4) W. Borsche and J. Niemann, *Ber.*, **66**, 1633 (1932); E. Späth, F. Wessely and E. Nadler, *ibid.*, **66**, 125 (1933).

THE CRYSTALLINE TRIHYDROCHLORIDES OF STREPTOMYCIN AND MANNOSIDOSTREPTOMYCIN

Sir:

The preparation of the crystalline reineckate, sulfate,[1] helianthate,[2] and the calcium chloride double salt[2] of streptomycin and the reineckate of mannosidostreptomycin[3] has been reported. To date, there has been no published information on the crystallization of a simple mineral acid salt of either of these antibiotics. We now wish to report that, starting with relatively pure material, we have obtained the trihydrochlorides of streptomycin and mannosidostreptomycin in the crystalline state from methanol solution.

The streptomycin trihydrochloride crystallizes with two molecules of water of crystallization as monoclinic prisms showing birefringence. The crystalline material was shown to be a single substance by a modification of the Craig countercurrent distribution technique[4] and thus to be free of mannosidostreptomycin. On heating on the hot-stage, the dihydrate decomposes gradually without melting. When the trihydrochloride was dried at 55° *in vacuo*, it had the following analytical composition: C, 34.86; H, 6.36; Cl, 14.25 (Calcd. for $C_{21}H_{39}N_7O_{12}\cdot 3HCl\cdot 2H_2O$: C, 34.54; H, 6.36; Cl, 14.57). After drying at 100° *in vacuo*, the anhydrous material showed $[\alpha]^{26.6}D$ −86.1° (1.0% in water) and the following analytical data were obtained: C, 36.27; H, 6.14; N, 14.29; Cl, 15.69 (Calcd. for $C_{21}H_{39}N_7O_{12}\cdot 3HCl$; C, 36.50; H, 6.13; N, 14.19; Cl, 15.40).

When assayed with *K. pneumoniae* in a broth-dilution test,[5] the trihydrochloride dihydrate had a potency of 820 units/mg. and on this basis the anhydrous material would have an activity of 891 units/mg.[6]

The trihydrochloride of mannosidostreptomycin crystallizes in the form of hexagonal plates which are isotropic. By means of the counter-current distribution method,[4] this material was also shown to be a single entity and to be free of streptomycin.

After drying at 55° *in vacuo*, the trihydrochloride was found to have the following analysis: C, 36.45; H, 6.26; Cl, 12.14 (Calcd. for $C_{27}H_{49}N_7O_{17}\cdot 3HCl\cdot 2H_2O$: C, 36.47; H, 6.35; Cl, 11.96). When dried at 100° *in vacuo*, the anhydrous material showed $[\alpha]^{26.6}D$ −54.1° (1.0% in water)

(1) J. Fried and O. Wintersteiner, *Science*, **104**, 273 (1946).

(2) R. L. Peck, N. G. Brink, F. A. Kuehl, Jr., E. H. Flynn, A. Walti and K. Folkers, THIS JOURNAL, **67**, 1866 (1945).

(3) J. Fried and E. Titus, *J. Biol. Chem.*, **168**, 391 (1947).

(4) A modification of the counter-current distribution described by Titus and Fried (*J. Biol. Chem.*, **174**, 57 (1948)) has been developed by our colleagues Drs. Plaut and McCormack which eliminates the appearance of the tautomers of the two streptomycins in the Craig diagram.

(5) R. Donovick, D. Hamre, F. Kavanagh and G. Rake, *J. Bact.*, **50**, 623 (1945).

(6) Based on the F. D. A. working standard. Spectrophotochemical assays based on a maltol method, similar to that published by G. F. Mueller (THIS JOURNAL, **69**, 195 (1947)), have confirmed these microbiological results.

and gave analytical data: C, 38.25; H, 6.22; N, 11.31; Cl, 12.52 (Calcd. for $C_{27}H_{49}O_7O_{17}\cdot 3HCl$: C, 38.01; H, 6.14; N, 11.49; Cl, 12.47).

When assayed with *K. pneumoniae* in a broth dilution test,[5] the anhydrous mannosidostreptomycin had a potency of *circa* 210 units/mg.[6]

Additional information on the properties and activities of these crystalline hydrochlorides will be published at a later date.

We wish to express our appreciation to Dr. R. Donovick, Mr. R. Blue, and Mr. D. Lapedes for the bio-assays, Mr. F. Russo-Alesi for the countercurrent distributions, and Mr. J. Alicino for the micro-analysis.

DIVISION OF CHEMICAL DEVELOPMENT LEON J. HEUSER
E. R. SQUIBB AND SONS MORRIS A. DOLLIVER
NEW BRUNSWICK, N. J. ERIC T. STILLER

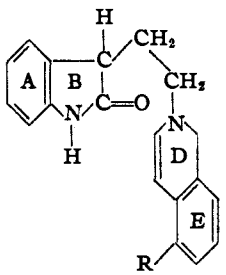
RECEIVED JULY 19, 1948

THE SYNTHESIS OF KETOYOBYRINE

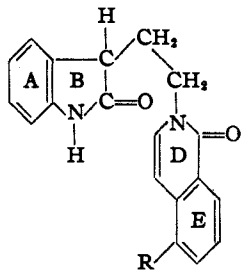
Sir:

For several years we have been investigating the possibility of synthesizing the basic ring structure of yohimbine by ring closure of isoquinolylethyl oxindoles of the type represented by formula (I).[1] The introduction of the double bond in ring D presented numerous difficulties because of the ease with which compounds of the type (I) (as well as the yohimbine molecule itself) suffer cleavage at the nitrogen atom of ring D. Moreover, we have pointed out[1] that 1,2-dihydroisoquinolines like (I) are virtually unknown.

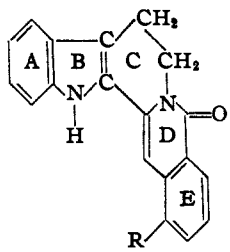
Accordingly, our efforts were later directed toward the preparation and ring closure of compounds of the type represented by formula (II)



(I) (R = CH_3 or H)



(II) (R = CH_3 or H)



(III) Ketoyobyrine (R = CH_3)

where the appropriate double bond of ring D could be introduced without difficulty. Ring closure of such a compound would lead to compounds of the structure (III), which type of structure has recently been proposed for ketoyobyrine, on the basis of an exhaustive study of its chemistry[2a] and likewise on the basis of a comparison of its absorption spectrum with that of rutaecarpine.[2b]

Pending more complete presentation of our various syntheses of the type of structure represented by (III), we wish to record our synthetic confirmation of this proposed structure for ketoyobyrine.

6-Methylhomophthalic acid, m. p. 196°, was prepared from *o*-tolylacetic acid[3] by conversion *via* the Arndt–Eistert reaction into *o*-tolylpropionic acid, which was then treated according to the method of Mercer and Robertson.[4] Condensation with tryptamine yielded N-(β-indolylethyl)-6-methylhomophthalimide, m. p. 228°. Conversion of the latter into the corresponding homophthalamic acid,[5] m. p. of picrate 147°, methylation of the acid with diazomethane, m. p. of methyl ester 222°, dec., followed by ring closure with phosphorus oxychloride, yielded ketoyobyrine, m. p. 316–318°, dec.

Anal. Calcd. for $C_{20}H_{16}ON_2$: C, 79.98; H, 5.37; N, 9.32. Found: C, 79.43; H, 5.55; N, 9.24. Comparisons of the ultraviolet absorption spectrum of synthetic ketoyobyrine with that of the product of natural origin showed the two to be identical. Absorption maxima for synthetic material: at 385, 366 and 340 mμ, log ε 4.40, 4.51 and 4.52, respectively.

(1) Julian, Magnani, Pikl and Karpel, THIS JOURNAL, **70**, 174 (1948).

(2) (a) Woodward and Witkop, THIS JOURNAL, **70**, 2409 (1948); (b) Raymond-Hamet, *Compt. rend.*, **226**, 137 (1948).

(3) Julian, Karpel, Magnani and Meyer, THIS JOURNAL, **70**, 180 (1948).

(4) Mercer and Robertson, *J. Chem. Soc.*, 288 (1936).

(5) *Cf.* Haworth, Perkin and Pink, *J. Chem. Soc.*, 1709 (1925).

THE GLIDDEN COMPANY PERCY L. JULIAN
RESEARCH LABORATORIES WILLIAM J. KARPEL
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RECEIVED JULY 24, 1948

THE OZONIZATION OF THE MALEIC ANHYDRIDE ADDUCT OF DEHYDROERGOSTERYL ACETATE

Sir:

A recent publication of Bergmann and Stevens[1] describes the preparation of the maleic anhydride adduct of 3(β)-acetoxy-9,11-oxidobisnor-5,7-choladienic acid (II) by the ozonization of 9,11-oxidoergosteryl acetate–maleic anhydride adduct. For some time previous a study of analogous reactions has been under way in our laboratories and we now wish to report the preparation of the maleic anhydride adduct of 3(β)-acetoxybisnor-5,7,9-cholatrienic acid (I) by the selective ozonization of 9,11-dehydroergosteryl acetate–maleic anhydride adduct (III).

A solution of the dehydroadduct (III) in methylene chloride was treated with two equiva-

(1) Bergmann and Stevens, *J. Org. Chem.*, **13**, 10 (1948).