Stereochemistry of α-Parinaric Acid from *Impatiens edgeworthii* Seed Oil[1]

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ABSTRACT

α-Parinaric acid is a major constituent fatty acid (48%) from *Impatiens edgeworthii* Hook F. seed oil. Partial hydrazine reduction of the tetraene gave products which permit defining the stereochemistry of α-parinaric acid. Its structure is *cis*-9, *trans*-11, *trans*-13, *cis*-15-octadecatetraenoic acid. The tetraene readily reacts with maleic anhydride to give the Diels-Alder product with no *trans*-unsaturation. The formation of this adduct is contrary to previous reports.

INTRODUCTION

FIRST REPORTED in 1933 to be a constituent of the seed oil *Parinarium laurinum* of the Rosaceae family (1), α-parinaric acid was initially considered a geometrical isomer of eleostearic acid. Two years later Farmer and Sunderland (2) designated it as a 9,11,13,15-octadecatetraenoic acid. Since then, numerous members of the genus *Impatiens* of the Balsaminaceae family have been reported to contain α-parinaric acid (3,4).

During the more than 30 years that α-parinaric acid has been known, efforts to learn its stereochemistry have been frustrated (5,6). Both Riley (5) and Kaufmann and Sud (6) failed to obtain Diels-Alder adducts by reaction with maleic anhydride. Kaufmann and Sud concluded that *trans*-double bonds could not be adjacent.

Impatiens edgeworthii Hook F. seed oil, not previously studied, has 48% of α-parinaric acid. This communication defines the stereochemistry of α-parinaric acid isolated from *I. edgeworthii* seed oil.

PROCEDURES AND DATA

General Methods

Gas-liquid chromatographic (GLC) analyses were carried out with a Burrell Kromatog K-5, and the retention values were treated as described by Miwa et al. (7). Free acids were identified by comparing retention times of observed peaks with those of components of a standard mixture. Quantities are reported as area percent. Infrared spectra were determined on carbon disulfide solutions with an Infracord Model 337 spectrophotometer; ultraviolet spectra, on isooctane solutions with a Beckman DK-2A far ultraviolet spectrophotometer; and melting points, on a Fisher-Johns block. Extractions and most reactions were performed in a nitrogen atmosphere either in the dark or in flasks wrapped with aluminum foil. Most products were maintained as petroleum ether solutions during storage or transfer manipulations.

Preparation of Mixed Methyl Esters

I. edgeworthii seed was flaked in a roller mill, and the resulting flakes were immediately immersed in pentane-hexane (bp 33–57C). The oil was removed by extraction in a Soxhlet apparatus.

I. edgeworthii seed oil (13.7 g) in ca. 2 liters of pentane-hexane was stirred 20 hr with 100 ml of 2 *N* ethanolic potassium hydroxide at room temperature. Water (100 ml) and ethyl ether (600 ml) were added, and the mixture was chilled in an ice bath. Free acid liberated by dropwise addition of *N* hydrochloric acid during nitrogen agitation was taken up in the organic phase. The aqueous phase was further extracted with two 250-ml portions of ethyl ether. The combined ethereal solution was washed with water and then dried over sodium sulfate. Almost all the solvent was removed at room temperature under a stream of nitrogen. The fatty acid concentrate was esterified by reaction with diazomethane (8).

GLC analyses gave the relative amounts of esters with retention characteristics like those of common esters, and ultraviolet analyses provided the amount of tetraene present. Table I shows the composition of the total ester mixture based on GLC and ultraviolet analyses.

Isolation of Methyl α-Parinarate

The mixture of esters was fractionated by countercurrent distribution (CCD) in a 200-tube Craig-Post apparatus with the solvent system hexane-acetonitrile. The esters were divided among the first 10 tubes of the apparatus, and 10 ml of upper phase was added

[1] Honorable Mention, AOCS Bond Award. Presented at the AOCS meeting, Cincinnati, Ohio, October 1965.
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TABLE I

Composition of Water-Insoluble Acids from *Impatiens edgeworthii* Seed Oil

Type of acid	Ester, %	Type of acid	Ester, %
14:0	trace	18:2	6
16:0	5	18:3	26
18:0	3	18:4 conj.	48[a]
18:1	11		

[a] Conjugated tetraene was determined by ultraviolet analyses by using λ max 302.5 mμ $E^{1\%}_{1\,cm}$ 2560 equal 100%.

at each transfer stage. After 190 transfers, upper phase was decanted into a fraction collector combining 4 transfers per fraction until 990 transfers had been made. Solvent was removed, in vacuo, from selected fractions to give the weight distribution plot shown in Figure 1.

Samples were taken at either side of the expected position of the tetraene peak to locate the peak and still conserve and preserve sample. With the exception of 870 through 873, transfers 634 through 906 were combined and stored in the dark as a 0.2% hexane solution at −18C. A sample taken after 6 weeks' storage had infrared and ultraviolet spectra like that of the initial preparation.

Spectral Data

Methyl Ester. The infrared spectrum of the tetraene in carbon disulfide solution has characteristic absorption at the 10–11 μ region (10.08 S, 10.30 W, 10.53 M, 10.74 W, and 10.88 μW). The ultraviolet spectrum of the tetraene has maxima at 278.5, 289.5, 302.5, and 317 mμ with $E^{1\%}_{1\,cm}$ = 870; 1,690; 2,560; and 2,240, respectively.

Impatiens Oil. The ultraviolet spectrum of *I. edgeworthii* seed oil has maxima at 280, 290, 303.5, and 318 mμ with $E^{1\%}_{1\,cm}$ = 410; 800; 1,230; and 1,100, respectively. The infrared spectrum of the oil in carbon disulfide has absorbance at 10.09 S, 10.32 W, and 10.53 M, and 10.75 μW.

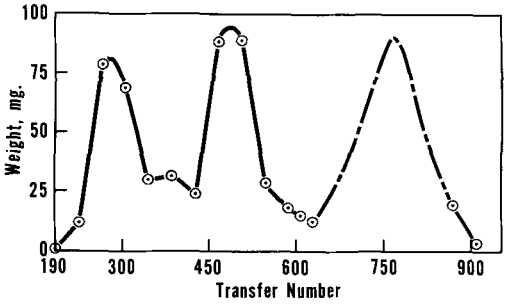

FIG. 1. Countercurrent distribution of methyl esters from *Impatiens edgeworthii* seed oil. Dashed portion of the plot is an estimate based on recovery.

Hydrogenation Studies

Impatiens Oil. A portion of the oil (0.179 g) was reduced as an ethanol solution in the presence of a platinum catalyst. A portion of the saturated oil was transesterified in 3 ml of methanol containing 2% of sulfuric acid. The esters were isolated in the usual manner, and GLC analyses showed 4.8% of methyl palmitate and 95.2% of methyl stearate. Some of the saturated oil was saponified; then the solvent was removed from the alkaline solution, and the remaining solid was chilled, covered with ethyl ether, and acidified with hydrochloric acid. Acetic acid was found in the ethereal solution by GLC analyses.

Methyl α-Parinarate. Pure methyl α-parinarate (0.094 g) was hydrogenated in ethanol with a platinum catalyst. The ester absorbed 3.9 mole equivalents of hydrogen to yield a white solid melting at 34.5–36.0C. GLC analyses indicated 97+% of methyl stearate and about 2% of methyl "oleate."

Oxidation of α-Parinarate

A portion (0.41 g) of the tetraene was oxidized with permanganate-periodate according to the method of von Rudloff (9). The resulting cleavage products were extracted with six 150-ml portions of ethyl ether. The combined ether extract was dried over sodium sulfate. Solvent was removed in vacuo at 0 C. A free acid sample was shown by GLC analysis to contain propionic and acetic acid (4:1). The acetic acid is probably the result of over oxidation. The remainder of the cleavage products was esterified with diazomethane. Solvent was removed in vacuo to leave 0.313 g of esters. GLC analyses indicated 90% of dimethyl azelate, 2% of dimethyl suberate, and lesser amounts of other products of shorter retention times.

Partial Hydrazine Reduction

A portion of the methyl α-parinarate (2.09 g) was reduced with hydrazine as previously described for the reduction of α-eleostearic acid (10). Aliquots were removed at predetermined time intervals to evaluate the progress of the reduction (Fig. 2). For ultraviolet determinations, arbitrarily selected ε-values were used for calculating conjugated diene (28,300), conjugated triene (47,000), and conjugated tetraene (74,400). Since the tetraene gives rise to a

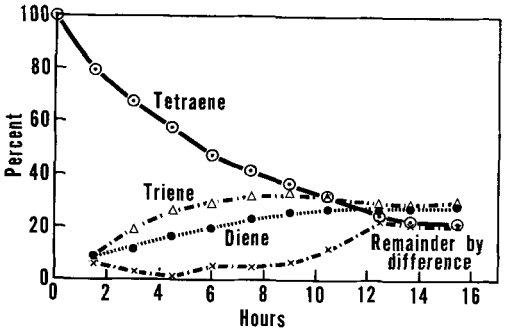

FIG. 2. Hydrazine reduction of *Impatiens edgeworthii* tetraene as estimated by ultraviolet analyses.

peak at about 279 mμ which overlaps the triene peak at 270 mμ, an empirical correction was applied in the determination for conjugated triene. The correction factor, optical density apparent triene—0.18 X optical density of the tetraene peak at 317 mμ = optical density of corrected triene, was deduced by determining the absorbance at 270 mμ for various concentrations of pure tetraene and relating the absorbance changes at 270 mμ to the absorbance of the secondary peak for tetraene at 317 mμ.

Fractionation of Hydrazine-Reduced Products

Methyl esters (1.926 g) of the hydrazine reduction product obtained after 15.5 hr were fractionated by CCD as described previously. The esters were added to tube O with 10 ml of hexane and 40 ml of acetonitrile. At each transfer, 10 ml of equilibrated hexane was added to tube O. After 200 transfers the upper phase was decanted into the fraction collector combining 2 transfers per fraction. Solvent was evaporated from selected fractions, and the

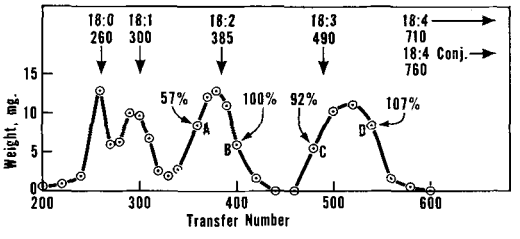

FIG. 3. Countercurrent distribution of a hydrazine-reduction product mixture from *Impatiens edgeworthii tetraene*. The numerals across the top indicate the position of expected maxima for nonconjugated fatty acid methyl esters. Fractions A, B, C, and D refer to selected fractions on which detailed spectral data were obtained. The percentages are approximate amounts of conjugation as determined by ultraviolet spectroscopy.

TABLE II

Spectral Data on Selected Countercurrent Distribution Fractions from Hydrazine-Reduced Tetraene[a]

Sample designation		Major chromaphore		
Fraction	Transfer No.	Infrared	Ultraviolet max.	ε
A	360	*trans,trans*	231.0	18,800
B	400	*cis,trans*	231.5	27,400
C	480	*cis,trans,trans*	268.5	43,000
D	540	*cis,trans,trans*	268.5	50,100

[a] See Fig. 3.

residues were weighed to give the data shown in Figure 3. Spectral analyses of selected fractions are summarized in Table II.

Maleic Anhydride Adduct of Methyl α-Parinarate

Preparation. The tetraene (0.057 g) was refluxed 2 hr with 5 ml of benzene and 0.049 g of maleic anhydride. Solvent was removed in vacuo. The product was dissolved in ethyl ether, washed with four 50-ml portions of water, and dried over sodium sulfate. Ether was evaporated in vacuo to give 0.067 g of product. Absorption bands at 5.39 and 5.64 μ (anhydride carbonyl) showed the presence of adduct, and ultraviolet spectral analysis showed about 32% of tetraene. After trituration with pentane-hexane, 0.027 g of insoluble purified adduct was obtained. The infrared spectrum of the purified adduct showed no trans-unsaturation. Adduct formed in a similar manner darkened while being dried in an Abderhalden apparatus. Elemental analyses of the product gave values about 2% low with respect to calculated carbon. Additional adduct was prepared, and the product was hydrogenated with a platinum catalyst. The saturated adduct was isolated from hydrogenated starting materials by chromatography on a silica column.

Anal. Calcd. for $C_{28}H_{38}O_5$: C, 70.01; H, 9.71. Found: C, 70.38; H, 9.68.

Oxidation. The tetraene Diels-Alder product was ozonized in methanol essentially by the procedure of Ackman et al. (11). The bulk of the formic acid solvent from the peracid cleavage of the ozonides was removed by distillation through a fractionating column. GLC analyses of the free acids showed propionic and formic acids. Methyl esters of the cleavage products gave GLC peaks corresponding to dimethyl azelate (90%), dimethyl suberate (5%), and lesser amounts of products with shorter retention times.

trans, trans-Diene Concentrate

Isolation. Transfers 372 through 379 and 382 through 389 from CCD of hydrazine-

reduction products (Fig. 3) were combined to give 0.104 g. As evidenced by infrared spectroscopy, a *trans,trans*-conjugated diene fraction (0.016 g) was isolated by crystallization from acetone at −70C.

Cleavage. The *trans,trans*-conjugated diene was oxidized with permanganate-periodate in 30% *t*-butanol (9). The fragments obtained correspond to those expected from cleavage of the mixture shown in Table III.

cis, trans-Diene Concentrate

Isolation. Transfers 392 through 399 from CCD of hydrazine-reduction products (Fig. 3) were combined to give 0.037 g of a mixture of dienes. The fraction had an infrared spectrum like that of a mixture of *cis,trans*- and *trans, trans*-conjugated dienes. The residue was reacted 3 hr with maleic anhydride as above to yield 0.044 g. A portion of the reaction product (0.034 g) was fractionated on a silica gel column with hexane-ethyl ether (49:1). A nonadducted fraction (0.010 g) was obtained, λ max 233 mμ, ϵ = 25,200. The infrared spectrum of the nonadduct indicated *cis,trans*-conjugated diene with ca. 10% of *trans,trans*-conjugated diene (12).

Cleavage. The *cis,trans*-conjugated diene was oxidized with permanganate-periodate as previously described. The cleavage fragments correspond to those expected from oxidation of the mixture shown in Table III.

Preparation of α-Parinaric Acid

A portion of the methyl α-parinarate hexane solution (30 ml) was stirred overnight at room temperature with 5-ml of 2*N* ethanolic potassium hydroxide. The saponification mixture was worked up as described for the saponification of *Impatiens oil.* Solvent was removed in vacuo to yield 0.072 g of α-parinaric acid. Its infrared spectrum at the 10–11 μ region is like that of the methyl ester, except the bands at 10.53, 10.74, and 10.88 μ are superimposed on the broad medium intensity OH deformation (out of plane) band from the carboxyl group (13). The absorbance of the band at 10.08 μ gave K = 1.44.

Attempts to obtain the melting point of α-parinaric acid between microscope cover slides emphasize the reactivity of this acid. When heating α-parinaric acid at our usual rate, ca. 2 to 3C per min, softening occurred at 60C. As the temperature continued to rise, the appearance of the sample changed to indicate apparent resolidification. At about 130C, the sample became bright yellow, but it darkened at higher temperatures. A fresh sample, placed on the melting point block at 55C and rapidly heated, melted at 65–70C. Upon standing overnight under nitrogen purge, α-parinaric acid gave a hard lump with no appreciable solubility in carbon disulfide. Even at a high rate of heating, this hard material failed to melt at temperatures up to 200C. The infrared spectrum of the tetraene artifact as a Nujol mull had little absorption in the 10–11 μ region.

DISCUSSION

Pure conjugated tetraene from *Impatiens edgeworthii* seed oil was isolated as its methyl ester by CCD. It readily absorbed 4 mole equivalents of hydrogen to give methyl stearate; thus, a normal C-18 skeleton was indicated. Oxidative cleavage of the ester gave fragments indicating that the unsaturation is in the 9,11,13,15-positions. Comparing absorbance for the band at near 10.1 μ (K = 1.44) with the absorbance values summarized in Table IV (14) indicates the presence of 2 *trans*-double bonds in a conjugated system. To determine the configuration of each double bond, the tetraene was partially reduced with hydrazine. The resulting mixture was resolved according to unsaturation by CCD. Spectral analyses of selected fractions, summarized in Table II, indicate that the two *trans*-double bonds are adjacent. This finding is contrary to the report by Kaufmann and Sud (6) on their studies with parinaric acid from other *Impatiens* species.

Kaufmann and Sud proposed that α-parinaric acid has one of the three possible structures in

TABLE III

Compositions of Dienes from Hydrazine Reduction[a]

Isomer	*trans,trans* %	*cis,trans* %
9,11	12	39
9,13	....	4
9,15	....	5
11,13	71	14
11,15	....	1
13,15	16	35

[a] Determined by GLC analyses of oxidative cleavage fragments.

TABLE IV

Infrared Absorbance Values of Known Conjugated Acids

Ester	Double bond configuration	K[a]
Punicate	*cis,trans,cis*	0.52
Diene	*cis,trans*	0.495
α-Eleostearate	*cis,trans,trans*	1.68
Diene	*trans,trans*	1.43
β-Eleostearate	*trans,trans,trans*	2.39

[a] Values determined for absorbance maxima at near 10.1 μ (14).

which no two *trans*-double bonds could be adjacent. The spectral evidence from the present study provides for two structural patterns; i.e., *trans,trans,cis,cis* or *cis,trans,trans,cis* for configuration of the double bonds. The conjugated trienes, which could result from partial reduction of these two possible structures, can be easily identified by infrared spectroscopy. The spectrum of a *cis,cis,trans*-conjugated triene isomer has been reported (15), and Tolberg et al. (14) have published the spectrum of a *cis,trans,trans*-conjugated triene. We have evidence for only the *cis,trans,trans-* (*trans, trans,cis-*) conjugated triene (Table II); therefore, provided hydrazine reduced the double bond in a manner analogous to α-eleostearic acid (10), the parent tetraene has the *cis,trans, trans,cis*-configuration of double bonds. This structure is further supported by data in Table III obtained by cleaving concentrates of *trans, trans-* and *cis,trans*-conjugated dienes. Conclusive proof of configuration was obtained by preparing the maleic anhydride adduct of the tetraene. Other authors (5,6) have reported attempts to prepare maleic anhydride adducts from α-parinaric acid, but they were not successful. The infrared spectrum of the Diels-Alder product showed no *trans*-unsaturation. Cleavage fragments from oxidation of the adduct were those expected for a product formed by adduction across the two central double bonds. Therefore, α-parinaric acid is *cis*-9, *trans*-11,*trans*-13,*cis*-15-octadecatetraenoic acid.

The hydrazine reduction of the tetraene proceeds essentially as it did with α-eleostearic acid (10). The bulk of the products were conjugated; however, evidence was obtained for the presence of some nonconjugated products.

The presence of acetic acid as a triglyceride constituent in *I. edgeworthii* seed oil is in accord with studies of seed oils from other *Impatiens* species (4). The infrared spectrum of α-parinaric acid from the current study is similar to the spectrum reported by Kaufmann and Sud (6) for impatienic acid. This acid was reported to isomerize to α-parinaric acid on standing overnight. This transformation was evidenced by spectral analyses and melting point data. Our attempts to repeat this isomerization phenomenon resulted in complete loss of α-parinaric acid during the overnight storage to give an intractable solid. The infrared and ultraviolet spectra of methyl α-parinarate were unchanged when stored in solvent and protected from light.

In recent publications Takagi (16) described studies with the tetraene from *Parinarium laurinum* of the Rosaceae family. Using the hydrazine approach, he obtained results similar to ours. Therefore, the acid from both *P. laurinum* and *I. edgeworthii* seed oils is identical. These findings are in accord with the theory recently advanced by Gunstone (17) for the possible biosynthesis pathway to conjugated polyenes.

ACKNOWLEDGMENTS

Gas-liquid chromatographic analyses by J. W. Hagemann. Microanalyses by Mrs. Clara McGrew. Translation of reference 16 by Dr. T. K. Miwa.

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