847. *Animal Fats. Part X.* Fulmar Oil.*

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The stomach oil of the fulmer petrel is shown to be a triglyceride mixture. Its component acids and the distribution of these between the 2- and the 1- and 3- position of glycerol are reported.

The fulmer petrel (*Fulmarus glacialis*) breed in enormous numbers in St. Kilda and the Orkney and Shetland islands. When disturbed, the bird ejects, as a defensive measure, with considerable force, some of the oil stored in its stomach. This clear yellow oil, also used by the bird for preening and courtship and to calm rough seas when landing on the ocean, has recently been used medicinally [1] and in view of discordant reports [2,3] on the composition of this oil it seemed to merit re-investigation.

Fulmar oil has been described as a liquid wax [2] and as a mixture of triglycerides; [3] the sample of oil used in this investigation, when examined by thin-layer chromatography,[4] behaved as a triglyceride. The glyceryl esters were therefore converted into methyl esters and examined, qualitatively and quantitatively, by gas liquid chromatography using non-polar and polar stationary phases. Over 20 peaks were observed on the two chromatographs and these were identified by comparison of retention times with those quoted by DeWitt [5] and, in some cases, by isolation and examination of individual esters.

Fulmer esters, eluted from a column of silica impregnated with silver nitrate,[6] gave concentrates of saturated (23%), monoenoic (56%), dienoic (1%), trienoic and tetraenoic (2%), and more highly unsaturated esters (18%). The monoenoic esters were subsequently separated according to chain length by preparative gas liquid chromatography and the acid derived from each ester fraction was degraded by von Rudloff oxidation.[7] The degradation fragments showed $16:1^{9}$, $18:1^{9}$, $20:1^{11}$, and $22:1^{11}$ † to be the major monoethenoid acids. These monoethenoid acids were recently shown to be present in the body oil of the Bluefin Tuna.[8] The highly unsaturated esters were similarly separated and oxidised. The C_{20} and C_{22} acids gave glutaric and succinic acids, respectively, as the only identified products suggesting the presence of eicosa-5,8,11,14,17-pentaenoic and docosa-4,7,10,13,16,19-hexaenoic acids. These two acids have been previously recognised in several fish oils.[9]

The quantitative results are given in Table 1 and resemble Lovern's earlier figures. The results obtained after hydrogenation, and those obtained in the preparative separation according to unsaturation, agree with those calculated from the composition of the methyl esters. The close resemblance, qualitative and quantitative, between fulmar oil and other marine oils is probably the result of the fishy diet of these birds and such resemblances have been noted before.[3] Marine oils have long been characterised by the high average unsaturation of their C_{20} and C_{22} acids, but now, through the use of gas liquid chromatography,

* Part IX, *J. Sci. Food Agric.*, 1957, **5**, 290.

† The symbol $16:1^{9}$ refers to the C_{16} acid with one double bond in the Δ^{9} position; other symbols of this type are interpreted in a similar way.

[1] Private communication from Dr. A. C. Gordon Ross (Glasgow).
[2] Rosenheim and Webster, *Biochem. J.*, 1927, **21**, 111.
[3] Lovern, *Biochem. J.*, 1938, **32**, 2142.
[4] Malins and Mangold, *J. Amer. Oil Chemists' Soc.*, 1960, **37**, 383, 576.
[5] DeWitt, *J. Sci. Food Agric.*, 1963, **14**, 92.
[6] Gunstone and Sealy, *J.*, 1963, 5772.
[7] von Rudloff, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 126.
[8] Roubal, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 213, 215.
[9] Whitcutt and Sutton, *Biochem. J.*, 1956, **63**, 469; Whitcutt, *J.*, 1957, **67**, 60; Klenk, with Eberhagen, *Z. physiol. Chem.*, 1957, **307**, 42; with Tomuschat, *ibid.*, 1957, **308**, 165; with Brucker-Voigt, *ibid.*, 1961, **324**, 1; with Montag, *Annalen*, 1957, **604**, 4; Stoffel and Ahrens *J. Lipid Res.*, 1960, **1**, 139.

TABLE 1.

Ester	Carbon numbers [10] Ap. L	PEGA	% (wt.)	Hydrogen % (wt.)	Lovern [3]
14 : 0	14·0	14·0	3·3	3·1	2·0
14 : 1	13·8	14·4	0·3	—	0·9
15 br	14·4	14·4	0·4	0·4	—
15 : 0	15·0	15·0	0·4	Tr	—
16 : 0	16·0	16·0	14·5	22·6	13·9
16 : 1	15·8	16·4	6·1	—	3·9
16 : 2	15·5	16·9	1·0	—	—
17 br	16·45	16·4	0·5	0·6	—
17 : 0	17·0	17·0	0·7	0·7	—
18 : 0	18·0	18·0	2·3	18·4	3·2
18 : 1	17·7	18·4	12·8	—	26·9(−2·8H)
18 : 2	17·5	18·9	0·2	—	
18 : 3	17·5	19·5	Tr	—	
18 : 4	17·2	19·9	2·3	—	
19 : 0	19·0	19·0	Tr	—	—
20 : 0	20·0	20·0	0·2	28·0	—
20 : 1	19·75	20·4	16·6	—	26·8(−4·0H)
20 : 3	19·2	20·9	0·2	—	
20 : 5	19·0	22·1	11·0	—	
22 : 0	22·0	22·0	—	26·2	—
22 : 1	21·65	22·4	18·2	—	22·1(−6·6H)
22 : 5	20·75	23·8	1·0	—	
22 : 6	20·55	24·0	8·0	—	

Iodine value of methyl esters 148 (observed), 137 (calculated from above composition), Lovern quotes 130 for the oil he examined.

TABLE 2.

	Triglyceride (% mol.)	2-Monoglyceride (% mol.)	Enrich. factor		Triglyceride (% mol.)	2-Monoglyceride (% mol.)	Enrich. factor
14 : 0 *	4·2	12·9	3·07	18 : 1	13·2	7·2	0·55
15 : 0	0·5	0·5	—	20 : 1	15·6	4·4	0·28
16 : 0	16·5	27·0	1·64	22 : 1	15·7	4·8	0·31
17 : 0	0·8	0·4	—	16 : 2	1·1	3·2	2·91
18 : 0	2·4	0·5	0·21	18 : 4	2·4	3·8	1·58
20 : 0	0·2	0	—	20 : 3	0·2	0·5	—
14 : 1	0·4	0·1	—	20 : 5	10·6	11·8	1·11
16 : 1	7·0	10·3	1·47	22 : 5	0·9	2·0	2·22
				22 : 6	7·1	11·5	1·62

* Some minor components have been omitted.

TABLE 3.

Source *	Polyethenoid acids (%-mol.)	Enrichment factor 22 : 6	20 : 5	14 : 0	16 : 0	16 : 1	18 : 1	20 : 1	22 : 1
a	9	2·54	2·14	1·50	1·53	0·84	0·38	0·69	0·75
b	11	1·94	2·33	1·80	1·83	0·86	0·58	0·69	0·60
c	12	2·00	1·80	2·00	1·04	1·62	1·07	0·22	0·60
d	17	2·25	2·12	1·15	1·13	0·88	0·83	0·40	0·20
e	20	1·67	2·14	1·50	1·11	1·05	0·74	0·43	0·33
f	20	2·00	1·70	1·29	1·00	0·89	0·67	0·60	0·43
g	22	2·08	1·46	0·71	0·57	1·29	1·00	0·68	0·33
h	22	1·62	1·11	3·07	1·64	1·47	0·55	0·28	0·31
i	23	2·15	2·42	1·50	0·46	1·42	1·26	0·82	0·67
j	23	1·84	1·00	1·42	0·89	0·86	0·91	1·00	1·29
k	31	1·76	1·00	1·67	1·16	0·93	0·43	0·45	0·60
l	32	1·94	1·05	1·40	1·18	0·88	0·35	0·60	0·20
m	42	1·59	1·50	0·60	0·93	0·84	0·88	0·94	0·23
n	12	0·50	0·60	1·33	0·87	1·47	1·30	0·35	0·12
o	17	—	0·38	1·71	1·29	1·47	1·10	0·29	0·30
p	22	—	0·30	2·20	1·55	1·56	1·18	0·37	0·14

* (a) Cod liver, (b) halibut liver (Atlantic), (c) seal liver, (d) herring, (e) eel, (f) halibut liver (Pacific), (g) salmon, (h) fulmar petrel, (i) scallop muscle, (j) cod muscle, (k) skate liver, (l) pilchard, (m) lobster liver, (n) whale blubber, (o) seal oil, (p) seal oil.

[All from reference 11, except (sourcc h).]

it is possible to recognise individual polyenoic acids. Our results, along with others,[5,8,9,11,12] indicate that marine oils contain palmitic acid as the major saturated acid, that most, if not all, contain C_{16}, C_{18}, C_{20}, and C_{22} monoethenoid acids, and that the polyethenoid acids are mainly 20 : 5 and 22 : 6, accompanied sometimes by 22 : 5 and, less commonly, by 20 : 4. The polyethenoid acids are mainly of the linolenic class, *i.e.*, they are methylene-interrupted acids containing the end group $CH_3 \cdot CH_2 \cdot CH{:}$.

We have also examined the lipolysis of the fulmar glycerides with pancreatic lipase.[13] The 2-monoglycerides resulting from lipolysis were isolated, converted into methyl esters, and examined by gas liquid chromatography. The results are given in Table 2 along with our suggested enrichment factor. This we define as the ratio of the molar concentration of an acid at C-2 to the molar concentration of that acid in the triglycerides. Enrichment factors between 0 and 1 indicate that the acid is being concentrated at the α (C-1 and C-3) positions; factors between 1 and 3 show enrichment at the β (C-2) position.

These results are in general agreement with those of Brockerhoff *et al.*[11] on other marine triglycerides (Table 3). The polyenoic acids (22 : 6 and 20 : 5) and the lower saturated acids (14 : 0 and 16 : 0) are concentrated at the β-position; the monoethenoid acids, in particular the C_{20} and C_{22} acids, are concentrated at the α-positions. These positional specificities are marked and consistent throughout the available results with the exception of the blubber oils of marine mammals.

Experimental

Light petroleum had boiling range 40—60°. Gas liquid chromatography was carried out with a Pye Argon chromatograph incorporating 4-ft. columns with Apiezon L (10%), QF-1 (10%), or poly(ethylene glycol adipate) (5%) as stationary phase on Celite; these were used at temperatures between 100 and 200° with an argon flow-rate of 33·3 ml./min. and a detector voltage of 1000 v. Preparative gas liquid chromatography was effected with a Perkin-Elmer fractometer fitted with Apiezon L (20%) column, operating at 220°, and using a catharometer detector. The eluate was collected in U-tubes packed with cotton wool and kept at −40°.

Hydrolysis was effected by boiling ethanolic potassium hydroxide (0·5 N, 100% excess) in an inert atmosphere.

Thin-layer Chromatography.—Chloroform solutions (10%; 10 μl.) of fulmar oil, a triglyceride, and an ester wax were applied to a thin layer (275 μ) of Merck's silica gel G and developed with light petroleum–ether–acetic acid (90 : 10 : 1). When the spots were made visible by exposure to iodine vapour it was clear that the fulmar oil was behaving as triglyceride and not like an ester wax.

Conversion of Fulmar Oil into Methyl Esters.—The oil (2 g.), collected during 1962 at the Bird Observatory, Fair Isle, Shetland, was hydrolysed, and the fatty acids (1·8 g.), free from unsaponifiable material (4%), were recovered in the usual way and converted into methyl esters (1·8 g.) with boiling methanolic hydrogen chloride (0·5N, 20 ml.). The esters were examined qualitatively and quantitatively, before and after catalytic hydrogenation (10% Pd/C), by gas liquid chromatography with the results given in Table 1.

Isolation of Individual Components.—A mixture of Celite (5 g.) and silica impregnated with silver nitrate (10 g.) [6] was introduced into a chromatographic tube along with light petroleum. Fulmar esters (250 mg.), placed on this column, were eluted with light petroleum containing increasing amounts of ether. Saturated esters (53 mg.; 23%) were eluted with 1% ether, monoenoic esters (127 mg.; 56%) with 3% ether, dienoic esters (3 mg.; 1%) with 5% ether trienoic and tetraenoic esters (6 mg.; 2%) with 10% ether, and pentaenoic and hexaenoic esters

[10] Woodford and van Gent, *J. Lipid Res.*, 1960, **1**, 188.
[11] Brockerhoff, Ackman, and Hoyle, *Arch. Biochem. Biophys.*, 1963, **100**, 9; Brockerhoff and Hoyle, *ibid.*, 1963, **102**, 452.
[12] Kayama, Tsuchiya, Nevenzel, Fulco, and Mead, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 499; Reiser, Stevenson, Kayama, Choudhury, and Hood, *ibid.*, p. 507.
[13] Coleman, *J. Amer. Oil Chemists' Soc.*, 1961, **38**, 685.

(40 mg.; 18%) with 100% ether. This fractionation was monitored by gas liquid chromatography.

The monoenoic esters (127 mg.) were separated by preparative gas liquid chromatography into C_{16} (9 mg.), C_{18} (15 mg.), C_{20} (21 mg.), and C_{22} (20 mg.) fractions. Difficulties were encountered with the polyenoic esters (40 mg.) which gave only poor yields of pentaenoic (3 mg.) and hexaenoic (4 mg.) fractions.

von Rudloff Oxidation of Olefinic Acids.[7]—To the olefinic acid (10 mg.), dissolved in aqueous potassium carbonate (180 mg. in 5 ml.), was added a solution of potassium periodate (70 mg.) and potassium permanganate (1 mg.) in water (20 ml.). The quantity of oxidant for the penta- and hexa-enoic acids was increased five and six times, respectively. The mixture was shaken at room temperature for 24 hr., excess of oxidant was reduced by sulphur dioxide, and the volume of the neutralised solution was reduced to one half on a rotary film evaporator. The solution was acidified, saturated with sodium chloride, and extracted with ether. The recovered acids (9 mg.) were esterified and examined by gas liquid chromatography on Apiezon L and QF-1 columns.

Each acid oxidised gave the products indicated in parentheses: 16 : 1 (heptanoic and azelaic acid), 18 : 1 (nonanoic and azelaic acid), 20 : 1 (nonanoic and undecanedioic acid), 22 : 1 (undecanoic and undecanedioic acid), 20 : 5 (glutaric acid), and 22 : 6 (succinic acid).

Lipolysis of Fulmar Oil.[13]—Fulmar oil (1 g.) was dispersed in an ammonium chloride–ammonium hydroxide buffer (1·2M; 10 ml.; pH 8·5) to which was added a solution of calcium chloride (22%; 2·0 ml.), sodium taurocholate (10 mg.), and pork pancreatic lipase (100 mg.) which had been homogenised with acetone, centrifuged, and dried in a vacuum desiccator. During hydrolysis at 40° the pH was kept at 8·5 by continuous addition of ammonia (s.g. 0·88) from a burette. When about two thirds of the acids had been liberated the pH was adjusted to 1·0 with 10N-hydrochloric acid. The ether extract (3 × 30 ml.), after passing through a column of IR400 " Amberlite " resin (30 g.) to remove free fatty acids, gave neutral glycerides (500 mg.). These were separated on a column (400 × 19 mm.) of silica gel (30 g.; Davidson Grade 923; 100—200 mesh). The gel had been kept at 120° overnight and water (5%) had been added thereafter. Triglycerides (4 mg.) were eluted with benzene, diglycerides (121 mg.) with benzene containing 10% of ether, and monoglycerides (269 mg.) with ether. This last fraction was hydrolysed, esterified, and examined by gas liquid chromatography (see Table 2).

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