comprised about 5% of the nitrated methyl oleate.

Some nitro derivatives obtained from nitrations using acetyl nitrate are unstable at elevated temperatures (2). Nitro-acetates derived from styrene, for example, were found to decompose into acetic acid and β-nitro styrene on attempted distillation (10). Although the derivatives described in this paper were also unstable at high temperature, no apparent decomposition occurred under normal handling conditions.

The principal impurity formed in reduction of the isomeric nitro derivatives was methyl stearate, thus indicating that hydrogenolysis was the predominant side-reaction. Methyl aminostearate was a colorless, odorless liquid, and unlike the nitro intermediates, appeared to be stable during distillation.

Unsaturated fatty acids have been nitrated previously with N_2O_4 and other nitric acid derivatives (11,12,13). Usually, these reagents are corrosive, hard to handle, and may lead to formation of large varieties of products that are difficult to resolve. In the present work, use of acetyl nitrate at room temperature has enabled us to nitrate methyl oleate without encountering these difficulties. This reagent is also being used for nitrating methyl linoleate, methyl linolenate, and fractions of methyl esters derived from menhaden oil (14). In these nitrations, nitro, acetoxy-nitro, and nitro-nitrato groups were introduced into the fatty acid chains using conditions similar to those described here.

In conclusion, acetyl nitrate is a convenient and effective reagent for nitrating a variety of unsaturated fatty acid derivatives. Compounds obtained from these nitrations are potentially useful intermediates for synthesizing derivatives having groups centrally located in the molecule such as amines, amino-acetates, and amino-alcohols.

ACKNOWLEDGMENTS

Infrared spectra provided by E. J. Gauglitz, Jr., technical assistance by John Wekell, and general assistance by E. H. Gruger, Jr.

REFERENCES

1. Harwood, H. J., JAOCS, *31*, 559–563 (1954).
2. Bordwell, F. G., and E. W. Garbisch, Jr., J. Am. Chem. Soc., *82*, 3588–3598 (1960).
3. Brown, J. F., Jr., *Ibid.*, *77*, 6341–6351 (1955).
4. Malins, D. C., and H. K. Mangold, JAOCS, *37*, 576–578 (1960).
5. Mangold, H. K., *Ibid.*, *38*, 708–727 (1961).
6. Malins, D. C., and C. R. Houle, Proc. Soc. Expl. Biol. and Med., *108*, 126–129 (1961).
7. Vandenheuvel, F. A., and D. R. Vatcher, Anal. Chem., *28*, 838–845 (1956).
8. "Organic Syntheses," Col. Vol. III, E. C. Horning, Editor, John Wiley and Sons, Inc., New York, 1955, pp. 181–183.
9. Siggia, S., and C. R. Stahl, Anal. Chem., *27*, 550–552 (1955).
10. Bordwell, F. G., and E. W. Garbisch, Jr., J. Org. Chem., *27*, 2322–2325 (1962).
11. Khan, N. A., J. Chem. Phys., *23*, 2447–2448 (1955).
12. Scaife, C. W., British Patent 587,992 (1947).
13. Vasil'ev, S. V., Trudy Moskov. Inst. Tonkoi Khim. Tekhnol. im. M. V. Lomonosova, 26–36 (1956); C. A. 53, 19413c.
14. Houle, C. R., and D. C. Malins, unpublished results (1962).

[Received September 4, 1962—Accepted December 6, 1962]

Hydrogenation of Linolenate. VII. Separation and Identification of Isomeric Dienes and Monoenes[1]

B. SREENIVASAN,[2] JANINA NOWAKOWSKA, E. P. JONES, E. SELKE, C. R. SCHOLFIELD, and H. J. DUTTON, Northern Regional Research Laboratory,[3] Peoria, Illinois

Abstract

Isomeric dienes and monoenes produced by partial hydrogenation of linolenic acid have been separated by the combined use of low-temp crystallization and countercurrent distribution. *Cis,trans* dienes have been separated from *cis,cis* dienes. *Cis,cis* conjugatable dienes have been partially separated from *cis,cis* nonconjugatable dienes. Dienes with one *trans* double bond were separated by gas chromatography into two groups: *cis,trans* and *trans,cis.* Individual positional isomers could not be separated.

Cis-9 monoene was separated from *cis*-12, *cis*-15, and *trans* monoenes by low-temp crystallization. Countercurrent distribution at 3,000 transfers only partially separated this mixture of *cis*-12, *cis*-15, and *trans* monoenes. The double bond in both *cis* and *trans* monoenes was found in all carbon positions, 7 through 16, showing for the first time that the 15,16 bond of linolenic acid had moved away from the carboxyl. The major *cis* bonds remained at carbons 9, 12, and 15.

Combination of countercurrent distribution fractions has produced samples containing 95% *cis,cis* dienes; 90% *cis,trans* or *trans,cis* dienes; 95% *cis* monoenes; and 90% *trans* monoenes.

Introduction

RESEARCH of the last decade on the hydrogenation of unsaturated fatty acids or their esters has stressed the formation of geometric and positional isomers (1–7). Highly unsaturated acids, such as linoleic and eleostearic, have been reduced to monoenes, and the mixture has been analyzed for both types of isomers. Allen (3) reported the separation of *cis* from *trans* monoenes but experimental details were not given. Lemon (8) hydrogenated linseed oil until no alkali isomerizable acids were present and isolated an "isolinoleic" acid having at least one double bond in *trans* configuration. Rabello and Daubert (9) hydrogenated linolenic acid to a similar degree and isolated (by low-temp crystallization) an "isolinoleic" acid concentrate composed of at least three positional isomeric dienes. This concentrate included only a part of the dienes formed since a majority was lost in the fractionation procedure.

Rebello and Daubert (9) have pointed out that 15 positionally isomeric dienes could be formed from linoleic acid on reduction of one of its double bonds if one assumes the migration of residual bonds by one carbon atom to either side of the original position. Geometric isomers containing either one or two *trans* bonds can also be formed so the total number of possible isomeric dienes in partly reduced linolenic acid becomes staggering.

The first paper in this series (10) showed that dienes formed by partial catalytic hydrogenation of

---

[1] Presented at the AOCS meeting at Chicago, Ill., 1961.

[2] Present address: The Tata Oil Mills Co., Ltd., Sewri, Bombay, India.

[3] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, U.S.D.A.

TABLE I

Analysis of Linolenate Concentrate and Hydrogenated Linolenate Concentrate

Substance	I.V.	Gas chromatography[c]			Alkali-isomerization			*Trans* as elaidate %
		Oleate monoene %	Linoleate diene %	Linolenate triene %	Oleate monoene %	Linoleate diene %	Linolenate triene %	
Linolenate concentrate[a]	251.2	3.4	21.4	75.2	3.1	20.4	76.5	......
Hydrogenated linolenate concentrate	169.6	21.8[b]	50.5	27.7	......	20.8	29.7	23.6

[a] Preconjugated diene 0.7%. Preconjugated triene nil.
[b] Includes 0.6% saturated.
[c] Relative peak area.

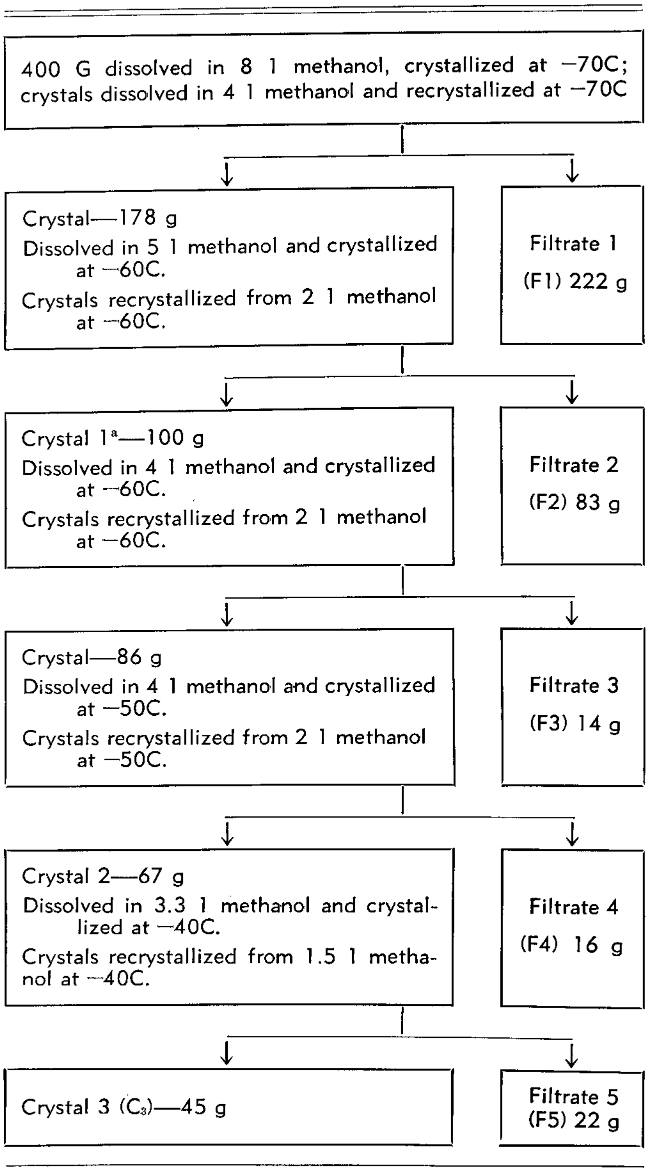

FIG. 1. Scheme of crystallization of hydrogenated linolenate.

[a] Solution of crystals in methanol at room temperature gave a turbid solution, presumably saturated triglycerides present in the catalyst; filtration gave a clear solution for subsequent treatment.

methyl linolenate could be separated into one major diene fraction by countercurrent distribution, and that this fraction could be partially resolved by subsequent fractional crystallization. This paper reports separation of (1) *cis,cis* from *cis,trans* (or *trans,cis*) and from *trans,trans* dienes; and (2) *cis* from *trans* monoenes by the use of fractional crystallization as the primary separation procedure, and countercurrent distribution (CCD) as the subsequent resolving tool.

Experimental

Hydrogenation. A linolenate concentrate was obtained as filtrate fraction by crystallizing methyl esters of linseed oil from a 5% solution in methanol at −70C. Approx 400 g of concentrate was hydrogenated in a Parr hydrogenation bomb at 140C at a gauge pressure of 0–5 psi using 0.5% nickel catalyst (Girdler G-15) until 1 mole of hydrogen was absorbed. A turbine-type stirrer assured thorough mixing. The course of hydrogenation was followed by observing the pressure drop in a hydrogen reservoir connected to the bomb. Table I gives analytical data for the linolenate before and after hydrogenation. Analytical methods used for fatty acid composition were by gas-liquid chromatography (GLC) and by alkali isomerization and for *trans* isomers were by infrared. Instruments used were substantially the same as previously (10) described. *Cis,trans* dienes were determined by the method proposed by Jackson (11)[4] et al.

Low-Temp Crystallization. The hydrogenated esters were separated into six major fractions by low-temp crystallization from methanol as detailed in Figure 1. Analytical data are presented in Table II.

Countercurrent Distribution. Filtrate 2 (20-g sample) was separated into monoenes, dienes, and trienes by countercurrent distribution (CCD) using 10 ml of pentane-hexane as upper phase and 40 ml of acetonitrile as lower phase (10). After the application of 250 transfers, samples were collected and the weight curve (Fig. 2a) was determined. Samples from the left and right side of the diene peak (arrows) were analyzed by GLC. The former gave two major partly

---

[4] Since initiation of this work a paper by W. E. Tolbert and D. H. Wheeler [JAOCS *35*, 385–388 (1958)], and one by J. R. Chipault, and J. M. Hawkins [JAOCS *36*, 535–539 (1959)] have disclosed improvements in the analysis of complex diene mixtures.

TABLE II

Analysis of Crystallization Fractions

Fraction	Yield %	I.V.	Gas chromatography[b]			Countercurrent distribution[c]			Alkali isomerization		
			Monoene %	Diene %	Triene %	Monoene %	Diene %	Triene %	Diene %	Triene %	*Trans* %
Fil. 1, −70C	55.4	202.5	2.2	51.2	46.6	3.1	50.4	46.5	20.6	42.6	22.4
Fil. 2, −60C	20.7	183.8	11.2	67.9	20.9	12.2	64.2	23.6	32.0	21.9	21.1
Fil. 3, −60C	3.4	150.2	36.0	54.7	9.3	......	......	......	27.0	7.0	27.5
Fil. 4, −50C	3.9	109.5	70.2	29.8	......	......	......	......	12.3	0.8	13.4
Fil. 5, −40C	5.4	86.9	100.0	......	......	......	......	......	2.0	......	17.3
Cryst. 3, −40C	11.3	79.6	94.3 5.7[a]	......	......	......	......	......	......	......	52.7

[a] Saturated.
[b] Relative peak area.
[c] Wt, %

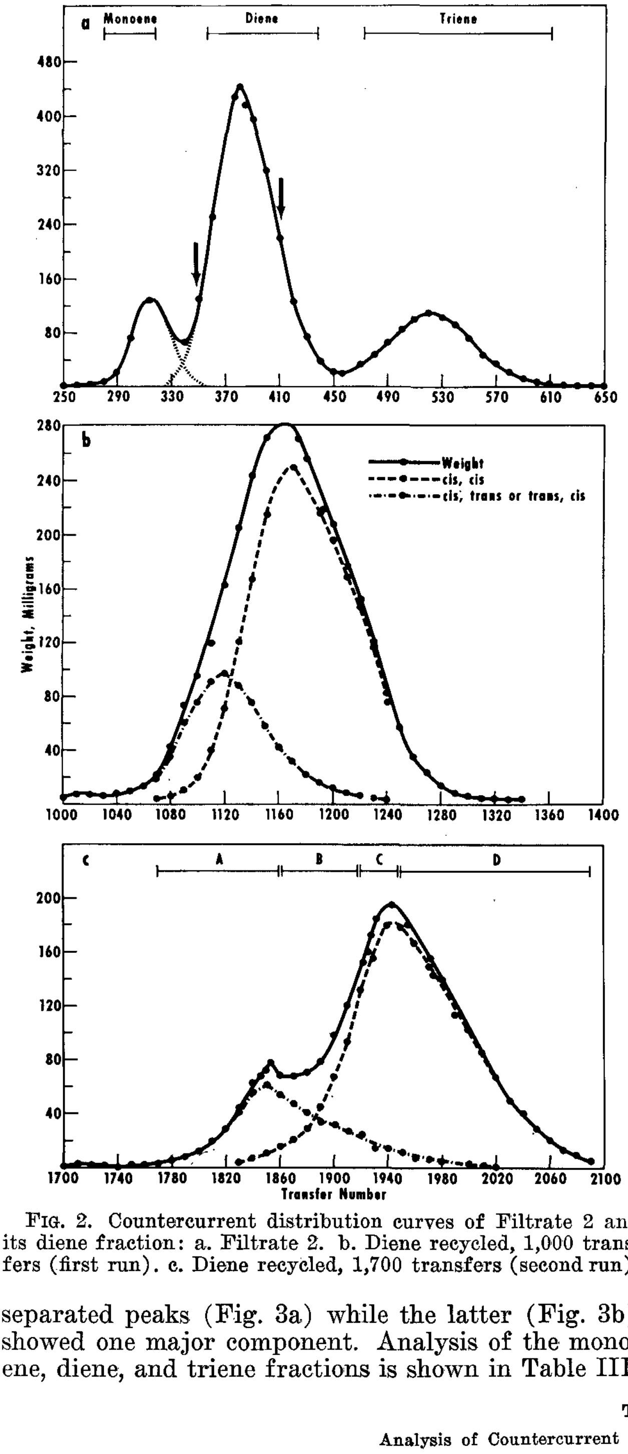

Fig. 2. Countercurrent distribution curves of Filtrate 2 and its diene fraction: a. Filtrate 2. b. Diene recycled, 1,000 transfers (first run). c. Diene recycled, 1,700 transfers (second run).

separated peaks (Fig. 3a) while the latter (Fig. 3b) showed one major component. Analysis of the monoene, diene, and triene fractions is shown in Table III.

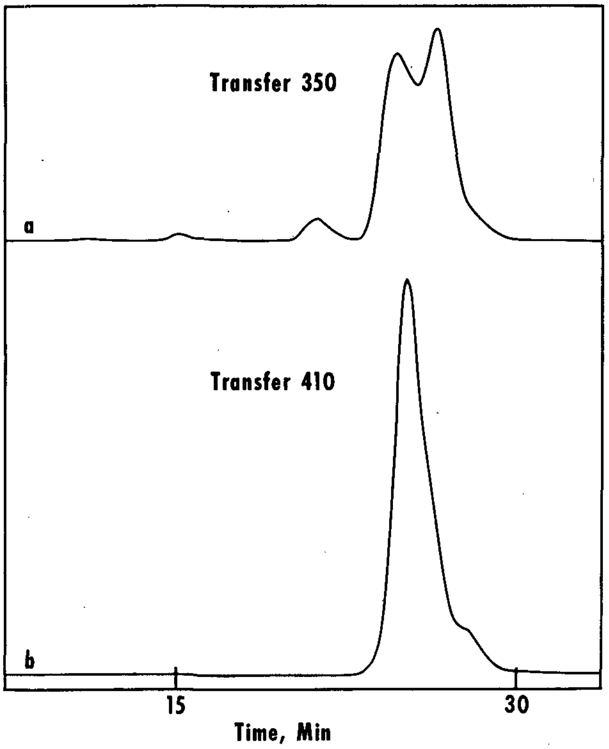

Fig. 3. Gas-liquid partition chromatograms of Filtrate 2 diene: a. Left side of CCD wt curve, transfer No. 350. b. Right side of CCD wt curve, transfer No. 410. Column: 4 ft × ¼ in. 10% polyvinyl acetate on 60–80 mesh chromosorb, T 169C, 50 ml/min Argon flow rate, 250-mm pressure, ionization detector.

In order to improve the separation of the dienes another sample of Filtrate 2 was separated. The monoene peak (transfers 1 to 349) was collected and combined with the monoene of Figure 2a, and the diene peak (transfers 350–441) was recycled until 1,000 transfers had been applied; collection of diene was then begun. Plot of the weight curve through 1,340 transfers appears in Figure 2b. Representative samples were analyzed by infrared for *trans* content, and from the data, curves for the isomeric dienes were calculated and plotted. From partition coefficients of K = 4.60 for *cis,trans* and K = 4.24 for *cis,cis* isomers it was calculated that about 1,700 transfers could be applied before mixing would occur in our 200-tube CCD instrument.

On recombination of the tubes of Figure 2b the dienes were subjected again to CCD. The partition coefficients (Fig. 2c) were 4.71 for the *cis,trans*

TABLE III

Analysis of Countercurrent Distribution Fractions for Filtrate 2

	Yield %	*Trans* %	U.V. analysis after alkali isomerization		Dicarboxylic acids by oxidative cleavage										
			Diene %	Triene %	C_5 %	C_6 %	C_7 %	C_8 %	C_9 %	C_{10} %	C_{11} %	C_{12} %	C_{13} %	C_{14} %	C_{15} %
Monoene,[b] Fig. 2a	12.2	9.6	0.8	......	...... 0.2	1.0 0.6	1.6 2.0	3.9 2.4	58.6 65.0	3.7 2.8	13.9 16.6	8.7 7.7	2.8 2.6	5.4	5.4 4.0
Diene,[b] Fig. 2a	64.2	22.1	47.5	......	......	......	......	......	......	......	......	......	......	......	......
A, Fig. 2c	7.6[a]	88.3	8.7	......	4.3 1.4	10.4 1.4	5.0 1.7	3.3 4.1	41.5 50.0	15.2 23.3	12.1 12.9	6.9 5.2	....	....	....
B, Fig. 2c	14.5[a]	39.7	33.3	......	2.6 1.2	9.5 5.8	4.0 3.8	3.5 6.6	56.0 61.6	5.6 6.0	15.2 6.0	2.1 8.9	....	....	....
C, Fig. 2c	13.0[a]	9.9	62.5	......	1.2	11.4 3.5	3.3 2.6	4.0 3.4	63.6 74.0	2.4 1.2	...... 1.9	13.0 12.8	1.1	....	....
D, Fig. 2c	29.1[a]	4.6	69.5	......	1.5	14.2 3.1	2.3 1.3	4.1 2.6	64.4 79.6	1.8 1.7	...... 4.0	11.1 7.7	0.7	....	....
Triene,[b] Fig. 2a	23.6	7.7	0.0	100.0	......	7.0 trace	2.9 1.0	5.2 4.0	81.9 93.0	1.4 2.0	....	....	....	1.7	1.7 ......

[a] Values based on the total filtrate and their sum equal 64.2%.
[b] Top row of figures are by LLC; bottom row of figures are by GLC.

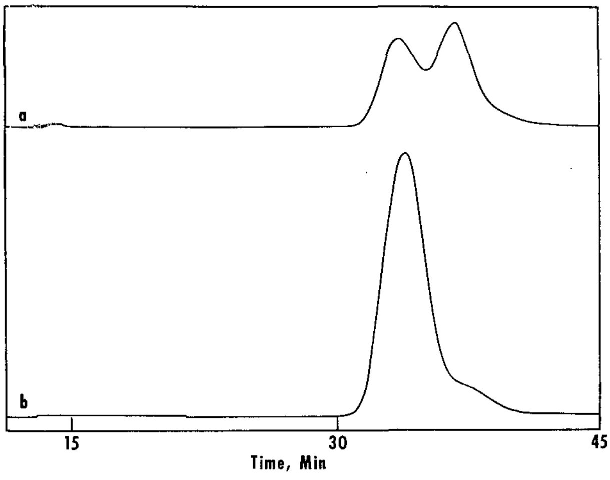

FIG. 4. Gas-liquid partition chromatograms of diene from Filtrate 2: a. Fraction A rich in *trans* isomers. b. Fraction D rich in *cis,cis* isomers. Column: 4 ft × ¼ in. 10% ethylene glycol succinate on 80–100 mesh chromosorb, T 150C, 40 ml/min Argon flow rate, 255-mm pressure, ionization detector.

and 4.24 for the *cis,cis* isomers. Fractions A, B, C, and D were obtained for analysis. Each was checked for conjugatable isomers, for *trans* content, and for positional isomers by alkali isomerization, by infrared spectra, and by oxidative cleavage, respectively. The cleaved acids were analyzed by liquid-liquid chromatography (LLC) and, after conversion to methyl esters with diazomethane, by GLC. Results are included in Table III. Typical GLC curves for Fraction A (rich in *cis,trans*) and of Fraction D (rich in *cis,cis*) are depicted in Figures 4a and 4b, respectively.

Filtrate 1 was fractionated by CCD in a manner comparable to that just described for Filtrate 2. Since results were similar, for brevity the data have been omitted.

Filtrate 5. The unsaturated components in Filtrate 5 were almost exclusively monoenes (by GLC) with a low *trans* content. Positional isomer results are listed in Table IV.

Crystal 3. This fraction was also predominantly monoene (94.3% by GLC) containing about 50% *trans* isomer. It, too, was separated by CCD and collection started at 200 transfers.

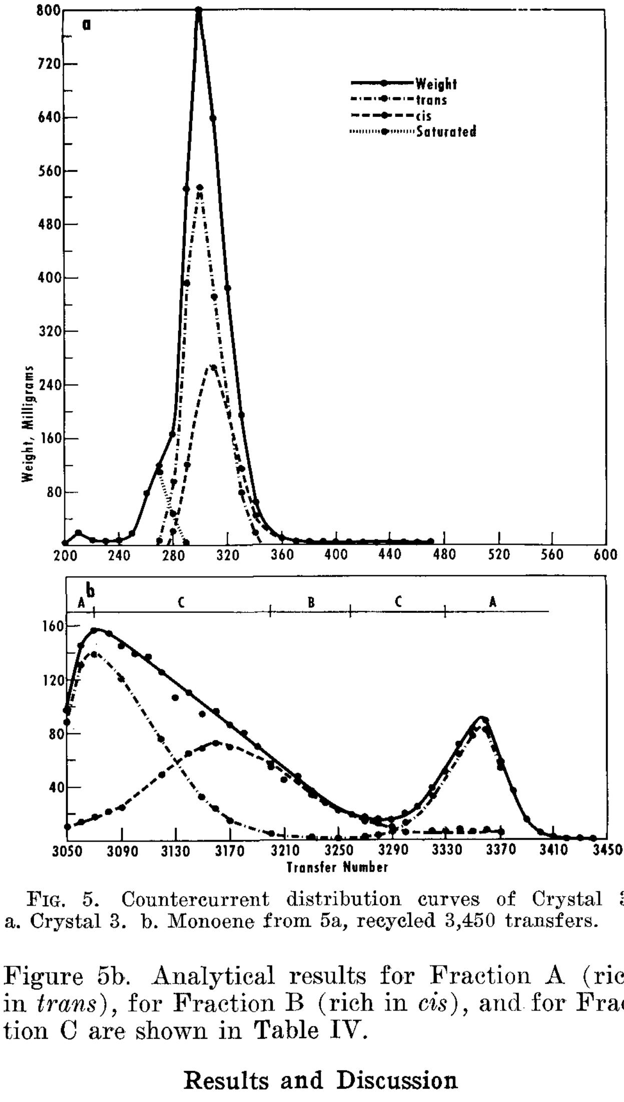

FIG. 5. Countercurrent distribution curves of Crystal 3: a. Crystal 3. b. Monoene from 5a, recycled 3,450 transfers.

Figure 5a showed that *cis* and *trans* isomers had peaks very close together with partition const of 8.0 and 7.6, respectively, which would require about 3,000 transfers for separation. Transfers 280–380 (Fig. 5a) were combined and then resubjected to 3,050 transfers. An increase in rate of movement of components in the CCD instrument was not as anticipated and part of the first peak was recycled into the instrument before withdrawal of tubes was begun at 3,050 transfers. The wt and isomer curves are shown in Figure 5b. Analytical results for Fraction A (rich in *trans*), for Fraction B (rich in *cis*), and for Fraction C are shown in Table IV.

Results and Discussion

Hydrogenation. The composition of the starting linolenate concentrate as determined by GLC was in agreement (Table I) with that determined by alkali isomerization. The two methods as anticipated failed to agree for the hydrogenated product; however, GLC analysis was more rapid and was considered to give a clearer picture of the type and approx proportion of the components. It is assumed that the area for a component is directly proportional to its molar concentration. When considered together the two methods reveal some details of importance. For example both methods were in agreement for the triene content. Contrariwise, the diene content by GLC was 2½-fold greater than the isomerization value, and suggested large amounts of nonconjugatable dienes.

TABLE IV

Analysis of Countercurrent Distribution Fractions for Filtrate 5 and Crystal 3

Fraction	Yield %	I.V.	*Trans* %	Dicarboxylic acid data after oxidative cleavage, mole %[a]								
				C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
Filtrate 5	5.4	......	17.3	2.7 1.2	44.2 36.5	8.5 7.2	4.5 3.9	24.9 32.0	...... 3.0	...... 4.0	13.3 13.0	1.9 ......
Cryst. 3	11.3	79.6	52.7	......	......	......	......	......	......	......	......	......
Monoene	92.5	......	59.7	......	......	......	......	......	......	......	......	......
A	27.5[a]	85.5	89.7	4.8 4.5	4.8 4.7	19.0 18.1	16.3 15.7	9.7 11.0	14.4 14.9	31.2 17.6	31.2 9.6	31.2 5.8
B	7.9[a]	83.7	5.8	...... 0.6	1.7 2.3	1.6 1.4	3.0 5.6	41.6 40.3	52.1 0.7	52.1 2.5	52.1 43.3	52.1 3.6
C	57.2[a]	84.6	55.2	3.0 1.7	4.2 3.9	14.4 10.2	12.5 11.4	16.9 17.9	7.0 7.7	42.0 7.8	42.0 30.7	42.0 7.9

[a] The sum of these monoene fractions equals 92.5%.

Because hydrogenation results in only a small change in mol wt, we shall consider wt per cent and mole per cent to be the same. Table I shows that hydrogenation reduced the linolenate by 48 mole %, increased the linoleate by 29 mole %, and increased the oleate by about 17 mole %. Published rates of hydrogenation of linolenate and linoleate (14,15,17, 18) indicate that linolenate reacts at about twice the rate of linoleate, but we are unable to calculate with certainty the source of the monoene increase because monoenes are known to be formed directly from trienes without the intermediate formation of dienes. For example, Bailey (14,15) concluded that linolenate reacts with 1 mole of hydrogen to produce a small amount of monoene without intermediate formation of diene. Hilditch (16) came to the same conclusion on partial hydrogenation of methyl eleostearate.

Low-Temp Crystallization of Hydrogenated Linolenate. Although crystallization procedures make it feasible to process either large or small quantities, it will be seen that separation of components in our partly reduced linoleate was very inadequate, making it necessary to resort to CCD for more complete separation. Observation of iodine values in Table II would suggest good separation, but when actual compositions were determined, anomalies became obvious. Trienes, although being most unsaturated, did not appear exclusively in Filtrate 1. This fraction was richer in dienes than expected and also contained some monoene. The alkali isomerizable diene of Filtrate 1 may be linoleate because its amount agreed with that in the original concentrate. The presence of nonconjugatable *cis,cis* diene seemed certain because the total diene exceeded the sum of the conjugatable and the *trans* isomers.

Filtrate 2 was obtained under conditions which permitted most of the linoleate to remain in solution. Conjugatable diene was greater than for Filtrate 1 suggesting that *cis,cis* conjugatable diene was linoleate. A considerable proportion of *cis,cis* nonconjugatable diene was also present because the total diene exceeded the sum of the conjugatable and *trans* isomers. The triene content in Filtrates 1 and 2 was about the same for the three methods: GLC, CCD, and alkali isomerization. This agreement may mean that the triene was all linolenate. This assumption was supported by the oxidative cleavage results which show that the principal dibasic acid was azelaic.

Filtrates 3 and 4 were minor intermediate fractions and were not studied. Filtrate 5 and Crystal 3 were chiefly monoenes. As expected, Crystal 3 showed a higher *trans* content than Filtrate 5, nevertheless one finds equal amounts of *cis* and *trans* isomer in Crystal 3.

Oxidative data for the monoenes of Crystal 3 (Table IV) reveal a wide distribution of unsaturated bonds from carbon atoms 8 through 12. While *cis* monoenes with double bonds at carbons 9 and 12 were expected, the presence of *cis* bonds at carbons 8 and 11 was due to migration of the 9 and 12 bonds.

Separation of Fractions by CCD. Data in Table III for Figure 2c show that geometric isomers were separated by CCD when a large number of transfers were applied. Calculations based on the separation of peaks in the weight curve (Fig. 2c) indicated the possibility of complete separation being achieved with a 500-tube CCD instrument. Our 200-tube apparatus limited the transfer number to 1,700 for these two components, beyond which head and tail fractions mix upon recycling. Alternately, an improved system of immiscible solvents would permit a smaller number of transfers. The CCD technique does permit the collection and analysis of separate transfers for subsequent combination of any desired fraction or peak. Diene fractions of Filtrate 2 were obtained in this manner. The *trans* isomer content of Fraction A (Fig. 2c) showed a fourfold increase as compared with the diene peak of Figure 2a. Fraction D (Table III) was composed almost entirely of *cis,cis* dienes. (The infrared spectrum had a shoulder at 10.34 μ and because the *trans* content had been calculated by the base line technique the 4.6% shown in Table IV may be too large.) As previously stated, the GLC curves for this fraction (Figs. 3a and 3b) were markedly different. Fraction A (Fig. 2c) showed at least two components while Fraction D showed a major peak and a tailing shoulder, signifying the presence of more than one component. The alkali isomerizable *cis,cis* dienes (Fraction D of Table III) failed to show separable components by GLC. Since *cis,trans* or *trans,cis* linoleate isomers were 80% isomerized using 6.6% KOH at 180C for 45 min, and since no evidence of the presence of *trans,trans* dienes was obtained, it is inferred that the low value for this type of diene in Fraction A might be due to the presence of small amounts of dienes containing the alkali isomerizable pentadiene system. Other dienes were, therefore, isomers with one *trans* bond whose positions were located by oxidative cleavage.

The recovery of dibasic acids by the periodate permanganate method used was 80–85% of theory for "pure" linoleic acid. Two dibasic acids are formed from each dienoic acid on oxidative cleavage. The "primary" one due to cleavage of the bond nearest the carboxyl and the "secondary" one due to cleavage at the other double bond. The third acid will be monobasic. Thus malonic acid will be considered to be "secondary." Dibasic acids of low-mol wt are quite soluble in water so quantitative recovery of these and of short chain monobasic acids by ether extraction from saturated aqueous sodium solution was not attained. LLC gave satisfactory results for dibasic acids actually applied to the column within the range of C_5–C_{13} but was inadequate for longer chain acids. GLC, however, was adequate for estimation of these and longer chain acids after formation of the diesters with diazomethane. (We subsequently ascertained that a solution of boron trifluoride in methanol is preferable for the preparation of diesters.)

LLC data in Table III for Filtrate 2 (Fractions A–D) show total dibasic acids of about 80–85 mole % recovery, which was considered normal for our cleavage method. Assuming that C_8 and higher dibasic acids are primary and that C_7 dibasics may be either primary or secondary, the data of Table III have been adjusted to 100% and incorporated into Table V.

Fraction D of Filtrate 2 was practically all *cis,cis* diene. Hydrogenation of linolenate should produce 9,12, 9,15, and 12,15 dienes which on oxidative cleav-

TABLE V

Primary Dicarboxylic Acid Data for Filtrate 2 by LLC Adjusted to 100%

Sample	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}
Filtrate 2						
Diene A	4.2	52.5	19.2	15.3	8.7	......
Diene B	4.2	68.0	6.8	18.4	2.6	......
Diene C	4.7	75.6	2.9	......	15.5	1.3
Diene D	5.0	78.4	2.2	......	13.5	0.9

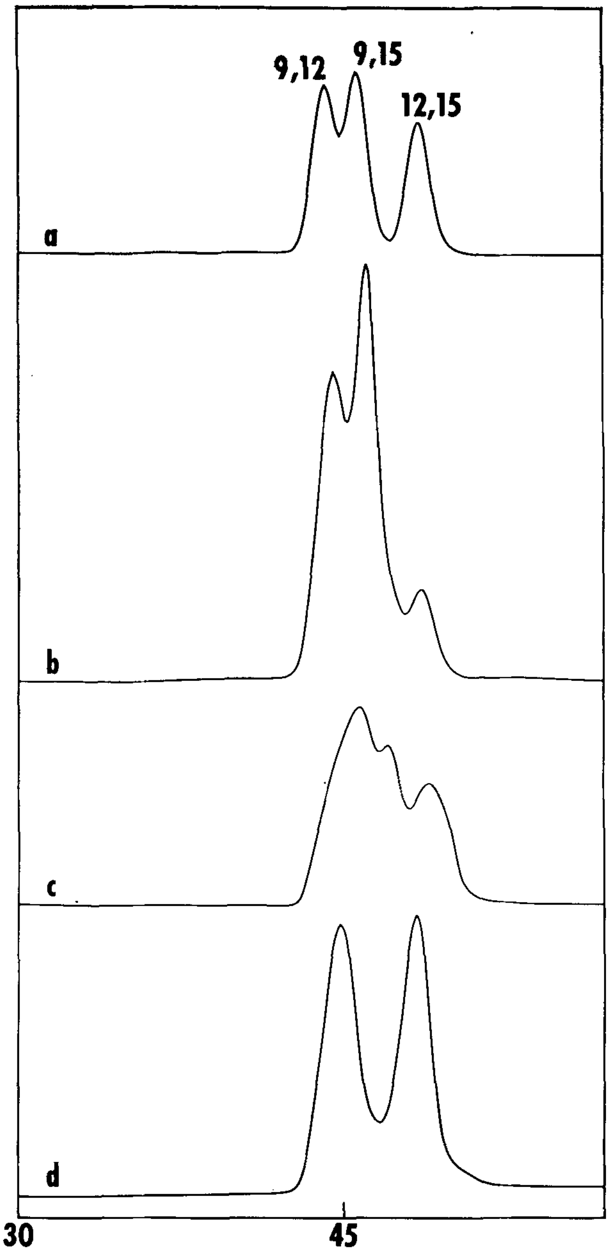

FIG. 6. Gas-liquid partition chromatograms of dienes: a. Dienes from hydrazine reduction of methyl linolenate—standard. b. Filtrate 2, diene C. c. Filtrate 2, diene B. d. Filtrate 2, diene A. Column: 200-ft capillary, 0.010-in. i.d. coated with Apiezon L. 200C, pressure 60 psi argon, ionization detector Sr^{90}.

TABLE VI

Diene Isomers of Filtrate 2

Sample	Per cent isomer			
	9,12	12,15	9,15	Other
Filtrate 2				
C	47.0	15.5	28.6	8.9
D	56.0	13.5	22.4	8.1

age should give C_9 and C_{12} primary and C_6 and C_3 secondary dibasic acids. Table V shows that the first three are the principal dibasic acids produced. Interpretation of the quantitative distribution of these dienes in this fraction follows: 13.5% of 12,15 is indicated by the C_{12} diacid; 56% of 9,12 is arrived at by subtracting the 12,15 diene content from the total isomerization value of 69.5%; 22.4% of 9,15 results from subtraction of the 9,12 content of 56% from the total diacid value of 78.4%. Their sum is about 92%, leaving 8% for distribution among the small amounts of *cis,trans* and other nonconjugatable *cis,cis* dienes. The total nonconjugatable diene of about 30.5%, when reduced by the 22.4% of 9,15 diene as estimated, gives 8% which is in excellent agreement with the 8% also estimated above. The other dibasic acids in Fraction D present in minor amounts are C_8 and C_{10}. Since bond movement was suspected, we suggest that dienes with one *trans* bond in these positions were present. However, the *cis,trans* content was lower than the total of the C_8 and C_{10} diacids, indicating the presence of *cis,cis* dienes with double bonds starting at these positions.

Fraction C was similar to Fraction D. Although the *cis,trans* content was greater in Fraction C than in D, no significant change in dibasic acid content was noted. It contained 15.5% of 12,15, 38.8% of 9,12, and 36.8% of 9,15 positional isomers if one assumes that all C_{12} dibasic acid came from *cis,cis* 12,15 diene, with about 9% attributed to *trans* dienes with one bond in each position.

Trans isomer content was much larger in Fractions A and B than in C and D. Analysis of Fractions A and B show a decrease in C_9 and C_{12} diacids and an increase in C_{10} and C_{11} diacids. The 9 and 12 bonds may have migrated to form conjugated 10,12 and 9,11 dienes which by 1,4 addition of hydrogen yielded dienes with double bonds at positions 10 and 11. The other bond in these dienes will be at the 15,16 position.

GLC results when coupled with isomerization data permit the comparison of the geometric isomers of Filtrate 2. Data in Table VI show that Fraction D was richer in *cis,cis* 9,12 diene and poorer in *cis,cis* 9,15 diene than Fraction C. Thus CCD caused some separation of *cis,cis* isomers.

Filtrate 5. Oxidative cleavage data show that the major monoenes of Filtrate 5 are C_9, C_{12}, and C_{15}, with their total about equal to the *cis* content. We assume that other isomers (Table IV) are *trans*. The greater amount of C_9 monoene in Filtrate 5 as compared with Crystal 3 shows that low-temp crystallization effectively separated oleate from other positional *cis* and *trans* monoenes.

Crystal 3. All three fractions of Crystal 3 show significant amounts of C_{16} monoene, denoting migrating of the 15,16 bond away from the carboxyl. Fraction B was almost entirely composed of C_{12} and C_{15} *cis* monoenes. They have a higher mp than oleic acid and thus resemble *trans* monoenes in solubility characteristics, a partial explanation of why they were found in Crystal 3 fraction.

Capillary Gas Chromatographic Identification of Isomers. Concurrently with this investigation, the partial chemical reduction of linolenic acid by hydrazine was shown to give *cis,cis* 9,12, 9,15, and 12,15 octadecadienoates (12). Capillary gas liquid chromatograms of these components simplifies the interpretation of the results previously discussed in this paper so this confirmatory evidence will be submitted. Dienes from hydrazine reduction were resolved into three well-defined peaks (Fig. 6a) identified as 9,12, 9,15, and 12,15 *cis,cis* dienes. This GLC cuve serves as a standard for comparison of emergence position with dienes produced by partial catalytic hydrogenation.

Capillary gas chromatograms were also useful in identifying the monoene ester fractions. Partial chemical reduction of linolenic acid with hydrazine also gave *cis* monoenes with double bonds exclusively at positions 9, 12, and 15. Capillary GLC for this mixture (Fig. 7a) showed elution in order of the 9, 12,

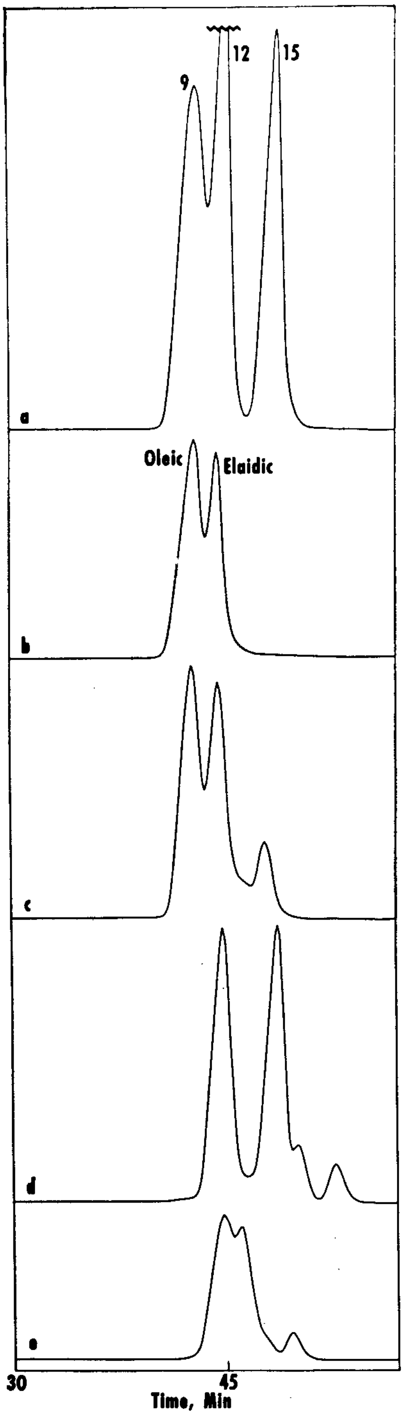

FIG. 7. Gas-liquid partition chromatograms of monoenes: a. Monoenes from hydrazine reduction of methyl linolenate—standard. b. Methyl oleate and methyl elaidate—standard. c. Filtrate 5. d. Crystal 3-B. e. Crystal 3-A. Column: 200-ft capillary, 0.010-in. i.d. coated with Apiezon L. 200C, pressure 60 psi argon, ionization detector Sr^{90}.

and 15 carbon positions and its chromatogram was used as another standard. Figure 7b shows a capillary GLC curve for a standard oleic-elaidic ester mixture, with elution peaks in this order.

Filtrate 2 Dienes. Fractions C and D gave a curve (Fig. 6b) similar to that of the standard and was composed presumably of the same *cis,cis* dienes. Although Fraction C contained 10% *trans* isomers, it gave no corresponding elution peak.

Fraction B (Fig. 6c) partially separated into three peaks with the first and second at the 9,12 and the 9,15 regions. The third appeared before the 12,15 region and may be *trans* isomers.

Fraction A (Fig. 6d) showed two well-defined peaks with a slight shoulder thereafter. Here again the first peak covered both the 9,12 and 9,15 region while the second was to the left of the 12,15 region. Since this fraction contained 88% *trans* isomers, these peaks could represent either *cis,trans* (or *trans,cis*) or positional isomers. The first peak suggests one of the double bonds was at the C_9 position. Moreover the relative area of the first peak (51%) and the C_9 dicarboxylic acid content of 52.5% in Table V were in agreement. The C_9 position should be a *cis* bond so we suggest that the first peak was *cis,trans* and that the second was *trans,cis* dienes. Cleavage definitely showed at least five positional isomers which the capillary column failed to separate. Positional isomers with one *trans* double bond are most difficult to resolve even with a 200-ft capillary column.

Filtrate 5 (Fig. 7c) showed three peaks plus a shoulder after the second peak. The first peak agreed with the 9 monoene region (Fig. 7a) and its relative area of 46% also agreed with the C_9 dicarboxylic content of 44% in Table IV. The second peak corresponded to the region of both the *cis* 12 monoene and methyl elaidate. This peak area was about 43%, whereas the C_{12} dibasic content was about 25%. However, if the values for the *trans* content (17.3%) and the C_{12} diacid content (24.9%) are totaled, good agreement is obtained. We deduce that the second peak included both the *cis* 12 and the *trans* monoenes. The last peak was *cis* 15 monoene with its area of about 11% in fair agreement with the 13% C_{15} dibasic content in Table IV.

Crystal 3. Fraction B of Figure 7d showed four peaks: the two major ones were *cis* 12 and *cis* 15 monoenes and gave areas of about 45% and 43% as compared with dibasic contents of 41% and 43%, respectively. The third peak or shoulder was probably a mixture of *trans* and *cis* monoenes while peak four could be a *trans* monoene mixture.

Fraction A (Fig. 7e) was partly resolved into three peaks with a shoulder between the second and third peak. The last peak area of 6% agreed with the C_{16} dibasic content (Table IV) of about 6%. The first peak eluted in the region of *cis* 12 monoene and elaidate and may represent all *trans* monoenes from C_8 to C_{12}, as well as *cis* 12 monoene.

REFERENCES

1. Feuge, R. O., M. B. Pepper, Jr., R. T. O'Connor, and E. T. Field, JAOCS, *28*, 420 (1951); Feuge, R. O., E. R. Cousins, S. P. Fore, E. F. DuPré, and R. T. O'Connor, *Ibid.*, *30*, 454 (1953).
2. Boelhouwer, C., J. Gerckens, Ong Tian Lie, and H. I. Waterman, *Ibid.*, *30*, 59 (1953).
3. Allen, R. R., and A. A. Kiess. *Ibid.*, *32*, 400 (1955); *Ibid.*, *33*, 355, 419 (1956).
4. Allen, R. R., *Ibid.*, *33*, 301 (1956).
5. Chahine, M. H., E. R. Cousins, and R. O. Feuge, *Ibid.*, *35*, 396 (1958).
6. Scholfield, C. R., E. P. Jones, J. A Stolp, and J. C. Cowan, *Ibid.*, *35*, 405 (1958).
7. Cousins, E. R., W. A. Guice, and R. O. Feuge, *Ibid.*, *36*, 24 (1959).
8. Lemon, H. W., Can. J. Res., *27B*, 607, 610 (1949).
9. Rebello, I., and B. F. Daubert, JAOCS, *28*, 177 (1951).
10. Scholfield, C. R., E. P. Jones, Janina Nowakowska, E. Selke, B. Sreenivasan, and H. J. Dutton, *Ibid.*, *37*, 579 (1960).
11. Jackson, J. E., R. F. Paschke, Wesley Tolberg, H. M. Boyd, and D. H. Wheeler, *Ibid.*, *29*, 229–234 (1952).
12. Scholfield, C. R., E. P. Jones, Janina Nowakowska, E. Selke, and H. J. Dutton, *Ibid.*, *38*, 208–211 (1961).
13. Ahlers, N. H. E., R. A. Brett, and N. G. McTaggart, J. Appl. Chem., *3*, 433 (1953).
14. Bailey, A. E., and G. S. Fisher, Oil & Soap, *23*, 14 (1946).
15. Bailey, A. E., JAOCS, *26*, 644 (1949).
16. Hilditch, T. P., and S. P. Pathak, Royal Soc. London, Proc. *A198*, 323 (1949).
17. Johnston, A. E., D. Macmillan, H. J. Dutton, and J. C. Cowan, Hydrogenation of Linolenate. VI. Survey of Commercial Catalysts. In press.
18. Scholfield, C. R., Janina Nowakowska, and H. J. Dutton, JAOCS, *39*, 1 (1962).

[Received August 23, 1962—Accepted October 30, 1962]