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Animal Fats

1. THE COMPONENT ACIDS OF DEER FAT AND OF CAMEL FAT

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Study of the detailed composition of seed fats has led to the realization that there is a close relationship between the classification of the parent plants on the basis of fat composition and that arrived at by botanists on more general grounds. Among land animals, however, only very tentative generalizations can be made because of the paucity of detailed analyses of animal fats. Hilditch (1947, p. 85) has drawn attention to this deficiency and it has been further emphasized by Barker & Hilditch (1950) who list the land animals, the fats of which have been satisfactorily analysed. Further analyses have since been reported for the fats obtained from hippopotamus (Barker & Hilditch, 1950), badger (Gupta, Hilditch & Meara, 1950), horse (Brooker & Shorland, 1950; Gupta & Hilditch, 1951), rabbit (Clément & Meara, 1951) and tiger (Pathak & Agarwal, 1952).

We are interested in this problem, and over the last few years have collected, from fifteen different animals, samples of fats which we hope to study. These vary in weight from 20 to 600 g. and cover an iodine-value range from 26 to 108. The fats from many of these animals have not previously been examined in any detail, and even in the few cases where satisfactory analyses have been reported it is considered worth while to repeat the work in order to study variation between different members of the same species. In the present paper we record our analyses of deer and of camel fat; these relatively simple examples were purposely selected as a convenient beginning to this work in order to gain experience for the study of more complicated fats. The methods of analysis may have to be varied

for different fats. Modifications have already been made in the light of experience and the methods reported in this paper are, therefore, not in every detail the most satisfactory ones. Improvements will be reported later as they are applied.

Reports on the composition of deer fat seem to be limited to the recording of physical data and characteristics (largely summarized by Lewkowitsch, 1922) and to the work of Baughman, Jamieson & McKinney (1929) and of Treadwell & Eckstein (1939). The former authors studied five samples of American reindeer fat of iodine value 33.7–39.4. Treadwell & Eckstein have reported the saponification value, iodine value and thiocyanogen value of fats obtained from various parts of five Virginia white-tailed deer (*Odocoileus virginianus borealis*). Apart from the fat isolated from the mammary gland there is little variation in these samples. These earlier results are summarized in Table 8.

Previous recorded data on camel fat appear to be limited to a report by Armstrong & Allan (1924), in which only the major component acids, palmitic (37%), stearic (16%) and oleic (47%) are recognized.

EXPERIMENTAL

Preliminary work

In planning this series of analyses, it was anticipated that some of the fatty-acid fractions to be examined would be rather smaller than usual, either on account of the relatively small amount of material available or because of the concentration of minor components in a small fraction. These fractions (6–12 g.) have been distilled through a Dixon 6 in.

still (Dixon, 1945), and the resulting fractions (0.7–1.0 g.) examined in the usual way. In devising a method of determining the saponification equivalent on about 0.6 g. of ester we were unable to get satisfactory results by the double indicator procedure of Marcali & Rieman (1946), and eventually found that the standard method on a reduced scale gave the best results. The ester was hydrolysed with 0.5 N-ethanolic KOH (10 ml.) for 30 min. and the excess alkali titrated against 0.5 N-HCl using a 5 ml. microburette. Table 1 gives the results of analysing various quantities of a mixture of crude myristic, palmitic and stearic acids. In Exp. A, the Towers (T. 117) column was used and equivalents determined by the standard procedure; in Exps. B and C, the Dixon 6 in. still was used and the equivalents were determined by the above method. The main difficulty lies in the satisfactory examination of the residue, the proportion of which tends to increase as the amount of material distilled decreases. These results are considered to show a fair agreement, particularly between the major components.

Table 1. *Results obtained by distilling various quantities of the same mixture*

Exp.	...	...	A	B	C
Amount distilled (g., approx.)			40	12	6
No. of distilled fractions			12	11	7
Calculated composition (%)					
Lauric			0.6	0.3	—
Myristic			10.2	10.0	10.1
Palmitic			40.1	39.0	39.0
Stearic			38.7	39.5	36.6
Arachidic			—	0.6	3.7
Tetradecenoic			0.4	0.4	0.5
Octadecenoic			10.0	10.2	10.1

As we have all the necessary facilities for doing low-temperature crystallizations, we decided to carry out all our separations by this method and to dispense with the lead salt separation, thus following the methods used by Brooker & Shorland (1950), Gupta & Hilditch (1951), and Clément & Meara (1951). It is, nevertheless, desirable that a method of crystallization be used which separates saturated and unsaturated acids as effectively as does the lead salt procedure. The former authors have used ether or acetone as solvents at -30° to -35° to do this, but as we were able to use a refrigerator maintained at -20° we tried to find a solvent which would separate saturated and unsaturated acids at this temperature. Preliminary results showed that equilibrium is attained in less than 18 hr. and that there is no advantage in using solutions more dilute than 10 ml. of solvent/g. of acids. Table 2 shows the results obtained on separating mixed tallow acids with various solvents at this dilution at -20° overnight. The results of a lead salt separation are included for comparison. Table 3 contains similar results obtained with a mixture of crude palmitic (12%),

stearic (13%) and oleic (75%) acids. Perusal of Table 2 shows that ethyl acetate, light petroleum or methanol give results comparable with those obtained in the lead salt separation and that even better results follow the use of methanol containing up to 5% of water. Similar satisfactory results were obtained with these solvents on the mixture containing only about 25% of saturated acids, though in this case aqueous methanol (5%) gave less satisfactory results.

Table 2. *Low-temperature crystallization of tallow acids*

(Iodine value 42.8; about 10 g.)

Solvent	Insoluble		Soluble	
	Wt. (%)	Iodine value	Wt. (%)	Iodine value
Lead salt separation	52.8	5.6	47.2	83.6
Acetone	46.1	2.7	53.9	75.5
Ether	35.1	2.8	64.9	62.3
Ethyl acetate	48.9	2.6	51.1	82.0
Light petroleum	51.7	6.0	48.3	80.9
Methanol	49.7	3.5	50.3	79.8
Methanol-water (1%)	49.2	3.4	50.8	74.3
Methanol-water (2%)	50.2	3.5	49.8	82.4
Methanol-water (5%)	52.3	3.9	47.7	85.8
Methanol-water (10%)	57.5	10.1	42.5	87.8
Methanol-water (15%)	55.1	6.9	44.9	86.4
Dichloromethane	47.8	2.0	52.2	79.3
Chloroform	Results so obviously inferior that the two fractions were not examined.			
Carbon tetrachloride				

Table 3. *Low-temperature crystallizations of mixed acids*

(Approx. 10 g. mixed acids, containing about 25% saturated acids.)

Solvent	Insoluble		Soluble	
	Wt. (%)	Iodine value	Wt. (%)	Iodine value
Light petroleum	24.9	12.3	75.1	93.8
Ethyl acetate	24.0	7.7	76.0	93.5
Methanol	25.5	8.8	74.5	95.2
Methanol-water (5%)	29.1	15.8	70.9	96.6

It was finally decided to use methanol as crystallizing solvent, the above data having shown that a small amount of water in this solvent does not significantly affect the results.

Source and preparation of fat

The deer fat (230 g.), obtained from a butcher in Perth, had come from a stag (*Cervus elaphus*) reared in Scotland. The crude material, which was very hard after being kept at

Table 4. *Characteristics of deer fat and camel fat*

	Fat			Mixed acids			
	Iodine value	Saponification equivalent	F.F.A.*	Iodine value	Saponification equivalent	$E_{1\text{ cm.}}^{1\%}$ 234 m μ .†	$E_{1\text{ cm.}}^{1\%}$ 268 m μ .†
Deer	35.5	284.9	2.0	32.4	266.2	20.3	6.9
Camel	35.1	282.2	2.1	36.8	270.4	22.2	4.6

* Free fatty acid as % oleic.

† Ultraviolet absorption after alkali isomerization (Hilditch, Morton & Riley, 1945; Hilditch, Patel & Riley, 1951).

0°, was crushed and extracted with light petroleum; removal of the solvent left 151 g. of fat, the characteristics of which are summarized in Table 4.

The camel fat was extracted from material kindly supplied by Mr G. T. Iles, Zoological Superintendent, Belle Vue, Manchester. The animal was a male bactrian camel (*Camelus bactrianus*) aged about 7 years and the fat was taken from the mesentery. Its diet consisted of meadow and clover hays supplemented by a daily feed of chopped vegetables and fruit along with chopped hay, oats, bran and horse-feed cubes mixed together with a little cod-liver oil; fresh grass was also available. The material supplied was autoclaved to render it sterile and then crushed and extracted with acetone. The characteristics of the fat are given in Table 4.

Method of analysis

In view of the relatively high proportion of saturated acids expected in these fats, the mixed acids were crystal-

lized first from methanol (10 ml./g.) at -20° and the soluble acids were subsequently crystallized from acetone (10 ml./g.) at -50 to -55°. This gave three fractions (*A*, *B* and *C* in order of increasing solubility) details of which are summarized in Table 5. Each fraction was subsequently methylated and distilled, the iodine value and saponification equivalent of each fraction being determined. The composition of each fraction and hence of the fat has been calculated by the methods described by Hilditch (1947, pp. 484-512) and is given in Tables 6 and 7. Spectroscopic measurements were made after isomerization of the acid fractions only; better results are obtained if selected ester fractions are used and this procedure is followed in all subsequent work. In these two fats, however, the amount of polyethenoid acids, other than C_{18} , is negligible in the case of deer fat and very small in the camel fat so that the error introduced in this way is hardly significant. Absorption measurements on unisomerized acids gave very small values (probably due to oxidation) which have been neglected.

Table 5. Low-temperature crystallization of deer and camel fatty acids

Fraction	Deer			Camel		
	<i>A</i>	<i>B</i>	<i>C</i>	<i>A</i>	<i>B</i>	<i>C</i>
Weight (g.)	61.18	20.73	15.97	38.70	21.94	8.43
Percentage of total	62.5	21.2	16.3	56.0	31.8	12.2
Iodine value	5.9	67.3	122.7	2.9	69.0	107.5
Absorption after isomerization						
$E_{1\text{cm}}^{1\%}$ at 234 m μ . (180°/60 min.)	—	16.6	209.2	—	12.4	148.7
$E_{1\text{cm}}^{1\%}$ at 268 m μ . (170°/15 min.)	—	2.0	80.9	—	1.5	37.5

Table 6. Component acids of deer fat

	Fraction			Total	% (wt.)*	% (mol.)*
	<i>A</i>	<i>B</i>	<i>C</i>			
Lauric	—	—	0.01	0.01	—	—
Myristic	1.09	2.26	1.08	4.43	4.4	5.3
Palmitic	22.32	2.42	0.32	25.06	25.1	26.7
Stearic	33.96	0.85	0.42	35.23	35.4	33.9
Arachidic	1.34	0.16	—	1.50	1.5	1.3
Tetradecenoic	—	0.09	0.40	0.49	0.5	0.6
Hexadecenoic	0.26	0.89	1.65	2.80	2.8	3.0
Octadecenoic	3.44	13.99	7.72	25.15	25.2	24.3
Octadecadienoic	—	0.34	2.26	2.60	2.6	2.5
Octadecatrenoic	—	0.08	2.38	2.46	2.5	2.4
Unsaponifiable	0.09	0.12	0.06	0.27	—	—

* Excluding unsaponifiable material.

Table 7. Component acids of camel fat

	Fraction			Total	% (wt.)*	% (mol.)*
	<i>A</i>	<i>B</i>	<i>C</i>			
Myristic	1.24	3.93	1.08	6.25	6.3	7.4
Palmitic	25.67	2.63	0.39	28.69	28.8	30.5
Stearic	25.81	1.51	0.04	27.36	27.4	26.2
Arachidic	1.45	0.09	—	1.54	1.6	1.3
Tetradecenoic	—	0.18	0.36	0.54	0.5	0.6
Hexadecenoic	0.14	1.22	1.83	3.19	3.2	3.4
Octadecenoic	1.55	20.08	4.67	26.30	26.4	25.3
Octadecadienoic	—	0.38	1.48	1.86	1.9	1.8
Octadecatrenoic	—	0.09	0.83	0.92	0.9	0.9
As eicosenoic†	0.17	1.57	1.27	3.01	3.0	2.6
Unsaponifiable	—	0.08	0.26	0.34	—	—

* Excluding unsaponifiable material.

† Includes all unsaturated acids higher than C_{18} . (Average unsaturation about -3H.)

Table 8. *Component acids of some 'stearic-rich' animal fats*

(Values quoted are % by wt.)

	Deer			Camel		Sheep	Ox	Pig	Hippo- potamus
	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)
Myristic	4.4	7	65-70	6.3	—	2-4	3.0	1.0*	2.3
Palmitic	25.1	35		28.8	37	23-28	29.2	28-30	27.1
Stearic	35.4	20		27.4	16	15-31	21.0	12-16	22.2
Arachidic	1.5	1		1.6	—	—	0.4	—	1.1
Tetradecenoic	0.5	—	—	0.5	—	—	0.6	0.2*	0.4
Hexadecenoic	2.8	—	—	3.2	—	1.0-2.5	2.7	2.5*	2.2
Octadecenoic	25.2	37	27-31	26.4	47	36-46	41.1	41-48	39.3
Octadecadienoic	2.6	—	3-5	1.9	—	4-6	1.8	6-7	3.5
Octadecatrienoic	2.5	—	—	0.9	—	—	—	—	1.5
C ₂₀₋₂₂ unsaturated acids	—	—	—	3.0	—	0.5*	0.2	2.0*	0.4

(a) and (d), present work. (b), Baughman, Jamieson & McKinney (1929). (c), Treadwell & Eckstein (1939). (e), Armstrong & Allan (1924). (f), (g) and (h), quoted by Hilditch (1947, p. 105). (j), Barker & Hilditch (1950).

* Approximate values.

Identification of acids

The results obtained by these methods have been qualitatively confirmed by the isolation of palmitic and stearic acids from appropriate fractions and by the preparation of derivatives of some of the unsaturated acids. For both fats, oleic, linoleic and linolenic acids were shown to be present by the isolation of the usual dihydroxy-, tetrabromo-, and hexabromo-stearic acids respectively. In all cases satisfactory melting points and mixed melting points with authentic specimens were recorded. Suitable fractions from the analysis of camel fat also gave myristic acid and dihydroxypalmitic acid (m.p. 123-124.5°; literature, 125°) as a derivative of hexadec-9-enoic acid. When a deer-fat fraction rich in hexadecenoic acid was similarly oxidized by the method of Lapworth & Mottram (1925), the hydroxy acid obtained analysed satisfactorily for dihydroxypalmitic acid (Found: C, 66.4; H, 11.4. Calc. for C₁₈H₃₂O₄: C, 66.6; H, 11.2%) but melted sharply at 116°. The quantity available did not permit further investigation of this interesting point but the hexadecenoic acid present in deer fat may be an isomer of the more common hexadec-9-enoic acid. Hilditch & Lovern (1928) have previously reported a dihydroxypalmitic acid, m.p. 115°, from the hexadecenoic acid present in sperm oil.

RESULTS AND DISCUSSION

The results obtained in the present work, along with some other relevant data, are summarized in Table 8. Comparing the present analyses with previous results for the same species it will be noticed that our results agree fairly well with the less detailed analyses of the Virginia white-tailed deer but less well with that of the American reindeer fat. Agreement between the figures for camel fat is only rough. These differences are not surprising in view of the approximate methods used in the earlier work.

Both deer and camel fats are more saturated than the average sheep or ox tallow, and this is reflected mainly in the increased content of stearic acid and decreased amount of oleic acid. In deer fat particularly the stearic figure is exceptionally high, whilst in both cases the amount of oleic acid is remarkably

low. In general, the results are those to be expected of 'stearic-rich animal depot fats'; the content of palmitic acid (26.7 and 30.5 % (mol.), respectively) lies practically within the accepted range of 30 ± 3 % (Banks & Hilditch, 1931; Hilditch & Longenecker, 1937); the remainder of the acids are almost entirely C₁₈; and smaller proportions of myristic, arachidic, tetradecenoic and hexadecenoic acids are also present. Deer fat does not contain any unsaturated acids higher than C₁₈, but the present sample of camel fat contains 3 % of higher unsaturated acids; these may originate in the cod-liver oil supplied in the camel's diet. In spite of the fact that the amount of oleic acid is lower than usual, both fats contain di- and tri-ethenoid acids of the C₁₈ series. These appear to be largely, if not entirely, the normal linoleic and linolenic acids present in seed fats.

SUMMARY

1. A systematic study of animal fats has been begun and the composition of deer fat and of camel fat are reported.

2. Satisfactory methods of studying smaller quantities of ester than is usual in this work are described.

3. Crystallization from methanol at -20° is an effective method of separating saturated and unsaturated acids.

4. Both deer and camel fat are 'stearic-rich' with palmitic (25 and 29 %), stearic (35 and 27 %) and oleic acids (25 and 26 %) as major components, and myristic, arachidic, tetradecenoic, hexadecenoic, octadecadienoic and octadecatrienoic acids as minor components. Camel fat also contains some higher unsaturated acids.

We wish to thank Mr G. T. Iles of Belle Vue, Manchester, for supplying us with the sample of camel fat used in this investigation, the University of Glasgow for a scholarship held by one of us (R. P. P.) and Imperial Chemical Industries Ltd. for a grant towards the purchase of apparatus.

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Animal Fats

2. THE COMPONENT ACIDS OF PYTHON FAT

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The need for more information on the composition of animal fats has been emphasized in the introduction to this series (Gunstone & Paton, 1953), and we now report the composition of python fat.

Previous reference to python fat is limited to an account by Tsujimoto & Kobayashi (1920) of a sample of python oil of acid value 0.6, saponification value 194.1, and iodine value 80.3, which yielded some ether-insoluble polybromides (2.1%) indicating the presence of highly unsaturated acids, probably of the C_{20} and C_{22} series. The characteristics of some other snake oils have been given by Pollard & McLaughlin (1950), but no analyses are reported. A few detailed analyses of the depot fats of Amphibia and Reptilia have been published, and from these few results some tentative generalizations have been drawn. More information is clearly needed.

EXPERIMENTAL

Source and preparation of the fat

The sample of python fat, kindly supplied by Mr G. T. Iles of Belle Vue, Manchester, had been obtained from a female reticulated python (*Python reticulatus*), 19 ft. long and 21 in. around its greatest girth. It had been fed on goats, ducks, hens and rabbits. The fat was probably attached to the intestines. The crude material (640 g.) was autoclaved to render it sterile, crushed and extracted with acetone. Removal of the solvent left the fat (580 g.) as a soft, white,

solid with the following characteristics; saponification equivalent 288.5, iodine value 73.0, free fatty acid 0.3% (as oleic). The mixed acids resulting on hydrolysis had saponification equivalent 275.9 and iodine value 76.1.

Method of analysis

The mixed acids were divided into four less complex fractions by crystallization under various conditions, the insoluble acids being recrystallized in each case. Crystallization was effected from acetone at -60° (twice), and from methanol at -40° and at -20° . Five fractions, *A*, *B*, *C'*, *C''* and *D* in order of increasing solubility were thus obtained, *C'* and *C''* being combined to form fraction *C*. The amounts and iodine values of each fraction are given in Table 1.

Concentrations of 10 ml. of solvent/g. of acids are used throughout.

Table 1. *Low-temperature crystallization of python fat*

Fraction	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Weight (g.)	54.06	66.61	26.14	35.26
Percentage of total	29.7	36.6	14.3	19.4
Iodine value	2.5	84.5	94.4	156.8

Each fraction was then methylated and fractionally distilled under reduced pressure, the iodine value and saponification equivalent of each fraction determined and certain fractions examined spectrographically, after