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The Branched-chain Fatty Acids of Butterfat

5. THE ISOLATION OF 12-METHYLTRIDECANOIC ACID

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Detailed analyses of a butterfat sample (G/43) recently yielded trace quantities of two isomeric C_{15} branched-chain fatty acids, (+)-12-methyltetradecanoic acid and 13-methyltetradecanoic acid (Hansen, Shorland & Cooke, 1954). In this present communication is reported the isolation from the same sample of a C_{14} iso-acid, 12-methyltridecanoic acid.

EXPERIMENTAL

As reported earlier (Hansen *et al.* 1954) the acids from hydrogenated butterfat after removal of part of the steam-volatile acids, were crystallized from 10 vol. of acetone at -33° , the 'liquids' converted into methyl esters (wt. 1876 g., sap. equiv. 223.8, iod. val. 4.7) and fractionated *in vacuo* in a 490×3.8 cm. stainless steel column packed with glass helices. Thirty-three fractions (KL1 to KL33) and a large residue resulted. For the purpose of this present investigation the following fractions were combined and denoted K27: KL24, wt. 35.24 g., sap. equiv. 232.5, iod. val. 2.0; KL25, wt. 38.38 g., sap. equiv. 238.6, iod. val. 2.5; KL26, wt. 34.78 g., sap. equiv. 240.2, iod. val. 3.5; KL27, wt. 35.99 g., sap. equiv. 241.4, iod. val. 2.9.

Fraction K27 was hydrolysed, unsaponifiable matter removed and the acids reconverted into methyl esters (sap. equiv. 235.6, iod. val. 0.9); 134 g. of these were refractionated in a 122×3.3 cm. column (column B, Shorland, 1952). Of the twenty-four fractions distilled over, those relevant to this paper are: K27L7, wt. 3.31 g., sap. equiv. 229.0, iod. val. 1.5, m.p. ester -5.2 to -4.8° , m.p. acid 38.8 – 39.7° ; K27L8, wt. 10.78 g., sap. equiv. 238.6, iod. val. 0.6, m.p. ester 3.8 – 4.5° , m.p. acid 50.5 – 50.8° ; K27L9, wt. 11.55 g., sap. equiv. 241.4, iod. val. 0.5, m.p. ester 4.0 – 4.8° , m.p. acid 43.8 – 45.0° . Fraction K27L8 (denoted K34) was refractionated in column E (Shorland, 1952) to yield eight fractions (K34L1 to K34L8) and a residue K34LR. The following fractions, K34L4, wt. 1.52 g., sap. equiv. 238.6, m.p. ester 4.2 – 5.1° ; K34L5, wt. 0.56 g., sap. equiv. 242.2, m.p. ester 4.2 – 5.1° ; K34L6, wt. 1.10 g., sap. equiv. 241.5, m.p. ester 4.2 – 5.1° ; K34L7, wt. 0.83 g., sap. equiv. 241.0, m.p. ester 4.5 – 5.3° ; were combined (K36) and converted

into fatty acids (m.p. 51.9 – 52.6° ; mixed m.p. with pure myristic acid gave m.p. 39.8 – 40.3°). Purification of K36 was then effected by repeated low-temperature crystallization from 40 vol. of the following solvents, the soluble fraction being removed after each crystallization: Light petroleum (b.p. 50 – 60°) at -40° , 5 crystallizations; methanol at -40° , 10 crystallizations; acetone at -70° , 1 crystallization; acetone at -60° , 1 crystallization; acetone at -50° , 1 crystallization. After the final crystallization the soluble fraction K36S17L (0.03 g.) had m.p. 50.0 – 51.1° while the insoluble fraction K36S17S (2.01 g.) had m.p. 53.7 – 54.0° .

The properties of fraction K36S17S were as follows: wt. 2.01 g., m.p. acid 53.7 – 54.0° , m.p. methyl ester 5.0 – 6.0° , sap. equiv. 227.8 (calc. for $C_{14}H_{28}O_2$, 228.4), iod. val. 0.0; X-ray long spacing $26.7 \text{ \AA}$. (Found: C, 73.9; H, 12.4%. Calc. for $C_{14}H_{28}O_2$: C, 73.6; H, 12.4%). C-methyl, 10.5% (equiv. to 1.6 mols. of acetic acid); n_D^{25} (acid) 1.4250; n_D^{25} (methyl ester) 1.4360; $[\alpha]_D^{25}$ 0.0° in CHCl_3 (c, 13.3). When mixed in equal proportions with pure myristic acid (m.p. 54.0 – 54.4°) it gave a mixed m.p. of 40.8 – 41.8° , and when mixed with pure synthetic 12-methyltridecanoic acid kindly supplied by Professor E. Stenhagen of Uppsala University (m.p. 53.3 – 53.6°) it gave a mixed m.p. of 53.5 – 53.8° .

Fraction K36S17S, as described above, was derived from fraction K27L8. Investigation of two adjacent fractions K27L7 and K27L9 by the same techniques yielded a number of fractions with corresponding characteristics. These were bulked together, denoted K56, and recrystallized from light petroleum (2 crystallizations), methanol (3 crystallizations), and acetone (6 crystallizations) respectively, using in each case 40 vol. of solvent at -40° . The resulting fraction K56S10S possessed the following chemical and physical properties: wt. 4.39 g., m.p. 53.5 – 54.0° , sap. equiv. 227.9, iod. val. 0.0; X-ray long spacing $26.7 \text{ \AA}$. Found C, 73.7; H, 12.2%; C-methyl, 10.3% (equiv. to 1.6 mols. acetic acid). When mixed with an equal quantity of pure myristic acid (m.p. 54.0 – 54.4°) it gave a mixed m.p. of 40.2 – 41.0° .

X-ray diffraction measurements recorded in this work were made with a Philips Geiger X-ray spectrometer using

manganese-filtered iron K α radiation. Samples were melted on a glass slide and cooled.

Combustion analyses and C-methyl determinations were made by Weiler and Strauss, Oxford.

Melting points were determined in closed capillaries and are uncorrected.

DISCUSSION

The two fractions isolated (K36S17S and K56S10S) have saponification equivalents, combustion analyses and iodine values in close agreement with the calculated requirements for a C₁₄ saturated fatty acid with empirical formula C₁₄H₂₈O₂. Both K36S17S and K56S10S possess an X-ray long spacing of 26.7 Å. This figure approximates to the values of 27.0 Å and 26.8 Å respectively recorded for 12-methyltridecanoic acid by Arosenius, Ställberg, Stenhagen & Tägtström-Eketorp (1949) and by Hougen, Ilse, Sutton & de Villiers (1953). At the same time the figure distinguishes it from *n*-tetradecanoic acid which has a long spacing of 31.6 Å (Francis & Piper, 1939). The C-methyl values (10.5 and 10.3 % respectively) when interpreted in accordance with the work of Ginger (1944) indicate the presence of two alkyl groups, one of which is a side chain. Further evidence for the identification of the two fractions as the C₁₄ *iso*-acid 12-methyltridecanoic acid (*isomyristic* acid) is provided by the melting points of 53.7–54.0° and 53.5–54° respectively (cf. Weitkamp, 1945 (53.3°); Arosenius *et al.* 1949 (53.3–53.6°); Fordyce & Johnson, 1933 (50.5–51.5°)) and by the absence of a depression in melting point when mixed in equal proportions with pure synthetic 12-methyltridecanoic acid. The melting points of these fractions are less than one degree below that of the corresponding normal acid (m.p. of *n*-tetradecanoic acid, 54.4°; Francis & Piper, 1939); they thus conform with the generalizations regarding *iso*-acids as stated by Weitkamp (1945) and by Cason & Winans (1950). The possibility of the methyl side chain being in any position other than the *iso* position is precluded by the investigations of Cason, Taylor & Williams (1951) on the relationship between melting point and structure of high molecular weight branched-chain acids. These investigators found that except for the *iso*-acid, methyl-branched isomers have melting points at least 10° below that of the normal isomer. Weitkamp (1945) observed that a further characteristic of *iso*-acids with an even number of carbon atoms was that their mixed melting points with corresponding normal acids were depressed 10–15°; this was found to be the case with fractions K36S17S and K56S10S when mixed with pure myristic acid.

The higher molecular weight branched-chain *iso*-acids formerly isolated from butterfat (Hansen & Shorland, 1951; Hansen *et al.* 1954), and from mutton fat (Hansen, Shorland & Cooke, 1953) all contained an odd number of carbon atoms. This present paper reports the occurrence in butterfat of an *iso*-acid with an even number of carbons. Even-numbered acids have already been found in wool grease (Weitkamp, 1945), although in that case odd-numbered *iso*-acids appear to be absent.

In an earlier paper (Hansen *et al.* 1954) it was noted that fractions rich in esters of the C₁₅ *iso*- and *anteiso*-acids distilled over together after the esters of myristic acid. In this present work the esters of *isomyristic* acid were not accompanied by esters of the C₁₄ *anteiso*-acid, and they distilled over immediately after methyl laurate and before methyl myristate.

The proportion of 12-methyltridecanoic acid present in butterfat is estimated to be of the order of 0.05 % of the total fatty acids.

SUMMARY

12-Methyltridecanoic acid has been isolated in trace amounts from butterfat by methods which included hydrogenation, fractional distillation, and low-temperature crystallization.

We are indebted to Mr M. Fieldes of the Soil Bureau, Department of Scientific and Industrial Research, Wellington, New Zealand, for the X-ray measurements reported in this paper, and to Professor E. Stenhagen, Uppsala University, for a pure synthetic sample of 12-methyltridecanoic acid.

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