Phthiocerol B, a Constituent of the Lipids of Tubercle Bacilli

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PREVIOUS investigations[1] indicated that phthiocerol is a mixture of two homologous β-diols having the structure (I) where n is 20 and 22, respectively. In the present studies unidimensional multiple chromatography[2] of crude phthiocerol (isolated from the lipids of tubercle bacilli as described previously[3]) on Kieselgel G plates [light petroleum (b.p. 60—80°)/acetone, 85 : 15; three passes] showed the presence of the diols (I), in the following named phthiocerol A, material now designated phthiocerol B, and ketonic material, presumed to be essentially phthiodiolone[4] (R_F values 0·61, 0·55, and 0·45, respectively). The presence of several more polar constituents was also observed.

The mixture of cyclic acetals formed by reaction of crude phthiocerol with acetaldehyde and toluene-*p*-sulphonic acid showed on unidimensional multiple chromatography the same sequence of polarity, thus indicating that phthiocerol A and B as well as phthiodiolone contain an analogous diol system. Column chromatography of the acetals (from 17·4 g. of crude phthiocerol) on neutral alumina gave a mixture of the acetals of phthiocerol A and B, followed by impure acetal of phthiodiolone (2·41 g.), and by more polar acetals (1·16 g.). The mixture of the acetals of phthiocerol A and B was chromatographed repeatedly (as above) to give phthiocerol A ethylidene acetal (7·4 g.), m.p. 40° (from ethanol) and phthiocerol B ethylidene acetal (0·165 g.), m.p. 35·5° (from ethanol). Decomposition of the acetals (ethanol, toluene-*p*-sulphonic acid) gave the corresponding diols, phthiocerol A, m.p. 73—73·5° (from light petroleum, b.p. 40—60°), $[\alpha]_{589}^{20}$ −4·5°, $[\alpha]_{578}^{20}$ −4·7°, $[\alpha]_{546}^{20}$ −5·4°, $[\alpha]_{436}^{20}$ −9·5° and phthiocerol B, m.p. 71—71·5° (from light petroleum, b.p. 40—60°), $[\alpha]_{589}^{20}$ −8·2°, $[\alpha]_{578}^{20}$ −8·2°, $[\alpha]_{546}^{20}$ −9·5°, $[\alpha]_{436}^{20}$ −16·4° (all in $CHCl_3$).

The infrared spectra of both diols as well as those of the corresponding acetals showed close agreement; all spectra included a band due to methoxyl (1090 cm.$^{-1}$).

The n.m.r. spectrum of phthiocerol A showed a triplet (J = 7·2 c./sec.) at τ9·09 (two terminal methyl groups), a doublet (J = 7·2 c./sec.) at τ9·18 (methyl branch), a quartet at τ7·10 (assigned to CH of the >CHOMe residue), and a singlet at τ 6·66 (methoxyl). The n.m.r. spectrum of phthiocerol B differed in two major respects. It showed an additional doublet (J = 7·2 c./sec.) at τ 8·97 (methyl group); secondly, the proton at the carbon atom bearing the methoxyl group was apparently displayed as a quintet at τ 6·84 overlapping the methoxyl singlet at τ 6·68.

The mass spectra of phthiocerol A and the corresponding acetal were in agreement with the structure (I) for phthiocerol A. The base peak in these spectra at m/e 73 due to $CH_3CH_2CH(OMe)^+$ was ~ 2·5 times larger than the next largest peak at m/e 43 ($C_3H_7^+$).

[1] R. Ryhage, S. Ställberg-Stenhagen, and E. Stenhagen, *Arkiv Kemi*, 1959, **14**, 247, 259, and references cited there.
[2] J. A. Thoma, *Analyt. Chem.*, 1963, **35**, 214.
[3] J. A. Hall, J. W. Lewis, and N. Polgar, *J. Chem. Soc.*, 1955, 3971.
[4] H. Demarteau-Ginsburg, A. Ginsburg, and E. Lederer, *Biochim. Biophys. Acta*, 1953, **12**, 587.

The mass spectra of phthiocerol B and the corresponding acetal showed a base peak of similar intensity to the above at *m/e* 59 presumably due to the $CH_3CH(OMe)^+$ ion. The molecular ions of the acetal derived from phthiocerol B (*m/e* 538, 566) were fourteen units lower in mass than those of the acetal derived from phthiocerol A (*m/e* 552, 580).

A detailed comparison of the intermediate fragments in the above spectra led to the conclusion that phthiocerol B is a mixture (59 : 41) of diols having the structure (II), where *n* is 20 and 22, respectively.

The biogenesis of phthiocerol B might be explained by the scheme proposed[5] for phthiocerol A by postulating the final incorporation of acetate rather than propionate. In this scheme phthiodiolone, suggested[5] to be a ketone with a structure

(I) $CH_3\cdot[CH_2]_n\cdot\underset{\displaystyle OH}{\underset{|}{CH}}\cdot CH_2\cdot\underset{\displaystyle OH}{\underset{|}{CH}}\cdot[CH_2]_4\cdot\underset{\displaystyle CH_3}{\underset{|}{CH}}\cdot\underset{\displaystyle OCH_3}{\underset{|}{CH}}\cdot CH_2\cdot CH_3$

(II) $CH_3\cdot[CH_2]_n\cdot\underset{\displaystyle OH}{\underset{|}{CH}}\cdot CH_2\cdot\underset{\displaystyle OH}{\underset{|}{CH}}\cdot[CH_2]_4\cdot\underset{\displaystyle CH_3}{\underset{|}{CH}}\cdot\underset{\displaystyle OCH_3}{\underset{|}{CH}}\cdot CH_3$

differing from that of phthiocerol A (I) by containing C=O in place of CHOMe, was considered to be a precursor of phthiocerol A. It appears probable that the precursor of phthiocerol B is an analogous ketone, and crude phthiocerol may thus be expected to contain a ketone having a terminal portion corresponding to that of phthiocerol B.

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[5] E. Lederer, *Pure Appl. Chem.*, 1961, **2**, 587.