Steric Configurations of Parinaric and Punicic Acids

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Abstract

GLC analysis of the scission products from the fractions separated by silver nitrate-silicic acid column chromatography of the hydrazine reduced products has shown that parinaric and punicic acids have (*cis*-9,*trans*-11,*trans*-13 and *cis*-15) and (*cis*-9,*trans*-11 and *cis*-13) configurations, respectively.

Introduction

THE STERIC CONFIGURATION of parinaric acid has been reported to contain either two *cis* and two *trans* or one *cis* and three *trans* double bonds (1). The configuration of punicic acid was suggested to be *trans*-9,*cis*-11,*cis*-15 in 1954 (2) but was revised by Crombie and Jacklin (3) in 1957 to *cis*-9,*trans*-11,*cis*-15 on the basis of infrared spectra of the natural and synthesized acids.

Recently Takagi and Craig studied hydrazine reduction of α- and β-eleostearic acids reported an initial attack at their ends of the conjugated system to produce a conjugated diene with no conversion of *cis* to *trans* structure (4). Takagi studied the separation of the monoenes and conjugated dienes resulting from the reduction with silver nitrate-silicic acid column chromatography, and proposed a method to determine the steric configuration of conjugated fatty acids (5).

The steric configurations of parinaric and punicic acids are reported in the present report. The result of the preliminary experiments has been reported concerning the steric configuration of parinaric acid (6).

Materials and Methods

Punicic acid, prepared from pomegranate seed oil by recrystallization of the fatty acids according to the procedure outlined by Crombie and Jacklin (3), showed a melting point 43–44C and the ethanol solution of the crystals exhibited the maximum at 274 mμ (with 50,100 as molar absorbtivity). Parinaric acid, prepared from garden balsam seed oil by recrystallization of the fatty acids according to the procedure outlined by Riley (7), showed a melting point 85–86C and the ethanol solution of the crystals exhibited the maximum at 305 mμ (with 72,400 as molar absorbtivity). Punicic acid 100 to 150 mg and parinaric acid 150 to 200 mg were added to 10% hydrazine (85% hydrazine hydrate in water)-ethanol (50 ml), respectively. After stirring for 3 hr at 50C, the solution was made acidic with dilute hydrochloric acid and extracted with ether. The extract was converted to the methyl ester with 2% sulfuric acid-methanol at room temperature for 15 hr under nitrogen atmosphere.

A GLC apparatus with flame ionization detector was used for analyses of methyl esters. The column was 2.25 m × 6 mm I.D. stainless steel containing diethyleneglycol succinate polyester on 60–80 mesh Celite 545 (1:6 w/w). The mole percentages of each esters were calculated on weight per cent content of active carbon (7). The method outlined by De Vries (9) was followed for the preparation of silver nitrate-silicic acid packing. A column 15 mm I.D. containing 20 g packing was used for the chromatography, and benzene-hexane solvent system were used for development and elution. During chromatographic runs 5 g fractions were collected and after evaporation of the solvent in a stream of nitrogen, the methyl ester contents were determined by GLC analysis using palmitate as an internal standard. The contents of the geometric isomers of the conjugated dienes were also estimated by the calculation of the area ratios of the peaks in the ordinary procedure, since Morris et al. have shown them to be stable under GLC conditions (12).

Permanganate-periodate oxidation were carried out according to the procedure of von Rudloff (10) as modified by Youngs (11) and the scission products were analyzed by GLC of the methyl esters

Results

Gas chromatographic charts (Fig. 1A, 1B) of the partially reduced punicic and parinaric acids show peaks for stearate, octadecenoate and *cis,trans*-conjugated octadecadienoate common to both acids. A major peak for *trans,trans*-octadecadienoate is shown for the reduced parinaric acid. Little loose peaks emerged near and at the position of conjugated dienes on the chart of parinarate due to the artifacts, but addition of parinarate to the reduced parinarate gave only little influence on the area ratios of the conjugated diene peaks to others. Separations of the partially reduced acids by silver nitrate-silicic acid column chromatography are compared to the separation for the partially reduced α-eleostearic acid (Fig. 2A, 2B, 2C). The separation of products from parinaric acid (Fig. 2C) show the *trans* monoene and *trans,trans*-conjugated diene peaks common with α-eleostearate. Further peaks containing *cis*-monoene and *cis,trans*-conjugated diene could not be resolved into individual fractions. The partially reduced punicic acid did not show a peak for *trans, trans*-conjugated diene by the column chromatography. Infrared spectra of the fractions of peak 2 in Figure 2 showed the maximum at 10.3 μ, but those of peak 5 did not. Ultraviolet spectra of the fractions of peak 3 and 4 showed the maximum at 231 and 233 mμ, respectively. The facts supported the assignment of the peaks shown in Figure 2. The oxidation and GLC analysis of fractions (Table I) were used to

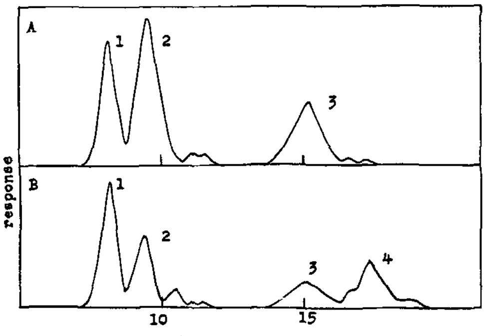

FIG. 1. GLC analysis of partially reduced punicic (A), and parinaric (B) acids. Peaks 1, 2, 3 and 4 are respectively methyl stearate, methyl octadecenoate, conjugated *cis,trans* methyl octadecenoate and conjugated *trans,trans* methyl octadecenoate.

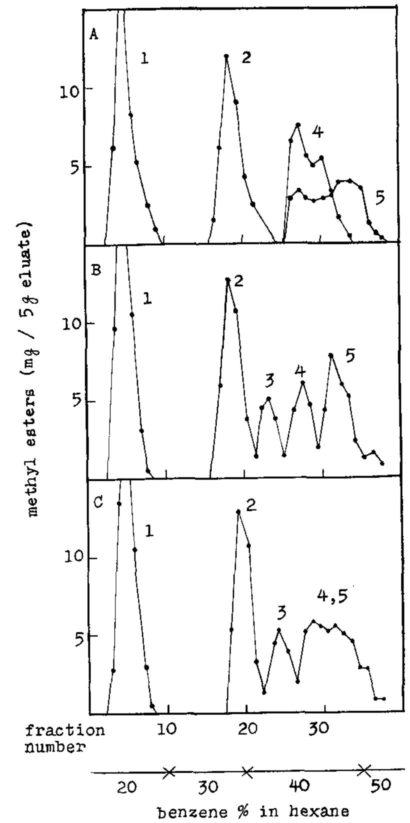

FIG. 2. Silver nitrate-silicic acid column chromatography of partially reduced punicic (A), α-eleostearic (B) and parinaric (C) acid methyl esters. Peaks 1, 2, 3, 4 and 5 are respectively methyl stearate, *trans* methyl octadecenoate, conjugated *trans, trans* octadecadienoate, conjugated *cis,trans* octadecadienoate and *cis* methyl octadecenoate.

estimate the double bond positions. Major components of each fraction shown in Table I were estimated from Figure 2. GLC analysis showed 2.6% of *cis,trans*-conjugated diene and 1.4% of monoene in the fraction 23 from parinaric acid, less than 1% of *trans,trans* diene in the fraction 18 from parinaric and punicic acids, and 3.9% of *cis,trans* diene in the fraction 34 from punicic acid as the impurities.

Discussion

The GLC separation of the methyl esters of the partially reduced punicic acid (Fig. 1A) show only a *cis,trans* diene peak, which is confirmed by the data (Fig. 2A) in which there is no peak for a *trans, trans* diene in the fractions separated by silver nitrate-silicic acid chromatography. The oxidation of the separated fractions (Table I) shows that fraction 18 is a *trans*-11 monoene and fraction 34 a *cis*-9 monoene. Fraction 26 to 33 contained the *cis,trans* conjugated diene admixed with a *cis* monoene. Since only a *cis,trans* diene peak was presented in the original mixture, the configurations of punicic acid is *cis*-9,*trans*-11,*cis*-13 octadecatrienoic acid. Accordingly fraction 26 is mainly *trans*-11,*cis*-13 diene mixed with *cis*-9,*trans*-11 diene and admixed with *cis*-13 monoene. The data on fractions 28 and 30 show that the *cis* double bond in position 13 decreases the retention time as compared to the *cis* bond in position 9 and this accounts for the admixture of *cis* monoene with *cis,trans* dienes.

TABLE I

Dicarboxylic Acids Produced by Oxidation of Conjugated Diethenoid and Monoethenoid Acid Fractions (Mole %)

Number of fraction	Number of C atoms 8	9	10	11	12	13	Major components
Punicic acid							
18	0.3	2.5	1.5	95.7	0	0	*Trans* monoene
26	0.9	17.6	1.3	52.6	1.6	26.0	*Cis* monoene and *cis,trans* conj. diene
28	1.5	34.3	1.2	40.6	1.9	20.5	
30	2.4	55.5	1.3	25.5	2.9	13.2	
34	3.0	89.5	0.7	3.5	0.9	2.4	*Cis* monoene
Parinaric acid							
18	0.4	3.1	1.8	46.2	3.0	45.5	*Trans* monoene
23	0.5	2.0	2.2	92.7	0.2	2.4	*Trans,trans* conj. diene
28[a]	0.8	25.5	1.9	6.5	1.4	37.0	*Cis* monoene and *cis, trans* conj. diene
31[b]	1.3	48.7	0.6	2.0	1.9	30.1	

Dicarboxylic acid %, [a] C-14 1.1, C-15 25.8 [b] C-14 1.5, C-15 13.9.

The GLC separations of methyl esters of the partially reduced parinaric acid (Fig. 1B) show both *cis,trans* and *trans,trans* diene peaks. Similarly the data (Fig. 2C) show a *trans,trans* and *cis,trans* peaks. The *cis,trans peaks* are expected to be mixtures of *cis,trans* dienes and *cis* monoenes the same as for punicic acid. The oxidation data (Table I) shows fraction 18 to be a mixture of *trans*-11 and *trans*-13 monoenes, while fraction 23 is the *trans,trans*-11,13 conjugated diene. The oxidation of the other fractions confirms the presence of *cis*-9 and *cis*-15 monoenes. Thus the structure of parinaric acid is *cis*-9,*trans*-11, *trans*-13,*cis*-15 octadecatetraenoic acid. It is noteworthy that the *cis* bonds in both of these conjugated acids are at the terminal ends of the conjugated system.

ACKNOWLEDGMENT

Advice by B. M. Craig, National Research Council of Canada.

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