The Branched-Chain Fatty Acids of Mutton Fat

3. THE ISOLATION OF 16-METHYLHEPTADECANOIC ACID (*iso*STEARIC ACID)*

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The presence of trace quantities of odd-numbered *iso* and *anteiso* branched-chain fatty acids in hydrogenated mutton fat has been established by the isolation of 13-methyltetradecanoic acid, (+)-12-methyltetradecanoic acid, (+)-14-methyl-hexadecanoic acid and 10-methyldodecanoic acid (Hansen, Shorland & Cooke, 1953, 1952, 1954*a*). In the present paper is reported the isolation from hydrogenated mutton fat of trace quantities of an even-numbered *iso* acid, 16-methylheptadecanoic acid (*iso*stearic acid), which has not formerly been found in any natural fat, although it has been isolated from wool grease (Weitkamp, 1945; Sheng-Lieh Liu, 1952) and has been synthesized (Fordyce & Johnson, 1933; Weitkamp, 1945; Stenhagen & Tägtström-Eketorp, 1945; Hougen, Ilse, Sutton & de Villiers, 1953; Milburn & Truter, 1954).

EXPERIMENTAL

As described earlier (Hansen *et al.* 1952, 1953, 1954*a*) the fat used in this investigation [sample G/48, saponification equivalent (sap.equiv.) 286·9, iodine value 46·6, unsaponifiable matter 0·56%, and free fatty acids 0·4%] was obtained by mincing and steam-rendering the external fatty tissues from the carcasses of old overweight ewes. The glycerides were converted into methyl esters (7·69 kg.) and hydrogenated at 180°. The hydrogenated methyl esters (iodine value 3·0) were then repeatedly crystallized at −30° from 10 vol. of acetone. Of the resulting acetone-insoluble methyl esters ('solids') 6·45 kg. was fractionated *in vacuo*, yielding 16 fractions and a large residue. The eleventh fraction of this series (denoted OS11, 530 g., sap.equiv. 297·2, iodine value 1·9) is the one pertaining to this paper. Of this fraction, 416 g. was crystallized at −40°, twice from 10 vol. and once from 12 vol. of acetone, yielding a soluble fraction (denoted O22, 18·91 g.) which was refractionated in column E (Shorland, 1952). Thirteen fractions and a residue resulted, the twelfth and thirteenth of which O22L12 (1·54 g., sap.equiv. 295·2, m.p. 17·5–18·5°) and O22L13 (2·95 g., sap.equiv. 299·0, m.p. 18·5–19·7°) were freed from unsaponifiable matter, combined as acids and denoted O23 (3·65 g., m.p. 57·5–58·5°).

By means of repeated low-temperature crystallization of O23 acids from various solvents (Table 1) two fractions O23S14S and O23S14LS3S, which possessed melting points approximating to that of *iso*stearic acid, were separated. Accordingly they were bulked and denoted O25. Fractions O23S5L–O23S11L (Table 1) were combined, denoted O24,

and as methyl esters were fractionated in a micro column, yielding four fractions and a residue. Two of these fractions (O24L2, 0·57 g., m.p. 21·5–22·5°, sap.equiv. 293·5; O24L3, 0·58 g., m.p. 21·0–22·5°, sap.equiv. 295·9) were combined and denoted O26. When converted into acids (m.p. 61·3–62·8°), O26 was subjected to a series of low-temperature crystallizations (Table 2) to yield fraction O26S11S.

The following are the chemical and physical properties of fractions O25 (0·25 g.) and O26S11S (0·22 g.). Fraction O25 had m.p. 68·0–68·4° [reported m.p.'s: 69·5° (Weitkamp, 1945); 67·6–68·2° (Fordyce & Johnson, 1933); 67·5–68·0° (Milburn & Truter, 1954); 67·8–68·5° (Stenhagen & Tägtström-Eketorp, 1945); 69·5–69·7° (Hougen *et al.* 1953)]; when mixed with an equal quantity of 16-methyl-heptadecanoic acid supplied by Weitkamp (1945) it gave a mixed m.p. of 69·0–69·4°; when mixed with an equal quantity of *n*-stearic acid (m.p. 69·4–69·8°) it gave a mixed m.p. of 58·1–58·7°; iodine value 1·7; X-ray long spacing 34·3 ± 0·5 Å [reported values: 33·8 Å (Velick, 1947); 33·8 Å (Arosenius, Ställberg, Stenhagen & Tägtström-Eketorp, 1949); 33·75 Å (Hougen *et al.* 1953)]; n_D^{70} 1·4331. (Found: C, 75·8; H, 12·5; *C*-Me, 5·8%; sap.equiv., 283·0. Calc. for $C_{18}H_{36}O_2$: C, 76·0; H, 12·8; *C*-Me, 5·3%; sap.equiv., 284·5.) Methyl ester: m.p. 25·8–26·0°; n_D^{40} 1·4372.

Fraction O26S11S had m.p. 68·2–68·7°; when mixed with an equal quantity of 16-methylheptadecanoic acid, supplied by Weitkamp (1945), it gave a mixed m.p. of 68·8–69·4°; when mixed with an equal quantity of *n*-stearic acid it gave a mixed m.p. of 58·5–59·1°; iodine value 2·2; X-ray long spacing 34·6 ± 0·5 Å; n_D^{70} 1·4338. (Found: C, 76·5; H, 12·5; *C*-Me, 5·9%; sap.equiv., 285·0. Calc. values given above.)

Melting points were determined in closed capillaries and are uncorrected. Combustion analyses and *C*-methyl determinations were made by Drs G. Weiler and F. B. Strauss, Oxford, England. X-ray measurements were made with a Philips Geiger X-ray spectrometer, in which manganese-filtered iron Kα radiation was used. In the preparation of samples for X-ray analysis samples were melted on a glass slide and cooled.

DISCUSSION

The presence of 16-methylheptadecanoic acid in mutton fat is established by the chemical and physical properties of fractions O25 and O26S11S. Probably the most conclusive evidence confirming the identity of these fractions is the fact that their melting points were not depressed when the fractions were mixed in equal proportions with pure 16-methylheptadecanoic acid supplied by Weitkamp (1945). Weitkamp's (1945) sample was of course identified by his ingenious method of structure elucidation based on the number of

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* Part 2: Hansen, Shorland & Cooke (1953).

Table 1. *Low-temperature crystallization of fatty acid fraction O23 (3·65 g.)*

The insoluble fraction (S) from one crystallization was used for the next crystallization, except in fraction O23S14L, where the soluble fraction (L) was used.

Fraction crystallized	Conditions of crystallization	No. of crystallizations	Soluble			Insoluble		
			Fraction	Wt (g.)	M.p.	Fraction	Wt. (g.)	M.p.
O23	Light petroleum (b.p. 40–60°), 40 vol., –40°	2	O23L + O23SL	0·60	13·2–25·8°	O23SS	3·02	59·5–60·8°
O23SS	Acetone, 40 vol., –40°	1	O23S2L	0·23	40·0–43·0°	O23S2S	2·79	61·1–62·0°
O23S2S	Hexane, 40 vol., –40°	1	O23S3L	0·03	38·3–40·3°	O23S3S	2·75	61·8–62·2°
O23S3S	Hexane, 100 vol., –40°	1	O23S4L	0·04	41·0–42·6°	O23S4S	2·71	61·5–62·2°
O23S4S	Ether, 40 vol., –40°	2	O23S5L + O23S6L	0·38	54·1–56·8°	O23S6S	2·30	62·3–63·1°
O23S6S	Methanol, 30 vol., room temp.	2	O23S7L + O23S8L	1·70	59·3–62·7°	O23S8S	0·59	66·8–67·6°
O23S8S	Ether, 40 vol., –40°	1	O23S9L	0·03	59·8–60·8°	O23S9S	0·56	66·4–67·0°
O23S9S	Ether, 100 vol., –40°	2	O23S10L + O23S11L	0·09	62·5–65·2°	O23S11S	0·46	67·6–68·1°
O23S11S	Acetone, 100 vol., –40°	1	O23S12L	Trace	—	O23S12S	0·44	67·6–68·1°
O23S12S	Methanol, 40 vol., –40°	1	O23S13L	Trace	—	O23S13S*	0·44	67·6–68·1°
O23S13S	Ether, 20 vol., +7°	1	O23S14L	0·28	66·3–67·0°	O23S14S*	0·14	68·4–69·0°
O23S14L	Ether, 20 vol., –15°	2	O23S14LL + O23S14LSL	0·13	64·3–65·7°	O23S14LSS	0·13	67·9–68·3°
O23S14LSS	Light petroleum (b.p. 40–60°), 20 vol., +9°	1	O23S14LS2L	Trace	—	O23S14LS2S	0·12	67·9–68·3°
O23S14LS2S	Ether, 20 vol., –18°	1	O23S14LS3L	Trace	—	O23S14LS3S*	0·11	67·9–68·3°

(The soluble fractions O23S7L+O23S8L, O23S9L and O23S10L+O23S11L were combined and denoted O24.)

* Combined and denoted O25.

Table 2. *Low-temperature crystallization of fatty acid fraction O26 (1·06 g.)*

Fraction crystallized	Conditions of crystallization	No. of crystallizations	Soluble			Insoluble		
			Fraction	Wt. (g.)	M.p.	Fraction	Wt. (g.)	M.p.
O26	Ether, 40 vol., –40°	1	O26L	0·11	54·4–55·7°	O26S	0·95	61·5–63·0°
O26S	Ether, 100 vol., –40°	1	O26SL	0·11	57·0–57·7°	O26SS	0·84	63·1–63·9°
O26SS	Acetone, 40 vol., –40°	1	O26S2L	0·01	—	O26S2S	0·83	—
O26S2S	Methanol, 30 vol., –14°	1	O26S3L	0·03	52·8–53·4°	O26S3S	0·81	63·0–64·2°
O26S3S	Methanol, 80 vol., –14°	1	O26S4L	0·05	55·4–56·7°	O26S4S	0·75	63·5–64·5°
O26S4S	Methanol, 100 vol., –14°	3	O26S5L + O26S7L	0·14	55·8–57·4°	O26S7S	0·60	65·0–65·5°
O26S7S	Ether, 20 vol., –14°	4	O26S8L + O26S11L	0·38	58·8–65·0°	O26S11S	0·22	67·5–69·4°

transitions appearing in the solidification-point curves of binary mixtures of branched-chain acids with normal fatty acids. As is characteristic of *iso* acids, the melting points of these fractions are about 1° below the melting points of their corresponding normal acids (Weitkamp, 1945; Cason, 1948). Further melting-point evidence consistent with the fractions being *iso*stearic acid is that when they are mixed in equal quantities with *n*-stearic acid their mixed melting points were depressed by approximately 10° (cf. Weitkamp, 1945).

Before the present investigation only two high-molecular-weight even-numbered *iso* acids had been isolated from natural fats, namely the C_{14} *iso* acid 12-methyltridecanoic acid from butterfat (Hansen, Shorland & Cooke, 1954*b*) and the C_{16} *iso* acid 14-methylpentadecanoic from ox-perinephric fat (Hansen, Shorland & Cooke, 1955). In wool grease, however, Weitkamp (1945) separated the complete series of even-numbered *iso* acids from C_{10} to C_{28} inclusive. Of the low-molecular-weight even-numbered *iso* acids, *iso*butyric acid has recently been identified in the steam-volatile acids of mutton fat (McInnes, Hansen & Jessop, 1956) by means of the gas–liquid chromatogram of James & Martin (1952). Similarly, *iso*butyric acid has been reported to be present in wool grease (Sheng-Lieh Liu, 1952).

It is estimated that in the sample of mutton fat investigated, 16-methylheptadecanoic acid represents not less than 0·05 % of the total fatty acids.

SUMMARY

Hydrogenated mutton fat has been found to contain trace quantities of 16-methylheptadecanoic (*iso*-stearic) acid.

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Studies on Sulphatases

13. THE HYDROLYSIS OF SUBSTITUTED PHENYL SULPHATES BY THE ARYLSULPHATASE OF *ALCALIGENES METALCALIGENES**

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In a previous paper (Dodgson, Spencer & Williams, 1955) the effect of variaton of pH on the affinity of enzyme and substrate (cf. Dixon, 1953) was examined for the arylsulphatase of *Alcaligenes metalcaligenes* and the substrates *p*-nitrophenyl, *p*-acetylphenyl and 2-hydroxy-5-nitrophenyl sulphates. The results indicated that the formation of the enzyme–substrate complex involved an electrostatic attraction between the substrate and positively and negatively charged groups in the enzyme.

Further information on the mechanism of the enzyme action has now been obtained by correlating the effect of structural change in the substrate molecule with the kinetics of the hydrolytic reaction.

* Part 12: Dodgson, Spencer & Wynn (1956).