The Mycolipodienic Acids, Constituents of the Lipids of Tubercle Bacilli

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IN the course of studies of the lipids of tubercle bacilli (human strains) we have isolated a series of homologous acids the main component of which is shown below to have the structure (II; R = H, $x + y = 15$), closely related to that of mycolipenic acid (I; R = H).[1] The isolation of these acids, designated mycolipodienic acids, was achieved as follows.

The methanol-soluble material obtained in the earlier work[1] on partial hydrolysis of the lipid extracts was esterified with methanolic sulphuric acid and the methyl esters (mixtures of saturated and unsaturated esters) separated from other products by chromatography on silica gel. Reaction with mercuric acetate in methanol[2] converted the esters containing nonconjugated unsaturated linkages into their mercuric acetate adducts which were separated from other esters (including methyl mycolipenate) by chromatography over silica gel. Thin-layer chromatography (t.l.c.) of the re-formed unsaturated esters showed the presence of the methyl mycolipodienoates as slightly less polar components which were isolated by repeated preparative t.l.c. of the mixture (1 g.) as an oil (65 mg.), $[\alpha]_D$ + 12·2° (CCl_4).

The mass spectrum (A.E.I. MS9) of this material shows the presence of homologues, the main constituent having a molecular weight of 420, as compared with a molecular weight of 422 for methyl mycolipenate (I; R = Me).[1]

The ultraviolet spectrum shows λ_{max} 217 mμ (ϵ 10,350) (in EtOH), to be compared with λ_{max} 217 mμ (ϵ 13,800) for methyl mycolipenate,[1] thus indicating the presence of $\alpha\beta$-unsaturation.

The n.m.r. spectrum (at 100 Mc./sec.) of the methyl mycolipodienoates closely resembles that of methyl mycolipenate. The spectra show close agreement in the region τ 8·9—9·3 (Me); moreover, both show a doublet at τ 8·15 (J 1 c./sec., CH=C*Me*–C=O), a broad singlet (1 H) at τ 7·4 (CH_2–C*H*Me–CH=CMe–C=O), a singlet at τ 6·28 (3 H) (CH_3–O–C=O), and a doublet at τ 3·50 (J 10 c./sec., CHMe–C*H*=CMe–C=O). The close conformity of the fine splitting of the signal at τ 8·15 in both spectra indicates the same stereochemistry in respect of the $\alpha\beta$-double bond which for methyl mycolipenate was assigned[1,3] the *trans*-configuration. The n.m.r. spectrum of the methyl mycolipodienoates shows in addition a signal at τ 4·67 (2 H) attributable to ethylidene C*H*, and a signal at τ 8·00 (4 H) attributable to protons α to a double bond.

Closer examination of the mass spectrum shows a peak at *m/e* 169 (5% of base peak) (base peak at *m/e* 55) which is characteristic[3] of 2,4,6-trimethyl-substituted $\alpha\beta$-unsaturated methyl esters and corresponds to a fragment arising by cleavage of the carbon chain between C-6 and C-7; it also shows a peak at 101 (33%), due to cleavage between C-3 and C-4 preceded by a double-bond shift.[3]

The above evidence indicated that the methyl mycolipodienoates have the structure (II; R = Me) with $x + y = 15$ for the main homologue; in view of the same sign and order of magnitude of their rotatory power as that reported[1] for methyl mycolipenate ($[\alpha]_D$ + 16·8° in $CHCl_3$) and the structural relationship with the latter the asymmetric centres are regarded as having the same (L)-configuration. It now remained to locate the nonconjugated double bond.

Recent studies[4] in this laboratory have shown that the mass spectra of epoxides derived from long-chain unsaturated esters give recognisable peaks due to cleavage α to the epoxy-group, corresponding to the oxonium fragments (IIIa) and (IIIb) (where R^1 and R^2 are alkyl), and, in addition, a strong peak 44 mass units below the peak corresponding to (IIIb). The esters (II; R = Me) were, therefore, converted into the epoxides (IV) by reaction with perlauric acid. The mass spectrum of these epoxides shows peaks at *m/e* 155 (cleavage at a) and 323 (cleavage at b), and an additional strong peak at 279 (323—44), thus suggesting that the main homologue of the dienoates has the structure (II; R = Me with $x = 7$ and $y = 8$). The spectrum also shows smaller peaks, differing from these main peaks by 14 and 28 mass units, which may be due either to positional isomerism of the nonconjugated double bond, or the presence of homologues. The n.m.r. spectrum of the above epoxides, with the protons of the epoxide ring appearing at τ 7·12 (while those of a *trans*-epoxide are found to absorb at τ 7·36), indicates that the nonconjugated double bond in the parent unsaturated esters has *cis*-configuration.

Gas–liquid chromatography, on both polar and nonpolar stationary phases, of the esters (II; R = Me) showed the presence of six homologues,

(I) $CH_3\cdot[CH_2]_{17}\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CH{=}CMe\cdot CO_2R$

(II) $CH_3\cdot[CH_2]_x\cdot CH{=}CH\cdot[CH_2]_y\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CH{=}CMe\cdot CO_2R$

(IIIa) $R^1\cdot CH\text{–}CH$ (O) +

(IIIb) $CH\text{–}CH\cdot[CH_2]_n\cdot CO_2R^2$ (O) +

(IV) $CH_3\cdot[CH_2]_x\cdot CH\text{–}CH\cdot[CH_2]_y\cdot\overset{L}{C}HMe\cdot CH_2\cdot\overset{L}{C}HMe\cdot CH{=}CMe\cdot CO_2Me$ (O; b, a)

the most abundant (C_{27}, 71%) of which had a retention time very similar to that of the C_{27}-constituent (I; R = Me) of a sample of methyl mycolipenate. The remaining homologues are present in the following proportions: C_{29}, 4%; C_{28}, 4%; C_{26}, 11%; C_{25}, 8%; C_{24}, 2%.

The structure (II; R = H with $x = 7$ and $y = 8$) for the main homologue of the mycolipodienic acids indicates that its biogenesis might be explained by the scheme suggested[5] for mycolipenic acid, postulating the incorporation of oleic acid (known to be present in the lipids of tubercle bacilli), in place of stearic acid.

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