The Methoxymycolic and Ketomycolic Acids from Human Tubercle Bacilli

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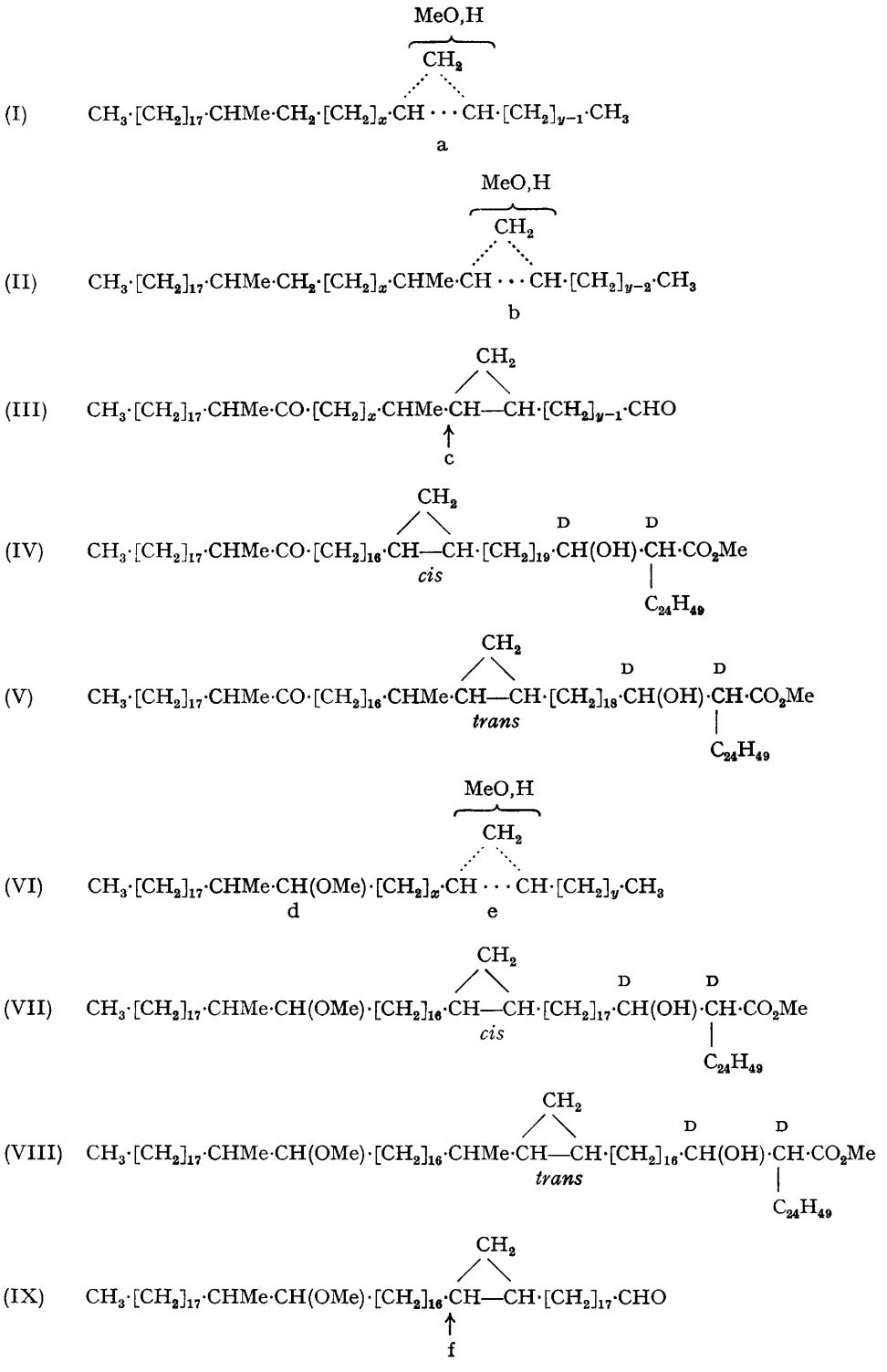
IN continuation of earlier studies,[1-3] the n.m.r. spectra of methyl mycolate-II and a new sample of methyl mycolate-III (m.p. 52—53°, $[\alpha]_D$ + 5·0°, obtained by chromatography of the crude mycolic esters from human tubercle bacilli) are both found to show signals attributable to *cis* (τ 9·4, 10·3) and *trans* (τ 9·8) cyclopropane protons.[4] The relative intensities of these signals suggest that these esters consist of mixtures of *cis*- and *trans*-cyclopropane compounds in the approximate ratios 5 : 1 and 1 : 2 for mycolate-II and -III, respectively.

The mass spectra of methyl mycolates-II and -III both show peaks which can be assigned to two separate homologous series of meroaldehydes[1] at *m/e* 768, 796, *824*, 852, 880, 908 (mycolate-IIa) (*M* — MeOH); 782, 810, *838*, 866, 894, 922 (mycolate-IIb) (*M* — MeOH), and *m/e*, 784, 812, 840, *868*, 896, 924 (mycolate-IIIa) (*M*); 798, 826, 854, *882*, 910, 938 (mycolate-IIIb) (*M*), respectively (the most abundant peak in any series is in italics). The relative intensities (6 : 1, 5 : 9) of these peaks corresponding to series a and b are similar to those of the n.m.r. signals, so it appears that mycolates-IIa and -IIIa have *cis*-cyclopropane rings while mycolates-IIb and -IIIb correspond to *trans* stereochemistry. The fact that the *trans*-compounds have molecular weights 14 mass units higher than the *cis*-compounds suggests the presence of an additional methyl branch (*cf.*, ref. 5) the location of which adjacent to a cyclopropane function is supported by the presence of a doublet (τ 9·01) in the n.m.r. spectrum of methyl mycolate-III (*cf.*, ref. 3).

Methyl mycolate-III was converted into its thioketal derivative which on pyrolysis gave, in addition to the corresponding meroaldehyde, 1-normeromycolane-III thioketal presumably formed by thermal loss of carbon monoxide. Desulphurization of the latter with Raney nickel gave 1-nordeoxymeromycolane-III. Meromycolal-II was also prepared and reduced with $LiAlH_4$ to the primary alcohol. The corresponding methanesulphonate on reduction with $LiAlH_4$ gave meromycolane-II; this compound and 1-nordeoxymeromycolane-III were treated with BF_3–MeOH [2] and the methoxylated products separated.

The mass spectrum of the methoxylated product derived from 1-nordeoxymeromycolane-III shows peaks at *m/e* 311, 325, 339; 577, 591, 605 due to cleavage at centre a of component-IIIa (I) and


MeO,H
CH2
(I) CH3·[CH2]17·CHMe·CH2·[CH2]x·CH···CH·[CH2]y−1·CH3
a
MeO,H
CH2
(II) CH3·[CH2]17·CHMe·CH2·[CH2]x·CHMe·CH···CH·[CH2]y−2·CH3
b
CH2
(III) CH3·[CH2]17·CHMe·CO·[CH2]x·CHMe·CH—CH·[CH2]y−1·CHO
c
CH2
D D
(IV) CH3·[CH2]17·CHMe·CO·[CH2]16·CH—CH·[CH2]19·CH(OH)·CH·CO2Me
cis
C24H49
CH2
D D
(V) CH3·[CH2]17·CHMe·CO·[CH2]16·CHMe·CH—CH·[CH2]18·CH(OH)·CH·CO2Me
trans
C24H49
MeO,H
CH2
(VI) CH3·[CH2]17·CHMe·CH(OMe)·[CH2]x·CH···CH·[CH2]y·CH3
d e
CH2
D D
(VII) CH3·[CH2]17·CHMe·CH(OMe)·[CH2]16·CH—CH·[CH2]17·CH(OH)·CH·CO2Me
cis
C24H49
CH2
D D
(VIII) CH3·[CH2]17·CHMe·CH(OMe)·[CH2]16·CHMe·CH—CH·[CH2]16·CH(OH)·CH·CO2Me
trans
C24H49
CH2
(IX) CH3·[CH2]17·CHMe·CH(OMe)·[CH2]16·CH—CH·[CH2]17·CHO
f

m/e 297, 311, 325; 591, 605, 619 attributable to cleavage at centre b of component-IIIb (II). The mass spectrum of methyl mycolate-III contains a large peak at *m/e* 321 which can be attributed to cleavage at position c (see below) of the meroaldehyde-IIIb having the structure (III). In the light of previous evidence[1,6] the above results lead to the structures (IV) and (V) for the main components of methyl mycolate-IIIa and -IIIb, respectively (*cf.*, ref. 5).

The mass spectrum of the methoxylated compound from addition of BF_3–MeOH to the mixture of meromycolane-IIa and -IIb shows peaks at *m/e* 325 and 297, 311, 325 due to cleavage at centres d and e, respectively, of component-IIa (VI) and peaks at *m/e* 561 and 561, 575, 589 due to cleavage also at centres d and e of this component but with elimination of methanol from centres e and d, respectively. Taking other evidence[1,6] into account it may thus be calculated that the main component of methyl mycolate-IIa has the structure (VII). The main component of mycolate-IIb is present in insufficient quantity to give significant fragments but by analogy with mycolate-IIIa and -IIIb, methyl mycolate-IIb probably has the structure (VIII). The structures suggested[1] previously were an attempt to represent mycolates-II and -III, each now shown to contain constituents belonging to two series, by single formulae taking into account the evidence then available.

Etémadi[7] has claimed that the structure (VII) can be derived on the basis of a peak at *m/e* 307 in the mass spectrum attributable to cleavage of the meroaldehyde (IX) at position f. However, in agreement with our recent results,[3] the mass spectra of methyl mycolate-II and meromycolal-II do not contain significant peaks at *m/e* 307, nor does the spectrum of methyl mycolate-III contain an analogous peak at *m/e* 335 due to cleavage of meromycolal-IIIa. As mentioned above, the latter spectrum does contain a large peak at *m/e* 321 which is attributable to cleavage of meromycolal-IIIb (III) and shows once more that specific fragmentation of long-chain aldehydes does not occur at a cyclopropane ring unless the ring is at a critical distance from the aldehyde group[2,3] or other structural features (*e.g.*, a methyl branch) are present near the cyclopropane function.[3]

The formulae (IV, V, VII, VIII) suggested above are compatible with structures suggested for olefinic dicarboxylic acids isolated from *M.phlei*.[8] The terminal chain of methyl mycolate-I[2,3] is two CH_2- units larger than those of mycolate-II and -III and thus a biosynthesis involving methylation with methionine of a common olefinic intermediate as suggested recently by Etémadi[5] is very unlikely. The obvious route to the function–CHMe·CO– would be the incorporation of propionic acid which is known to occur[9] in the biosynthesis of other compounds from human tubercle bacilli. Consequently the uptake of labelled propionic acid should be studied before analogies are drawn with the biosynthesis of mycolic acids from *M. avium* and *M. phlei*, in which this methyl branch is said[8] to be derived from methionine.

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