The Isolation of *iso*- and (+)-*anteiso*-Fatty Acids of the C₁₅ and C₁₇ Series from Shark (*Galeorhinus australis* Macleay) Liver Oil

By ISOBEL M. MORICE AND F. B. SHORLAND

*Fats Research Laboratory, Department of Scientific and Industrial Research,
Wellington, New Zealand*

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Recent investigations in this Laboratory (cf. Shorland, 1956) have shown that trace amounts of a series of saturated even- and odd-numbered *iso*-acids and odd-numbered *anteiso*-acids, together with saturated and Δ^9 -unsaturated *normal* odd-numbered acids, occur in the fats of ruminants. The odd-numbered *normal* acids, including *n*-pentadecanoic and *n*-heptadecanoic acids, have also been found in shark-liver oil (Morice & Shorland, 1955). However, apart from the occurrence of *iso*valeric acid in the fats of dolphins and porpoises (André, 1924) and of a C₁₈ saturated acid with two side methyl groups in shark (*Galeorhinus australis* Macleay) liver oil (Morice & Shorland, 1952), branched-chain fatty acids have not been reported as constituents of non-ruminant fats. In this paper we report the isolation of 13-methyltetradecanoic, (+)-12-methyltetradecanoic, 15-methylhexadecanoic and (+)-14-methylhexadecanoic acids from shark (*G. australis* Macleay) liver oil.

EXPERIMENTAL

Appropriate fractions obtained during high-vacuum fractional distillation of the methyl esters of shark-liver oil previously described (Morice & Shorland, 1955) were used.

C₁₅ branched-chain acids

The C₁₅ branched-chain acids were concentrated by the efficient and systematic fractionation *in vacuo* of fractions A1 (82 g., saponification equiv. 622.6, iodine value 24.1) and A2 (680 g., saponification equiv. 265.0, iodine value 31.2), with a column (150 cm. $\times$ 2.5 cm.) packed with stain-

less-steel gauze rings $\frac{1}{8}$ in. in diameter (Dixon, 1949) and column E (Shorland, 1952). As previously described (Shorland, Gerson & Hansen, 1955), this permitted a partial separation of straight- and branched-chain isomers. The final selection of fractions for the isolation of the branched-chain fatty acids was made from the soluble fractions obtained during the isolation of the corresponding *normal* acids previously described (Morice & Shorland, 1955), and from the lower-boiling-point fractions of methyl esters of C₁₅ acids, giving a total of 19.03 g. of acids, denoted F.

13-Methyltetradecanoic acid. Crystallization of F seven times from 40 vol. of light petroleum (b.p. 40–60°) gave an insoluble fraction FI7 (8.70 g.) and soluble fractions FS1–FS7, total wt. 10.30 g. Fraction FI7 was converted into methyl esters and fractionally distilled, giving five further fractions and a residue. Of these fractions, FI73 [0.91 g., saponification equiv. 255.8, m.p. (acid) 47–48°] and FI74 [1.44 g., saponification equiv. 257.0, m.p. (acid) 47–49°] were combined, denoted G and refractionated by distillation (Table 1).

Fractions G1–G3 were combined, giving 1.79 g., denoted H, and crystallized once from 40 vol. of acetone at –40° and then five times from 40 vol. of light petroleum (b.p. 40–60°) at temperatures from –50 to –70°. Further crystallization of the insoluble fraction HI6 [0.30 g., m.p. 8.0–8.3° from 40 vol. of light petroleum (b.p. 40–60°)] at –70° yielded precipitate HI7 (0.22 g., m.p. 8.5–8.7°) and a soluble fraction HS7 (0.08 g., m.p. 8.1–8.3°). The m.p. was not depressed when HI7 was mixed with authentic methyl 13-methyltetradecanoate, m.p. 8.5–8.7°. The corresponding acid showed m.p. 52.0–52.4°, which was not depressed on admixture with authentic 13-methyltetradecanoic acid, m.p. 52.0–52.4°. When the acid was mixed with authentic *n*-pentadecanoic acid, m.p. 52.2–52.7°, however, the melting point was lowered to 46.0–50.5°.

(+)-12-Methyltetradecanoic acid. The soluble fractions FS1–FS4 were combined, giving 9.89 g., and crystallized systematically from light petroleum (b.p. 40–60°), 5–20 vol. at –40° being used to remove the higher- and lower-melting-point constituents. Those fractions melting within the range 13.0–22.0° were combined, giving 3.33 g., and further purified by similar procedures, as well as by chromatography of the methyl esters on silica gel (Isherwood, 1946). From the resulting fractions those melting within the range –16.0 to –12.0° were combined, giving 1.28 g. and denoted J. Further purification was effected by fractional distillation (Table 2) and crystallization (Table 3).

Fraction J2I3, when mixed with authentic (+)-12-methyltetradecanoic acid, m.p. 22.7–24.5°, melted at 24.0–26.0°.

Table 1. Fractionation of methyl esters G by distillation at 0.1 mm.

Fraction	Wt. (g.)	M.p.	
		Alone	Mixed with 13-methyltetra- decanoate (m.p. 8.5–8.8°)
G1	0.44	3.5–4.5°	4.4–6.2°
G2	1.04	3.5–3.8	5.0–7.5
G3	0.31	1.0–1.4	2.1–2.7
GR	0.26	4.5–5.4	0.5–2.0

Table 2. *Fractionation of methyl esters J by distillation at 0.1 mm.*

Fraction	Wt. (g.)	Saponification equiv.	M.p.	$[\alpha]_D^{15}$ in CHCl_3
J1	0.29	257.1	-12.0 to -10.5°	+2.10°
J2	0.69	259.9	-14.0 to -13.0	+2.78
JR	0.36	269.9	-5.0 to -1.0	—

Table 3. *Purification of acids J2 by crystallization*

Fraction J2 is the acid obtained from methyl ester J2 (Table 2). The fractions were crystallized from 40 vol. of light petroleum at -40°.

Fraction crystallized	Soluble fraction			Insoluble fraction		
	No.	Wt. (g.)	M.p.	No.	Wt. (g.)	M.p.
J2	J2S1	0.26	17.2-20.0°	J2I1	0.42	25.7-26.4°
J2I1	J2S2	0.10	23.7-25.2	J2I2	0.31	26.2-27.0
J2I2	J2S3	0.06	25.2-26.0	J2I3	0.25	26.6-27.3

Table 4. *Fractional distillation of methyl esters KI4 at 0.1 mm.*

Fraction	Wt. (g.)	Saponifica- tion equiv.	M.p.
KI41	1.82	280.6	12-14°
KI42	2.34	283.1	13-15
KI43	1.88	284.4	13-15
KI44	1.20	283.4	12-14
KI4R	1.67	290.4	22-23

Table 5. *Fractional distillation of methyl esters M at 0.1 mm.*

Fraction	Wt. (g.)	Saponification equiv.	M.p.	$[\alpha]_D^{19}$ in CHCl_3
M1	0.30	281.4	1.0-2.4°	+1.93°
M2	0.68	284.4	1.0-1.7	+2.28
M3	0.65	284.9	1.2-1.9	+2.56
MR	0.25	310.6	8.0-13.0	—

C₁₇ branched-chain fatty acids

Fractions A3 (667 g., saponification equiv. 277.2, iodine value 36.9) and A4 (637 g., saponification equiv. 286.9, iodine value 52.4) were refractionated, and fractions whose equivalents approximated to 284 were combined and denoted D as described earlier (Morice & Shorland, 1955). Crystallization of D, 99 g., from 10 vol. of acetone twice at -78° gave a soluble fraction DS1 (15.2 g., iodine value 124.2). Recrystallization of the insoluble fraction DI1 under similar conditions yielded a further soluble fraction DS2 (12.5 g., iodine value 111.7). The precipitate DI2 yielded, after three crystallizations from 15 vol. of acetone at -40°, a precipitate DI3 (30.9 g., iodine value 1.3) and a soluble fraction DS3 (38.5 g., iodine value 84.1).

15-Methylhexadecanoic acid. As mentioned in a previous paper (Morice & Shorland, 1955) DI3 was fractionally distilled to separate methyl *n*-heptadecanoate from the branched-chain isomers. A concentrate of methyl esters of *C₁₇* branched-chain acids denoted K was obtained by combining the appropriate fractions from the distillation of DI3 and of DS3. The acids from K (18.05 g.) were crystallized from light petroleum (b.p. 40-60°) by using successively 60 and 80 vol. at -40°, followed by two crystallizations

with 40 vol. at -20°. The final precipitate KI4 (8.78 g.) melted at 53-55°. The corresponding methyl esters were fractionated at 0.1 mm. (Table 4).

KI42 and KI43 were combined and denoted L. The corresponding acids were repeatedly crystallized successively from light petroleum (b.p. 40-60°), ethyl ether and acetone by the use of 40 vol. of solvent at -40°, finally to yield an insoluble fraction LI9 (2.04 g., m.p. 60.2-60.6°) and a soluble fraction LS9 (0.09 g., m.p. 56.5-57.5°). The m.p. of LI9 was undepressed when the compound was mixed with authentic 15-methylhexadecanoic acid, m.p. 60.2-60.4°, but when mixed with *n*-heptadecanoic acid it was lowered to 53.5-54.5°.

(+)-14-Methylhexadecanoic acid. The soluble fractions KS3 and KS4 from the crystallization of K described above were combined and converted into acids, giving 6.7 g., m.p. 32-42°, $[\alpha]_D^{19} + 1.36^\circ$ in CHCl_3 .

By systematic low-temperature crystallization as described by Hansen, Shorland & Cooke (1952), followed by chromatography of the methyl esters on silica gel (Isherwood, 1946), a series of fractions were obtained. Of these, fractions within the m.p. range 1.0-3.5° and $[\alpha]_D^{19} + 1.72$ -1.82° in CHCl_3 were combined, giving 1.89 g., denoted M and fractionated (Table 5).

Table 6. *Crystallization of fatty acid fraction N at -40°*

Fraction	Conditions of crystallization		Sol. fraction	Wt. (g.)	M.p.	Insol. fraction	Wt. (g.)	M.p.
	Solvent	Vol.						
N	Light petroleum	40	NS1	0.08	20.4-25.1°	NI1	1.18	34.0-35.0°
NI1	Acetone	100	NS2	0.16	32.8-33.2	NI2	1.02	34.0-35.0
NI2	Acetone	100	NS3	0.17	33.0-34.0	NI3	0.84	34.0-35.0
NI3	Ether	40	NS4	0.74	34.0-34.5	NI4	0.10	34.0-37.0

Table 7. *Chemical and physical properties of purified acids*

Fraction	Wt. (g.)	Saponification equiv.	M.p.	Combustion analysis (%)		[α] _D ²⁰ in CHCl ₃	X-ray long spacing (Å)
				C	H		
HI7	0.21	243.6	52.0-52.4°	74.7	12.3*	Nil	29.4
J2I3	0.25	244.3	26.7-28.4	74.5	12.4*	+2.65°	31.1
LI9	2.04	269.6	60.2-60.6	75.9	12.5†	Nil	32.9
NS4I	0.57	270.7	35.3-35.8	75.6	12.4†	+2.81°	30.7

* C₁₅H₃₀O₂ requires C, 74.4, H, 12.5%, saponification equiv. 242.4.† C₁₇H₃₄O₂ requires C, 75.5, H, 12.7%, saponification equiv. 270.4.

Fractions M2 and M3 were combined, converted into acids, 1.28 g., m.p. 33.0-33.5°, denoted N and crystallized (Table 6). Fraction NS4, when mixed with authentic 14-methylhexadecanoic acid, m.p. 34.5-35.5°, melted at 34-35°. Further purification by chromatography of the methyl ester, followed by crystallization of the acid, gave NS4I (0.57 g., m.p. 35.3-35.8°) (Table 7).

The X-ray long spacings were determined with a Philips-Geiger X-ray spectrometer, manganese-filtered iron K α radiation being used. The samples were melted on a glass slide and quickly cooled. Melting points are uncorrected. The optical rotations were determined in 0.5 dm. cells, and unless otherwise stated pure fractions were used. The C and H analyses were by Drs G. Weiler and F. B. Strauss, Oxford.

DISCUSSION

The analytical data in Table 7, together with the evidence of mixed melting points, enable fractions HI7, J2I3, LI9 and NS4I to be identified as 13-methyltetradecanoic, (+)-12-methyltetradecanoic (cf. Hansen, Shorland & Cooke, 1953, 1954), 15-methylhexadecanoic (cf. Hansen, Shorland & Cooke, 1955) and (+)-14-methylhexadecanoic acids (cf. Hansen *et al.* 1952) respectively. The melting point of (+)-12-methyltetradecanoic acid isolated in this work (26.6-27.3°) was somewhat higher than that of the authentic specimen (m.p. 22.7-24.5°; Hansen *et al.* 1954). These latter investigators obtained from butterfats fractions melting at 23.7° and 27.6°, with properties corresponding to those of (+)-12-methyltetradecanoic acid, suggesting the possibility of two modifications of this acid. The values for the optical rotations are somewhat lower than have been found for other naturally occurring *anteiso*-acids. Weitkamp (1945), for example, reported [α]_D²⁰ +4.7° for (+)-12-methyltetradecanoic acid, and [α]_D²⁰ +5.0° in acetone for (+)-14-methylhexadecanoic acid isolated from wool grease. The

lower values found in the present work indicate the probability of racemization of the acids in the living tissues or subsequently during isolation. However, the procedures used were similar to those previously employed.

The proportions of branched-chain fatty acids when present in small amounts are difficult to assess from the amounts isolated, as purification involves considerable losses. Based on the optical rotations of relevant fractions as compared with the purified acids (cf. Shorland *et al.* 1955) the percentages of *anteiso*-acids were calculated as C₁₅, 0.11, and C₁₇, 0.18%. No reliable estimate of the *iso*-acids could be made, but from the amounts isolated it appears that they are present in proportions similar to those of the *anteiso*-acids.

The isolation from shark-liver oil (Morice & Shorland, 1955) of 0.28% of *n*-pentadecanoic and 0.17% of *n*-heptadecanoic acids, together with 0.1-0.2% of *iso*- and *anteiso*-C₁₅ and -C₁₇ acids, indicates the presence of a series of odd-numbered saturated *normal*, *iso*- and *anteiso*-acids, as has been found in the milk and depot fats of ruminants (cf. Shorland, 1956).

In ruminants the odd-numbered *iso*- and *anteiso*-acids in the depot and milk fats were considered by El-Shazly (1952) to be formed by the addition of acetate units to *isovaleric* and (+)-2-methylbutyric acid derived from leucine and isoleucine by the action of bacteria in the rumen. Shorland *et al.* (1955) have suggested that the *normal* odd-numbered fatty acids might similarly arise from propionic and valeric acids present in the rumen. The origin of the odd-numbered *normal*, *iso*- and *anteiso*-acids of shark-liver oil cannot at present be determined. The possibility remains that they could be derived from the lower *normal*, *iso*- and *anteiso*-acids formed by the action of micro-organisms on

the appropriate amino acids or, in common with the greater part of the depot fat of fishes, derived from direct assimilation from the diet (cf. Shorland, 1955).

SUMMARY

The liver oil of the New Zealand school shark (*Galeorhinus australis* Macleay) has been shown to contain traces (0.1–0.2%) of 13-methyltetradecanoic, (+)-12-methyltetradecanoic, 15-methylhexadecanoic and (+)-14-methylhexadecanoic acids.

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Studies in the Biochemistry of Micro-organisms

98. TRITISPORIN, AN ANTHRAQUINONE COLOURING MATTER OF *HELMINTHOSPORIUM TRITICI-VULGARIS* NISIKADO: STRUCTURE AND SYNTHESIS*

By S. NEELAKANTAN, ANNA POCKER AND H. RAISTRICK

Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London

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Helminthosporium tritici-vulgaris Nisikado is a micro-fungus which is pathogenic to wheat (*Triticum vulgare* Vill.), on the leaves of which it produces a yellow-spot disease which occurs fairly frequently in Japan. Nisikado (1929*a, b*) first isolated the fungus from this source and described it. In the course of an extensive investigation on the production of hydroxyanthraquinones by species of *Helminthosporium*, Raistrick, Robinson & Todd (1934) showed that the dried mycelium of laboratory cultures of *H. tritici-vulgaris*, grown on Czapek–Dox solution, contains three such substances, i.e. catenarin, helminthosporin and tritisporin.

Catenarin, $C_{15}H_{10}O_6$, was also isolated from other species of *Helminthosporium*, e.g. *H. gramineum* Rabenhorst by Charles, Raistrick, Robinson & Todd (1933) and *H. catenarium* Drechsler—hence its name—and *H. velutinum* Link by Raistrick *et al.* (1934), who presented evidence which indicated that catenarin is a β -hydroxymethyl-1:4:5-trihydroxyanthraquinone with the β -hydroxymethyl group in position 2, 3 or 6, position 6 being preferred. On this basis catenarin would be 1:4:5-trihydroxy-6-

hydroxymethylanthraquinone (structure I). Later it was proved by Anslow & Raistrick (1940) by analytical methods that catenarin has a CH_3 - and not a $CH_2.OH$ - side chain and has in fact structure (II), and this conclusion was confirmed by the synthesis of catenarin by Anslow & Raistrick (1941).

Tritisporin, $C_{15}H_{10}O_7$, of which only a small amount was available for investigation at the time, was believed by Raistrick *et al.* (1934) to be a derivative of 1:4:5:7-tetrahydroxyanthraquinone, and they suggested that either structure (III) or (IV) should be attributed to tritisporin, which was

* Part 97: Raistrick & Rudman (1956).