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CHEMICAL STUDIES ON AMPHOTERICIN B

II. 2-METHYLHEPTADECANEDIOIC ACID FROM PERHYDROGENATED AMPHOTERICIN B

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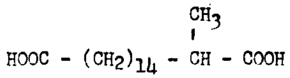
Amphotericin B is a heptaenic macrolide antifungal antibiotic with a tentative empirical formula $C_{46}H_{73}O_{20}N$ and molecular weight of 960 (1,2). It yields mycosamine on hydrolysis (2) and belongs to the non-aromatic structural subgroup of heptaenes (3).

In the course of our studies on the structure of this substance, oxidative degradation of perhydrogenated amphotericin B was carried out in order to isolate a large fragment which would contain the hydrocarbon chain corresponding to the original heptaenic portion of the antibiotic. Perhydroamphotericin B (2,4) was oxidized with nitric acid (d = 1.4) in acetic acid at 100 C or with potassium permanganate under alkaline conditions at room temperature followed by further oxidation with chromic acid in acetic acid at 65 - 70 C. The crude products were first purified by cellulose column chromatography in the solvent system, tetrahydrofuran: 3 N ammonia (4:1), and crystallized from 80 percent acetic acid. The final purification was achieved by means of preparative thin layer chromatography on kieselgel G with double development. The first solvent mixture was cyclohexane: ethyl ether: acetic acid (60:40:2), and this was followed by the mixture cyclohexane: ethyl ether: acetic acid (70:30:1). The product was recrystallized from ethylene chloride: Colorless crystals, m.p. 88.5° and $[\alpha]_D^{20} = 16 \pm 2°$ (ethyl acetate). Calcd. for $C_{18}H_{34}O_4$: C, 68.74; H, 10.99. Found: C, 68.35; H, 10.98.

This substance was identified as 2-methylheptadecanedioic acid, I,

(I)



$$HOOC - (CH_2)_{14} - \overset{\overset{\displaystyle CH_3}{|}}{CH} - COOH$$

by comparison with reference material(*) in the following manner: IR spectrum (Fig. 1); NMR spectrum (Fig. 2); paper and thin layer chromatography in a number of solvent systems; and vapor phase chromatography of the dimethyl ester at 191° (PYE instrument) using a straight standard

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(*) The synthesis of racemic 2-methylheptadecanedioic acid, I, was accomplished according to the following scheme: The methyl ester of erucic acid was converted to the glycol with performic acid and the glycol cleaved to yield, as one of the products, methyl 12-formyl-dodecanoate. From triphenyl phosphine and 3-bromotiglic acid methyl ester, the phosphonium bromide derivative was obtained. The reaction of these two substances under the conditions of the Wittig reaction yielded the dienic-dicarboxylic acid ester which, upon hydrogenation with Raney nickel catalyst and subsequent saponification, afforded the free 2-methylheptadecanedioic acid, m.p. 99-100°. The analytical values, including molecular weight and neutralization equivalent, were in good accord with the calculated values (5).

column (4') packed with PEGA, in which the retention time was 60.0 min., identical with that of an authentic sample of dimethyl 2-methylheptadecanedioate. The mixed melting point determination was carried out employing optically active material obtained by similar oxidative degradation of perhydrocandidin (6). No depression was observed.

It is of interest that the same branched chain acid has been obtained by degradation of perhydronystatin (7) although in that case the product was not optically active as a result of the hydrogenolysis of a tertiary hydroxyl group during the hydrogenation step. The asymmetric nature of the amphotericin B product indicates that a similar grouping must not exist in the amphotericin B structure. Homologous branched acids have also been obtained as degradation products of the polyenic antifungal antibiotics fungichromin and filipin (8,9) but not from pimaricin or trichomycin (10,11), findings which suggest deviations in the biogenesis of these substances.

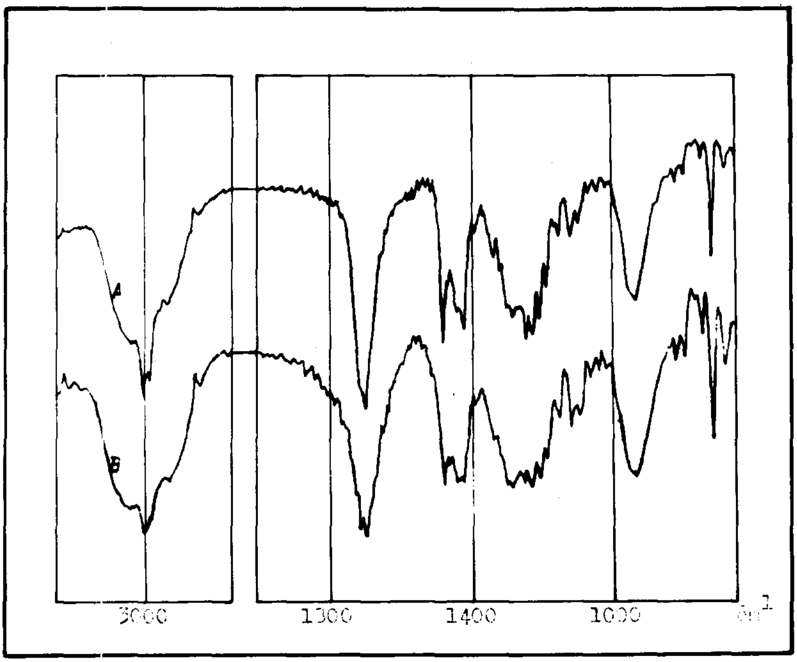

FIGURE I

Infrared Spectra of Synthetic 2-Methylheptadecanedioic Acid (A) and Product I from Amphotericin B (B). Film technique. Ordinate is transmittance on arbitrary scale. Unicam SP 200.

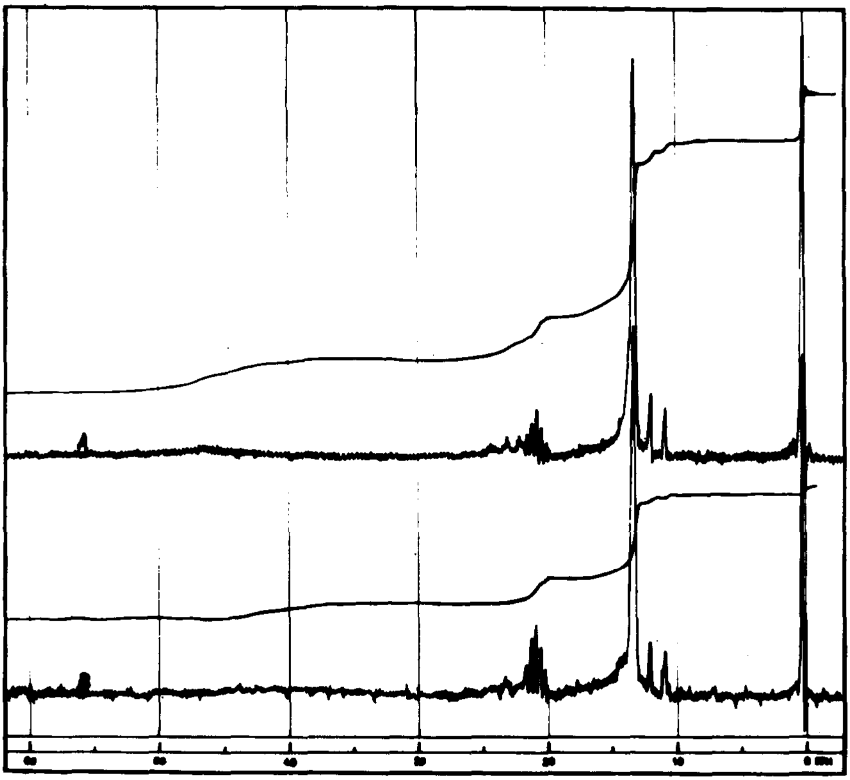

FIGURE 2

N.M.R. Spectra of Synthetic 2-Methylheptadecanedioic Acid (A) and Product I from Amphotericin B (B).[1] 60 Mc. in D-acetone. Field increases from left to right.

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