Countercurrent Distribution of Alkali-Isomerized Methyl Linolenate with an Argentation System[1]

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Abstract

The isomerized product obtained by heating linolenic acid with potassium hydroxide in ethylene glycol at 165C for 30 min was separated into urea-adduct-forming (AF) and nonurea-adduct-forming (NAF) materials. Both were converted to methyl esters and fractionated by countercurrent distribution (CCD) between hexane and 0.2 *N* silver nitrate in 90% methanol. Some of the CCD fractions were further fractionated by low-temperature crystallization from acetone.

The AF material consisted largely of trienoate containing triene conjugation and of trienoate containing diene conjugation. The NAF material was composed of the same two components and cyclic esters.

Introduction

At this laboratory countercurrent distribution (CCD)[3] with a silver nitrate-containing system (argentation) has been used to study monoene, diene and triene fractions from partially hydrogenated fatty esters (14,16). For aid in interpretation of this work on hydrogenated products, it was desirable to investigate separations of other mixtures of isomeric fatty esters. A previous paper (15) showed that *cis* and *trans* monoenoic esters were separated and that separation of isomeric dienoic esters resulted from differences in silver complex formation which were caused both by *cis* and *trans* configuration of double bonds and by differences in number of methylene groups between double bonds. Isomerized methyl linoleate was separated into unconjugated and conjugated fractions, and the *trans,trans*-conjugated esters were separated from mono-*trans* (15). In other related work (17) argentation CCD of geometric isomers of linolenate has been described.

Our first application of this argentation solvent system was the fractionation of the methyl esters of cyclic acids obtained by prolonged treatment of linolenic acid with alkali (13). We describe here the argentation CCD of products formed in an earlier stage of alkali isomerization of linolenic acid where large amounts of diene and triene conjugation are still present.

Experimental

Isomerization of Linolenate

The linolenic acid was prepared by solvent fractionation in a Podbielniak apparatus (1,18) and was approximately 90% linolenic with linoleic acid as the main impurity. To a solution of 100 g of potassium hydroxide in 420 ml of ethylene glycol, which had been heated to 165C under nitrogen, 103 g of linolenic acid was added. After the mixture was heated at 160–166C for 30 min and cooled to 115C, 200 ml of water was added. After cooling to room temperature the solution was acidified and the fatty acids were recovered in ether. The ether solution was treated with Darco G-60 carbon and the solvent was removed to yield 100 g of isomerized acids ($a_{267} = 47.5$, $a_{234} = 64.1$).

A preliminary fractionation was made by adding the acids to 160 g of urea in 500 ml of methanol. The mixture was warmed to dissolve the urea and allowed to cool to room temperature. The adduct was filtered and washed with ether. In the adduct-forming material (AF) 31.8 g of acids was recovered; in the nonadduct-forming (NAF) material, 66.0 g. Both were esterified with methanol containing sulfuric acid catalyst.

The AF material was characterized by a strong absorption maximum ($a_{267} = 97.7$) and smaller maxima at 278, 257, 247 and 235 mμ. There was a strong infrared absorption band at 10.1 μ with a = 0.934 and weak bands at 10.4 and 10.6 μ. For the NAF esters, absorptivities were $a_{268} = 21.7$ and $a_{234} = 67.5$; in the infrared, $a_{10.2} = 0.285$, $a_{10.4} = 0.162$ and $a_{10.6} = 0.231$. The AF material contained only traces of cyclic esters; the NAF, 16%.

Analytical Procedures

Ultraviolet absorptions were measured in isooctane solutions. Infrared absorptions were measured in carbon disulfide solutions, and absorptivities corresponding to *trans* bonds were calculated with transmissions measured from a base line tangent to the curve at approximately 9.3 and 10.75 μ. A procedure similar to that of Black and Eisenhauer (2) was used to

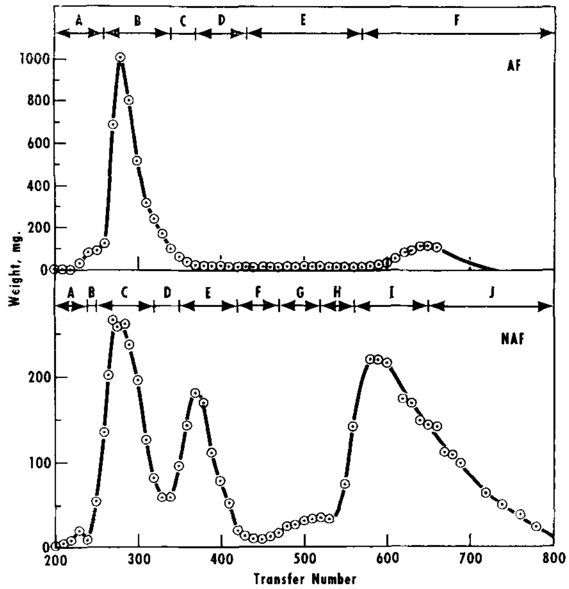

FIG. 1. Countercurrent distribution of urea-adduct-forming (AF) and nonadduct-forming (NAF) material from methyl esters of alkali-isomerized linolenic acid. Solvents are 40 ml of 0.2 N $AgNO_3$ in 90% methanol in each tube with 10 ml portions of hexane. Single withdrawal technique is used. Transfer number where fraction eluted from the instrument is plotted against weight.

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[3] Abbreviations: AF, urea-adduct forming; NAF, nonurea-adduct forming; CCD, countercurrent distribution; GLC, gas-liquid chromatography; ECL, equivalent chain length; NMR, nuclear magnetic resonance.

TABLE I

Analyses of Fractions from Countercurrent Distribution of Adduct-Forming Material in Figure 1

Fraction	Weight, %	Ultraviolet absorption		Infrared absorption		
		a_{267}	a_{233}			
A	3.8	... .	19.0	$a_{10.2} = 0.219$		
B	66.7	118.5	....	$a_{10.1} = 1.25$		
C	3.0	91.0	....	$a_{10.1} = 0.515$,	$a_{10.4} = 0.144$,	$a_{10.7} = 0.078$
D	2.0	....	38.6	$a_{10.2} = 0.299$,	$a_{10.4} = 0.215$,	$a_{10.6} = 0.133$
E	2.3	....	63.5	$a_{10.2} = 0.348$,	$a_{10.4} = 0.296$,	$a_{10.6} = 0.161$
F	22.2	....	79.5	$a_{10.2} = 0.408$		$a_{10.6} = 0.334$

determine cyclic ester content. Samples were hydrogenated at atmospheric pressure in ethyl acetate solution with palladium-on-alumina catalyst. The proportion of cyclic esters in the resulting mixture of cyclics and stearate was determined by gas-liquid chromatography (GLC).

CCD of AF and NAF Material

CCD of 30 g of the AF material is shown in Figure 1; CCD fractions were combined as shown in the figure. Ultraviolet and infrared data are given in Table I. CCD of 28 g of NAF material is also shown in Figure 1. The remainder of the NAF (30 g) was similarly distributed and the corresponding fractions from the two distributions were combined as shown in the figure. Weights and spectral data are given in Table II.

These distributions, and all others, were made in a 200-tube automatic CCD apparatus with 40-ml portions of 0.2 N $AgNO_3$ in 90% methanol as the lower layer and with 10-ml portions of hexane as the upper layer. The single withdrawal technique (5) was used with the eluate from two CCD transfers combined in one collector tube. In Figure 1, the transfer number at which a fraction eluted from the apparatus is plotted against weight in the corresponding collector tube.

As seen in Figure 1, CCD of AF produced two main bands; NAF produced three bands two of which were at the same locations as those in AF. The flow sheet in Figure 2 summarizes this fractionation and identifies the principal components which will be shown to occur in the main fractions.

Fractions AF-B and NAF-C are center cuts of the first bands eluted from the CCD instrument with the AF and NAF material, respectively. They are similar in that they were eluted from the CCD apparatus in approximately the same number of transfers and in that they both contain triene configuration with only traces of cyclic esters. To secure better separation, AF Fraction B (Fig. 1) was put back into the CCD apparatus and recycled to 830 transfers before withdrawal in the fraction collector, but only a single broad band was obtained. Fractions from the center of the band extending from transfer number 1120 to 1140 and weighing 8.8 g were distilled to yield 7.2 g of material, which was crystallized from acetone (5 ml/g sample) at −40C; the solid fraction was recrystallized twice at −50C to yield 1.8 g of a final crystal fraction corresponding to a *trans,trans,trans* triene conjugated ester (mp 40C, $a_{267} = 194.3$, $a_{10.5} = 1.93$).

Similarly, NAF Fraction C was put back into the CCD apparatus and recycled to 780 transfers before withdrawal in the fraction collector. No pure esters were obtained, but analyses of fractions from the instrument indicate a mixture of triene conjugated geometric isomers with small amounts of conjugated diene probably from the linoleic impurity in the original linolenic acid.

The second band eluted from NAF is absent in AF material. Analysis of NAF-E from this band by capillary gas chromatography (0.01 in. × 200 ft) on Apiezon L at 200 C gave a peak at equivalent chain length (11) (ECL) 18.0, equal to about 46% of the area under the GLC curve, and a broad poorly resolved band equal to 47% with peaks at ECL of 18.3 and 18.5. There was a smaller peak (5%) at ECL 17.6, the position for linolenate and linoleate, and another (2%) at 16.6.

The absence of complex formation with urea, the absorption at 262 mμ without the side bands characteristic of conjugated trienes and the absence of strong bands in the 10–11 μ region are all indicative of cyclic esters.

The last bands eluted from the CCD instrument include AF Fraction F and NAF Fractions I and J. These fractions were found to be much of the same. AF-F was distilled to yield 5.1 g of product ($a_{233} = 88.2$, $a_{10.2} = 0.420$, $a_{10.6} = 0.287$). Capillary GLC on Apiezon L produced 5 peaks with ECL (equivalent chain length) and approximate percentages as follows: 18.0 (40%), 18.2 (21%), 18.3 (18%), 18.4 (13%), and 18.7 (8%).

NAF Fractions I and J were combined. Capillary GLC on Apiezon L was similar to that of AF-F. The combined fractions were distilled at approximately 148C and 0.2 mm pressure to yield 24 g product with $a_{233} = 97.6$, $a_{10.2} = 0.448$ and $a_{10.6} = 0.291$.

With both the samples from AF and NAF the ratio of absorptivity at 10.2 μ to 10.6 μ was higher in the distilled material, indicating that some *cis,trans*

TABLE II

Analyses of Fractions from Countercurrent Distribution of Nonadduct-Forming Materials in Figure 1

Fraction	Weight, %	Ultraviolet absorption		Infrared absorption		
A	0.3	....			....	
B	0.2	....			....	
C	22.8	$a_{234} = 43.5$,	$a_{268} = 66.8$	$a_{10.1} = 0.348$, $a_{10.2}$ 0.277,		$a_{10.4} = 0.530$ $a_{10.6}$ weak
D	3.5	$a_{272} = 44.4$		$a_{10.2} = 0.185$,		$a_{10.4} = 0.109$ $a_{10.6}$ weak
E	14.1	$a_{234} = 11.7$,	$a_{262} = 12.7$[a]	10–11 μ, no significant bands		
F	1.1	....			....	
G	2.3	$a_{232} = 71.3$		$a_{10.2} = 0.363$,		$a_{10.4} = 0.368$ $a_{10.6} = 0.268$
H	4.0	$a_{233} = 80.8$		$a_{10.2} = 0.374$,		$a_{10.6} = 0.300$
I	31.2	$a_{233} = 91.0$		$a_{10.2} = 0.429$,		$a_{10.6} = 0.376$
J	20.4	$a_{233} = 72.1$		$a_{10.2} = 0.431$,		$a_{10.6} = 0.370$

[a] No triene structure.

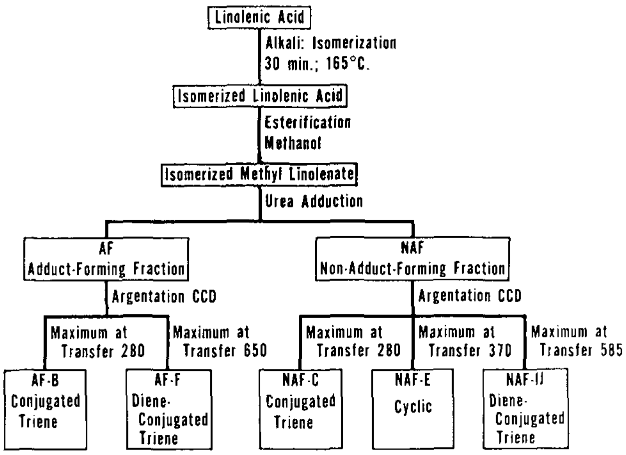

FIG. 2. Main fractions from alkali-isomerized linolenate.

conjugated ester had been converted to *trans,trans* during the distillation. To reisolate material with a higher *cis,trans* conjugated ester content, distilled NAF IJ was again fractionated by CCD and recycled to 511 transfers before withdrawal in the fraction collector. Only one broad band was obtained, the fractions of which were combined in the samples shown in Table III. All these samples from Table III when reduced as described for cyclic ester determination showed only stearate by GLC with no cyclic impurities.

An attempted low-temperature crystallization of NAF IJ-1 (Table III) from acetone (5 ml/g) produced no solid at −70C, and the sample itself with no solvent remained a viscous liquid with no solid material when cooled to −70C.

It seemed likely that all these samples were made up largely of esters in which the 12 double bond had moved to produce diene conjugation and to leave the remaining double bond separated by two methylene groups from the conjugated system. Evidence for this structure was obtained by alkali isomerization, by oxidative cleavage of the samples and of a maleic anhydride adduct and by nuclear magnetic resonance (NMR).

Analytical alkali isomerization of AF-F at 180C for 45 min (3) gave $a_{233} = 103$ and $a_{267} = 10.8$. Thus, little increase in triene conjugation was produced by alkali isomerization.

AF-F and NAF IJ-1 and 4 (Table III) were oxidized by the permanganate periodate procedure (8). The dibasic acids, which were analyzed by GLC as shown in Table IV, contained the expected succinic acid from between the isolated double bond and the conjugated system.

A 0.54-g portion of AF-F, 0.30 g malic anhydride, 30 ml toluene, and 5 mg iodine were refluxed 20 hr. The solution was washed with 15% potassium iodide to remove iodine and then with water; the solvent was evaporated leaving 0.71 g of maleic anhydride adduct. One-half gram of the adduct was saponified with potassium hydroxide in ethylene glycol and 0.52 g of acids was recovered. After oxidation of the adduct acids (8) GLC of the dibasic esters showed that 89% of the volatile dibasic esters was dimethyl azelate. Therefore, maleic anhydride is assumed to have added at the 13 and 15 double bonds and the presence of 9,13,15 linolenate is indicated. By using C_{12} dibasic ester as an internal standard, it was shown that about 53% of other material not volatile under the conditions of the GLC analysis was also present in the esters of the original cleaved acids. It is assumed that this nonvolatile material resulted from addition of maleic anhydride to the 9,11,15-isomer and that the amount of 9,11,15-linolenate was approximately equal to that of 9,13,15.

TABLE IV

Dibasic Acids After Oxidation of Diene Conjugated Trienoic Acids

Carbon number	Adduct-forming fraction F	Nonadduct-forming fraction 1	Nonadduct-fraction 4
2	4.53	3.26	2.37
3	1.36	0.92	1.17
4	31.23	30.20	38.62
5	1.88	1.66	1.93
6			
7	0.89	0.58	0.57
8	3.83	3.27	3.29
9	5.72	58.91	50.93
10	0.56	1.19	1.13

NMR spectrum of methyl linolenate has been discussed in a previous paper (6). In Figure 3 are shown NMR spectra for NAF Fractions IJ-1 and 4 (Table III) in carbon tetrachloride with tetramethyl silane and benzene references. Spectra for NAF-IJ-2 and 3, and AF-F were similar. In none of the samples was there a visible signal at 7.2 τ that would be observed for the di-α-olefinic methylene protons if double bonds separated by a single methylene group were present. However an integral curve showed a small area equivalent to 0.2 proton for NAF-IJ-3 and 0.4 proton for NAF-IJ-4. A signal was found in the 7.85 τ region equivalent to 10 protons adjacent to olefinic and carboxylic groups. This signal implies a separation of at least two methylenes between the isolated olefinic bond and conjugated system. There was no signal at 9.10 τ which would be there for the terminal methyl group if the 15,16 double bond were saturated or moved toward the carboxyl. Instead, the signal was displaced toward lower tau values as in linolenate. Also, the triplet for the methyl group of the 9,13,15-isomer is displaced from that of the 9,11,15-isomer forming two A_3B_2X systems with apparent coupling constant $J_{AB} = 8.0$ cps and $\delta = 0.053$ ppm between the two isomers.

By analogy with other fatty esters the signal at 9.05 τ—approximately the same location as that from the terminal methyl of linolenate—is probably from the 9,11,15-isomer and the signal at 9.00 τ is from the 9,13,15-isomer in which the conjugated system has an additional effect on the methyl protons. As shown in Figure 2, the areas associated with this signal from the two isomers vary for the CCD fractions in Table III. The isomer believed to be 9,11,15-octadecatrienoate was eluted slightly faster and was concentrated slightly in Fraction 1.

TABLE III

Analyses of Fractions from Countercurrent Distribution of Distilled Nonadduct-Forming Fractions I and J

Fraction	Transfers	Weight, %	Ultraviolet absorption a_{232}	Infrared absorption	
1	511–580	30.7	90.1	$a_{10.1} = 0.562$,	$a_{10.6} = 0.199$
2	581–630	31.6	96.9	$a_{10.2} = 0.424$,	$a_{10.6} = 0.296$
3	631–680	19.3	91.7	$a_{10.2} = 0.402$,	$a_{10.6} = 0.283$
4	681–800	18.3	91.7	$a_{10.2} = 0.367$,	$a_{10.6} = 0.320$

Discussion

The products formed by alkali isomerization of linolenate depend largely upon conditions. In previous work (13) at this Laboratory, linolenic acid was heated 7 hr at 200C with potassium hydroxide in ethylene glycol to produce a high concentration of cyclic acids. For this investigation a lower temperature and shorter reaction time were used to yield a product with large amounts of acids containing diene and triene conjugation.

The urea separation produced AF and NAF materials differing greatly in ultraviolet and infrared absorption. The maxima at 278, 267 and 257 mμ and the strong band at 10.1 μ indicate the presence of *trans,trans,trans,* conjugated ester in the AF fraction. In the NAF material, the higher absorption at 234 mμ, the lack of structure in the triene ultraviolet absorption region and the infrared absorption pattern suggest *cis,trans* diene conjugated and cyclic esters, in addition to triene conjugation. These conclusions are confirmed by argenation CCD.

CCD of the AF material produced two main bands, whereas NAF gave three bands including two at the same positions as AF. Because of similarities between the AF and NAF bands with like elution positions an alternate, and possibly better, fractionation procedure would be argentation CCD of the whole alkali-isomerized esters followed by recycle CCD, urea fractionation and low-temperature crystallization of esters from the individual bands.

As shown by IR and UV absorption, the first band in both AF and NAF was made up largely of triene conjugated esters. Only traces of cyclic material were present. Additional fractionation of these samples was achieved by the use of CCD, recycling to the maximum amount possible in our 200-tube instrument. Low-temperature crystallization from acetone yielded a fraction from AF material with properties corresponding to methyl pseudo-eleostearate (methyl *trans* 10, *trans* 12, *trans* 14 octadecatrienoate). Kass and Burr (9) report melting point 41C compared to our 40C and Paschke et al. (12) report a strong IR absorption band at 993 cm^{-1} (10.07 μ). Hoffman and co-workers (7) report for β-eleostearic acid—which should have quite similar UV absorption—$a_{269} = 201.8$. This corresponds to 191.5 for the methyl ester compared to $a_{267} = 194.3$ for our compound.

Infrared absorption of other fractions from this AF material (fractions from the sides of the broad band obtained by recycle CCD and filtrates from low-temperature crystallization) and of the NAF material indicated *trans,trans,trans* conjugated esters mixed with triene conjugated esters containing *cis* double bonds and absorbing at about 10.1 and 10.4 μ.

The material in the band found only in the NAF material is mainly cyclic esters as shown by the weak ultraviolet maximum at 262 mμ with no conjugated triene structure and the absence of strong absorption bands in the 10-11 μ infrared region (13). Capillary GLC on Apiezon L produced chromatograms with several peaks, but the chromatograms were less complex than those obtained when linolenic acid was heated under more drastic conditions to produce high concentrations of cyclic acids.

The composition of the last band eluted is quite similar in AF and NAF materials. The UV absorption at 233 mμ and the IR bands at 10.2 and 10.6 μ are indicative of *cis,trans* diene conjugation. NAF Fraction G is similar except for the absorption at 10.4 μ, which is believed caused by isolated *trans* double bonds since no triene conjugation, which would absorb at the same wavelength, is present. Capillary GLC shows one main peak with several smaller peaks, which probably represent *cis* and *trans* isomers. The geometric configuration of the conjugated double bonds as indicated by ratio of absorptivity at 10.2 μ to 10.6 μ proved to be quite labile. The AF fraction had a ratio $a_{10.2}/a_{10.6}$ of 1.22 and the NAF fraction of 1.16. After distillation the ratios were 1.46 and 1.54, respectively. The ratios of absorptivities before distillation are similar to the value for $a_{10.16}/a_{10.55}$ of 1.198 found by Chippault and Hawkins (4) for *cis, trans* conjugated diene. It was possible to recover esters with a ratio like that of the original by CCD with recycling as shown in Table III.

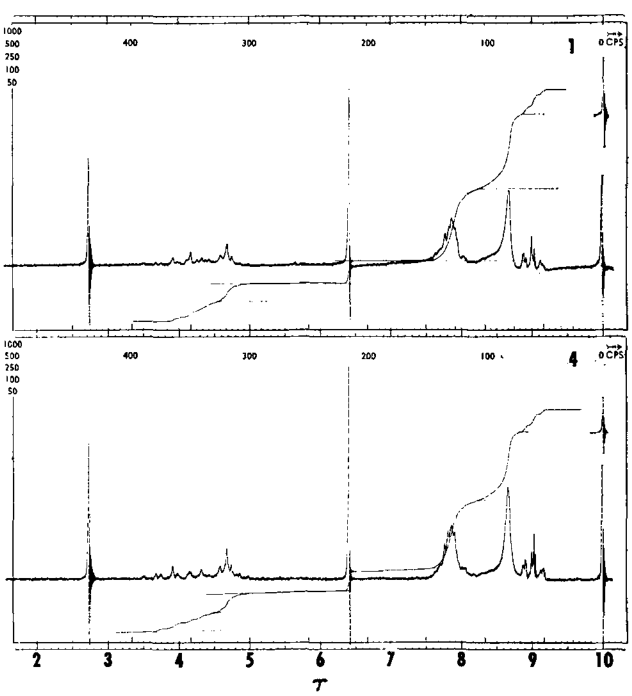

FIG. 3. NMR spectra for diene conjugated trienoic esters from NAF material Band 600 (NAF Fractions IJ-1 and 4, Table III).

Kass and Skell explained the conjugated products resulting from alkali isomerization of linolenic acid in terms of Ingold's concept of the three-carbon prototropic mechanism (10). They state the 9,11,15- and 9,13,15- linolenic acid isomers are the conjugated diene-containing acids which may be shown to occur. Evidence that this CCD band is largely these 9,11,15- and 9,13,15-isomers was obtained by alkali isomerization, oxidative cleavage and NMR spectra. Analytical alkali isomerization produced only a small amount of triene conjugation. The increase in diene absorption may be in part or completely caused by change of *cis,trans* to *trans,trans* configuration. Any unchanged linolenate would also be in these fractions (15). The isolation of azelaic acid in about 50% yield following oxidative cleavage of the maleic anhydride adduct is evidence for the 9,13,15-isomers, and we assume that the 9,11,15-isomer is probably present in like amount. As shown in Table IV, oxidative cleavage produces a large amount of the expected C_4 dibasic acid. The lower values, as compared to the long chain dibasic acids result from difficulties in quantitative recovery of the short chain dibasic acids. NMR shows that there are three olefinic bonds, two of them conjugated and the other at least two methylenes removed. The location of the terminal methyl signal shows that the

15,16 position is unsaturated. The position of this signal is slightly different for the two isomers and shows that a small amount of fractionation occurs between them in argentation CCD.

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