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Animal Fats

3. THE COMPONENT ACIDS OF OSTRICH FAT*

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In continuation of our study of animal fats (Gunstone & Paton, 1953a, b) we now report the composition of ostrich fat, which has not been examined previously.

EXPERIMENTAL

The fat used in this investigation was obtained from an adult male ostrich (*Struthio camelus*) which had been in captivity in Edinburgh Zoo. Its diet consisted of pasture supplemented with whole maize. In its natural state the ostrich eats small mammals, birds, snakes, lizards and insects, as well as grass, leaves, fruits, berries and seeds. 'A post-mortem revealed a knotted mass of grass filling the proventriculus and gizzard. A varied bacterial flora was isolated from the bone marrow and there was severe congestion of the duodenum and a fatty infiltration of the liver.' (Information kindly supplied by Mr E. C. Appleby.)

The crude fatty tissue (830 g.) was autoclaved at 120° and

broken up in a homogenizer before extraction with light petroleum (b.p. 40–60°). The fat (705 g.) was obtained as a white low-melting solid of iodine value 80.4, saponification equivalent 282.8, and free acid 2.1% (as oleic acid), whilst the mixed acids obtained on hydrolysis had iodine value 83.4 and equivalent 273.3.

The mixed acids (197.6 g.) were divided into three fractions by crystallization from methanol (10 ml. per g.) at –40° overnight and by recrystallization of the insoluble portion at –20° from methanol (10 ml. per g.). The results are shown in Table 1.

Each fraction was methylated by treatment with methanol and either HCl at room temperature (B and C) or conc. H₂SO₄ at the reflux temperature of the solution (A) and the resulting esters distilled through an electrically heated and packed column (Towers, Widnes T. 117) under reduced pressure. From the iodine value and saponification equivalent of each fraction and the spectrographic examination, after alkali isomerization, of selected fractions the composition of the mixed acids was calculated by the methods previously described (Gunstone & Paton, 1953b). The results are shown in Table 2.

* Paper 2 of this series: Gunstone, F. D. & Paton, R. P. (1953b), *Biochem. J.* **54**, 621.

Table 1. *Low-temperature crystallization of fatty acids from ostrich fat*

Fraction	Acids			Esters	
	Wt. (g.)	(%, w/w)	Iodine value	Iodine value	Sap. equiv.
A. Insoluble at –20°	77.5	39.2	21.1	20.3	280.2
B. Soluble at –20°, insoluble at –40°	60.0	30.4	94.4	88.8	292.5
C. Soluble at –40°	60.1	30.4	154.9	146.6	284.2

Table 2. *Component acids of ostrich fat*

All values except in last column are %, w/w, of total.

	Fraction			Total	% (wt.)*	% (mol.)*
	A	B	C			
Myristic	0.14	0.05	0.68	0.87	0.9	1.0
Palmitic	23.76	0.69	0.26	24.71	24.8	26.3
Stearic	5.93	—	—	5.93	5.9	5.7
Arachidic	0.45	—	—	0.45	0.4	0.4
Tetradecenoic	—	0.13	0.77	0.90	0.9	1.1
Hexadecenoic	0.44	1.86	3.30	5.60	5.6	6.0
Hexadecadienoic	—	—	0.49	0.49	0.5	0.5
Octadecenoic	8.12	24.65	6.88	39.65	39.8	38.3
Octadecadienoic	0.33	2.67	14.03	17.03	17.1	16.6
Octadecatrienoic	—	—	3.83	3.83	3.8	3.8
As eicosenoic†	—	0.31	—	0.31	0.3	0.3
Unsaponifiable	0.03	0.04	0.16	0.23	—	—

* Excluding unsaponifiable material.

† Includes all unsaturated acids higher than C_{18} (average unsaturation -2.1 H).

The following acids were identified in appropriate fractions: palmitic acid (hexadecanoic acid), m.p. 62°; stearic acid (octadecanoic acid), m.p. 68°; hexadecenoic acid as dihydroxypalmitic acid, m.p. 126–127°; oleic acid as dihydroxystearic acid, m.p. 130–131°; linoleic acid as tetrabromostearic acid, m.p. 111–113°; and linolenic acid as hexabromostearic acid, m.p. 181–182°. An attempt to prepare dihydroxymyristic acid from the fraction richest in tetradecenoic acid gave a very small quantity of a product m.p. 118–119°. Bromination of a fraction considered to contain hexadecadienoic acid gave a derivative, m.p. 176–179°, which was insoluble in light petroleum, and which appeared to be hexabromopalmitic acid. (Found: Br 66.0; calc. for $C_{16}H_{20}O_2Br_6$, Br 65.7%.)

RESULTS AND DISCUSSION

Unsaturated C_{18} acids. Spectrographic analysis, after alkali isomerization, of a fraction consisting mainly of C_{16} esters indicated an appreciable $E_{1\text{cm}}^{1\%}$ value (223.7) at 234 $m\mu$, and a much smaller value (15.0) at 268 $m\mu$. After allowing for the small quantity of polyethenoid C_{18} acids present, these

reported the presence of hexadecadienoic and hexadecatrienoic acids in rabbit fat on the basis of spectrographic evidence following alkali isomerization.

Unsaturated C_{18} acids. As noted previously (Gunstone & Paton, 1953b) the use of spectrographic constants obtained from polyethenoid acids known to be wholly *cis* may not be justified for calculating the composition of animal fats in which the structure of the polyethenoid acids is doubtful. In python fat (Gunstone & Paton, 1953b) and crocodile fat (Gunstone & Russell, 1954) the C_{18} polyethenoid acids appear to be essentially linoleic and linolenic acids, but this is less certain in the present case for the following reasons:

(i) Herb & Riemenschneider (1952) suggest that replacement of the potassium hydroxide-glycol (7.5%) by a more concentrated solution (21%) gives larger constants, thereby increasing the sensitivity of this procedure. When a fraction rich in C_{18} unsaturated acids was examined in this way the results differed from those obtained by the standard procedure thus (all values are % (w/w) of fraction):

Glycol reagent (%)	Octadecatrienoic acid	Octadecadienoic acid	Octadecenoic acid	Total
7.5	15.2	55.7	27.3	98.2
21	10.6	59.0	34.7	104.3

values were 121.8 and 0.5 respectively. The former was taken to indicate the presence of hexadecadienoic acid (12.1%) in this fraction. Attempts to confirm this by the isolation of tetrabromopalmitic acid from a similar fraction containing no C_{18} acids gave only hexabromopalmitic acid. The small amount of 'hexadecadienoic acid' reported in Table 2 should therefore be regarded as an unidentified mixture of unsaturated C_{18} acids. Clément & Meara (1951) have

(ii) Bromination of a fraction rich in C_{18} unsaturated acids (Markley, 1947) showed the presence of linoleic acid (22%) and linolenic acid (9%), whilst alkali isomerization gave values of 46.4 and 10.8% for octadecadienoic and octadecatrienoic acids respectively. The large difference in the values for linoleic acid and octadecadienoic acid is probably significant and casts further doubt on the homogeneity of the octadecadienoic acid.

Table 3. *Component acids of some bird fats*

All values %, w/w, except for iodine values.

	Grey-goose (abdominal)	Emu (subcutaneous)	Light Sussex hen (abdominal)	Ostrich
Iodine value	57.1	65.8	78.5	80.4
Lauric	12.3	—	—	—
Myristic	8.2	0.9	1.2	0.9
Palmitic	20.3	17.5	24.0	24.8
Stearic	5.6	10.1	4.1	5.9
Arachidic	—	0.6	—	0.4
Tetradecenoic	0.6	0.9	—	0.9
Hexadecenoic	2.5	2.1	6.7	6.1
Octadecenoic	41.6	62.2	42.5	39.8
Octadecadienoic	6.6	5.2	20.8	17.1
Octadecatrienoic	—	—	—	3.8
Unsaturated C ₂₀₋₂₂	2.3	0.5	0.7	0.3

Component acids of bird fats

The ostrich is reported to be the largest living bird and belongs to the subclass Ratitae. There are very few detailed analyses of bird fats and it is of interest to compare the results obtained in this investigation with those quoted by Hilditch, Sime & Maddison (1942). These are summarized in Table 3. Some sea-bird fats, which are rather different in composition (Lovern, 1938), have not been included in this discussion.

In several respects the figures for the grey-goose are rather unusual and as Hilditch *et al.* (1942) suggest this may be due to the presence of coconut oil in the diet. These figures are accordingly neglected in making the following generalizations.

(i) Saturated acids account for 29–32 % of the total. This value, which is surprisingly constant in view of the variation in iodine value, lies between the corresponding value for amphibians and reptiles (20–30 %) and that for rodents (35–40 %).

(ii) The content of palmitic acid is only slightly below the value of 30 ± 3 % (mol.) said to be characteristic of higher land animals (Banks & Hilditch, 1931; Hilditch & Longenecker, 1937), though the value for the emu seems to be somewhat low.

(iii) The lower values for stearic acid parallel those noted for reptiles, amphibians and rodents, and are in marked contrast to the greater values of many land animals. The results for the emu are again anomalous.

(iv) Unsaturated C₁₆ acids attain a value (6–7 %) which recurs frequently in bird and rodent fats.

(v) Unsaturated C₁₈ acids comprise 60–70 % of the total acids of bird fats. The unsaturation of these fats is controlled largely by the relative amounts of the various C₁₈ unsaturated acids, an increase in iodine value being accompanied by an increase in polyethenoid acids and a decrease in the monoethenoid acid content. Winter & Nunn (1953) have reported similar observations in the C₂₀ and C₂₂ acids in seal oils.

(vi) The amount of C₂₀₋₂₂ unsaturated acids is small in each case and contrasts with the larger values recorded for amphibian and reptile fats.

The number of bird fats examined is too small to permit of any but tentative conclusions, but the present results confirm that, so far as fat composition is concerned, this class of animals falls between the reptiles and the rodents and is somewhat closer to the latter.

SUMMARY

1. A quantitative study of the component acids of ostrich fat is reported, and the results are correlated with those previously reported for other bird fats.

2. Palmitic (24.8 %), oleic (39.8 %), and octadecadienoic acid (17.1 %) are the major components, whilst myristic, stearic, arachidic, hexadecenoic, polyethenoid C₁₆, octadecatrienoic and higher unsaturated acids are also present.

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