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The Isolation of *n*-Pentadecanoic and *n*-Heptadecanoic Acids from Shark (*Galeorhinus australis* Macleay) Liver Oil

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Although the occurrence in natural fats of normal odd-numbered fatty acids, including *n*-pentadecanoic and *n*-heptadecanoic acids, has been reported by various investigators, subsequent work has invariably shown that the acids described were in fact mixtures of adjacent members of even-numbered fatty acids. The generally accepted view until recently is indicated by Hilditch (1947) as follows: 'with the solitary exception of *iso*-valeric acid (found only in the depot fats of the dolphin and porpoise) the molecules of all natural straight-chain fatty acids, saturated or unsaturated, contain an even number of carbon atoms.' This view, however, now requires modification as, after the isolation of pure *n*-heptadecanoic acid from hydrogenated mutton fat (Hansen, Shorland & Cooke, 1954*a*, *b*), *n*-pentadecanoic acid has been found in hydrogenated mutton fat (Hansen, Shorland & Cooke, 1954*c*), in hydrogenated shark-liver oil (Morice & Shorland, 1954) and in butterfat (Shorland, Gerson & Hansen, 1955). In addition, *n*-undecanoic acid was shown to be present in hydrogenated butterfat (Hansen, Shorland & Cooke, 1955); also, by means of liquid–gas partition chromatography (James & Martin, 1952), Hansen & McInnes (1954) demonstrated the occurrence in hydrogenated ox tallow of all the odd-numbered carbon acids from C_3 to C_9 inclusive.

In this paper is described the isolation of *n*-pentadecanoic and *n*-heptadecanoic acids from shark-liver oil by the combined processes of fractional distillation and crystallization without recourse to hydrogenation.

EXPERIMENTAL

The X-ray spacings were determined with a Philips Geiger X-ray spectrometer, Fe-filtered Kα radiation being used. The samples were melted on a glass slide and quickly cooled. Melting points are uncorrected. The specific rotations were determined on pure fractions, except where these melted above 24°. Such fractions were diluted with $CHCl_3$. C and H analyses were by Drs G. Weiler and F. B. Strauss, Oxford.

The oil (saponification equiv. 320·0, iodine value 161·9, unsaponifiable matter 8·4%, acid value 5·2) was prepared by solvent extraction (cf. Shorland, Bruce & Jessop, 1952) of the fresh livers of thirty-one male sharks caught in Cook Strait on 21 June 1944. The crude methyl esters prepared from the oil were distilled under high vacuum (at approx. 0·001 mm.) in a falling-film still at 140–160°, the residue being recycled twice to separate the volatile methyl esters

(6300 g.) from the non-volatile unsaponifiable matter. The final residue thus obtained weighed 800 g. and contained 45·5% unsaponifiable matter.

The methyl esters (6290 g.) were fractionated at 0·1 mm. in a Vigreux column (208 cm. × 6·5 cm.), giving ten fractions denoted A 1–A 10.

n-*Pentadecanoic acid*

Fractional distillation at approx. 0·1 mm. of A 2 (680 g., saponification equiv. 265·0, iodine value 31·2) in a column (150 cm. × 2·4 cm.) packed with stainless-steel gauze rings $\frac{1}{8}$ in. in diameter (Dixon, 1949) resulted in a series of 16 fractions (A 2, 1–16) of which those shown in Table 1 were used for the isolation of *n*-pentadecanoic acid, being subjected to systematic fractionation in column *E* (Shorland, 1952) whereby methyl esters of C_{14}, C_{15} and C_{16} acids were separated. In addition, as in the butterfat sample earlier examined by Shorland *et al.* (1955), the methyl esters of the C_{15} acids were resolved into low melting-point fractions possessing optical activity and high melting-point fractions from which optical activity was absent. These higher melting-point fractions were combined giving 21·5 g. of esters. The corresponding acids (14·0 g.) were crystallized at −40° three times from 40 vol. and once from 100 vol. light petroleum (b.p. 40–60°). The insoluble acids were again crystallized twice from 100 vol. acetone giving 10·2 g. precipitate denoted C, m.p. 52·5–53·0°. For further purification the acids were converted into methyl esters (10·7 g.) and fractionated in column *E* (Shorland, 1952), as shown in Table 2. The acids (1·41 g.) from C2 (Table 2) were crystallized from 20 vol. of acetone at −5°, giving 0·90 g. precipitate (C2I), m.p. 52·5–53·0° and a soluble fraction (C2S), 0·50 g., m.p. 50·5–51·0°. Of the acids from C3, 1·25 g. were similarly crystallized, but at −18°, resulting in 1·04 g. precipitate (C3I), m.p. 52·2–52·7°, and a soluble fraction (C3S), 0·21 g., m.p. 50·0–51·5°. C3I on admixture with authentic pentadecanoic acid, fraction O16S7S (cf. Hansen *et al.* 1954*c*), showed no depression in melting point.

Table 1. *Characteristics of fractions used for isolation of* n-*pentadecanoic acid*

Fraction	Wt. (g.)	Saponification equiv.	Iodine value (Wijs)	M.p.
A 2, 4	27·2	243·6	6·4	15–16°
5	23·6	255·5	14·9	0–4
6	18·1	261·3	19·0	14–15
7	24·7	265·3	65·9	−10–3

Table 2. *Fractionation of methyl esters* (C) *at* 0·1 *mm.*

Fraction	Wt. (g.)	Saponification equiv.	M.p. Esters	M.p. Acids
C1	2·0	255·2	17·9–18·2°	51·0–51·5°
C2	2·4	256·4	18·5–18·7	51·5–52·5
C3	2·3	256·4	18·6–18·8	51·5–52·0
C4	4·0	257·7	18·0–18·2	51·5–52·5

The chemical and physical properties of fraction C3I were as follows: saponification equiv., 243·0; iodine value, 0·13; C, 74·1, H, 12·4% (calc. for $C_{15}H_{30}O_2$: saponification equiv., 242·4; iodine value, 0·0; C, 74·3; H, 12·5%), X-ray long spacing 35·55 Å, n_D^{60} 1·4328.

n-*Heptadecanoic acid*

To obtain a concentrate of methyl esters of C_{17} acids fractions A 3 (667 g., saponification equiv. 277·2, iodine value 36·9) and A 4 (637 g., saponification equiv. 286·9, iodine value 52·4) were refractionated. Fractions A 3, 10 and 11, and A 4, 13–16, whose equivalents approximated to 284, were combined with fractions 1 and 2 obtained from refractionation of A 3, 12, and A 4, 17 (108·8 g.) and denoted D. Crystallization of the acids (99·0 g.) from acetone, using 10 vol. four times at −78° and 15 vol. three times at −40°, gave 30·9 g. insoluble acids (DI). DI was converted into methyl esters (31·8 g.) and fractionated in column *E* (Shorland, 1952) at 0·1 mm. as shown in Table 3.

From previous experience with the isomers of pentadecanoic acid (cf. Shorland *et al.* 1955) it was assumed that methyl *n*-heptadecanoate would follow the methyl esters of the branched-chain fatty acid isomers during fractional distillation. This tendency is indicated by the sequence of the melting points (see Table 3), though the relative constancy of the optical rotation in the various methyl heptadecanoate fractions shows that the resolution of the isomers is incomplete. To isolate methyl *n*-heptadecanoate, therefore, fraction DI, 8 was converted into acids (4·5 g.) and crystallized at −40°, using successively 40 vol. acetone (twice), 100 vol. acetone and finally 40 vol. (twice) of ether. The final precipitate, denoted E (3·3 g.), melted at 60·5–61·0° (undepressed on admixture with authentic heptadecanoic acid), while the soluble portion (0·2 g.) melted at 53–55°. Further purification was effected as shown in Table 4 by fractional distillation at 0·1 mm. of the methyl esters.

Table 3. *Fractional distillation of methyl esters containing mainly* C_{17} *acids*

Fraction	Wt. (g.)	Saponification equiv.	Iodine value (Wijs)	M.p.	$[\alpha]_D^{24}$
DI, 1	2·1	265·6	0·5	24–26°	0·08
DI, 2	3·4	268·7	0·2	29–30	0·12
DI, 3	5·0	273·5	0·9	24–25	0·12
DI, 4	5·3	284·6	1·5	10–11	0·38
DI, 5	1·9	283·3	1·6	9–10	0·44
DI, 6	5·0	284·4	0·9	10–12	0·48
DI, 7	3·4	283·1	1·9	15–19	0·40
DI, 8	5·4	293·9*	6·8	24–25	0·44

* Saponification equiv. of methyl esters freed from unsaponifiable matter, 285·3.

The acids from E2 (0·85 g.) and E3 (0·84 g.) were crystallized from 20 vol. of acetone at −5°, giving insoluble fractions E2I, 0·73 g., m.p. 60·5–61·5°, and E3I, 0·71 g., m.p. 61·0–61·3°. The soluble portions E2S, 0·12 g., and E3S, 0·13 g., melted respectively at 54–58° and 59·0–59·5°.

Table 4. *Purification of methyl* n*-heptadecanoate by fractional distillation*

Fraction	Wt. (g.)	Saponification equiv.	M.p.
E1	1·22	284·2	28·4–29·3°
E2	0·89	284·5	29·7–30·2
E3	0·90	286·7	29·2–30·2
E4	0·44	303·2*	29·7–30·1

* Saponification equiv. of methyl esters freed from unsaponifiable matter, 293·2.

The chemical and physical properties of fraction E3I were as follows: saponification equiv., 270·7; iodine value, 0·92; C, 75·4; H, 12·5% (calc. for $C_{17}H_{34}O_2$: saponification equiv., 270·4; iodine value, 0·0; C, 75·5, H, 12·7%); X-ray long spacing 39·57 Å, n_D^{70} 1·4320.

Fractions 3–7 obtained by refractionation of A3, 12 and A4, 17, and fractions A4, 18 and 19 were combined and crystallized from methanol (40 vol. at −20°), giving 8·96 g. precipitate. This on fractional distillation yielded: (1) 4·4 g., saponification equiv., 284·4, m.p. of acid 60·0–60·5° undepressed by pure heptadecanoic acid, and another fraction (2) 1·9 g., saponification equiv. 289·7, containing by calculation 1·2 g. methyl heptadecanoate. Crystallization of fraction DI, 7 gave 0·7 g., the acid from which melted at 60·0–60·5° (undepressed by pure heptadecanoic acid).

DISCUSSION

The C and H analyses and saponification equivalents of fractions C3I and of E3I correspond to those required respectively for a C_{15} and a C_{17} saturated acid. The melting point of C3I (52·2–52·7°) agrees with those reported for *n*-pentadecanoic acid, 52·1° (Francis, Piper & Malkin, 1930), 53·0° (Levene & West, 1914), 52·3–53·3° (Links & de Groot, 1953), 54·4° (Coops, quoted by Links & de Groot, 1953), 52·4° (Weitkamp, 1945) and 52·26° (Meyer & Reid, 1933). The melting point of E3I (61·0–61·3°) corresponds with the values recorded for *n*-heptadecanoic acid, 61·3° (Francis & Piper, 1939) and 59·0–60·0° (Levene & West, 1914). The X-ray long spacings of C3I (35·55 Å) and of E3I (39·57 Å) respectively approximate to that reported by Slagle & Ott (1933) for pentadecanoic acid (35·75 Å) and with the values of 40·45 and 40·05 Å for heptadecanoic acid reported respectively by Francis & Piper (1939) and by Slagle & Ott (1933). The refractive indices of fractions C3I and E3I of n_D^{60} 1·4328 and n_D^{70} 1·4320, correspond with those given by Dorinson, McCorkle & Ralston (1942) for pure *n*-pentadecanoic (n_D^{60} 1·4329) and *n*-heptadecanoic (n_D^{70} 1·4324) acids respectively.

Whereas normal-even-numbered fatty acids on solidfication adhered closely to the wall of the flask, the acids isolated in this work, on solidification, moved away from the walls of the flask. Francis *et al.* (1930) have noted that the normal-odd-numbered fatty acids shrink away from the walls of the flask.

From these results it is clear that both *n*-pentadecanoic and *n*-heptadecanoic acids occur in the shark-liver oil examined.

The 10·1 g. of practically pure *n*-pentadecanoic acid isolated in the present work, after correction for losses due to manipulation and withdrawal of samples for analysis, becomes 17·1 g. which, based on the weight of fatty acids taken (6010 g.), is 0·28%. Similarly, the weight of pure *n*-heptadecanoic acid isolated (7·8 g.), together with that calculated on the basis of the equivalents from two slightly impure fractions (1·2 g.), after correction, becomes 10·3 g., which is 0·17% of the total fatty acids.

Hansen *et al.* (1954*a*, *c*) estimated *n*-pentadecanoic and *n*-heptadecanoic acids to be present in hydrogenated mutton fat to the extent of 0·15 and 1·2% respectively, while Shorland *et al.* (1955) calculated that the fatty acids of butterfat contained 0·82% pentadecanoic acid. Thus it would appear that the proportions of these acids in shark-liver oil and ruminant fats are comparable. Whereas in mutton fat heptadecanoic acid was present in greater amounts than pentadecanoic acid, in the shark-liver oil examined the reverse was true.

The origin of the odd-numbered carbon fatty acids in the shark-liver oil studied is at present uncertain, but it is perhaps possible that, as in ruminants, the amino acids of the proteins are broken down to lower fatty acids, including propionic acid, which forms valeric acid in the rumen (Gray, Pilgrim, Rodda & Weller, 1951). By the operation of a similar mechanism to that involved in the building up of even-numbered carbon fatty acids, the long-chain odd-numbered carbon fatty acids could be formed by successive addition of acetate units to propionic acid.

SUMMARY

From the liver oil of the New Zealand school shark (*Galeorhinus australis* Macleay) *n*-pentadecanoic and *n*-heptadecanoic acids have been isolated in amounts corresponding respectively to 0·28 and 0·17% of the total fatty acids.

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Studies in the Biochemistry of Micro-organisms

96. THE COLOURING MATTERS OF *PENICILLIUM HERQUEI* BAINIER & SARTORY*

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One of the four major sections—the Biverticillata–Symmetrica—into which Raper & Thom (1949) divide all species in the genus *Penicillium* was designed by them to include species having, among other characteristics, biverticillate conidial structures which are usually symmetrical. They state (p. 558): 'Penicillia can almost invariably be placed here if they produce...aerial hyphae more or less yellow pigmented and encrusted and colony reverse in yellow, orange or red to purplish red shades.'

Workers in this laboratory have isolated in pure crystalline form the colouring matters produced, by a number of well-defined species of Biverticillata–Symmetrica from three of the six series into which this major section is divided. These colouring matters, although often showing clear signs of chemical inter-relationship, belong to a number of different chemical types. Thus stipitatic acid, $C_8H_6O_5$, from the bright-yellow *P. stipitatum* Thom in the *P. luteum* series (Birkinshaw, Chambers & Raistrick, 1942) is β-hydroxytropolone-β'-carboxylic acid, which is pale yellow in colour. Another bright-yellow species in the same series, *P. wortmanni* Klöcker, afforded two crystalline colouring matters—rugulosin, $C_{30}H_{22}O_{10}$, forming bright-yellow cubes or prisms, and skyrin, $C_{30}H_{18}O_{10}$, reddish orange rods (Breen *et al.* 1955). Seven different pigments were isolated from strains of *P. islandicum* Sopp in the *P. funiculosum* series, colonies of which vary in colour from yellow-orange through orange-red to red. They were: islandicin, bright-red plates, $C_{15}H_{10}O_5$, 1:4:5-trihydroxy-2-methylanthraquinone (Howard & Raistrick, 1949); chrysophanol, golden plates, $C_{15}H_{10}O_4$, 4:5-dihydroxy-2-methylanthraquinone (Howard & Raistrick, 1950); skyrin and flavoskyrin, $C_{15}H_{12}O_5$, light-yellow needles, of unknown structure but converted almost quantitatively into chrysophanol on heating (Howard & Raistrick, 1954*a*); iridoskyrin, deep-red plates, $C_{30}H_{18}O_{10}$; rubroskyrin, dark-red plates, $C_{30}H_{22}O_{12}$; and erythroskyrin, dark-red micro-crystals, and the only pigment in this series containing nitrogen, $C_{24}H_{31}O_6N$ (Howard & Raistrick, 1954*b*). Rubroskyrin is readily converted, by treatment with concentrated sulphuric acid at room temperature, into iridoskyrin and, *inter alia*, into islandicin, by thermal decomposition. Finally, rugulosin and skyrin were also isolated from *P. rugulosum* Thom, in the *P. rugulosum* series, colonies of which have yellow to orange-brown, but seldom a red, reverse (Breen *et al.* 1955). It will

* Part 95: Breen, Dacre, Raistrick & Smith (1955).