replacement with another fatty acid, may have an equally important effect on the physiological function of the cell. This possibility obviously justifies further investigation.

ACKNOWLEDGMENT

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Preparation of 9,15-Octadecadienoate Isomers[1]

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Abstract

Linolenic acid was reduced with hydrazine to produce a mixture containing a max of dienoic acids. After methylation this mixture was separated into trienoic, dienoic, monoenoic, and saturated esters by countercurrent distribution (CCD) with acetonitrile and hexane. The dienoic ester was further fractionated by CCD with methanolic silver nitrate and hexane to separate pure *cis,cis*-9,15-octadecadienoate and the equimixture of *cis,cis*-9,12- and 12,15-octadecadienoates.

Following isomerization of the *cis,cis*-9,15-octadecadienoate with selenium, the geometric isomers were fractionated by CCD with methanolic silver nitrate and hexane. Pure *trans,trans* and pure *cis,cis* isomers were isolated, as well as an unresolved mixture of *cis,trans* and *trans,cis* isomers. The characteristics of these isomers and related compounds are compared as determined by CCD, IR absorption, and capillary gas-liquid chromatography (GLC).

Introduction

THE HYDRAZINE REDUCTION of linolenic acid produces seven fatty acids of which *cis,cis*-9,15-octadecadienoic acid is one (6). In this comparatively simple reaction neither *trans* isomers nor positional isomers are formed as they are in a catalytic reduction (4,7). If the reduction is stopped at max concn of dienoic acids, the *cis,cis*-9,15-octadecadienoic acid is one-third of the dienoic acids or ca. 16%. After methylation the dienoic esters can be fractionated from saturates, monoenes, and trienes by CCD in an acetonitrile-hexane system (6,8). Subsequently, by using an argentation system of hexane and 0.2*N* silver nitrite in 90% methanol, the 9,15-dienoate can be separated by CCD from the 9,12 and 12,15 isomers. This latter system separates compounds by degree of unsaturation, by geometric configuration of double bonds, and by the number of methylene groups between double bonds for polyunsaturated esters (5). In the present work, the preparation of pure *cis,cis*-9,15-octadecadienoate is described, together with its isomerization by selenium to produce geometric isomers. This mixture of methyl esters is separated by using CCD with the argentation system to give pure *trans,trans* and *cis,cis*-9,15-octadecadienoates, as well as an unresolved mixture of mono *trans* isomers (*cis, trans* and *trans, cis*) 9,15-octadecadienoates.

Experimental

Hydrazine Reduction. A mixture of 87.3% linolenic, 9.8% linoleic, and 2.9% oleic acids obtained from a urea crystallization of linseed oil fatty acids was reduced as follows: to 204 g was added 2 liters 95% ethanol; the solution was warmed to 50C before adding 174 ml hydrazine hydrate. Compressed air was bubbled through the mixture to provide stirring and the necessary oxygen (6). The reaction was stopped after 6 hr and 20 min by shutting off air flow and adding 2.4 liters dilute HCl (1:5). The sample was extracted with pentane-hexane, washed, and dried. After solvent evaporation, the sample was esterified with 1,040 ml methanol and 2 ml H_2SO_4. Methyl esters were extracted with pentane-hexane after refluxing for 7.5 hr, washed, dried, and distilled under vacuum to yield 183.3 g of the following composition: 21.3% triene, 47.3% diene, 25.8% monoene, and 5.6% stearate.

Countercurrent Distribution Procedure. A 200-tube automatic CCD apparatus in which each tube contained 40 ml of lower solvent layer was used. The distributions were made according to the single-

[1] Presented at the AOCS meeting in Minneapolis, 1963.
[2] A laboratory of the No. Utiliz. Res. and Dev. Div., ARS, USDA.

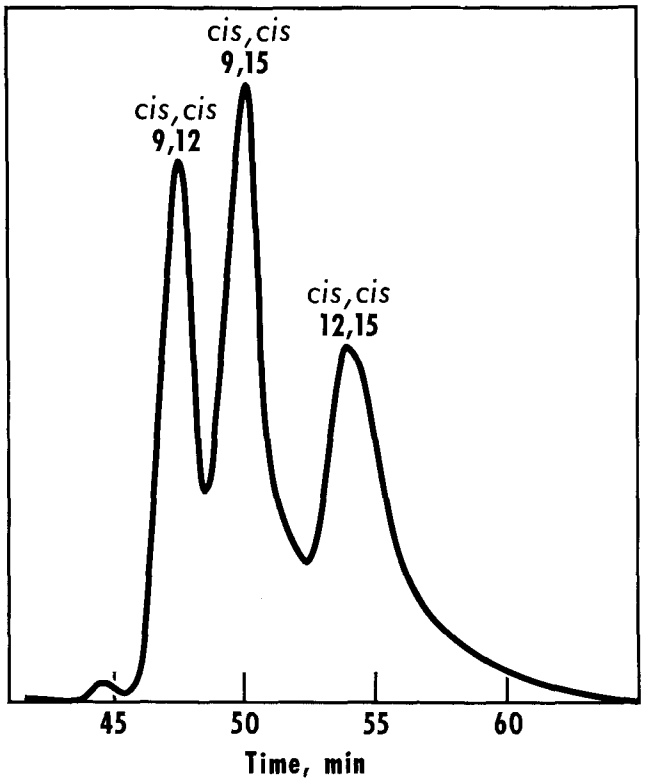

FIG. 1. Gas chromatogram of a mixture of 9,12-, 12,15-, and 9,15-methyl linoleates on DEGS, 200-ft capillary column at 160C.

withdrawal procedure (1,8) using 10-ml portions of upper layer, and solutions from two transfers were collected in each collection tube. The wt of material after evaporation of solvents was plotted against the transfer number.

Selenium Isomerization. *cis,cis*-9,15-Octadecadienoate was isomerized by heating under nitrogen at 190C for 9 hr with 2.0% selenium. Selenium was removed from the product by stirring with mercury (9). The product contained 145% *trans* by IR measurement, calculated on the basis of methyl elaidate as 100%.

Analysis for trans *Double Bonds.* *trans* Double bonds were determined by IR absorption of carbon disulfide solutions in a 0.5-mm cell. The absorption band at 10.4 μ was measured from a base line drawn tangent to the absorption curve at ca. 9.4 and 10.7 μ. All *trans* percentages were calculated on the basis of methyl elaidate as 100%.

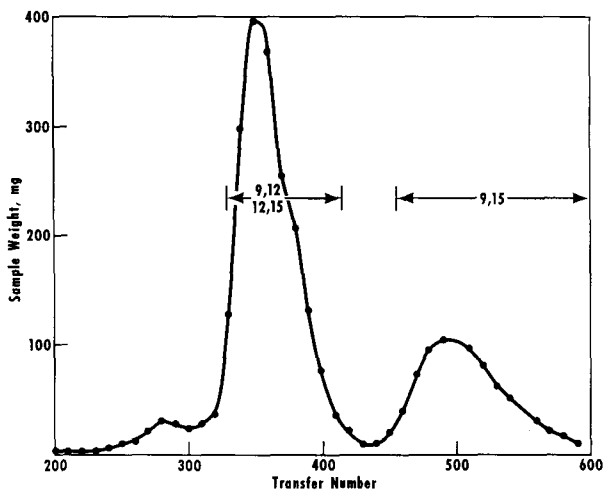

FIG. 2. CCD of a mixture of 9,12-, 12,15-, and 9,15-methyl linoleates.

TABLE I

Dibasic Acid Cleavage Products from Octadecadienoate Isomers (Mole Per Cent)

Fraction	Internal				Terminal		
	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
cis,cis	2.3	6.9	88.9	1.9	3.5	96.5	0
trans,trans A	1.6	9.0	83.7	5.7	4.9	93.0	2.1
trans,trans B	1.3	7.0	87.4	4.3	4.3	93.6	2.1
Mono *trans* C	2.2	10.8	80.3	6.7	5.1	92.3	2.6
Mono *trans* D	1.9	7.5	85.8	4.8	5.1	92.3	2.6

Gas Chromatograms. Chromatograms were run on three capillary columns, all 0.01 in. diam: a 200-ft capillary coated with diethylene glycol succinate (DEGS) at 160C, a 100-ft capillary coated with 100% cyanoethyl silicone at 170C (3), and a 200-ft capillary coated with Apiezon L at 200C. A radium D ionization detector was used. Order of elution was determined by adding either known standards or separated isomers to the original mixtures.

Discussion and Results

The diene recovered from the acetonitrile CCD had no *trans* absorption, and GLC showed only three peaks (Fig. 1) from a DEGS capillary column on which the 9,12 and 9,15 isomers eluted before the 12,15. The same separation has been reported by Scholfied et al. with an Apiezon L capillary column (6). A 100% cyanoethyl silicone capillary gives the same elution pattern but not as good separation.

The *cis,cis*-9,15-octadecadienoate prepared in the first argentation CCD (combined as indicated in Fig. 2) showed one peak on capillary GLC. The sample was cleaved by the periodate-permanganate oxidation procedure of Jones and Stolp (2). Table I contains an analysis calculated on the assumption that eight carbon or longer dibasics are terminal, that is from the carboxyl end of the molecule, and that seven carbon or shorter dibasics are internal, from between the double bonds. The *cis,cis* isomer double bonds are mainly in the 9- and 15-positions.

The selenium-isomerized 9,15-octadecadienoate contained 145% *trans* as elaidate. This would be a mixture of 52.6% *trans,trans*, 39.9% mono *trans*, and 7.6% *cis,cis* if the *trans* bonds are distributed at random and are additive in their absorption. The integrated areas under the argentation CCD for this material (Fig. 3) gave 54.4% *trans,trans*, 37.7% mono *trans*, and 7.9% *cis,cis*. Calculating *trans* value for the mixture from these percentages give 146.5% *trans*, which compares excellently with the 145% *trans* by IR. The agreement indicates that the isomerization is random and that the *trans* absorption is additive.

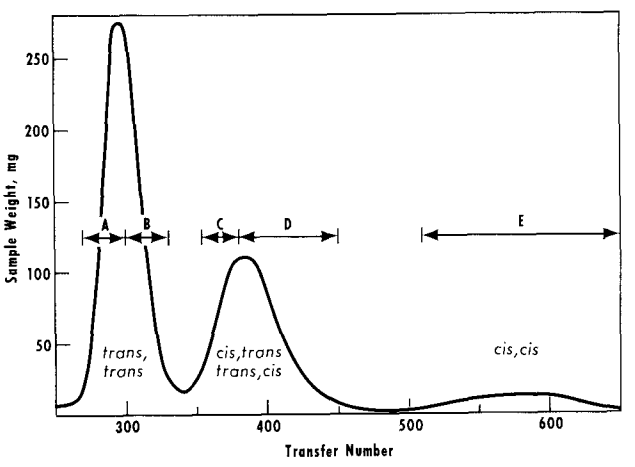

FIG. 3. CCD of selenium-isomerized methyl 9,15-octadecadienoate.

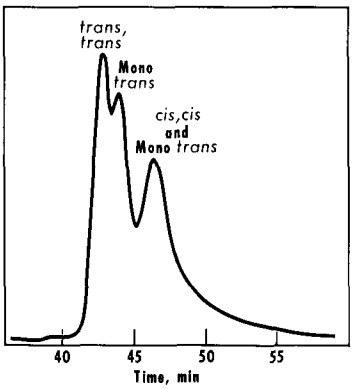

FIG. 4. Gas chromatogram of selenium-isomerized methyl 9,15-octadecadienoate on DEGS, 200-ft capillary column at 160C.

Table I shows that selenium isomerization moved ca. 3 or 4% of the double bonds.

Using a 200-ft DEGS capillary column to separate 9,15 isomers, one obtains an elution pattern of three peaks, which are a *trans,trans* isomer, a mono *trans* isomer, and a mixed peak of mono *trans* and *cis, cis* isomers (Fig. 4). A 100-ft cyanoethyl silicone capillary column (a polar column like DEGS) gives the same order of elution but separates both mono *trans* isomers from the *cis,cis* isomer (Fig. 5). The 200-ft Apiezon L capillary column does not give the same order of elution; the *trans,trans* and *cis,cis* isomers elute together and mono *trans* isomers come before and after the *trans,trans* and *cis,cis* peak (Fig. 6).

The separation of 9,15 isomers by argentation CCD requires no recycling as it does for 9,12 isomers (5) because one double bond in a 9,15 isomer has little effect in reducing the complexing characteristics of the other double bond and because 9,15 isomers have lower partition coefficients. In a 9,12 isomer the complexing of one double bond decreases the tendency for the other bond to complex (5); thus, the differences in the isomer complexes are not as great for 9,15 isomers as for 9,15.

Both the *trans,trans* peak and the mono *trans* peak of argentation CCD were split into two fractions as shown in Figure 3. There were only small differences in the two *trans,trans* fractions as shown by capillary GLC (Fig. 7A,7B) and by oxidative cleavage (Table I). The IR absorption for the 9,15 *trans,trans* is ca. twice that of the elaidate shown in Table II. The absorption for 9,12 *trans,trans* is less because of interaction between the double bonds (5).

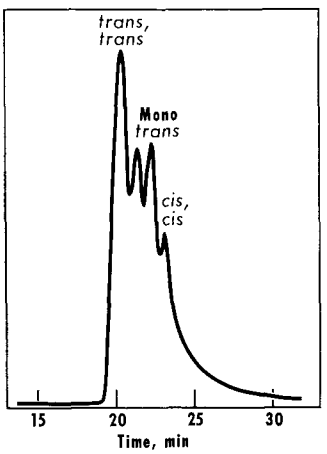

FIG. 5. Gas chromatogram of selenium-isomerized methyl 9,15-octadecadienoate on 100% cyanoethyl silicone, 100-ft capillary column at 170C.

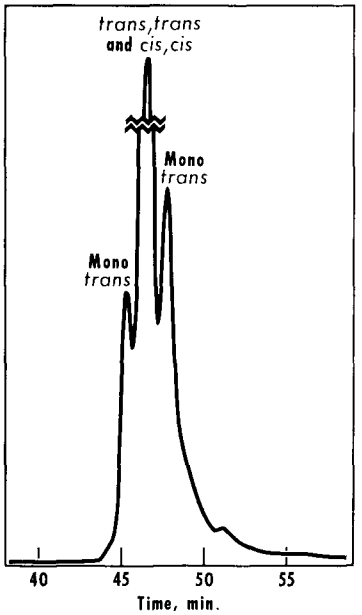

FIG. 6. Gas chromatogram of selenium-isomerized methyl 9,15-octadecadienoate on Apiezon L, 200-ft capillary column at 200C.

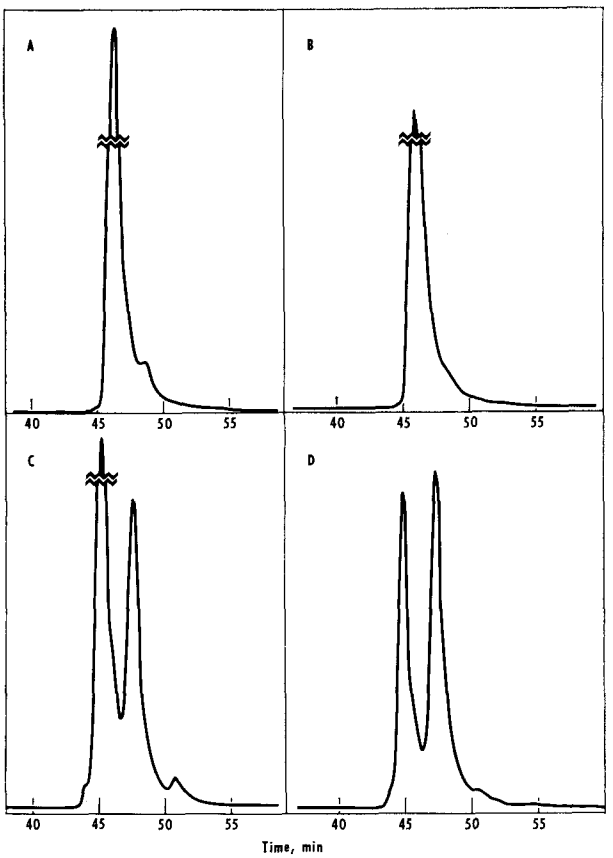

FIG. 7. Gas chromatograms of CCD fractions from selenium-isomerized methyl 9,15-octadecadienoate on Apiezon L, 200-ft capillary column at 200C.

TABLE II

Comparison of Infrared Absorbance for Isomers

Fraction	Percentage *trans* as methyl elaidate
9,15 *trans,trans* A	189.7
9,15 *trans,trans* B	207.3
9,15 Mono *trans* C	110.5
9,15 Mono *trans* D	104.2
9,12 *trans,trans*	166[a]
9,12 Mono *trans*	85[a]

[a] From literature reference 5.

The mono *trans* fractions differ in the ratio of the two isomers as shown by capillary GLC (Fig. 7C,7D). Fraction C might have a small amount of *trans,trans* isomer explaining its higher *trans* absorption. The mono *trans* oxidative cleavage shows little difference between the two fractions. The IR absorption for the 9,15 mono *trans* is a little more than that of the elaidate where a 9,12 mono *trans* is ca. 85% of the elaidate (5).

This procedure for preparing 9,15-octadecadienoates is involved due in part to the small percentage of desired product in the starting material and in part to the number of CCD's that have to be performed. The procedure does, however, provide valuable, pure isomers for standardization of GLC and IR analyses.

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Catalysts for Selective Hydrogenation of Soybean Oil.[1] II. Commercial Catalysts

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Abstract

A survey of commercial hydrogenation catalysts demonstrated the higher selectivity (S_L = 2.4–2.7) of certain platinum, palladium and rhodium catalysts for hydrogenating linolenic components in soybean oil. Nickel catalysts generally showed selectivities below $S_L = 2.0$ although skeletal nickel achieved higher values. *Trans*-isomers were in the range 7.8–15.4% for the above noble metal catalysts. Nickel catalysts provide a lesser degree of isomerization, 5.2–7.4% of *trans*-isomers for the most selective catalysts.

Introduction

IN A PREVIOUS publication (1), a method was outlined for determining the selectivity of catalysts for the hydrogenation of linolenic components in soybean oil. In the present paper, various commercial catalysts were secured from several manufacturers and the method was applied. The object of the survey was primarily for orientation purposes. Presumably, many of the catalysts had proved commercially successful for hydrogenation processes and it was felt that selectivity might be inherent in a particular class of such materials. The survey should not be considered as a means of obtaining relative ratings, since time did not permit optimization studies for any particular catalyst. However, it was felt that the broad spectrum of products which could be obtained in this way might afford valuable clues in suggesting improved or new catalysts.

Catalysts were received from: the Baker & Co. Div., Engelhard Industries, Inc., Newark; the Davison Chemical Div., W. R. Grace & Co., Baltimore; Girdler Catalysts, Chemical Products Div., Chemetron Corp., Louisville; Harshaw Chemical Co., Cleveland; and Nikko & Co., Ltd., Tokyo. Source and typical compositions, where known, are given in appropriate tables.

Experimental Procedure

The soybean oil used throughout the work was a refined bleached product obtained from Swift & Co. The following acid composition applied for most of the work:

Component	Amount, %
Palmitic	10.0
Stearic	3.0
Oleic	27.1
Linoleic	54.9
Linolenic	5.0
Total	100.0

The apparatus, experimental procedure, and evaluation method were previously described (1).

Commercial Platinum Catalysts

Platinum catalysts provided good selectivities as evidenced by the selectivity index S_L in Table IA. The selectivity appears to be somewhat temp dependent, i.e., as the temp was raised, the selectivity generally improved. Concurrent with greater linolenic hydrogenation, the formation of *trans*-isomers also increased. The total hydrogenation rate did not increase appreciably with temp, indicative of a high activation energy, a diffusion-controlled process, or both. Since the selectivity improved with temp, the selective nature of the catalyst would appear to play a dominant role. In these experiments, approximately 50% of the linolenic constituents were hydrogenated with the most effective catalysts; this, in general, was found to be a point beyond which further removal proved quite difficult. Since the linolenic concn was then down to 2.5% under such conditions, the selectivity of the catalyst indeed had to be exceptional to obtain further decreases.

The concn of platinum was 0.025%, based on the oil; the dispersion of this concn on different carriers appeared to have little effect in obtaining selectivity. There is slight evidence that the more dilute metal concn (0.5 and 1%) afford somewhat less *trans*-isomerization.

The inclusion of a small amount of organic acid (approximately 5% concn acetic acid) in one test afforded no significant effects.

[1] Presented at the AOCS Meeting at Toronto, 1962.