New Members of the Phthiocerol and Phenolphthiocerol Families from *Mycobacterium marinum*

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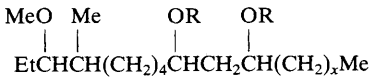
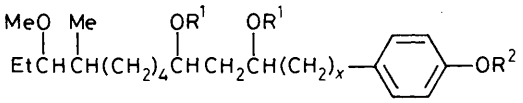
Phthiocerols and phenolphthiocerols from *Mycobacterium marinum* have *erythro*, instead of the more usual *threo*, configuration of their diol units.

The pathogenic mycobacteria *Mycobacterium tuberculosis*, *M. bovis*, *M. leprae*, *M. kansasii* and *M. marinum* produce waxes and glycolipids which are diesters of phthiocerol A (**1a**) and related diols and 'phenolic' glycolipids based on phenolphthiocerol A (**2a**), respectively.[1—4] This communication reports that the diols (**1a**, **2a**) from *M. marinum* have *erythro* configuration in contrast to those studied from other mycobacteria.

Non-polar lipids were extracted from *M. marinum* ATCC 927 and waxes and phenolic glycolipids isolated by t.l.c.[2] The phenolic glycolipids were degraded by mild acid methanolysis[3] and the free phenol was methylated. Proton n.m.r. (200 MHz) of the diacyl phthiocerol A (**1b**) and diacyl methyl phenolphthiocerol A (**2b**) both showed resonances at δ 4.90 (> CH-OR), distinct from those at δ 4.84 in diacyl phthiocerol A from *M. tuberculosis*. Reduction of (**1b**) and (**2b**) with $LiAlH_4$[1] gave methyl-branched alcohols and the diols (**1a**, **2c**), the latter having R_f values on t.l.c. (petroleum ether/acetone 82 : 18, three times) of 0.55 and 0.50 in contrast to 0.49 and 0.44 for standard diols from *M. tuberculosis* and *M. kansasii*, respectively. I.r. spectra in dilute CCl_4 (*ca.* 0.005 M) of the diols (**1a**, **2c**) both gave absorptions at 3621 and 3533 cm^{-1} in the relative ratio 0.68. These ratios of free to bonded hydroxy absorptions correlate well with that (0.70) recorded for *erythro*-decane-



(1) **a**; R = H, x = 16—22
b; R = $Me(CH_2)_{16-20}[CH_2CH(Me)]_{2-4}CO$, x = 16—22
c; R = H, x = 14



(2) **a**; $R^1 = R^2$ = H, x = 16—22
b; $R^1 = Me(CH_2)_{16-20}[CH_2CH(Me)]_{2-4}CO$, R^2 = Me, x = 16—22
c; R^1 = H, R^2 = Me, x = 16—22
d; $R^1 = R^2$ = H, x = 18—22

4,6-diol; the *threo*-isomer of this compound and phthiocerol A from *M. tuberculosis* showed ratios of 1.16 and 1.19, respectively.[5] The diols (**1a**, **2c**) were converted to cyclic acetals by acid-catalysed reaction with 3,5-di(trifluoromethyl)-benzaldehyde and the ^{1}H n.m.r. spectra (200 MHz) both showed resonances at δ 5.57 assigned to the CH of the substituted benzylidene group. The corresponding acetal from the *M. tuberculosis* phthiocerol A had a signal at δ 5.82 again indicating, by comparison with model ethylidene acetals,[5] that the diols (**1a**, **2a**) have *erythro*-configuration. Electron impact m.s. showed that the phthiocerol A and phenolphthiocerol A components had the structures (**1c**) and (**2d**), respectively.[1,4]

The novel *erythro*-diols (**1c**, **2d**) from *M. marinum* can be distinguished from the *threo*-diols, common in other mycobacteria, by t.l.c., i.r. spectra and n.m.r. spectra of both cyclic acetal derivatives and the intact waxes and glycolipids. It should be noted, however, that the current stereochemical assignments were made on mixtures of homologues. The n.m.r. spectra did not show any evidence of stereochemical inhomogeneity in the mixtures. When larger amounts of the diols become available, pure molecular species may be obtained by reverse-phase chromatography and analysed individually.

The multimethyl-branched acyl components, esterified to these lipids, have L absolute configuration in contrast to those, the mycocerosic (mycoceranic) acids, from *M. tuberculosis, M. bovis, M. leprae*, and *M. kansasii* which have D-chiral centres.[2,4] It is apparent, therefore, that the lipids from *M. marinum* are composed of structurally similar, but stereochemically distinct, long-chain components. The methyl-branched acids from *M. ulcerans* also have L-configuration[4] and it is likely, therefore, that the diols to which they are linked have *erythro*-stereochemistry.

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References

1 D. E. Minnikin, G. Dobson, M. Goodfellow, M. Magnusson, and M. Ridell, *J. Gen. Microbiol.*, 1985, **131**, 1375.
2 G. Dobson, D. E. Minnikin, S. M. Minnikin, J. H. Parlett, M. Goodfellow, M. Ridell, and M. Magnusson, in 'Chemical Methods in Bacterial Systematics,' eds. M. Goodfellow and D. E. Minnikin, Academic Press, London, 1985, pp. 237—265.
3 D. E. Minnikin, G. Dobson, J. H. Parlett, M. Goodfellow, and M. Magnusson, *Eur. J. Clin. Microbiol.*, 1987, **6**, 703.
4 M. Daffé and M-A, Lanéelle, *J. Gen. Microbiol.*, 1988, **134**, 2049.
5 K. Maskens, D. E. Minnikin, and N. Polgar, *J. Chem. Soc.* (*C*), 1966, 2113.