The Isolation of *cis*- Δ^9 -Heptadecenoic Acid from Butterfat

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Butterfat contains a wide range of fatty acids extending from C_2 to C_{26} (cf. Hilditch, 1956; Jack & Smith, 1956; Shorland & Hansen, 1957). Although the better-known constituents are the even-numbered saturated homologues from C_4 to C_{18} , and the even-numbered mono-unsaturated acids from C_{10} to C_{22} , it has been established in recent years that trace quantities of saturated branched-chain acids, saturated *normal* odd-numbered acids and saturated high-molecular-weight (C_{20} to C_{26}) components are also present. In addition, there is evidence for the occurrence in butterfat of C_{18} conjugated and unconjugated di- and poly-unsaturated acids, and C_{20} and C_{22} di- and poly-unsaturated non-conjugated acids (cf. Hilditch, 1956; Riemenschneider, 1957), as well as *trans* C_{16} and C_{18} acids (Bertram, 1928; Cornwell, Backderf, Wilson & Brown, 1953; Backderf & Brown, 1958).

In the present paper is reported the isolation from butterfat of an odd-numbered mono-unsaturated fatty acid: *cis*- Δ^9 -heptadecenoic acid. Odd-numbered unsaturated fatty acids have not formerly been isolated from butterfat.

EXPERIMENTAL

The butterfat used in this investigation was prepared from fresh butter supplied by the Awahuri Dairy Co., Palmerston North, New Zealand, and was considered a typical sample of the butter made in early February 1959, with cream from predominantly Jersey-breed herds. The butterfat was prepared by repeatedly washing the melted butter in hot water, centrifuging and drying *in vacuo*.

Fatty acids were prepared by hydrolysing 3502.5 g. of butterfat by the conventional method recommended by Hilditch (1956). The fatty acids were steam-distilled and yielded 3099.0 g. of steam-non-volatile acids which in turn were separated into 'liquid' and 'solid' acids by crystallizing three times from acetone at -31° . For this investigation the 'liquids' were converted into methyl esters and 1469 g. was fractionally distilled *in vacuo* in a 460 cm. $\times$ 5 cm. glass vigreux column yielding 24 fractions (N2LL1–N2LL24) and a residue. One of these methyl ester fractions (N2LL14) with constants suggesting the presence of the methyl esters of C_{17} fatty acids (wt. 38.71 g.; m.p. -18.9 to -16.3° ; saponification equiv. 288.8; iodine value 77.0) was denoted G1 and refractionated in a 50 cm. $\times$ 1.8 cm. column fitted with a coiled spring (column E, Shorland, 1952). Thirteen ester fractions (G1L1–G1L13) and a residue resulted, and the following, which were relevant to this

investigation, were bulked as fatty acids and denoted G6: G1L3, 1.97 g., m.p. -7.5° to -2.5° , saponification equiv. 281.1; G1L4, 2.26 g., m.p. -9.5° to -8.6° , saponification equiv. 284.4; G1L5, 1.79 g., m.p. -7.7° to -6.9° , saponification equiv. 283.4; G1L6, 2.46 g., m.p. -7.2° to -6.3° , saponification equiv. 283.3; G1L7, 2.52 g., m.p. -7.2° to -6.3° , saponification equiv. 282.7; G1L8, 2.53 g., m.p. -13.5° to -12.0° , saponification equiv. 285.4.

Eighteen low-temperature crystallizations of G6 fatty acids from solvents, the final two being from 10 vol. of acetone and methanol respectively at -50° , yielded fraction G11S3S (wt. 1.42 g., m.p. 11.4 – 12.2°). Further purification of this fraction was effected by eluting the methyl esters through a silica-gel chromatographic column (wt. 20.0 g.) with 50 ml. charges of light petroleum containing 1% of ethyl ether. Of the 14 fractions obtained, the sixth to the eleventh inclusive, which had almost identical melting points, were combined and denoted G15 (wt. 0.80 g., m.p. -26.3 to -25.2°). Fraction G15, after conversion into fatty acids, possessed the following chemical and physical properties which identified it as *n*-heptadecenoic acid: m.p. 11.4 – 12.2° [reported values: 9 – 10° (Chisholm & Hopkins, 1957); 13.0 – 13.5° (Ames & Bowman, 1951) for synthetic *cis*- Δ^9 -heptadecenoic acid; 14.5° (Appel, Böhm, Keil & Schiller, 1947)]; saponification equiv. 268.1 (Calc. for $C_{17}H_{33}O_2$: 268.4); combustion analysis: found: C 76.2; H 11.7. Calc. for $C_{17}H_{33}O_2$: C 76.1; H 12.0%; iodine value 83.1 (Calc. for *n*-heptadecenoic acid 94.6); methyl ester m.p. -26.3° to -25.2° (Appel *et al.* 1947, report -26.0°). Hydrogenation of G15 methyl esters in ethanol with Pt oxide as catalyst yielded a fraction which after purification by crystallization from 20 vol. of ethyl ether at -40° had m.p. 27.2 – 28.0° [Francis & Piper (1939) reported m.p. 29.7° for pure *n*-methyl heptadecanoate]. When the hydrogenated methyl ester (m.p. 27.2 – 28.0°) was mixed in equal proportions with authentic *n*-methyl heptadecanoate (m.p. 28.5 – 29.0°) it gave a mixed m.p. of 28.0 – 28.6° . The methyl ester when converted into fatty acid had m.p. 60.6 – 60.9° [Francis & Piper (1939) report m.p. 61.3° for pure *n*-heptadecanoic acid], and when mixed in equal proportions with pure *n*-heptadecanoic acid (m.p. 61.3 – 61.6°) gave a mixed m.p. of 60.7 – 60.9° .

Gas-liquid-chromatographic analyses. With the original model of the Pye Argon Chromatograph, which incorporates the ionization detector introduced by Lovelock, James & Piper (1959), the methyl esters of fraction G15 were chromatographed first at 150° through a Celite column impregnated with 20% of polydiethylene glycol succinate and secondly at 200° through a Celite column containing 20% of Apiezon M as liquid phase. The mean of two R_F determinations with the polyester column was 3.68 (mean of five values found for authentic *n*-methyl heptadecanoate, R_F 3.67, s.d. ± 0.06). With the Apiezon M column the R_F was 3.04.

Gas-liquid analysis of the hydrogenated methyl ester of fraction G15, with a Celite column containing 20% of polydiethylene glycol succinate at 150°, gave R_F 3.19 (mean of six values found for authentic *n*-methyl heptadecanoate, R_F 3.18, s.d. ± 0.12). R_F values were determined relative to *n*-methyltetradecanoate.

Location of double bond. Oxidation of 0.17 g. of G15 methyl esters with KMnO_4 in acetic acid by the method described by Haverkamp Begemann, Keppler & Boekennoogen (1950) yielded 0.08 g. of monobasic acids soluble in light petroleum and 0.06 g. (m.p. 101–103°) of dibasic acids insoluble in light petroleum. The weight of unoxidized neutral material soluble in ethyl ether after washing with Na_2CO_3 was 0.11 g. The dibasic acids were crystallized three times from water and yielded 0.03 g. of azelaic acid, m.p. 104.0–105.2°, and gave a mixed m.p. of 104.5–105.7° with equal proportions of pure azelaic acid (m.p. 105.1–105.7°). The acids soluble in light petroleum when subjected to gas-liquid-chromatographic analysis in the apparatus described by James & Martin (1952) were shown to contain the following constituents (expressed in moles %): *n*-hexanoic (?) acid 2.7%; *n*-heptanoic (?) acid 8.0%; *n*-octanoic acid 81.3%; *n*-nonanoic acid 8.0%. In order to determine the weight of *n*-octanoic acid in the total acids soluble in light petroleum, 0.0726 g. of acids was made up to 5 ml. in ethyl ether and a sample (0.25 ml.) was applied to the gas-liquid chromatogram by means of a hypodermic syringe. The analysis showed that 0.001921 g. of *n*-octanoic acid and 0.0001985 g. of *n*-nonanoic acid were detected from the 0.00363 g. applied, indicating that 58.9% by weight of the charge passed through the column and was detected.

Infrared analysis carried out with a Perkin-Elmer model 21 spectrophotometer showed fraction G15 to contain no *trans* acids.

Fraction G11S3S(l). A further fraction G11S3S(l) obtained as the twelfth fraction after chromatographing through silica gel, and referred to earlier, appears to be purer than G15. This methyl ester fraction (wt. 0.08 g.), m.p. -23.5° to -22.2°, on gas-liquid-chromatographic analysis with the polyester column was shown to contain 96.4 moles % of *n*-methylheptadecanoate, 1.4 moles % of 14- or 15-methylhexadecanoate and 2.2 moles % of *n*-methyloctadecanoate.

Combustion analysis and melting points. Combustion analyses were carried out by Dr A. D. Campbell, Chemistry Department, University of Otago, New Zealand. Melting points were determined in closed capillaries and are uncorrected.

DISCUSSION

The chemical and physical properties of fraction G15 isolated in this work identify it as the straight-chain C_{17} unsaturated fatty acid *cis*- Δ^9 -heptadecenoic acid. Its *cis* configuration is established by infrared analysis, which revealed an absence of *trans* acids. The position of the double bond was determined by oxidation of 0.17 g. of sample G15 with KMnO_4 in acetic acid and subsequent gas-liquid-chromatographic analysis (James & Martin, 1952) of the resulting 0.08 g. of monobasic fatty acids soluble in light petroleum. It was found that 58.9% by weight of a sample of the acids soluble in

light petroleum was detected by gas-liquid chromatography and *n*-octanoic acid constituted 90.6% by weight of these detected volatile acids. Based on the total weight (0.08 g.) of acids soluble in light petroleum produced in the oxidation, 58.9% represents 0.047 g. of volatile fatty acids including 0.043 g. of *n*-octanoic acid. Gas-liquid-chromatographic analysis of the methyl esters of fraction G15 showed it to contain only 90.4% of *n*-heptadecenoic acid, there being present as impurities 6.2 moles % of 14- or 15-methylhexadecanoic acid and 3.4 moles % of *n*-octadecenoic acid. After allowing for this 9.6% of impurities, the calculated weight of *n*-octanoic acid produced on oxidation of the original 0.17 g. of the fraction is 0.079 g. and the weight of *n*-octanoic acid found (0.043 g.) represents approximately 54% of the calculated yield. This establishes most of fraction G15 as having the double bond in the 9:10 position as is characteristic of animal fats.

Further confirmation of the location of the double bond in fraction G15 is provided by the yield of azelaic acid on oxidation with potassium permanganate in acetic acid.

Although odd-numbered unsaturated *normal* fatty acids have not formerly been isolated from butterfat, a straight-chain C_{17} mono-unsaturated fatty acid (*n*-heptadecenoic acid) has been found in human-hair fat (Weitkamp, Smiljanic & Rothman, 1947), lamb-caul fat (Shorland & Jessop, 1955), musk-ox body fat (Chisholm & Hopkins, 1957), shark-liver oil (I. M. Morice & F. B. Shorland, unpublished work) and sheep perinephric fat (R. P. Hansen, F. B. Shorland & N. J. Cooke, unpublished work). Weitkamp *et al.* (1947) provided evidence to show that the trace amounts of C_{17} unsaturated acids present in human-hair fat yielded *n*-undecanoic and *n*-nonanoic acids on oxidation with potassium permanganate in acetone and comprised a mixture of 80% of Δ^8 and 20% of Δ^9 isomers of *n*-heptadecenoic acid.

Based on the weight of pure *n*-heptadecenoic acid isolated in this work, together with the amounts estimated to be present in accompanying impure fractions, the content of this fatty acid in the sample of butterfat investigated is estimated to be 0.06% of the total weight of fatty acids. Gas-liquid-chromatographic analysis, however, suggests the total content of *n*-heptadecenoic acid in this sample to be approximately 0.2% of the total weight of fatty acids. In the body fat of musk-ox the amount of *n*-heptadecenoic acid present was estimated by Chisholm & Hopkins (1957) to be 0.9% of the weight of fatty acids.

The acknowledged complexity of the fatty acid composition of butterfat is further illustrated by the present isolation of *cis*- Δ^9 -heptadecenoic acid, which represents the fourth C_{17} fatty acid to be isolated from butterfat, the others being *n*-hepta-

decanoic (margaric) acid (Hansen, Shorland & Cooke, 1957) and the branched-chain (+)-14-methylhexadecanoic acid and 15-methylhexadecanoic acid (Hansen & Shorland, 1951).

SUMMARY

1. *cis*- Δ^9 -Heptadecenoic acid has been isolated from butterfat and conclusively identified.
2. The weight of *cis*- Δ^9 -heptadecenoic acid isolated from the sample of butterfat under investigation represented approximately 0.06 % of the total weight of fatty acids.
3. Odd-numbered unsaturated fatty acids have not formerly been found in butterfat.

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The Effects of some Ions and Chelating Agents on the Oxidase Activity of Caeruloplasmin

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Caeruloplasmin, the blue cuproprotein of plasma, has oxidase activity against a variety of substrates. Holmberg & Laurell (1951) showed that the activity was markedly affected by anions. Complex effects were obtained, activity being increased by anions in low concentration and decreased by the same anions in higher concentration. Broman (1958) reported inhibition of crude caeruloplasmin by dicarboxylic acids and ethylenediaminetetraacetic acid. It was suggested that the effect of the latter was due not to a direct interaction with caeruloplasmin, but to the removal of catalytically active metal-protein complexes present as contaminants.

In the work described below, the effects of some

anions, cations and chelating agents on the oxidase activity of purified caeruloplasmin were investigated.

METHODS AND MATERIALS

The apparatus was washed and the solutions were made up as described by Curzon & Vallet (1960).

Chemicals. A.R. grades were used whenever obtainable. *NN*-Dimethyl-*p*-phenylenediamine dihydrochloride (British Drug Houses Ltd., Poole, Dorset, England), supplied in sealed ampoules, was subdivided into 100 mg. lots and resealed into ampoules under vacuum and stored at -25° . Purity was 99 % with respect to free base. Trace-metal contamination was 10 p.p.m. of Ba, 4 p.p.m. of Al, Ca, Fe, Mg, Na and Ni, and 0.4 p.p.m. of Cr and Mn. These figures are rough estimates obtained from the estimated