The Mycolic Acids of *Mycobacterium phlei*

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Summary The mycolic acids from *Mycobacterium phlei* consist of homologous series of various keto- and carboxy-mycolic acids in addition to those containing the β-hydroxy-acid system as sole oxygen functions.

THE mycolic acid fraction from *Mycobacterium phlei*† was separated, by chromatography of the methyl esters over alumina, into esters derived from (a) mycolic acids devoid of oxygen functions other than the β-hydroxy-acid system, (b) ketomycolic acids, and (c) dicarboxylic acids. Each of these fractions, represented by the general formula (I; $R^2 = n\text{-}C_{22}H_{45}$ for the main homologues) gave on pyrolysis meromycolic aldehydes (II) which on reduction, followed by acetylation of the resulting alcohols, gave the meromycolyl acetates (III); the latter were then subjected to argentation chromatography.

$$\underset{(I)}{R^1CH(OH)\cdot CHR^2\cdot CO_2Me} \longrightarrow \underset{(II)}{R^1CHO} \longrightarrow \underset{(III)}{R^1CH_2OAc}$$

For the ketomycolic acids, which have the same general structural features as those from other mycobacteria,[1,2] the reduction of the aldehydes (II) also resulted in reduction of the keto-group to a secondary hydroxy-group. The

† Extracts of *Mycobacterium phlei* supplied by Dr. G. A. Snow, Imperial Chemical Industries, Limited, Alderley Park, Cheshire.

subsequent acetylation gave, therefore, diacetates which, by argentation chromatography, were separated into three fractions, diacetates -(I), -(II), and -(III) in the order of increasing polarity.

The main part of the diacetate-(I), *m/e* 772, 800, 828 [*M* − 60 (HOAc)], 712, 740, 768 [*M* − 120 (2 HOAc)], 509, 537, 565, 579, 593, 607 [*M* − 281 (loss of the terminal alkyl chain)], showing in its n.m.r. spectrum the presence of terminal and branched methyl, has been assigned structure (IV) where $x = 31$ for the main homologue; the parent mycolate is then (I) with R = $Me\cdot[CH_2]_{17}\cdot CHMe\cdot CO\cdot[CH_2]_x\cdot$. In the same mass spectrum, another series of peaks of lower intensity corresponded to minor components of the structure (V); the mass spectrum of the methoxylated products[3] indicated for the main homologue $y = 14$ and $z = 17$.

The mass spectrum of the diacetate-(II) [*m/e* 812, 840, 868 (*M* − 60); 591, 619, 647 (*M* − 281)] together with the application of a procedure[4] involving oxidation of the corresponding epoxides with periodic acid indicated the structure (VI) with $x = 17$, $y = 14$, and $z = 16$ for the most abundant homologue. The position of the signals given by the epoxide protons[5] established *trans*-configuration for the double bond.

The diacetate-(III) was found, by the same procedures, to have the structure (VII) in respect of its main homologue.

In studies of the remaining fractions by the above procedures, the acids of fraction (a) were found to include, in addition to the *cis*-dienic and dicyclopropane acids reported[6] their monoenoic monocyclopropane analogues; minor components containing *trans*-double bonds are also present. Among the dicarboxylic acids of fraction (c) there are in addition to the unsaturated acids previously described[7] cyclopropanoid analogues of the latter.

These results give, for the first time, an indication of the whole complexity of the mycolic acids from *M. phlei*. A comparison of the structures of these acids present in the same organism is of interest for establishing the biosynthetic route to the various types of mycolic acids.

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(IV) $Me\cdot[CH_2]_{17}\cdot CHMe\cdot CH(OAc)\cdot[CH_2]_x\cdot CH_2OAc$

(V) $Me\cdot[CH_2]_{17}\cdot CHMe\cdot CH(OAc)\cdot[CH_2]_y\cdot \underset{c}{CH(CH_2)CH}\cdot[CH_2]_z\cdot CH_2OAc$

(VI) $Me\cdot[CH_2]_x\cdot CHMe\cdot CH(OAc)\,[CH_2]_y\cdot CHMe\cdot \underset{t}{CH{=}CH}\cdot[CH_2]_z\cdot CH_2OAc$

(VII) $Me\cdot[CH_2]_{17}\cdot CHMe\cdot CH(OAc)\cdot[CH_2]_{14}\cdot \underset{c}{CH{=}CH}\cdot[CH_2]_{17}\cdot CH_2OAc$

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