FATTY ACID COMPOSITION OF SOME CUCURBITACEAE SEED OILS¹

MARY J. CHISHOLM AND C. Y. HOPKINS

Division of Pure Chemistry, National Research Council, Ottawa, Canada Received October 15, 1963

ABSTRACT

Seed oils of nine species of Cucurbitaceae were analyzed by gas chromatography and other techniques. The oil of *Ecballium elaterium* yielded 22% of punicic acid; *Cucumis dipsaceus* and *Cucurbita ficifolia* had the ordinary palmitic-oleic-linoleic composition; a variety of *Momordica charantia* had 57% of α -eleostearic acid. Occurrence of punicic acid as the major acid of *Trichosanthes anguina* was confirmed. No evidence could be found for the existence of an isomer of linoleic acid in *Telfairia pedata* oil. The fatty acid composition was determined quantitatively for the above oils and for those of *Cucumis melo*, *Lagenaria siceraria*, and *Luffa cylindrica*. The 4-bromophenacyl esters of α -eleostearic and punicic acids were prepared.

Seed oils of the Cucurbitaceae family fall into two distinct groups, one having the ordinary palmitic-oleic-linoleic composition, the other having a considerable proportion of conjugated triene fatty acids in the glycerides. The first group includes the common cultivated cucurbits. The second or conjugated oil group consists of certain species of *Cucurbita*, *Momordica*, *Telfairia*, and *Trichosanthes*.

Nine species of the family were examined in the present work to ascertain or confirm the identity of the conjugated acids and to resolve certain anomalies in previous studies. *Ecballium elaterium, Cucumis dipsaceus,* and *Cucurbita ficifolia* had not been analyzed previously. The percentage composition of each oil was determined by gas-liquid chromatography (GLC) of the methyl esters, in conjunction with ultraviolet absorption analysis. The conjugated acids, when present in substantial amount, were isolated and purified by crystallization at low temperatures. They were identified by orthodox chemical methods.

RESULTS AND DISCUSSION

Preliminary data are shown in Table I. The high refractive indices of the first three oils indicate conjugated unsaturation and this was confirmed by their ultraviolet spectra and by isolation of conjugated triene fatty acids (see Table II). None of the oils had an absorption peak in the region of 233 m μ ; thus they contained no appreciable amount of conjugated diene acid.

In the analysis of the methyl esters by GLC, neither myristic nor linolenic acid was observed, although these two acids had been reported in some species by earlier workers (11, 15, 16). The results suggest that seed oils of Cucurbitaceae contain little or no linolenic acid. No acids other than those shown in Table II were observed. The infrared spectra did not disclose any evidence of oxygenated acids and GLC analysis showed that C_{20} and C_{22} acids were absent.

There was evidently some loss of conjugated triene ester on the GLC column during the analysis. Accordingly, the content of conjugated triene was determined by ultraviolet absorption analysis and the amount of each non-conjugated acid was calculated as a percentage of the remainder.

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TABLE I Seed oils of Cucurbitaceae

Species	Oil in seed,* wt.%	Refractive index, 25°	Iodine value
Oils with conjugated unsaturation Echallium elaterium A. Rich. Momordica charantia L. var. Long green	33.3	1.4880	
monsoon	25.4	$(1.4990)^{\dagger}$	
Trichosanthes anguina L.	29.1	1.4979	
Oils with ordinary unsaturation Cucumis dipsaceus Ehrenb. Cucumis melo L. Cucurbita ficifolia Bouché Lagenaria siceraria Standl. Luffa cylindrica Roem. Telfairia pedata Hook	28.8 29.8 32.9 26.8‡ 20.1 38.3§	$1.4742 \\ 1.4748 \\ 1.4722 \\ 1.4721 \\ 1.4741 \\ 1.4730 \\ 1.4671$	$139.0 \\ 132.6 \\ 121.8 \\ 134.3 \\ 126.6 \\ 83.2$

†At 37°; solid at 25°. ‡In kernel, 51.1%. §In kernel, 61.0%.

	TAB	LE I	I
Estimated	fatty	acid	composition

Species	Fatty acid, wt. % of total acids					
	Conjugated trienoic	16:0*	18:0	18:1	18:2	
Ecballium elaterium	22.2†	9	3	9	57	
Momordica charantia	56.9‡	2	34	4	3	
Trichosanthes anguina	42.8^{+}	6	7	23	21	
Cucumis dipsaceus	0.8	10	5	6	78	
Cucumis melo	0.5	11	4	15	70	
Cucurbita ficifolia	0.1	12	4	26	57	
Lagenaria siceraria	0.3	14	. 3	7	76	
Luffa cylindrica	0.2	13	6	15	- 66	
Telfairia pedata	0.4	35 `	14	7	44	

*The figures indicate chain length and number of double bonds. †Punicic acid. ‡α-Eleostearic acid.

The conjugated triene esters gave rise to a broad composite peak in the C_{22} region. This peak undoubtedly represents a mixture of the original conjugated ester and one or more of its geometric isomers, produced by cis-trans isomerization on the column, as observed with methyl α -eleostearate by Morris, Holman, and Fontell (1).

Some degree of uniformity can be seen in the fatty acid composition of this group of oils. In seven of the nine species, in spite of the variation in individual acids, the sum of the unsaturated acids fell within the range 81-88% of the total acids. (The exceptions were Momordica charantia and Telfairia pedata.) This suggests that the unsaturated acids, both conjugated and non-conjugated, may arise from a common substrate.

From a taxonomic viewpoint, it is noted that all species of *Momordica* and *Trichosanthes* reported to date have conjugated oils (three species of each) and all species of Citrullus, *Cucumis*, and *Luffa* studies so far have non-conjugated oils (three, three, and four species). On the other hand, one species of *Telfairia* has a conjugated oil (2) and one has not. Similarly, two species of *Cucurbita* have an appreciable amount of conjugated fatty acid (3) while three species have a negligible amount.

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Ecballium elaterium

Punicic acid was isolated from the oil of this seed and identified by its spectral absorption and by mixed melting point with an authentic sample. Punicic acid constituted 22% of the total fatty acids but the largest component was linoleic acid.

Momordica charantia

Occurrence of α -eleostearic acid as the major acid of *M. charantia* seed oil has been established (4, 5). The present sample, variety "Long green monsoon", had a noticeably higher content of α -eleostearic acid (57%) than that of the unnamed varieties reported previously (40–48%). This was reflected in the higher refractive index and higher melting point of the oil. The oil is unusual in composition, since the fatty acids consist almost entirely of α -eleostearic acid (57%) and stearic acid (34%). There is less than 5% each of palmitic, oleic, and linoleic acids.

Trichosanthes anguina

The major acid of *T. anguina* was reported to be an isomer of α -eleostearic acid (6, 7) and was identified unequivocally by Crombie and Jacklin in 1957 as punicic acid (8). However, in 1960, Chowdhury (9) re-examined the oil of this species and found the major component to be α -eleostearic acid; conclusive evidence of its identity was given. Our sample of this seed yielded punicic acid as the major component of the oil (43% of the total acids) and there was no evidence of α -eleostearic acid. It is possible that Chowdhury had an unusual variety of *T. anguina*.

The strong infrared band at 752 cm⁻¹ appears to be characteristic of the cis,trans,cis grouping in punicic acid and was not observed in the spectrum of α -eleostearic acid (cis,trans,trans).

Telfairia pedata

An early report of the occurrence of an isomer of linoleic acid in T. pedata oil (10) was refuted by several authorities (11, 12). However, in view of the recent discovery of linoleic acid isomers in a seed oil (13, 14), T. pedata was re-examined. The infrared spectrum of the oil showed no evidence of trans unsaturation, thus ruling out the presence of geometric isomers. The total mixed acids were subjected to oxidative splitting and the fragments were identified by gas chromatography over a wide range of column temperatures. The expected acids, hexanoic, nonoic, and azelaic (from the oxidation of oleic and linoleic acids), were found in quantity. The major dienoic acid is, therefore, linoleic acid. Other fragments were few and very small in amount. It was judged from the GLC data that traces of valeric, heptanoic, and glutaric acids were produced. It is possible, therefore, that traces of isomers of linoleic acid were present but it is estimated that the amount of any one isomer would not exceed 1% of the total fatty acids.

T. pedata oil differs markedly in composition from other Cucurbitaceae oils, having a much higher content of saturated acids (49%). The major acid is linoleic (44%). The data confirm the earlier analyses by Smit and van Loon (11) and by Hilditch and Meara (15) except that linolenic acid was not present in our sample.

Other Species

The other species in this study had the ordinary palmitic-oleic-linoleic composition, with minor amounts of stearic acid and less than 1% of conjugated triene acid. No other fatty acids were detected and it is judged that any minor component acid that may be present does not exceed 0.5% of the total. All of the non-conjugated oils had a relatively

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high content of octadecadienoic acid. The fatty acids of *Cucumis dipsaceus* oil contained 78% of linoleic acid, identified as its tetrahydroxy derivative, and it is presumed that the dienoic acid in the other species is also linoleic.

A high refractive index, reported earlier for *Cucumis melo* oil (16), suggested the presence of conjugated acids. However, the present sample had a normal refractive index and contained only 0.5% of conjugated acids.

EXPERIMENTAL

Seed of *Telfairia pedata* (oyster nut) was supplied and its identity was verified through the kindness of Dr. D. H. S. Horn. The other seeds were obtained from reliable commercial sources. Ultraviolet spectra were determined in cyclohexane solution with a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulphide in a Perkin-Elmer model 21 spectrophotometer with sodium chloride prism. Melting points were determined in capillary tubes and were corrected. The oils were obtained by Soxhlet extraction with petroleum ether (b.p. 30-60°) except where noted otherwise. Iodine values were determined by the Wijs method (30 minutes).

The oils were converted to methyl esters by transesterification in methanol with hydrogen chloride catalyst. The esters were examined by GLC and the composition was estimated by measuring the peak areas. The GLC analyses were made using a copper column at 185° and a thermal conductivity (thermistor) detector. The liquid phase was a diethylene glycol – succinic acid polyester. The average of three determinations for each oil is reported in Table II. A slightly different procedure was followed with oils containing much conjugated acid, as explained above.

Ecballium elaterium

The seeds were ground and extracted by stirring with petroleum ether at 25° and the solvent was removed in a current of nitrogen at 25–30°. The oil had ultraviolet absorption λ_{max} 266, 275, 287 mµ and absorptivity $E_{1\,cm}^{1\%}$ 382 at 275 mµ, equivalent to 22.2% of conjugated triene acid. The oil was hydrolyzed by refluxing under nitrogen for 30 minutes with aqueous 6% potassium hydroxide. The acids (8.5 g) were dissolved in 85 ml of acetone and cooled. Crystals were taken off at -10, -30, and -55°. Ultraviolet absorption analysis showed that the -30° fraction had 68% and the -55° fraction 23% of conjugated triene acid. The -55° fraction (3.65 g) was crystallized three times from pentane at -25°, yielding 0.41 g of punicic acid, m.p. 43.5-44°. After one crystallization from acetone it had m.p. 44.5-45.5°, alone and mixed with authentic punicic acid of m.p. 45.0-45.5°. The ultraviolet spectrum was the same as that of punicic acid, λ_{max} 265, 275, 287 mµ.

Momordica charantia

Seed of *M. charantia*, var. Long green monsoon (from Poona, India), was hulled and the kernel oil was extracted at 25°. The oil had λ_{max} 262, 272, 283 m μ and negligible absorption at 233 m μ . The absorptivity, $E_{1cm}^{1\%}$, was 1007 at 272 m μ , equivalent to 56.9% of conjugated triene acid, calculated as α -eleostearic. Infrared maxima were at 987 (vs) and 959 (w) cm⁻¹. The mixed fatty acids were crystallized fractionally from acetone at low temperatures. There was a large yield of crystals at 10°, mainly stearic acid. After one crystallization from ethanol it had melting point and mixed m.p. 68–69°. Conjugated triene acid was collected at -15° . After further crystallization from acetone it melted at 45.5–46.5°. It formed an adduct readily with maleic anhydride by the usual method (17). The adduct melted at 63.5–64.5°, alone and mixed with the maleic anhydride adduct of α -eleostearic acid.

Trichosanthes anguina

Seed of *T. anguina* (from Poona, India) yielded an oil having λ_{max} 265, 275, 287 m μ and $E_{1cm}^{1\%}$ 739 at 275 m μ , equivalent to 42.8% of conjugated triene fatty acid. Absorption at 233 m μ was negligible. Infrared maxima were at 982 (s) and 931 (s) cm⁻¹ (cis,trans,cis-triene), also at 752 cm⁻¹. There were no absorption bands attributable to hydroxy, keto, or isolated trans unsaturated groupings.

Twenty grams of the oil was hydrolyzed and the mixed acids were crystallized from acetone. Saturated acids were removed at -15° and the conjugated acid (6.5 g) at -38° . The latter, after three crystallizations from pentane melted at 44.5-45.5°, alone and mixed with puncic acid from pomegranate seed oil.

4-Bromophenacyl Esters

A solution of 0.5 g of punicic acid in 20 ml of ethanol was treated with 2,4'-dibromoacetophenone by the method of Neish and Lemieux (18). The crude product (0.8 g) was crystallized from ethanol at 0°, giving 4-bromophenacyl punicate, m.p. 61–63°. The ester is unstable like the acid. Its identity was confirmed by hydrogenation to the saturated ester by means of Adams catalyst in ethyl ether. The product, 4-bromophenacyl stearate, melted at 88–89° after two crystallizations from ethanol. The melting point was unchanged in admixture with an authentic sample of m.p. 88.5–89.0° (lit., 90°).

 α -Eleostearic acid was converted to its 4-bromophenacyl ester in the same way. The ester had m.p. 53–54° and was unstable. Anal. Calc. for C26H35O3Br: C, 65.67; H, 7.42. Found: C, 65.33; H, 7.40.

Telfairia pedata

Oil from seed kernels of T. pedata (origin South Africa) was hydrolyzed and the mixed fatty acids were subjected to oxidative splitting by permanganate-periodate (19). The unchanged saturated acids were filtered off and esterified with methanol (A). The water-soluble monobasic and dibasic acids were extracted from the filtrate with ether in a continuous extractor. They were converted to methyl esters (B) by a method that avoids loss by evaporation of any component (20).

The A esters, examined by GLC at column temperatures of 140°, 160°, and 185°, gave peaks corresponding to palmitate and stearate. The B esters, examined at temperatures from 50° to 195°, gave major peaks corresponding to hexanoate, nonoate, and azelate. The minor peaks were very small. They indicated traces of valerate, heptanoate, and glutarate. There was no indication of other monobasic acids from Ca to C15 inclusive, nor of dibasic acids from C3 to C14 inclusive.

The infrared spectrum of T. pedata oil showed no absorption characteristic of a terminal double bond (ca. 910 cm⁻¹), nor of an $\alpha\beta$ -unsaturated acid (ca. 1275 cm⁻¹).

Cucumis dipsaceus

The dienoic acid of the oil was identified as linoleic acid as follows. The mixed fatty acids (1.2 g) were oxidized by aqueous alkaline permanganate for 10 minutes at 10°. The solid product (0.92 g) was washed with hexane, then with ethyl acetate, and finally crystallized from ethanol, giving di-erythro-9,10,12,13tetrahydroxystearic acid, m.p. 173-174°, alone and mixed with an authentic sample.

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