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STUDIES ON THE MYCOLIC ACIDS FROM HUMAN TUBERCLE BACILLI

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In continuation of previous studies (1) on the mycolic acids isolated from human tubercle bacilli (strains D.T., P.N. and C.) unidimensional multiple chromatography (2) showed the presence of three esters in comparable amounts, subsequently referred to as methyl mycolate -I, -II, and -III, respectively, in order of increasing polarity [the products described previously (1) and named mycolic esters A and B have now been found to be partially separated mixtures of the above three esters]; the ester -III was found to contain a keto group.

The mixture of esters was partially separated by chromatography over alumina into fractions containing only ester -I and -II, and fractions containing all three esters. Ester -I and -II were separated from each other (on alumina) as their acetyl-derivatives, then hydrolysed, and the acids converted into methyl esters. Each of these esters contained a small quantity of their C_2-epimers. Removal of the latter by chromatography on alumina gave methyl mycolate -I, m.p. 49-50°, $[\alpha]_{589}^{20}$ +3.7° (all rotations in $CHCl_3$), and methyl mycolate -II, m.p. 48-48.5°, $[\alpha]_{589}^{20}$ +0.1°. Ester -III was isolated as its acetoxy-oxime by chromatography on silica gel. Liberation of the keto-ester and purification by chromatography on alumina gave methyl acetoxymycolate -III, m.p. 43.5-44°, $[\alpha]_{589}^{20}$ +6.2°. The chromatography of the above esters on alumina also gave some methyl anhydromycolate -I, -II, and -III, m.p. 39-41°, 32-35°, and

52-52.5° respectively.

The infra-red spectra of the mycolic esters and their acetoxy-derivatives showed the expected carbonyl absorptions, and, in the case of methyl mycolate -II, a band at 1090 cm.$^{-1}$ due to methoxyl. In accordance with the findings of Gastambide-Odier et al. (3) all three esters gave a peak at 1020 cm.$^{-1}$ indicating the presence of cyclopropane groups in the molecules. Moreover, a band at 3060 cm.$^{-1}$ indicated in all cases the presence of a cyclopropylmethylene group (4), the band in ester -I having about twice the intensity of those in ester -II and ester -III.

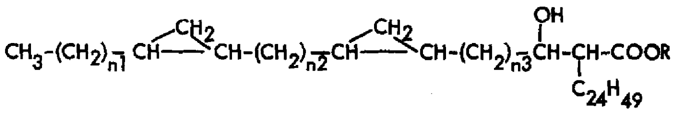
The n.m.r. spectrum (all spectra in $CDCl_3$) of methylmycolate -I showed bands at τ 10.3 (2 H) and τ 9.4 (6 H) which are attributable to two cis-1,2-disubstituted cyclopropane groups [cf. (5)]. The absorption at τ 10.3 is presumably due to the cyclopropylmethylene proton trans to the two substituent groups thus being closely shielded by three ring protons. A band at τ 9.12 showed the presence of two terminal methyl groups.

The n.m.r. spectrum of methylmycolate -II showed a singlet at τ 6.67 confirming the presence of a methoxyl group. A band at τ 10.3 (1 H) suggested a cis-1,2-disubstituted-cyclopropane group, but bands at τ 9.4 (2 H) and τ 9.8 (1 H), as compared with a single band at τ 9.4 in the case of methyl mycolate -I, pointed to a different environment of the cyclopropane group. The possible presence of a methoxyl group at an adjacent carbon atom which might shield one of the ring protons and shift its signal to higher field seemed to be supported by the appearance of the CH-OMe proton signal (τ 7.10) (ill-defined multiplet). A doublet at τ 9.17, superimposed on triplets (τ 9.12) due to two terminal methyl groups, indicated the possible presence of a methyl branch (6).

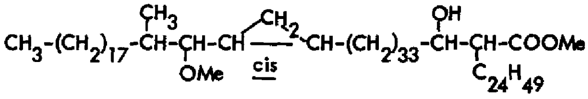
The n.m.r. spectrum of methyl acetoxy mycolate -III (benzene as internal standard) showed a broad band at τ 9.78 (4 H) (sharper band after reduction with $LiAlH_4$); there was no signal at τ 10.3, [a synthetic sample of methyl trans-9,10-methylene-

octadecanoate showed a multiplet at τ 9.80 (4 H)]. Triplets at τ 9.07 showed the presence of two terminal methyl groups, and a doublet at τ 8.91 the group $-\underset{|}{CH}(CH_3)-\overset{O}{\overset{\|}{C}}-$ (6) (LiAl H_4 reduction removed this feature of the spectrum); the ultraviolet spectrum showed absorption at 213 mμ (ε 309). The above evidence suggested that methyl acetoxymycolate -III might contain the unit $-CH(CH_3)-C(=O)-CH\overset{CH_2}{—}CH-$ (trans) (the trans- form presumably arising via the enol during the alkaline hydrolysis of the lipid extracts).

The low-resolution mass spectrum (A.E.I., MS9 for all spectra; the most abundant peak in any series is underlined) of methyl anhydromycolate -I showed peaks due to molecular ions (M) at m/e 1104, 1132, 1160, 1188 and 1216. The spectrum of methyl mycolate -I contained rearrangement peaks at m/e 712, 740, 768, 796, 824 (meroaldehydes) (7), and 382, 410 (C_{24}- and C_{26}-esters derived from the α-branch) and also a series of peaks at m/e 431, 459, 487, 515, 543 (aldehyde -281). These results, together with the other data described above, indicate that methyl mycolate -I is essentially the same as methyl α-mycolate (Test) described recently to which the following general formula was assigned (7) (R = CH_3)



The mass spectrum of methyl anhydromycolate -II showed ten homologous peaks (M-32) at m/e 1188 to 1314, that at 1216 being the most abundant. Cleavage α to the CH-OMe grouping gave peaks at m/e 297, 325 and homologous peaks at m/e 907 to 1033 (M-32-281), that at m/e 935 predominating. In the light of the n.m.r. evidence these fragments point to a structure

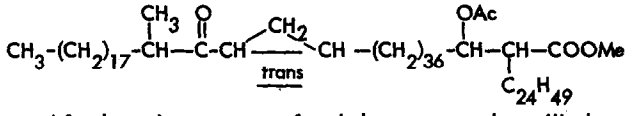


for the main component of methyl mycolate -II. The spectrum of methyl mycolate -II

itself had rearrangement peaks at m/e 712, 740, 768, 796, 824, 852, 880 (mero-aldehydes -32) and 382, 410 (α-branch). A further series of peaks was observed at m/e 519, 547, 575, 603, 631 (aldehydes-281), and also a corresponding series after loss of methanol (aldehydes -281 -32). Simple cleavage of the aldehyde to give the hydrocarbon fragment $CH_3-(CH_2)_{17}-\overset{CH_3}{\underset{|}{C}}H^+$ (m/e 281) can account for the above series of peaks. It is interesting to note that loss of a fragment of equal mass is apparently involved in the spectrum of methyl mycolate -I (m/e 740 →459); however no such loss occurs in the case of methyl anhydromycolate -I in contrast to that observed above in the spectrum of anhydromycolate -II (m/e 1216 →935). The mass spectra of the ethylidene acetals of the 1,3-diols formed by lithium aluminium hydride reduction of the hydroxy esters -I and -II both showed fragments attributable to meroaldehydes. The spectrum of the cyclic acetal -II showed the expected peaks (m/e 519 to 631 as above) due to loss of 281 mass units from the aldehyde, but the spectrum of the cyclic acetal-I showed no significant peaks in this region of the spectrum. This suggests that the series of peaks at m/e 431 to 543 in the spectrum of methyl mycolate -I are not due to simple cleavage of meromycolic aldehyde -I (accordingly the mass spectrum of methyl trans-9,10-methyleneoctadecanoate is found to show no significant peaks due to cleavage adjacent to the cyclopropane group). It is thus apparent that the fragments at m/e 459 to 543, which have been shown (7) in the case of α-mycolate (Test) to contain an oxygen atom, may be formed by some other route from methyl mycolate -I (M-410-281). An analogous pattern (M-382-253) can be discerned in the published spectra of methyl α-kansamycolate (6,8).

The mass spectrum of methyl anhydromycolate -III showed ten homologous peaks (M-32) at m/e 1172 to 1298, that at 1242 being the most abundant. Cleavage α to the carbonyl group gave peaks at m/e 281, 309 and homologous peaks at m/e 891 to 961.

This series was apparently continued but swamped by fragments due to cleavage β to the carbonyl group at m/e 920 to 1046, that at 990 predominating. The difference in mass (29 units) between the products of α- and β-cleavage establishes that there is a methyl branch adjacent to the carbonyl group on the side remote from the ester group. Peaks at m/e 393, 421 due to cleavage at the β-carbon of the αβ-double bond confirm that the α-chains are the same as in esters -I and -II. The structure



may be proposed for the main component of methyl acetoxymycolate -III; by analogy with phthiocerol A and phthiodiolone A (9,10) it is to be expected that methyl mycolate -II and -III are biogenetically related.

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