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The Branched-Chain Fatty Acids of Ox Fat

2. THE ISOLATION OF 15-METHYLHEXADECANOIC ACID*

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Earlier investigations on the minor constituents of ox suet fat indicated the presence of trace quantities of the C₁₇ *anteiso*-acid 14-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1952). Examination of a further sample of the fat surrounding the kidneys of oxen has shown that the C₁₇ *iso*-acid, 15-methylhexadecanoic acid, also is present.

EXPERIMENTAL

Approximately 14.5 kg. ox perinephric fat (sample J/21, comprising the fatty tissue surrounding the kidneys and extending about 6 in. along the body) were steam-rendered

to yield 11.6 kg. glycerides (acidity 0.83% expressed as oleic acid). After the free fatty acids had been neutralized with aqueous NaOH and the soaps removed by repeated washing with water, the glycerides (sap. equiv. 285.0, iodine value 33.3) were hydrogenated at 180° and at atmospheric pressure with the aid of a nickel catalyst supported on kieselguhr. Part of the hydrogenated fat (iodine value 0.3, m.p. 59.0–61.5°) was saponified, converted into fatty acids and steam-distilled. Of the fatty acids non-volatile in steam, 7150 g. were crystallized four times from 10 vol. acetone at approximately –20°, resulting in 734.5 g. 'liquids' and 6285 g. 'solids'. The 'liquid' acids were converted into methyl esters and 727.2 g. were fractionated in a Stedman column, yielding 22 fractions and a residue as shown in Table 1.

* Part 1: Hansen, Shorland & Cooke (1952).

Table 1. Fractional distillation of the 'liquid' methyl esters (727.2 g.) of ox perinephric fat

Fraction	Wt. of methyl esters (g.)	Sap. equiv. methyl esters	Iodine value of methyl esters	M.p. methyl esters (°)	Sap. equiv. acids†	M.p. acids† (°)
J/21, L1	5.01	237.2	4.5	–25.0 to –23.1	169.5	18.0–21.2
J/21, L2	6.27	252.3	3.4	–9.5 to –8.5	185.7	23.0–26.5
J/21, L3	9.10	245.1	2.0	–1.8 to –0.3	199.7	36.8–38.2
J/21, L4	14.70	252.5	0.9	11.5–12.7	222.9	44.8–47.0
J/21, L5	31.33	245.4	0.0	10.0–15.0	228.9	52.0–52.4
J/21, L6	30.01	254.6	0.0	17.1–17.6	229.2	52.8–53.2
J/21, L7	38.00	243.2	0.2	15.2–19.5	229.2	53.8–54.1
J/21, L8	37.97	245.0	0.1	16.3–17.0	230.5	52.2–52.8
J/21, L9	38.61	248.5	0.0	13.7–14.5	233.5	47.7–49.3
J/21, L10	40.48	258.3	0.6	–1.0–0.2	239.7	37.0–39.0
J/21, L11	35.36	266.6	0.0	5.0–6.5	244.0	40.0–40.7
J/21, L12	38.06	272.1	0.9	22.8–23.8	254.2	55.0–56.3
J/21, L13	41.59	272.3	0.0	23.9–25.9	256.8	57.2–59.3
J/21, L14	41.72	273.1	0.0	25.8–26.5	257.7	58.0–59.8
J/21, L15	40.78	275.2	0.0	17.2–19.2	260.8	57.3–58.2
J/21, L16	38.83	282.2	1.4	10.4–13.5	265.7	47.8–49.0
J/21, L17	40.42	288.6	1.8	4.8–8.2	271.1	37.8–39.5
J/21, L18	34.94	298.6	4.0	22.0–22.9	280.7	42.0–48.0
J/21, L19	36.95	302.6	4.8	28.9–30.6	288.0	60.0–61.1
J/21, L20	42.69	299.2	15.5	22.0–24.0	283.4	61.8–63.9
J/21, L21	32.61	299.4	32.3	26.8–29.0	275.8	46.3–48.5
J/21, L22	6.05	375.4	40.3	16.8–17.2	282.1	49.0–52.5
J/21, LR	31.90	723.5	58.6	—	283.3	—

† Freed from unsaponifiable matter.

Two of these fractions, J/21, L16 and J/21, L17, are relevant to this paper. When freed of unsaponifiable matter, combined as acids (denoted U1) and converted into methyl esters, they were refractionated in column C (Shorland, 1952). Seventeen fractions (U1L1–U1L17) and a residue resulted, of which the following were further investigated for the purpose of this paper: U1L7, 4.56 g., m.p. 4.2–5.7°, sap. equiv. 281.5; U1L8, 6.82 g., m.p. 2.0–4.0°, sap. equiv. 283.1; U1L9, 4.35 g., m.p. 1.2–3.0°, sap. equiv. 283.2; U1L10, 5.47 g., m.p. 1.5–3.5°, sap. equiv. 283.3; U1L11, 6.73 g., m.p. 1.2–3.0°, sap. equiv. 283.4; U1L12, 3.52 g., m.p. 0.0–1.7°, sap. equiv. 283.6; U1L13, 3.11 g., m.p. 1.0–3.0°, sap. equiv. 283.3.

Fractions U1L7–U1L13 inclusive were combined (denoted U6), and refractionated in column E (Shorland, 1952) as shown in Table 2.

Table 2. Fractional distillation of U6 methyl esters (34.0 g.)

Fraction	Wt. (g.)	Sap. equiv.	M.p. (°)	
U6L1	1.07	275.5	10.0–10.4	
U6L2	1.02	275.2	9.5–10.0	
U6L3	1.18	280.7	6.0–6.8	
U6L4	0.85	280.1	5.5–6.5	
U6L5	3.40	282.1	3.0–4.0	Denoted U12
U6L6	4.66	283.5	2.5–3.5	
U6L7	2.86	283.3	2.7–3.5	
U6L8	4.32	282.6	2.5–3.5	
U6L9	2.64	283.9	1.8–2.5	
U6L10	3.16	283.4	2.2–3.0	
U6L11	2.99	283.7	0.2–1.7	
U6L12	3.31	283.2	–0.3–+6.0	
U6LR	2.59	290.3	—	

Of the resulting fractions, U6L5–U6L11 were combined (denoted U12) and the acids (22.59 g.) were crystallized successively from 40 vol. of the following solvents at –40°: light petroleum b.p. 40–60° (twice), acetone (twice), ether (six times). After each crystallization the soluble fraction was removed and the insoluble material recrystallized. The final crystallization from 40 vol. of ether at –40° yielded a soluble fraction U12S9L, wt. 0.21 g., m.p. 59.0–59.6° and an insoluble fraction U12S9S, wt. 3.21 g., m.p. 60.2–60.4°.

The chemical and physical properties of U12S9S were as follows: acid, sap. equiv. 270.4; iodine val. 0.0; combustion analysis, C, 75.4; H, 12.5%; m.p. 60.2–60.4°; when mixed in equal proportions with a sample of synthetic 15-methylhexadecanoic acid supplied by Stenhagen (Arosenius, Stållberg, Stenhagen, Tägtström-Eketorp, 1949, m.p. recorded as 59.3–59.8° but uncorrected m.p. observed here with a standardized thermometer 60.2–60.9°) gave mixed m.p. 60.4–60.7°; when mixed in equal proportions with a sample of *n*-heptadecanoic acid from mutton fat (O2S3LSS, m.p. 61.3–61.4°, Hansen, Shorland & Cooke, 1954a) gave mixed m.p. 54.8–55.4°; X-ray long-spacing 33.0±0.5Å; *C*-methyl value 7.07%, equivalent to 1.28 mol. acetic acid; n_D^{20} 1.4315 (value observed here for sample of 15-methylhexadecanoic acid supplied by Stenhagen (Arosenius *et al.* 1949) n_D^{20} 1.4317). Methyl ester, m.p. 18.3–18.9°; n_D^{20} 1.4419.

The following are the corresponding data for 15-methylhexadecanoic acid (calc. for $C_{17}H_{34}O_2$), sap. equiv. 270.4, iodine val. 0.0, combustion analysis, C, 75.4%, H, 12.5%; m.p. 60.2° (Weitkamp, 1945), 59.3–59.8° (Arosenius *et al.*

1949), 59.5–60.0° (Weitzel & Wojahn, 1951); X-ray long-spacing 33.35Å (Arosenius *et al.* 1949).

X-ray measurements were made with a Philips Geiger X-ray spectrometer using manganese-filtered iron K α radiation.

Samples were melted on a glass slide and quickly cooled. All melting points were determined in closed capillaries and are uncorrected. Combustion analysis and *C*-methyl determinations were made by Weiler & Strauss, Oxford.

DISCUSSION

The chemical and physical properties of fraction U12S9S agree closely with the recorded data for the C_{17} *iso*-acid 15-methylhexadecanoic acid, and the absence of mixed melting point depression is further evidence that the two substances are identical. When, however, fraction U12S9S was mixed in equal proportion with a sample of *n*-heptadecanoic acid, the melting point was depressed by 5° as would be expected with a mixture of an odd-numbered *iso*-acid and its corresponding normal acid (cf. Hansen, Shorland & Cooke, 1954b).

Although it was earlier suspected that the C_{17} *iso*-acid 15-methylhexadecanoic acid was present in butterfat (Hansen & Shorland, 1951), the investigation reported in this paper represents the first irrefutable isolation of this acid from a natural fat. As hydrogenation was employed in the procedure adopted, this work does not preclude the possibility that the acid was formed, in whole or in part, by the addition of hydrogen to an unsaturated C_{17} acid. However, as (+)-12-methyltetradecanoic acid and 13-methyltetradecanoic acid (Shorland, Gerson & Hansen, 1955) and a multi-branched C_{20} acid (Hansen & Shorland, 1953) have been extracted from butterfat by methods which did not involve hydrogenation, it is thought probable that 15-methylhexadecanoic acid also occurs as such in the natural ox fat.

Although the melting points of *iso*-acids are less than 1° below those of the corresponding normal acids (cf. Weitkamp, 1945; Cason, 1948; Hansen, Shorland & Cooke, 1954c, d), a relatively big difference exists between the melting points of the methyl esters of *iso*- and normal-acids. The methyl ester of the C_{17} *iso*-acid has m.p. 18.3–18.9°, while that of the normal C_{17} acid has m.p. 28.6° (Francis & Piper, 1939), a difference of 9.7°. Similar differences exist between the methyl ester of the C_{15} *iso*-acid (m.p. 9.4°, Hansen *et al.* 1954c) and that of C_{15} normal-acid (m.p. 18.5°, Ralston, 1948) and between the methyl ester of the C_{14} *iso*-acid (m.p. 5.0–6.0°, Hansen *et al.* 1954d) and that of the C_{14} normal-acid (m.p. 18.8°, Francis & Piper, 1939).

In this investigation on ox perinephric fat 15-methylhexadecanoic acid is estimated to be present to the extent of about 0.06% of the total fatty acids.

SUMMARY

The C₁₇ branched-chain fatty acid 15-methylhexadecanoic acid has been isolated in trace quantities (approximately 0.06 % of total fatty acids) from hydrogenated ox perinephric fat.

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The Composition of Collagen and Acid-Soluble Collagen of Bovine Skin

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The partial dissolution of collagen in dilute solutions of weak acids, such as formic and acetic, has been known for some years, and has been extensively studied by Nageotte (1927a, b, 1928, 1930, 1933), Nageotte & Guyon (1933), Leplat (1933), and Fauré-Fremiet (1933). Interest in this soluble protein has been revived in recent years by the work of Orekhovich and his colleagues in the U.S.S.R., who report the extraction of a soluble collagenous-type protein from the skin of various animals using dilute citrate buffers, and which they suggest is a soluble precursor of collagen (Plotnikova, 1947; Tustanovskii, 1947; Orekhovich, Tustanovskii, Orekhovich & Plotnikova, 1948; Chernikov, 1949; Orekhovich, 1950, 1952). The information regarding these various soluble collagens has recently been reviewed by Harkness, Marko, Muir & Neuberger (1954). These authors also report the presence of a small amount of a protein of collagen type which is extracted from skin by dilute phosphate, pH 9.0 (alkali-soluble collagen). On the basis of experiments on the feeding of labelled glycine to rabbits they conclude that this is a true precursor of collagen, whereas the metabolic role of the acid-soluble collagen de-

scribed by Orekhovich is less certain, and it is not necessarily an intermediate in the formation of all the insoluble collagen of the skin.

Harkness *et al.* (1954) determined the hydroxyproline and tyrosine content of the alkali-soluble and acid-soluble collagen, and also of the gelatin obtained from the remaining insoluble collagen. Both soluble collagens contained less tyrosine and more hydroxyproline than the insoluble collagen, and the acid-soluble had a higher hydroxyproline and tyrosine content than the alkali-soluble collagen. Similar differences have been observed by Bowes, Elliott & Moss (1953) between the hydroxyproline and tyrosine content of the acid-soluble collagen of calf skin and the adult collagen of ox hide; and between the acetic acid-soluble and insoluble fractions of tendon collagen.

A complete analysis of the acid-soluble collagen of calf skin has now been carried out in order that a more detailed comparison may be made with that of adult collagen. A preliminary account of some of these results has already been given (Bowes *et al.* 1953). The chromatographic method of Moore & Stein (1951) was used for the determination of the amino acids, and it was thought