Kamlolenic Acid and Other Conjugated Fatty Acids in Certain Seed Oils[1]

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Abstract

The major fatty acid of the seed oil of *Trewia nudiflora* is shown to be α-kamlolenic acid, not α-eleostearic acid as believed earlier. Other conjugated acids were found and identified in seed oils not previously studied, viz., α-eleostearic acid in *Parinari insularum* and *Ricinodendron rautanenii*; *trans*,8-*trans*,10-*cis*,12-octadecatrienoic acid in *Calendula stellata*.

The identity of the conjugated acids in four other seed oils was established, viz., α-eleostearic acid in *Prunus yedoensis* and *Valeriana officinalis*; punicic acid in *Cucurbita digitata* and *C. palmata*.

Introduction

The occurrence in seed oils of fatty acids with conjugated ethenoid unsaturation was believed, until recent years, to be limited to relatively few plant genera and to involve about a half dozen conjugated acids, mainly with a triene grouping. The list of such acids has been greatly extended in the last decade with the addition of new dienoic, hydroxy-dienoic and trienoic acids, all with conjugated unsaturation.

Conjugated acids are easily detected in oils by ultraviolet or infrared spectroscopy and the spectra give some indication of the structure of the acid. However, identification cannot be made from spectroscopic data alone. Further evidence is needed to differentiate between positional isomers, now known to exist, such as 8,10-12- and 9,11,13-octadecatrienoic acids, or geometric isomers, e.g. *cis*-9,*trans*-11,*trans*-13- and *trans*-9,*trans*-11,*cis*-13-octadecatrienoic acids. The absorption spectra do not distinguish between the individual acids in these pairs (1).

In the present work, conjugated acids have been found and identified in the seed oils of three species not previously studied. In addition, five species known to have conjugated acids have been reexamined and the identity of the conjugated acid in each has been established with certainty. Proof of the structures was based on isolation of the acids or on the preparation of suitable derivatives or both.

The content of conjugated acid in each oil was calculated from the ultraviolet absorption of the oil and the coefficient $E^{1\%}_{1\,cm}$ of the pure acid, determined in this laboratory. The values for the pure acids were as follows (in cyclohexane except as noted): α-eleostearic, 1766 at 272 mμ; punicic, 1694 at 275 mμ; calendic, 1816 at 272 mμ; α-kamlolenic, 1810 at 270 mμ (in methanol).

Table I lists the species included in this study and shows the distinctive conjugated acid of each oil.

TABLE I

Conjugated Fatty Acids in Seed Oils

Family and species	Oil content, % (air-dry basis) Seed	Oil content, % (air-dry basis) Kernel	Refractive index, n^{25}_{D}	Conjugated acid: Identity	Conjugated acid: Amount in oil, %
Rosaceae					
Parinari insularum A.Gr.	......	65.2	1.5111	α-Eleostearic	63.7
Prunus yedoensis Matsum.	......	31.9	1.4947	α-Eleostearic	35.3
Euphorbiaceae					
Ricinodendron rautanenii Schinz	......	42.0	1.4872	α-Eleostearic	23.8
Trewia nudiflora L.	19.3	......	1.5082	α-Kamlolenic[b]	39.5
Valerianaceae					
Valeriana officinalis L.	28.4	......	1.5020	α-Eleostearic	44.6
Cucurbitaceae					
Cucurbita digitata A.Gr.	27.1	......	1.4820	Punicic	17.2
Cucurbita palmata S.Wats.	26.0	......	....	Punicic	11.0
Compositae					
Calendula stellata Cav.	12.2[a]	......	1.5070	Calendic[c]	49.6

[a] Seed + pericarp.
[b] α-Kamlolenic acid is 18-hydroxy-9,11,13-octadecatrienoic acid.
[c] Calendic acid is *trans*,8-*trans*,10-*cis*,12-octadecatrienoic acid.

Results and Discussion

Parinari insularum seed (origin Fiji) contained an oil whose absorption spectra showed the presence of a conjugated triene acid. The acid was identified as α-eleostearic acid by treating the total mixed acids with maleic anhydride. The unreacted acids were removed by petroleum ether and the α-eleostearic-maleic anhydride adduct was purified by crystallization and identified by comparison with an authentic sample.

The content of α-eleostearic acid (63.7% of the oil) was considerably higher than in other *Parinari* (Parinarium) species (2). Neither licanic nor parinaric acids appeared to be present.

The oil from the seed kernels of *Prunus yedoensis* was shown by Tachibana and Toyama to contain a conjugated triene component (3). In the present work this component was concentrated by low-temperature crystallization and identified as α-eleostearic acid by preparation of its maleic anhydride adduct.

Seed of *Ricinodendron rautanenii* (origin Zambia) yielded an oil containing a substantial amount of conjugated trienoic acid, as shown by its ultraviolet spectrum. The acid was isolated and identified as α-eleostearic acid by its melting point and the melting point of its adduct with maleic anhydride. Two other species of *Ricinodendron* have been studied; both have a substantial content of α-eleostearic acid (2).

Trewia nudiflora seed (origin India) gave an oil having ultraviolet and infrared spectra typical of a conjugated trienoic acid with one *cis* and two *trans* double bonds. Sarkar and Chakrabarty reported, on the basis of the ultraviolet spectrum, that the oil of this species contained α-eleostearic acid (4). However, the trienoic acid isolated from our sample melted at 76–77C and was nearly insoluble in petroleum ether. The melting point was unchanged in admixture with authentic α-kamlolenic acid isolated from seed of *Mallotus philippinensis*.

On hydrogenation, the *Trewia* acid gave 18-hydroxystearic acid, mp and mixed mp 99–100C. The major acid of our sample of *Trewia* seed oil was thus α-kamlolenic acid (18-hydroxy-9,11,13-octadecatrienoic acid). There was little or no evidence of any

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other conjugated acid. Kamlolenic acid has not been found until now in any seed oils except those of the genus Mallotus (family Euphorbiaceae) (5).

The infrared spectrum of *Trewia* oil showed only a very weak hydroxyl band, at 3620 cm^{-1}, presumably because nearly all of the hydroxy acid is combined in the form of estolides. It is similar to *Mallotus* oil in this respect.

The oils of the wild gourds, *Cucurbita digitata* and *C. palmata* (origin USA) were examined earlier by Ault, Swain and Curtis (6), who reported 10–17% of conjugated trienoic acid by ultraviolet absorption analysis. These authors suggested, on the basis of the position of the absorption maxima, that the trienoic acid was punicic (trichosanic) acid. This indication has been confirmed in the present work by isolating the conjugated acid from each oil and determining its melting point in admixture with an authentic sample. The infrared spectra were also in agreement. There was estimated to be 17% of punicic acid in the fatty acids of *C. digitata* and 11% in the acids of *C. palmata,* in good agreement with the results of the earlier work (6).

Punicic acid is known to occur in three other genera of the Cucurbitaceae family (7,8) but it has not been identified previously in *Cucurbita.* The ordinary cultivated species of *Cucurbita* apparently do not produce conjugated fatty acids (2). *C. digitata* and *C. palmata* are believed to be the only plants native to North America that have been shown to produce punicic acid.

Seed of *Calendula stellata,* obtained from various botanical gardens, yielded an oil containing much conjugated trienoic acid. The mixed acids, treated with maleic anhydride, gave an adduct which was identical with the corresponding adduct of authentic calendic acid (*trans*,8-*trans*,10-*cis*,12-octadecatrienoic acid) obtained from the oil of *Calendula officinalis* (9). The results of Earle and co-workers (10) indicate that this acid occurs in various genera of Compositae but positive identification has been made only in the above-mentioned two species of *Calendula* and in one species of *Osteospermum* (11).

The seed oil of *Valeriana officinalis* was shown by Earle and co-workers to contain a conjugated trienoic acid reported as eleostearic acid (12). The present work confirms its identity as α-eleostearic acid by preparation of the maleic anhydride adduct of the acid. The amount in the oil (44.6%) is close to that found by Earle (42%). Other species of this family also contain α-eleostearic acid (8,11).

Experimental

Certain seeds were supplied through the kindness of the following: W. C. Ault, U.S. Department of Agriculture; W. P. Bemis, University of Arizona; Forestry Department, Zambia; Forestry Department, Fiji; Agriculture Department, Canada. Other seeds were obtained from established commercial suppliers. Melting points were determined in capillary tubes and are corrected. Ultraviolet spectra were determined in cyclohexane solution with a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulfide solution (except where noted) in a Perkin-Elmer model 21 spectrophotometer with sodium chloride prism. Petroleum ether was the fraction of bp 30–60C.

The oils were extracted by shaking with petroleum ether at 25C (except where noted) and the solvent was removed in a current of nitrogen at 25–35C. Hydrolysis of the oils was carried out in 1 N ethanolic potassium hydroxide for 16 hr at 25C. The acids were extracted with petroleum ether and the solvent was removed as above.

Parinari insularum

The hard, woody nuts were broken up and the small kernels with adhering yellow fibers were removed. The oil from the kernels had λ_{max} 262, 272, 283 mμ and $E^{1\%}_{1cm}$ 1125 at 272 mμ. There was no absorption in the conjugated tetraene region and no peak in the region of 233 mμ. Infrared maxima were at 960 (W) and 987 (VS) cm^{-1}.

The mixed acids from the oil, heated with maleic anhydride in benzene (1), gave an adduct, mp 64–65C after crystallization from ether: petroleum ether (1:4). It did not depress the melting point of the adduct of maleic anhydride with α-eleostearic acid.

Prunus yedoensis

The seed kernels yielded 31.9% of oil, n_D^{25} 1.4947, λ_{max} 262, 272, 283 mμ and $E^{1\%}_{1cm}$ 624 at 272 mμ. The oil (10 g) was hydrolyzed and the mixed acids were crystallized from acetone. Crystals deposited at −22C were discarded and crystals obtained at −36C (1.9 g) were reacted with maleic anhydride in benzene as described above. The resulting adduct melted at 62–64C and did not depress the melting point of the adduct of maleic anhydride with α-eleostearic acid.

Ricinodendron rautanenii

The kernels (20% of the whole nut) yielded an oil with λ_{max} 262, 272, 283 mμ and $E^{1\%}_{1cm}$ 420 at 272 mμ. The infrared spectrum had ν_{max} 960 (W) and 988 (VS) cm^{-1}. There were no hydroxyl peaks.

The mixed acids (15.0 g) were crystallized fractionally from acetone. Crystals collected at −30C (4.1 g) were crystallized from pentane at −5C, giving 2.0 g of α-eleostearic acid, mp 47–48C. Its adduct with maleic anhydride (1) had mp 63–64C, alone and mixed with an authentic sample.

Trewia nudiflora

The seeds as received were roughly spherical, about 6–7 mm in diameter, with a fairly hard shell and a whitish kernel about 5 mm in diameter. The weight of 100 seeds was 18.5 g. The oil was extracted from the crushed seeds with petroleum ether in a Soxhlet apparatus. The oil had λ_{max} 262, 272, 283 mμ in cyclohexane and $E^{1\%}_{1cm}$ 715 at 272 mm. The infrared spectrum had ν_{max} 960 (W) and 987 (VS) cm^{-1}. There was a very weak band at 3620 cm^{-1}.

The mixed acids (7 g) were crystallized from acetone (70 ml). Saturated acids, which separated at −10C, were removed and the filtrate was cooled to −20C, yielding 2.1 g of acid, mp 70–73C. After crystallizing once from acetone and once from ethyl ether, it had mp 76–77C, alone and mixed with authentic α-kamlolenic acid. The *Trewia* acid (0.2 g) was hydrogenated in methanol with Adams catalyst. The product, on crystallizing from ethyl ether, had mp 99–100C, alone and mixed with 18-hydroxystearic acid. Its infrared spectrum (in Nujol mull) had a strong hydroxyl band at 3260 cm^{-1}.

Valeriana officinalis

The oil had λ_{max} 262, 271, 282 mμ and $E^{1\%}_{1cm}$ 789. The mixed acids (9 g) were crystallized fractionally from acetone (90 ml). Crystals taken at −30C (1.3 g) had mp 40–43C (α-eleostearic acid, 49C). This fraction was heated with maleic anhydride in benzene for 3 hr. The resulting adduct, crystallized from ether: petroleum ether, had mp 63–64C, alone and mixed with the adduct of maleic anhydride with authentic α-eleostearic acid.

Cucurbita digitata

The oil had λ_{max} 265, 275, 287 mμ and $E^{1\%}_{1\,cm}$ 291 at 275 mμ. Infrared maxima were at 933 and 984 cm^{-1}, characteristic of a *cis,trans,cis* conjugated linkage. There was no absorption at 3500–3600 cm^{-1} (no alcoholic hydroxyl). The mixed acids (13.8 g) were dissolved in 138 ml of acetone and cooled. Crystals were removed at −10, −30, and −45C. The product obtained at −45C (4.8 g) was crystallized 3 times from pentane at −30 to −40C, giving punicic acid, mp 44–45C, alone and mixed with an authentic sample.

Cucurbita palmata

The oil had λ_{max} 265, 275, 286 mμ and $E^{1\%}_{1\,cm}$ 187 at 275 mμ. There was very little absorption in the region of 233 mμ. The mixed acids (8.0 g) were crystallized fractionally from 80 ml of acetone. Saturated acids were removed at −10C and monoenoic acids at −30C. The fraction taken at −55C (2.4 g) was crystallized repeatedly from pentane, giving punicic acid, mp 42–44C alone and mixed with an authentic sample.

Calendula stellata

The oil had λ_{max} 262, 272, 283 mμ and $E^{1\%}_{1\,cm}$ 902 at 272 mμ. The mixed acids, freed from unsaponifiable matter, were heated with maleic anhydride in benzene for 3 hr. The resulting adduct had mp 71–73C, alone and mixed with the maleic anhydride adduct of calendic acid (*trans*,8-*trans*,10-*cis*,12-octadecatrienoic acid).

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