602. *Experiments on the Synthesis of Mycolipenic Acid. Part I.*

By A. S. Bailey, N. Polgar, and Sir Robert Robinson.

The structure advanced for mycolipenic acid (Polgar and Robinson, *Chem. and Ind.*, 1951, 685) has been confirmed in respect of the length of the normal chain.

The synthesis of the optically inactive acid (or of a stereoisomeride) is now described.

Mycolipenic acid * is a dextrorotatory acid from the lipids of tubercle bacilli which has already been shown to have the structure (I), where n was stated to be probably 17 (Polgar and Robinson, *Chem. and Ind.*, 1951, 685). The stepwise degradations of mycolipenic acid already reported resulted in the isolation of a straight-chain methyl ketone which gave a semicarbazone of m. p. 124·5° (the m. p. 122°, given in the earlier communication rose on further crystallisations). The melting point of this semicarbazone was not depressed on admixture with n-eicosan-2-one semicarbazone, m. p. 126—126·5°, or n-nonadecan-2-one semicarbazone, m. p. 125·5—126°. The absence of depression of melting points on admixture does not provide sufficient proof of identity in this series, and we are greatly indebted to Mrs. D. M. Crowfoot Hodgkin for identifying the degradation product as n-eicosan-2-one by comparing the X-ray diffraction powder photographs of the above semicarbazones. Mrs. Hodgkin reported as follows:

"A specimen of the semicarbazone of the degradation product was compared with specimens of the semicarbazones of eicosan-2-one and nonadecan-2-one. All three crystallised in very similar small plates which were hardly distinguishable by measurement under the microscope. Powder photographs, taken with a 3-cm. camera, showed that the derivative of the degradation product could be identified with that of eicosan-2-one. The spacings of the lines observed for these two specimens (cf. Table) were all identical within the limits of experimental error and a few small differences in the appearance of the lines could be accounted for by different degrees of orientation of the fragments in the specimens used for photography. The photograph of the semicarbazone of nonadecan-2-one, although very similar in general appearance, differed in the spacings of many of the weaker lines. The identity of the longer spacings of the semicarbazone of eicosan-2-one and that of the degradation product were further checked by photographs on plates at greater crystal-to-film distances."

Principal wide-angle lines on powder photographs.

Semicarbazone of nonadecan-2-one		Semicarbazone of the degradation product		Semicarbazone of eicosan-2-one	
Spacing	Intensity	Spacing	Intensity	Spacing	Intensity
6·05	w	6·34	w	6·40	w
5·59	w +	4·43	m	4·42	m
5·02	w +	4·08	vw	4·09	vw
4·60	m	3·90	w +	3·90	w
4·38	w	3·73	s	3·72	s
3·71	s	3·44	w	3·55	?vw
2·21	w	2·44	w	2·46	w
2·12	w	2·40	w	2·40	w
		2·14	vw	2·14	Blurred
		2·12	w		w Blurred

The provisional formulation of the degradation product as eicosan-2-one (Polgar and Robinson, *loc. cit.*) is thus confirmed. It follows that in formula (I) n is 17, *i.e.*, mycolipenic acid is 2 : 4 : 6-trimethyltetracos-2-enoic acid.

At this point the opportunity is taken to comment briefly on "phthioic acid," obtained by Spielman and Anderson (*J. Biol. Chem.*, 1935—36, **112**, 759) from the lipids of human tubercle bacilli. This acid, formulated by them as $C_{26}H_{52}O_2$, was isolated by a procedure

* The suffix I is now omitted because there is no evidence of the natural occurrence of a second, closely related, unsaturated isomeric acid.

involving hydrogenation, but the value given for its specific rotation (+12·5°) suggests that the hydrogenation was incomplete (cf. Chanley and Polgar, *Nature*, 1950, **166**, 693). It is doubtless partly due to this partial hydrogenation that Cason and Sumrell (*J. Biol. Chem.*, 1951, **192**, 405), on fractionation of the methyl esters of " phthioic acid " originating from the work of Anderson and his collaborators, obtained a variety of products. These were claimed to comprise at least twelve components, including αβ-unsaturated dextro- and lævo-rotatory acids.

Whilst awaiting with interest the more precise elaboration of these findings we can say at once that they do not accord with the impressions gained from investigations made in these laboratories.

In regard to the dextrorotatory acid which the American authors termed C_{27}-phthienoic acid, it should be noted that Cason, Freeman, and Sumrell (*ibid.*, p. 415) have provided no evidence of its structure by degradation, nor any clear proof of the presence of methyl substituents. The physical methods employed led these authors to assign a methyl group to $C_{(5)}$. Freeman (*J. Amer. Chem. Soc.*, 1953, **75**, 1859), ignoring our disclosure of the full constitution of mycolipenic acid in 1951, has now found by comparison of infra-red spectra that one methyl branch is on $C_{(4)}$.

There can be very little doubt but that C_{27}-phthienoic acid and mycolipenic acid are identical.

The molecule of mycolipenic acid contains two asymmetric carbon atoms and the acid is therefore one of four possible optically active forms. The acid is dextrorotatory, and the same applies to the α-methyl-substituted acids obtained as degradation products (Polgar and Robinson, *loc. cit.*) of which the α-carbon atom corresponds to $C_{(4)}$ and $C_{(6)}$, respectively, of mycolipenic acid. These considerations will be helpful in synthesising optically active intermediates corresponding in configuration to the natural product. Experiments in this direction are in progress. Meanwhile, in the present communication a synthesis of the optically inactive acids is placed on record.

The starting point was 2-methyleicosanoic acid (II), obtained by a malonic ester synthesis employing octadecyl iodide and ethyl methylmalonate. Reduction of (II) with lithium aluminium hydride afforded 2-methyleicosan-1-ol. The latter was converted into the iodide which by condensation with ethyl methylmalonate and the usual subsequent procedure gave 2 : 4-dimethyldocosanoic acid (III). This was transformed by repetition of the above processes into 2 : 4-dimethyldocosan-1-ol, and 2 : 4 : 6-trimethyltetracosanoic acid (IV). Bromination of the acid (Hell–Volhard–Zelinsky method), followed by reaction of the α-bromo-acid bromide with methanol and dehydrobromination of the resulting bromo-ester by means of pyridine, and hydrolysis of the product, gave 2 : 4 : 6-trimethyltetracos-2-enoic acid (I; $n = 17$).

(I) $CH_3{\cdot}[CH_2]_n{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CH{:}CMe{\cdot}CO_2H$ $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CO_2H$ (II)

(III) $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CO_2H$ $CH_3{\cdot}[CH_2]_{17}{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CH_2{\cdot}CHMe{\cdot}CO_2H$ (IV)

In earlier experiments preparation of 2 : 4-dimethyldocosanoic acid (III) was attempted by a shorter route, namely, by oxidation of the appropriate alkyl-substituted *tert.-cyclo*hexanol (cf. Fieser and Szmuszkovicz, *J. Amer. Chem. Soc.*, 1948, **70**, 3352). For this purpose, 3 : 5-dimethyl-1-*n*-octadecyl*cyclo*hexan-1-ol was prepared by a Grignard reaction between *n*-octadecyl bromide and 3 : 5-dimethyl*cyclo*hexanone, obtained by hydrogenation of 1 : 3 : 5-xylenol, and oxidation of the resulting alcohol to the ketone. Oxidation of the product with chromic acid was expected to yield 2 : 4-dimethyl-6-oxodocosanoic acid; however, only small quantities of acidic material were obtained and this approach was not pursued further.

The physiological properties of 2 : 4 : 6-trimethyltetracos-2-enoic acid and related acids have been examined by Dr. J. Ungar of Glaxo Laboratories Ltd. The preliminary results suggest that the acid is a marked irritant which causes fibrotic changes of non-specific character in the organs of the peritoneal cavity. On the other hand, 2 : 4-dimethyldocosanoic acid (III), obtained as an intermediate in the above synthesis, exhibited a moderate specific activity similar to that seen previously with 3 : 12 : 15-trimethyldocosanoic acid. It produced multiple white nodules of cheesy appearance on the organs

within the peritoneal cavity and on the diaphragm; in particular, the spleen and omentum showed multiple granulomatous lesions with central necrosis. 2 : 4-Dimethyldocos-2-enoic acid (forthcoming publication) showed only very low activity of a non-specific character, and it thus appears that the introduction of an αβ-double bond decreases the activity.

Experimental

Nonadecan-2-one.—Hexadecyl iodide (3 g.; prepared from hexadecan-1-ol by interaction with iodine and red phosphorus) was added to ethyl sodioacetoacetate [from sodium (0·2 g.), ethanol (10 c.c.), and ethyl acetoacetate (2 c.c.)], and the mixture refluxed for 4 hr. Next day potassium hydroxide (2·5 g.) in water (30 c.c.) was added, and the mixture kept for 1 hr. with frequent shaking. The resulting solution was then refluxed with 5N-hydrochloric acid (20 c.c.) for 2 hr. The product was collected with ether, and the ethereal solution washed successively with 5% aqueous potassium hydroxide, and water, and dried (Na_2SO_4). Evaporation then gave the crude *ketone* (2·4 g.) which, after crystallisation from ethanol, had m. p. 53·5° (Found : C, 81·1; H, 13·5. $C_{19}H_{38}O$ requires C, 80·9; H, 13·5%). Its *semicarbazone* had m. p. 125·5—126° after crystallisation from ethanol (Found : C, 71·0; H, 12·2; N, 12·5. $C_{20}H_{41}ON_3$ requires C, 70·8; H, 12·1; N, 12·4%).

n-*Eicosan-2-one.*—This ketone was prepared according to the procedure of Churchward, Gibson, Meakins, and Mulley (*J.*, 1950, 959). Its semicarbazone had m. p. 126—126·5° after crystallisation from ethanol (Found : C, 71·1; H, 12·2. Calc. for $C_{21}H_{43}ON_3$: C, 71·4; H, 12·2%).

2-*Methyleicosanoic Acid* (II).—Iodine (32 g.) was added in small portions during 1 hr. to a mixture of octadecan-1-ol (61 g.) and red phosphorus (2·6 g.) at 130—140° (bath). The temperature was then raised to 170—180° and the heating continued for another 3 hr. After cooling, the product was taken up in ether, and the ethereal solution filtered through glass wool. It was then washed, successively, with water, aqueous sodium hydrogen sulphite, and water, dried ($MgSO_4$), and distilled, affording octadecyl iodide, b. p. 156—160°/0·1 mm. This (134 g.) was added with stirring during 10 min. to the sodio-derivative of ethyl methylmalonate, obtained from sodium (12·3 g.), *n*-butanol (200 c.c.; dried over K_2CO_3 and distilled), and ethyl methylmalonate (92 g.; Blatt, *Org. Synth.*, Coll. Vol. II, 1943, pp. 272, 279), and the mixture was refluxed for 7 hr. Next day potassium hydroxide (60 g.) in water (75 c.c.) was added, and the mixture refluxed with stirring for 10 hr. The butanol was then removed by distillation, water being added at the same rate as the butanol distilled. When most of the butanol had been removed, the liquid frothed considerably. Excess of hydrochloric acid was added at this stage, and the distillation continued for another hour, to remove the remaining butanol. When the mixture was cooled the crude substituted malonic acid solidified. It was collected, washed with a little ether, dried, and then decarboxylated at 160—170° (bath) under reduced pressure for 3 hr. The product was taken up in benzene, and the solution filtered. Removal of the solvent and crystallisation of the residual product from light petroleum (b. p. 60—80°) afforded 2-methyleicosanoic acid, m. p. 61·8—62° (95 g., 83%). Schneider and Spielman (*J. Biol. Chem.*, 1942, **142**, 345) give m. p. 61·5—62°; Stenhagen and Tagstrom (*Svensk Kem. Tidskr.*, 1942, **54**, 145; *Chem. Abs.*, 1944, **38**, 2314) give m. p. 61·7—62°. The p-*bromophenacyl* ester crystallised from ethanol in clusters of needles, m. p. 83—84° (Found : C, 67·0, 66·1; H, 9·2, 9·3. $C_{29}H_{47}O_3Br$ requires C, 66·6; H, 9·0%).

2-*Methyleicosan*-1-*ol.*—2-Methyleicosanoic acid (45 g.) was refluxed with ethanol (500 c.c.) and concentrated sulphuric acid (22 c.c.) for 6 hr. After removal of most of the ethanol by heating on a steam-bath under reduced pressure, the residual product was extracted with ether. The extract was washed with water and dried ($MgSO_4$), and the ether removed. A solution of the resulting crude ester in dry ether (100 c.c.) was then added to a suspension of lithium aluminium hydride (4 g.) in ether (350 c.c.) at such a rate that the ether refluxed steadily. After 3 hr.' refluxing the excess of lithium aluminium hydride was decomposed by the addition of ethyl acetate; water was then added, followed by the addition of dilute sulphuric acid. The aqueous layer was extracted with ether. The combined ethereal extracts were washed, successively, with dilute sulphuric acid, water, 5% aqueous potassium hydroxide (no acidic material was found in this washing), and water, and dried ($MgSO_4$). After evaporation of the ether, the residual oil (41·5 g.) solidified on cooling and had m. p. 42—44°. On crystallisation from light petroleum (b. p. 40—60°), 2-*methyleicosan*-1-*ol* was obtained as clusters of blades, m. p. 48·5—49° (Found : C, 80·2, 80·3; H, 14·0, 13·8. $C_{21}H_{44}O$ requires

C, 80·8; H, 14·1%). The *phenylurethane* crystallised from light petroleum (b. p. 40—60°) as colourless needles, m. p. 61—62° (Found: C, 78·1; H, 11·4. $C_{28}H_{49}O_2N$ requires C, 77·9; H, 11·4%).

1-*Iodo*-2-*methyleicosane*.—A mixture of 2-methyleicosan-1-ol (20 g.) and red phosphorus (0·92 g.) was heated at 120° (bath), and iodine (12·2 g.) added during 30 min. The bath-temperature was gradually raised to 160—180° during 30 min. and maintained thereat for another 3 hr. After cooling, ether was added, and the ethereal solution filtered through glass wool, then washed successively with water, aqueous sodium hydrogen sulphite, 5% aqueous potassium hydroxide, and water, and dried ($MgSO_4$). Distillation afforded 1-*iodo*-2-*methyleicosane* (22 g.), b. p. 166—170°/0·03 mm., m. p. 21—22° (thermometer in melt) (Found: C, 59·8; H, 10·2; I, 29·0. $C_{21}H_{43}I$ requires C, 59·8; H, 10·2; I, 30·0%).

2 : 4-*Dimethyldocosanoic Acid* (III).—The last mentioned iodide (40 g.) was added during 5 min. to the sodio-derivative of ethyl methylmalonate [from sodium (3·32 g.), *n*-butanol (80 c.c.), and ethyl methylmalonate (25 g.)], and the mixture refluxed for 7 hr. Next day the product was hydrolysed by refluxing it with potassium hydroxide (16 g.) in water (20 c.c.) for 7 hr., and worked up by the procedure given for a previous example except that the crude malonic acid (35·4 g.) was isolated by ether-extraction. Crystallisation of a small quantity from light petroleum (b. p. 40—60°) gave 2-*carboxy*-2 : 4-*dimethyldocosanoic acid* as clusters of fine needles, m. p. 66—68° (Found: C, 73·2; H, 11·7. $C_{25}H_{48}O_4$ requires C, 72·8; H, 11·7%). The remainder of the crude acid was decarboxylated by heating it at 160° (bath) for 2 hr. On distillation 2 : 4-*dimethyldocosanoic acid*, b. p. 190—195°/0·04 mm., m. p. 28—29°, was obtained. After resolidification, it melted at 38—41°. It crystallised from light petroleum (b. p. 40—60°) as fine needles, m. p. 50—51° (Found: C, 78·1; H, 13·3. $C_{24}H_{48}O_2$ requires C, 78·3; H, 13·1%). The p-*bromophenacyl* ester formed fine needles (from ethanol), m .p. 57—58° (Found: C, 78·1; H, 13·3. $C_{24}H_{48}O_2$ requires C, 78·3; H, 13·1%).

2 : 4-*Dimethyldocosan*-1-*ol*.—A solution of 2 : 4-dimethyldocosanoic acid (10 g.) in dry ether (100 c.c.) was added dropwise to lithium aluminium hydride (1·8 g.) in ether (100 c.c.), and the mixture refluxed for 4 hr. The product was worked up as described for a previous example. Distillation afforded 2 : 4-*dimethyldocosan*-1-*ol* (9·6 g.), b. p. 172—176°/0·1 mm., m. p. 36—39° (Found: C, 81·7; H, 13·9. $C_{24}H_{50}O$ requires C, 81·4; H, 14·1%).

2 : 4 : 6-*Trimethyltetracosanoic Acid* (IV).—Iodine (6·1 g.) was added to a mixture of the above alcohol (9·5 g.) and red phosphorus (0·45 g.), heated at 110—120° (bath), during 30 min.; the mixture was then heated at 160—180° (bath) for 3 hr., and the product worked up as described for previous examples. The resulting iodide (10 g.) had b. p. 190—200°/0·03 mm.

This iodide (9 g.) was added to the sodio-derivative of ethyl methylmalonate [from sodium (0·96 g.), *n*-butanol (20 c.c.), and ethyl methylmalonate (7 g.)], and the mixture refluxed for 7 hr. Potassium hydroxide (8 g.) in water (8 c.c.) was then added, and the mixture refluxed for 5 hr. After working up by the usual method, the crude malonic acid was collected by means of ether. Crystallisation from light petroleum (b. p. 40—60°) gave 2-*carboxy*-2 : 4 : 6-*trimethyltetracosanoic acid* as fine needles, m. p. 62—65° (Found: C, 74·5; H, 12·2. $C_{28}H_{54}O_4$ requires C, 74·1; H, 11·9%). The remainder of the crude acid was decarboxylated at 160° (bath). Distillation of the product afforded 2 : 4 : 6-*trimethyltetracosanoic acid* (5·5 g.), b. p. 210—215°/0·08 mm. (Found: C, 79·4; H, 13·1. $C_{27}H_{54}O_2$ requires C, 79·1; H, 13·2%).

2 : 4 : 6-*Trimethyltetracos*-2-*enoic Acid* (I; $n = 17$).—Trimethyltetracosanoic acid (2·1 g.) was heated with bromine (3·5 g.) in the presence of red phosphorus (0·17 g.) in a boiling water-bath for 8 hr. After cooling, dry methanol (20 c.c.) was added, and the mixture refluxed for 2 hr. Dilution with water and extraction with ether afforded the crude bromo-ester. This was refluxed with pyridine (20 c.c.) for 18 hr. After addition of dilute hydrochloric acid, the product was isolated by means of ether, and the ethereal extract washed successively with dilute hydrochloric acid and water. The ethereal solution was then evaporated, and the residual crude ester hydrolysed by refluxing it with a solution of potassium hydroxide (1 g.) in water (2 c.c.) and ethanol (8 c.c.) for 1·5 hr. Acidification of the product furnished 2 : 4 : 6-*trimethyltetracos*-2-*enoic acid* (1·2 g.), b. p. 216—218°/0·25 mm. (Found: C, 79·3; H, 12·5. $C_{27}H_{52}O_2$ requires C, 79·4; H, 12·8%). Light absorption: Max., 2140 Å; log ε, 4·12. Its *amide* had m. p. 40° after crystallisation from methanol (Found: N, 3·6. $C_{27}H_{53}ON$ requires N, 3·4%).

3 : 5-*Dimethylcyclohexanone*.—3 : 5-Dimethyl*cyclo*hexanol, prepared by hydrogenation of 1 : 3 : 5-xylenol over Raney nickel at 180°/80 atm., was oxidised by means of potassium dichromate and dilute sulphuric acid in the usual manner. 3 : 5-Dimethyl*cyclo*hexanone, b. p. 178—180°, formed a semicarbazone, m. p. 206—207°, in agreement with the records but the 2 : 4-dinitrophenylhydrazone, which crystallised from ethanol in clusters of orange-yellow rods,

had m. p. 166—167° (Birch, *J.*, 1947, 1642, reports m. p. 155—157°). The ketone can occur in stereoisomeric forms and this is a possible cause of the divergence.

3 : 5-*Dimethyl*-1-n-*octadecyl*cyclo*hexan*-1-*ol*.—3 : 5-Dimethyl*cyclo*hexanone (15·1 g.) in ether (20 c.c.) was added to an ice-cold Grignard solution from octadecyl bromide (33·3 g.; prepared by the procedure described by Blatt, *Org. Synth.*, Coll. Vol. II, 1943, p. 246, except that hydrogen bromide was passed into the reaction mixture for a total time of about 6 hr.), magnesium (2·4 g.), and ether (150 c.c.), and the mixture was refluxed for 1·5 hr. Next day, ice and aqueous ammonium chloride were added. The ethereal layer was filtered to remove hexatriacontane, washed with water, and dried (K_2CO_3). On distillation, the fraction of b. p. 180—210°/0·1 mm. (20·4 g.) was collected. This solidified on cooling, and had m. p. 43—45°. The *alcohol* crystallised from ethyl acetate–methanol (4 : 1) in fine needles, m. p. 50—51° (Found : C, 82·4; H, 13·6. $C_{26}H_{52}O$ requires C, 82·1; H, 13·7%). Heating it with iodine gave the corresponding *olefin*, m. p. 36—37° (Found : C, 85·9; H, 14·0. $C_{26}H_{50}$ requires C, 86·2; H, 13·8%).

Attempted oxidations of the alcohol (4-g. portions) with chromium trioxide in acetic acid, following the procedure of Fieser and Szmuszkovicz (*J. Amer. Chem. Soc.*, 1948, **70**, 3352), failed to yield any significant amounts of acidic material.

DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, May 28th*, 1953.]