5,11,14-Eicosatrienoic Acid in *Podocarpus nagi* Seed Oil[1]

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Abstract

Eicosatrienoic acid, the unique constituent acid of *Podocarpus nagi* seed oil is characterized as all-*cis*-5,11,14-eicosatrienoic acid. Eicosadienoic acid found in the same oil is shown to be *cis,cis*-11,14-eicosadienoic acid.

Introduction

THE UNIQUE eicosatrienoic acid was found in *Podocarpus nagi* seed oil by Koyama and Toyama (1), the first trienoic acid of natural origin which showed only diene conjugation by treatment with alkali. It was separated by fractionation of the bromide and then debrominated. Although the acid value (A.V.), iodine value (I.V.) and hydrogenation indicated an eicosatrienoic acid, the alkali isomerization showed absorption due to a conjugated diene. The unusual double bond distribution in this acid from *Podocarpus nagi* seed oil was reconfirmed by other authors (2). Recently, it was suggested that the acid should be assigned either the structure 9,15,18- or 9,12,18-eicosatrienoic acid (3).

In the present study, structural work on the eicosatrienoic acid was carried out using gas-liquid chromatography (GLC) for analysis of fatty acids and scission products, and the structure differed from that described before. Methyl esters obtained from the original oil were subjected to GLC analysis and two unusual peaks emerged on the chart in addition to peaks for methyl palmitate, stearate, oleate and linoleate. The emergence times of the unusual peaks correspond approximately with those expected for methyl eicosadienoate and methyl eicosatrienoate. The proportion of chain lengths, e.g., C_{16}, C_{18} and C_{20}, were in agreement with those obtained by a nonpolar column, SE-30 on Celite. Methyl esters of the oil were distilled and the C_{20} acid fraction was separated into fractions A and B by adsorption chromatography using silicic acid. The I.V. and saponification values (S.V.) indicated that fraction A and B consisted chiefly of methyl eicosadienoate and methyl eicosatrienoate, respectively. Hydrogenation yielded arachidic acid and alkaline isomerization gave products with the absorption max of a conjugated diene in the UV region. IR and UV spectra did not indicate the presence of a *trans* double bond, a terminal double bond or a conjugated double bond in fractions A and B. The characteristics of the eicosatrienoic acid described above were in accord with those described by the other authors (1) although the presence of the eicosadienoic acid in this oil has not been mentioned previously. GLC of the scission products from oxidation of the fatty acid mixture indicated the presence of caproic, nonanoic, glutaric, adipic, azelaic and undecanedioic acids. Comparison of the composition of the original fatty acid mixture and the scission products indicated that caproic, glutaric and adipic acids were likely produced from eicosatrienoic acid, and caproic and undecanedioic acids from eicosadienoic acid. On this evidence the eicosadienoic acid could be assigned the structure of *cis,cis*-11,14-eicosadienoic acid and the eicosatrienoic acid could have four possible structures, e.g., 5,11,14-, 5,8,14- 6,9,14- or 6,11,14-. The fatty ester mixture was reduced to the alcohols by lithium aluminum hydride, and subjected to periodate-permanganate oxidation followed by GLC of the scission products. Adipic acid was a major dicarboxylic acid component in the scission products which indicated that the part between the carboxyl group and the first double bond in the eicosatrienoic acid contained five carbon atoms, and distribution of double bonds could be either 5,11,14- or 5,8,14. To determine the structure definitely, several samples of the eicosatrienoic acid were isolated by distillation, adsorption chromatography, urea adduct formation, low temp crystallization and preparative GLC. The first sample was oxidized by periodate-permanganate reagent. The dicarboxylic acid in the product consisted approx of equivalent amt glutaric and adipic acids, the the monocarboxylic acid consisted chiefly of caproic acid. Further structural confirmation was sought by the hydrazine reduction method (4) reported for determining the structure of the octadecatrienoic acid by Bagby et al. (5). The acids expected in the scission products from partially hydrogenated 5,11,14-eicosatrienoic acid show in Table I. The hydrazine reduction of the eicosatrienoic acids yielded products containing 75–80% of monoenoic and dienoic acids. Methyl, butyl and decyl esters of the scission products were analyzed by GLC and all acids in Table I were detected definitely. If the structure were 5,8,14-eicosatrienoic acid, the scission products would contain suberic and dodecanoic acid instead of undecadioic and nonanoic acid, respectively.

The 5,11,14-eicosatrienoic acid contains a butylene-interrupted unsaturation as a structural unit. It is noteworthy that no other fatty acids with butylene-interrupted unsaturation appear to have been reported as constituents of natural fatty oils. Recently, Bagby, Smith and their coworkers found *trans*-5-octadecenoic acid and *trans,cis,cis*-5,9,12-octadecatrienoic acid in *Thalictrum polycaprum* seed oil (5); *cis*-5-eicosenoic acid, *cis*-5-docosenoic acid (6) and *cis,cis*-5,13-docosadienoic acid (7) in *Limnanthes douglasii* seed oil. The structure of the second acid contains an ethylene-interrupted unsaturated system and that of the last acid contains a hexylene-interrupted unsaturation. The second acid is similar to the eicosatrienoic acid in the present work since both have a 5-double bond which can not be isomerized to a conjugated system with the other double bonds by the usual treatment with alkali; the major component, monocarboxylic acid, obtained from both by the oxidative cleavage was caproic acid. The structure of the eicosatrienoic acid differed from that of arachidonic acid only in lacking the double bond in the 8-position. The *cis*-5-double bond is com-

TABLE I

Scission Products from Partially Hydrogenated 5,11,14-Eicosatrienoic Acid

Position of double bond			Dicarboxylic acid (no. of carbon)	Monocarboxylic acid (no. of carbon)
5	11	14		
X	X		5, 6	9
X		X	5, 9	6
	X	X	11	6
X			5	15
	X		11	9
		X	14	6

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mon to C_{20}-polyethenoid acids of animal origin; i.e., 5,8,11-eicosatrienoic acid and 5,8,11,14-eicosatetraenoic acid (arachidonic acid) in brain (8), ox liver (9) and and menhaden oil (10), and 5,8,11,14,17-eicosapentaenoic acid in ox liver (9), cod liver oil (11), pilchard oil (12) and menhaden oil (10).

The *cis,cis*-11,14-eicosadienoic acid found in *Podocarpus nagi* seed oil has also been found in brain (8), ox liver (9) and menhaden oil (10). However, its presence in vegetable sources seems not to have been confirmed although wide distribution of small amt of eicosadienoic acid in the oils of Cruciferae have been reported (13,14).

Experimental

Podocarpus nagi Oil. Seeds of *Podocarpus nagi* (650 g) were coarsely ground and extracted with methanol and ether, to yield a yellowish liquid oil (230 g, yield 38.0%) which showed a trace of precipitation by addition of acetone and had the following characteristics: d_4^{20} 0.9232, n_D^{20} 1.4781, A.V. 3.5, S.V. 185.9, I.V. 159.7, unsaponifiable matter 0.51%. I.V. in this report were measured by Wijs method, 1 hr reaction time.

Preparation of Methyl Esters. The oil (100 g) was saponified by refluxing with 1 liter 1 *N* ethanolic potassium hydroxide. Unsaponifiable material was removed and the free fatty acids (94 g) obtained in the usual manner had the following characteristics: A.V. 192.7, I.V. 164.0. The mixed fatty acids (92.5 g) were esterified by refluxing with 500 cc 2% H_2SO_4 in methanol for 1 hr. The unchanged acids were removed by washing the ethereal solution with 5% potassium carbonate.

GLC Analysis. The methyl esters were analyzed by GLC using a polyester column made up with succinate ethylene glycol on Chromosorb W, 60–80 mesh in the ratio of 1:6 (w/w) in an 8-ft x $\frac{3}{16}$ in. O.D. copper column. The apparatus used was described in previous publications (15). The analysis in mole per cent indicated 5.4% palmitic acid, 1.5% stearic acid, 20.7% oleic acid, 37.6% linoleic acid, 1.4% eicosenoic acid, 12.9% eicosadienoic acid and 20.5% eicosatrienoic acid (Fig. 1A). The calculations of the compositions from GLC were done by the ratio of the peak areas and the percentages shown as mole percentage except where otherwise noted. The analysis of the same sample by a silicone column showed C_{16} 4.3%, C_{18} 57.0%, C_{20} 38.7% in wt percentage which compared favorably to the results from the polyester column which were C_{16} 4.8%, C_{18} 58.2% and C_{20} 37.0% in wt percentage.

Isolation of Eicosadienoic and Eicosatrienoic Acid Fractions.

1) *Fractional Distillation and Column Chromatography.* Distillation of the methyl ester (94.8 g) under pressure at 1 mm Hg yielded a C_{20} ester fraction having the following characteristics: n_D^{20} 1.4685, S.V. 176.9, I.V. 189.0. The methyl ester (2.9 g) was subjected to elution chromatography. The column (2.5 cm I.D.) was made up with silicic acid (150 g) (Mallinchrodt chromatographic grade, 100 mesh) as a hexane slurry and was developed and eluted with 0–1.0% ether-hexane. A fraction 0.89 g (n_D^{20} 1.4610 to 1.4619) was rechromatographed in the same manner using a column of 1.5 cm I.D. packed with silicic acid (25 g). The middle fraction (A) 0.3 g eluted showed the following characteristics: n_D^{20} 1.4615, S.V. 177.3, I.V. 165.1. Calculated for $C_{21}H_{38}O_2$: S.V. 174.0, I.V. 157.4. Another fraction 1.07 g (n_D^{20} 1.4695 to 1.4718) from the first chromatography was also rechromatographed in the same manner using silicic acid (30 g). The middle fraction (B) 0.43 g eluted showed the following characteristics: n_D^{20} 1.4710, S.V. 176.5, I.V. 241.2. Calculated for $C_{21}H_{36}O_2$: S.V. 175.0, I.V. 237.6, and purity 96.1% (wt) by GLC. Fractions A and B were presumed to consist chiefly of methyl eicosadienoate and methyl eicosatrienoate respectively. Ethanol solutions of fraction A and B (0.1 g) were hydrogenated with platinum black catalyst. After removal of catalyst by hot filtration followed by saponification, the solution was acidified and extracted with ether. After recrystallization from hexane, the products melted at 74–75C and 73.5–74.5C, respectively, and both showed no depression of melting point on admixture of pure specimen of arachidic acid. Fractions A and B were isomerized with 6.6% potassium hydroxide-ethylene glycol under nitrogen at 180C for 25 min (16). UV spectra of the ethanol solution of the products exhibited the max at 234 mμ (with 81.0 and 79.3 as specific extinction coefficients). IR spectra of fractions A and B by the liquid film and carbon disulfide solution methods indicated that *trans* double bond, triple bond and terminal bond were not present in either.

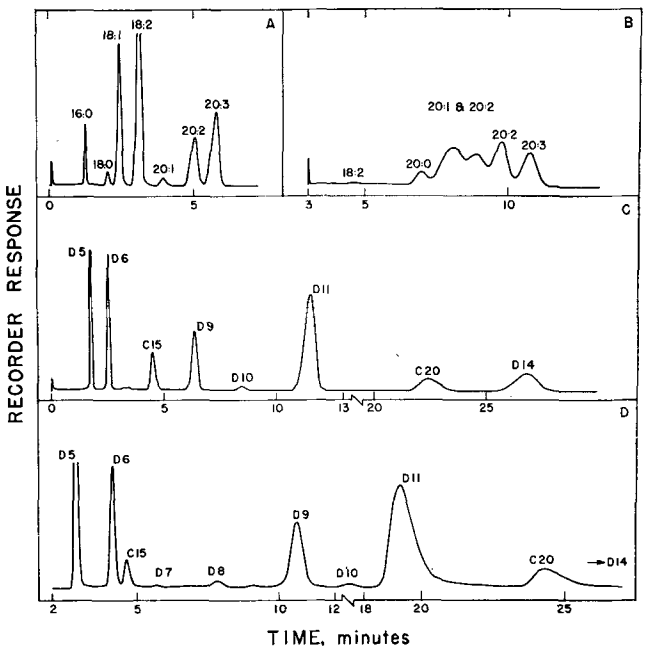

FIG. 1. GLC analyses. A. Fatty Acid methyl esters from *Podocarpus nagi* seed oil. B. Analysis of partially hydrogenated eicosatrienoic acid. C. Analysis of methyl esters of oxidative scission products from partially hydrogenated eicosatrienoic acid. D. Analysis of butyl esters of oxidative scission products from partially hydrogenated eicosatrienoic acid; D: dicarboxylic acid, C: monocarboxylic acid, and numbers indicate carbon atoms in component acids.

TABLE II

Constitutions of Scission Products from Fatty Acid Mixture

Mole %	Glutaric	Adipic	Suberic	Azelaic	Undecanedioic	Palmitic	Stearic	Caproic	Nonanoic
A Found	11.4	8.1	1.4	59.9	13.8	4.1	1.3		
B Found	.....	....	1.7	74.4	17.1	5.1	1.6		
Calculated	.....	....	0	73.3	18.0	6.8	1.8		
C Found								76.3	23.7
Calculated								75.6	24.4

TABLE III

Gas Chromatographic Analysis of Scission Products from Alcohol Mixture

	Glutaric	Adipic	Suberic	Azelaic	Undecanedioic	Palmitic	I	II
Emergence time (min)	4.00	5.47	9.20	12.10	19.78	9.86	15.96	26.03
Area ratio	4.2	18.8	1.8	25.3	6.1	3.7	35.8	4.3

2) *Fractionation by Urea Adducts and Absorption Chromatography.* Urea (10 g) was added to the methanol solution of the distilled methyl esters (9 g) containing C_{20} acid 68% and C_{18} acid 32%. After boiling, the solution was cooled to room temp and urea adducts were filtered off. The same procedures were repeated twice for the filtrate. The urea adducts were combined and crystallized from 150 cc methanol. The nonadducts fraction (2.1 g) from the filtrate contained methyl eicosatrienoate 73.4% pure by GLC. This fraction was chromatographed on a column (1.0 cm I.D.) made up of 25 g silica gel (Davison 60–200 mesh dried in 110C oven) as a Skellysolve B slurry with 0–5% ether-Skellysolve B. The last eluted fraction contained methyl eicosatrienoate, 94.2% pure by GLC. Yield: 0.28 g (fraction C).

3) *Preparative Gas-Liquid Chromatography and Low Temperature Crystallization.* A portion of the original fatty acid mixture (72 g) was esterified with 250 cc methanol and a few drops boron trifluoride etherate complex by boiling for 1 hr (17). The methyl ester (70 g) was dissolved in 500 cc acetone and kept at −30C overnight and filtered. The filtrate was kept at −50C for 6 hr in a dry ice-acetone bath. Filtration yielded 42 g with a C_{20} acid content of 43.0%. Preparative GLC of this fraction using a silicone column gave a C_{20} fraction with methyl eicosatrienoate content of 62.5% by GLC which by fractional crystallization from Skellysolve F −70C yielded the fraction (D) 0.41 g, methyl eicosadienoate 30.2%, methyl eicosatrienoate 69.8%.

4) *Hydrazine Reduction of Eicosatrienoic Acid.* The fatty acids from fraction C (0.13 g) were added to 10% hydrazine (85% hydrazine hydrate in water, Matheson Coleman & Bell)—ethanol solution (5 ml). After stirring for 4 hr at 50C, the solution was made acidic with dilute HCl and extracted with ether. The products (E) 0.12 g were esterified with diazomethane and analyzed by GLC using 8-ft x $^3/_{16}$ in. O.D. column packed with butanediol succinate C-22 (60–80 mesh) 1:4. Analysis indicated approx trienoic acid 14%, dienoic and monoenoic 79%, saturated acid 3% and C_{18} acid 4%. The fatty acid from fraction D (0.30 g) was reduced using 15 cc of the 10% hydrazine-ethanol solution in the same manner as E. The reduced products (F) (0.29 g) showed approx the following: trienoic acid 19%, monoenoic acid and dienoic acid 75%, and saturated acid 6% by GLC (Fig. 1B).

Permanganate-Periodate Oxidations.

1) *Oxidation of Mixed Acids.* Oxidative scission was done by the permanganate-periodate method (18, 19). The dicarboxylic acids were analyzed as methyl esters on a polyester column (20) and monocarboxylic acids were analyzed as the decyl esters (21). The results of the analysis show in Table II. The calculated values in column B were obtained from the fatty acid composition considering undecanedioic acid as produced from eicosenoic acid and eicosadienoic acid, and glutaric acid, adipic acid and the cleaved products of eicosatrienoic acid were neglected in column B. The calculated values in column C were obtained from the fatty acid composition regarding caproic acid produced from linoleic acid, eicosadienoic acid and eicosatrienoic acid, and nonanoic acid from oleic acid and eicosenoic acid. The good agreement in Table II showed that assumptions described above were reasonable, except the structure of the eicosenoic acid could not be deduced as its content was too small.

2) *Oxidation of Methyl Eicosatrienoate.* Fraction C (60 mg) was subjected to the periodate-permanganate oxidation followed by GLC of the methyl esters and the decyl ester is the same manner as described before. GLC indicated 51.8% glutaric acid, 43.6% adipic acid and 4.6% azelaic acid by analysis of the methyl ester and 93.2% caproic acid, 2.8% caprylic acid and 4.0% nonanoic acid by analysis of the decyl ester.

3) *Oxidation of the Alcohols.* Lithium aluminum hydride (8.8 mg) and ether (15 cc) were placed in an Erlenmeyer flask attached to a condenser protected with a calcium chloride trap. After a few min stirring, the ether solution of 50 μl methyl ester of fatty acid mixture was added. After a further 30 min stirring, 10% cold H_2SO_4 was added and the contents stirred for several min until bubbling ceased. The product was saponified and extracted with ether. Ether extract was oxidized in the same manner as above and the scission products were analyzed as methyl esters by GLC using a phthalate ethylene glycol C-22 firebrick (1:4.5) column. The results show in Table III, where peaks I and II were presumed to correspond with ω-hydroxypelargonic acid and ω-hydroxyundecanoic acid respectively.

4) *Oxidation of the Reduced Products.* The reduced products E and F were cleaved by the periodate-permanganate reagent as described before. The products from E and were esterified to methyl and decyl esters and analyzed by GLC. The ether extract by liquid-liquid extraction from the scission products of F was evaporated on a water bath. The residue was refluxed with 10 cc butanol containing a few drops of boron trifluoride etherate complex for 1 hr and the butanol was distilled from the solution. The butyl ester was analyzed by GLC using a phthalate ethylene glycol C-22 firebrick column. The results show in Table IV, Fig. 1C and Fig. 1D. The methyl esters of glutaric, adipic, azelaic and undecanedioic acids were collected using traps containing Skellysolve B cooled

TABLE IV

Gas Chromatographic Analysis of Scission Products from Acid Reduced by Hydrazine

Mole %	Dicarboxylic acid										Monocarboxylic acid						
	5	6	7	8	9	10	11	12	13	14	6	7	8	9	15	18	20
From E methyl ester	31.3	18.7	....	0.7	17.1	0.5	12.9	1.2	....	4.7					8.1	0.7	4.1
From E decyl ester											68.2	2.5	....	26.7	2.6		
From F methyl ester	16.7[a]	13.5[a]	....	....	10.8	1.0	29.4	....	....	9.5					4.6	....	5.2
From F butyl ester	35.7	16.4	0.5	0.7	8.2	0.7	22.4	....	....	6.0					4.6	....	4.8

[a] The per cent of methyl glutarate and methyl adipate in the analysis of II are low probably due to incomplete extraction from the aqueous solution.

with dry ice-acetone. Sodium soaps of glutaric and adipic acids, and barium soaps of the other acids were prepared for infrared spectroscopy (22). Ethanol solutions of the free acids were titrated with 1 *N* sodium hydroxide to a phenolphthalein end point. The solutions were evaporated until thick slurries of salt remained. Acetone was added to the slurries and the insoluble material was filtered off. The soaps were washed twice by acetone and dried at 110C. To prepare the barium soaps, several mg of the free acids were dissolved in about 3 ml methanol and 1 ml 1% barium chloride dihydrate methanol solution was added. Coned aqueous ammonia (28%) was added dropwise to the hot methanol solutions until no additional precipitate was formed. The precipitate was centrifuged, washed twice with 3 ml portions hot methanol and dried at 110C. The soaps were used to make potassium bromide pellets and the IR spectra were identical with those of the pure specimens.

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• *Letters to the Editor*

Surface Activity of Symmetrical and Unsymmetrical Mono- and Di-glycerides

MIXTURES of mono-, di- and triglycerides prepared by glycerolysis of fats find application in producing oil in water emulsions for various purposes (edible, pharmaceutical, cosmetic, etc.). In the past 1-monoglycerides were considered the only active ingredients in such preparations, the presence of 2-monoglycerides being as a rule disregarded. However, it seems that commercially produced partial glycerides contain appreciable amounts of 2-monoglycerides (1,2,3). It appears also that diglycerides invariably present in commercial products comprise both 1,2- and 1,3-isomers (4,5). This could be expected since Crossley et al. (6) have shown that 1,2-diglycerides are by no means as unstable as was hitherto assumed and that the heating of symmetrical diglycerides leads to an appreciable formation of 1,2-isomers. The purpose of the present work was to compare the effects of symmetrical and unsymmetrical mono- and diglycerides on the interfacial tension of fat and hydrocarbon-water systems which could assist in the evaluation of the emulsifying properties of commercial monoglyceride preparations. At the same time this would be tantamount to a comparison between the effects of free primary and secondary hydroxyl groups of glycerol on the surface activity of partial glycerides.

Experimental

The measurements of the interfacial tension were carried out with a du Noüy tensiometer using a platinum ring 3.96 cm in circumference. The ratio of the diam of the ring to that of the wire was 41.0. The following partial glycerides were investigated: pure 1-monostearin, mp 81.5C prepared by a modified isopropylidene-glycerol method (7); 2-mono-stearin, mp 74.3C prepared essentially by Martin's method (8) and containing approx 3% of the 1-monoester; 1,3-dipalmitin mp 74.0, and 1,3-distearin, mp 79.5, prepared by direct esterification of glycerol with fatty acid chlorides in N,N dimethyl formamide-chloroform solution (9), 1,2-dipalmitin, mp 62.5 and 1,2-distearin, mp 68.0, prepared by heat isomerisation of

TABLE I

Interfacial Tension in dynes/cm at 50C of oil (hydrocarbon)-water systems with added glycerides (0.5% glyceride solution in non-aqueous medium)

Non-equeous medium	Control	Type of glyceride added						
		1-mono-stearin	2-mono-stearin	1,3-Di-stearin	1,2-Di-stearin	1,3-Di-palmitin	1,2-Di-palmitin	Tri-palmitin
Soybean oil	29.6	22.4	21.4	29.0	28.6	29.0	28.4	29.5
Benzene	35.6	22.1	20.4	34.1	32.8	33.8	33.3	35.2
Kerosene	50.0	5.1	2.9	39.7	27.3	38.1	24.9	46.4
n-Heptane	45.8	6.9	5.3	37.8	24.3	36.5	22.4	43.8
Paraffin oil	48.0	2.6	2.2	39.2	25.6	38.7	24.6	46.3