tion and dehydrogenation reactions taking place. Further study should reveal other related hydrocarbons, some more complex, some simpler, if this is the case.

Further work is proceeding on the resolution of fraction 2, and of other fractions. After considerable subfractionation, yields become small and it is apparent that characterization of components will have to depend mainly on spectroscopic identification.

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Preparation and Purity of Linoleic Acid from Commercial Corn, Cottonseed, and Safflower Oils[1]

B. SREENIVASAN and J. B. BROWN, Department of Physiological Chemistry, Ohio State University, Columbus, and E. P. JONES, V. L. DAVISON, and JANINA NOWAKOWSKA, Northern Regional Research Laboratory,[2] Peoria, Illinois

Abstract

Linoleic acid from commercial corn, cottonseed, and safflower oils was prepared by low temperature crystallization using acetone and petroleum ether as solvents; temperatures ranged between −70 and 50C. This method has the advantages of simple equipment and of flexibility in preparatory capacity. The crystalline fraction obtained at −55C was shown to be "pure" linoleic acid.

Isomerization with potassium tertiary butoxide, oxidative cleavage by periodate-permanganate, and analysis by liquid-liquid and gas-liquid partition chromatography were used to ascertain the purity and the presence of isomers in the final product. This fraction was found to contain 90 to 95%, 9,12 dienoic acid; approximately 5% of dienes with the first double bond at the C_8 position and the second bond either at the C_{12} or C_{13} positions; and small amounts of nonconjugatable 9,15 *cis,cis* dienes. Linoleic acid from these oils was similar in composition, except that from corn oil showed the presence of diene with the first double bond at the C_{11} position.

LINOLEIC ACID is the most widely distributed and abundantly occurring dienoic acid of nature. Among its best known sources are corn, cottonseed, and safflower oils. It has been shown to have the structure, *cis,cis*-9,12-octadecadienoic acid (1). Isolation of linoleic acid in its native form by low temperature crystallization from corn, cottonseed, grape seed, poppy seed, and sesame oils was first described by Frankel, Stoneburner, and Brown (2). Their preparations were about 93–97% pure, using the tetrabromide number as the criterion of purity. Swift, Rose, and Jamieson (3) prepared methyl linoleate by adsorption chromatography on alumina columns and used iodine value to estimate purity.

After introduction of the alkali isomerization technique (4) it was used to determine the purity of methyl linoleate obtained by adsorption chromatography on silicic acid columns (5). Swern and Parker (6) employed urea complex precipitation procedures for preparation of large quantities of linoleic acid concentrate of about 95% purity as shown by alkali isomerization. Beal and Brekke (7) used liquid-liquid partition technique for separation of linoleic acid and gas-liquid chromatography (GLPC) for analysis. Ozonolysis, followed by liquid-liquid partition chromatography (LLPC) of dibasic acids, was used by Allen and Kiess (8), and by Cousins *et al.* (9) to judge the purity of their linoleic acid preparations from safflower oil. While Allen and Kiess reported small amounts of an isomer with a double bond at the 8 position, Cousins and his coworkers attributed the presence of such small amounts of isomers, with double bonds in positions other than 9,12-, to the shift of these bonds caused by alkali during the saponification of the oil for preparing the fatty acids.

Scholfield, Nowakowska, and Dutton (10) describe a countercurrent distribution procedure to prepare methyl linoleate from soybean and safflower oil methyl esters, and by using GLPC they indicate the possible presence in their preparations of palmitoleate, which has a similar partition coefficient to linoleate.

Of the procedures mentioned, only adsorption chromatography on silicic acid columns (5) and countercurrent distribution (10) are reported to yield methyl linoleate of greater than 99.0% purity. However, the investigators who used the two methods assumed that their preparations were the 9,12-octadecadienoate and did not investigate the possible presence of other isomers. Besides, adsorption chromatography yields only small amounts of products, and countercurrent distribution needs special apparatus.

In the present investigation low temperature crystallization technique was chosen not only because of its easy adaptability when solid carbon dioxide is available, but also because its flexible preparatory capacity is limited only by the size of cooling baths and crystallization cylinders. Since GLPC is the most versatile of the available analytical techniques in revealing the presence and identity of individual compounds in mixtures, all samples were analyzed by this method. Isomerization with potassium tertiary butoxide (11) was used to estimate the amounts of linoleic and trienoate acids in some fractions, mainly to confirm the quantitative aspects of GLPC. The

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[2] A laboratory of the Northern Utilization Research and Development Division, U.S.D.A.

TABLE I

Analysis of Corn, Cottonseed, and Safflower Oil Acids and Their Crystallization Fractions

Sample	Yield[a] %	Trienate %	Linoleate %	Oleate %	Stearate %	Palmitoleate %	Palmitate %	Other lower esters %
Corn Oil Acids	.....	0.7(0.7)[b]	57.0(56.8)[b]	28.6	1.7	0.3	11.7	.....
Crystal —70C	37.1	.....	5.3	59.0	4.1	.....	31.0	0.6
Filtrate 1 —70C	8.1	6.7(6.4)	80.7(67.4)	10.2	.....	2.4	.....	.....
Filtrate 2 —70C	54.8	.....	90.7(90.0)	8.4	.....	0.9	.....	.....
Crystal 1 —60C	39.9	.....	95.6(96.2)	4.4	.....	.....	.....	.....
Filtrate 1 —60C	14.9	3.1	74.2	20.6	.....	2.1	.....	.....
Crystal 2 —60C	31.2	.....	98.7	1.3	.....	.....	.....	.....
Filtrate 2 —60C	8.7	2.6	83.8	13.6	.....	.....	.....	.....
Crystal —55C	17.6	.....	99.5(100.0)	0.5	.....	.....	.....	.....
Filtrate —55C	13.6	.....	96.9	3.1	.....	.....	.....	.....
Cottonseed Oil Acids	.....	trace (0.2)	57.6(55.6)	14.8	1.6	.....	25.0	1.0
Crystal —70C	36.0	.....	11.5	22.4	3.9	.....	61.6	0.6
Filtrate 1 —70C	10.1	trace(0.7)	85.4(65.7)	10.3	.....	3.0	0.6	0.7
Filtrate 2 —70C	53.9	trace	88.3(89.0)	11.7	.....	trace	.....	trace
Crystal —50C	38.2	.....	91.7(92.0)	8.3	.....	trace	.....	trace
Filtrate —50C	15.7	.....	76.7	19.8	.....	1.5	.....	2.0
Crystal —60C	30.0	.....	95.8(95.4)	4.2	.....	.....	.....	.....
Filtrate —60C	8.2	.....	80.3	16.8	.....	1.7	.....	1.2
Crystal —55C	16.0	.....	100.0(100.0)	.....	.....	.....	.....	.....
Filtrate —55C	14.0	.....	93.3	6.7	.....	.....	.....	.....
Safflower Oil Acids	.....	—(0.7)	75.4(75.2)	14.2	2.3	.....	8.1	.....
Crystal —70C	20.8	.....	9.8	34.4	12.4	.....	43.3	.....
Filtrate 1 —70C	10.2	4.2(4.0)	83.5(68.0)	11.1	.....	1.1	.....	.....
Filtrate 2 —70C	69.0	.....	92.4(93.6)	7.6	.....	.....	.....	.....
Crystal —50C	54.9	.....	95.3(94.7)	4.8	.....	.....	.....	.....
Filtrate —50C	14.1	.....	81.7	18.3	.....	.....	.....	.....
Crystal —60C	42.7	.....	97.8(98.4)	2.2	.....	.....	.....	.....
Filtrate —60C	12.2	.....	85.8	14.2	.....	.....	.....	.....
Crystal —55C	25.6	.....	100.0(100.0)	.....	.....	.....	.....	.....
Filtrate —55C	17.1	.....	94.6	5.4	.....	.....	.....	.....

[a] All yield percentages calculated on the mixed fatty acids of the oils.

[b] All figures in parentheses obtained by the tertiary butoxide isomerization procedure (11). Others for component acids are by gas-liquid chromatography and represent area percentages under respective peaks.

purity and the presence of other isomers in the final product have been ascertained by GLPC on a column packed with polyvinyl acetate and on a capillary column coated with Apiezon L: oxidative cleavage by periodate-permanganate (12,13) followed by LLPC of dibasic acids formed; and GLPC of diethyl esters of these dibasic acids and also of monobasic acids (14).

Experimental

Materials. Corn and cottonseed oils are commercially refined samples. Safflower oil is an alkali-refined sample, NRRL No. 699, processed at the Northern Laboratory. We realize these oils may have been altered by storage or by the processing operations involved in their recovery, but they are considered to be normal commercial oils.

Preparation of Fatty Acids. Five hundred g of oil was saponified by the standard procedure with KOH in an atmosphere of nitrogen. Fatty acids were lib-

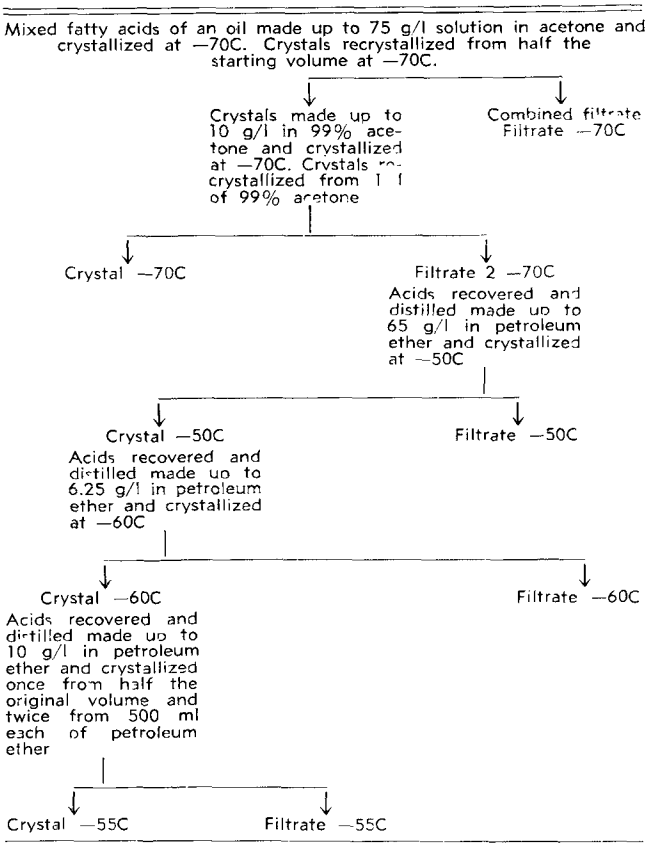

FIG. 1. Flow sheet for crystallization of fatty acids from corn, cottonseed, and safflower oils.

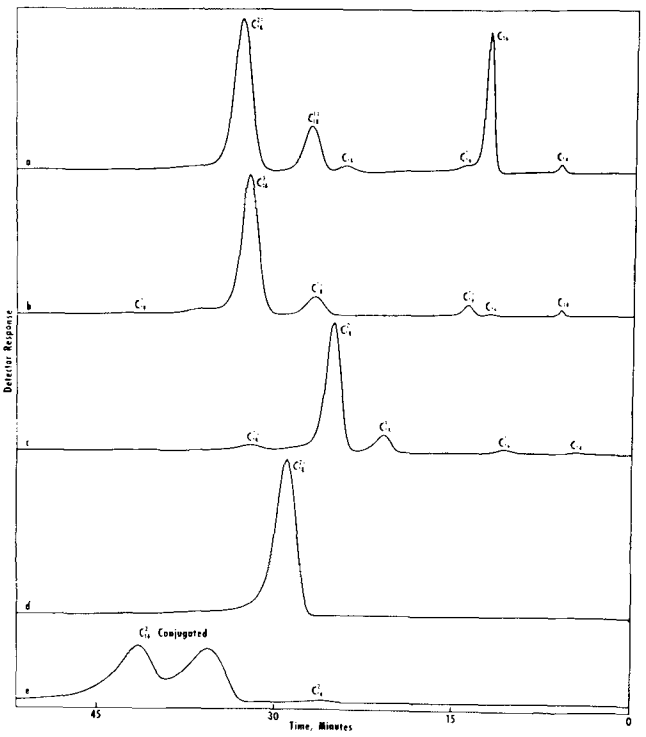

FIG. 2. Gas-liquid partition chromatograms. Methyl esters of:

(a) Cottonseed acids
(b) Cottonseed acids, Filtrate 1 —70C fraction
(c) Corn oil acids, Filtrate 1 —70C fraction
(d) Cottonseed acids, Crystal —55C fraction
(e) Cottonseed acids, Crystal —55C fraction isomerized

Column: 4 ft x ¼ inch, 10% polyvinyl acetate on 60–80 mesh Chromosorb; T 164–168C, 30 ml/min argon flow rate; ionization detector.

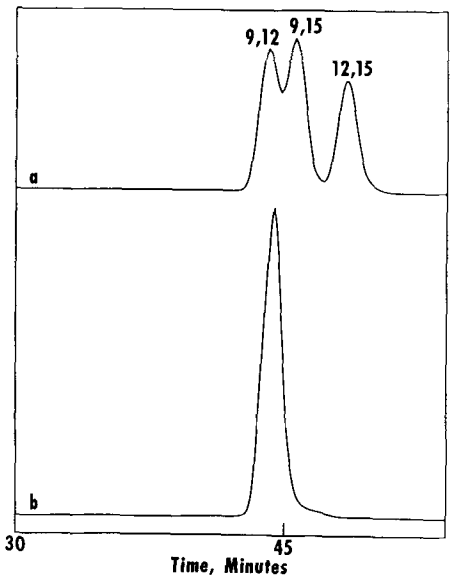

FIG. 3. Gas-liquid partition chromatograms.

(a) Dienes from hydrazine reduction of methyl linolenate—reference
(b) Methyl esters of cottonseed acids, Crystal −55C fraction

Column: 200-ft stainless-steel capillary 0.010 inch ID coated with Apiezon L; T 200C, pressure 60 psi argon; *ionization detector.*

erated with HCl, washed, dried under vacuum, and distilled at approximately 0.2 to 0.5 mm pressure. The distilled acids were used in crystallization.

Crystallization Solvents. Distilled acetone (100%) was used in the first crystallization. In the subsequent crystallization steps the solvents were: 99% acetone prepared by adding 8 ml of water to 1 l of distilled acetone; petroleum ether of the low-boiling type, 30–60C. Pentane (99 mole %), hexane (95 mole %), heptane (99 mole %), and pentane-hexane, bp 35–60C gave identical results.

Gas-Liquid Partition Chromatography. Acids obtained from fractional crystallization were converted by diazomethane in ether solutions to methyl esters, and these were applied on the columns. A Pye-Argon gas chromatograph with 4-ft column packed with

TABLE II

Analysis of Linoleic Acid from Different Oils

	Crystal −55C			References
	Corn oil	Cotton-seed oil	Safflower oil	
Physical constants				
Melting point °C	−5.2	−5.1	−5.4	−5.2 to 5.0 (22)
$N_D^{20°}$	1.4691	1.4689	1.4690	1.4699 (22)
$k_{10.36}$ μ	0.146	0.148	0.144	
Iodine value Wijs 1 hr	180.0	180.0	180.4	181.1 (Theory)
Tertiary Butoxide Isomerization 140C[a]				
U.V.				
2 hr k_{232} mμ	98.1	98.5	98.1	98 (11)
4 hr k_{232} mμ	97.9	98.3	98.2	
GLPC	%	%	%	
Conjugated				
2 hr	98.2	98.7	98.8	
4 hr	97.1	98.0	98.0	
Nonconjugated				
2 hr	1.5	1.3	1.2	
4hr	2.0	1.5	1.3	
Ethylene Glycol—KOH Isomerization 180C 25 min				
U.V.				
k_{232} mμ	91.2	91.1	90.5	91.5 (21)
GLPC	%	%	%	
Conjugated	96.1	96.8	97.5	
Nonconjugated	3.9	3.2	2.5	
Oxidative Cleavage	(LLPC)	(GLPC)	(LLPC)	(GLPC)	(LLPC)	(GLPC)
Dibasic acids	%	%	%	%	%	%
C_5 }						
C_6 }	4.1	.....	4.2		4.6	.....
C_7 }				1.5		
C_8	6.9	5.6	7.2	6.0	6.5	4.5
C_9	81.0	88.2	83.2	92.5	82.6	95.5
C_{10}	2.5	Trace	2.5	.....	.....	.....
C_{11}	.....	6.2	.....	.....	.....	.....
C_{12}	5.1	.....	2.5	.....	4.0	.....
C_{14}	0.4	.....	0.9	.....	.....	.....
Monobasic acids						
C_3	.....	Trace	.....	Trace	.....	Trace
C_4	.....	Trace	.....	Trace	.....	Trace
C_5	.....	3.9	.....	3.2	.....	3.1
C_6	.....	96.1	.....	96.8	.....	96.9

[a] Preconjugated dienes negligible.

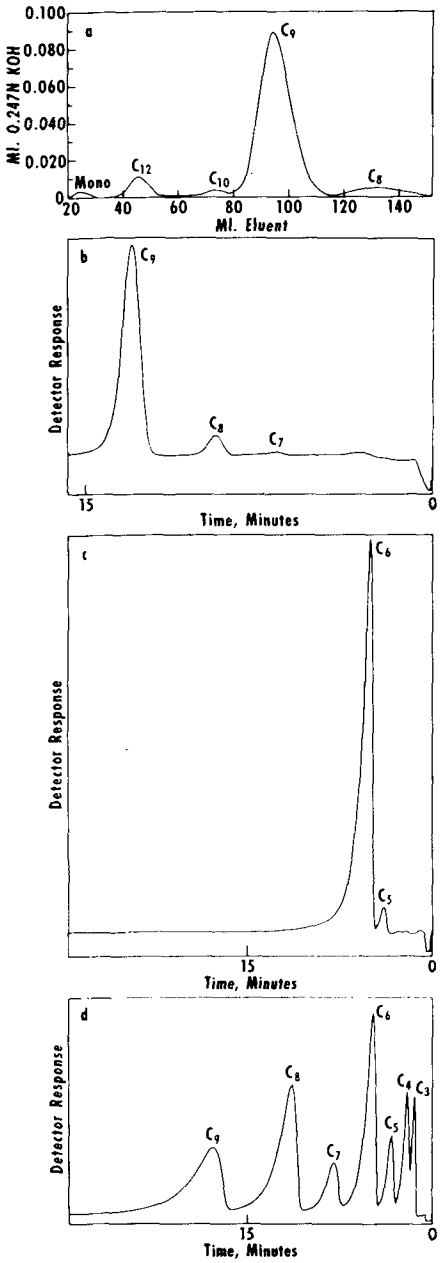

FIG. 4. Liquid-liquid and gas-liquid partition chromatograms.

(a) Liquid-liquid chromatogram of dibasic acids from cottonseed acids, Crystal −55C fraction
Column: Silicic acid using equilibrated mixture of water, methanol, ethanol, and benzene
(b) Gas-liquid chromatogram of diethyl esters from cottonseed acids, Crystal −55C fraction
Column: 4 ft x ¼ inch 8% polyvinyl acetate on 60–80 mesh Chromosorb; T 170C, 30 ml/min argon flow rate; ionization detector
(c) Gas-liquid chromatogram of monobasic acids from cottonseed acids, Crystal −55C fraction
(d) Monobasic acids—standard reference
Column: 4 ft x ¼ inch 10% butanediol succinic polyester on 60–80 mesh Chromosorb; T 150C, 30 ml/min argon flow rate; ionization detector.

10% w/w of polyvinyl acetate on Chromosorb W (60–80 mesh) and a Barber-Coleman gas chromatograph with a 200-ft capillary column coated with Apiezon-L, were used under conditions designated in Figures 2–4.

Oxidative Cleavage. Crystal −55C fractions, the final product, were oxidized according to the method of Lemieux and von Rudloff (12) modified by Jones and Stolp (13). Fatty acid (0.156 g) was added to 5 ml of 0.2 *N* NaOH, and the soap was diluted with 1 l of water before adding 250 ml of 1.0% solution of sodium metaperiodate. This solution was adjusted to pH 8.0 with a 20% solution of sodium carbonate and cooled to 0C with crushed ice. One ml of 0.5 M potassium permanganate in 50 ml water was slowly added with stirring, and the total volume was adjusted to 1.8 l. When the solution approached room temperature, the pH was raised to 9.2 with sodium carbonate, and oxidation was allowed to proceed for 24 hr. Thereafter the solution was acidified with HCl and SO_2 gas was introduced to reduce the remaining permanganate and periodate. The acids were converted into salts with strong NaOH, the volume was reduced on the steam bath in a current of air to about 50 ml, the salts were acidified with strong phosphoric acid, and the volatile monobasic acids were steam distilled into a dilute NaOH solution.

The nonvolatile fraction was successively extracted 4 times with 100 ml of diethyl ether and the extracts combined. After removal of ether on a rotary evaporator, the solids were dissolved for analysis by LLPC on a silicic acid column. This method fails to recover dibasic acids of less than 6 carbon atoms; the adjusted mole % recovery of the acids found is listed in Table II.

The diesters were prepared with absolute ethanol and p-toluene sulfonic acid and were analyzed by GLPC (Table II).

Monobasic acids formed in oxidative cleavage were recovered as follows: after evaporating the alkaline solution to dryness, the residue was acidified with phosphoric acid, and the monobasic acids were extracted with petroleum ether. The extracts were evaporated in conical bottom centrifuge tubes on a rotary evaporator under water pump vacuum at room temperature. Frost which formed on the outside of the centrifuge tubes minimized the loss of the lower members, such as propionic acid. When the solvent was reduced to a negligible amount, the sample was analyzed by GLPC.

Crystallization and Identification of Crystal Fractions. The scheme of crystallization shown in Figure 1 was followed with slight modifications for each of the three samples. Crystallization at −70C from pure acetone eliminated the trienoate impurity from all the oils as shown in Table I, and illustrated by a typical gas chromatographic curve, Figure 2b. Conversely the concentration of less soluble impurities such as palmitic and oleic acid was increased in the crystal fraction by this crystallization. The hexadecenoate peak in cottonseed esters of Filtrate 1 ($C_{16}^{1=}$), Figure 2b, was better defined than in the original oil (Fig. 2a). The identity of this peak was confirmed by running a chromatogram of this fraction mixed with an authentic specimen of hexadecenoate. Since cottonseed oil has only traces of trienoate, if any, no peak appears in the region corresponding to linolenate either in the original cottonseed oil or in its Filtrate 1 −70C fraction. However in Filtrate 1 −70C fractions of corn oil and of safflower oil, a peak (Fig. 2c) appears in the trienoate region; thus, "trienoic" acid occurred to a small extent in these samples. The gas chromatographic and alkali isomerization values for this acid are in good agreement (Table I), but they do not prove it to be linolenic acid.

The second step in the crystallization procedure incorporated a significant improvement over the older crystallization technique (2) and gave a filtrate containing 88 to 92% linoleic acid. A solvent composed of 1% water and 99% acetone was used. The choice of this solvent resulted from an observation that acetone recovered from previous crystallizations retained more linoleic acid in solution and gave filtrate fractions richer in linoleic acid than did pure acetone; see Table I. Filtrate 2 −70C has a higher content of linoleic acid, although Filtrate 1 −70C was obtained by crystallization from pure distilled acetone at higher concentration of fatty acids than for Filtrate 2 −70C. The slight disparity in the results for linoleate content obtained by GLPC and by alkali isomerization will be explained later. Because the acetone recovered from the first filtrate contained about 1% water, it was evident that this moisture condensed from the air on the surface of the cold acetone and thus diluted the solvent. This small amount of water, probably by association with the fatty acids, caused greater differences in the solubilities of linoleic and oleic acids. A solvent containing more water, 2 to 3%, did not further improve the separation. In the Filtrate 2 −70C fractions from the three oils, 85 to 87% of the original linoleic acid were found.

Since the Filtrate 2 −70C fraction is composed mostly of linoleic and oleic acids, and with trace amounts of possibly palmitoleic (corn oil), this fraction would be an excellent starting material for laboratory-scale preparations of pure methyl linoleate by adsorption chromatography (5), or by countercurrent distribution (10), or pure linoleic acid by liquid-liquid fractionation (7).

In the subsequent crystallizations where a hydrocarbon solvent is used, the crystal fractions (Crystal −50C, Crystal −60C, and Crystal −55C) are richer in linoleic acid than in oleic acid, whereas the contrary would be expected from the solubilities of linoleic and oleic acids (15). This apparent anomaly can be partially explained on the basis of association of these acids: carboxylic acids are known to exist as associated dimers in hydrocarbon solvents at low temperatures (16); therefore with fractions (Filtrate 2 −70C, Crystal −50C, Crystal −60C) rich in linoleic acid, the molecular entities in solution in hydrocarbon solvents are linoleic-linoleic, linoleic-oleic, and oleic-oleic dimers. The linoleic-oleic dimer probably has greater solubility than the oleic-oleic dimer and thus stays in solutions under the present conditions of crystallization. Since the concentration of linoleic-linoleic dimer should exceed that of the linoleic-oleic dimer, the former could crystallize from solution and give a product richer in linoleic acid. This hypothesis fails to account for the oleic-oleic dimer which should be the least soluble dimer, and thus exert a harmful effect on separation.

Discussion

Crystal −55C represents the final product in all preparations and would, by conventional practice, be called pure linoleic acid. Both physical and chemical constants given in Table II agree well with those previously reported in the literature. Infrared spectrum for these samples did not show any band at about 10.36 μ, indicating the absence of any isolated

trans bonds; the $k_{10.36\mu}$ constant in Table II is used for corrections of background adsorption due to *cis* bonds when the content of *trans* isomers in mixtures (17) is calculated. GLPC of the methyl esters of these samples show that they are pure C_{18} dienes (Fig. 2d). Because these chromatograms were run at different times (sometimes as much as 3 months apart), elution times are not the same for the same component, e.g., diene ($C_{18}^{2=}$), due to changes in column characteristics. However, this discrepancy does not seriously interfere with qualitative identification.

Isomerization of several samples (Table I) shows all Crystal −55C fractions to be pure linoleic acid. However, when the esters of the isomerized acids were analyzed by GLPC, a small peak occurred in the region where methyl linoleate is usually eluted (Fig. 2e) followed by two well-separated peaks of conjugated esters. The conjugatable and nonconjugatable contents reported in Table II were calculated from the areas under these peaks. The small peak might be due either to esters that were not completely conjugated, or to esters that were nonconjugatable. To clarify this point, isomerizations with tertiary butoxide were run for a longer time, i.e., for 4 hr. The results recorded in Table II give within experimental limits the same values for conjugatable and nonconjugatable acids as found before. It can be concluded therefore that the small peak represents nonconjugatable *cis,cis* dienes. This conclusion explains the disparities referred to earlier between GLPC and tertiary butoxide isomerization for linoleic acid contents of Filtrate 1 −70C fractions. The disparities are obviously due to the presence of such nonconjugatable dienoic acids. The content of nonconjugatable esters is higher when isomerizations are carried by the ethylene-glycol potassium hydroxide procedure (18) (Table II), indicating that with this reagent the isomerization of conjugatable dienoic acids is not complete in 25 min.

To learn more about the isomers that are present, two lines of investigation were chosen, namely, GLPC on capillary columns and oxidative cleavage, followed by analysis of the resulting mono- and dibasic acids. Recent study (19) on the isomeric dienes produced by hydrazine reduction of linolenic acid show that the esters of the *cis,cis* dienes are eluted from a capillary column coated with Apiezon-L in the order: 9,12; 9,15; and 12,15 (Fig. 3a). A comparison of this curve with that for linoleic acid from cottonseed oil (Crystal −55C, Fig. 3c) shows that the major peak is 9,12-diene followed by a shoulder in the 9,15 diene region. Thus there is additional evidence for the presence of nonconjugatable *cis,cis* dienes in these preparations. Crystal −55C fractions of corn and safflower oil acids had similar chromatograms.

LLPC of the dibasic acids formed by oxidative cleavage gave three peaks in the regions of C_8, C_9, and C_{12} dibasic acids with the C_9 being the largest (Fig. 4a). Acidic artifacts have been reported to cause peaks in LLPC analysis of dibasic acids obtained by ozonolysis (20). For this reason the identity of the dibasic acids from Crystal −55C fractions were confirmed by converting them into diethyl esters and by analyzing with GLPC. With the exception of linoleic acid from corn oil in which a C_{11} peak could be identified, only two well-defined peaks, C_8 and C_9, were observed in the other oils (Fig. 4b). Consequently, the C_{12} peak obtained with LLPC is probably due to an artifact.

Since dibasic acids give the position of the double bond nearest the carboxyl group of the parent acid, monobasic acids were analyzed to locate the other bond. Short chain monobasic acids (up to C_9) are volatile; therefore they were applied as acids on the column. Only four monobasic acids C_3–C_6 (Fig. 4c) were detectable, with C_5 and C_6 having measurable peaks. (Fig. 4d is a standard monobasic acid curve.) Since the C_9 dibasic and C_6 monobasic acids were always the major components, consequently linoleic acid, i.e., 9,12-dienoic acid, was the chief component as expected. It is present to the extent of 90–95% in all Crystal −55C fractions, which also contain significant amounts of dienoic acids with the first double bond at the C_8 position (about 5%) and the second bond either at the C_{12} or C_{13} positions as indicated by the presence of C_6 and C_5 monobasic acids. Conceivably, a diene with double bonds at 9,13 positions was also present. The absence of a C_7 monobasic acid precludes the possibility of a 8,11-conjugatable dienoic acid.

Nonconjugatable dienes are present to the extent of 1–2%. Of the possible isomers, the 8,13-diene is the most difficult to isomerize since the double bonds are separated by three methylene groups. The 8,12- and 9,13-dienes are separated by only two methylene groups, and if one of these is attacked by alkali, the resulting rearranged diene offers the readily isomerizable pentadiene system. Because tertiary butoxide is a very strong base, it can conceivably cause the isomerization of double bonds separated by two methylene groups. The content of nonconjugated dienes (Table II) was always higher by the glycol-KOH method than by the tertiary butoxide method, which offers some basis for the belief that isomerization, by tertiary butoxide, of double bonds separated by two methylene groups occurs. With linoleic acid from corn oil, there is evidence that a diene with the first double bond at C_{11} position is present, but the position of the other double bond is not clear.

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