REFERENCES

- Auerbach, C. & Robson, J. M. (1942). Report to Ministry of Supply, 4 June.*
- Auerbach, C. & Robson, J. M. (1946). *Nature, Lond.*, **157**, 302.
- Bell, E. V., Bennett, G. M. & Hock, A. L. (1927). *J. chem. Soc.* p. 1803.
- Berenblum, I. (1940a). Report to Ministry of Supply, 19 June.*
- Berenblum, I. (1940b). Report to Ministry of Supply, 15 July.*
- Berenblum, I. & Schoental, R. (1947). *Nature, Lond.*, **159**, 727.
- Berenblum, I. & Wormall, A. (1939). *Biochem. J.* **33**, 75.
- Bournsnell, J. C., Francis, G. E. & Wormall, A. (1946). *Biochem. J.* **40**, 737.
- Cashmore, A. E. & McCombie, H. (1923). *J. chem. Soc.* **123**, 2884.
- Clarke, H. T. (1912). *J. chem. Soc.* **101**, 1583.
- Claudatus, I. (1939). *Mikrochemie*, **126**, 305.
- Cohen, B. & Harris, J. (1942). U.S. National Defence Research Council Report, 25 April.*
- Creeth, J. M., Gulland, J. M. & Jordan, D. O. (1947). *J. chem. Soc.* p. 1141.
- Davidson, J. N. & Waymouth, C. (1944-5). *Nutr. Abstr. Rev.* **14**, 1.
- Doering, W. E., Linstead, R. P. & Ball, E. G. (1942). Report to Ministry of Supply, 9 Dec.*
- Embden, G. (1921). *Hoppe-Seyl. Z.* **113**, 138.
- Fell, H. B. & Allsopp, C. B. (1943). Report to Ministry of Supply, 27 July.*
- Fletcher, W. E., Gulland, J. M. & Jordan, D. O. (1944). *J. chem. Soc.* p. 33.
- Fletcher, W. E., Gulland, J. M., Jordan, D. O. & Dibben, H. E. (1944). *J. chem. Soc.* p. 30.
- Ford-Moore, A. H. (1940). Report to Ministry of Supply, 23 Jan.*
- Frampton, V. L. (1939). *J. biol. Chem.* **129**, 233.
- Gulland, J. M. (1944). *J. chem. Soc.* p. 208.
- Gulland, J. M. (1947). *Symposia Soc. exp. Biol.* **1**, 1.
- Gulland, J. M., Barker, G. R. & Jordan, D. O. (1945). *Ann. Rev. Biochem.* **14**, 175.
- Gulland, J. M., Jordan, D. O. & Taylor, H. F. W. (1947). *J. chem. Soc.* p. 1131.
- Gulland, J. M., Jordan, D. O. & Threlfall, C. J. (1947). *J. chem. Soc.* p. 1129.
- Helfrich, O. B. & Reid, E. E. (1920). *J. Amer. chem. Soc.* **42**, 1208.
- Hoffmann, W. S. & Osgood, B. (1938). *J. biol. Chem.* **124**, 347.
- Hughes, E. D. (1941). *Trans. Faraday Soc.* **37**, 603.
- Jordan, D. O. & Taylor, H. F. W. (1946). *J. chem. Soc.* p. 994.
- Koller, P. C. (1943). Report to Ministry of Supply by J. M. Robson, October.*
- Lawson, W. E. & Reid, E. E. (1925). *J. Amer. chem. Soc.* **47**, 2821.
- Mohler, H. & Hartnagel, J. (1941). *Helv. chim. Acta*, **24**, 564.
- Moore, S., Stein, W. H. & Fruton, J. S. (1946). *J. org. Chem.* **11**, 675.
- Niederl, J. B. & Niederl, V. (1938). *Micromethods of Quantitative Organic Elementary Analysis*. New York: John Wiley and Sons.
- Ogston, A. G. (1941). Report to Ministry of Supply, 14 July.*
- Robson, J. M. (1943). Biochemical Subcommittee, Chemical Board, Ministry of Supply, 1 July.*
- Rydon, H. N. (1943a). Report to Ministry of Supply, 19 Feb.*
- Rydon, H. N. (1943b). Report to Ministry of Supply, 12 Oct.*
- S.D. (1942). Min. of Home Security Booklet. *S.D. Method for the Detection of Mustard Gas Vapour*. London: H.M.S.O.
- Sevag, M. G., Lackmann, D. B. & Smolens, J. (1938). *J. biol. Chem.* **124**, 425.
- Watson, H. B. (1938). *Rep. Progr. Chem.* **38**, 208.

* Information available on application to Ministry of Supply, London.

The Component Acids of Herring Visceral Fat

BY T. P. HILDITCH AND S. P. PATHAK

Department of Industrial Chemistry, University of Liverpool

(Received 25 July 1947)

The component acids of North Sea herring flesh fats laid down at different seasons, including those of intensive feeding when much fat is deposited, and those of relative inanition when the body fat content falls to a low level, have been discussed by Lovern (1938). Similar data for the flesh fat of Icelandic herring have been given by Bjarnason & Meara (1944). The component acids of fats from other parts of the herring

have not been studied in detail hitherto. Some time ago Mr J. R. Bruce, of the Marine Biological Station, Port Erin, Isle of Man, sent to us a quantity of oil extracted from the viscera of herrings taken locally, and we have now had an opportunity to examine the nature of the component acids of the visceral fat, which displays interesting differences from the characteristic composition of herring flesh fats.

METHODS

The visceral oil studied was from herrings caught off the south-western coast of the Isle of Man during the first week of August, 1942. The herrings had been gutted by hand in a local factory prior to conversion into kippers. The offal would have contained intestines, stomach, liver, mesentery, etc., and also the milt or roe, together with a very small number of heads and of broken fish (these forming, however, only a minute percentage of the whole). It was rendered by boiling with water in the Port Erin laboratory. The separated oil was filtered and then sent to us. It was stored in a full bottle in a refrigerator for a long period before we had an opportunity to examine it, but had remained fresh and free from oxidation. The oil was pale yellow in colour, had saponification equivalent 296.2 and iodine value 146.8, and contained 5.7% of free fatty acid (as oleic) and 0.75% of unsaponifiable matter.

Its component acids were determined by fractional distillation in a vacuum of their methyl esters through an electrically-heated packed column. Before conversion to esters the mixed acids from 205 g. of the oil were first resolved into three groups by crystallization from acetone and from ether at low temperature. It has been shown recently (Hilditch & Pathak, 1947) that low temperature crystallization from solvents affords much more rapid and efficient preliminary separation of the mixed acids of marine animal oils than the procedures hitherto employed, in which use was made successively of the different solubility of their lithium salts in acetone and of their lead salts in ethanol (Hilditch & Maddison, 1942; Bjarnason & Meara, 1944).

In the present instance the herring visceral oil mixed fatty acids (190 g.), after removal of the bulk of the unsaponifiable matter present, were first crystallized from acetone (1900 ml.) at -60° for 5 hr., when 66.9 g. (iodine value 277.0) remained in solution; recrystallization of the deposited acids under the same conditions left a further 17.7 g. (iodine value 236.1) in solution. These two soluble portions were united as fraction *C* (84.6 g., iodine value 268.4). The portion (105 g.) which had remained insoluble in acetone at -60° was then crystallized from ether (1050 ml.) at -30° for 5 hr. when 53.8 g. (*B*, iodine value 89.8) remained in solution and 51.2 g. (*A*, iodine value 23.9) deposited. Nearly all the polyethenoid constituents of the acids were thus obtained in the most soluble portion *C*, whilst the low iodine value of the least soluble portion *A* indicated that only monoethenoid acids were present therein:

Fraction	(g.)	(%)	Iodine value
<i>A</i> Insoluble in ether at -30°	51.2	27.0	23.9
<i>B</i> Soluble in ether at -30°	53.8	28.4	89.8
<i>C</i> Soluble in acetone at -60°	84.6	44.6	268.4

Each group of acids was separately converted into methyl esters, which were fractionally distilled as described above, the most unsaturated group *C* being dealt with first, fractionation and analysis of the ester fractions being carried out as rapidly as possible to minimize chances of autoxidation and polymerization. From the iodine values and equivalents of the ester fractions the composition of the latter was calculated by methods which have been described elsewhere (Hilditch, 1947). The mean unsaturation, ex-

pressed by the fractional number of hydrogen atoms short of saturation, e.g. -2.0 (monoethenoid), was determined as usual by interpolation or extrapolation from the respective ester fractions in each of the groups *B* and *C*, from which the mean equivalent of each of the homologous ester groups (C_{16} , C_{18} , C_{20} and C_{22}) followed (the values employed are listed in Table 2).

RESULTS

The ester-fractionation data obtained for each of the three groups of herring visceral oil fatty acids are summarized in Table 1.

Table 1. *Fractionation of methyl esters of herring visceral oil acids A, B and C*

Fraction	(g.)	Saponification equivalent	Iodine value
Methyl esters of acids <i>A</i>			
<i>A</i> 1	2.50	246.0	0.8
<i>A</i> 2	2.64	250.8	0.9
<i>A</i> 3	3.68	263.6	0.9
<i>A</i> 4	3.89	268.0	1.0
<i>A</i> 5	4.72	268.3	1.2
<i>A</i> 6	5.41	268.5	1.3
<i>A</i> 7	4.44	268.9	1.5
<i>A</i> 8	4.23	270.5	3.0
<i>A</i> 9	4.30	292.0	29.1
<i>A</i> 10	3.74	301.6	58.3
<i>A</i> 11	4.45	302.1	71.3
<i>A</i> 12	4.95	310.9*	68.7
	48.95		
Methyl esters of acids <i>B</i>			
<i>B</i> 1	3.34	247.8	5.4
<i>B</i> 2	3.59	252.8	31.4
<i>B</i> 3	4.56	281.3	73.1
<i>B</i> 4	4.65	292.3	87.9
<i>B</i> 5	4.72	293.3	88.9
<i>B</i> 6	5.10	297.7	93.8
<i>B</i> 7	4.83	299.1	102.3
<i>B</i> 8	4.94	301.3	107.0
<i>B</i> 9	5.13	302.1	113.7
<i>B</i> 10	4.36	303.5	118.2
<i>B</i> 11	2.25	305.9	115.4
<i>B</i> 12	3.28	320.9*	112.2
	50.75		
Methyl esters of acids <i>C</i>			
<i>C</i> 1	3.40	250.5	85.9
<i>C</i> 2	3.94	266.4	123.8
<i>C</i> 3	3.97	271.4	128.8
<i>C</i> 4	3.82	277.8	134.4
<i>C</i> 5	4.04	290.1	156.7
<i>C</i> 6	5.44	301.8	158.2
<i>C</i> 7	5.38	307.5	212.2
<i>C</i> 8	6.24	313.5	318.4
<i>C</i> 9	5.20	319.4	346.4
<i>C</i> 10	4.86	327.3	362.9
<i>C</i> 11	4.03	332.4	377.4
<i>C</i> 12	5.31	335.9	371.8
<i>C</i> 13	4.47	351.4*	308.7
	60.10		

* Equivalents of esters (freed from unsaponifiable matter): *A* 12, 307.4; *B* 12, 314.8; *C* 13, 340.8.

Table 2. *Equivalents, iodine values and mean unsaturation employed in calculating the composition of unsaturated methyl-ester fractions from groups B and C*

Methyl esters of acids	B			C		
	Saponification equivalent	Iodine value	Mean unsaturation	Saponification equivalent	Iodine value	Mean unsaturation
C ₁₄	240.0	105.8	-2.0	240.0	105.8	-2.0
C ₁₆	268.0	94.8	-2.0	267.4	123.5	-2.6
C ₁₈	295.9	90.1	-2.1	294.3	159.7	-3.7
C ₂₀	321.3	185.8	-4.7	317.6	335.0	-8.4
C ₂₂	—	—	—	343.5	388.3	-10.5

The percentage composition (by weight) of each group of acids A, B, C was then calculated (Table 3) and therefrom that of the total fatty acids of the herring intestinal oil can be obtained (Table 4).

Table 3. *Composition (% w/w) of the acids in groups A, B and C*

Acid	A (%)	B (%)	C (%)
Myristic	11.2	7.6	1.4
Palmitic	53.1	4.6	—
Stearic	9.2	1.2	—
Arachidic	1.2	—	—
Unsaturated C ₁₄	Trace	1.4	2.3
Unsaturated C ₁₆	1.2	6.5	18.6
Unsaturated C ₁₈	17.5	62.3	20.8
Unsaturated C ₂₀	6.5	16.3	35.9
Unsaturated C ₂₂	—	—	20.8
Unsaponifiable	0.1	0.1	0.2

Table 4. *Component acids of herring visceral oil*

Acid	Fatty acids					
	A (27.0%)	B (28.4%)	C (44.6%)	Total	(%, w/w)	(%, by mol.)
Myristic	3.0	2.2	0.6	5.8	5.8	7.1
Palmitic	14.4	1.3	—	15.7	15.7	17.0
Stearic	2.5	0.3	—	2.8	2.8	2.7
Arachidic	0.3	—	—	0.3	0.3	0.3
Unsaturated C ₁₄	—	0.4	1.0	1.4 (-2.0)	1.4	1.7
Unsaturated C ₁₆	0.3	1.9	8.3	10.5 (-2.5)	10.5	11.5
Unsaturated C ₁₈	4.7	17.7	9.3	31.7 (-2.6)	31.8	31.4
Unsaturated C ₂₀	1.8	4.6	16.0	22.4 (-7.1)	22.4	20.4
Unsaturated C ₂₂	—	—	9.3	9.3 (-10.5)	9.3	7.9
Unsaponifiable	Trace	Trace	0.1	0.1	—	—

DISCUSSION

The composition of the herring visceral oil shows marked differences both from that of body (flesh) fats of North Sea herrings (Lovern, 1938) and of Iceland herrings (Bjarnason & Meara, 1944), and from that of the fat in one of the main foods of the herring, the copepod *Calanus finmarchicus* (Lovern, 1935). These differences are illustrated in Table 5.

Mr W. C. Smith, of Port Erin Marine Biological Station, has informed us that *Calanus* is an important item in the diet of herring in Manx waters, and also reminded us that of course the fish do not feed actively at or near the spawning season (early autumn).

The circumstance that the specimen of visceral fat studied probably contained an uncertain amount of herring milt and roe introduces a possibly complicating factor, since Lovern (private communication

to the authors) has observed that herring roe glycerides have iodine values of 170 and somewhat higher, and thus may contain large proportions of C₂₀ and C₂₂ acids of high unsaturation (Lovern, unfortunately, was unable to undertake full analyses of the roe component acids). Since, however, Manx herring at the time of year concerned often include a fair proportion of immature fish, it is on the whole likely that the proportion of milt and roe fat in the total visceral fat which we have examined may not have been very substantial, and that the component acid data in Table 5 represent for the most part those for the fats of the herring viscera.

The chief differences between these visceral fatty acids and those of the herring body fats are in the much lower content of unsaturated C₂₂ acids in the former, coupled with much higher mean unsaturation of the acids of both the C₂₂ and C₂₀ series as compared with those of the body fats; further, the pro-

portion of unsaturated C_{18} acids is much larger in the visceral than in the body fats, although their mean unsaturation is considerably lower. Subordinate differences may include a somewhat lower content of unsaturated C_{20} acids, and somewhat higher contents of total saturated acids and, perhaps, of unsaturated C_{16} acids as well as of palmitic acid.

limits in each case, whilst the only significant differences in proportions of acids present are a larger amount of unsaturated C_{18} and a smaller amount of unsaturated C_{22} acids.

On the other hand, although the mean unsaturation of the various groups (except in the C_{18} acids) is much the same in herring visceral fat and in the

Table 5. *Component acids (% w/w) of herring body and visceral fats and of copepod fat*

	Body (flesh) fats				
	North Sea			Visceral fat Isle of Man (August)	Copepod fat (<i>Calanus fin- marchicus</i>)
	July	October	Iceland		
Iodine value	152.5	138.6	140.0	146.8	177.6
Component acids:	(%)	(%)	(%)	(%)	(%)
Myristic	8.3	7.3	7.1	5.8	8.3
Palmitic	12.1	13.0	11.7	15.7	10.6
Stearic	0.3	Trace	0.8	2.8	1.3
Arachidic	—	—	0.1	0.3	—
Unsaturated C ₁₄	0.5	0.8	1.2	1.4	1.6
Unsaturated C ₁₆	6.4	4.9	11.8	10.5	11.8
Unsaturated C ₁₈	21.0	20.7	19.6	31.8	16.8
Unsaturated C ₂₀	28.3	30.1	25.9	22.4	24.5
Unsaturated C ₂₂	23.1	23.2	21.7	9.3	25.1
Mean unsaturation of:					
Unsaturated C ₁₄	-2.0	-2.0	-2.0	-2.0	-2.0
Unsaturated C ₁₆	-3.4	-2.7	-2.4	-2.5	-2.4
Unsaturated C ₁₈	-4.5	-4.2	-3.5	-2.6	-5.1
Unsaturated C ₂₀	-5.5	-4.6	-5.2	-7.1	-7.8
Unsaturated C ₂₂	-4.6	-4.3	-4.3	-10.5	-8.1

Lovern (1938) has suggested that characteristics of herring body fats include an unusually high content of unsaturated C_{22} acids, unusually low unsaturation in the C_{20} and C_{22} acids, and unusually high unsaturation in the C_{16} and C_{18} acids, with lower contents than usual of unsaturated C_{16} acids (although the amounts of total C_{16} acids fall within the usual range). These conclusions were based on his previous statistical survey of the average composition of marine fish fats (Lovern, 1937), in which, however, the data referred to many more liver fats than body fats from marine fish. The statistical data indicated the following approximate amounts and unsaturation of unsaturated acids as being characteristic for marine fish in general:

	(%)	Mean unsaturation
Unsaturated C_{16}	10	-2.0 to -2.5
Unsaturated C_{18}	25	-2.6 to -3.3
Unsaturated C_{20}	25	-5.5 to -6.5
Unsaturated C_{22}	15	-7.0 to -9.5

Turning to the herring visceral fat now under discussion, it will be seen that its component acids align themselves much more closely, both in their proportions and in their mean unsaturation, with Lovern's 'average values' for marine fish fats than with the observed values for herring flesh (depot) fats. The mean unsaturation for the various groups falls within or approximates to Lovern's average

dietary *Calanus* fat, the respective differences in content of unsaturated C_{18} and unsaturated C_{22} acids are as well marked, or even more so, than those between the same groups of acids in the herring visceral and body fats. Lovern (1938) has put forward the view that the herring body fat, with component acids in much the same proportions as in *Calanus* fat, has been stored as a result of ingestion in quantity in the herring diet of the latter fat, but that prior to laying down in the herring fat depots the *Calanus* fat has been modified by considerable hydrogenation of its unsaturated C_{20} and C_{22} components and some dehydrogenation of its unsaturated C_{16} components.

At first sight the observed composition of the herring visceral fat would seem to be at variance with Lovern's explanation, but in our opinion this does not necessarily follow. The visceral fat may be considered, in the herring, to be more of the nature of an organ fat than a depot fat; indeed, its similarity in composition to the 'average marine fish fats' (which as already pointed out were mainly liver fats) may be due to this circumstance. (Fish livers, of course, often function simultaneously as depots, but very broadly speaking the main characteristics of the component acids of fish liver fats are similar, whether the liver is rich or poor in fat.) If the visceral fat of the herring is considered to be an organ fat rather than a depot fat, its dissimilarity to

the specific depot fat laid down by the herring is not necessarily surprising. Thus the liver lipids of land animals (pig, sheep, ox) which have been examined in detail are found, either in their glycerides or their phosphatides, to be respectively broadly similar in composition (Hilditch & Shorland, 1937), and to have some features (e.g. presence of unsaturated C_{16} , C_{20} and C_{22} acids) which are not only common to all these animal liver lipids but are also reminiscent of the glyceride fats of aquatic organisms: but neither the glyceride nor the phosphatide fractions of these liver lipids have any resemblance to the depot fats laid down by the different animals, the depot fats of which moreover differ considerably in composition from one species to another.

We therefore confine ourselves to the conclusion that our present results show that herring visceral fat differs materially in composition from the typical fat laid down in the herring fat depots, and that its component acids are very similar in content and in their degree of unsaturation to those of what appear to be typical marine fish liver fats. It is also evident that much more data could still profitably be sought as to the detailed composition of the fatty

acids in different organs and depots from a wide range of species of fish.

SUMMARY

1. The component acids of herring visceral fat have been determined by the ester-fractionation procedure.

2. Preliminary resolution of marine animal fatty acids into groups differing in unsaturation, by means of crystallization from suitable solvents at low temperatures, is shown to be preferable to their separation in the form of lithium and lead salts.

3. The herring visceral oil fatty acids differ from those of herring body fats (and of *Calanus* fat, a fat largely ingested by the herring) in their much lower content of unsaturated C_{22} acids and much higher content of unsaturated C_{18} acids, together with much higher degree of average unsaturation of the C_{20} and C_{22} acids and lower average unsaturation of the C_{18} acids.

We wish to express our thanks to Mr J. R. Bruce for affording us the opportunity to study the specimen of herring visceral fat.

REFERENCES

- Bjarnason, O. B. & Meara, M. L. (1944). *J. Soc. chem. Ind., Lond.*, **63**, 61.
Hilditch, T. P. (1947). *Chemical Constitution of Natural Fats*, 2nd ed., pp. 506-9. London: Chapman and Hall.
Hilditch, T. P. & Maddison, L. (1942). *J. Soc. chem. Ind., Lond.*, **61**, 169.
Hilditch, T. P. & Pathak, S. P. (1947). *J. Soc. chem. Ind., Lond.*, **66**, 421.
Hilditch, T. P. & Shorland, F. B. (1937). *Biochem. J.* **31**, 1499.
Lovern, J. A. (1935). *Biochem. J.* **29**, 847.
Lovern, J. A. (1937). *Biochem. J.* **31**, 755.
Lovern, J. A. (1938). *Biochem. J.* **32**, 676.