The Branched-Chain Fatty Acids of Butterfat

1. THE ISOLATION FROM BUTTERFAT OF BRANCHED-CHAIN FATTY ACIDS WITH SPECIAL REFERENCE TO THE C₁₇ ACIDS

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(Received 16 May 1951)

Although branched-chain saturated fatty acids have been found in the lipids of certain bacteria as well as in wool wax, they have not hitherto been reported as existing in natural glycerides, with the solitary exception of *isovaleric* acid found by Chevreul in 1817 (cf. Hilditch, 1947) in the head and jaw oil of the dolphin.

Recently, Hansen & Shorland (1950) observed that the methyl ester concentrates of C₁₈ unsaturated acids of butterfat when oxidized with finely ground potassium permanganate in acetone solution (Armstrong & Hilditch, 1925) yielded a residual neutral saturated product which was liquid at room temperature. Concentrates of other C₁₈ unsaturated acids, such as those from pig fat, had been found to yield a solid neutral product corresponding to methyl stearate. Further purification of this liquid neutral product from butterfat was effected by recrystallization from acetone at -30° followed by fractionation in an electrically heated column packed with glass helices (cf. Longenecker, 1937). A combustion analysis of one of the fractions suggested the presence of a C₁₉ branched-chain acid contaminated with traces of oxygenated acids (cf. Hansen & Shorland, 1950).

Subsequent to this preliminary investigation it was found that the suspected branched-chain constituents could be separated by hydrogenation of the methyl esters of the unsaturated acids and subsequent crystallization from acetone at -30°, under which conditions the methyl esters of the branched-chain acids remained in solution.

The purpose of this paper is, first, to describe the general technique employed in the isolation from butterfat of two isomeric C₁₇ branched-chain fatty acids; and, secondly, to record their properties.

EXPERIMENTAL

A concentrate (4.4 kg.) of C₁₈ methyl esters prepared from butterfat was crystallized three times from 10 vol. of acetone at -30°. (The butterfat used in this investigation was prepared from butter churned on 16. v. 47 and supplied to this Laboratory through the courtesy of Dr F. H. McDowall, Dairy Research Institute, Palmerston North, by the Rangitaiki Plains Dairy Co. Ltd., Whakatane, New Zealand.) The resulting acetone-soluble 'liquids' were hydrogenated at 150° and at atmospheric pressure, using as catalyst nickel supported on kieselguhr. Repeated crystallizations from acetone at -30° removed the 'solid' products of hydrogenation, and the acetone-soluble material (87.6 g. iodine value 8.3) was fractionated at about 0.01 mm. in an 8 ft. (2.4 m.) Vigreux column containing approximately twenty-two theoretical plates (cf. Table 1).

Fractions EL3, EL4 and EL5 were bulked together as methyl esters (33.0 g.), denoted Ea, and fractionated in the 8 ft. Vigreux column. Fractionation data are shown in Table 2.

Analysis of liquid methyl ester fraction EaL5 by Dr T. S. Ma, Microchemical Laboratory, Otago University, Dunedin, New Zealand. (Found: C, 75.8; H, 12.9. Calc. for C₁₈H₃₆O₂: C, 76.0; H, 12.8%.)

As a means of aggregating methyl esters of molecular weight similar to that of EaL5 (methyl esters of suspected C₁₇ acids), selected fractions derived from EL1 to EL6 by repeated distillation were bulked together with EaL5, and the whole refractionated in the 8 ft. Vigreux column. On

Table 1. *Fractionation of 87.6 g. C₁₈ liquid methyl esters*

Fraction	Wt. (g.)	Saponification equiv.	Iodine value	
EL1	2.25	269.7	7.3	Liquid
EL2	8.42	272.1	2.4	Solid
EL3	10.91	277.5	1.9	Semi-solid
EL4	10.38	283.1	3.2	Liquid
EL5	13.44	290.9	3.7	Liquid
EL6	14.05	308.2	4.9	Liquid
EL7	12.28	310.3	8.6	Liquid
EL8	2.45	317.6	23.0	Semi-solid
ELR*	14.03	263.4	25.7	—

* Residue (non-volatile). For esters in this fraction, excluding unsaponifiable matter, saponification equiv. 241.7; iodine value, 17.8.

Table 2. *Fractionation of Ea methyl esters*

Fraction	Wt. (g.)	Saponification equiv.	Iodine value	
EaL1	2.16	273.0	2.4	Solid
EaL2	4.83	275.5	1.1	Solid
EaL3	5.42	278.1	0.5	Solid
EaL4	3.61	280.3	0.6	Liquid
EaL5	8.19	284.7	1.4	Liquid
EaL6	6.35	291.4	3.1	Liquid
EaLR*	2.57	302.6	15.3	—

* Residue (non-volatile). For esters in this fraction, excluding unsaponifiable matter, saponification equiv. 299.9; iodine value, 12.8.

Table 3. *Chemical and physical characteristics of fraction Es*

	M.p.	Saponification equiv.	Iodine value	Refractive index
Es methyl ester	8.5–9.0°	284.9	1.5	$n_D^{20.5}$ approx. 1.4419
Es acid	47.3°	—	—	$n_D^{50.2}$ 1.4400

Table 4. *Fractional crystallization of Es acids from 40 vol. of methanol*

Fraction	Wt. (g.)	M.p. (°)	Temp. of crystallization (°)	Saponification equiv.
Es	1.59	47.3	—	270.9 (acid)
EsL	0.21	28.1	-37	—
EsS	1.37	48.6		
EsSL	0.26	37.0	-30	—
EsSS	1.12	48.7		
EsS2L	0.10	34.5	-42	—
EsS2S	1.01	51.0		
EsS3L	0.07	33.3	-45	—
EsS3S	0.93	50.5		
EsS4L	0.14	36.0	-39	—
EsS4S	0.78	50.9		
EsS5L	0.17	39.7	-35	—
EsS5S	0.60	53.3		
EsS6L	0.18	48.5	-35	—
EsS6S	0.41	53.9		
EsS7L	0.14	51.7	-35	269.5 (acid)
EsS7S	0.28	54.4		

completion of eight systematic distillations, a series of fractions was obtained whose saponification equivalents approximated 284. These fractions, namely, EFL1 (285.3), EFL2 (282.9), EFL3 (281.9), EIL1 (284.4), EIL2 (283.0), EIL3 (284.4) and EIL4 (284.5), when bulked together and denoted Ep, provided 5.47 g. of methyl esters. Ep was crystallized from 20 vol. methanol at -70° , and the portion insoluble in methanol at -70° was then recrystallized from 20 vol. of methanol at -60° , yielding 3.27 g. of 'solids' (Er2), saponification equivalent 283.4 (methyl ester), n_D^{20} , 1.4443.

Chromatographic adsorption of Er2 on activated alumina removed traces of impurities and provided sixteen fractions; of these, the following four, which had been eluted with light petroleum (b.p. 50–60°) were bulked together as methyl esters to constitute Es: Er2b 0.87 g., $n_D^{22.5}$ 1.4421; Er2c 0.54 g., $n_D^{22.5}$ 1.4418; Er2d 0.25 g., $n_D^{22.5}$ 1.4418; and Er2e 0.17 g., $n_D^{22.7}$ 1.4420.

Properties of fraction Es are shown in Table 3.

Purification of Es fatty acids was effected by repeated low-temperature crystallization from 40 vol. of methanol of successive fractions insoluble in this solvent at the temperatures recorded in Table 4.

The chemical and physical characteristics of the residual fatty acid fraction, Es7S, insoluble in methanol at -35° , are shown in Table 6.

Table 5. *Fractional crystallization of Et acids from 40 vol. of light petroleum (b.p. 50–60°)*

Fraction	Wt. (g.)	M.p. (°)	Temp. of crystallization (°)
Et	0.90	—	—
EtL	0.21	1	-45
EtS	0.67	42	
EtSL	0.01	—	-45
EtSS	0.65		
EtS2L	0.15	37.2	-30
EtS2S	0.50	40.5	
EtS3L	0.05	35.8	-30
EtS3S	0.45	39.2	
EtS4L	0.05	37.6	-30
EtS4S	0.39	40.2	

Table 6. *Chemical and physical characteristics of fatty acids EuS and EsS7S*

Fraction	Saponification equivalent	M.p. (°)	C-methyl* (%)	Refractive index	Combustion* analysis	Iodine value	X-ray diffraction analysis (A.)	
							Long spacing	Side spacings†
EuS (acid)	270.1	39.8	8.06	$n_D^{60.8}$ 1.4333	C 75.6, H 12.8	2.0	33.1	4.78, 4.45, 4.18, 4.03, 3.84, 3.70
EuS (methyl ester)	—	3.5	—	n_D^{30} 1.4422	—	—	—	—
EsS7S (acid)	269.5	54.4	6.68	n_D^{72} 1.4268	C 75.8, H 12.3	2.4†	33.9	4.91, 4.66, 4.38, 4.18, 3.94, 3.68
EsS7S (methyl ester)	—	18.0‡	—	n_D^{20} 1.4438	—	—	—	—

* Analyses by Drs G. Weiler and F. B. Strauss, Oxford, England.

† Side spacings include the 7th, 8th and 9th order of the long spacings.

‡ Determinations made after 0.01 g. of EsS7S had been removed by crystallization from light petroleum and melting point had been raised to 54.5°.

Those fractions soluble in methanol at the temperatures given in Table 4, were bulked together to constitute Et (0.90 g.). Fractional crystallizations of Et and of successive 'solid' fractions derived from it were carried out using 40 vol. of light petroleum (b.p. 50–60°) at temperatures ranging from –45 to –30°, as shown in Table 5.

Residual fraction EtS4S (0.39 g.), subsequently denoted Eu, was crystallized from 40 vol. acetone at –40°, yielding an acetone-soluble fraction weighing 0.04 g., m.p. 35.2°. The fraction insoluble in acetone at –40° (EuS) weighed 0.35 g. Its chemical and physical properties are recorded in Table 6.

Using a Philips Geiger X-ray spectrometer, diffraction measurements were made on the two substances EsS7S and EuS. Samples were melted on a glass slide and quickly cooled.

DISCUSSION

The combustion analyses, together with the saponification equivalents of EuS and EsS7S (Table 6), agree closely with the theoretical values for $C_{17}H_{34}O_2$, viz. C, 75.5; H, 12.7%; saponification equivalent 270.4; while the C-methyl values afford evidence of the presence of two methyl groups in each acid (cf. Ginger, 1944). X-ray diffraction measurements show that the long spacings of EsS7S (33.9 A.) and of EuS (33.1 A.) cannot be considered to correspond within the experimental error (± 0.5 A.) to a straight chain C_{17} saturated acid (cf. Table 7).

On the other hand, the long spacing of EsS7S is consistent with this fraction, being a methyl branched-chain C_{17} or C_{18} saturated acid, and the value for EuS corresponds to that of a C_{17} methyl branched-chain saturated acid. A study of the side spacings for long-chain *iso* acids published by Arosenius, Ställberg, Stenhagen & Tägtström-Eketorp (1949), reveals that the value of 4.16 appears in three odd-numbered homologues, namely, the C_{13} , C_{15} and C_{17} acids. A corresponding value of 4.18 was obtained in the X-ray analyses of both the samples EsS7S and EuS.

It has been observed by Cason (1948), that the only form of single methyl side-chain fatty acid

which has a melting point less than 10° below that of the corresponding normal isomer is the *iso* acid (branching group on the carbon next to the end of the chain). Weitkamp (1945), in his investigations on the fatty acids of wool grease, presents evidence to show that *iso* acids with fourteen or more even-numbered carbons always melt one degree or less below the normal isomer. Accepting the value of 59.2–60.5° (Cason & Winans, 1950) for the melting point of normal heptadecanoic acid, it appears that

Table 7. *X-ray long spacings of straight-chain, methyl iso, and methyl anteiso saturated fatty acids*

Carbon content of acid	Long spacings (A.)		
	Straight-chain saturated fatty acids*	Methyl <i>iso</i> saturated fatty acids†	Methyl <i>anteiso</i> saturated fatty acids‡
C_{13}	30.0	26.2	—
C_{14}	31.60§	27.0	—
C_{15}	34.2	29.8	—
C_{16}	35.60§	30.4	—
C_{17}	38.6	33.35	33.4
C_{18}	39.75§	33.8	—
C_{18} (d)	—	—	34.2
C_{18} (d-1)	—	—	28.0
C_{19}	43.15	36.2	—
C_{20}	44.15§	—	—

* Data by Francis, Piper & Malkin (1930).

† Data by Arosenius, Ställberg, Stenhagen & Tägtström-Eketorp (1949).

‡ Data by Velick (1947).

§ Data by Francis & Piper (1939).

|| Data by Velick (1944).

EsS7S (m.p. 54.4) is an impure *iso* acid, 15-methyl-hexadecanoic acid. A further attempt at purification of EsS7S by fractional crystallization from light petroleum (b.p. 50–60°) at –40° removed in the soluble fraction only 0.01 g. and raised the melting point of the insoluble fraction 0.1°. Location of the position of the CH_3 side chain in fraction EuS is

under investigation, the melting point evidence (m.p. 39·8°), however, is consistent with the presence of a methyl side chain in the antepenultimate position (cf. Weitkamp, 1945) suggesting that EuS may be identified as 14-methylhexadecanoic acid (m.p. 36·8°).

Although data which have so far been obtained in this work are limited to the C₁₇ acids, accompanying evidence indicates the presence in butterfat of a wide range of branched-chain fatty acids. Investigation of these acids is in progress.

Prior to the present investigation, no naturally occurring *iso* acid with an odd number of carbon atoms has been reported (cf. Arosenius *et al.* 1949), although two such odd-numbered *iso* acids, a C₁₉ (Cason, 1942; Weitkamp, 1945), and a C₁₇ (Weitkamp, 1945) have been synthesized.

The butterfat from which the two C₁₇ branched-chain acids have been isolated was drawn from a mid-May (1947) churning of cream, representative of numerous cows in the Whakatane district. In our preliminary experiments, when the presence of branched-chain acids was suspected, the bulked C₁₈ unsaturated methyl esters which yielded liquid saturated products on oxidation were derived from several churnings made during different months, suggesting that methyl side-chain acids are not peculiar to one butterfat sample only.

For the elucidation of the mode of combination of branched-chain acids in butterfat, further work is required. It has been shown by Morice (1951), that cholesterol comprises about 0·40 % of the total 0·65 % of unsaponifiable matter in butterfat. Of the

cholesterol present, about 0·26 % is in the free form (Morice, 1951). Branched-chain acids probably comprise more than 0·8 % of the total fatty acid content of the butterfat, this quantity having been extracted from a concentrate of the C₁₈ unsaturated acids alone. Accordingly, combination of branched-chain acids with cholesterol only is not reconcilable with the proportions of these substances present. It is probable, therefore, that the branched-chain acids are present in the neutral fat as glycerides, or are combined with some water-soluble compound such as the disaccharide trehalose, as is the case in certain bacillary lipids (Anderson, 1941).

SUMMARY

1. Branched long-chain fatty acids formerly unidentified in natural glycerides have been isolated from butterfat.

2. Two C₁₇ methyl branched-chain fatty acids, isomeric with normal heptadecanoic acid are shown to be present in butterfat.

3. Based solely on the melting point evidence of branched-chain fatty acids as presented by Cason (1948), one of the two C₁₇ fatty acids isolated (m.p. 54·4°) appears to be the *iso* acid, while the other (m.p. 39·8°) appears to be the *anteiso* acid.

We are indebted to Miss N. J. Cooke for technical assistance. The X-ray diffraction analyses, together with their interpretation, were made by Mr M. Fieldes of the Soil Bureau, Department of Scientific and Industrial Research, Wellington, New Zealand.

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