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.

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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.

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BETHESDA, MARYLAND WENDELL E. DETTY

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(2) Unpublished results of Joseph Leiter and Faith Jouvenal.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