IDENTIFICATION OF CONJUGATED TRIENE FATTY ACIDS IN CERTAIN SEED OILS[1, 2]

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ABSTRACT

Seed oils were hydrolyzed under mild conditions and the major conjugated fatty acid of each oil was isolated and identified. In two families, species which were closely related botanically contained different but isomeric acids. Thus, in the Bignoniaceae, *Jacaranda chelonia* had *cis,trans,cis*-8,10,12-octadecatrienoic acid as a major acid while *Catalpa speciosa* had *trans,trans,cis*-9,11,13-octadecatrienoic acid. In the Cucurbitaceae, *Momordica charantia* had the ordinary *cis,trans,trans*-9,11,13-octadecatrienoic (α-eleostearic) acid while *M. balsamina* had *cis,trans,cis*-9,11,13-octadecatrienoic (punicic) acid. *M. balsamina* is a new and convenient source of punicic acid. α-Eleostearic acid was identified as a major acid in examples of Valerianaceae and Rosaceae. Further proof was obtained that the fatty acid of *Calendula officinalis* (Compositae) is *trans,trans,cis*-8,10,12-octadecatrienoic acid.

Until the year 1956 only two simple conjugated triene fatty acids were known to occur in seed oils, viz. α-eleostearic and punicic acids. Three additional acids of this type have been discovered since then (1, 2, 3, 4). All five of these simple conjugated acids have an 18-carbon straight chain; in two of them the unsaturated grouping occupies the 8,10,12 position but in the other three it is in the 9,11,13 position. The present work reveals the occurrence of four of these acids in plant species in which they were not known previously (Table I). Positive identification of each acid was made by orthodox chemical procedures.

TABLE I
Occurrence of conjugated octadecatrienoic acids
$CH_3(CH_2)_xCH{=}CH{-}CH{=}CH{-}CH{=}CH(CH_2)_yCOOH$

Species	Formula	Unsaturated grouping: Position	Unsaturated grouping: Configuration	Reference
Jacaranda chelonia (Bignoniaceae)	I	8,10,12	cis,trans,cis	*
Catalpa speciosa (Bignoniaceae)	II	9,11,13	trans,trans,cis	*
Momordica charantia (Cucurbitaceae)	III	9,11,13	cis,trans,trans (α-eleostearic)	5
Momordica balsamina (Cucurbitaceae)	IV	9,11,13	cis,trans,cis (punicic)	*
Calendula officinalis (Compositae)	V	8,10,12	trans,trans,cis	1, 2
Centranthus ruber (Valerianaceae)	III	9,11,13	cis,trans,trans	*
Cotia chestnut (Rosaceae, see text)	III	9,11,13	cis,trans,trans	*
Prunus mahaleb (Rosaceae)	III	9,11,13	cis,trans,trans	6

*Present work

Seed of *Jacaranda chelonia* Griseb. (Bignoniaceae) yielded an oil containing a substantial amount of conjugated triene acid, as shown by its ultraviolet spectrum. The acid was isolated and identified as *cis,trans,cis*-8,10,12-octadecatrienoic acid (I) by melting point, mixed melting point, and ultraviolet spectrum. The only previously known occurrence of this acid is in the oil of *Jacaranda mimosifolia* D. Don (4).

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The seed oil of *Catalpa speciosa* Warder, also a member of the Bignoniaceae family, yielded a different triene acid. It melted at 31–32° and gave an adduct with maleic anhydride which melted at 72–73°. Its ultraviolet spectrum indicated a triene grouping of two trans and one cis bond (cf. ref. 7). The acid was identified as *trans,trans,cis*-9,11,13-octadecatrienoic acid (II) by mixed melting point with an authentic sample. This was confirmed by mixed melting point of the maleic anhydride adducts. The acid is known to occur in the oil of *Catalpa ovata* Don (3) but has not been observed elsewhere. Occurrence of this trans,trans,cis acid nullifies an earlier suggestion that the 9:10 double bond in natural triene acids is always cis (8).

The existence of acid I in two species of *Jacaranda* and of acid II in two species of *Catalpa* provides rather striking confirmation of the botanical classification of these plants. It is notable that one genus produces the 8,10,12-triene while the other produces the 9,11,13-triene, also that the two acids differ in their geometric configuration. Although it cannot be taken for granted that a given species of plant will always produce the same isomer of a conjugated fatty acid, we are not aware of any evidence to the contrary.

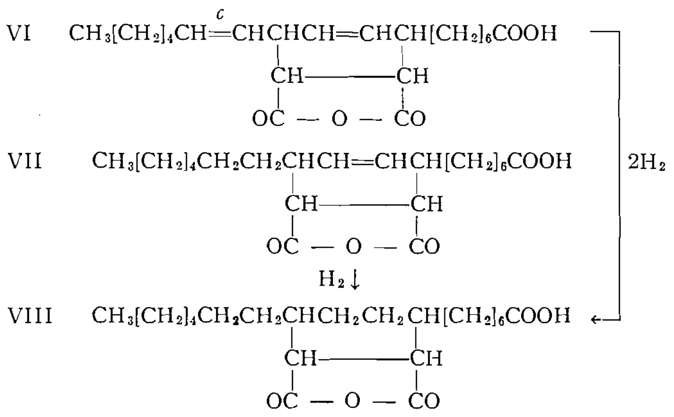
The oil of *Momordica charantia* L. (Cucurbitaceae) has been studied in the past and the presence of α-eleostearic acid has been reported. Claims that the oil contained trichosanic (punicic) acid have apparently not been substantiated. Verma and Aggarwal (5) isolated the acid and identified it as α-eleostearic by melting point, ultraviolet absorption, and conversion to the all-trans form. However, they did not report any mixed melting points. Our sample of *Momordica charantia* seed yielded α-eleostearic acid (III) as the major acid and its identity was proved conclusively, thus confirming the work of Verma and Aggarwal.

Another species of the same genus, *M. balsamina* L., contained a different acid. Earle and co-workers (9) reported that the oil of this species contained 50% of a conjugated triene acid and listed it as trichosanic acid, although evidence of its identification was not given. Our sample of the oil was estimated to contain 58% of a conjugated triene acid which was identified unambiguously as punicic acid (synonymous with trichosanic), i.e. *cis,trans,cis*-9,11,13-octadecatrienoic acid (IV). Thus it appears that two species of the same genus produce different major fatty acids, indicating a marked specificity of fatty acid biosynthesis. The two acids from these species differ only in geometric configuration (cis,trans,trans and cis,trans,cis).

Punicic acid has been available hitherto only from *Punica granatum* (Punicaceae) and from *Trichosanthes* sp. (Cucurbitaceae). *Momordica balsamina* constitutes a useful new source of the acid since the seed is available commercially and the oil has a high content of punicic acid.

The oil of *Calendula officinalis* L. (Compositae) was shown by McLean and Clark to contain an 8,10,12-octadecatrienoic acid (1). Later, the configuration of this acid was found to be trans,trans,cis (V) by infrared studies and by degradation of its maleic anhydride adduct (2). The trans,trans,cis configuration was confirmed in the present work by hydrogenating the adduct (VI) and comparing the product with the compound (VIII) obtained by hydrogenating the adduct (VII) of *trans,trans*-8,10-octadecadienoic acid; both melted at 105.5–106.5° and a mixed melting point determination showed that they were identical. Hence, the two trans double bonds of the triene acid must be at positions 8 and 10.

Seed oil of *Centranthus ruber* DC. (Valerianaceae) was found to contain a conjugated triene acid which was isolated and identified as α-eleostearic acid. This appears to be the first identification of a conjugated triene acid in this family although Earle and co-



workers (9) reported infrared evidence for a triene acid in *Valeriana officinalis* that was listed as eleostearic acid. *Valerianella olitoria*, of the same family, was shown by Steger and van Loon to contain no conjugated acid (10) and we have confirmed this.

Two species of Rosaceae were examined. The kernel oil of the cotia chestnut was reported earlier to contain a conjugated acid on the basis of its diene value and drying properties (11). Our sample of this nut, obtained from the vicinity of Tefe, Amazonas, Brazil, yielded an oil containing about 23% of α-eleostearic acid, identified by means of its maleic anhydride adduct. There was no evidence of an hydroxy acid. The cotia chestnut is considered to be a species of *Parinarium* or *Licania* (family Rosaceae) but there is still some doubt concerning its classification.

The oil of *Prunus mahaleb* L. was re-examined. It was studied by Alpar and Esin (6), who isolated the triene acid and prepared derivatives which showed fairly conclusively that it was α-eleostearic acid. In the present work its identity has been confirmed by means of spectral analysis and mixed melting points of the acid and derivatives.

These results, taken in conjunction with previous data, indicate that α-eleostearic is the most commonly occurring conjugated triene acid; it is found in species of Rosaceae, Euphorbiaceae, Cucurbitaceae, and Valerianaceae. Punicic acid occurs in three species of Cucurbitaceae and one of Punicaceae. Other positional or geometric isomers of octadecatrienoic acid are found in four species of Bignoniaceae and one of Compositae.

EXPERIMENTAL

Cotia chestnuts were supplied through the kindness of Dr. K. S. Markley. Other seeds were obtained from commercial sources. Melting points were determined in capillary tubes and are corrected. Ultraviolet spectra were determined in cyclohexane solution, except where noted, with a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulphide in a Perkin–Elmer model 21 spectrophotometer with sodium chloride prism.

Jacaranda chelonia

Seed of *J. chelonia* Griseb. (10 g) was ground and extracted at room temperature by stirring with 30–60° petroleum ether. It yielded 19% of oil. The solvent was removed at 25–30° under nitrogen. The oil (1.9 g) had ultraviolet absorption λ_{max} 265, 275, 287 mμ and absorptivity $E_{1\,cm}^{1\%}$ 540 at 275 mμ, equivalent to about 31% of conjugated triene fatty acid. The oil was hydrolyzed, the unsaponifiable matter removed, and the acids recovered by methods described previously (3). The mixed acids were crystallized from acetone and then from pentane in the usual way (3), yielding 0.38 g of pure conjugated acid, m.p. 43.5–44°, λ_{max} 265, 275, 287 mμ. The melting point was unchanged in admixture with an authentic sample of *cis,trans,cis*-8,10,12-octadecatrienoic acid prepared from the oil of *J. mimosifolia* (4).

Catalpa speciosa

Seed of *C. speciosa* Warder, treated as described above, yielded 22% of oil having λ_{max} 262, 272, 283 mμ and absorptivity $E_{1\,cm}^{1\%}$ 604 at 272 mμ, equivalent to about 34% of conjugated triene fatty acid. The mixed acids were crystallized at low temperature, giving a triene acid, m.p. 31–32°, λ_{max} 262, 272, 283 mμ. It reacted readily with maleic anhydride, giving an adduct, m.p. 72.5–73.5°. The melting point was unchanged in admixture with the corresponding adduct of *trans,trans,cis*-9,11,13-octadecatrienoic acid, prepared from the oil of *C. ovata* (3).

Momordica charantia

Seed of *M. charantia* L. was extracted and the fatty acids of the oil were crystallized in the usual way (3). A triene acid was obtained. It melted at 46–47°, alone and mixed with authentic α-eleostearic acid prepared from tung oil. Its maleic anhydride adduct melted at 63–64°, alone and mixed with the maleic anhydride adduct of α-eleostearic acid.

Momordica balsamina

Seed of *M. balsamina* L. yielded an oil having λ_{max} 266, 275, 286 mμ and absorptivity at 275 mμ equivalent to about 58% of conjugated triene fatty acid. Crystallization of the fatty acids yielded a triene acid, m.p. 44–45°, unchanged in admixture with punicic acid prepared from *Punica granatum*. The pure *Momordica* acid had λ_{max} 265, 275, 287 mμ; absorptivity $E^{1\%}_{1\,cm}$ 1694 at 275 mμ; infrared absorption, ν_{max} 933 (s), 983 (s) cm^{-1}. The absorption maxima were identical with those of punicic acid. The *Momordica* acid did not form an adduct with maleic anhydride under the normal conditions for this reaction. It formed a *p*-phenylphenacyl ester, m.p. 61.5–63.5°, alone and mixed with an authentic sample of the *p*-phenylphenacyl ester of punicic acid (4).

Calendula officinalis

trans,trans-8,10-Octadecadienoic acid was prepared from oleic acid by the method of Gupta and Kummerow (12). The diene acid (0.24 g), m.p. 55–56°, was refluxed with benzene (20 ml) containing maleic anhydride (0.21 g) for 6 hours. The product was crystallized from ether:petroleum ether (3:4) at 0°, giving 0.18 g of the pure adduct, 3-6′-carboxyhexyl-6-heptylcyclohexene-4,5-dicarboxylic anhydride (VII), m.p. 109–110°. Von Mikusch gives m.p. 110° (13). Calcd. for $C_{22}H_{34}O_5$: C, 69.8; H, 9.05. Found: C, 70.1; H, 9.06.

The adduct (VII) was hydrogenated in methanol with Adams catalyst and the product was crystallized from methanol at −30°, giving 3-6′-carboxyhexyl-6-heptylcyclohexane-1,2-dicarboxylic anhydride (VIII), m.p. 105.5–106.5°. Calcd. for $C_{22}H_{36}O_5$: C, 69.4; H, 9.5. Found: C, 69.5; H, 9.7.

The triene acid of *Calendula officinalis* L., 8,10,12-octadecatrienoic acid, and its adduct with maleic anhydride were prepared as described previously (2). The adduct, m.p. 73–74°, considered to be 3-6′-carboxyhexyl-6-*cis*-1′-heptenylcyclohexene-4,5-dicarboxylic anhydride (VI), was hydrogenated in methanol with Adams catalyst; it absorbed 2 moles of hydrogen. The product was crystallized from methanol at 0°. It melted at 105.5–106.5° and the melting point was not depressed in admixture with compound VIII, prepared from *trans,trans*-8,10-octadecadienoic acid. Hence the identity of VI is confirmed.

Centranthus ruber

Seed of *C. ruber* DC. yielded an oil having λ_{max} 262, 271, 282 mμ and absorptivity at 271 mμ equivalent to about 43% of conjugated triene fatty acid. The mixed fatty acids were crystallized, giving a triene acid that was somewhat difficult to purify. It melted at 42–43°. On treatment with iodine in pentane it gave β-eleostearic acid, m.p. and mixed m.p. 70–71°. The unisomerized acid reacted readily with maleic anhydride to give an adduct, m.p. 63.5–64.5° alone and mixed with the adduct of α-eleostearic acid (14).

Oil of *Valerianella olitoria* Poll. had negligible ultraviolet absorption in the conjugated triene region.

Cotia chestnut

Kernels of the cotia chestnut were extracted, yielding 61% of oil. The oil had λ_{max} 261, 270, 282 mμ in isooctane; absorptivity equivalent to about 24% of conjugated triene fatty acid; ν_{max} 957 (w), 986 (s) cm^{-1}. The triene acid was concentrated by crystallization but retained some saturated fatty acid. The concentrate was allowed to react with maleic anhydride, giving an adduct, m.p. 63–64° alone and mixed with the adduct of α-eleostearic acid.

Prunus mahaleb

Whole seeds of *P. mahaleb* L. yielded 15% of oil. The oil had λ_{max} 261, 270, 282 mμ in isooctane and absorptivity equivalent to about 29% of conjugated triene fatty acid. The mixed methyl esters of the fatty acids had infrared maxima at 960 (w) and 988 (vs) cm^{-1}. A concentrate of the triene acid was prepared and a portion was converted to its maleic anhydride adduct. The adduct melted at 63–64°, alone and mixed with the adduct of α-eleostearic acid. Another portion was isomerized by iodine in pentane solution to the all-trans form, m.p. and mixed m.p. with β-eleostearic acid, 70–71°.

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