[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

Mold Metabolites. I. Isolation of Several Compounds from Clinical Penicillin

BY DONALD J. CRAM[1] AND MAX TISHLER

During studies of the purification of clinical penicillin a number of microbiologically inactive compounds were isolated. Two of the substances described in the present report were separated from the residue obtained when crude sodium penicillin was dissolved in acetone. The remaining compounds were isolated from the acetone soluble fractions by chromatographic adsorption and fractional elution using acid washed alumina as the adsorbent.

Six of the compounds were identified and include tiglic acid, *d*-α-methylbutyric acid, furoic acid,[2] β-indoleacetic acid, phenylacetic acid[2] and 2-decenedioic acid. The isolation of the last substance from natural sources is noteworthy for 2-decenedioic acid is a homolog of traumatic acid, a plant hormone.

Three pigments were also isolated, two of them in the pure state. The properties of pigment I (including the ultraviolet absorption spectrum recorded in Fig. 1) agree very well with those reported for β-penitrin, a substance obtained by Stodola, *et al.*,[3] by the alkaline hydrolysis of penitrinic acid which these workers isolated from *Penicillium notatum* culture liquors. Pigment II has been characterized, and its ultraviolet absorption spectrum is recorded in Fig. 1. This spectrum bears a resemblance to that of penitrinic acid,[3] and this fact as well as the presence of one nitrogen atom and a carboxyl group in both molecules suggests a structural similarity between the two substances.

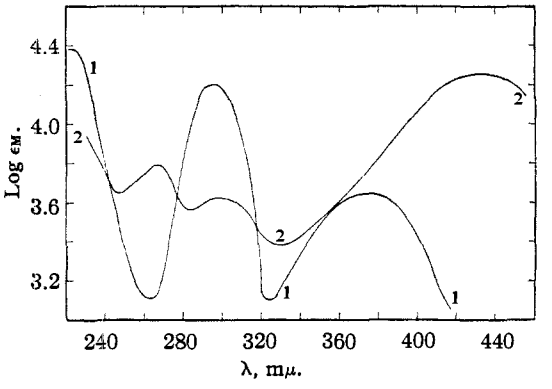

Fig. 1.—Ultraviolet absorption spectra in absolute ethanol: curve 1, pigment I; curve 2, pigment II.

Pigment III was isolated in somewhat larger quantities and has therefore been characterized more completely. The compound has the molecular formula $C_{14}H_{16}O_3$ and titrates as a monobasic acid of a strength comparable to phenol. A Zerewitinoff determination disclosed two active hydrogens, and a C-methyl determination indicated the presence of three such groups in the molecule. The substance contains no methoxy groups and forms no carbonyl derivatives; however a monoacetate as well as a monomethyl ether of pigment III were formed without difficulty. Both of these derivatives gave a characteristic coloration with ferric chloride and possessed the properties of a very weak acid, as shown by a slightly enhanced solubility in dilute alkali over that in water. These facts suggest that pigment III contains a normal phenolic hydroxyl, and another phenolic (or enolic) hydroxyl masked in some way, possibly by chelation with a carbonyl group. The ultraviolet absorption spectrum of pigment III in both neutral and acid medium is recorded in Fig. 2. The determination of the structure of pigment III is in progress.

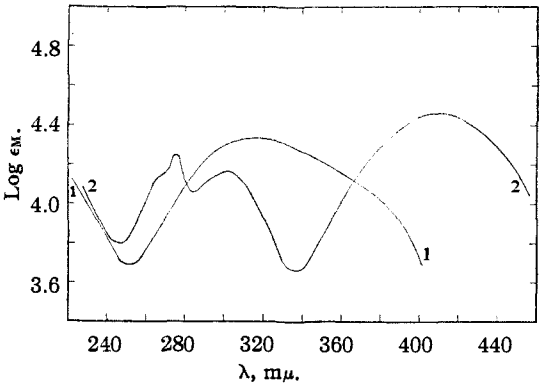

Fig. 2.—Ultraviolet adsorption spectrum of pigment III: curve 1, in absolute ethanol; curve 2, in 50% ethanol–50% 0.05 *N* sodium hydroxide solution.

Experimental

Tiglic Acid.—Tiglic acid was obtained from the acetone insoluble fraction of crude sodium penicillin by distillation and crystallization, m. p. 63–64° (lit.[4] 64.5°).

Anal. Calcd. for $C_5H_8O_2$: C, 59.99; H, 8.06. Found: C, 59.98; H, 7.89.

The dibromide was prepared, m. p. 86–87° (lit.[4] 86°).

***d*-α-Methylbutyric Acid.**—This acid was distilled from the concentrate of the mother liquors of the tiglic acid crystallization, b. p. 175° (lit.[5] 174°); neut. equiv., 102 (calcd. 103); $[\alpha]^{20}D$ +15.2° (lit.[6] $[\alpha]^{20}D$ 16.66°). The amide was prepared, m. p. 106° (lit.[7] 111°); $[\alpha]^{20}D$ +14.7 in water, 1% (lit.[6] $[\alpha]D$ +18.9° in water).

Anal. Calcd. for $C_5H_{11}ON$: N, 14.20. Found: N, 13.95.

(1) Present address: Department of Chemistry, University of California, Los Angeles, California.

(2) The isolation of these acids is recorded in Penicillin Monograph, Chapter IV, "Status of Research on the Structure of Benzylpenicillin in December, 1943," by R. L. Peck and K. Folkers.

(3) Stodola, Wachtel, Moyer and Coghill, *J. Biol. Chem.*, **159**, 67-70 (1945).

(4) Wislicenus, *Ann.*, **272**, 1, 1893.

(5) Marckwald, *Ber.*, **32**, 1093 (1899).

(6) Marckwald, *ibid.*, **37**, 1045 (1904).

(7) Taverne, *Rec. trav. chim.*, **13**, 197 (1894).

Furoic Acid.—Furoic acid, m. p. 129–130°, was isolated from the first chromatographic fraction of the acetone-soluble portion of crude sodium penicillin. A mixed melting point with an authentic sample gave no depression.

β-Indoleacetic Acid.—The pure acid was crystallized from the second chromatographic fraction, m. p. 167°. A mixed melting point with an authentic specimen gave no depression.

Phenylacetic Acid.—This acid was isolated from the third chromatographic fraction, m. p. 76–77°. Admixture of the substance with an authentic specimen of phenylacetic acid produced no depression of melting point.

2-Decenedioic Acid.—This dicarboxylic acid was isolated from the chromatograph filtrate of the acetone soluble portion of sodium penicillin, m. p. 172–173°.[8]

Anal. Calcd. for $C_{10}H_{16}O_4$: C, 59.99; H, 8.06. Found: C, 60.46; H, 8.11.

The dianilide was prepared by the usual procedure, m. p. 211–212°.

Anal. Calcd. for $C_{22}H_{27}O_2N_3$: C, 75.40; H, 7.48; N, 8.00. Found: C, 75.19; H, 7.08; N, 8.08.

The decenedioic acid was unequivocally identified by oxidation with alkaline permanganate to suberic acid, m. p. 140–141° (lit.[9] 140°), and by catalytic hydrogenation to sebacic acid, m. p. 133–134° (lit.[9] 133–133.5°).

Pigment I.—Pigment I (β-penitrin[3]) was isolated from the mother liquors from the crystallization of β-indoleacetic acid, m. p. 207° (lit.[3] 204–205°). Since only about 2 mg. of this substance was obtained, it was not possible to obtain an analysis. However, the ultraviolet absorption spectra (see Fig. 1) is practically identical with that of β-penitrin.[3]

Pigment II.—This substance was isolated as a red-orange benzylamine salt, m. p. 128–129°. The salt was crystallized by the addition of benzylamine to an ether solution prepared by acidification and ether extraction of the acetone insoluble fraction of crude sodium penicillin. The free pigment was obtained by ether extraction of an acidulated aqueous suspension. Recrystallization from an ether–petroleum ether mixture gave orange prisms, m. p. 105–106°.

Anal. Calcd. for $C_{19}H_{11}NO_6$: C, 49.79; H, 4.51; N, 5.88; neut. equiv., 117 (for 2 acidic groups). Found: C, 49.84; H, 4.24; N, 6.15; neut. equiv., 120.

The pigment is slightly soluble in water, and very soluble in sodium bicarbonate, in which it produces a deep red-orange coloration that is discharged on acidification of the solution. The substance is no more soluble in dilute acid than in water and exhibits no ordinary basic properties. An aqueous solution of the pigment does not give a color with ferric chloride, but rapidly decolorizes potassium permanganate. When dissolved in an aqueous solution of sodium hydrosulfite the pigment was decolorized, and ether extraction of the mixture with subsequent evaporation of the ether produced white needles, m. p. 129–130°. This substance turned a dilute ferric chloride solution deep red. The pigment is optically inactive.

Pigment III.—Pigment III crystallized directly as a sodium salt from an aqueous solution of crude sodium penicillin in water. The free pigment was obtained by acidification of an aqueous solution of the sodium salt. Several recrystallizations from ether gave orange plates, m. p. 113–114° (the melt partially resolidified and melted again at 129–130°).

Anal. Calcd. for $C_{14}H_{16}O_3$: C, 72.38; H, 6.95; mol. wt., 232; neut. equiv., 232. Found: C, 72.49, 72.41; H, 6.84, 6.95; mol. wt. (in camphor), 214; neut. equiv., (potentiometric titration against a hydrogen electrode), 229, pH $\frac{1}{2}$ 8.38; active hydrogen, 2.03 gram atoms; C-methyl determination, 2.38 and 2.41.

The substance is almost insoluble in water and sodium bicarbonate solution, soluble in sodium hydroxide solution (deep orange coloration) from which an orange sodium salt separates. The molecule exhibits no optical activity and contains no methoxyl groups. A trace of ferric chloride turns an aqueous methanolic solution of the substance a permanent black color.

Acetate of Pigment III.—Pigment III (200 mg.) was heated for five minutes with 2 cc. of acetic anhydride and a trace of sodium acetate, cooled and diluted with water. The yellow solid that separated was recrystallized from ethanol to give fine yellow needles, wt. 180 mg., m. p. 103–104°. This substance gives a black coloration with a trace of ferric chloride in a solution of methanol and water.

Anal. Calcd. for $C_{16}H_{18}O_4$: C, 70.05; H, 6.61. Found: C, 70.23; H, 6.68.

Methyl Ether of Pigment III.—To a solution of 100 mg. of pigment III in 10 cc. of methanol was added 1 cc. of freshly distilled dimethyl sulfate, and a 1 *N* sodium hydroxide solution was added to the mixture dropwise over a period of half an hour at such a rate as to keep the solution on the basic side. At the end of this time the mixture was acidified, diluted with water and ether extracted. When dried and evaporated the ether layer produced long flat yellow needles, m. p. 105–106° (weight 125 mg.). Further recrystallization of the substance from ether did not alter the melting point. This substance was slightly acidic as shown by its greater solubility in alkali than in neutral or acid solution. A methanol water solution of the compound gave a black color with a trace of ferric chloride.

Anal. Calcd. for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 73.18; H, 7.65.

Repeated attempts to introduce more than one methoxyl group into pigment III never resulted in a product that did not show a greater solubility in alkali than in neutral aqueous medium.

Summary

Nine microbiologically inactive compounds have been isolated from clinical penicillin, and six of these have been identified as tiglic acid, *d*-α-methylbutyric acid, phenylacetic acid, β-indoleacetic acid, furoic acid and 2-decenedioic acid. Three pigments have also been isolated and characterized, the first of which has been identified as β-penitrin.

RECEIVED JUNE 9, 1948

(8) English [THIS JOURNAL, **63**, 941–943 (1941)] prepared this sustance for testing as a wound plant hormone, m. p. 172–173°. No derivatives were reported.

(9) Kraft and Nordlinger, *Ber.*, **22**, 818 (1889).