Animal Fats

4. THE COMPONENT ACIDS OF CROCODILE FAT

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The order Crocodilia comprises the crocodiles proper, alligators, caimans, and gerials. Information on the fat obtained from animals of this group appears to be limited to reports of some characteristics of the oils (Kobayashi, 1922; Luhr, 1932; Nembrot & Cadrobbi, 1936; Bennett, Brown Coomes, Morton & Raymond, 1950). In this paper we report the investigation of samples of fat from two species of crocodile living in different environments.

EXPERIMENTAL

One sample of fat was obtained from an adult Estuarine Crocodile (*Crocodylus porosus*) of about 20 years. This animal had been kept in captivity in Edinburgh Zoo where its diet consisted of whole dead piglets. The crude fatty material (690 g.) after autoclaving at 120° was broken up in a homogenizer and extracted with light petroleum (b.p. 40–60°) giving a semi-solid fat (585 g.). The second sample of fat, obtained through the kindness of the staff of the Colonial Products Advisory Bureau, came from a crocodile (*C. niloticus*) in Tanganyika living (presumably) in its natural state. Some characteristics of these two fats and of the acids obtained from them are set out in Table 1.

The method of analysis was similar to that already described for python (Gunstone & Paton, 1953) and for ostrich fat (Gunstone & Russell, 1954). The mixed acids were separated into three fractions by crystallization at –40 to –45° and at –20°, and each fraction esterified and then distilled. The results are summarized in Tables 2–4.

From both fats palmitic and stearic acid were isolated from appropriate fractions, and the presence of hexadecenoic, oleic, linoleic and linolenic acids was confirmed by the preparation of the usual derivatives. In addition, myristic acid was isolated from *C. niloticus*.

Bromination of fractions rich in polyethenoid C_{18} acids was effected quantitatively (Markley, 1947) and the results compared with those obtained by the alkali isomerization procedure (see Table 5).

Further evidence about the nature of these C_{18} unsaturated acids was obtained in the case of *C. porosus* fat by observing the spectrum after alkali isomerization at 180° for

0.5, 1.0 and 4.5 hr. The values of $E_{1\text{ cm.}}^{1\%}$ at 234 m μ . were 453, 529 and 486 respectively (see Discussion for the significance of these results).

Fractions from *C. niloticus* rich in C_{20} and C_{22} unsaturated acids were also brominated, and the solid products extensively crystallized from a variety of solvents. Two compounds were obtained and these were probably hexabromoeicosanoic acid (decomposes at 255°; found: Br, 61.4; calc. for $C_{20}H_{34}O_2Br_6$, 61.0%), and decabromodocosanoic acid (melts with decomposition at 260°; found: Br, 70.6; calc. for $C_{22}H_{34}O_2Br_{10}$, 70.7%).

DISCUSSION

Unsaturated acids

Evidence of the presence of polyethenoid C_{18} acids is derived only from spectroscopic data, and it is probable that these acids are a complex mixture (cf. discussion of similar acids in ostrich fat, Gunstone & Russell, 1954).

For the C_{18} acids the agreement in the values obtained by bromination and by spectroscopic measurements after alkali isomerization (see Table 5) suggests that the octadecadienoic and octadecatrienoic acids of *C. porosus* are essentially the *all-cis* isomers found in vegetable oils. This conclusion is borne out for the dienoic acid by the fact that maximal absorption was reached after isomerization for 1 hr., whereas Jackson, Paschke, Tolberg, Boyd & Wheeler (1952) report longer times for the *trans-trans* acid (360 min.) and *cis-trans* acids (150 min.). The results obtained in the bromination of *C. niloticus* fractions are less definite, and though linoleic and linolenic acids are present they may be accompanied by isomeric compounds.

The unsaturated acids higher than C_{18} are rather complex. The unsaturated C_{20} acids of *C. porosus* fat appear in fractions B and C. That portion which is present in fraction B must be largely monoethenoid

Table 1. *Characteristics of crocodile fat*

	Fat			Mixed acids	
	Iodine value	Sapon. equiv.	Free acid (as % oleic)	Iodine value	Sapon. equiv.
<i>C. porosus</i>	80.5	278.9	3.4	83.9	267.4
<i>C. niloticus</i>	93.3	283.2	0.4	96.8	271.5

Table 2. *Low-temperature crystallization of crocodile fatty acids*

Fraction	<i>C. porosus</i>			<i>C. niloticus</i>		
	<i>A</i>	<i>B</i>	<i>C</i>	<i>A</i>	<i>B</i>	<i>C</i>
Weight (g.)	68.5	58.1	80.0	62.3	37.9	102.4
% (w/w) of total	33.2	28.1	38.7	30.8	18.7	50.5
Iodine value	10.3	85.8	143.5	9.4	89.5	153.1

Table 3. *Component acids of Crocodylus porosus fat*

All values %, w/w, except last column.

	Fractions			Total	% (wt.)*	% (mol.)*
	<i>A</i>	<i>B</i>	<i>C</i>			
Lauric	—	—	0.44	0.44	0.4	0.6
Myristic	0.80	0.83	1.22	2.85	2.9	3.4
Palmitic	23.58	2.51	0.43	26.52	26.6	28.1
Stearic	4.75	—	—	4.75	4.8	4.5
Arachidic	0.56	—	—	0.56	0.6	0.5
Dodecenoic	—	—	0.17	0.17	0.2	0.2
Tetradecenoic	—	0.18	0.90	1.08	1.1	1.3
Hexadecenoic	0.17	1.17	4.87	6.21	6.2	6.6
Hexadecadienoic	—	—	0.32	0.32	0.3	0.3
Octadecenoic	3.06	19.24	11.15	33.45	33.5	32.2
Octadecadienoic	0.25	1.43	15.29	16.97	17.0	16.4
Octadecatrienoic	—	—	2.61	2.61	2.6	2.6
As eicosenoic†	—	2.67	1.08	3.75	3.8	3.3
Unsaponifiable	0.03	0.07	0.22	0.32	—	—

* Excluding unsaponifiable material.

† Average unsaturation - 3.5H.

Table 4. *Component acids of Crocodylus niloticus fat*

	Fractions			Total	% (wt.)*	% (mol.)*
	<i>A</i>	<i>B</i>	<i>C</i>			
Myristic	0.79	1.14	1.98	3.91	3.9	4.7
Palmitic	22.25	1.32	0.35	23.92	24.0	25.6
Stearic	3.40	—	—	3.40	3.4	3.3
Arachidic	1.34	—	—	1.34	1.3	1.2
Tetradecenoic	—	0.27	0.71	0.98	1.0	1.2
Hexadecenoic	0.29	1.36	12.68	14.33	14.4	15.4
Hexadecadienoic	—	—	0.32	0.32	0.3	0.3
Hexadecatrienoic	—	—	0.26	0.26	0.3	0.3
Octadecenoic	2.60	12.91	15.13	30.64	30.8	29.7
Octadecadienoic	0.12	0.65	5.67	6.44	6.5	6.3
Octadecatrienoic	—	—	3.05	3.05	3.1	3.0
Eicosenoic†	—	1.00	5.72	6.72	6.8	5.7
Docosenoic‡	—	—	4.22	4.22	4.2	3.3
Unsaponifiable	0.01	0.05	0.41	0.47	—	—

* Excluding unsaponifiable material.

† Average unsaturation - 5.8H.

‡ Average unsaturation - 7.9H.

Table 5. *Polyethenoid C₁₈ acids*Values are %, w/w, of fractions containing only C₁₈ acids.

	Bromination		Alkali isomerization	
	Diene	Triene	Diene	Triene
<i>C. porosus</i>	48.7	12.9	44.4	14.3
<i>C. niloticus</i>	16.9	17.9	12.8	23.8

Table 6. *Component acids of some amphibian and reptile fats*

All values %, w/w. Values are taken from Hilditch (1947) apart from the results for turtle (ii) (Giral & Marquez, 1948), python (Gunstone & Paton, 1953) and crocodile (present work). Values in parentheses are mean unsaturations.

	Frog	Tortoise	Turtle		Crocodile		Lizard		Python
			(i)	(ii)	(i)	(ii)	(i)	(ii)	
Lauric	—	—	13.3*	—	0.4	—	—	—	—
Myristic	4	1	10.6	6.6	2.9	3.9	4	4	1.3
Palmitic	11	14	17.0	21.8	26.6	24.0	18	29	19.7
Stearic	3	4	4.1	15.5	4.8	3.4	7	10	10.8
Arachidic	—	—	—	1.9	0.6	1.3	—	—	1.2
Tetradecenoic	—	—	1.3	3.5	1.1†	1.0	—	—	0.5
Hexadecenoic	15	9	7.8	18.0	6.5	15.0	10	12	3.9
Octadecenoic	52	65	39.6	31.4	33.5	30.8	56	40	47.0
Octadecadienoic					17.0	6.5	(-2.4H)	(-2.7H)	10.7
Octadecatrenoic					2.6	3.1			0.8
'Eicosenoic'					3.8	6.8	5	5	4.1
'Docosenoic'	(-6H)	(-4H)	(-6.3H)	(-8.6H)	—	4.2	(-5H)	(-5.5H)	—

* Also 0.2% decanoic acid.

† Also 0.2% dodecenoic acid.

in view of its low iodine value (approx. 90), its small absorption at 234 m μ . (approx. 40) after alkali isomerization, and the fact that saturated acids should not be present in this fraction. The homologous acids in fraction *C* are more unsaturated, and since they show appreciable absorption, after alkali isomerization, at 234, 268, 300 and 315 m μ . they must contain a tetraethenoid acid, possibly accompanied by less unsaturated C₂₀ acids. The higher unsaturated acids of *C. niloticus* are present in greater amounts, and include C₂₂ as well as C₂₀ acids. Both occur almost entirely in fraction *C* and are highly unsaturated (iodine value 220–300). Alkali isomerization of the C₃₀ fraction gives absorption maxima at 234, 268, 300, 315 and 346 m μ ., showing that pentaethenoid acids are present, whilst bromination experiments indicated that eicosatrienoic and docosapentaenoic acids occur in the C₂₀ and C₂₂ fractions.

Depot fat of amphibians and reptiles

Klenk (1933) and Klenk, Ditt & Diebold (1935) have drawn attention to the fact that the fatty acid composition of certain amphibian and reptile fats studied were intermediate in nature between the fats of sea and of land animals. Results obtained later have not made it necessary to contradict this, nor do the present results on crocodile fat. The quantitative differences in the values recorded in Table 6 make it difficult to point to any generalization, though it is apparent that in all cases hexadecenoic acid makes an appreciable contribution (4–15%) whilst the C_{20–22} unsaturated acids are present in similar amounts (4–15%). It can be seen further that in any fat the amounts of these two groups of acids are very similar, small amounts of hexadecenoic acid being associated with small amounts of the C_{20–22} unsaturated acids and vice versa.

Another factor which makes it difficult to draw comparisons lies in the differences in the two analyses of lizard fat, turtle fat, and of crocodile fat. In the present case the two samples are from different species and, in addition, one had been kept in captivity for several years whilst the other had lived in its natural state. In comparing the compositions of the two crocodile fats two points are worthy of note: (i) despite the difference in iodine value between these two samples there is little difference in the content of saturated acids, all the differences being in the relative amounts of the unsaturated acids; and (ii) the more unsaturated fat, obtained from *C. niloticus* living in its natural state, contained a greater proportion of C₁₆ and of C_{20–22} unsaturated acids, whilst the *C. porosus* sample contained more octadecadienoic acid which was more certainly linoleic acid than in the former case. These differences may reflect differences in diet, since the captive animal was fed on whole dead piglets, whilst crocodiles in their natural state are reported to eat mainly fish. Fish fats are known to contain high proportions of hexadecenoic and of C_{20–22} unsaturated acids.

Bennett *et al.* (1950) have suggested that crocodile oil might find a use alongside turtle oil in cosmetic preparations. Comparison of our results for *C. niloticus* (this animal was in its natural state and belongs to the species potentially available) with those given by Giral & Marquez (1948) for turtle oil shows a marked similarity apart from the greater proportion of unsaturated C_{20–22} acids in the crocodile oil and somewhat less stearic acid.

SUMMARY

1. The component acids of two samples of crocodile fat, *Crocodylus porosus* and *C. niloticus* are reported.

2. Each oil contained similar quantities of saturated acids (33–35 %), mainly palmitic (24–27 %), but differed in the proportions of the various unsaturated acids. These differences are particularly evident in the content of hexadecenoic acid, octadecadienoic acid, and the unsaturated C_{20-22} acids.

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REFERENCES

Bennett, H., Brown, E., Coomes, T. J., Morton, D. & Raymond, W. D. (1950). *Colonial Plant and Animal Products*, 1, 315.

Giral, F. & Marquez, A. (1948). *Arch. Biochem.* **16**, 187.
 Gunstone, F. D. & Paton, R. P. (1953). *Biochem. J.* **54**, 621.
 Gunstone, F. D. & Russell, W. C. (1954). *Biochem. J.* **57**, 459.
 Hilditch, T. P. (1947). *The Chemical Constitution of Natural Fats*, 2nd ed. p. 69. London: Chapman and Hall.
 Jackson, J. E., Paschke, R. F., Tolberg, W., Boyd, H. M. & Wheeler, D. H. (1952). *J. Amer. Oil Chem. Soc.* **29**, 229.
 Klenk, E. (1933). *Hoppe-Seyl. Z.* **221**, 264.
 Klenk, E., Ditt, F. & Diebold, W. (1935). *Hoppe-Seyl. Z.* **232**, 54.
 Kobayashi, S. (1922). *J. Chem. Ind., Tokyo*, **25**, 691.
 Luhr, W. (1932). *Chem. Umsch. Fette*, **39**, 85.
 Markley, K. S. (1947). *Fatty Acids, their Chemistry and Physical Properties*, pp. 605–8. London: Interscience Publishers Ltd.
 Nembrot, A. & Cadrobbi, B. (1936). *Ann. Chim. Anal.* **26**, 571.

The Sulphatase of Ox Liver

3. FURTHER OBSERVATIONS ON SULPHATASE B AND AN INVESTIGATION OF THE ORIGIN OF FRACTIONS A AND B

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The previous communications of this series have described the occurrence of two fractions exhibiting sulphatase activity in an aqueous extract of an acetone powder of ox liver, and the purification of one of these fractions, sulphatase A (Roy, 1953*a*, *b*). The present paper describes the further purification of the second fraction, sulphatase B, as a preliminary to the study of the relationship between the two fractions, more especially the possibility of artifact formation during their preparation. This is of particular interest as Dodgson, Spencer & Thomas (1953) have not detected two fractions in their preliminary study of rat liver sulphatase, although unpublished work from this laboratory has shown that two fractions, comparable to fractions A and B of ox liver, can be isolated from an acetone powder of rat liver by the methods already described (Roy, 1953*a*).

EXPERIMENTAL

Preparation of the enzyme

The general methods of preparing the acetone-powder extract and of fractional precipitation with acetone or $(NH_4)_2SO_4$ were as described previously (Roy, 1953*a*, *b*). Protein estimations were kindly performed by Dr L. M. H. Kerr using a slight modification of the Folin phenol-method

of Lowry, Rosenbrough, Farr & Randall (1951). In what follows, one sulphatase B unit (s.u.) is defined as the amount of enzyme which will liberate 1 μ g. nitrocatechol under the standard conditions described below.

Stage B. This was obtained by the precipitation of 800 ml. aqueous extract with 43% (v/v) acetone in phosphate buffer, pH 7, at -9° as described by Roy (1953*a*). The precipitate so obtained was dissolved in water and dialysed overnight against running tap water at room temperature, giving 400 ml. of sulphatase B (700 000 s.u., 25 s.u./mg. protein).

Stage B-1. To 400 ml. sulphatase B were added 26 ml. 0.5M sodium acetate, pH 6.5, and 4.5 ml. 0.3M- $CaCl_2$. The enzyme was then precipitated by the addition of 220 ml. acetone (34%, v/v, final concentration), the temperature being lowered to -9° during the process. After equilibration at that temperature the precipitate was centrifuged off, dissolved in 100 ml. water and dialysed as above. A copious inactive precipitate was separated, giving 250 ml. sulphatase B (stage B-1; 500 000 s.u., 36 s.u./mg. protein).

Stage B-2. 250 ml. B-1 were made 0.3 saturated with respect to $(NH_4)_2SO_4$ by the slow addition of 55 g. solid $(NH_4)_2SO_4$. After standing a few hours the precipitate was centrifuged off and discarded. The supernatant was made 0.5 saturated with respect to $(NH_4)_2SO_4$ by the addition of the calculated amount of solid and after equilibration the active precipitate was centrifuged off, dissolved in water and dialysed overnight. This $(NH_4)_2SO_4$ fractionation was then repeated on the dialysate, giving a clear solution of sulphatase B-2 (200 000 s.u., 190 s.u./mg. protein).