Constituents of the Lipids of Tubercle Bacilli. Part IV. Mycoceranic Acid.*

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Degradative studies of a lævorotatory acid isolated from the lipids of tubercle bacilli are reported. It is found that the acid, now termed mycoceranic acid, has the structure $CH_3\cdot[CH_2]_n\cdot CHMe\cdot CH_2\cdot CHMe\cdot CH_2\cdot CHMe\cdot CO_2H$ where *n* is probably 21 (cf. *Chem. and Ind.*, 1953, 353).

The isolation of a lævorotatory acid from the lipids of tubercle bacilli (human type) was described in Part II.* The present communication concerns degradative studies of this acid.

The acid was already shown to be saturated; titration gave a result in close agreement with $C_{31}H_{62}O_2$. A stepwise degradation described in Part II (*loc. cit.*), involving oxidation of the derived α-hydroxy-acid by means of lead tetra-acetate, gave a ketone; the same ketone was also obtained by a Wieland–Barbier degradation. This indicated that the parent acid has one alkyl substituent in the α-position to the carboxyl group.

Bromination of the lævorotatory acid (Hell–Volhard–Zelinsky method) and dehydrobromination of the α-bromo-derivative with pyridine gave an αβ-unsaturated acid ($\lambda_{max.}$ 2180 Å; log ε 4·06) which on ozonization yielded pyruvic acid.

These experiments indicated that the parent saturated acid has the terminal group $\cdot CHMe\cdot CO_2H$.

The methyl ester of the αβ-unsaturated acid exhibited $[\alpha]_D$ −10·2°, which indicated the presence of an asymmetric centre adjacent to the αβ-double bond (Part III, *loc. cit.*; cf. also Jocelyn and Polgar, *J.*, 1953, 132), and this was confirmed by oxidation of the ester, by potassium permanganate in acetone, to an acid having $[\alpha]_D$ −5·8°, and a by-product ketone. Both oxidation products were closely analogous to those resulting on oxidation of mycolipenic acid, already shown to be 2 : 4 : 6-trimethyltetracos-2-enoic acid (Part III), the acid forming a *p*-bromophenacyl ester of m. p. 68°, and the ketone yielding a semicarbazone, m. p. 92·5° (as compared with the previous m. p.s 68°, and 94—95°, respectively). This suggested that the above αβ-unsaturated acid must have the structure (II) analogous to that of mycolipenic acid, and differing from the latter only in the sign of the rotation, and by the length of the normal chain. It then follows that the acid and the ketone, obtained on oxidation, have the structures (III) and (IV), respectively, analogous to those of the oxidation products of mycolipenic acid.

In further experiments, the acid (III) has been subjected to the same sequence of degradative reactions which, in case of mycolipenic acid, resulted in the isolation of eicosan-2-one. Bromination of the acid, followed by reaction of the α-bromo-acid bromide with methanol, and dehydrobromination by means of pyridine, gave an αβ-unsaturated ester, with $[\alpha]_D$ −9·1°, the magnitude of the rotation again indicating the presence of an asymmetric centre adjacent to the αβ-double bond. This was oxidized, by means of potassium permanganate in acetone, to an acid (with negative rotation) and a by-product ketone. The semicarbazone of the latter had m. p. 126° (compared with 124—125° for the semicarbazone of *n*-eicosan-2-one), which indicated that the ketone has an unbranched chain.

(I) $CH_3\cdot[CH_2]_n\cdot CHMe\cdot CH_2\cdot CHMe\cdot CH_2\cdot CHMe\cdot CO_2H$
(II) $CH_3\cdot[CH_2]_n\cdot CHMe\cdot CH_2\cdot CHMe\cdot CH{:}CMe\cdot CO_2H$
(III) $CH_3\cdot[CH_2]_n\cdot CHMe\cdot CH_2\cdot CHMe\cdot CO_2H$
(IV) $CH_3\cdot[CH_2]_n\cdot CHMe\cdot CH_2\cdot CO\cdot CH_3$

These results showed that the lævorotatory acid, now termed mycoceranic acid, has the structure (I), where *n* is probably 21. The length of the normal chain requires confirmation by an *X*-ray crystallographic study of the preceding ketone.

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* Parts II and III, preceding papers. An account of this work was included in a lecture at the VIth International Congress for Microbiology, Rome, September, 1953.

A point of interest is that mycoceranic acid (I), as well as the αβ-unsaturated acid (II), and the acidic degradation products having an asymmetric centre in the α-position to the carboxyl group, exhibit lævorotation. Mycolipenic acid itself, and also its degradation products with an asymmetric centre in the α-position to the carboxyl grouping (structurally differing from those of mycoceranic acid only by the length of the normal chain) are dextrorotatory. Thus mycoceranic and mycolipenic acid possess opposite configurations.

EXPERIMENTAL

Ultra-violet absorption spectra were determined in methanol by Dr. F. B. Strauss, with the assistance of Mr. F. Hastings. Specific rotations were measured in ether, 0·5-dm. tubes being used.

Wieland–Barbier Degradation.—The methyl ester of the lævorotatory acid (described in Part II, *loc. cit.*) (2·2 g.) in benzene (15 c.c.) was added dropwise to a Grignard solution from bromobenzene (20 g.) and magnesium (3· g.) in ether (45 c.c.), and the mixture refluxed for 3 hr. The solution was worked up, and the resultant alcohol dehydrated as described in Part III. The product, $[\alpha]_D^{14}$ $-27·6°$ (*c*, 8·55), was then ozonized in carbon tetrachloride (20 c.c.) for 2 hr. On working up as previously described a ketone (0·9 g.) resulted which gave a semicarbazone, m. p. 68—69° (Found: C, 75·4; H, 12·5; N, 8·8. Calc. for $C_{31}H_{63}ON_3$: C, 75·5; H, 12·8; N, 8·5%), undepressed an admixture with the semicarbazone (m. p. 69—70°) of the ketone obtained by the stepwise degradation described in Part II (*loc. cit.*).

Bromination of the Lævorotatory Acid and Dehydrobromination of the α-Bromo-ester.—The lævorotatory acid (0·4 g.) was heated with bromine (slight excess) in the presence of red phosphorus (0·03 g.) for 6 hr. as previously described, and the resulting crude acid bromide converted into the methyl ester by means of methanol (10 c.c.). This bromo-ester, in refluxing anhydrous pyridine (6 c.c.; 24 hr.), afforded an αβ-unsaturated *ester*, b. p. 215—220° (bath)/0·05 mm. (0·31 g.), $[\alpha]_D^{13}$ $-10·2°$ (*c*, 6·25) (Found: C, 80·5; H, 12·9. $C_{32}H_{62}O_2$ requires C, 80·3; H, 13·0%). Light absorption: Max., 2180 Å; log ε 4·06. The acid, obtained by hydrolysis with ethanolic potassium hydroxide, gave on ozonization according to the procedure described in Part III (*loc. cit.*) pyruvic acid, isolated as its 2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p. 214—215° (microscope hot-stage).

Oxidation of the αβ-Unsaturated Ester.—The above ester (0·3 g.) in acetone (25 c.c.) was oxidized with potassium permanganate and the product worked up as described in Part III. The main product was an acid (III), $[\alpha]_D^{13}$ $-5·8°$ (*c*, 12·09), which formed a p-*bromophenacyl* ester, m. p. 68° after crystallization from ethanol (Found: C, 70·2; H, 9·7. $C_{36}H_{61}O_3Br$ requires C, 69·6; H, 9·8%). As a by-product, small amounts of a ketone (IV) were isolated which was characterized as its *semicarbazone*, m. p. 92·5° (sintering at 91°) after one crystallization from ethanol (the m. p. may rise somewhat on further crystallizations) (Found: C, 74·0; H, 12·3. $C_{28}H_{57}ON_3$ requires C, 74·5; H, 12·6%).

Degradation of the Acid (III).—This was carried out by the procedure given in Part III for the degradation of the acid oxidation product of mycolipenic acid. Bromination and dehydrobromination, followed by treatment of the α-bromo-acid bromide with methanol, gave an αβ-unsaturated ester, $[\alpha]_D^{17}$ $-9·1°$ (*c*, 10·81), which was oxidized with potassium permanganate in acetone. The oxidation products were an acid yielding a p-*bromophenacyl* ester, m. p. 82—83° (from ethanol) (Found: C, 68·7; H, 9·4; Br, 13·9. $C_{33}H_{55}O_3Br$ requires C, 68·4; H, 9·5; Br, 13·8%), and a ketone, which formed a semicarbazone of m. p. 126° after crystallization from ethanol.

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