Constituents of the Lipids of Tubercle Bacilli. Part III. Mycolipenic Acid.*

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Degradative studies of mycolipenic acid, a dextrorotatory acid from the lipids of tubercle bacilli, are reported which have shown that this acid is (+)-2 : 4 : 6 - trimethyltetracos-2-enoic acid † (cf. Polgar and Robinson, *Chem. and Ind.*, 1951, 685).

In the preceding Part,* the separation of the dextrorotatory acids of the lipids of tubercle bacilli (human type) into a solid and a liquid acid was described, both of which were shown to contain an αβ-unsaturated acid. The degradative studies of the αβ-unsaturated constituent of the solid acid are now reported.

Ozonization of the solid acid yielded pyruvic acid (isolated as its 2 : 4-dinitrophenylhydrazone). This showed that the αβ-unsaturated acid has the terminal grouping $\cdot C{:}CMe\cdot CO_2H$.

Oxidation of the methyl ester derived from the solid acid, by means of potassium permanganate in acetone, indicated that another main constituent of this material is an ester of a saturated branched-chain acid. This was unaffected by the oxidation, whereas the methyl ester of the αβ-unsaturated acid, now termed mycolipenic acid, was converted into an acid (II), $C_{24}H_{48}O_2$, forming a *p*-bromophenacyl ester of m. p. 68°, and small amounts of a methyl ketone (III), $C_{21}H_{43}\cdot CO\cdot CH_3$ (positive iodoform test), characterized as its semicarbazone, m. p. 94—95°. The same ketone was obtained on a Wieland–Barbier degradation of the acid (II), thus indicating that this acid has one alkyl (methyl) substituent in the α-position to the carboxyl group. These results disclosed that mycolipenic acid has the terminal grouping $\cdot CHMe\cdot CH{:}CMe\cdot CO_2H$.

The acid (II) had $[\alpha]_D$ +7·1°. Bromination (Hell–Volhard–Zelinsky method), followed by reaction of the α-bromo-acid bromide with methanol, and dehydrobromination of the resulting bromo-ester by refluxing pyridine, gave an αβ-unsaturated ester (IV) ($\lambda_{max.}$ 2160 Å; log ε 4·14) which exhibited $[\alpha]_D$ +13·3°. The rotatory power of this ester already indicated (cf. Jocelyn and Polgar, *J.*, 1953, 132) that there is an asymmetric centre adjacent to the αβ-double bond, *i.e.*, at $C_{(4)}$. This was confirmed by oxidation of the ester, by means of potassium permanganate in acetone, which, similarly to the oxidation of the initial αβ-unsaturated ester (see above), yielded an acid (V) and a by-product ketone (VI). The acid (V) had $[\alpha]_D$ about +7° and gave an amide of m. p. 107—108° which corresponded to the m. p. (108°) recorded (Schneider and Spielman, *J. Biol. Chem.*, 1942, **142**, 345) for (±)-2-methyleicosanoamide. The ketone (VI), yielding a semicarbazone of m. p. 124—125°, was identified as *n*-eicosan-2-one by *X*-ray crystallographic studies, kindly carried out by Mrs. D. M. Crowfoot Hodgkin; an account of these studies has already been given (Bailey, Polgar, and Robinson, *J.*, 1953, 3031).

* Part II, preceding paper. An account of this work has been included in a lecture at the VIth International Congress for Microbiology, Rome, September, 1953.

† Geneva nomenclature (CO_2H = 1) is used throughout.

These results showed that mycolipenic acid is (+)-2 : 4 : 6-trimethyltetracos-2-enoic acid (I).

(I) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CH{:}CMe{\cdot}CO_2H$
(II) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CO_2H$
(III) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH_2{\cdot}CO{\cdot}CH_3$
(IV) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH{:}CMe{\cdot}CO_2Me$
(V) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CO_2H$
(VI) $CH_3{\cdot}[CH_2]_{17}{\cdot}CO{\cdot}CH_3$

An early oxidation experiment involving a mixture of the dextrorotatory acids is described in the Experimental section.

Experimental

Ultra-violet absorption spectra were determined in methanol, specific rotations in ether, 0·5-dm. tubes being used.

Ozonization of the Solid Dextrorotatory Acid.—Ozonized oxygen was passed into an ice-cooled solution of the solid dextrorotatory acid (Part II, *loc. cit.*) (0·5 g.) in carbon tetrachloride (10 c.c.) for 15 min. Water (10 c.c.) was then added, and the product heated at 100° for 0·5 hr. (the carbon tetrachloride was allowed to evaporate during this procedure). After cooling, the water-insoluble products were filtered off, and a 0·1% solution of 2 : 4-dinitrophenylhydrazine in aqueous hydrochloric acid was added to the filtrate. The precipitated product was collected and triturated with aqueous 5% sodium hydrogen carbonate; the solution was then filtered and the filtrate acidified with dilute hydrochloric acid. The product had m. p. 213—214° (microscope hot-stage) (Found : N, 20·8. Calc. for $C_9H_8O_6N_4$: N, 20·9%), undepressed by an authentic specimen of pyruvic acid 2 : 4-dinitrophenylhydrazone. In an earlier experiment (cf. Part II) in which ozonized oxygen was passed through a solution containing the dextrorotatory solid acid for 3·5 hr., only acetic acid was found in the aqueous solution of the ozonization products.

Oxidation of the Methyl Ester of the Solid Dextrorotatory Acid.—To a solution of the ester (1 g.; obtained by refluxing the acid with 5% methanolic sulphuric acid) in dry acetone (100 c.c.), heated under reflux with stirring, powdered potassium permanganate was added in small portions until the rate of oxidation became very slow (about 4 hr.). The precipitated manganese dioxide was filtered off and washed with dry ether. The combined filtrates were evaporated, and the residue, after addition of water and acidification (hydrochloric acid), was extracted with ether. The product (0·6 g.) obtained on evaporation of the ethereal extract contained only little acidic material (it required 1·1 c.c. of 0·1N-sodium hydroxide for neutralization). The neutralized (phenolphthalein) solution was extracted with ether, the ethereal solution evaporated, and the product separated into a ketone and a non-ketone fraction with the aid of Girard's P reagent (1 g.). The ketone fraction (4-methyldocosan-2-one), isolated in the usual manner, distilled at 205—210° (bath)/0·01 mm., and yielded a *semicarbazone*, m. p. 94—95° (Found : C, 72·85; H, 12·3; N, 10·5. $C_{24}H_{49}ON_3$ requires C, 72·9; H, 12·4; N, 10·6%). The non-ketone fraction (about 0·5 g.) (Found : C, 78·6; H, 13·2%) exhibited no high-intensity absorption in the ultra-violet, and had $[\alpha]_D$ +0·8° (*c*, 12·6).

The above manganese dioxide precipitate was dissolved by the addition of sodium hydrogen sulphite and dilute hydrochloric acid, and the product extracted with ether. Re-extraction of the ethereal solution with 5% aqueous potassium hydroxide, followed by acidification of the aqueous extract, afforded 2 : 4-*dimethyldocosanoic acid* (II) (0·3 g.), m. p. 34—35° (crude product), $[\alpha]_D^{16}$ +7·1° (*c*, 5·9) (Found : C, 78·3; H, 13·3%; equiv., by titration with aqueous-methanolic 0·1N-sodium hydroxide, 369·9. $C_{24}H_{48}O_2$ requires C, 78·3; H, 13·1%; equiv., 368). Its p-*bromophenacyl* ester had m. p. 68° after crystallization from ethanol (Found : C, 67·8; H, 9·4; Br, 14·1. $C_{32}H_{53}O_3Br$ requires C, 68·0; H, 9·4; Br, 14·2%).

No oxalic acid was found in the aqueous mother-liquors of the above oxidation products.

Wieland–Barbier Degradation of the Preceding Acid Oxidation Product (II).—The methyl ester of this acid (0·23 g.; obtained by refluxing the acid with methanolic sulphuric acid) in benzene (10 c.c.) was added to a Grignard solution from bromobenzene (6·7 g.) and magnesium (1 g.) in ether (20 c.c.), and the mixture refluxed for 2 hr. The solvent was then distilled off on a steam-bath as far as possible, and benzene (5 c.c.) added to the residue, and then slowly distilled off. After being heated on the steam-bath for a further hour, the residue was cooled, and decomposed by means of ammonium chloride and ice. The product, collected in ether, was then distilled in steam (to remove diphenyl). The non-volatile part was extracted with ether, the ethereal solution evaporated, and the resulting crude alcohol dehydrated by refluxing formic acid (98%; 5 c.c.) for 1·5 hr. After dilution with water, the product was extracted with

ether, and the ethereal extract washed successively with water, 5% aqueous potassium hydroxide, and water, and dried (Na_2SO_4). The product obtained on evaporation of the ether was dissolved in carbon tetrachloride (5 c.c.), and a stream of ozonized oxygen passed into the solution for 10 min., while cooling with ice. The solvent was then evaporated under reduced pressure at 30° (bath), water (10 c.c.) added to the residue, and the mixture slowly heated to 100° and kept there for further 20 min. The product, collected with ether, was kept with a solution of powdered potassium permanganate in acetone (5 c.c.) at room temperature for 10 min. Sodium hydrogen sulphite and dilute hydrochloric acid were then added. The product was extracted with ether, and the ethereal solution washed successively with water, 5% aqueous potassium hydroxide, and water. Evaporation of the dried (Na_2SO_4) ethereal extract, followed by purification of the residual product with the aid of Girard's P reagent (1 g., in 10 c.c. of methanol) yielded a ketone (III) forming a semicarbazone, m. p. 92—93° (from ethanol) (Found: N, 10·8%), undepressed on admixture with the semicarbazone (m. p. 94—95°) of the ketone obtained by the oxidation of the methyl ester of the solid dextrorotatory acid (see preceding section). The parent ketone, regenerated by heating of the semicarbazone with aqueous oxalic acid, gave a positive iodoform test.

Bromination of the Acid Oxidation Product (II) *and Dehydrobromination of the α-Bromo-ester.*—To a mixture of the acid (0·27 g.) and red phosphorus (0·04 g.) at 90° (bath) was added dropwise dry bromine (slight excess) with frequent shaking. After a further 3 hours' heating some more bromine (few drops) was added, and heating continued for another 3 hr. The product was cooled, dry methanol (10 c.c.) added, and the mixture refluxed for 1 hr. Dilution with water and extraction with ether gave the crude bromo-ester. This ester (0·3 g.) and dry pyridine (7 c.c.) were refluxed for 17 hr. After addition of dilute hydrochloric acid, the product was collected with ether and distilled. The unsaturated ester (IV) distilled at 200—210° (bath)/0·02 mm., and had $[\alpha]_D^{21}$ +13·3° (*c*, 3·16), n_D^{18} 1·4590 (Light absorption: Max., 2160 Å; log ε 4·14).

Oxidation of the Unsaturated Ester (IV).—The preceding ester (about 0·1 g.) in acetone (25 c.c.) was oxidized with potassium permanganate as above. The precipitated manganese dioxide was filtered off and washed with ether. The combined filtrates were evaporated, and the residual product was refluxed with ethanolic potassium hydroxide for 1 hr. After acidification, the mixture was extracted with ether, and the ethereal extract washed with 5% potassium hydroxide solution. The non-acid material remaining in the ethereal layer distilled at 160—170° (bath)/0·07 mm. It formed a semicarbazone which had m. p. 122—123° after one crystallization from ethanol; further crystallizations from the same solvent raised the m. p. to 124—125°. Only a few mg. resulted which were reserved for *X*-ray crystallographic studies (cf. Bailey, Polgar, and Robinson, *loc. cit.*).

The precipitated manganese dioxide was treated with sodium hydrogen sulphite and dilute hydrochloric acid, and the mixture extracted with ether. Re-extraction of the ethereal solution with 5% aqueous potassium hydroxide, followed by acidification of the aqueous extract, yielded 2-methyleicosanoic acid (V), $[\alpha]_D$ about +7° (*c*, 1·6). It was characterized as the amide, m. p. 107—108° after crystallization from methanol (Found: C, 77·7; H, 12·8. Calc. for $C_{21}H_{43}ON$: C, 77·5; H, 13·2%).

Oxidation of a Mixture of the Dextrorotatory Acids.—In early experiments preceding the work described in Part II (*loc. cit.*) a mixture of the dextrorotatory acids (0·5 g.), isolated by distillation of the methyl esters essentially as described in Part I (Polgar, *Biochem., J.*, 1948, **42**, 206) and having $[\alpha]_D^{16}$ +8·3° (*c*, 11·8) (Found: C, 78·2; H, 12·4%), was subjected to an oxidation with potassium permanganate in acetone in the manner already described. After evaporation of the solvent, the precipitated manganese dioxide was removed by the addition of aqueous sodium hydrogen sulphite and dilute sulphuric acid, and the product extracted with ether. Re-extraction of the ethereal solution with 5% aqueous potassium hydroxide, and then with water, followed by acidification of the aqueous phase, afforded a mixture of acids which yielded a *p*-bromophenacyl ester, m. p. 65—66° after crystallization from ethanol (Found: C, 68·2; H, 9·2; Br, 13·9%). The non-acid material which remained in the ethereal layer gave, with the aid of Girard's P reagent, a ketone, forming a semicarbazone, m. p. 89—90° (Found: C, 73·0; H, 12·1; N, 10·8%). An acid yielding the same *p*-bromophenacyl ester as above (Found: C, 67·85; H, 9·6; Br, 14·15%) was obtained on ozonization (2 hr.) of the above mixture of the dextrorotatory acids (0·3 g.) in carbon tetrachloride (7 c.c.), followed by the action of hydrogen peroxide (30% w/v) and formic acid (98%) on the ozonization product.

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