Hydrogenation of Linolenate. I. Fractionation and Characterization Studies[1]

C. R. SCHOLFIELD, E. P. JONES, JANINA NOWAKOWSKA, E. SELKE, B. SREENIVASAN, and H. J. DUTTON, Northern Regional Research Laboratory,[2] Peoria, Illinois

Methyl linolenate hydrogenated at 140°C. with 0.5% Ni catalyst and 1.1 mole of hydrogen at atmospheric pressure was separated into octadecenoate, octadecadienoate, and octadecatrienoate fractions by countercurrent distribution. Gas chromatography on a 200-ft. capillary Apiezon L column revealed one component in the triene fraction, four in the diene fraction, and nine in the monoene fraction. These components were partially fractionated by low-temperature crystallization, and their solubilities were correlated with alkali conjugation results, with infrared data for *cis* and *trans* configuration of bonds and with dibasic acids isolated from the fractions after oxidative cleavage. Approximately 45% of *trans* acids were present in both the monoene and diene fractions. Considerable migration of double bonds from the original 9, 12, and 15 positions occurred. *Cis,cis* dienes which could not be conjugated by alkali were formed. Little alteration of the residual methyl linolenate was observed. The results demonstrate the applicability and utility of new techniques of fractionation and analysis to the study of the hydrogenation mechanism.

CONSIDERABLE EVIDENCE supports the belief that flavor stability of soybean salad oil would be improved by removing the 9% of linolenyl groups present in the oil. This removal of linolenate might be accomplished by hydrogenation if sufficiently small alteration of other fatty acyl groups occurred so that a liquid oil was retained. However upon catalytic hydrogenation of fatty materials, isomers of the naturally-occurring fatty acids are formed.

Much current research on hydrogenation of vegetable oils is on the formation of position and geometric isomers by side reactions that are inherent in the hydrogenation process. Not only are some double bonds saturated, but others are transferred from *cis* to *trans* configuration, from nonconjugated to conjugated positions, and are moved up and down the carbon chain (1). Because these isomers differ in their subsequent reactivity with hydrogen, their presence complicates the interpretation of kinetic studies (2). Furthermore isolinoleate isomers probably contribute to undesirable odors (3) in addition to the original linolenate.

Lemon found that the isolinoleates from hydrogenated linseed and perilla oil contained *trans* bonds (4); Rebello and Daubert found a mixture of at least three isomers, the 8,14-, 9,15-, and 10,14-isolinoleic acids (5). Much of the information on isomers formed during hydrogenation is necessarily inferential because techniques of fractionation have not been commensurate in resolving power with the complexity of mixtures encountered.

In recent years new techniques for analysis of methyl esters of fatty acids have been discovered and older ones have been improved. Among them are a) the improved procedures for double bond location (6); b) infrared analysis of geometric isomers (7); c) ultraviolet spectrophotometric measurement of conjugation both before and after alkali isomerization (8,9); d) countercurrent distribution to separate octadecanoates, octadecenoates, octadecadienoates, and octadecatrienoates (10); and e) gas chromatography, where in the separation of geometric isomers a 200-ft. capillary column has been used with a sensitive ionization detection system (11). The present paper describes the application of these new and improved techniques to a study of partially hydrogenated methyl linolenate.

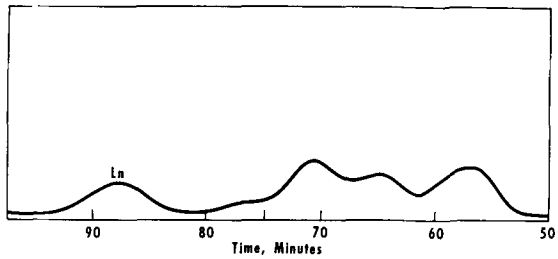

FIG. 1. Gas chromatogram of hydrogenated methyl linolenate from a 4-ft. x ¼-in. column packed with 10% Resoflex 296 on chromosorb and operated at 150°C., 35.5 ml. argon per min., using a radium D ionization detector.

Experimental

Methyl linolenate, prepared by countercurrent distribution between pentane-hexane and acetonitrile (10), was hydrogenated in glass manometric equipment by using a magnetic stirrer at atmospheric pressure and 140°C. To 15.2 g. of methyl linolenate containing 0.5% nickel (a commercial catalyst consisting of nickel on kieselguhr in hardened oil) 1.1 moles of hydrogen per mole of ester were added.

Analytical data on the hydrogenated material are shown in Table I. The measurement of alkali-isomerized acids employed the 45-min. heating procedure of Brice *et al.* (9). The gas-liquid chromatographic values were obtained from the chromatogram shown in Figure 1. A 4-ft. by ¼-in. glass column packed with 10% Resoflex 296 on chromosorb was operated at 150°C. and 35.5 ml. per min. argon gas flow, using radium D ionization detector. Under these conditions methyl oleate, linoleate, and linolenate would be completely separated. However the chromatogram of Figure 1 was so complex that only the amount of triene could be calculated with confidence.

TABLE I
Analysis of Hydrogenated Methyl Linolenate

Method of analysis	Monoene	Diene	Triene
	%	%	%
Alkali conjugation	.....	7.1	18.5
Gas liquid chromatography	.....	.....	16.4
Countercurrent distribution	36.9	43.5	20.6

The hydrogenated ester was fractionated by a 600-transfer countercurrent distribution between pentane-hexane and acetonitrile, using 10-ml. portions of up-

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[2] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

per layer and 40-ml. portions of lower layer (Figure 2). The solvent system has been found to separate esters into fractions based upon the number of double bonds in the molecule. Areas under the curve in the monoene, diene, and triene regions were measured to give the values in Table I.

Fractions from the countercurrent distribution were combined, as shown in Figure 2, to give monoene,

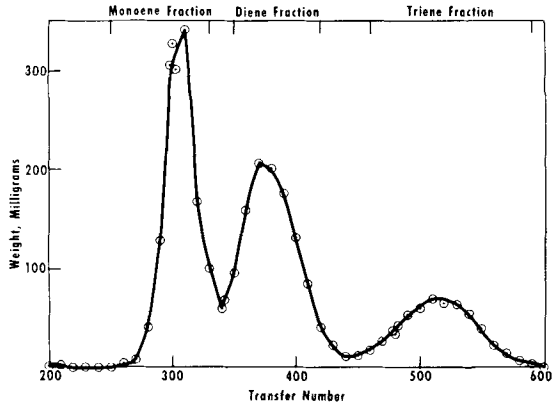

FIG. 2. Countercurrent distribution of hydrogenated methyl linolenate in a 200-tube instrument, using 40 ml. acetonitrile for lower layer and 10 ml. of pentane-hexane for upper layer.

diene, and triene fractions. Analysis of these fractions is shown in Table II. Ultraviolet absorption was measured in methanol solutions in a Cary recording spectrophotometer. The absorptivities given are the maxima in the diene region. No preformed triene conjugation was found. Esters containing *trans* bonds were measured by infrared spectroscopy as 4% solutions in carbon disulfide and were calculated by using methyl elaidate as a standard.

Oxidative cleavage and identification of the resultant dibasic acids were carried out according to the procedure of Jones and Stolp (6), using a periodate-permanganate oxidation mixture for cleavage and using liquid partition chromatography for separation and identification. Also methyl esters of the dibasic acids were formed, and these esters were separated by gas chromatographic procedures (12), using a 4-ft. column packed with 10% Craig succinate polyester on chromosorb. The values obtained from both liquid-liquid partition chromatography (LLPC) and gas liquid chromatography (GLPC) are given in Figures 3 and 4.

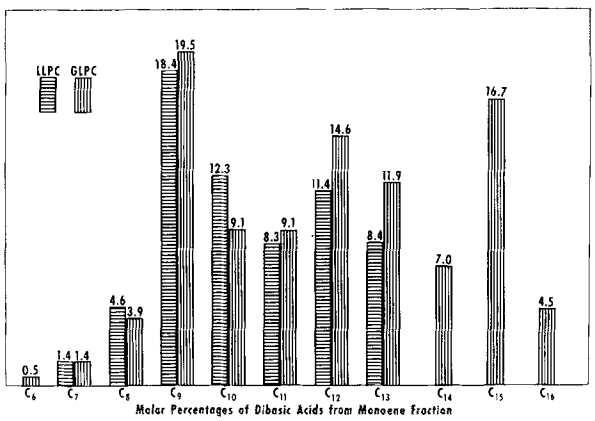

FIG. 3. Molar percentages of dibasic acids obtained by oxidation of monoene portion of hydrogenated methyl linolenate. Dibasic acids analyzed by liquid partition chromatography and by gas chromatography.

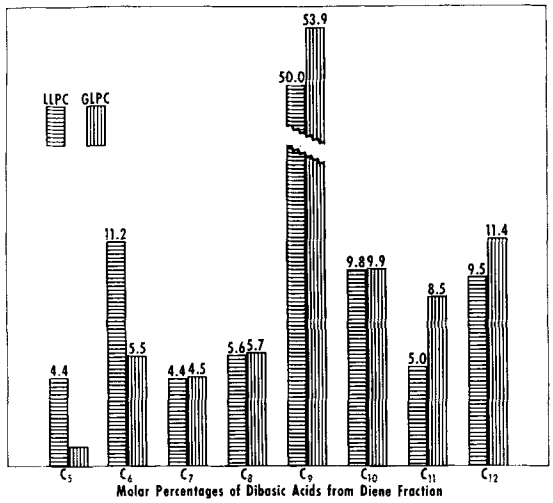

FIG. 4. Molar percentages of dibasic acids obtained by oxidation of diene portion of hydrogenated methyl linolenate. Dibasic acids analyzed by liquid partition chromatography and by gas chromatography.

Gas chromatographic analysis of the monoene and diene fractions was carried out at 215°C. in a 200-ft. capillary column coated with Apiezon L, followed by a β-ionization detector with strontium 90 (Figures 5 and 6). The monoene and diene fractions were further fractionated by crystallization from methanol solutions (20 ml./g.) at −35°C. and−60°C., respectively (Tables III and IV and Figures 5 and 6). The original monoene chromatogram was run at a different time and under slightly different conditions from the filtrate and crystal fractions. Its time scale is shown at the top of Figure 5. For comparison, Component A in the original monoene has been aligned with the same component in the filtrate.

Results and Discussion

Partial hydrogenation of methyl linolenate produces a complex mixture of monoene and diene isomers. The gas chromatographic curve of Figure 1 indicates a number of partially resolved monoene and diene components. In Table I the amount of triene found by gas chromatography agrees fairly well with amount of alkali isomerization and by countercurrent distribution. In the diene fraction the value found by alkali isomerization is much less than the total found by countercurrent distribution. This difference indicates that a large amount of diene isomers formed by hydrogenation is not conjugated by alkali. These dienes may be position isomers formed both by hydrogenation of the 12,13-double bond and by movement of double bonds to positions separated by more than one methylene group; also the dienes may be geometric isomers containing *trans* bonds. Under these isomerization conditions as shown by Jackson *et al.* (13), *cis*-9, *trans*-12 linoleate would give a value of about 85% of the normal value for *cis.cis* diene and *trans,trans* linoleate, a value of about 35%.

As shown in Table II, the monoene fraction contains 46.1% of *trans* esters and the amount of these *trans* esters is reflected in the melting point of 34°C. for the free acid. The gas chromatographic behavior of the monoene fraction on the capillary column is shown in Figure 5, where at least 9 components are present. Mixed chromatograms of methyl oleate and elaidate with the monoene fraction show chromato-

TABLE II

Analysis of Fractions from Countercurrent Distribution of Hydrogenated Methyl Linolenate

Fraction	Monoene	Diene	Triene
Iodine value (Wijs)	86.9	174.6	250.3
Preformed diene conjugation azai	.....	0.56	1.88
Alkali conjugation	0.4% diene	19.7% diene	97.3% triene 3.1% diene
Trans esters	46.1%	44.7%	.....
M.P. acids (°C.)	34	14	.....

graphic identity of the oleate with peak B and the elaidate with peak C. Since the 11 positional isomers determined by the dibasic acid composition (Figure 3) may occur in either *cis* or *trans* forms, some of the gas chromatographic peaks must correspond to two or more monoenes. Further study with known monoenes is necessary to identify all peaks.

The dibasic acids from C-7 to C-15 were obtained by oxidative cleavage of the monoene fraction with permanganate-periodate reagent. They were fractionated by liquid partition chromatography, and their molar percentages are given in Table II as well as the percentages obtained for the same acids after conversion to methyl esters with diazomethane and separation by gas chromatography on the 4-ft. column. The dibasic acid composition shows that, although there has been considerable movement of double bonds, the largest amount of dibasic acids still corresponds to the original 9, 12, and 15 positions.

Solvent crystallization of the monoene fraction containing 46.1% of *trans* acids from methanol at −35°C. resulted in the recovery of 67% of the esters in a crystal fraction and of 33% in the filtrate. The filtrate contained 19% of *trans* acids and the crystal fraction, 64.8% (Table III).

A comparison of chromatograms for the filtrate and solid fractions (Figure 5) tends to confirm that the peak B is *cis* octadecenoate, "oleate," and that the fraction E is *trans* octadecenoic, "elaidate." Because the remaining peaks tend to concentrate in the crystal fraction, it is inferred that these acids also are probably *trans* acids. Peaks C, D′, E′, and F′ in the filtrate curve are displaced from the corresponding peaks in the other curves and do not necessarily represent the same components.

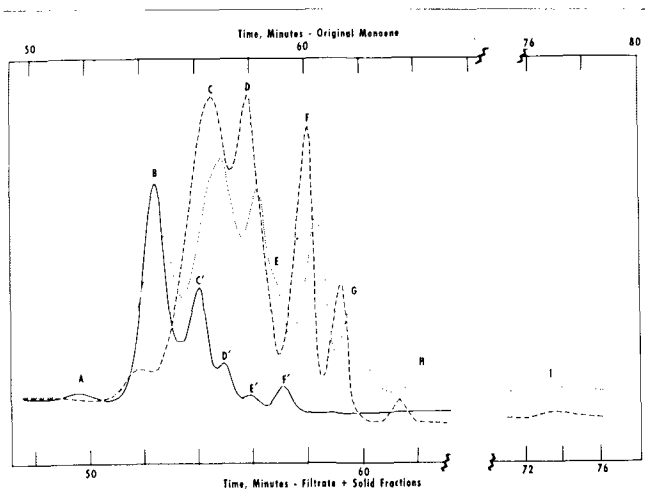

FIG. 5. Gas chromatogram of monoene portion of hydrogenated methyl linolenate and its liquid and crystal fractions from methanol at −35°C. from a 200-ft. capillary column coated with Apiezon L, operated at 215°C., and using a strontium 90 ionization detector. Dotted line, monoene; dash line, crystal; solid line, filtrate.

TABLE III

Gas Chromatographic Analysis of Methyl Octadecenoate and of Its Crystal and Filtrate Fractions

Total monoene *trans* 46.1%		Fractions			
		Crystalline (66.8%) *trans* 64.8%		Filtrate (33.2%) *trans* 19.0%	
Peak	% Wt.	Peak	% Wt.	Peak	% Wt.
A	1.5	A	.....	A	3.3
B (oleate)	20.5	B (oleate)	3.5	B (oleate)	56.8
C (elaidate)	33.2	C (elaidate)	39.8	C (elaidate)	23.9
D + E	22.7	D + E	30.2	D′	8.8
.....	.....	.....	.....	E′	2.8
F	13.6	F	17.2	F′	4.4
G	6.5	G	7.3	.....	.....
H	1.3	H	1.4	.....	.....
I stearate	0.7	I	0.6	.....	.....

From the area under curve B of the monoene fraction it is estimated that the oleate amounts to 20.5% of the sample. This figure agrees rather well with the amount of C_9 dibasic acids formed on oxidative cleavage of 19.5%.

In the diene fraction the small amount of preformed diene conjugation is equivalent to only about 0.5% of esters. After alkali conjugation a value of 19.7% diene was obtained. As stated, this low value is probably caused both by positional and geometric isomerism. The value of 44.7% *trans* ester is calculated with methyl elaidate standard. This calculation assumes that only *cis,trans* dienes are present. The value would be lower if *trans,trans* esters are assumed to be present. Since at least 55% *cis,cis* esters are present and only 19.7% esters which can be conjugated by alkali, it is clear that *cis,cis* positional isomers which are not conjugated by alkali are formed.

Of the four major peaks shown for the diene fraction (Figure 6), peak A appears to be *cis,cis* linoleate by mixed chromatogram. On fractional crystallization 28% of the diene was recovered in the crystal fraction and 72% in the filtrate. A less dramatic fractionation of the *trans* esters occurred among the diene esters than among the monoenes (Figure 4 and Table IV). In part this difference is caused by the

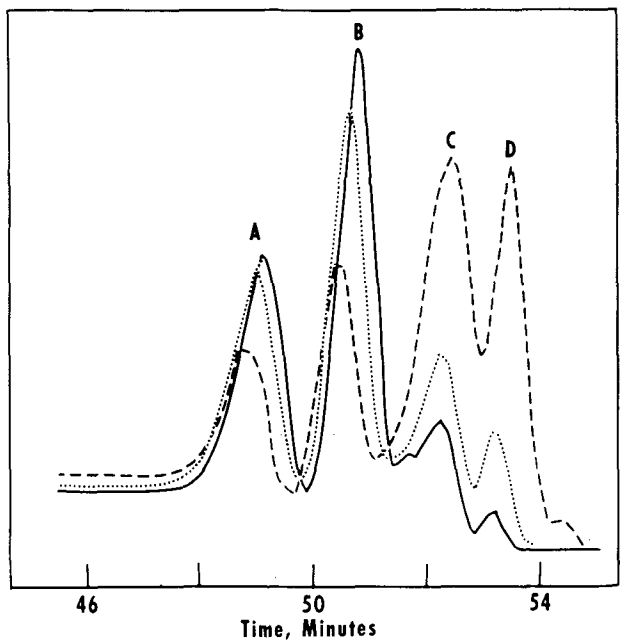

FIG. 6. Gas chromatogram of diene portion of hydrogenated methyl linolenate and its liquid and crystal fractions from methanol at −60°C. from a 200-ft. capillary column coated with Apiezon L, operated at 215°C., and using a strontium 90 ionization detector. Dotted line, diene; dash line, crystal; solid line, filtrate.

TABLE IV
Gas Chromatographic Analysis of Octadecadienoate and of Its Crystal and Filtrate Fractions

Total diene *trans* 44.7% Conjugatable diene 19.7%		Fractions			
		Crystalline (28%) *trans* 52.1% Conjugatable diene 26.6%		Filtrate (72%) *trans* 37.9% Conjugatable diene 14.6%	
Peak	% Wt.	Peak	% Wt.	Peak	% Wt.
A	30.8	A	14.1	A	34.0
B	42.0	B	19.9	B	47.7
C	20.1	C	40.7	C	15.7
D	7.1	D	24.1	D	2.6
....	.....	E	1.2	....	.....

complex relation of *cis,trans* isomers of octadecadienoates to their solubility characteristics. Thus the proportions of *trans* esters change only slightly on fractional crystallization. Isomers corresponding to peaks C and D tend to concentrate in the crystal fraction, and the *trans* content in this fraction also increases somewhat.

Oxidative cleavage of the dienoic acids results in two dibasic acids, one that includes the original carboxyl and the other that is formed from the carbon atoms between the double bonds. The shorter dibasic acids are largely formed from this group between the double bonds. For example, C_6 dibasic acid is formed from 9,15 dienoic acid which results from hydrogenation of the 12 bond. The 50% value for C_9 acids results from the large amount of acids in which the 9 bond is still in the original position.

The triene fraction appears to undergo comparatively little change during hydrogenation. Its iodine value is 250, and it contains about 2% conjugated diene but no conjugated triene. Alkali conjugatable triene comprises 97.3% of the fraction.

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

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Some Characteristics of the Membranes Protecting Oil Emulsions in Protein Solutions[1]

JUAN M. MARTÍNEZ MORENO, C. GÓMEZ HERRERA, E. MÁRQUEZ DELGADO, C. JANER DEL VALLE, and L. DURAN HIDALGO, Instituto de la Grasa y Sus Derivados, Seville, Spain

Expressed olive juice contains emulsified globules of olive oil. Electron photomicrographs of these globules show a rough membrane protecting them against coalescence. Some features of the membranes, such as the existence of "poles" and deposited microcrystals, are disclosed by the photographs and by electron diffraction patterns. Heavy metals appear to concentrate in the membranes and not in the aqueous medium.

WHEN AN OIL PHASE is dispersed into a water phase, or conversely, the droplets formed tend to coalesce because of surface forces, cohesion, and differences in density. The emulsion is stabilized when these forces are counteracted by other forces preventing the droplets from coming into direct contact with one another. The viscosity of the continuous phase plays a role here, but a more effective prevention is the formation of a barrier of electric charges surrounding the droplets or of a thin film of a third substance with affinity for both phases. Both may act simultaneously.

Natural emulsions of the "oil-in-water" type are often stabilized by lipoproteins, which form a very thin layer surrounding the droplets. This discovery was made by Ascherson who proposed the name "haptogen membranes" for these films in 1840. Milk is a typical example of this kind of emulsion and the one most extensively studied to date. Palmer and coworkers devoted 20 years to the study of the membranes protecting the fat droplets in milk, and the techniques developed by him for the isolation of these membranes have been used by many other investigators to study milk and other natural emulsions. A recent book on this subject (1) constitutes a very useful up-to-date study with many references.

Our interest in these studies was aroused because an oil-in-water emulsion also forms when olives are ground in the first step for the extraction of their oil. In this emulsion the olive oil droplets are protected by lipoproteic membranes formed at the expense of the substances dissolved in the juice of the olives. The extraction consists of two steps: separation of the emulsion from the solids present in the ground paste, and settling or centrifugation to free

[1] Presented at the 50th annual spring meeting, American Oil Chemists' Society, New Orleans, La., April 20–22, 1959.