## 746. *Constituents of the Lipids of Tubercle Bacilli. Part VIII.* Studies on Mycolic Acid.*

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A scheme for a stepwise degradation of mycolic acid is presented. Oxidation of methyl anhydromycolate, obtained *via* the toluene-*p*-sulphonyl ester of methyl mycolate, with potassium permanganate in acetone gave *n*-pentacosanoic acid together with a methoxy-acid, named *O*-methylmeromycolic acid, having 26 carbon atoms less than the parent acid. Demethylation of this methoxy-acid with acetic anhydride and toluene-*p*-sulphonic acid afforded an acetoxy-acid which, on pyrolysis of its methyl ester, yielded an unsaturated ester. This ester on oxidation with potassium permanganate in acetone is shown to give a straight-chain acid, presumed to be heptadecanoic acid, and the half-ester of a branched-chain dicarboxylic acid. The structural features of mycolic acid in the light of the present and of earlier results are discussed.

MYCOLIC ACID was isolated from the wax fractions of human tubercle bacilli (strain H 37) by Stodola, Lesuk, and Anderson; it was assigned the formula $C_{88}H_{176}O_4$ and shown to contain one carboxyl, one hydroxyl, and one methoxyl group.[1] Asselineau and Lederer,[2] in examining samples of mycolic acid from different strains of human tubercle bacilli, found variations in the composition and physical properties of the products. In these studies they subjected the crude acids to chromatography over alumina and found that small amounts of the material were more strongly adsorbed than the main component, and had different m. p.s. They distinguished the products by Greek-letter prefixes indicating the order of elution and designated the acid which was first eluted α-mycolic acid. Most of the acids isolated from human strains (those from other strains are not considered here)

* Part VII, *J.*, 1956, 2036.

[1] Stodola, Lesuk, and Anderson, *J. Biol. Chem.*, 1938, **126**, 505; Lesuk and Anderson, *ibid.*, 1940, **136**, 603.

[2] For a general review see Asselineau and Lederer, *Fortschr. Chem. org. Naturstoffe*, 1953, **10**, 170, and references cited there.

were shown by Asselineau and Lederer to undergo, in agreement with earlier findings,[1] pyrolytic decomposition with the formation of *n*-hexacosanoic acid; dehydration with a mixture of acetic anhydride and potassium hydrogen sulphate gave an αβ-unsaturated acid, named anhydromycolic acid. From these facts together with other evidence, including the isolation of *n*-pentacosanoic acid on ozonolysis of anhydromycolic acid (from α-mycolic acid, m. p. 55—56°, isolated from the strain "Test"), the French investigators inferred that mycolic acid from human tubercle bacilli contains a *n*-tetracosyl branch in α-position and a hydroxyl group in β-position to the carboxyl group.[3]

The acid studied in the present work was obtained from human tubercle bacilli (strains D.T., P.N., and C.[4]) in the course of investigations earlier described.[5] It had, after purification by precipitation of its benzene solution with methanol, m. p. 54—55°. Chromatography over alumina resulted in loss of material, the recovered acid (about 80%) showing no change of m. p. When the corresponding methyl ester, m. p. 43—44·5°, was subjected to chromatography over alumina, most of the material was recovered with the same m. p., except the most strongly adsorbed portion (about 7%) which had a lower m. p., but showed no difference in its infrared spectrum. In agreement with earlier statements the acid, hereafter called simply mycolic acid, was found to contain one carboxyl, one hydroxyl, and one methoxyl group, and gave on pyrolysis hexacosanoic acid.

The infrared spectra of mycolic acid and methyl mycolate (paraffin mulls or natural films) show bands at 3484 (bonded hydroxyl) and 1094 cm.$^{-1}$ (alkyl ether). The carbonyl stretching band appears at 1681 cm.$^{-1}$ (shoulder at 1706 cm.$^{-1}$) for the acid, and at 1709 cm.$^{-1}$ (shoulder at 1730 cm.$^{-1}$) for the ester, *i.e.*, at lower frequencies than usually associated with saturated acids and esters, presumably owing to hydrogen-bonding between the carbonyl and hydroxyl group (cf. Gordy [6]); the spectrum of the acetyl derivative of methyl mycolate shows the normal value of 1742 cm.$^{-1}$.

Chemical studies of mycolic acid are complicated by the fact that degradation products of comparable size differ but little in physical properties, and it is difficult to separate the desired product from any unchanged starting material or from by-products; moreover, there is a lack of reliable criteria for the homogeneity of such substances. With these difficulties in mind we adopted the following scheme which permitted a systematic breakdown of mycolic acid, and the isolation of the resulting degradation products.

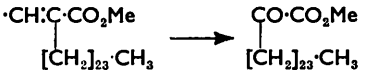
We decided to convert mycolic acid into methyl anhydromycolate, and to oxidise the latter with potassium permanganate in acetone. If there were any saturated esters (including unchanged methyl mycolate) present, they would not interfere, since these esters are unaffected by potassium permanganate in acetone; any acidic products obtained would, therefore, represent oxidation products of methyl anhydromycolate. We were aware of the difficulties to be expected in attempting to separate the acidic oxidation products from non-acid material by the usual procedures, but such separations can be readily achieved by taking advantage of the retention of acidic products by the manganese dioxide precipitate resulting on oxidation with potassium permanganate in acetone. The opportunity is taken to point out the general usefulness of this procedure, already employed in an earlier case, namely, for the oxidation of methyl mycolipenate, but only mentioned in the Experimental section of the paper.[7] Small amounts of manganese dioxide can absorb appreciable quantities of acidic oxidation products, and the latter are readily liberated on dissolving the manganese dioxide (after being washed to remove non-acid material), *e.g.*, by the addition of aqueous sodium hydrogen sulphite and hydrochloric acid. The presence of any water-insoluble acidic oxidation products is immediately seen after the manganese dioxide has dissolved, and even small amounts can thus be readily detected.

[3] Ref. 2, p. 198.
[4] Green, *Veterinary J.*, 1946, **102**, 267.
[5] Chanley and Polgar, *J.*, 1954, 1003.
[6] Gordy, *J. Chem. Phys.*, 1940, **8**, 516.
[7] Polgar, *J.*, 1954, 1008.

The first step was the preparation of methyl anhydromycolate. Attempts to dehydrate mycolic acid by means of acetic anhydride and potassium hydrogen sulphate under the conditions previously described [8] gave low yields of αβ-unsaturated material, and also resulted in partial loss of the methoxyl group (see also Asselineau [9]). In our work the toluene-*p*-sulphonyl derivative of methyl mycolate, obtained by reaction of methyl mycolate with toluene-*p*-sulphonyl chloride in pyridine, was heated with benzene–methanolic potassium hydroxide (cf. Linstead *et al.*;[10] Pudles and Lederer[11]); acidification then readily gave a product which, as indicated by its ultraviolet absorption (ε 12,700 at 2180 Å), characteristic of an αβ-unsaturated acid, was essentially anhydromycolic acid. This with diazomethane gave the methyl ester which was oxidised with potassium permanganate in acetone.

In preliminary experiments the resulting manganese dioxide precipitate, after being washed with acetone, was still contaminated with appreciable amounts of neutral material, owing to the sparing solubility of the latter in acetone. Attempts to separate it from the acidic material by chromatography resulted in considerable loss of acids. It was then found that the non-acid material could be satisfactorily removed by refluxing the manganese dioxide with ether, then centrifuging the ethereal suspension to separate colloidal manganese dioxide. Further, it was found advantageous to introduce the potassium permanganate gradually from a Soxhlet apparatus.

The non-acid material isolated after the oxidation appeared to consist only of methyl mycolate and a little methyl anhydromycolate. Treatment of the acidic product, obtained from the manganese dioxide precipitate, with urea[12] removed pentacosanoic acid as a complex, and left a branched-chain methoxy-acid, now named *O*-methylmeromycolic acid. Methyl 2-oxohexacosanoate which might be expected to arise on oxidation of methyl anhydromycolate according to the scheme




has been found[13] to be oxidised to pentacosanoic acid under the conditions employed.

*O*-Methylmeromycolic acid, which in the above experiments resulted from mycolic acid by the loss of twenty-six carbon atoms, distilled unchanged in a high vacuum at about 300°. This thermal stability appeared to rule out the presence of an α-methoxyl group since an α-methoxy-acid should give an aldehyde of one carbon atom less at the high temperature required for the distillation (cf. Darzens and Levy[14]). Moreover, the methoxyl group was retained on reaction with thionyl chloride under conditions which are found[15] to result in the loss of the elements of methanol from a β-methoxy-acid.

The methoxyl group was removed by the action of acetic anhydride in the presence of toluene-*p*-sulphonic acid (cf. Huffmann and Lott[16]), followed by refluxing of the product (mixture of acetoxy-acid and -anhydride) with glacial acetic acid; this gave the acetoxy-acid, *O*-acetylmeromycolic acid, which on hydrolysis with benzene–methanolic potassium hydroxide afforded the hydroxy-acid, meromycolic acid. The infrared spectrum of the latter showed no γ-lactone-carbonyl absorption (1760 cm.$^{-1}$ region), indicating that the hydroxyl group is not in γ-position to the carboxyl group; hence the methoxyl group in *O*-methylmeromycolic acid must be beyond the γ-position. Further, since removal of

[8] Asselineau and Lederer, *Biochim. Biophys. Acta*, 1951, **7**, 126.
[9] Asselineau, *Bull. Soc. chim. France*, 1952, 557.
[10] Linstead, Owen, and Webb, *J.*, 1953, 1211.
[11] Pudles and Lederer, *Bull. Soc. Chim. biol.*, 1954, **36**, 759.
[12] Cf. Schlenk, *Annalen*, 1949, **565**, 204; Linstead and Whalley, *J.*, 1950, 2987; Skellon and Taylor, *J.*, 1953, 1433.
[13] Boast and Polgar, *J.*, 1957, 3800.
[14] Darzens and Levy, *Compt. rend.*, 1933, **196**, 348.
[15] Morgan and Polgar, unpublished work.
[16] Huffmann and Lott, *J. Biol. Chem.*, 1948, **172**, 789.

the methoxyl group did not lead to an unsaturated acid the methoxyl group was probably not attached to a tertiary carbon atom.

(I) $CH_3{\cdot}[CH_2]_{n-1}{\cdot}CH{:}CH{\cdot}R{\cdot}CO_2Me$

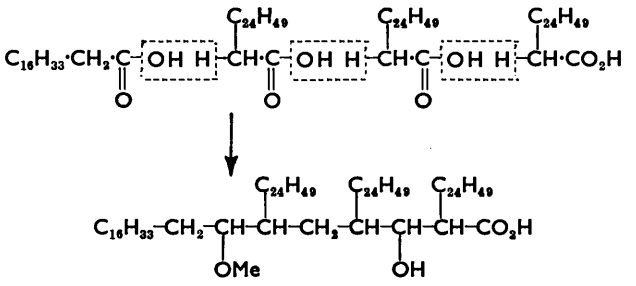
(II) $CH_3{\cdot}[CH_2]_n{\cdot}CH(OX){\cdot}R{\cdot}CO_2H$

$CH_3{\cdot}[CH_2]_n{\cdot}CH(OMe){\cdot}R{\cdot}CH{:}C(CO_2H){\cdot}[CH_2]_{23}{\cdot}CH_3$ (III)

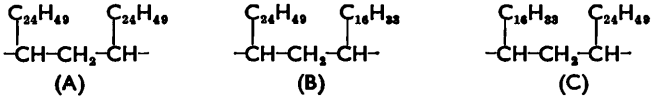
$CH_3{\cdot}[CH_2]_n{\cdot}CH(OMe){\cdot}R{\cdot}CH(OH){\cdot}CH(CO_2H){\cdot}[CH_2]_{23}{\cdot}CH_3$ (IV)

Pyrolysis of methyl *O*-acetylmeromycolate, followed by oxidation of the resulting unsaturated product, methyl anhydromeromycolate, gave a straight-chain acid, presumed to be heptadecanoic acid, together with the half-ester of a branched-chain dicarboxylic acid. Since pyrolysis of an acetate has been shown [17] to give only the least substituted olefin, with no rearrangement, methyl anhydromeromycolate may be formulated as (I), and *O*-acetylmeromycolic acid as (II; X = Ac), where *n* is probably 16, and R represents a branched-chain alkyl residue; meromycolic acid is then (II; X = H), and *O*-methylmeromycolic acid has structure (II; X = Me). Accordingly, the structures of anhydromycolic and mycolic acid are formulated as (III) and (IV), respectively.

The analytical figures obtained for some of the above degradation products agree better with a formula $C_{97}H_{194}O_4$ for mycolic acid than with the $C_{88}$ formula proposed previously, but in view of the high molecular weight such formulæ are only an approximation; if a composition $C_{97}H_{194}O_4$ is assumed for mycolic acid, R in the above formulæ (I) to (IV) is $C_{51}H_{102}$. The structural features so far established point to a biogenesis of



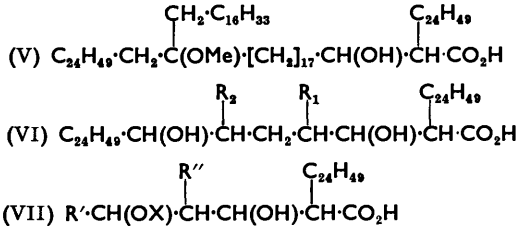
mycolic acid from hexacosanoic and stearic acid units,[18] with reduction of carbonyl groups to methylene or to the *sec.*-alcohol stage, and with or without *O*-methylation of the latter, as in the annexed scheme. On this hypothesis formulation of mycolic acid as (IV) where R is (A), (B), or (C) deserves consideration; the formula with R = (A) corresponds to a composition $C_{97}H_{194}O_4$, and those with R = (B) or (C) represent $C_{89}H_{178}O_4$.



Asselineau and Lederer, from studies of a complex mixture of hydroxy-, methoxy-, and unsaturated acids resulting on reaction of " α-mycolic acid " from the strain " Test " with acetic anhydride and potassium hydrogen sulphate, inferred structure (V) for the mycolic acid.[19] Asselineau proposed for " $\alpha_2$-mycolic acid," isolated from the strain $R_1$ and

[17] Bailey and King, *J. Amer. Chem. Soc.*, 1955, **77**, 75.
[18] Cf. ref. 2, p. 213.
[19] Asselineau and Lederer, *Bull. Soc. chim. France*, 1953, 335.

regarded as having the composition $C_{87}H_{174}O_4$, the structure (VI) with the portion between the hydroxyl groups stated to be hypothetical.[20] Another structure was advanced by



Aebi, Vilkas, and Lederer [21] for the principal acid from the strain " Brévannes " which they suggested was a mixture of (VII; X = Me) and (VII; X = H), but it is difficult to reconcile these formulæ with the reported formation, by the action of acetic anhydride, of a diene-acid with only one double bond conjugated with the carboxyl group. Evidently, more chemical evidence is needed to clarify the position. This is also desirable in view of the recent controversy [22] on " cord factor " which has been claimed to be toxic and to consist of a trehalose ester of mycolic acid.[23]

## Experimental

Ultraviolet spectra were determined for purified *cyclo*hexane solutions and infrared spectra for natural films or paraffin mulls. The alumina used for chromatography (Spence, type H) was acid-washed and activated at 110°, and its activity tested according to the method of Brockmann and Schodder.[24] Petrol refers to light petroleum, b. p. 40—60°.

*Isolation of Mycolic Acid.*—The crude acid was obtained in earlier studies [5] from the *iso*propyl ether extract (B) of the bacterial cells. The methanol-insoluble product resulting from partial hydrolysis of the lipids was subjected to a more vigorous hydrolysis by refluxing benzene–methanolic potassium hydroxide for 215 hr. (cf. ref. 5). Addition of methanol to a benzene solution of the liberated acids precipitated crude mycolic acid, m. p. 52—54°. This acid (15 g.) was dissolved in hot benzene (90 c.c.) and filtered while warm; methanol (180 c.c.) was then added slowly with stirring, and next day the precipitated mycolic acid collected and washed with methanol. The product (14·5 g.) was a pale yellow powder, m. p. 54—55° (Found: C, 81·8; H, 13·8; OMe, 2·2. Calc. for $C_{97}H_{194}O_4$: C, 81·7; H, 13·7; OMe, 2·2. Calc. for $C_{89}H_{178}O_4$: C, 81·5; H, 13·6; OMe, 2·4%). The *amide*, prepared *via* the acetyl derivative by partial reduction of the crude acetylmycolamide with lithium aluminium hydride, had m. p. 73—75° (Found: C, 81·4; H, 13·7; N, 1·0. $C_{97}H_{195}O_3N$ requires C, 81·7; H, 13·8; N, 1·0%).

Mycolic acid, purified by the above procedure, was used for the degradation experiments described later. Five successive precipitations from benzene did not raise the m. p. above 54—55°. Fractional precipitation from benzene, by adding to purified mycolic acid (6 g.) in benzene (30 c.c.) 20-c.c. portions of methanol, gave fractions which all had m. p. 54—55°.

A portion (0·5 g.) of mycolic acid was chromatographed in petrol on alumina (10 g.; activity II). The following fractions were obtained: (i) 0·02 g., eluted by petrol–benzene (1 : 1), (ii) 0·03 g. eluted by ether, (iii) 0·33 g. eluted by ether–acetic acid (9 : 1), and (iv) 0·01 g. eluted by ether–acetic acid (4 : 1). The total amount recovered was 0·39 g. (78%), the main fraction (iii) having m. p. 54—55°.

Methyl mycolate (1·02 g.; obtained from the acid by means of diazomethane as a wax, m. p. 43—44·5°) gave on chromatography in petrol on alumina (35 g.; activity II) fractions: (i) 325 mg., eluted by petrol–benzene (3 : 1), (ii) 430 mg., eluted by petrol–benzene (1 : 1), (iii) 165 mg., eluted by benzene, and (iv) 65 mg., eluted by ether (97% recovery). Fractions (i) to (iii) were almost colourless products of m. p. 43—44·5° and appeared to be identical; fraction (iv) which

[20] Asselineau, *ibid.*, 1953, 427.
[21] Aebi, Vilkas, and Lederer, *ibid.*, 1954, 79.
[22] Spitznagel and Dubos, *J. Exp. Med.*, 1955, **101**, 291; Bloch, Defaye, Lederer, and Noll, *Biochim. Biophys. Acta*, 1957, **23**, 312.
[23] Noll, Bloch, Asselineau, and Lederer, *ibid.*, 1956, **20**, 299.
[24] Brockmann and Schodder, *Ber.*, 1941, **74**, 73.

was deep yellow melted at 40—41°, but its infrared spectrum was indistinguishable from that of methyl mycolate.

*Pyrolysis of Mycolic Acid and its Derivatives.*—Pyrolytic distillation of mycolic acid (3·2 g.) gave, in addition to higher-boiling material, a product (0·9 g.), b. p. 200—250° (bath)/0·1 mm., which after purification by formation of a urea complex crystallised from acetone as a white solid, m. p. 85—87° (Found: C, 78·5; H, 13·2. Calc. for $C_{26}H_{52}O_2$: C, 78·8; H, 13·2%). Francis and Piper [25] give m. p. 87·7° for hexacosanoic acid.

Mycolamide gave on pyrolytic distillation a product, b. p. 210—230° (bath)/0·07 mm., m. p. 110—111·5° (Found: C, 79·0; H, 13·2; N, 3·3. Calc. for $C_{26}H_{53}ON$: C, 79·0; H, 13·5; N, 3·5%). Marie [26] records m. p. 109° for cerotic amide.

On distillation of methyl mycolate (3 g.; prepared as stated above) (Found: C, 81·5; H, 13·4. Calc. for $C_{98}H_{196}O_4$: C, 81·8; H, 13·7%) two distinct fractions were obtained, *viz.*, (i) 0·8 g., b. p. 170—190° (bath)/0·06 mm., and (ii) 1·9 g., b. p. 300—360° (bath)/0·1 mm. Fraction (i), after purification *via* its urea complex and crystallisation from acetone, had m. p. 61·5—62° (Found: C, 79·0; H, 13·1. Calc. for $C_{27}H_{54}O_2$: C, 79·0; H, 13·2%); Francis and Piper [25] give m. p. 62·9° for methyl hexacosanoate. Fraction (ii) showed in its infrared spectrum a band at 1733 cm.$^{-1}$ which suggested the presence of some ester. A sample (1 g.) was hydrolysed by refluxing benzene–methanolic potassium hydroxide, and the product resulting on acidification chromatographed on alumina. Petrol eluted a white wax (0·85 g.), m. p. 36—40° (Found: C, 84·5; H, 13·8; OMe, 1·8%), which was not acidic (titration). The product which was obviously a mixture showed in its infrared spectrum a carbonyl band at 1695 cm.$^{-1}$. Ultraviolet light absorption: max. 2080 and 2300 Å (ε 4380 and 3420, respectively).

*Anhydromycolic Acid* (III).—Toluene-*p*-sulphonyl chloride (0·3 g.) (purified by shaking its benzene solution with aqueous sodium carbonate and by crystallisation from dry benzene) was added to a solution of methyl mycolate (0·5 g.) in dry pyridine (2 c.c.), and the mixture refluxed for 5 hr. Acidification of the product, followed by ether-extraction, gave the crude toluene-*p*-sulphonyl ester of methyl mycolate. This ester (0·4 g.) in benzene (10 c.c.) was refluxed with 5% methanolic potassium hydroxide (5 c.c.) for 6 hr.; the mixture was then acidified and extracted with ether. The product, m. p. 39—42° (Found: no S), was largely anhydromycolic acid (ultraviolet absorption: max. 2100 and 2180 Å; ε 12,000 and 12,700, respectively). Reaction with diazomethane gave methyl anhydromycolate (max. 2140 and 2200 Å; ε 11,900 and 11,420, respectively).

When methyl mycolate (0·6 g.) was refluxed with acetic anhydride (30 c.c.) and potassium hydrogen sulphate (3 g.; freshly fused) for 3 hr. (cf. ref. 8), the product showed in its infrared spectrum no band in the 1093 cm.$^{-1}$ region (alkyl ether), and the analytical values indicated the presence of two acetyl groups (Found: C, 80·3; H, 12·9. Calc. for $C_{101}H_{198}O_6$: C, 80·4; H, 13·2%).

*Oxidation of Methyl Anhydromycolate.*—Potassium permanganate was introduced continuously into a refluxing solution of methyl anhydromycolate (10 g.) in dry acetone (150 c.c.) with the aid of a Soxhlet extractor (the usual paper thimble was replaced by a wad of glass wool on which was placed 1 g. of powdered potassium permanganate). When the rate of oxidation became very slow (the pink colour persisted for 30 minutes' refluxing), the hot solution was filtered, and the manganese dioxide precipitate washed with hot acetone, then refluxed with dry ether (50 c.c.). After centrifuging of the cooled mixture for 0·5 hr. the ethereal solution was decanted; this prodecure was repeated twice with fresh portions of dry ether.

The combined acetone and ether filtrates were evaporated; examination of the residue (2·8 g.) by chromatography on alumina indicated that most of the material was methyl mycolate and anhydromycolate.

The manganese dioxide precipitate was dissolved by the addition of sodium hydrogen sulphite and dilute hydrochloric acid, and the mixture extracted with ether. Evaporation of the ethereal extract afforded a mixture of the acidic oxidation products. Preliminary studies of this material showed that chromatography on alumina gave no separation, the same mixture being eluted by 49 : 1 and 19 : 1 ether–acetic acid. Chromatography on silica (The British Drug Houses Ltd., " silica gel 30—120 mesh ") gave a number of fractions, but no distinct separation. Distillation of a sample (0·5 g.) gave two fractions, *viz.*, (i) b. p. 160—200° (bath)/0·02 mm., and (ii) b. p. 260—300° (bath)/0·02 mm. Fraction (i) was obtained as a

[25] Francis and Piper, *J. Amer. Chem. Soc.*, 1939, **61**, 577.
[26] Marie, *Ann. Chim. Phys.*, 1896, **7**, 208.

white solid (0·2 g.), m. p. 79°; it formed a urea complex. Fraction (ii) was a soft yellow wax (0·2 g.), m. p. 45—60°, which did not form a urea complex. Finally, the procedure described below was adopted.

*Separation of the acidic oxidation products.* The above mixture of acids (5·3 g.) was dissolved in light petroleum (b. p. 60—80°; 200 c.c.), and urea (6 g.), moistened with a few drops of methanol, added. The mixture was stirred and heated to boiling, then allowed to cool. The urea complex was filtered off and washed with light petroleum, then dissolved in water; the straight-chain acid separated as a white solid. The light petroleum filtrate and washings were combined, concentrated (to 200 c.c.), and treated again with urea (8 × 2 g.) as above, until no more straight-chain acid was removed. This procedure gave the normal-chain acid (1·76 g.), m. p. 71—73°, as a pure white solid. The branched-chain acid, recovered from the light petroleum solution, was a pale yellow wax (2·82 g.), m. p. 44—47°.

The normal-chain acid, after several crystallisations from acetone, had m. p. 76°. Further purification *via* the urea complex, followed by crystallisation from acetone, and finally from benzene gave the acid as plates, m. p. 78·5° (Found: C, 78·6; H, 13·0. Calc. for $C_{25}H_{50}O_2$: C, 78·4; H, 13·2%). Its infrared spectrum was identical with that of a sample of pentacosanoic acid, obtained by oxidation of methyl 2-oxohexacosanoate; [13] the band progressions in the region 1333—1176 cm.$^{-1}$ (cf. ref. 27) were identical, and differed from those of hexacosanoic acid (obtained by pyrolysis of mycolic acid), and of tetracosanoic acid (kindly provided by Dr. J. C. Smith).

The branched-chain acid, O-*methylmeromycolic acid*, had $[\alpha]_D^{20}$ +1·4° (*l.* 1; *c* 3·559 in $CHCl_3$), and melted, after crystallisation from acetone, at 45—47° to a clear plastic which suddenly became mobile at 60°. It distilled unchanged at 300—320° (bath)/0·06 mm. (Found: C, 82·0, 81·5; H, 13·3, 13·5; OMe, 2·7. $C_{71}H_{142}O_3$ requires C, 81·7; H, 13·7; OMe, 3·0%); it showed no high-intensity absorption in the ultraviolet region. Infrared absorption: bands at 1709 (CO) and 1093 cm.$^{-1}$ (alkyl ether).

The *amide* was prepared by refluxing *O*-methylmeromycolic acid (0·3 g.) in benzene (5 c.c.) with thionyl chloride (1·5 c.c.) for 3 hr.; the benzene and excess of thionyl chloride were removed by distillation, and the residual crude acid chloride taken up in a little dry ether, then poured on ice-cold aqueous ammonia. The product crystallised from acetone as a white solid, m. p. 64—70° (Found: C, 81·5; H, 13·5; N, 1·7. $C_{71}H_{143}O_2N$ requires C, 81·6; H, 13·8; N, 1·4%); its infrared spectrum contained a band at 1093 cm.$^{-1}$ (alkyl ether).

In another experiment, the acid chloride was prepared as above, and, after addition of a little ether, poured into water. The product was unchanged *O*-methylmeromycolic acid; it showed no high-intensity ultraviolet absorption, and the infrared spectrum indicated that the methoxyl group was intact.

*Demethylation of* O-*Methylmeromycolic Acid.*—*O*-Methylmeromycolic acid (1 g.) was refluxed with acetic anhydride (20 c.c.) and toluene-*p*-sulphonic acid (0·3 g.) for 0·5 hr. The mixture was extracted with ether, and the extract washed with water, then evaporated, leaving a dark yellow wax. Examination of the infrared spectrum showed the presence of acetyl, some free acid carbonyl, and also two anhydride bands at 1821 and 1754 cm.$^{-1}$, thus indicating that the product was a mixture of the acetoxy-acid and the acetoxy-anhydride. It was then refluxed with glacial acetic acid (20 c.c.) for 2 hr. to decompose the anhydride. The product was poured into water and extracted with ether. The ether layer, after being washed with water and dried ($MgSO_4$), was evaporated, to give O-*acetylmeromycolic acid* (II; X = Ac), m. p. 39—40° and 45° after crystallisation from acetone (Found: C, 80·9; H, 13·0. $C_{72}H_{142}O_4$ requires C, 80·7; H, 13·3%). Its infrared spectrum showed the absence of methoxyl and anhydride, and the presence of acid carbonyl (1709 cm.$^{-1}$) and acetyl (1739 and 1238 cm.$^{-1}$).

*O*-Acetylmeromycolic acid (0·1 g.) was refluxed with 5% benzene–methanolic (1 : 1) potassium hydroxide (20 c.c.) for 4 hr. Acidification and ether-extraction gave *meromycolic acid* (II; X = H) which crystallised from acetone as a white powder, m. p. 40° (Found: C, 81·5; H, 13·3. $C_{70}H_{140}O_3$ requires C, 81·6; H, 13·7%).

*Pyrolysis of Methyl* O-*Acetylmeromycolate and Oxidation of the Product.*—*O*-Acetylmeromycolic acid (0·7 g.) was converted, by reaction with diazomethane, into methyl *O*-acetylmeromycolate, m. p. 33—35°. This ester was pyrolysed by distillation at 300—400° (bath)/14 mm. The whole distilled, and the distillate was redistilled twice at the same pressure; a small charred residue remained. The distilled product was refluxed with acetone (50 c.c.) and powdered

[27] Jones, McKay, and Sinclair, *J. Amer. Chem. Soc.*, 1952, **74**, 2575.

potassium permanganate until the rate of oxidation became very slow (8 hr.). The acid and the neutral fraction were then separated as described before. The neutral fraction was found to be unoxidised ester. The acid fraction (0·32 g.) gave on distillation two fractions, (*a*) b. p. 140—180° (bath)/0·05 mm., and (*b*) b. p. 200—300° (bath)/0·05 mm.; no residue remained.

Fraction (*a*) was treated in light petroleum (b. p. 60—80°) with urea, moistened with a little methanol. A complex was formed, decomposition of which with water gave the acid as a soft wax, crystallising from methanol as a fine powder, m. p. 52—53° (Found: C, 75·4; H, 12·4. Calc. for $C_{17}H_{34}O_2$: C, 75·4; H, 12·7%).

Fraction (*b*) did not form a urea complex; it was redistilled at 280—300° (bath)/0·02 mm., to give a soft, pale yellow wax, m. p. 30—35°. Its infrared spectrum showed an acid carbonyl band at 1709 $cm.^{-1}$, and an ester carbonyl as a shoulder on the former band at 1736 $cm.^{-1}$. The product was obtained in too small amounts for further studies.

In another experiment, meromycolic acid on distillation at 300—350°/0·05 mm. gave a product which contained some unsaturated material. It was oxidised with potassium permanganate as above, but no attempt was made to separate acid from neutral products, and the whole material was distilled. Fraction (i), a pale yellow wax, was collected at 140—200°/0·05 mm. Fraction (ii), collected at 220—320°/0·05 mm., crystallised from methanol as a white powder, m. p. 43—47°, and from analysis appeared to be a dicarboxylic acid (Found: C, 79·4; H, 12·5. $C_{54}H_{106}O_4$ requires C, 79·1; H, 13·0%); its infrared spectrum showed a normal acid carbonyl frequency at 1709 $cm.^{-1}$.

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