329. *Methyl-substituted Long-chain Acids. Part III.*

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Previous studies indicated that 12 : 15-dimethyltricosoic acid (Part I, *J.*, 1945, 389) * exhibited some activity in stimulating the formation of pseudo-tuberculous lesions. It is now shown that this activity was due to the presence of a 2-methyl-substituted isomer of the acid. The substance, namely 2 : 11 : 14-*trimethyldocosoic acid*, is notably active, whereas a purified specimen of 12 : 15-dimethyltricosoic acid is found to be inactive.

In further experiments, designed to establish the essential structural features for the biological activity, 3 : 12 : 15-*trimethyltricosoic*, 2 : 12 : 15-*trimethyldocosoic*, and 1 : 11 : 14-*trimethyldocosoic acid* have been synthesised. All these acids are active.

2 : 11 : 14-*Trimethyldocos*-11-*enoic acid* appears to exhibit only weak activities.

Part I of this series (Polgar and Robinson, *loc. cit.*) recorded *inter alia* the preparation of 12 : 15-dimethyltricosoic acid.* The method of preparation involved at one stage the addition

* For numbering etc., see p. 1541.

of hydrogen bromide to an intermediate diene under peroxide-catalysed conditions, a process which may give rise to the formation, as a by-product, of a secondary bromide resulting from the addition of hydrogen bromide according to Markownikoff's rule. The acid exhibited some activity in producing lesions similar to those of tuberculosis, and the possibility that it may have contained some of its isomer, namely 2 : 11 : 14-trimethyldocosoic acid, suggested the examination of the last-mentioned acid, and also of a purified specimen of 12 : 15-dimethyltricosoic acid.

2 : 11 : 14-*Trimethyldocosoic acid* (I) was obtained by the procedure employed for the preparation of 12 : 15-dimethyltricosoic acid (Part I) with the modification that hydrogen iodide was used in place of hydrogen bromide, in which process there is no peroxide effect.

The purification of a specimen of 12 : 15-dimethyltricosoic acid, prepared by the procedure described in Part I, was accomplished by converting it into its acetol ester and thence into the semicarbazone (cf. Polgar, *Biochem. J.*, 1948, **42**, 207). After crystallisation of the latter from methanol, the pure acid was recovered by hydrolysis with hydrochloric acid in glacial acetic acid. The acid thus obtained melted at 40—42° (after crystallisation from methanol), whilst the original specimen had m. p. 14—15°.

Results of the biological tests with guinea-pigs, carried out by Mr. C. E. Coulthard, Dr. L. Dickinson, and Dr. J. Ungar, showed that the purified specimen of 12 : 15-dimethyltricosoic acid was inactive, whereas 2 : 11 : 14-trimethyldocosoic acid possessed considerable biological activity in respect of stimulating the formation of tuberculous-like tissue. Hence the activity of the original specimen of 12 : 15-dimethyltricosoic acid must have been due to the presence of 2 : 11 : 14-trimethyldocosoic acid.

(I.) $CH_3{\cdot}[CH_2]_6{\cdot}CHMe{\cdot}[CH_2]_2{\cdot}CHMe{\cdot}[CH_2]_8{\cdot}CHMe{\cdot}CH_2{\cdot}CO_2H$

(II.) $CH_3{\cdot}[CH_2]_6{\cdot}CHMe{\cdot}[CH_2]_2{\cdot}CHMe{\cdot}[CH_2]_8{\cdot}CHMe{\cdot}[CH_2]_2CO_2H$

(III.) $CH_3{\cdot}[CH_2]_5{\cdot}CHMe{\cdot}[CH_2]_2{\cdot}CHMe{\cdot}[CH_2]_9{\cdot}CHMe{\cdot}CH_2CO_2H$

(IV.) $CH_3{\cdot}[CH_2]_6{\cdot}CHMe{\cdot}[CH_2]_2{\cdot}CHMe{\cdot}[CH_2]_9{\cdot}CHMe{\cdot}CO_2H$

In order to obtain further information as to the structural requirements for the biological activity, several other acids, closely related to 2 : 11 : 14-trimethyldocosoic acid, were prepared.

3 : 12 : 15-*Trimethyltricosoic acid* (II) was obtained from 2 : 11 : 14-trimethyldocosoic acid by Arndt–Eistert homologation.

2 : 12 : 15-*Trimethyldocosoic acid* was synthesised by interaction of tridec-12-en-2-one with a Grignard reagent from 3-methylnonyl bromide; treatment of the product with hydrogen iodide, followed by condensation with ethyl sodiomalonate and the usual successive steps, afforded the required acid (III).

For the synthesis of 1 : 11 : 14-*trimethyldocosoic acid* (IV) the general procedure followed for the previous acids was modified by using a bromo-ketone in place of the usual unsaturated one. The yield of 10 : 13-*dimethyleicos*-10-*enyl bromide*, obtained by the reaction between 3-methyldecylmagnesium bromide and 11-*bromundecan*-2-*one* was somewhat lower than in similar reactions with unsaturated ketones.

2 : 11 : 14-*Trimethyldocos*-11-*enoic acid* was prepared by the procedure described for the corresponding saturated acid, omitting the reduction of an unsaturated intermediate. It distilled with slight decomposition and failed to give satisfactory analytical figures.

In view of statements in the literature (cf. Buu-Hoï and Ratsimamanga, *Compt. rend. Soc. Biol.*, 1943, **137**, 189) that 1 : 1-dimethyl-substituted acids such as αα-dimethylstearic acid produce characteristic tuberculous tissue when injected into experimental animals, a specimen of this acid was prepared for comparison. The acid has been previously synthesised by Birch and Robinson (*J.*, 1942, 488), and by Buu-Hoï and Cagniant (*Z. physiol. Chem.*, 1943, **279**, 76); in both cases Haller's method was employed, which depends on the preparation of ω-trialkylacetophenones and their subsequent decomposition by sodamide. In the present work αα-dimethylstearic acid was prepared from methyl α-methylstearate by interaction with sodium triphenylmethyl and methyl iodide (cf. Hudson and Hauser, *J. Amer. Chem. Soc.*, 1941, **63**, 3156; Polgar and Robinson, *J.*, 1943, 615). The acid was found to be biologically inactive.

Dr. J. Ungar summarises the physiological action of the acids studied as follows:

12 : 15-Dimethyltricosoic acid (original specimen, probably containing 2 : 11 : 14-trimethyldocosoic acid	weak at 100 mg.
2 : 11 : 14-Trimethyldocosoic acid	active at 25 mg.
2 : 12 : 15-Trimethyldocosoic acid	active at 25 mg.
1 : 11 : 14-Trimethyldocosoic acid	active at 25 mg.
3 : 12 : 15-Trimethyltricosoic acid	active at 50 mg.
2 : 11 : 14-Trimethyldocos-11-enoic acid	weak at 100 mg.
αα-Dimethylstearic acid	inactive

Dr. Ungar states in respect of the foregoing table :

" The classification is purely arbitrary and as a criterion of potency we used the minimum quantity of fatty acid, which, when injected intraperitoneally in guinea-pigs, caused granulomatous lesions macroscopically similar to lesions caused by tubercle bacilli or similar organisms."

It is not possible at this stage to define precisely the structural features essential for the biological activity, since many points remain to be determined. For example, whether acids with all three methyl branches near the middle of the chain, such as 9 : 12 : 15-trimethyltricosoic acid, are active, is as yet unknown.

From comparison of the acids studied it appears that, with two of the methyl branches at about $C_{(11)}$ and $C_{(14)}$, it makes little or no difference whether the third branch is at $C_{(1)}$, $C_{(2)}$, or $C_{(3)}$; however, it may be mentioned that the activity of 3 : 12 : 15-trimethyltricosoic acid is to be treated with some reserve, since its mode of preparation does not preclude the possibility of its being contaminated with some of the active lower homologue (2 : 11 : 14-trimethyldocosoic acid).

Comparison of the acids described in Part II indicates that acids with only two methyl branches, of which one is near the carboxyl group, such as 2 : 12-dimethyltricosoic acid, are slightly active. On the other hand, a purified specimen of 12 : 15-dimethyltricosoic acid (both methyl branches in the middle of the chain) was found inactive. Hence it may be concluded that three methyl branches are necessary in order to produce extensive lesions, and one of these must be near the carboxyl group. As to the other two branches, it may be stated that both 2 : 12 : 15-trimethyldocosoic acid and 2 : 12 : 18-trimethyltricosoic acid are active, although differences in the degree of activity of these acids may exist; there are no examples yet available of acids with all the three methyl branches near the carboxyl group.

Another point of interest emerging from these studies is that 1 : 11 : 14-trimethyldocosoic acid is active, whereas 1 : 12 : 16 : 20-tetramethyldocosoic acid (cf. Part I) was found to be inactive. The constitutional features distinguishing the last-mentioned acid are the presence of the $C_{(20)}$-methyl, further methyl branches at $C_{(12)}$ and $C_{(16)}$ (as compared with $C_{(11)}$ and $C_{(14)}$, or $C_{(12)}$ and $C_{(15)}$, and $C_{(12)}$ and $C_{(18)}$, respectively, in case of the active acids), and also the fact that it has been synthesised from an optically active starting material [(+)-citronellal]. In order to determine the cause of the biological inactivity further investigation is necessary.

The comparison of 2 : 11 : 14-trimethyldocos-11-enoic acid with the corresponding saturated acid seems to suggest that the introduction of an olefinic linkage results in a distinct diminution of the biological potency. It is to be noted, however, that there seems to be no diminution of activity in the case of 2 : 12 : 18-trimethyltricosa-12 : 18-dienoic acid (cf. Part II), as compared with 2 : 12 : 18-trimethyltricosoic acid.

The methyl substituent near to the carboxyl group may plausibly be assumed to function as protection against destruction by β-oxidation. Thus the acid containing it may have a better survival value and a more persistent effect.

The most likely function of the more remote methyl groups is to promote dehydrogenation.

Both these hypotheses are speculative, but are mentioned because they do afford some guidance in the selection of acids which are to be synthesised and biologically tested.

Experimental.

αα-Dimethylstearic Acid.—Methyl α-methylstearate (b. p. 143—147°/0·1 mm.; acetol ester semicarbazone of the acid, m. p. 86—88°) (10 g.) was methylated by means of sodium triphenylmethyl (from 14 g. of triphenylchloromethane and 150 g. of 1·5% of sodium amalgam in 120 c.c. of ether) and methyl iodide (6 g.). The resulting ester was hydrolysed, and the isolated crude acid neutralised with alcoholic sodium hydroxide (phenolphthalein) and esterified by heating it under reflux with an excess of chloroacetone. The resulting acetol ester gave a *semicarbazone*, m. p. 66—68° (from methanol) (Found : C, 67·6; H, 11·2. $C_{24}H_{47}O_3N_3$ requires C, 67·75; H, 11·05%) (yield, 7 g.). The semicarbazone (6 g.) was heated on the steam-bath with acetic acid and hydrochloric acid as described below for the purification of 12 : 15-dimethyltricosoic acid, but the product obtained on distillation did not solidify when cooled with ice-water; it was subsequently boiled for one hour with 10% aqueous methanolic potassium hydroxide. Isolation and distillation of the acid gave an almost colourless oil, b. p. 177—180°/0·1 mm., which easily solidified and melted at 46—48°; the m. p. was raised to 48—49° after one crystallisation from methanol (Buu-Hoï and Cagniant, *loc. cit.*, crystallised this acid from light petroleum and give m. p. 50—51°, but very considerable losses of material occurred when this solvent was used).

12 : 15-*Dimethyltricosoic Acid.*—This acid (9 g.; prepared by the procedure described by Polgar and Robinson, *loc. cit.*) was converted into the semicarbazone of its acetol ester in the manner already described; after crystallisation from methanol, the semicarbazone was obtained as colourless crystals, m. p. 70—71° (4·2 g.) (Found : C, 70·3; H, 11·4; N, 8·8. Calc. for $C_{29}H_{57}O_3N_3$: C, 70·3; H, 11·5; N, 8·5%). The semicarbazone (4 g.) was heated with glacial acetic acid (17 c.c.) and concentrated hydrochloric acid (3 c.c.) on the steam-bath; the solution darkened and an oil separated on the surface. After one hour

the mixture was cooled, diluted with water (40 c.c.), and extracted with light petroleum (b. p. 40—60°). Distillation gave a colourless oil, b.p. 203—205°/0·3 mm., which solidified on cooling and separated from hot methanol as colourless scales (2·6 g.), m. p. 40—42°.

2 : 11 : 14-*Trimethyldocosoic Acid* (I).—11 : 14-Dimethylheneicosa-1 : 11-diene (10 g.) was added to an ice-cold, saturated solution of anhydrous hydrogen iodide in dry benzene (80 c.c.); after being kept over-night, the solution was evaporated under reduced pressure in a bath at 35—40°. The residue was then added to a solution of ethyl malonate (25 g.) and sodium (2·5 g.) in alcohol (40 c.c.), and the mixture heated under reflux for 4 hours. *Ethyl* 1-*carbethoxy*-2 : 11 : 14-*trimethyldocos*-11-*enoate* (10 g.) was thus obtained as a pale yellow oil, b. p. 212—216°/0·15 mm., $n_D^{19°}$ 1·4612 (Found: C, 74·6; H, 11·6. $C_{30}H_{56}O_4$ requires C, 75·0; H, 11·7%). Hydrogenation of this ester (9·5 g.) with palladised strontium carbonate in alcohol at the atmospheric pressure, followed by alkaline hydrolysis and decarboxylation, gave 2 : 11 : 14-*trimethyldocosoic acid*, a colourless oil, b. p. 201—203°/0·2 mm.; on redistillation it had b. p. 189—191°/0·02 mm., $n_D^{15°}$ 1·4616 (Found: C, 78·4; H, 13·0. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The 2 : 4-*dinitrophenylsemicarbazone* of its acetol ester was a crystalline powder (from alcohol) which became sticky when filtered, and melted at 128—132° after sintering at 110° (Found: N, 10·4. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

2 : 11 : 14-*Trimethyldocos*-11-*enoic Acid*.—Ethyl 1-carbethoxy-2 : 11 : 14-trimethyldocos-11-enoate (see preceding section) was hydrolysed, and the free acid decarboxylated in the usual manner. The product, which was an oil, could not be distilled without decomposition. It was esterified with ethanol and acetyl chloride, and the ester distilled; b. p. 188—192°/0·15 mm. On hydrolysis the unsaturated acid was obtained as a colourless liquid, b. p. 201—204°/0·1 mm., $n_D^{16°}$ 1·4662, which failed to give satisfactory figures on analysis (Found: C, 78·0, 77·8; H, 12·3, 12·4. $C_{25}H_{48}O_2$ requires C, 78·95; H, 12·6%).

3 : 12 : 15-*Trimethyltricosoic Acid* (II).—2 : 11 : 14-Trimethyldocosoic acid (3 g.) was converted into its chloride by means of phosphorus trichloride (0·5 c.c.) and a drop of pyridine in benzene solution; the solution was decanted and evaporated under reduced pressure, and a solution of the residue in ether mixed with an ethereal solution of diazomethane (from 10 g. of nitrosomethylurea). Next day the excess of the reagent and the ether were evaporated (bath at 50°), finally under diminished pressure. The residue was dissolved in dry methanol (30 c.c.) and decomposed on the steam-bath by the addition of silver oxide (from 4 c.c. of a 10% solution of silver nitrate) during one hour. The filtered solution was heated under reflux with 10% potassium hydroxide (20 c.c.) for 30 minutes. The *acid*, isolated by means of ether, was a colourless oil, b. p. 198—200°/0·3 mm., $n_D^{18°}$ 1·4599 (Found: C, 78·9; H, 13·4. $C_{26}H_{52}O_2$ requires C, 78·8; H, 13·1%) (yield, 1·6 g.). The 2 : 4-*dinitrophenylsemicarbazone* of the acetol ester of this acid sintered at 110° and melted at 129—132° (Found: N, 9·9. $C_{36}H_{61}O_7N_5$ requires N, 10·3%).

1 : 11 : 14-*Trimethyldocosoic Acid* (IV).—ω-Hydroxydecoic acid was obtained by the procedure of Grün and Wirth (*Ber.*, 1922, **55**, 2206) with the following modifications in detail. The temperature during the addition of potassium permanganate was kept at about 50°; when the oxidation had been completed, the mixture was diluted with twice its volume of water, and the manganese sludge dissolved by passing in sulphur dioxide. In this way the yield was increased to 90%, based upon the undec-10-enol employed as starting material. The hydroxy-acid was then converted into ω-bromodecoic acid by heating it under reflux with concentrated hydrobromic and sulphuric acid (Chuit and Hausser, *Helv. Chim. Acta*, 1929, **12**, 474, used a 50% solution of hydrogen bromide in glacial acetic acid).

The action of ω-bromodecoyl chloride on methylzinc iodide (Blaise method) afforded 11-*bromundecan*-2-*one* in excellent yield. It was a colourless liquid, b. p. 148—152°/8 mm., $n_D^{18°}$ 1·4692 (Found: C, 53·5; H, 8·0. $C_{11}H_{21}OBr$ requires C, 53·0; H, 8·4%). Its 2 : 4-*dinitrophenylhydrazone* sintered at 82° and melted at 89—91° (Found: N, 13·5. $C_{17}H_{25}O_4N_4Br$ requires N, 13·05%).

The foregoing ketone (10 g.) was mixed with a Grignard solution from 3-methyldecyl bromide (12 g.) and magnesium (1·2 g.). Dehydration of the product by heating it with a crystal of iodine, followed by distillation, gave, after separation of some lower boiling fractions, 10 : 13-*dimethyleicos*-10-*enyl bromide* as a colourless liquid, b. p. 170—176°/0·2 mm.; on redistillation it had b. p. 172—174°/0·2 mm., $n_D^{12°}$ 1·4758 (Found: C, 67·8; H, 11·2. $C_{22}H_{43}Br$ requires C, 68·2; H, 11·1%) (yield, 8·5 g.).

This unsaturated bromide (8 g.) was boiled under reflux for 6 hours with a solution of ethyl methylmalonate (10·5 g.) and sodium (1·4 g.) in absolute alcohol (30 c.c.). After the product had been worked up in the usual way, *ethyl* 1-*carbethoxy*-1 : 11 : 14-*trimethyldocos*-11-*enoate* (8 g.) was obtained as an almost colourless oil, b. p. 206—210°/0·05 mm., and this, on hydrogenation (palladised strontium carbonate as catalyst), hydrolysis, and decarboxylation, afforded 1 : 11 : 14-*trimethyldocosoic acid* (5·7 g.), b. p. 201—203°/0·08 mm., $n_D^{17°}$ 1·4572 (Found: C, 78·7; H, 13·25. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). The 2 : 4-*dinitrophenylsemicarbazone* of its acetol ester, crystallised from alcohol containing a little light petroleum (b. p. 60—80°), had m. p. 136—138° (sintered at 120°) (Found: N, 11·0. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

2 : 12 : 15-*Trimethyldocosoic Acid* (III).—Tridec-12-en-2-one was prepared by the Blaise method, which afforded a somewhat shorter route than that used in a previous preparation (Polgar and Robinson, *loc. cit.*). Zinc–copper couple (50 g.), toluene (25 c.c.), ethyl acetate (12 c.c.), and methyl iodide (5 c.c.), with a trace of iodine, were heated on the steam-bath until a reaction began; undecenyl iodide (50 g.) was then added in 4 portions during one hour. Heating on the steam-bath was continued for 3 hours, and the mixture then gently boiled under reflux in an oil-bath at 120° for a further 3 hours. After cooling with ice-water, the solution was decanted, the residual zinc–copper couple rinsed with toluene (25 c.c.), and this toluene added to the decanted solution. Freshly distilled acetyl chloride (14 c.c.) was then slowly added in small portions with cooling (ice-water) and shaking, and the product worked up in the usual way. Yield, 26·8 g.

The above ketone (9·8 g.) was added to a Grignard solution from 3-methylnonyl bromide (13 g.). Distillation of the product gave 11 g. of a colourless liquid, b. p. 152—155°/0·25 mm., whose analysis showed it to consist of a mixture of a diene and the corresponding unsaturated carbinol. This mixture was submitted to the addition of hydrogen iodide and the malonic ester synthesis in the manner already described, and gave a liquid which could not be distilled without decomposition. It was, therefore,

hydrolysed and decarboxylated, and the product re-esterified by boiling it with a mixture of ethanol (20 c.c.) and acetyl chloride (2 c.c.). *Ethyl* 2 : 12 : 15-*trimethyldocos*-12-*enoate* (6 g.), b. p. 185—187°/0·25 mm., $n_D^{20°}$ 1·4585 (Found : C, 79·6; H, 12·65. $C_{27}H_{52}O_2$ requires C, 79·4; H, 12·75%), was thus obtained; this, when reduced catalytically (palladised strontium carbonate) and then hydrolysed, yielded 4·2 g. of 2 : 12 : 15-*trimethyldocosoic acid*, b. p. 202—204°/0·3 mm., $n_D^{18°}$ 1·4562 (Found : C, 78·8; H, 12·9. $C_{25}H_{50}O_2$ requires C, 78·5; H, 13·1%). Its acetol ester gave a 2 : 4-*dinitrophenylsemicarbazone*, m. p. 128—130° (sintered at 115 (Found : N, 10·6. $C_{35}H_{59}O_7N_5$ requires N, 10·6%).

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