565. *Mycoceranic Acid. Part III.*[1]

By N. Polgar and W. Smith.

In continuation of earlier investigations the lævorotatory acid fraction from the lipids of tubercle bacilli (" mycoceranic acid ") has been subjected to studies involving a series of stepwise degradations and examination of the degradation products by vapour-phase chromatography. The results of these studies indicate that the above fraction is a mixture containing homologous 2,4,6-trimethyl- and 2,4,6,8-tetramethyl-substituted long-chain acids, with 2,4,6-trimethylhexacosanoic and 2,4,6,8-tetramethyloctacosanoic acid (both regarded as having *D*-configuration * in respect of all asymmetric centres) as the major components.

In previous studies[1,3,4] " mycoceranic acid," the lævorotatory acid fraction from the lipids of tubercle bacilli, was assigned the structure (I) where n, on the basis of analytical data, was stated to have the probable value 21; however, attempts[1] to confirm the length of the carbon chain in mycoceranic acid by comparing two of the degradation products with synthetic compounds gave ambiguous results. Asselineau *et al.*,[5] from mass-spectrometric studies of a specimen of " methyl mycocerosate," isolated by Ginger and Anderson[6] and shown[5] to have infrared spectroscopic properties identical with those of methyl mycoceranate, concluded that the empirical formula of the acid was $C_{32}H_{64}O_2$ [corresponding to $n = 22$ in structure (I)]. More recently, as a result of investigations involving vapour-phase chromatography of the methyl ester derived from the natural product,

* The symbols *D* and *L* are used in the sense defined by Linstead *et al.*[2]

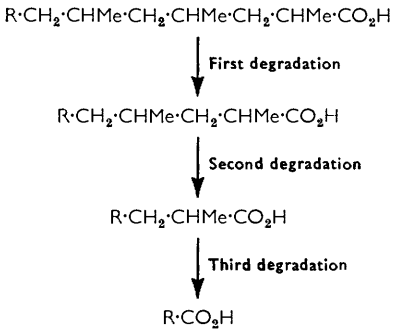
[1] Part II, Marks and Polgar, *J.*, 1955, 3851.
[2] Linstead, Lunt, and Weedon, *J.*, 1950, 3333.
[3] Chanley and Polgar, *J.*, 1954, 1003.
[4] Polgar, *J.*, 1954, 1011.
[5] Asselineau, Ryhage, and Stenhagen, *Acta Chem. Scand.*, 1957, **11**, 196.
[6] Ginger and Anderson, *J. Biol. Chem.*, 1954, **157**, 203.

followed by mass-spectrometric studies of the resulting fractions, Asselineau *et al.*[7] suggested that the naturally occurring acid was a mixture of C_{22}, C_{24}, C_{26}, and C_{28} straight-chain acids, 2,4,6-trimethyl-substituted C_{25}, C_{27}, and C_{29} acids, and 2,4,6,8-tetramethyl-substituted C_{30}, C_{32}, and C_{34} acids, with 2,4,6,8-tetramethyloctacosanoic acid as the main component.

The present paper reports a further study of mycoceranic acid (a preliminary report [8] has been published). The acid was obtained from the lipids of human tubercle bacilli (strains D.T., P.N., and C.[9]) through the mixture of barium salts which resulted as a by-product in the procedure described earlier [10] for the isolation of phthiocerol. The purified methyl ester (for details of isolation, see Experimental section) having $[\alpha]_D$ $-7{\cdot}35°$ gave on hydrolysis the acid with $[\alpha]_D$ $-5{\cdot}62°$.

Vapour-phase chromatography of the above methyl ester indicated the presence of two major and five minor components. From a comparison of the retention times of these components with those of synthetic methyl esters it appeared that they were derived from 2,4,6-trimethyl-substituted acids with 25, 26, 27, 28, 29, 30, and 31 carbon atoms and/or from 2,4,6,8-tetramethyl-substituted acids with 26, 27, 28, 29, 30, 31, and 32 carbon atoms; with the chromatographic apparatus employed it was not possible to differentiate between a C_n-tetramethyl-substituted and a C_{n-1}-trimethyl-substituted ester. It is of interest to note that there was no indication of the presence of a methyl ester derived from a 2,4,6,8-tetramethyl-substituted C_{34} acid as has been previously [7] suggested for methyl mycocerosate.

In further studies mycoceranic acid was subjected to three successive stepwise degradations by procedures already described,[4] each degradation involving conversion of the acids into the corresponding αβ-unsaturated esters and oxidation of the latter by potassium permanganate. These degradations are conveniently represented for a 2,4,6-trimethyl-substituted acid by the scheme shown below.



Since each degradation removed three carbon atoms (including the carbon atom of a methyl branch), the three degradations resulted in the removal of nine carbon atoms. If, therefore, mycoceranic acid is a mixture of 2,4,6-trimethyl-substituted and 2,4,6,8-tetramethyl-substituted acids (cf. ref. 7), the acidic products resulting from three successive degradations should be a mixture of straight-chain and 2-methyl-substituted acids.

After each degradation the methyl esters of the resulting acids were compared, by vapour-phase chromatography, with methyl esters of known structures. The results of these studies may be summarised as follows.

Examination of the methyl esters resulting from the first and second degradations gave ambiguous results, because it was not possible to differentiate, after the first degradation, between 2,4,6-trimethyl-substituted C_n esters and 2,4-dimethyl-substituted C_{n-1} esters, and,

[7] C. Asselineau, J. Asselineau, Ryhage, Ställberg-Stenhagen, and Stenhagen, *Acta Chem. Scand.*, 1959, **13**, 822.
[8] Polgar and Smith, *Chem. and Ind.*, 1961, 1958.
[9] Green, *Veterinary J.*, 1946, **102**, 267.
[10] Hall, Lewis, and Polgar, *J.*, 1955, 3971.

after the second degradation between 2,4-dimethyl-substituted C_n esters and 2-methyl-substituted C_{n-1} esters. After the third degradation, the retention times of the components indicated the presence of the methyl esters of straight-chain C_{16}, C_{17}, C_{18}, and C_{20} acids, and of 2-methyl-substituted C_{21}, C_{22}, and C_{23} acids, together with an additional component

(I) $Me\cdot[CH_2]_n\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CO_2H$

(II) $Me\cdot[CH_2]_{19}\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CO_2Me$

(III) $Me\cdot[CH_2]_{19}\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CH_2\cdot\overset{D}{C}HMe\cdot CO_2Me$

having a retention time intermediate between that of methyl nonadecanoate and methyl 2-methylnonadecanoate. Since nine carbon atoms have been removed, it follows that the specimen of mycoceranic acid investigated was essentially a mixture of 2,4,6-trimethyl-substituted C_{25}, C_{26}, C_{27}, and C_{29} acids, and 2,4,6,8-tetramethyl-substituted C_{30}, C_{31}, and C_{32} acids, together with an additional component which may have been 2,4,6-trimethylpentacosanoic, and/or 2,4,6,8-tetramethylpentacosanoic acid. Furthermore, an examination of the chromatogram obtained for methyl mycoceranate indicated that the major components of the mixture of esters were methyl 2,4,6-trimethylhexacosanoate and 2,4,6,8-tetramethyloctacosanoate. Since the asymmetric centres at C-2, C-4, and C-6 have been shown [1] to belong to the *D*-series, these esters are regarded as having the structures (II) and (III), respectively; their synthesis is described in the following communication.[11]

The isolation of methyl mycoceranate involved, at one stage, the removal of straight-chain methyl esters as urea complexes. An examination of these straight-chain esters by vapour-phase chromatography indicated the presence of esters derived from acids with 16, 18, 20, 22, 24, 25, 26, 27, and 28 carbon atoms.

Experimental

Petrol refers to light petroleum, of b. p. 40—60°. The alumina used for chromatography was standardised according to Brockmann and Schodder.[12] Optical rotations were measured in chloroform ($l = 1$).

Isolation of "Mycoceranic Acid."—In the procedure previously described [10] for the isolation of phthiocerol the methanol-insoluble product (about 500 g.), resulting from a partial hydrolysis of the lipids of tubercle bacilli, was subjected to a prolonged alkaline hydrolysis; the hydrolysate was acidified and extracted with ether. From the mixture of products resulting on evaporation of the ethereal extract "mycolic acid" was isolated by virtue of its insolubility in benzene–methanol (2 : 5); the remaining material, in benzene solution, was treated with methanolic barium hydroxide. The precipitated barium salts were collected by filtration and acidified with hydrochloric acid. Extraction of the resultant mixture with ether afforded the crude lævorotatory acids (73 g.) which were used as the starting material in the present work. The acids were converted by the action of 5% methanolic sulphuric acid into their methyl esters. The esters were chromatographed in petrol on alumina (activity II), and the material eluted by petrol (54 g.; further elution with ether gave material containing keto- and hydroxy-esters) was distilled. Treatment of the fraction, b. p. 200—230°/0·1 mm. (39·8 g.), $[\alpha]_D^{20}$ −7·22° (*c* 10·8), in petrol with urea [13] (moistened with methanol) removed straight-chain esters (together with some lævorotatory esters) as a complex, and left esters (37·2 g.) having $[\alpha]_D^{22}$ −7·35° (*c* 23·45). Hydrolysis of this product by refluxing aqueous-ethanolic (3 : 7) 9% potassium hydroxide for 1 hr. gave acids with $[\alpha]_D^{21}$ −5·62° (*c* 8·9). It should be noted that hydrolysis by refluxing aqueous-methanolic (3 : 7) 10% potassium hydroxide for 2·25 hr. gave acids with the same rotatory power, thus indicating that little, if any, inversion in respect to the centre at C-2 occurred under these conditions.

[11] Polgar and Smith, Part IV, following paper.
[12] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.
[13] Cf. Schlenk, *Annalen*, 1949, **565**, 204; Linstead and Whalley, *J.*, 1950, 2987; Skellon and Taylor, *J.*, 1953, 1433.

Stepwise Degradations.—(i) *First degradation.* The preceding acids (4 g.) were heated, with stirring, with bromine (7·4 g.) in the presence of red phosphorus (0·35 g.) at 100° (bath) for 6 hr., and the resulting crude acid bromides converted into the bromo-esters by means of methanol (20 ml.) in the manner already described.[4] This material was refluxed with pyridine (30 ml.) for 18 hr., and the product, isolated in the known manner, was passed in petrol over alumina (activity II; 20 g.). Elution with petrol gave the αβ-unsaturated esters as a wax (3·02 g.), $[\alpha]_D^{24}$ −13·45° (*c* 6·7), $\lambda_{max.}$ 2125 Å (ε 11,250, calc. for *M* 400). This product (2·75 g.) was oxidised with potassium permanganate (9 g., added in small portions) in refluxing acetone (150 ml.) for 6 hr., and the product worked up as described previously.[4] The resulting acids (1·93 g.) were esterified with 5% methanolic sulphuric acid, and the methyl esters (1·87 g.) chromatographed in petrol on alumina (activity III; 10 g.). Elution with petrol gave the esters as a wax (1·53 g.), $[\alpha]_D^{28}$ −6·55 (*c* 9·8); hydrolysis with 10% aqueous-methanolic potassium hydroxide afforded the corresponding acids.

(ii) *Second degradation.* Bromination and dehydrobromination, as above, followed by oxidation of the αβ-unsaturated esters with potassium permanganate, and esterification of the resulting acids with ethereal diazomethane, gave methyl esters, in the following named " esters from the second degradation."

(iii) *Third degradation.* A repetition of the above processes afforded the " esters from the third degradation."

Vapour-phase Chromatography.—A Pye Argon Chromatograph fitted with a 4-ft. column containing 10% of " Apiezon L " on " Embacel " was used. Since only a limited number of methyl esters of 2-methyl-, 2,4,6-trimethyl-, and 2,4,6,8-tetramethyl-substituted acids was available for comparison, the retention times of other members of these series were estimated by extrapolation in the following way.

The graphs plotted for the logarithms of the retention times (determined under identical experimental conditions) against the number of carbon atoms for methyl esters of (i) authentic straight-chain acids, and (ii) 2,4-dimethyl-substituted acids (dimethyl-eicosanoic,[14] -docosanoic,[15] -tricosanoic,[16] -pentacosanoic,[11] -hexacosanoic,[1] and -heptacosanoic [17]) gave straight parallel lines. Lines parallel to the foregoing ones were drawn through the points plotted for methyl esters of (iii) 2-methyl-substituted acids (methyloctacosanoic [14] and methyleicosanoic [18]), and (iv) 2,4,6-trimethyl-substituted acids (trimethylhexacosanoic [11] and trimethyloctacosanoic [1]), and for (v) methyl 2,4,6,8-tetramethyloctacosanoate,[11] and the retention times of unknown members of each homologous series estimated by reference to the graph. The retention times estimated by extrapolation are in the following quoted in parentheses; mono-, di-, tri-, and tetra-methyl denote 2-methyl, 2,4-dimethyl, 2,4,6-trimethyl, and 2,4,6,8-tetramethyl, respectively.

(a) " *Methyl mycoceranate.*" Vapour-phase chromatography (250°; flow-rate 30 ml./min.) indicated the presence of two major components with retention times of 23·2 and 38·9 min., and five minor components having retention times of 7·9, 10·3, 13·6, 17·6, and 29·7 min. (there were no components with retention times longer than 38·9 min.). The following are the retention times of methyl esters of trimethyl- and tetramethyl-substituted acids for the same conditions: trimethyldocosanoate, (7·9); tetramethyldocosanoate, (7·9); trimethyltricosanoate, (10·2); tetramethyltricosanoate, (10·4); trimethyltetracosanoate, (13·7); tetramethyltetracosanoate, (13·8); trimethylpentacosanoate, (17·8); tetramethylpentacosanoate, (17·9); trimethylhexacosanoate, 23·2; tetramethylhexacosanoate, (23·3); trimethylheptacosanoate, (29·6); tetramethylheptacosanoate, (29·8); trimethyloctacosanoate, 39·1; tetramethyloctacosanoate, 39·1.

(b) *Methyl esters of the acids resulting from the first degradation.* Vapour-phase chromatography (250°, flow rate 30 ml./min.) showed the presence of major components with retention times of 13·5 and 22·7 min., and minor components with retention times of 4·5, 5·9, 7·7, 10·2, and 17·5 min., to be compared with the following retention times for methyl esters of dimethyl- and trimethyl-substituted acids: dimethyleicosanoate, 4·6; trimethyleicosanoate, (4·6); dimethylheneicosanoate, (5·9); trimethylheneicosanoate, (5·8); dimethyldocosanoate, 7·8; trimethyldocosanoate, (7·6); dimethyltricosanoate, 10·2; trimethyltricosanoate, (10·1);

[14] Bailey, Polgar, Tate, and Wilkinson, *J.*, 1955, 1547.
[15] Fray and Polgar, *J.*, 1956, 2036.
[16] Bailey, Brice, Horne, and Polgar, *J.*, 1959, 661.
[17] Brettle, Polgar, and Smith, *J.*, 1960, 2802.
[18] Bailey, Polgar, and Robinson, *J.*, 1953, 3031.

dimethyltetracosanoate, (13·3); trimethyltetracosanoate, (13·3); dimethylpentacosanoate, 17·3; trimethylpentacosanoate, (17·4); dimethylhexacosanoate, 22·7; trimethylhexacosanoate, 22·6.

(c) *Methyl esters from the second degradation.* Vapour-phase chromatography at 251° (flow-rate 30 ml./min.) showed the presence of major components with retention times of 7·3 and 12·6 min., and minor components with retention times of 2·62, 3·52, 4·22, 5·45, and 9·6 min., to be compared with the following retention times for methyl esters of monomethyl- and dimethyl-substituted acids: methyloctadecanoate, 2·6; dimethyloctadecanoate, (2·67); methylnonadecanoate, (3·2); dimethylnonadecanoate, (3·3); methyleicosanoate, 4·2; dimethyleicosanoate, 4·3; methylheneicosanoate, (5·5); dimethylheneicosanoate, (5·7); methyldocosanoate, (7·2); dimethyldocosanoate, 7·4; methyltricosanoate, (9·4); dimethyltricosanoate, 9·7; methyltetracosanoate, (12·3); dimethyltetracosanoate, (12·6).

Component	Retention time (min.)	Methyl ester	Retention time (min.)
1	7·3	n-hexadecanoate	7·3
		methylhexadecanoate	(7·6)
2	10·25	n-heptadecanoate	10·25
		methylheptadecanoate	(10·6)
3	14·3	n-octadecanoate	14·4
		methyloctadecanoate	14·9
4	21	n-nonadecanoate	(20·6)
		methylnonadecanoate	(21·4)
5	29	n-eicosanoate	29·2
6	30·2	methyleicosanoate	30·1
7	44	n-heneicosanoate	(41·1)
		methylheneicosanoate	(43·2)
8	65	n-docosanoate	60·5
		methyldocosanoate	(64)

(d) *Methyl esters from the third degradation.* Vapour-phase chromatography at 216° (flow-rate 32 ml./min.) showed the presence of eight components with the retention times listed in the Table together with retention times for methyl esters of straight-chain and monomethyl-substituted acids.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, November 27th*, 1962.]