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The Branched-Chain Fatty Acids of Ox Fat

1. THE ISOLATION FROM OX SUET OF A C_{17} BRANCHED-CHAIN SATURATED FATTY ACID

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The occurrence of high molecular weight branched-chain saturated fatty acids has been reported in certain bacterial lipids (Anderson, 1927, 1941; Velick, 1944; Hofmann & Lucas, 1950) and in wool wax (Weitkamp, 1945). Recent investigations in this laboratory have revealed the presence in butterfat of (*a*) two isomeric C_{17} saturated fatty acids, each with a methyl side chain (Hansen & Shorland, 1951), (*b*) a multi-branched C_{20} fatty acid (Hansen & Shorland, 1952), (*c*) a C_{18} methyl branched-chain fatty acid (Hansen, Shorland & Cooke, 1951).

In this paper is described a C_{17} branched-chain acid isolated from ox suet by the processes of hydrogenation, fractional distillation *in vacuo*, and fractional crystallization.

EXPERIMENTAL

The fat used in this work was extracted from the suet (15·5 lb.) of a 4-year-old 'prime' heifer (dressed weight approximately 600 lb.). Following the determination of the fatty acid composition of a sample of the fat, 162 g. of the fractionated methyl esters of the C_{18} unsaturated acids were hydrogenated at 150° and at atmospheric pressure, using a nickel catalyst supported on kieselguhr.

Repeated crystallization from 15 vol. acetone at −30° removed the solid products of hydrogenation and yielded

Table 1. *Fractionation of ox-fat liquid methyl esters in micro column*

(Pressure approx. 0·05 mm. Hg.)

Fraction	Wt. (g.)	b.p. (°)	Saponification equiv.	Iodine value (Wijs)	
FL1	0·20	117–122	290·7	3·4	Liquid
FL2	0·60	122–128	290·5	1·6	Liquid
FL3	0·93	128–130	300·7	1·2	Liquid
FL4	0·46	130–131	319·3	2·2	Liquid
FLR	0·44	—	—	—	Viscous liquid

Table 2. *Fractional crystallization of* FL2 *acids*

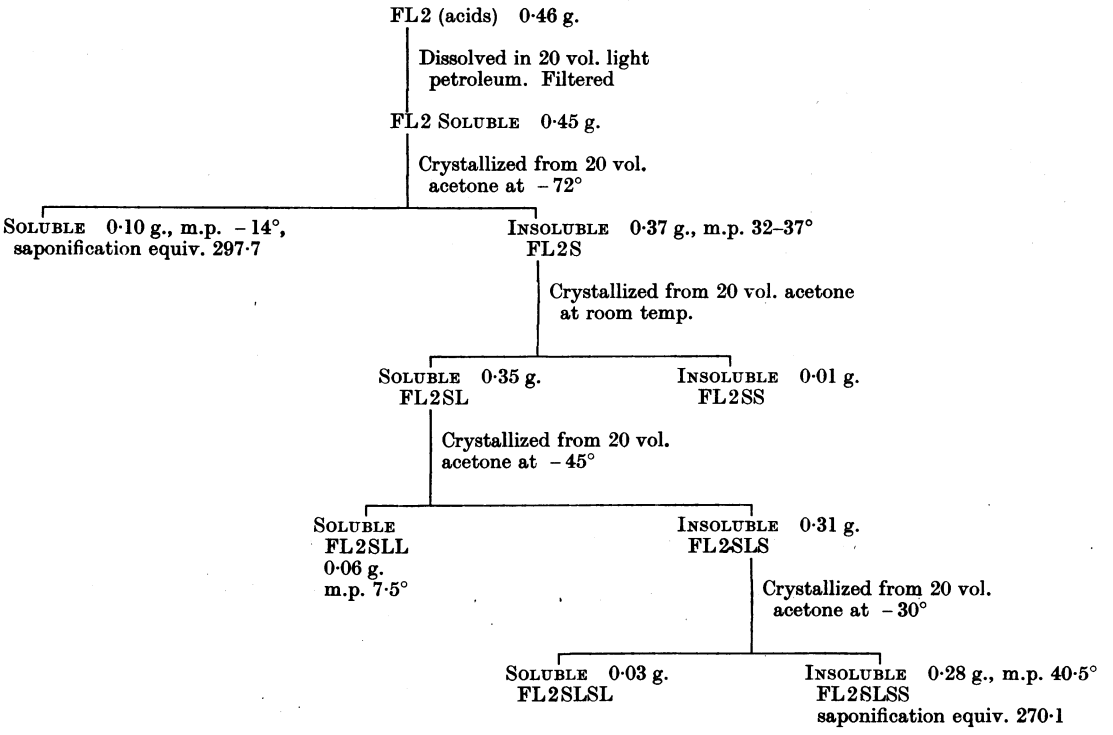

Table 3. *Properties of the* C_{17} *branched-chain fatty acid fraction FL2SLSS*

Fraction	Wt. (g.)	m.p. (°)	Saponification equiv.	Iodine* value (Wijs)	X-ray long spacing (A.)	*C*-Me (%)	Combustion analysis† (%)
FL2SLSS	0·28	40·5	270·1	3·3	33·5	7·0	C 75·5 H 12·5

* $E^{1\%}_{1\,cm.}$ 268 mμ. = 2·2; $E^{1\%}_{1\,cm.}$ 234 mμ. = 5·7.
† Analysis by Dr G. Weiler and Dr F. B. Strauss, Oxford.

6·3 g. (iodine value 2·7) of acetone-soluble 'liquids'. Further separation of 'solid' material was effected by two crystallizations from 10 vol. acetone at −30°, providing 2·7 g. of acetone-soluble 'liquids' (FL) which were fractionated in a micro column. Fractionation data are shown in Table 1.

Fractional crystallization of FL2 acids at varying temperatures, as illustrated in Table 2, resulted in the isolation of fraction FL2SLSS.

X-ray diffraction measurements were made on fraction FL2SLSS, a Philips Geiger X-ray spectrometer being used. Samples were melted on a glass slide and quickly cooled.

The chemical and physical characteristics of fraction FL2SLSS are recorded in Table 3.

DISCUSSION

The results of combustion analysis of the fraction reported in this paper agree closely with the theoretical values required by the formula $C_{17}H_{34}O_2$, namely, C, 75·5, H, 12·7 %. Further confirmation of the molecular weight of the substance is provided by the saponification equivalent of 270·1 which corresponds, within experimental error, with the theoretical value of 270·4 for a C_{17} saturated fatty acid. Evidence for the presence of a side chain is afforded by the *C*-methyl value, which is equivalent to the liberation of 1·26 mol. of acetic acid (cf. Ginger, 1944). Comparison of X-ray diffraction measurements of straight-chain and branched-chain fatty acids distinguishes fraction FL2SLSS, with long spacing 33·5A. (experimental error ± 0·5A.), from normal heptadecanoic acid which has a long spacing of 38·6A. (Francis, Piper & Malkin, 1930.) The long spacing of FL2SLSS does, however, conform with the value of 33·35A. (Arosenius *et al.* 1949) and 33·4A. (Velick, 1947), as reported for C_{17} *iso* and *ante-iso* acids, respectively. The chemical and physical characteristics of the branched-chain acid reported here are almost identical with those of a C_{17} fraction

EuS (saponification equiv. 270·1, m.p. 39·8°, *C*-methyl 8·06 %) recently isolated from butterfat (Hansen & Shorland, 1951). A melting-point determination made on an equimolecular mixture of fractions FL2SLSS from ox suet (m.p. 40·5°) and EuS from butterfat (m.p. 39·8°) gave a value of 40·0°. This evidence is consistent with the two fractions being identical.

In order to confirm the purity of this fraction and to identify conclusively other branched-chain acids whose presence has been indicated by examination of the fractions described in Table 1, further quantities of acetone-soluble 'liquids' from ox fat are in the process of investigation.

Although a comprehensive search for branched-chain acids has not been made on the total fatty acid constituents of ox fat, this investigation indicates that their presence in the C_{18} unsaturated acid fraction alone is of the order of 1 % of the total fatty acids.

SUMMARY

A C_{17} methyl branched-chain saturated fatty acid isomeric with *n*-heptadecanoic acid has been shown to be present in the fat extracted from ox suet.

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The Fate of Certain Organic Acids and Amides in the Rabbit

13. CHLORO- AND FLUORO-BENZOIC ACIDS AND AMIDES

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The primary object of this investigation was to determine the effect of chloro- and fluoro-groups upon the stability *in vivo* of the carbamyl group as it occurs in benzamide. When it was found that all the chloro- and fluoro-benzamides were readily converted into the corresponding acids by the rabbit, there was the added interest of comparing the proportion of the dose excreted conjugated with glucuronic acid when an amide was administered, with that excreted when the corresponding acid was given. It had been noted previously (Bray, Neale & Thorpe, 1946) that a greater proportion of a dose was excreted as benzoylglucuronide when benzoic acid itself was given, than when benzamide was administered. (Benzamide was readily hydrolysed *in vivo* to benzoic acid.) This was subsequently explained as a result of a study of the kinetics of this reaction (Bray, Thorpe & White, 1951).

The metabolism of chloro- or fluoro-benzamides does not appear to have been studied previously, but some investigations of the fate of the acids have been reported. Graebe & Schultzen (1867) isolated a compound which was probably the calcium salt of *m*-chlorohippuric acid from human *m*-chlorobenzoic acid urine. Novello, Miriam & Sherwin (1926) failed to detect glycine conjugation of the chlorobenzoic acids in the rabbit, and found that only the para isomer was excreted as a hippuric acid derivative by the dog. Schübel & Manger (1929) observed that 10–70 % of doses of *p*-chlorobenzoic acid were excreted unchanged by the rabbit. Quick (1932) found that all three isomers were conjugated with both glycine and glucuronic acid in the dog, glycine conjugation occurring to a much smaller extent with the ortho isomer than with the others; the extent of glucuronic acid conjugation was similar for all three. Only *o*-chlorobenzoic acid was excreted unconjugated to an appreciable extent. Hildebrandt (1903) had also found that the rabbit excreted this acid unchanged, and investigated the formation of chlorobenzoic and hippuric acids from chlorotoluenes. *p*-Chlorohippuric acid was isolated as a