Partial Reduction of *a*-Eleostearic Acid with Hydrazine

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Abstract

The following products are formed during partial reduction of α-eleostearic acid with hydrazine: *cis,trans*-9,11-octadecadienoic and *trans,trans*-11, 13-octadecadienoic acids; *cis*-9-, *trans*-11- and *trans*-13-octadecenoic acids; and stearic acid. The double bonds are reduced individually in the conjugated triene and also in the conjugated dienes that are formed. However, the reduction is selective since the *trans*-11 double bonds in the conjugated triene is reduced only slightly to yield the isolated 9,13-diene.

The *trans* double bond of the *cis,trans* conjugated diene reduces at a faster rate than the *cis* bond. No differences were observed in the rate of reduction of the *cis*-9 and *trans*-13 bonds in the triene or of the bonds in the *trans,trans* conjugated diene.

Introduction

A NUMBER OF WORKERS (2,4,12,14) have reported the partial reduction of various fatty acids with hydrazine. They have found that *cis* and *trans* double bonds usually reduce at the same rate and that no geometric isomerization or bond migration occurs. Aylward and Rao (3) have been the only ones to report a partial hydrazine reduction of α-eleostearic (*cis,trans,trans*-9,11,13-octadecatrienoic) acid and our results differ considerably from theirs. In 1963, Schilling stated the reduction rates of conjugated and terminal double bonds were different than those of isolated double bonds (13).

The results reported here follow the formation of various products throughout the reduction period and also give the complete composition of the final partial reduction mixture (15).

Experimental Procedures and Results

Gas Chromatographic Analysis. In general, all samples were analyzed on the same type of columns and under the same conditions as described earlier (9). All percentages are given in area per cent.

Preparation of α-Eleostearic Acid. α-Eleostearic acid was isolated from tung oil and purified essentially as described by Hoffman et al. (7). The melting point of the purified product was 46.5–47.0C. The lit. value is 48C (5). UV analysis indicated 103.6% of α-eleostearic acid [ϵ for α-eleostearic acid $= 47{,}000$; λ max 270.5 mμ (5)]. This high figure is probably due to the presence of a small amt of β-eleostearic acid.

Partial Reduction of α-Eleostearic Acid. A 2.05-g sample of α-eleostearic acid was dissolved in 150 ml of absolute ethanol. Aqueous hydrazine (2.0 ml, 64%) was added and the solution was heated at 52C ± 3 for 7.5 hr. Air was bubbled through the solution continuously to increase the rate of reduction (2,14). At 1.5-hr intervals, 2.0-ml aliquots were removed, acidified with dilute HCl and extracted with diethyl ether. The ether extract was washed well with water and the ether was removed *in vacuo*. The residues, assumed to be 0.025 g, were each dissolved in 25 ml of absolute ethanol to give stock solutions. UV determinations of α-eleostearic acid made on appropriate dilutions of the stock solutions are shown in Table I. These data were used only as a guide to show the extent of reduction as the reaction proceeded.

The remaining portions of the stock solutions were recovered individually and esterified by refluxing with 1% H_2SO_4 in methanol for 45 min. These esters were analyzed by GLC to obtain the percentages of the different reduction products present at various time intervals (Table I). The final reduction product was recovered and esterified by the same procedure as the aliquots. The overall recovery of material (including aliquots) was 91%.

Countercurrent Distribution of the Reduction Products. Methyl esters of the hydrazine reduction products were subjected to countercurrent distribution (CCD) between *n*-hexane (5 ml) and acetonitrile (40 ml) in an automatic 200-tube Craig-Post (15) apparatus. Collection of upper phase was begun (3 transfers/tube) after 200 transfers had been completed. A total of 840 transfers were collected. This procedure separated the esters according to their degree of unsaturation.

The solvent was removed from a sufficient number of collection tubes to obtain a wt distribution plot and the contents of selected tubes were analyzed by GLC to determine the location of the various products in the fraction collector.

The material from all the monoene-containing tubes was combined and the solvent was removed *in vacuo*. This fraction (90% yield based on original GLC analysis) had the following composition by GLC: $C_{16:0}$, 0.3; $C_{18:0}$, 13.5; $C_{18:1}$, 86.1; and $C_{18:2}$ (nonconjugated), 0.2. This mixture was shown to contain 47.6% of isolated *trans* unsaturation by quantitative IR analysis (1). A corrected value of 55.3% was obtained by taking into account the saturated esters present.

The conjugated dienes were obtained in the same manner as the monoenes except that center cut of the wt peak was taken for further work. Its composition by GLC was as follows: $C_{18:2}$ (nonconjugated), 3.4; $C_{18:2}$ (conjugated *cis,trans*), 44.1; $C_{18:2}$ (conjugated *cis,cis*), 1.3; $C_{18:2}$ (conjugated *trans,trans*) 50.5; and an unknown, 0.7. The IR spectrum confirmed that this fraction was a mixture of geometric diene isomers and that no isolated *trans* double bonds were present. No quantitative IR analysis was done because it was felt that the values obtained by GLC were sufficient and reliable (10). The presence of *cis,cis* conjugation in the mixture is probably the result of partial reduction of an unknown conjugated triene in tung oil, which has this configuration. A less likely possibility is that the *cis,cis* conjugated diene is formed by geometric isomerization during analysis by GLC. The recovery of conjugated dienes from CCD was not determined accurately since the recovered material contained 10–15% of short chain residues from evaporation of the *n*-hexane. In addition, the small amt of unconjugated diene produced during the reduction was concentrated at the edge of the conjugated diene peak and most of this was excluded from the cut taken.

Separation of cis and trans Monoenes. The combined monoene methyl esters were separated into a *cis* and a *trans* fraction (6) by preparative TLC on 10 x 40 cm plates spread with silver nitrate impregnated silica

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gel G (20:80). The solvent-free methyl esters were spotted directly using 10 spots of 1 μl each/plate. The spotted plate was placed in a "sandwich" arrangement similar to that described by Honegger (8) and developed with benzene in a closed chromatographic jar. This arrangement allowed the solvent front to migrate ca. 30 cm in two hr, whereas nearly four hr were required when the single-plate methods was used.

The spots were visualized by spraying the plates with an ethanolic solution of 2,7-dichlorofluorescein and viewing them under long-wavelength UV light. The bands containing the *trans* monoenes ($R_f = 0.60$) and the *cis* monoenes ($R_f = 0.43$) were marked on the plates and these bands of adsorbant and sample were scraped directly into a flask containing benzene. The monoenes were then thoroughly extracted from the silica gel-silver nitrate powder with benzene. Since the dye is insoluble in benzene, it remains with the silica gel. In this manner 0.033 g of *cis* monoenes and 0.045 g of *trans* monoenes were prepared. These amt represent recoveries of ca. 85–90%.

The *cis* fraction showed no *trans* absorption and the *trans* fraction assayed 99.7% of isolated *trans* double bonds by quantitative IR analysis (1).

Oxidative Cleavage of the cis and trans Monoenes. Both the *cis* and *trans* monoene fractions were cleaved by permanganate-periodate oxidation according to the method of von Rudloff (11). The alkaline residues (obtained by removal of *t*-butanol and water) were acidified with 12–15 ml of coned HCl and the resulting saturated salt solutions were extracted with diethyl ether (10 x 20 ml). The extracts were washed once with 10 ml of saturated salt solution, dried over sodium sulfate and filtered. The ether was distilled through a Vigreux column and the final 4–5 ml were evaporated by a gentle stream of nitrogen. The residues were esterified by refluxing one hr with 1% H_2SO_4 in methanol. After the methanolic solutions were diluted with water, they were extracted with ethyl ether. The extracts were washed with water and coned with a stream of nitrogen (no heat applied) to a volume of ca. 0.2 ml.

Analysis of the esterified cleavage products from the *cis* monoene by GLC showed 49.1% methyl nonanoate, 50.6% methyl nonanedioate and 0.2% of an unknown material. The amt of monobasic acids from cleavage of the *trans* monoenes were not obtained because of some loss due to ester volatility. The dibasic acid methyl esters were composed of 1.2% of nonanedioate, 56.5% of undecanedioate and 42.3% of tridecanedioate. Therefore, the *cis* fraction is entirely the Δ 9-monoene and the *trans* fraction consists almost exclusively of the Δ 11- and Δ 13-monoenes.

The calculated composition of the original monoene fraction (obtained by combining analytical results for the *cis* and *trans* fractions) is as follows: *cis*-9-octadecenoic acid, 45%; *trans*-9-octadecenoic acid, 2%; *trans*-11-octadecenoic acid, 30%; and *trans*-13-octadecenoic acid, 23%.

Hydrazine Reduction of the Conjugated Dienes. The combined conjugated diene fractions (44.1% *cis, trans* and 50.5% *trans,trans*) were heated with hydrazine for 16 hr, and the products were recovered in the same manner as the reduction products from α-eleostearic acid. The mixture was esterified with diazomethane and the esters were analyzed by GLC. The *cis,trans* conjugated diene content was 16.3% and the *trans,trans* diene content was 21.8%. These percentages represent a lowering of the *cis,trans*:*trans,trans* ratio from 0.87–0.75 during the partial reduction.

TABLE I

Partial Reduction Products of α-Eleostearic Acid (Area % by GLC)

Component (as methyl ester)	Reaction time, hr				
	1.5	3.0	4.5	6.0	7.5
$C_{18:0}$	1.2	2.8	6.1	9.8	14.4
$C_{18:1}$	3.4	9.0	14.5	17.3	20.6
$C_{18:2}$ (nonconj.)	1.6	2.2	2.4	2.0	1.5
$C_{18:2}$ (conj. *cis,trans/trans,cis*)	12.2	15.4	15.3	15.0	13.7
$C_{18:2}$ (conj. *cis,cis*)	3.0	2.6	2.5	2.4	0.7
$C_{18:2}$ (conj. *trans,trans*)	11.2	15.3	16.6	16.7	15.0
$C_{18:3}$ (eleostearic), by GLC	67.4	51.9	41.7	36.0	33.3
$C_{18:3}$ (eleostearic), by UV[a]	73	58	48	41	38
Unknown		0.8	0.9	0.8	0.8

[a] ϵ for α-eleostearic acid = 47,000 λ max 270.5 mμ.

The percentages of the various geometric isomers of the conjugated dienes reported here and in Table I are reliable since Morris et al. have shown them to be stable under GLC conditions (10) and since the absence of monoenes, other than those expected, shows that no isomerization of conjugated triene or diene occurred.

Discussion

Aylward (3) has theorized that the hydrazine reduction of α-eleostearic acid follows two routes: a) simultaneous addition of two moles of hydrogen to give a *cis* monoene, which is then reduced to stearic acid and b) the addition of one mole of hydrogen to give a *trans,trans* conjugated diene, which is then converted directly to the saturated acid by the simultaneous addition of 2 moles of hydrogen. Hence Route "a" would result eventually in *cis*-9-octadecenoic and stearic acids, whereas Route "b" would result in *trans,trans*-11,13-octadecadienoic and stearic acids. Neither route would produce *trans*-11- or *trans*-13-octadecenoic acid, but our work shows both of these acids are present in large quantities in the reduction mixture. Aylward's theories also preclude formation of *cis,trans* conjugated diene, which is also present in quantity (Table I).

The most likely reduction pathway suggested by our data is that the *cis*-9 and *trans*-13 double bonds are reduced selectively at approx the same rate to give nearly equal amt of *cis,trans* and *trans,trans* conjugated dienes. Table I shows that the *trans*-11 double bond is reduced only slightly to give a 9,13-diene, whereas the reduction of linolenic acid by this method produces a large amt of the comparable 9,15-diene (12,14). Therefore, it appears that the hydrazine reduction of a conjugated triene system proceeds in a manner which preserves conjugation. This influence is not present in the reduction of linolenic acid and the three double bonds are reduced at nearly the same rate yielding all three possible dienes.

The *trans* double bond of the *cis,trans* conjugated diene appears to reduce somewhat faster than either the *trans* bonds in the *trans,trans* diene or the *cis* bond in the *cis,trans* diene. This observation is supported by the fact that the amt of *cis,trans* isomer decreases more rapidly than the *trans,trans* isomer when a mixture of these two dienes is reduced with hydrazine. The relative amt of *cis*-9- and *trans*-11-monoenes formed in the reduction of the original triene support this conclusion. These conclusions support Schilling's statement that hydrazine reduction rates for conjugated double bonds differ from those for isolated double bonds (13).

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Composition and Positional Distribution of Fatty Acids in Phospholipids Isolated from Pork Muscle Tissues[1,2]

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Abstract

The composition and positional distribution of fatty acids in phospholipids isolated from four locations of a hog carcass is presented. Variations in fatty acid composition of phospholipids were found depending upon the location in the carcass. The total unsaturated fatty acid content averaged 34.3 mole % for lecithin, 52.5 mole % for phosphatidylethanolamine, 40.3 mole % for phosphatidylserine and 41.3 mole % in sphingomyelin. The cephalins had a much higher percentage of polyunsaturated fatty acids than lecithin. The chief saturated fatty acid in lecithin and sphingomyelin was palmitic and in cephalins it was stearic. A snake venom enzyme preparation *(Crotalus adamanteus)* hydrolyzed primarily unsaturated fatty acids in phosphoglycerides and the higher the percentage of unsaturation within the fatty acid the higher percentage of hydrolysis occurred. The unsaturated fatty acids were found chiefly at the the β-position and the saturated fatty acids at the α-position in the phosphoglycerides.

Introduction

THE PHOSPHOLIPIDS of blood and of neural and organ tissue of many animals have been investigated relative to kind, composition and, in some cases, to positional location of the fatty acids. Relatively little attention has been given to the phospholipid content of skeletal tissue and especially to their presence in such tissues of meat animals, although it is suspected that phospholipids may be important in keeping quality and flavor. Hornstein et al. (4) determined the phospholipids, as general classes, in pork and beef, and Kuchmak and Dugan (1) determined the specific phospholipids in four locations in a hog carcass. Younathan and Watts (10) showed that the bound lipids in pork and Zipser et al. (11) showed the bound lipids in mullet were implicated in oxidative deteriorations.

This study was aimed first at elucidation of the fatty acid composition of the phospholipids of pork muscle tissue as influenced by carcass location and subsequently at the elucidation of the positional location of the fatty acids in the phosphoglyceride moieties.

Experimental

Materials. Silicic acid, cp, precipitated, was obtained from Fisher Scientific Company. Silicic acid was washed with methanol and reactivated at 120C. All solvents used were freshly redistilled.

Crotalus adamanteus venom, used as a source of phosphotidase A (lecithinase A), was obtained from the Ross Allen Reptile Institute, Silver Springs, Fla.

Methyl esters of fatty acids, used as reference compounds in GLC, were obtained from the California Corp. for Biochemical Research and from Applied Science Laboratories.

Origin of Samples. Samples of muscle tissue were taken from the center belly section, a ham cross section, loin center cut and the 4–6 rib section of a hog of known breed and feeding history (Yorkshire-Hampshire castrate male, five months old, 190 lb standard growth feeding ration.)

Isolation of Phospholipids. Lipid extraction, silicic acid chromatography and procedures for establishment of identity of isolated phospholipid classes have been reported previously (1).

Enzymatic Hydrolysis of Lecithin. Enzymatic hydrolysis of lecithin with snake venom in diethyl ether solution and separation of hydrolysis products were achieved by the procedure of Tattrie (2). The precipitate which resulted after hydrolysis was removed by centrifuging and the ether solution was tested for the presence of phosphorus. If phosphorus was present in the ether solution, indicating the presence of unreacted lecithin, the enzymatic hydrolysis was repeated on another sample. When enzymatic hydrolysis was complete, the ether phase contained fatty acids only. Lysolecithin was recovered from the precipitate by dissolving it in chloroform and centrifuging out the protein. Complete enzymatic hydrolysis was achieved only with the lecithin sample from belly muscles and the best results on the samples from the other three locations on the hog carcass

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