567. *Studies on the Mycolic Acids. Part II.*[1]

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Further investigations of the material studied in Part I [1] indicate the presence of (i) at least three hydroxy-methoxy-acids containing as substituents in the α-position, normal alkyl chains with 20, 22, and 24 carbon atoms, and (ii) at least two hydroxy-acids (containing no methoxyl groups) having in the α-position normal alkyl chains with 22 and 24 carbon atoms. The hydroxy-methoxy-acids are shown to possess branches of normal alkyl chains at position 4; some further studies of these acids concerning the alkyl chains attached at the carbon atom bearing the methoxyl group are also described.

In Part I [1] degradative studies of "mycolic acid," isolated from human tubercle bacilli (strains D.T., P.N., and C[2]) were described. The results of these studies, together with the earlier work by Asselineau and Lederer,[3] pointed to the structure (I) for the acid, where n was probably 16, and R represented a branched-chain alkyl residue. Some extensions of these studies are now reported.

In the present work the methyl ester of the acid (obtained after purification of the crude acid by chromatography over alumina of activity III—IV) when chromatographed over alumina (activity III—IV) afforded (i) ester *A* consisting essentially of β-hydroxy-esters with a methoxyl group, and (ii) ester *B* which was largely a mixture of β-hydroxy-esters containing no methoxyl.

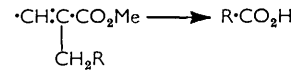
Ester A.—Pyrolysis of this ester as described previously [4] for mycolic acid and methyl mycolate, followed by vapour-phase chromatography of the resulting straight-chain esters, indicated the presence of two major and a minor component. Comparison of their retention times with those of authentic straight-chain esters showed that the major components were methyl tetracosanoate and hexacosanoate (in the approximate ratio of 1 : 3), and the minor component (about 2—3% of the mixture) was methyl docosanoate. These results in the light of the previous studies of Asselineau and Lederer [3] indicated that the ester *A* is a mixture of β-hydroxy-esters containing, as substituents in the

[1] Part I, Morgan and Polgar, *J.*, 1957, 3779.
[2] Green, *Veterinary J.*, 1946, **102**, 267.
[3] For a general review see Asselineau and Lederer, *Fortschr. Chem. Org. Naturstoffe*, 1953, **10**, 170, and references cited there.
[4] Stodola, Lesuk, and Anderson, *J. Biol. Chem.*, 1938, **126**, 505; Lesuk and Anderson, *ibid.*, 1940, **136**, 603.

α-position, normal alkyl chains with 20, 22, and 24 carbon atoms, with the α-C_{22}- and α-C_{24}-esters as major components.

The corresponding αβ-unsaturated esters (methyl anhydromycolate *A*), obtained from the ester *A* through the toluene-*p*-sulphonyl derivative, gave on oxidation with potassium permanganate in acetone, as described in Part I [1] for the oxidation of methyl anhydromycolate, a mixture of branched-chain methoxy-acids, now named *O*-methylmeromycolic acid *A*, together with straight-chain acids. The latter, on examination of their methyl esters by vapour-phase chromatography, were found to consist of docosanoic and tetracosanoic acid (in an approximate ratio of 1 : 3) as major constituents, together with small amounts of acids with 18, 19, 20, 21, 23, and 25 carbon atoms.

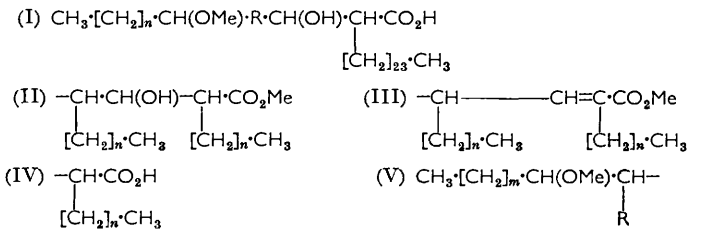
Comparison of the straight-chain acids arising on oxidation of the above αβ-unsaturated ester with the products obtained on pyrolysis of ester *A* indicates that the oxidation of the αβ-unsaturated ester proceeds essentially according to the scheme:



where R for the components of the αβ-unsaturated ester is $C_{19}H_{39}$, $C_{21}H_{43}$, and $C_{23}H_{47}$, respectively. It is noted at this point that in Part I [1] the oxidation of methyl anhydromycolate was considered to result in the formation of pentacosanoic acid. That suggestion was based upon a comparison of the oxidation product with an acid isolated in earlier studies [5] as an oxidation product of methyl 2-oxohexacosanoate (the latter expected to arise as an intermediate on oxidation of methyl anhydromycolate). In the earlier studies [5] the oxidation product of methyl 2-oxohexacosanoate was considered to be pentacosanoic acid; it is now, however, found, on vapour-phase chromatography of the corresponding methyl ester, that this oxidation product was largely tetracosanoic acid.

Demethylation of *O*-methylmeromycolic acid *A* by means of acetic anhydride in the presence of toluene-*p*-sulphonic acid (cf. Part I [1]), followed by pyrolysis of the resulting acetoxy-acid (*O*-acetylmeromycolic acid *A*), gave a mixture of unsaturated acids which was hydrogenated in the presence of platinic oxide, affording deoxymeromycolic acid *A*. Bromination of the latter (Hell–Volhard–Zelinsky method), followed by reaction of the α-bromo-acid bromide with methanol and dehydrobromination of the resulting α-bromo-ester by means of pyridine, gave the corresponding αβ-unsaturated ester. Oxidation of this ester by means of potassium permanganate in acetone gave, among other products, a mixture of straight-chain acids, found, on examination of their methyl esters by vapour-phase chromatography, to consist of docosanoic and tetracosanoic acid (in an approximate ratio of 1 : 3) as main constituents, together with small amounts of other acids (see Experimental section).

These results indicated that deoxymeromycolic acid *A* has the partial structure (IV) where *n* for the main constituents is 21 and 23, respectively; the ester *A* has then the structure (II) and the corresponding αβ-unsaturated ester the structure (III).



In further experiments the mixture of unsaturated esters, obtained on pyrolysis of the methyl ester derived from *O*-acetylmeromycolic acid *A*, was oxidised with potassium

[5] Boast and Polgar, *J.*, 1957, 3800.

permanganate in acetone, and the resulting straight-chain acids were investigated by vapour-phase chromatography of their methyl esters. The retention times of the components indicated the presence of major amounts of the acids with 16—20 carbon atoms with a preponderance of the C_{18}- and C_{19}-acids. Since pyrolysis of esters is now known [6] to yield a mixture of olefins, the above results do not admit any safe conclusions as to the accurate length of the alkyl chains attached at the carbon atom bearing the methoxyl group. In view of the preponderance of the C_{18}- and C_{19}-acids among the oxidation products the terminal portion of ester *A* is provisionally regarded as having the structure (V), where *m* for the main component is about 17; since the oxidation of the above unsaturated esters also appears to yield keto-esters (see Experimental section), R in formula (V) probably represents an alkyl branch.

Ester B.—Preliminary studies involving pyrolysis of this ester and oxidation of the corresponding αβ-unsaturated ester, as described for the ester *A*, indicated that ester *B* is a mixture of β-hydroxy-esters containing, as substituents in the α-position, normal alkyl chains with 22 and 24 carbon atoms, with an approximate ratio of 1 : 4 for the α-C_{22}- and α-C_{24}-esters.

Experimental

Petrol refers to light petroleum, of b. p. 40—60°. The alumina used for chromatography was acid-washed and standardised according to Brockmann and Schodder.[7] Vapour-phase chromatography was carried out on a Pye Argon Chromatograph fitted with a 4 ft. column containing, except where otherwise stated, 3% of silicone oil (E 301, Imperial Chemical Industries Limited) on " Embacel."

Isolation of Ester A *and* B.—Crude mycolic acid (30·5 g.; obtained as described in Part I [1]) was chromatographed on alumina (activity III—IV; 500 g.). The following fractions were taken: (1) ether (2 l.) (3·7 g.); (2) ether–acetic acid (99·5 : 0·5—99 : 1; 3 l.) (23 g.); (3) ether–acetic acid (95 : 5; 2 l.) (2 g.) (94% recovery). Fraction (1) contained non-acid material; only fraction (2) was used for the following studies. When, in other experiments, the crude mycolic acid was chromatographed over alumina of activity II or II—III, the recovered material amounted to only 74 and 78%, respectively.

A 9 g. portion of fraction (2) was esterified with ethereal diazomethane, and the resulting methyl ester chromatographed on alumina (activity III—IV; 250 g.). The following fractions were collected: (*a*) petrol (2 l.) (3·3 g.); (*b*) petrol (2 l.) (1 g.); (*c*) petrol (4 l.) (1 g.); (*d*) ether (4 l.) (3·8 g.). Fraction (*a*) showed in its infrared spectrum bands at 3484 (OH) and 1730 cm.$^{-1}$ (ester) with a weaker band at 1709 cm.$^{-1}$ (ester); there was no band at 1903 cm.$^{-1}$ (MeO). Fractions (*b*) and (*c*) showed bands at 3484 and 1709 with a shoulder at 1730 cm.$^{-1}$ (no band at 1093 cm.$^{-1}$), whilst fraction (*d*) showed bands at 3484, 1709 (with a shoulder at 1730), and 1093 cm.$^{-1}$ (MeO).

Fraction (*d*), now named ester *A*, had m. p. 44—45° (Found: C, 81·8; H, 13·2. Calc. for $C_{98}H_{196}O_4$: C, 81·8; H, 13·7. Calc. for $C_{90}H_{180}O_4$: C, 81·5; H, 13·7%).

Fractions (*a*), (*b*), and (*c*) were combined and chromatographed on alumina (activity II; 100 g.). The following fractions were obtained: (i) petrol (1500 ml.) (1·1 g.); (ii) petrol (600 ml.) (0·6 g.); (iii) ether (500 ml.) (3·4 g.). Fraction (i) showed in its infrared spectrum only a weak hydroxyl band (3484 cm.$^{-1}$), and carbonyl bands at 1709 and 1730 cm.$^{-1}$ (the latter being stronger); fractions (ii) and (iii) showed, in addition to the hydroxyl band, a carbonyl band at 1709 cm.$^{-1}$ with a shoulder at 1730 cm.$^{-1}$. Comparison of the spectra indicated that a partial separation of the initial mixture had been obtained, fraction (iii) containing most of the hydroxy-ester; this fraction (Found: C, 82·6; H, 13·3. Calc. for $C_{80}H_{160}O_3$: C, 82·1; H, 13·8. Calc. for $C_{90}H_{180}O_3$: C, 82·5; H, 13·85%) is in the following named fraction *B*.

Pyrolysis of Ester A.—The ester (1 g.) was subjected to pyrolytic distillation by heating it at 200—300° (bath)/0·1 mm. The whole distillate (0·2 g.), when treated in petrol with urea, moistened with a little methanol, formed a complex. The recovered ester, isolated by addition of water and ether-extraction, showed on vapour-phase chromatography (197°/260 mm.;

[6] DePuy and King, *Chem. Rev.*, 1960, **60**, 431; Froemsdorf, Collins, Hammond, and DePuy, *J. Amer. Chem. Soc.*, 1959, **81**, 643.

[7] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

column packed with 10% of silicone oil on " Embacel ") the presence of two major components in the approximate ratio 1 : 3 having retention times identical with those of methyl tetracosanoate (11·3 min.) and hexacosanoate (22 min.), respectively, together with about 2—3% of an ester with a retention time (6·3 min.) corresponding to that of methyl docosanoate.

Methyl Anhydromycolate A.—Toluene-*p*-sulphonyl chloride (25 g.) in dry pyridine (140 ml.) was added to ester *A* (23 g.), and the mixture set aside at room temperature, under anhydrous conditions, for 7 days. The product, isolated in the usual fashion, was dissolved in benzene (300 ml.) and hydrolysed with a refluxing solution of potassium hydroxide (30 g.) in methanol (400 ml.) (24 hr.). The liberated acid was isolated and chromatographed on alumina (activity III—IV; 500 g.). Ether eluted the αβ-unsaturated acid showing bands at 1681 (conjugated CO_2H) and 1639 cm.$^{-1}$ (conjugated C=C) [further elution with ether–acetic acid (98 : 2) gave a product containing hydroxy-acid].

The above αβ-unsaturated acid (16 g.) was esterified with ethereal diazomethane, and the resulting ester chromatographed on alumina (activity III—IV, 350 g.). Petrol eluted the αβ-unsaturated ester (15 g.), $[\alpha]_D^{21\cdot5}$ −1·75° (*c* 17·2 in $CHCl_3$; *l*, 0·2) (photoelectric polarimeter), 31—32° (Found: C, 82·9; H, 13·6. Calc. for $C_{98}H_{194}O_3$: C, 82·8; H, 13·8%); the infrared spectrum showed a single carbonyl band at 1715 cm.$^{-1}$, together with bands at 1639 (conjugated C=C) and 1093 cm.$^{-1}$ (methoxyl).

Oxidation of Methyl Anhydromycolate A.—This ester (10 g.) was oxidised with potassium permanganate (15 g.; added in small portions during 10 hr.) in refluxing acetone (150 ml.). After addition of sodium hydrogen sulphite and dilute hydrochloric acid, the product was isolated by ether-extraction, and then chromatographed on alumina (activity III—IV; 300 g.). Elution with ether gave a product (1·7 g.) which according to its infrared spectrum was largely the unchanged αβ-unsaturated ester. Further elution with ether–acetic acid (95 : 5) gave a mixture of the acidic oxidation products (7 g.). Treatment of the latter with urea, as described previously,[1] removed normal-chain acids, and left *O*-methylmeromycolic acid A (4 g.), m. p. 38—40° (Found: C, 81·5; H, 13·7. Calc. for $C_{71}H_{142}O_3$: C, 81·7; H, 13·7%). The straight-chain acids, obtained from the urea complex, were esterified with ethereal diazomethane, and the resulting esters distilled at 200—270° (bath)/0·1 mm. Vapour-phase chromatography (204°/260 mm.) of the distillate (2 g.) showed the presence of major components, in the approximate ratio of 1 : 3, having retention times corresponding to those of methyl docosanoate (20·9 min.) and tetracosanoate (41·6 min.), together with minor components having retention times corresponding to those of methyl octadecanoate (5·9 min.), nonadecanoate (8·4 min.), eicosanoate (10·9 min.), heneicosanoate (15 min.), tricosanoate (29·4 min.), and pentacosanoate (55·9 min.).

Oxidation of Methyl 2-Oxohexacosanoate.—The acid which resulted in earlier studies [5] on oxidation of methyl 2-oxohexacosanoate with potassium permanganate in acetone, was converted, by means of ethereal diazomethane, into the corresponding methyl ester. Vapour-phase chromatography (204°/260 mm.) of the latter showed that the main constituent had a retention time identical with that of methyl tetracosanoate; there were minor constituents having retention times corresponding to those of methyl docosanoate, tricosanoate, and pentacosanoate.

Deoxymeromycolic Acid A.—*O*-Methylmeromycolic acid *A* (4 g.) was converted into the corresponding *O*-acetyl derivative as described previously [1] for " *O*-methylmeromycolic acid." The product was pyrolysed at 380° (bath)/0·1 mm. (45 min.), and the resulting unsaturated acid hydrogenated in dioxan (100 ml.) in the presence of platinic oxide (5 hr.). Evaporation of the filtered solution gave the saturated acid, m. p. 48—50° (Found: C, 82·9; H, 13·4. Calc. for $C_{70}H_{140}O_2$: C, 83·0; H, 13·8%).

Bromination of Deoxymeromycolic Acid and Dehydrobromination of the α-Bromo-ester.—The deoxy-acid (0·47 g.) was refluxed with thionyl chloride (20 ml.) for 3 hr. A solution of bromine (0·1 g.) in thionyl chloride (1 ml.) was then added during 2 hr., and, after addition of more bromine (about 0·01 g. in 0·2 ml. of thionyl chloride) the mixture was set aside at room temperature. Next day the excess of thionyl chloride was removed under reduced pressure, and the product refluxed with methanol (10 ml.) and benzene (10 ml.) for 1 hr. The resulting bromo-ester, isolated by addition of water and ether-extraction, was refluxed with dry pyridine (20 ml.) overnight. The product (0·33 g.), isolated in the usual manner, showed $\lambda_{max.}$ 2150 Å (ε 7540, calc. for *M* 1000).

Oxidation of the Preceding αβ-Unsaturated Ester.—This ester (0·25 g.) was oxidised with potassium permanganate (3 g.) in refluxing acetone (50 ml.; 4 hr.). The product was distilled

at 200—250° (bath)/0·1 mm., and the distillate esterified with ethereal diazomethane. Vapour-phase chromatography (204°/260 mm.) of the resulting esters showed the presence of major constituents with retention times corresponding to those of methyl docosanoate and tetracosanoate in an approximate ratio of 1 : 3, together with minor amounts of the esters of acids containing 16, 17, 18, 19, 20, 21, 23, and 25 carbon atoms.

Pyrolysis of Methyl O-Acetylmeromycolate A *and Oxidation of the Product.*—*O*-Methylmeromycolic acid *A* was esterified with ethereal diazomethane, and the methyl ester (2·4 g.) converted into the *O*-acetyl derivative as described previously.[1] A 0·8 g. portion of this ester was pyrolysed at 380° (bath)/20 mm. (45 min.), and the resulting unsaturated ester oxidised with potassium permanganate (5 g., added in small portions) in refluxing acetone (75 ml.) (5 hr.). The product, isolated as before, was distilled at 200—250° (bath)/0·1 mm., and the distillate (0·15 g.) (all of it formed a urea complex) esterified with ethereal diazomethane. Vapour-phase chromatography (174°/410 mm.) of the resulting esters showed the presence of major components having retention times corresponding to those of methyl hexadecanoate (3·4 min.), heptadecanoate (5 min.), octadecanoate (7·3 min.), nonadecanoate (10 min.), and eicosanoate (15 min.), together with minor amounts of the esters derived from the acids containing 14, 15, 21, 22, and 23 carbon atoms. The residue from the above distillation was chromatographed on alumina (activity III—IV; 20 g.). Ether eluted a solid, m. p. 37—40°, which showed in its infrared spectrum carbonyl bands at 1709 and 1739 cm.$^{-1}$; it gave on reaction with 2,4-dinitrophenylsemicarbazide [8] a product, m. p. 42—44° (from petrol) which appeared to be largely the dinitrophenylsemicarbazone of a keto-ester (Found: N, 5·7. Calc. for $C_{59}H_{107}N_5O_7$: N, 7·0%). Further elution with ether–acetic acid (97 : 3) gave material, m. p. 34—37°; its infrared spectrum showed carbonyl bands at 1701 and 1739 cm.$^{-1}$.

Pyrolysis of Ester B.—The ester (0·2 g.) was heated at 200—300° (bath)/0·1 mm. The distillate (0·07 g.), all of which formed a urea complex, showed on vapour-phase chromatography (204°/260 mm.) the presence of two components, in the approximate ratio 1 : 4, having retention times identical with those of methyl tetracosanoate (40·8 min.) and hexacosanoate (82·5 min.).

Methyl Anhydromycolate B.—Ester *B* (18 g.) was added to a solution of toluene-*p*-sulphonyl chloride (20 g.) in dry pyridine (90 ml.), and the mixture kept at 45°, under anhydrous conditions, for 2 days. The product, isolated by acidification, followed by ether-extraction, was dissolved in benzene (125 ml.) and heated under reflux with potassium hydroxide (30 g.) in methanol (200 ml.) for 72 hr. The resulting acid, isolated by acidification and ether-extraction, was chromatographed on alumina (activity III—IV; 400 g.). Ether eluted the αβ-unsaturated acid (10 g.) showing bands at 1681 (conjugated CO_2H) and 1639 cm.$^{-1}$ (conjugated C=C). This acid was esterified with ethereal diazomethane, and the resulting ester chromatographed on alumina (activity III—IV; 150 g.). Petrol eluted the αβ-unsaturated ester (9·5 g.), m. p. 34—36°, $[\alpha]_D^{21\cdot5}$ −1·63° (*c* 8·45 in $CHCl_3$; *l*, 0·2) (photoelectric polarimeter) (Found: C, 83·3; H, 13·3. Calc. for $C_{80}H_{158}O_2$: C, 83·4; H, 13·7. Calc. for $C_{90}H_{178}O_2$: C, 83·7; H, 13·8%), $\lambda_{max.}$ 2170 Å (ε 11,240, calc. for *M* 1150).

Oxidation of Methyl Anhydromycolate B.—This ester (8 g.) was oxidised with potassium permanganate in acetone as described for previous examples. The product was chromatographed on alumina (activity III—IV; 100 g.). Elution with ether gave a product (3 g.) which according to its infrared spectrum was largely the unchanged αβ-unsaturated ester; further elution with ether–acetic acid (95 : 5) gave a mixture of the acidic oxidation products (4·5 g.). Treatment of the latter with urea removed straight-chain acids and left a branched-chain acid (no band progressions in the region 1333—1176 cm.$^{-1}$). The acids isolated from the urea complex were esterified with ethereal diazomethane, and the resulting esters distilled at 200—300° (bath)/0·1 mm. Vapour-phase chromatography (204°/260 mm.) of the distillate showed the presence of two major components, in the approximate ratio of 1 : 4, having retention times corresponding to those of methyl docosanoate and tetracosanoate.

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[8] McVeigh and Rose, *J.*, 1945, 713.