372. *The Seed and Fruit-coat Fats of* Celastrus Paniculatus.

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Both of these fats are peculiar in containing appreciable proportions of formic, acetic and benzoic acids in addition to the usual higher fatty acids (palmitic, stearic, oleic, linoleic, linolenic). Quantitative determinations have been made of the component acids present in each fat, and the seed fat has been separated into two

portions, one mainly glyceridic, and the other (which contained the greater part of the acids of low molecular weight) consisting of a mixture of glycerides and esters of a tetrahydroxylic water-soluble alcohol (approximate composition $C_{15}H_{26}O_5$), the nature of which was not definitely ascertained. It is shown that the formic, acetic, and benzoic acids are not part of the glycerides, but are esters of the tetrahydroxylic compound which are soluble in the fat and therefore accompany it during its extraction.

Celastrus paniculatus (natural family *Celastraceæ*) is a large deciduous shrub of the outer Himalayas, East Bengal, Bihar, and South India. The oil from the seeds is used medicinally in rheumatic diseases. From previous reports, it appears that *Celastraceæ* seed oils contain appreciable amounts of lower acids (acetic and formic). Barkenbus and Krewson (*J. Amer. Chem. Soc.*, 1932, **54**, 3993) reported 15·7% of water-soluble acids (formic, acetic, and traces of hexoic) in the seed fat of the American species *C. scandens*, for which they give the following total composition : glycerides of palmitic 8·4%, stearic 1·9%, linoleic 38·5%, linolenic 21·0%, and soluble acids (as acetic) 15·7%, with 3·0% of unsaponifiable matter. Kumaraswamy and Manjunath (*J. Indian Chem. Soc.*, 1936, **13**, 353) observed the presence of acetic and benzoic (but not formic) acids in addition to palmitic, stearic, lignoceric, cerotic, oleic, linoleic, and linolenic acids, in the seed oil of *C. paniculatus*. Godbole and Gunde (*Fette u. Seifen*, 1936, **43**, 249) found 15·3% of water-soluble acids in the same oil, but did not give details of their nature.

In the present communication we give a quantitative statement of the component acids in both the seed and the fruit-coat fats of *C. paniculatus*. The seeds, obtained from the United Provinces, India, were freed carefully from the thin fruit coat and pressed in a hand press. The fat thus obtained was liquid at room temperature and was greenish-brown. It had the following characteristics : saponification equivalent 217·3, acid value 22·9, iodine value 103·9, unsaponifiable matter 3·1% (iodine value 214·5). The husk or fruit coat of the seeds when extracted with benzene gave a dark-coloured semi-solid fat, with saponification equivalent 244·1, acid value 70·1, iodine value 95·0, and unsaponifiable matter 6·6% (iodine value 206·8). Both fats were submitted to detailed analysis as described below.

Component Acids of Celastrus paniculatus *Seed Fat.*—The seed fat (300 g.) was hydrolysed and the liberated mixed acids were distilled in steam until no more acid appeared in the distillate (the examination of which is described later). The non-steam-volatile mixed fatty acids (251 g., sap. equiv. 255·6, iod. val. 121·6) were separated into " solid " acids (22·6%) and " liquid " acids (77·4%) by means of the differing solubility of their lead salts in alcohol. Each group of acids was then converted into the corresponding methyl esters, which were fractionally distilled at 0·1 mm. pressure from a Willstätter bulb. The fractionation data are as follows :

Fraction No.	Wt., g.	Sap. equiv.	Iod. val.	Fraction No.	Wt., g.	Sap. equiv.	Iod. val.
		(i) *Methyl esters of " solid " acids.*					
S1	4·49	270·7	1·0	S5	4·83	274·6	1·4
S2	5·52	271·3	1·1	S6	5·02	276·6	1·9
S3	5·02	273·1	1·0	S7	5·67	281·2	3·5
S4	5·59	273·9	1·1	S8	6·01	340·9	62·4
					42·15		
		(ii) *Methyl esters of " liquid " acids. Primary fractionation.*					
L1	0·39	135·6	—	L6	7·93	292·8	169·3
L2	23·83	288·9	148·9	L7	7·18	296·9	164·4
L3	14·65	288·2	160·8	L8	5·13	292·9	163·9
L4	6·79	292·8	165·1	L9	8·90	396·4	126·9
L5	6·21	292·8	168·5				
					81·01		
		(iii) *Refractionation of the primary fraction L2.*					
L21	2·43	250·4	90·5	L24	4·71	293·9	162·4
L22	4·73	287·4	120·8	L25	4·31	295·0	168·9
L23	4·93	290·1	146·7				
					21·11		

Another portion of the esters of the " liquid " acids was also distilled in order to obtain both iodine and thiocyanogen values for the mixture of unsaturated C_{18} esters :

Methyl esters of " liquid " acids (second analysis).

Fraction No.	Wt., g.	Sap. equiv.	Iod. val.	SCN val.	Fraction No.	Wt., g.	Sap. equiv.	Iod. val.	SCN val.
2L1	0·85	—	—	—	2L4	9·80	296·4	169·5	106·8
2L2	32·13	289·7	148·9	93·6	2L5	9·50	358·2	207·2	103·6
2L3	10·34	295·8	168·9	102·1					
						62·62			

Qualitative examination of individual ester fractions. The acids present in certain of the ester fractions were characterised as follows :

Fraction L1. The acids, dissolved in hot water, separated on cooling as white glistening crystals (m. p. 121°, unaltered when mixed with pure benzoic acid).

Fractions L6 and L7. The acids from these fractions (5·35 g.) were united, dissolved in ether, and treated with bromine; 1·77 g. of acids (m. p. 174·5°; Br, 62·3%) insoluble in ether then separated ($C_{18}H_{30}O_2Br_6$ requires Br, 63·3%). When the bromo-adducts soluble in ether were recovered and crystallised from light petroleum, only 0·12 g. of crystalline products (m. p. 105—106°) separated; the small amount present of the form of linoleic acid which yields a tetrabromostearic acid of m. p. 114° is very unusual, and it appears as though the octadecadienoic acid, present in quantity in this seed fat, differs from the form usually encountered in seed fats. The bromo-adducts which remained in solution in light petroleum obviously consisted largely, from their bromine content (48·4%), of tetrabromostearic acids ($C_{18}H_{32}O_2Br_4$ requires Br, 53·3%).

The residual fraction L9. This contained acids which, when freed from unsaponifiable matter, had sap. equiv. 262·9, iod. val. 113·4.

The residual fraction S8. This contained acids of mean molecular weight 282·1 (after as complete removal as possible of the somewhat difficultly soluble unsaponifiable matter). No evidence was obtained for the presence of the lignoceric or cerotic acids reported by Kumaraswamy and Manjunath (*loc. cit.*).

Examination of the acids volatile in steam. The aqueous distillate was thoroughly extracted with ether, and the ethereal solution dried over anhydrous sodium sulphate for about a week.

(i) *Ether-soluble acids.* The residue, obtained after removal of the ether, was distilled at atmospheric pressure; only a small amount passed over :

Distillation of ether-soluble, steam-volatile acids.

Fraction No.	Wt., g.	B. p./760 mm.	Sap. equiv.
E1	0·21	60—96°	86·5
E2	0·67	96—100—80	92·9
E3	4·54	Residue	136·7

Fractions E1 and E2 were soluble in cold water, and consisted of formic and acetic acids with a little water.

The residue E3 was soluble only in hot water; the solution, on cooling, deposited a crystalline solid (equiv. 122·0) which melted at 121° (unchanged on admixture with benzoic acid).

(ii) *Water-soluble acids.* The aqueous distillate, after extraction with ether, amounted to 7865 c.c. A portion was neutralised with potash and concentrated and, from its reduction of solutions of potassium permanganate and of mercuric chloride, evidently contained formic acid. (Steam-distillation of the neutralised aqueous distillate gave a further distillate which possessed no reducing properties, showing that aldehydes or other non-acidic reducing compounds were not present.) The presence of acetic, as well as formic, acid was proved by the formation of ethyl acetate on heating the evaporated neutral salts with ethyl alcohol in presence of sulphuric acid.

The total acidity, and the amount of N/10-potassium permanganate required for oxidation, of separate aliquot portions of the original ether-extracted distillate were then determined, and the results calculated in terms of a mixture of formic and acetic acids. In this way it was found that the aqueous distillate (7865 c.c.) contained 15·6 g. of formic and 5·0 g. of acetic acid.

From the preceding quantitative data, coupled with the qualitative examination of the various acids, it is calculated that the component acids of the seed fat had the following composition :

Acid.	Steam-volatile acids. Ether-soluble, 1·95%.	Steam-volatile acids. Water-soluble, 7·45%.	Non-steam-volatile acids. "Solid" acids, 20·5%.	Non-steam-volatile acids. "Liquid" acids, 70·1%.	Total.	Total acids. % (wt.).	Total acids. % (mol.).	Fatty acids. % (wt.).	Fatty acids. % (mol.).
Formic	0·13	5·65	—	—	5·78	6·0	25·7	—	—
Acetic	0·04	1·80	—	—	1·84	1·8	6·2	—	—
Benzoic	1·46	—	—	0·50	1·96	2·0	3·3	—	—
Palmitic	—	—	15·75	3·68	19·43	20·1	15·5	22·3	23·8
Stearic	—	—	3·79	—	3·79	3·9	2·7	4·3	4·2
Oleic	—	—	0·42	14·45	14·87	15·3	10·7	17·0	16·6
Linoleic	—	—	—	37·65	37·65	38·8	27·3	43·0	42·2
Linolenic	—	—	—	11·73	11·73	12·1	8·6	13·4	13·2
Unsaponifiable, etc.	0·32	—	0·54	2·09	2·95	—	—	—	—

Non-glyceridic Components of C. paniculatus *Seed Fat.*—The occurrence of formic, acetic, and benzoic acids in combination in a fatty oil is very unusual. Moreover, the proportion by weight (9·8%) of these three acids in the total acids is extremely large in comparison with the observed amount (3%) of " unsaponifiable matter " (*i.e.*, non-acidic components soluble in ether but insoluble in water). In order to ascertain whether the low molecular weight acids were present as glycerides or as esters of some other alcohol, the seed oil was submitted to diphasic separation between light petroleum and aqueous (80%) methyl alcohol.

The seed oil (300 g.), dissolved in light petroleum (1200 c.c., b. p. 40—60°), was washed twenty times with 200 c.c. portions of 80% methyl alcohol, which removed 83 g. of material (M1), leaving 217 g. in the light petroleum solution (P1). The extract M1 (83 g.) was again dissolved in light petroleum (1660 c.c.) and washed five times with 350 c.c. portions of 80% methyl alcohol, which removed 56·4 g. (M2), and left 26·6 g. (P2) in the light petroleum. The general characteristics of these fractions of the seed oil were as follows :

	G.	Sap. equiv.	Iod. val.	Unsap. (%).	Acids (ether-soluble) freed from unsaponifiable matter. Equiv.	Acids (ether-soluble) freed from unsaponifiable matter. Iod. val.
Original oil	300	217·3	103·9	3·1	260·0	120·2
P1 (soluble in light petroleum)	217	230·8	120·1	3·5	270·8	131·6
P2 (,, ,, ,,)	26·6	248·5	106·5	2·4	268·5	115·3
M1 (first fraction from methyl alcohol)	83	182·8	63·9	3·3	218·0	96·5
M2 (final fraction from methyl alcohol)	56·4	164·4	41·9	1·8	(see below)	

Although only a partial separation was achieved by this means, the figures show that the acids of low molecular weight are present in the form of compounds relatively soluble in methyl alcohol, and it is therefore unlikely that the latter were glycerides. This was confirmed by a semi-quantitative examination of the acids present in the methyl alcohol-soluble portion (M2) :

Volatile in steam, water-soluble, ether-insoluble	formic	6·0 g.
	acetic	2·4 g.
,, ,, ,, ,, ether-soluble (equiv. 128·3, mainly benzoic)		8·3 g.
Non-steam volatile (equiv. 232·5*; iod. val. 99·2)		23·2 g.

* After boiling with water, the equivalent of the insoluble (fatty) acids was 265·6.

Formic, acetic, and benzoic acids thus formed together about 40% of the total acids in the fraction M2, as compared with 10% in the whole seed oil. The total weight of acids (39·9 g.) obtained is equivalent to about 45 g. of glycerides, so that (with about 1 g. of " unsaponifiable matter " also present) there would remain about 10 g. of the fraction M2 unaccounted for, on the assumption that glycerides were the only esters present.

The aqueous liquors from the hydrolysis and separation of the non-steam-volatile acids were therefore neutralised and evaporated to dryness, and the powdered residue thoroughly extracted with acetone in a Soxhlet apparatus. On removal of the acetone there were left nearly 20 g. of a dark syrup which, when acetylated, was only partly soluble in water. An ethereal solution of the acetylated product was washed repeatedly with water in order to remove triacetin as far as possible, and the substance left in solution in the ether was recovered and distilled under 1 mm.; the bulk then distilled at 180—185° and formed, on cooling, a transparent, pale yellow, resinous solid [sap. equiv. 110·3; C, 60·3; H, 7·4%; *M* (Rast), 441]. The nature of this compound has not been further determined, but it evidently contains four hydroxylic groups

in the molecule and is non-benzenoid in character (the tetra-acetates of, for example, substances of the formula $C_{14}H_{24}O_5$ or $C_{15}H_{26}O_5$ would require, respectively : C, 60·0, 60·8; H, 7·3, 7·5%; *M*, 440, 454; equiv., 110·0, 113·5).

On the assumption that, in the fraction M2 (sap. equiv. 164·4), the higher fatty acids are wholly in combination as glycerides, the mean equivalent of the non-glyceridic portion would be about 130. This is consistent with the view that the lower acids (formic, acetic, benzoic) present are in the form of acyl derivatives of the tetrahydroxylic compound which has just been described. We consider that the evidence we have obtained is sufficient to justify the conclusion that these lower molecular weight acids do not form an essential part of the seed fat of *Celastrus* species, but are present as esters of a tetrahydroxylic compound which are soluble in the fat and therefore accompany it during its extraction. It may be, of course, that the specific medicinal properties attributed to these fats are connected with the presence of these substances.

Component Acids of Celastrus paniculatus *Fruit-coat Fat.*—The acids liberated from the hydrolysed fat (81·5 g.) were steam-distilled till the distillate was free from acid. After removal of unsaponifiable matter (5·39 g.) the non-steam-volatile acids (60·5 g., equiv. 269·6, iod. val. 108·3) were separated into " solid " (28·9%) and " liquid " (71·1%) acids. The fractional distillation of the methyl esters of the " solid " and of the " liquid " acids, and the examination of the acids volatile in steam, followed the same course as in the corresponding portions of the seed-fat acids (cf. p. 1981), except that the esters of the " liquid " acids were distilled through an electrically-heated and packed column.

The ester fractionation data were as follows :

(i) *Methyl esters of " solid " acids (distilled from Willstätter bulb).*

Fraction No.	Wt., g.	B. p./0·1 mm.	Sap. equiv.	Iod. val.	Fraction No.	Wt., g.	B. p./0·1 mm.	Sap. equiv.	Iod. val.
S1	2·48	110—125°	269·9	0·7	S4	3·00	126°—falling	275·3	1·6
S2	2·87	125—126	271·4	0·8	S5	3·77	Residue	281·8	10·6
S3	3·81	125—126	273·0	1·2					
						15·93			

(ii) *Methyl esters of " liquid " acids (distilled through electrically-heated and packed column).*

Fraction No.	Wt., g.	B. p./0·1 mm.	Sap. equiv.	Iod. val.	SCN val.	Fraction No.	Wt., g.	B. p./0·1 mm.	Sap. equiv.	Iod. val.	SCN val.
L1	0·20	26—90°	196·3	—	—	L5	6·84	132—134°	293·1	159·2	111·1
L2	1·96	90—118	257·8	38·2	—	L6	7·76	132—134	293·6	158·9	111·4
L3	3·07	118—132	285·2	119·2	—	L7	5·12	134—falling	294·4	160·6	109·9
L4	6·07	132	292·5	146·4	109·1	L8	4·24	Residue	288·4	122·5	—
							35·26				

Examination of acids volatile in steam. Ether extraction of the aqueous distillate (6510 c.c.) yielded 2·52 g. of acids (equiv. 166·6, iod. val. 36·9) which contained some unsaponifiable matter and also benzoic and a small quantity of oleic acid. The ether-extracted aqueous distillates contained formic (1·6 g.) and acetic (2·7 g.) acids.

The quantitative composition of the mixed acids present in the fruit-coat fat is calculated from the above data to be approximately as follows :

	Steam-volatile acids.		Non-steam-volatile acids.			Total acids.		Fatty acids.	
Acid.	Ether-soluble, 3·7%.	Water-soluble, 6·4%.	"Solid" acids, 26·0%.	"Liquid" acids, 63·9%.	Total.	% (wt.).	% (mol.).	% (wt.).	% (mol.).
Formic	—	2·38	—	—	2·38	2·4	11·0	—	—
Acetic	—	4·02	—	—	4·02	4·0	14·1	—	—
Benzoic	2·36	—	—	0·10	2·46	2·5	4·3	—	—
Myristic	—	—	—	2·72	2·72	2·7	2·5	3·0	3·6
Palmitic	—	—	21·31	2·47	23·78	23·8	19·6	26·2	27·8
Stearic	—	—	3·66	—	3·66	3·7	2·7	4·0	3·9
Oleic	1·15	—	1·03	25·58	27·76	27·8	20·8	30·5	29·4
Linoleic	—	—	—	17·36	17·36	17·4	13·1	19·1	18·5
Linolenic	—	—	—	15·67	15·67	15·7	11·9	17·2	16·8
Unsaponifiable	0·19	—	—	—	0·19	—	—	—	—

The fruit-coat oil resembles that from the seeds in containing nearly 10% (wt.) of combined formic, acetic, and benzoic acids; examination of the non-acidic, water-soluble products of hydrolysis disclosed the presence, in addition to glycerol, of a syrupy compound with the same properties as that isolated from the products of hydrolysis of the seed fat. The higher fatty acids in the glyceridic portion of the fruit-coat fat are very similar to those of the seed fat, except that, of the unsaturated C_{18} acids, oleic is most abundant in the fruit-coat fat, and linoleic in the seed fat.

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