

4. *S*-Alkyl derivatives of some mercaptoglyoxalines show weak activity.

5. Ergotamine administration reduces thyroidal iodine uptake, a sex difference sometimes being observed.

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## The Branched-Chain Fatty Acids of Butterfat

### 6. FURTHER INVESTIGATIONS ON THE C<sub>15</sub> SATURATED ACIDS

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In work previously reported (+)-12-methyltetradecanoic and 13-methyltetradecanoic acids were isolated from mutton fat (Hansen, Shorland & Cooke, 1953), and from butterfat (Hansen, Shorland & Cooke, 1954*a*), while *n*-pentadecanoic acid was isolated from mutton fat (Hansen, Shorland & Cooke, 1954*b*). As, however, the isolation procedure in each case involved hydrogenation, it was not established with certainty that these acids were originally present in the fats as saturated constituents.

In this paper it is shown that without hydrogenation, and by use of a highly efficient fractionating column, two fractions were obtained from butterfat, the first of which contained mainly a mixture of the methyl esters of (+)-12-methyltetradecanoic and 13-methyltetradecanoic acids and the second consisted essentially of methyl *n*-pentadecanoate.

#### EXPERIMENTAL

Optical rotations were measured on the pure liquids without the use of solvents.

*Preliminary fractionation.* The butterfat used in this work was supplied by the Rangitaiki Plains Dairy Co. Ltd., Whakatane. The crude methyl esters (51 620 g.) prepared from the butterfat were heated on the water bath under the

vacuum of a water pump to remove traces of water and solvents which were unavoidably contaminated with a small proportion of the more volatile esters. The residue was distilled in a falling-film still at approximately 2.5 mm. and 135° yielding distillate (V1 + V2, 7323 g.) of which the more volatile part (V1, 372 g.) was collected in a dry-ice trap. The residue R1 (44584 g.) was redistilled in a falling-film still at 130° in high vacuum to give a distillate (V3, 42453 g.) and a residue (R2, 1841 g.).

V3 (15889 g.) was distilled *in vacuo* in a Vigreux column (210 × 6 cm.) and the first fraction redistilled in another Vigreux column (480 × 4.5 cm.). From the latter fractionation, fraction 1,6 the saponification equivalent of which approached that of pentadecanoic acid, was selected for the investigation reported in this paper. This concentrate was refractionated in column *G* patterned on column *E* (Shorland, 1952) (cf. Table 1). Column *G* (250 × 3.5 cm. with 3.5 mm. diameter coil) when tested with a 1:1 mixture of heptane-methylcyclohexane at atmospheric pressure was found to possess more than ninety theoretical plates.

*C<sub>15</sub> Fractions.* Fractions 1,6,1 to 1,6,3 were not further examined, as they appeared to consist essentially of methyl myristate. The remaining fractions corresponded in saponification equivalent to methyl esters of C<sub>15</sub> acids. Fractions 1,6,4-1,6,6 which were optically active and possessed low melting points, were combined and denoted A, while fraction 1,6,7 with relatively high melting point and with no detectable optical rotation was denoted B.

Table 1. *Fractional distillation of fraction 1,6*

Fraction	Wt. (g.)	B.p. (ca. 0.1 mm.)	Sap. equiv.	Iodine value (Wijs)	M.p. (°)	$\alpha_D^{18}$ (1 dm.)
1,6,1	30.41	93	241.7	23.0	18.0-18.5	Not detectable
1,6,2	29.95	93-94	242.9	3.5	18.5-19.0	Not detectable
1,6,3	19.01	94-98	243.6	2.9	16.8-17.3	Not detectable
1,6,4	31.75	98	258.0	4.2	-10.8 to -8.6	+1.80
1,6,5	34.68	98	257.6	3.1	-13.0 to -11.4	+2.84
1,6,6	11.81	98-99	257.2	11.3	-13.4 to -10.5	+2.84
1,6,7	44.09	—	257.3	5.4	17.8-18.0	Not detectable

Table 2. *Chemical and physical properties of fractions A 1-5S, BS and CS*

Fraction	Wt. (g.)	Sap. equiv.	Iodine value (Wijs)	Refractive index $n_D^{20}$	M.p. (°)	Combustion analysis (%)		C-methyl groups	$[\alpha]_D^{18}$	X-ray long spacing (Å)
						C	H			
A 1-5S	12.76	241.5	0.2	1.4318	52.2	74.3	12.4	1.27	Not detectable	29.7
BS	17.77	242.3	0.2	1.4327	53.3	74.3	12.3	—	Not detectable	35.61
CS	19.63	241.6	0.0	1.4328	24.4	73.7	12.3	1.42	+4.65	30.71 and 27.72
Calc. for $C_{15}H_{30}O_2$ :	242.4	—	—	—	—	74.4	12.5	—	—	—

Fraction A was further resolved in column G to give fractions A1 to A5 with observed rotations  $\alpha_D^{18}$  (1 dm.) ranging from +1.70° to +2.30° and A6 and AR with observed rotations  $\alpha_D^{18}$  (1 dm.) +2.61° and +3.00° respectively.

The combined fractions A1-A5 (denoted A1-5) were converted into acids (38.0 g.) and crystallized 9 times from 40 vol. of light petroleum (b.p. 50-60°) at -40° yielding 12.76 g. of insoluble acids (A1-5S, m.p. 52.2°; see Table 2) which, when mixed with an equal amount of 13-methyltetradecanoic acid, supplied through the courtesy of Professor E. Stenhagen, (Arosenius, Ställberg, Stenhagen & Tägtström-Eketorp, 1949), showed no depression in melting point.

Fraction B, which appeared to consist mainly of methyl *n*-pentadecanoate, was purified by fractional distillation in column G. Of the resulting fractions those with melting points ranging from 18.0 to 19.1° were combined, converted into acids (20.53 g.) and crystallized 5 times from 40 vol. of light petroleum at -40° giving 17.77 g. insoluble acids (BS) m.p. 53.3° (see Table 2) and 1.98 g. soluble acids (BL).

Soluble fractions A1-5 as well as A6, and BL were combined, giving 53.9 g. of acids (denoted C). Fraction C was crystallized from 10 vol. of light petroleum at -40° and the soluble fractions containing unsaturated acids were twice submitted to this process giving each time diminishing proportions of precipitate. The precipitates were combined and crystallized at -40° from light petroleum, using 10 vol., then 30 vol. and finally they were crystallized 4 times using 40 vol., the insoluble fractions being crystallized in each instance. The soluble fractions, except the first, were combined giving 30.77 g. acids. These were recrystallized, first from 10 vol. of light petroleum, and then twice from 40 vol. at -40° giving 19.63 g. of precipitate (denoted CS, m.p. 24.4°; see Table 2) and 11.41 g. soluble fraction.

Further amounts of  $C_{15}$  esters were isolated by fractional distillation of V2 and fractions 1,7 and 1,8 from V3.

*Proportions of  $C_{15}$  acids.* After identification of the  $C_{15}$  acids their proportions in the original esters were calculated on the basis that the  $C_{15}$  acids were separated sharply from

$C_{14}$  and  $C_{16}$  acids by ester fractionation (cf. Table 1). Further, the  $C_{15}$  acids themselves were separated by this procedure into two groups containing respectively (a) mixtures of *iso* and *ante-iso* acids, and (b) *normal* acids only. The proportions of *ante-iso* acids present in (a) were determined by means of optical rotation assuming that the pure acid had  $[\alpha]_D^{18} + 4.65^\circ$  and the *iso* acid was determined by difference. In all cases allowance was made for the small proportions of unsaturated constituents which are still under investigation. In this way percentages of  $C_{15}$  acids in the total fatty acids of the butterfat were calculated as *normal*, 0.82; *iso*, 0.37, and *ante-iso*, 0.43.

The X-ray long spacings were determined with a Philips Geiger X-ray spectrometer, iron-filtered  $K\alpha$  radiation being used. The samples were melted on a glass slide and quickly cooled. Melting points were determined in closed capillaries and are uncorrected.

C, H, and C-methyl analyses were by Drs G. Weiler and F. B. Strauss, Oxford.

## DISCUSSION

The analytical data shown in Table 2 demonstrate that fraction A 1-5S consists of 13-methyltetradecanoic acid and fraction CS consists of (+)-12-methyltetradecanoic acid (cf. Hansen, Shorland & Cooke, 1953, 1954a). It should be noted that reliable X-ray long spacings are difficult to obtain for *ante-iso* acids (Velick, 1947).

Fraction BS (cf. Table 2) when admixed with equal proportions of pure *n*-pentadecanoic acid showed no depression in melting point. Further evidence that fraction BS consists of pure *n*-pentadecanoic acid is given by the X-ray long spacing (35.61 Å) as compared with 35.75 Å found by Slagle & Ott (1933) for this acid.

In this laboratory's work on branched-chain fatty acids, the fat has usually been hydrogenated

to convert the unsaturated fatty acids into saturated, and thereby facilitate their subsequent separation from the branched-chain fatty acids by low-temperature crystallization. Under those circumstances it could not be assumed that the branched-chain acids isolated were actually present originally as saturated acids. Moreover, there existed the possibility that hydrogenation might open up a cyclopropane ring if present in the original acids (cf. Hofmann, Lucas & Sax, 1952). The present work, therefore, establishes that the *iso*, *ante-iso* and *normal* pentadecanoic acids were present as such in the butterfat, and were not formed from unsaturated or cyclic acids. The isolation, without recourse to hydrogenation, of a C<sub>20</sub> multibranched fatty acid fraction by distillation and chromatography of the methyl esters of butterfat supports the view that all the branched-chain fatty acids so far isolated from natural fats were present at least in part as saturated constituents.

Saturated straight-chain acids containing an odd number of carbon atoms have not generally been accepted as constituents of natural fats (cf. Hilditch, 1947; Ralston, 1948). However, recent work in this laboratory has led the present authors to abandon this view. Hansen & McInnes (1954) have presented evidence for the occurrence in ox perinephric fat of volatile fatty acids with an odd number of carbon atoms, while Hansen, Shorland & Cooke have isolated *n*-pentadecanoic (1954*b*) and *n*-heptadecanoic acids (1954*c, d*) from hydrogenated mutton fat. In addition, unpublished evidence by Morice & Shorland shows the presence of *n*-pentadecanoic acid in shark liver oil. The present work, however, affords the first conclusive evidence that a straight-chain fatty acid with an uneven number of carbon atoms (*n*-pentadecanoic acid) occurs as such in a natural fat. Gray, Pilgrim, Rodda & Weller (1951) have shown by use of isotopic labelling that propionic acid in the rumen is converted into valeric acid, and this result is consistent with our view that saturated acids with an odd number of carbon atoms may well be derived by the successive addition of acetate to propionic acid. It is hoped to test this view experimentally.

The quantity of C<sub>15</sub> acids isolated amounts to approximately 1.6% of the total fatty acids and does not necessarily represent the total amount present in butterfat, as traces of these acids are likely to occur in other fractions which have not been fully investigated. From the previous papers in this series it is obvious that further investigations

now in progress will lead to the isolation of other branched-chain acids and acids with an odd number of carbon atoms and their total quantity will be greater than was hitherto anticipated. Accordingly, earlier results for the fatty acid composition of butterfat, as well as probably those of many other fats, will need to be modified in the light of the present findings.

#### SUMMARY

1. Two C<sub>15</sub> branched-chain acids, (+)-12-methyltetradecanoic acid and 13-methyltetradecanoic acid, together with the straight-chain odd-numbered acid *n*-pentadecanoic acid, have been isolated from butterfat by methods which did not involve hydrogenation.

2. Expressed as a percentage of the total fatty acids, the amounts of (+)-12-methyltetradecanoic acid, 13-methyltetradecanoic acid and *n*-pentadecanoic acid were 0.43, 0.37 and 0.82% respectively.

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