The Branched-chain Fatty Acids of Butterfat

3. FURTHER INVESTIGATIONS ON A MULTIBRANCHED C_{20} SATURATED FATTY ACID FRACTION

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In a previous paper (Hansen & Shorland, 1951) the isolation from butterfat of a C_{20} multibranched saturated fatty acid fraction was recorded. As the methods then used involved hydrogenation the possibility that this fraction was unsaturated in the original butterfat was not excluded. In the investigation now reported, which was undertaken primarily to elucidate the nature of the unsaturated acids of butterfat, a fraction with properties similar to those recorded in the above-mentioned paper has been isolated without hydrogenation by the combined techniques of fractional distillation *in vacuo*, chromatographic adsorption on activated aluminium oxide, and low temperature crystallization.

EXPERIMENTAL

From 36·7 kg. of butterfat a concentrate of methyl esters (8·87 kg.) of the C_{18} unsaturated acids of butterfat was separated from the total unsaturated acids by distillation at about 0·1 mm. in a 208 × 6·5 cm. Vigreux column. Systematic crystallization of this concentrate of methyl esters from acetone at low temperatures yielded at −45° a soluble fraction (1·44 kg., iodine val. 129·5) which was converted to acids and further crystallized from acetone at −78°. The resulting soluble fraction was distilled as methyl esters (58·3 g., iodine val. 162·8) in an efficient 60 × 1·2 cm. fractionating column of the type described by Longenecker (1937). Fractionation data are shown in Table 1.

Fractions 2, 3 and 4 above were combined (denoted A) and chromatographed as shown in Table 2, using 800 g. activated Al_2O_3 in a 60 mm. diam. tube. In order to recover further quantities of the less unsaturated constituents to add to fractions A 1 to A 4, fractions A 5 to A 8 inclusive were combined (denoted B) and rechromatographed using 300 g. activated Al_2O_3 packed in a 30 mm. diam. tube.

Fractions A 1–A 4 and B 1–B 4 were combined (denoted *C*) and submitted to chromatographic separation on 300 g. of activated Al_2O_3 packed in a tube of 30 mm. diam. Of the eight resulting fractions, the first, C 1 (5·99 g., iodine val. 6·6) which had been eluted with 1200 ml. light petroleum (b.p. 50–60°) was distilled at 0·1 mm. in a 50 × 1·8 cm. fractionating column (*E*) described by Shorland (1952) and yielded the following fractions: D 1, 0·79 g., saponification equivalent 331·2, iodine value 7·6, unsaponifiable matter 15·4%; D 2, 1·22 g., saponification equivalent 347·8, iodine val. 2·8, unsaponifiable matter 10·2%; D 3, 2·18 g., saponification equivalent 375·4, iodine val. 3·9, unsaponifiable matter 19·7%; D 4, 1·01 g., saponification equivalent 401·5, iodine val. 4·4, unsaponifiable matter 21·5%; DR, 0·75 g., saponification equivalent 271·2, iodine val. 30·7.

Table 1. *Fractional distillation of methyl esters soluble in acetone at* −78°

Fraction	Wt. (g.)	B.p(0·1 mm.) (°)	Saponification equiv.	Iodine value (Wijs)
1	4·20	68–134	291·7	86·9
2	13·77	134	308·3	138·2
3	14·16	134	304·8	141·2
4	14·00	134–137	310·8	152·7
5	4·36	137–147	329·0	252·0
Residue	6·86		393·6	159·3
Total	57·35			

Table 2. *Chromatographic separation of methyl esters (fractions A and B)*

(Each fraction 1200 ml.; L.P. = light petroleum, b.p. 50–60°.)

Fraction	Wt. (g.)	Eluant	Iodine value
		Fraction A. 37·3 g.	
A 1	0·33	L.P.	28·5
A 2	1·53	L.P.	14·7
A 3	5·81	L.P.	25·9
A 4	3·70	L.P.	75·4
A 5	2·44	L.P.	107·2
A 6	2·58	L.P.:ether (98:2)	132·3
A 7	7·59	L.P.:ether (98:2)	168·1
A 8	3·66	L.P.:ether (98:2)	182·1
A 9	2·79	L.P.:ether (98:2)	203·8
A 10	3·35	L.P.:ether (90:10)	220·0
A 11	2·04	L.P.:ether (90:10)	234·0
A 12	1·48	Ether	205·9
A 13	0·55	Ether:ethanol (50:50)	129·1
Total	37·85		
		Fraction B. 15·88 g.	
B 1	1·19	L.P.	68·2
B 2	2·64	L.P.	92·9
B 3	2·60	L.P.	131·6
B 4	1·34	L.P.	143·2
B 5	1·02	L.P.:ether (98:2)	142·8
B 6	3·68	L.P.:ether (98:2)	188·2
B 7	1·82	L.P.:ether (98:2)	190·7
B 8	1·43	L.P.:ether (90:10)	166·4
B 9	0·39	Ether	124·5
Total	16·11		

Table 3. *Comparison of properties of fraction E 2 (from unhydrogenated butterfat) and Eq 1 bL (from hydrogenated butterfat)*

Fraction	Saponification equiv.	Analysis* (%)	Iodine value (Wijs)	C-methyl content* (%)	Refractive index	Optical rotation
E 2	312·0	C, 76·6 H, 12·4	0·0	12·7	n_D^{20} 1·4533	$[\alpha]_D^{18·5}$ +1·1 ($CHCl_3$)
Eq 1 bL (Hansen & Shorland, 1951)	314·5	C, 77·2 H, 13·1	0·0	13·4	n_D^{20} 1·4513	Nil†
Calculated for $C_{20}H_{40}O_2$	312·5	C, 76·8 H, 12·9				

* By Weiler and Strauss, Oxford.

† The specific rotation $[\alpha]_D$ of this fraction had been determined as +0·58°, using 0·17 g. material in 5 ml. chloroform, but as the observed mean rotation α_D of 0·01° was within experimental error, a zero activity was recorded.

Fractions D 1–D 4 were combined (denoted E), saponified, and after removal of unsaponifiable matter, reconverted to methyl esters. The methyl esters (3·46 g.) were then distilled at approx. 0·1 mm. in a 40 × 1·0 cm. column (*D*) described by Shorland (1952) and yielded the following fractions: E 1, 0·68 g., saponification equivalent 319·9, iodine val. 0·5; E 2, 2·07 g., saponification equivalent 324·1, iodine val. 0·0; ER, 0·62 g., saponification equivalent 319·0, iodine val. 9·3.

The properties of the E 2 acids are given in Table 3. The molecular extinction (ϵ) at 219 mμ. = 175, was determined with a Beckman spectrophotometer model DU.

DISCUSSION

The saponification equivalent and combustion analysis of E 2 are in agreement with the requirements for a fatty acid with empirical formula $C_{20}H_{40}O_2$, while the *C*-methyl determination which is equivalent to 2·64 mol. acetic acid points to the presence of three and possibly four methyl or homologous groups (cf. Ginger, 1944). Evidence indicating this fraction to be saturated is provided by the zero iodine value. The molecular extinction value (ϵ) of 175 at 219 mμ. as compared with a value of about 14 000 for an $\alpha\beta$-unsaturated ester as reported by Cason & Sumrell (1951) indicates that no $\alpha\beta$-unsaturated material is present. Although no true melting point could be observed for this liquid it was found that its viscosity increased with decreasing temperatures and at −65° it assumed the appearance of a glassy solid. The acid reported earlier (Hansen & Shorland, 1951) on re-examination showed a similar behaviour when reduced to an equally low temperature.

As only the most soluble portion of the crude C_{18} acids was investigated, the weight of the C_{20} branched-chain acid isolated (about 0·006 % of the butterfat) probably represents only part of the total amount present.

The physical and chemical properties of E 2 acids correspond closely with those of a fraction Eq 1 bL which was earlier isolated (Hansen & Shorland, 1951) from butterfat by methods which included hydrogenation (see Table 3).

As fraction Eq 1 bL was isolated from hydrogenated butterfat it was not ascertained if it existed in the original butterfat in the saturated or unsaturated state. The present work, however, was carried out without recourse to hydrogenation, and apart from substantiating the former investigation, it establishes that at least part and possibly all of this C_{20} multibranched fatty acid is present in butterfat as a saturated constituent.

SUMMARY

The present paper confirms the occurrence in butterfat of a multibranched C_{20} fatty acid fraction, and establishes that this acid exists in the original butterfat (in part or in whole) as a saturated constituent.

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