

The Occurrence of *n*-Heptadecanoic Acid (Margaric Acid) in Hydrogenated Mutton Fat

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Although the isolation of *n*-heptadecanoic acid (margaric acid) from natural fats has often been reported (cf. Ralston, 1948), there appears to be no case on record where the identification of the acid from these sources has been irrefutably established. Ralston (1948) states: 'All the naturally occurring heptadecanoic acids which have been described and subsequently investigated have been shown to consist of mixtures of palmitic and stearic acids. Because of the frequent mutual occurrence of palmitic and stearic acids in fats, and since they are often the chief saturated acids, it is not surprising that a mixture of them has frequently been identified as a pure compound.' Not only has the presence of margaric acid in fats been unsubstantiated, but so also has the presence of the other straight-chain odd-numbered fatty acids with the exception of the recently reported steam-volatile acids of ox perinephric fat, which were shown to contain trace amounts of all the straight-chain acids (both odd and even) from C₃ to C₁₀ (Hansen & McInnes, 1954). This latter work has prompted further investigations into the minor components of fats, and as a preliminary to a comprehensive search for odd-numbered straight-chain acids the 'solids' from a sample of hydrogenated mutton fat (external carcass fat) have been investigated and have yielded margaric acid. Brief reference to this finding has already been made (Hansen, Shorland & Cooke, 1954). From the same original material

used in this investigation and by similar techniques which included hydrogenation, fractional distillation and low-temperature crystallization, were isolated small quantities of three branched-chain fatty acids, namely, 12-methyltetradecanoic acid, 13-methyltetradecanoic acid (Hansen, Shorland & Cooke, 1953) and 14-methylhexadecanoic acid (Hansen, Shorland & Cooke, 1952).

EXPERIMENTAL

The fat used in this investigation (Lab. no. G/48, sap. equiv. 286.9, iodine value 46.6, unsaponifiable matter 0.56% and free fatty acids 0.4%) was obtained, as described earlier (Hansen *et al.* 1952, 1953), by mincing and steam-rendering the external fatty tissues from the carcasses of old overweight ewes. The washed glycerides were converted into methyl esters (7.69 kg.) and hydrogenated at 180°, using as catalyst N; supported on kieselguhr. 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. were fractionated *in vacuo* in a 490 × 3.8 cm. stainless-steel column packed with 3-4 mm. diameter single-turn glass helices. Sixteen fractions and a large residue resulted. For the purpose of this paper the tenth fraction (denoted 0S10, 232.8 g.; sap. equiv. 281.5, iodine value 0.8) was selected for further investigation. A portion of fraction 0S10 (denoted 01, 22.0 g.) was then refractionated at approximately 0.1 mm. pressure in a 50 × 1.8 cm. column fitted with a closely coiled spring as packing (column E, Shorland, 1952). Fractionation data are shown in Table 1.

Table 1. *Fractional distillation of methyl esters (01)*

Wt. 22.0 g.; sap. equiv. 281.5; iodine value 0.7. Pressure approx. 0.1 mm.				
Fraction	Wt. (g.)	Sap. equiv.	M.p. of ester (°)	M.p. of acid (°)
01S1	0.75	279.3	27.5-28.9	—
01S2	1.73	271.4	29.4-29.8	—
01S3	0.99	268.2	29.5-30.0	—
01S4	1.75	273.4	23.9-27.0	—
01S5	0.91	285.0	20.5-21.7	—
01S6	1.48	283.8	21.0-23.9	53.0-54.0
01S7	0.70	283.5	24.5-24.7	
01S8	3.12	284.1	25.5-26.6	57.0-57.8
01S9	1.20	284.0	27.0-29.6	60.3-60.7
01S10	1.45	282.5	29.8-30.5	60.8-61.0
01S11	0.75	282.8	28.2-30.6	60.6-60.7
01S12	2.42	284.7	29.6-30.2	
01S13	1.73	288.7	28.1-29.5	—
01S14	0.55	288.9	31.6-33.3	—
01SR	2.59	319.5	—	—

Those fractions with saponification equivalents approximating 284 were then examined in detail. Typical of the procedure adopted was the case of methyl ester fraction 01S9 (1.20 g.; sap. equiv. 284.0, m.p. 27.0–29.6°), which was converted into fatty acids (denoted 02, 1.03 g.; m.p. 60.3–60.7°) and submitted to low-temperature crystallization from light petroleum (b.p. 50–60°), methanol, acetone and ether respectively, to yield fraction 02S3LSS. The chemical and physical properties of fraction 02S3LSS (0.85 g.) were as follows: m.p. 61.3–61.4°; sap. equiv. 270.2; X-ray long spacing 40.8 Å; combustion analysis, C, 75.5; H, 12.5%; iodine value 0.0; n_D^{25} 1.4312 (a pure sample of *n*-heptadecanoic acid m.p. 61.1–61.2° under identical conditions gave n_D^{25} 1.4312). Fraction 02S3LSS, when mixed in equal proportions with pure *n*-heptadecanoic acid (m.p. 61.1–61.2°), gave mixed m.p. 61.0–61.3°. By similar processes of low-temperature crystallization the following acid fractions were obtained: 08S9S (derived from bulked fractions 01S6 and 01S7 acids (08)) 0.65 g.; m.p. 61.0–61.3°, sap. equiv. 269.0. 03S8S (derived from 01S8) 1.77 g.; m.p. 61.1–61.3°. 04S3S (derived from 01S10) 1.05 g.; m.p. 61.0–61.3°. 05SS (derived from bulked fractions 01S11 and 01S12 acids (05)) 1.66 g.; m.p. 61.2–61.6°.

The above fractions (08S9S, 03S8S, 04S3S and 05SS) were bulked together (denoted 010) and further purified by low-temperature crystallization to yield 010S3S (4.54 g.) with the following properties: m.p. 61.3–61.6°, sap. equiv. 270.3; C, 75.8; H, 12.6%; iodine value 0.0; X-ray long spacing 40.5 Å; n_D^{25} 1.4310.

The X-ray measurements reported in this paper were made with a Philips Geiger X-ray spectrometer using nickel-filtered Cu K α radiation for fraction 02S3LSS and manganese-filtered Fe K α radiation for fraction 010S3S.

Melting points were determined in closed capillaries and are uncorrected.

Combustion analyses were made by Drs G. Weiler and F. B. Strauss, Oxford.

DISCUSSION

The two fractions reported in this paper 02S3LSS and 010S3S possess saponification equivalents (270.2 and 270.3) carbon and hydrogen contents (C, 75.5; H, 12.5%; and C, 75.8; H, 12.6%) and iodine values (0.0 and 0.0) which signify a C₁₇ saturated fatty acid. (Calculated requirements for a saturated fatty acid with empirical formula C₁₇H₃₄O₂: sap. equiv. 270.4; C, 75.5; H, 12.7%; iodine value, 0.0.) The X-ray long spacing of each of the fractions (40.8 Å and 40.5 Å) agrees within the experimental error (± 0.5 Å) with the values of 40.45 Å (Francis & Piper, 1939) and 40.05 Å (Slagle & Ott, 1933) for *n*-heptadecanoic acid. These X-ray diffraction measurements show the two fractions to be almost identical and at the same time distinct from two of the methyl-branched isomers, 15-methylhexadecanoic acid and 14-methylhexadecanoic acid, which have long spacings of 33.35 Å (Arosenius, Ställberg, Stenhagen & Tägström-Eketorp, 1949) and 29.9 Å (Nunn, 1952) respectively. The melting points of the two fractions (61.3–61.4° and 61.3–61.6°) are in close agreement with the

Table 2. Purification of fatty acids by fractional crystallization of fraction 02

Fraction	Wt. (g.)	Conditions of crystallization	Soluble		Insoluble	
			Fraction	Wt. (g.)	Fraction	Wt. (g.)
02	1.03	Light petroleum (40 vol.), -40°	02L	0.03	02S	1.00
02S	1.00	Methanol (40 vol.), -40°	02SL	0.02	02SS	0.99
02SS	0.99	Acetone (40 vol.), -40°	02S2L	0.02	02S2S	0.97
02S2S	0.97	Light petroleum (100 vol.), +18°	02S3L	0.96	02S3S	0.01
02S3L	0.96	Ether (40 vol.), -40°	02S3LL	0.05	02S3LS	0.91
02S3LS	0.91	Ether (40 vol.), -40°	02S3LSL	0.05	02S3LSS	0.85
						(Saponification equiv. 270.2)

For details see text.

recorded figures for *n*-heptadecanoic acid (61.3°, Francis & Piper, 1939; 59.0–60.0°, Levene & West, 1914). Fraction 02S3LSS when mixed in equal proportions with pure margaric acid (m.p. 61.1–61.2°) did not depress the melting point. Evidence which precludes these two fractions consisting of equimolecular mixtures of palmitic and stearic acids is furnished by the data of Schuette & Vogel (1940), Smith (1936) and Francis, Collins & Piper (1937), which indicate a maximum solidification point of about 56° for such a mixture. Margaric acid is reported by Waterman & Bertram (1927) to have a refractive index of n_D^{60} 1.4319, and this value is similar to that obtained for fractions 02S3LSS (n_D^{60} 1.4312) and 010S3S (n_D^{60} 1.4310). The chemical and physical characteristics of 02S3LSS and 010S3S as recorded above establish the two fractions isolated from hydrogenated mutton fat as being the C_{17} straight-chain fatty acid *n*-heptadecanoic acid.

It is estimated that *n*-heptadecanoic acid constitutes about 1.2% of the total fatty acids of hydrogenated mutton fat. The two fractions 02S3LSS and 010S3S (5.39 g.) were separated from a 22.0 g. portion of the original fraction 0S10 (232.8 g.), and this proportion represents a total weight of 57.1 g. of pure margaric acid in the original fraction.

As the methyl esters in the present investigation had been submitted to hydrogenation, it has not been determined whether margaric acid exists as such (in part or in whole), or as a C_{17} unsaturated acid, in the unhydrogenated fat. The possibility that the heptadecanoic acid now isolated could have originated from a C_{17} acid containing a cyclopropane ring is also not excluded. Hofmann & Lucas (1950) and Hofmann, Lucas & Sax (1952) have shown that there occurs in the non-glyceridic lipids of *Lactobacillus arabinosus* a C_{19} saturated acid with a cyclopropane ring which is opened on hydrogenation to yield *n*-nonodecanoic acid and a methyloctadecanoic acid.

Whatever may be the chemical structure of the C_{17} acids in the original external carcass fat of sheep, it is probable that corresponding acids exist in other

natural glycerides also. Furthermore, it is reasonable to presume that not only C_{17} acids are present, but also all odd-numbered members in the range characteristic of the fat. In order to elucidate this problem, work on the nature of C_{17} and other odd-numbered straight-chain fatty acids is already in progress in this laboratory.

SUMMARY

Hydrogenated mutton fat has been found to contain approximately 1.2% of *n*-heptadecanoic acid.

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