the oleate eluted from the column, some linoleate appeared in the eluant. The difficulty was even more pronounced in the linoleate-linolenate separation.

Woelm neutral alumina of activity II was used in these experiments because further deactivation resulted in less separation of the oleate-linoleate mixture.

The lack of complete recovery, particularly of the more unsaturated esters, is believed to be a result of irreversible adsorption rather than loss during adduct decomposition and subsequent handling. Tests with the linoleate derivative including all the experimental procedure except passage through the column showed 99% recovery of the ester.

Acknowledgment

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REFERENCES

1. Hilditch, T. P., "The Chemical Constitution of Natural Fats," 2nd ed., John Wiley and Sons, Inc., New York, 1956, p. 574.
2. Dutton, H. J., and C. L. Reinbold, JAOCS *25*, 120–124 (1948).
3. Kurtz, F. E., J. Am. Chem. Soc. *74*, 1902–1909 (1952).
4. Riemenschneider, R. W., S. F. Herb, and P. L. Nichols, Jr., JAOCS *26*, 371–374 (1949).
5. Bergstrom, S., and K. Paabo, Acta Chem. Scand. *8*, 1486–1487 (1954).
6. Savary, P., and P. Desnuelle, Bull. Soc. Chim. France *20*, 939–945 (1953).
7. Kuemmel, D. F., JAOCS *35*, 41–45 (1958).
8. Crombie, W. M. L., R. Comber, and S. G. Boatman, Biochem. J. *59*, 309–315 (1955).
9. Simmons, R. O., and F. W. Quackenbush, JAOCS *30*, 614–616 (1953).
10. Kishimoto, Y., and N. S. Radin, Lipid Res. *1*, 72–78 (1959).
11. Jantzen, E., and H. Andreas, Angew. Chem. *70*, 656 (1958).
12. Jantzen, E., and H. Andreas, Chem. Ber. *94*, 628–633 (1961).
13. Quackenbush, F. W., and M. D. Pawlowski, J. Nutr. *72*, 196–202 (1960).
14. White, H. B., Jr., and F. W. Quackenbush, JAOCS; accepted for publication.
15. Brockmann, H., and H. Schodder, Chem. Ber. *74*, 73–78 (1941).
16. Snyder, F., and N. Stephens, Biochem. Biophys. Acta *34*, 244–245 (1959).
17. Stearns, E. M., Jr., H. B. White, Jr., and F. W. Quackenbush, JAOCS *39*, 61–62 (1962).

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Chromatographic Analysis of Seed Oils. Fatty Acid Composition of Castor Oil[1]

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Abstract

The fatty acid composition of a number of domestic and foreign castor oils was determined by consecutive column and gas-liquid chromatographic analysis. After saponification of the oils and removal of the unsaponifiables, the nonhydroxy, monohydroxy, and dihydroxy acids were fractionated by partition chromatography on silicic acid. The amount of acid in each fraction was determined by titration or weighing. Gravimetric data were in good agreement with the titrimetric data. The acids obtained by saponification were converted to methyl esters with diazomethane and similarly subjected to partition chromatography. The methyl esters from various fractions were analyzed by gas-liquid chromatography. Components were tentatively identified by their comparative retention times and confirmed by their behavior following hydrogenation and their ultraviolet spectra following alkali isomerization. Details concerning characteristics of the oils examined, of the procedures used, and of the results obtained are presented.

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

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Introduction

THE DETERMINATION of the fatty acid composition of castor oil presents a number of difficulties that are not experienced with most other vegetable oils. The presence of a very large proportion (about 90%) of a hydroxylated acid, ricinoleic acid, makes the accurate determination of the minor component acids rather difficult. The accurate determination of ricinoleic acid itself presents problems because it is a secondary alcohol mixed with other secondary alcohols; it is unsaturated mixed with other unsaturates; and as a hydroxy acid it is subject to interesterification. Methods used previously require lengthy and tedious procedures, and some components must be obtained by difference.

TABLE I
Fatty Acid Composition of Castor Oils[a]

Investigators	Palmitic %	Stearic %	Oleic %	Linoleic %	Linolenic %	C_{20} %	Ricinoleic %	Dihydroxy-stearic %
Eibner and Münzing (2)	......	3[b]	9	3	......	......	80	3[b]
Myddleton et al. (3)	......	8	......	......	7	......	84	1
Heiduschka and Kirsten (4)	......	3.6	7.2	1.4	......	......	86.4	1.4
Panjutin and Rapoport (5)	......	0.3	7.2	3.6	......	......	87.8	1.1
Steger et al. (6)	......	1.1[b]	......	6.6	......	......	92.3[c]	1.1[b]
Kaufmann and Bornhardt (7)	......	2.4[d]	7.4	3.1	......	......	87.0	0.6
Riley (8)	......	0.9[d]	nil	5.4	......	......	92.6	0.6
Gupta et al. (9)	0.5[e]	0.8[c]	0.1–6.8	3.9–5.0	......	<0.1[e]	85.9–94.9	0.5–1.0
Achaya and Saletore (10)	......	......	......	4.5	......	......	......	~1
Bolley (11)	......	......	......	......	......	......	90–93	......
Bergier (12)	......	......	......	......	......	......	82.2	......
Narayan and Kulkarni (13)	......	0.9[d]	1.1	6.3	......	......	91.7	......
Sreenivasan et al. (1)	......	3.0–3.5[d]	5.1–5.8	3.4–3.5	......	......	85.5–86.0	1.6–2.4
Vézinet and Naudet (14)	......	......	......	......	......	......	85.5–87.9	......
Present study	0.8–1.1	0.7–1.0	2.0–3.3	4.1–4.7	0.5–0.7	0.3–0.8[f]	87.7–90.4	0.6–1.1

[a] Wt % of total fatty acids except Eibner and Münzing gave % based on oil.

[b] Stearic plus dihydroxystearic reported as saturated acids.

[c] Includes oleic acid.

[d] Reported as saturated acids.

[e] Saturated acids range 0.2–2.5%. Suggested composition was 3% myristic, 37% palmitic, 57% stearic, and 5% arachidic (or dihydroxystearic).

[f] Mostly eicosenoic acid.

A summary of previous castor oil analyses can be found in the paper by Sreenivasan et al. (1). Fatty acid compositions of castor oil as reported by various investigators are given in Table I.

The best approach to the determination of the fatty acid composition of castor oil would appear to be chromatographic separation of the hydroxy acids from the nonhydroxy acids and analysis of each group. Previously, this was done in part. The ricinoleic acid content of castor oil was determined by Bergier (12) who chromatographed methyl esters on a silicic acid column wetted with aqueous methanol, and by Vézinet and Naudet (14) who used reverse phase chromatography on a swelled rubber column. In our work, silicic acid chromatography according to the procedure reported by Frankel et al. (15) was used to separate the nonhydroxy, monohydroxy, and dihydroxy acids into three portions which were titrated or weighed. Gas-liquid chromatography was then used to determine the relative proportions of the nonhydroxy acids.

Experimental

Castor Oils. The castor oils used are listed below with regard to their source and some of their analytical characteristics.

1. Angola No. 1 oil, unbleached. Imported May 1961. Acid value, 2.69; sap. value, 179.6; I.V., 86.1; OH value, 163.2; unsap., 0.42%.[3]
2. Angola No. 1 oil. Similar to sample No. 1 except plant bleached. Acid value, 2.92; sap. value, 178.7; I.V., 86.2; OH value, 161.5; unsap., 0.27%.[3]
3. Brazil No. 1 oil, unbleached. Imported Nov. 1960. Acid value, 0.73; sap. value, 178.7; I.V., 85.8; OH value, 164.4; unsap. 0.50%.[3]
4. Brazil No. 1 oil. Similar to sample No. 3 except from a different source. Imported May 1961. Acid value, 1.74; sap. value, 178.5; I.V., 85.8; OH value, 164.3; unsap., 0.34%.[3]
5. Brazilian oil. Acid value, 2.11; sap. value, 178.1; H.I.V. 84.1; OH value, 163.6; unsap., 0.41%.
6. No. 1 oil, clay bleached, from Brazilian seed. Pressed in U.S. in 1959 from seed harvested in the spring of 1959. Acid value, 2.08; sap. value, 179.8; I.V., 87.9; OH value, 161.9; unsap., 0.43%.[3]

[3] Samples and values supplied by Baker Castor Oil Co.

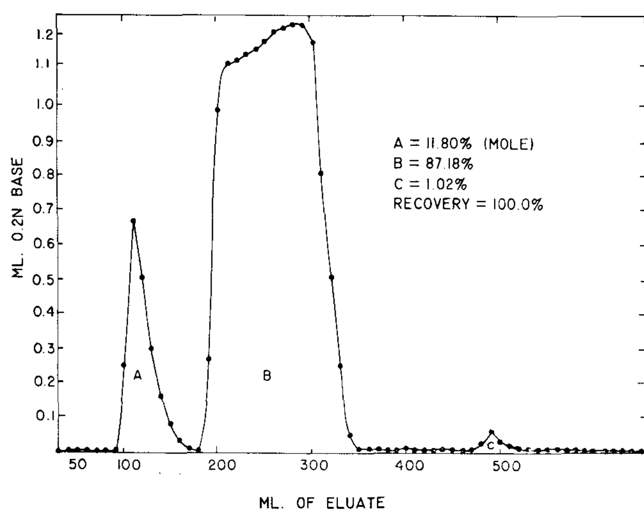

FIG. 1. Titrimetric analysis of the nonhydroxy (A), monohydroxy (B), and dihydroxy (C) acids obtained by fractionation of the acids from an Indian castor oil (No. 7).

7. Indian commercial oil, bleached. Acid value, 3.81; sap. value, 178.6; H.I.V., 84.4; OH value, 159.3; unsap., 0.39%.
8. Indian commercial oil, bleached. Imported Jan. 1960. Acid value, 2.24; sap. value, 178.0; I.V., 86.2; OH value, 161.4; unsap., 0.43%.[3]
9. Domestic No. 1 oil, bleached. Domestic seed, principally from the High Plains of Texas, harvested in 1960. Acid value, 1.07; sap. value, 180.2; I.V. 86.0; OH value, 164.4; unsap., 0.37%.[3]
10. Domestic oil, bleached. From seed grown in Texas in 1959. Acid value, 0.68; sap. value, 178.3; H.I.V., 84.7; OH value, 164.6; unsap., 0.45%.
11. Domestic oil. Seed grown near Plainview, Texas in 1960. Acid value, 0.55; sap. value, 179.7; H.I.V., 85.8; OH value, 165.9; unsap., 0.41%.

Preparation of Acids and Methyl Esters. For a typical saponification, a mixture of 35 g of the castor oil, 35 ml of 95% ethanol, 35 ml of water and 10.2 g of potassium hydroxide pellets was heated two hr on a steam bath. The alkaline solution was cooled and then transferred to a separatory funnel with 135 ml of cold water; the unsaponifiables were extracted into three portions of ether: 165 ml, 35 ml, and 35 ml. The soap solution was acidified with 90 ml of cold 3N HCl, the layers were separated, and the aqueous layer was extracted twice with 50-ml portions of ether. Traces of HCl were eliminated by washing the combined ether extracts with two 50-ml portions of water. The ether solution was dried with sodium sulfate, about 0.02% of butylated hydroxytoluene (BHT) was added as inhibitor, and the ether was removed under reduced pressure below 35C. About 33 g of acids per 35 g of oil was obtained.

The methyl esters were prepared by dissolving two g of castor acids in 25 ml of 12% methanol in ether and adding a solution of diazomethane in ether (16) until a yellow color persisted. The mixture was allowed to stand 2½ days at −34C, then 0.05% BHT was added as an inhibitor, and the solvent was removed under reduced pressure.

Silicic Acid Chromatography. The silicic acid column was prepared essentially according to Frankel and co-workers (15). A stock solution of castor acids was made by diluting a 2.5-g sample of acids to 25 ml with 2% methanol in benzene. A 10-ml aliquot of this solution was pipetted onto the column. It was allowed to sink in and was washed in with two 5-ml portions of 2% methanol in benzene. Then 330 ml of 2% methanol in benzene was added and the elution was started at a rate of 3–3.5 ml of eluate per min. This required a pressure (applied with purified nitrogen) of about 5 psi. Ten-ml. fractions were collected. When the last of the solvent reached the top of the silicic acid, 300 ml of 6% methanol in benzene was added in order to elute the dihydroxystearic acid, and the elution was continued as before.

For the chromatography of the methyl esters, the silicic acid column was prepared differently. Fifty g of dried silicic acid was blended with 40 ml of 20% methanol in benzene as before, but the slurry was made with 100 ml of 1% methanol in benzene. A sample of 0.9 g of methyl esters in 2 ml of 1% methanol in benzene was pipetted onto the column and washed in with a 3- and a 5-ml portion of solvent. Then nonhydroxy and monohydroxy esters were eluted with 350 ml of 1% methanol in benzene. Chromatostrips (17) were used to monitor the elutions. For this, a chromatostrip was spotted with 2

μl from each 10-ml fraction, sprayed with 2′,7′-dichlorofluorescein solution, and examined under ultraviolet light.

Analysis of Acid Fractions. The acid in the 10-ml fractions was titrated under nitrogen with 0.2 N potassium hydroxide in 95% ethanol. A Gilmont microburette, magnetic stirring, and thymol blue indicator were used. To ensure rapid development of the color at the endpoint, 1 ml of 95% ethanol was added to each fraction. The results from one run, using acids from an Indian castor oil, are shown in Figure 1. The percent recovery is calculated on the basis of titration of a separate aliquot from the 25 ml of stock solution. Recoveries are generally very close to 100%. Replicate analyses using another Indian castor oil gave these results: nonhydroxy acid, 11.56, 11.36, and 11.60%; monohydroxy acid, 87.80, 87.98, and 87.76%; and dihydroxy acid, 0.64, 0.66, and 0.64%.

An alternative to titrating the fractions is to weigh them (18). For this, each fraction was emptied into a tared aluminum dish, the solvent evaporated in a hood, and the dish reweighed.[4] A gravimetric analysis of the acids from an Indian castor oil gave the following results: 11.4% nonhydroxy, 87.8% monohydroxy, and 0.8% dihydroxy acid.

The 9,10-dihydroxystearic acid from castor oil (19, 20) was identified in the fractions eluted with 6% methanol in benzene. The residue from the evaporated fractions was taken up in chloroform and the solution chilled and filtered. The dried precipitate melted at 140–142C: Lit. mp 141C (19).

Gas-Liquid Chromatography (GLC). The apparatus used was an Aerograph, model A-90-C, equipped with a 1-mv Leeds and Northrup recorder and a disc integrator. Nonhydroxy esters were analyzed with a 6-ft, 0.25-inch, stainless steel column packed with 20% diethylene glycol succinate (DEGS) on 60–80 mesh firebrick. The column temp was 212–218C with a helium flow rate of 44 ml/min. Two-fold attenuation and a bridge current of 250 ma were used while 1-μl samples were injected. A 6-ft column of 20% Apiezon L on 60–80 mesh acid-washed Chromosorb W was also used, with temp at 260C and a flow rate of 68 ml/min for nonhydroxy esters and temp at 252C and a flow rate of 70 ml/min for monohydroxy esters.

Peaks were tentatively identified by comparison of their retention times with those of known compounds or by use of a plot of log retention time vs. carbon chain length (21,22). Figure 2 is a chromatogram of the nonhydroxy esters on DEGS with their equivalent chain length (ECL) indicated. On this chromatogram, an ECL of 16 corresponds to a retention time of 9.0 min and one of 21 to 33.5 min. The major peaks are for methyl palmitate, stearate, oleate, linoleate, linolenate, eicosenoate, and a material subsequently found to be the methyl ether of methyl ricinoleate. This last compound was identified by comparison with an authentic sample of methyl 12-methoxyoleate synthesized from methyl ricinoleate. The comparison was made by gas-liquid chromatography, thin-layer chromatography, and infrared analysis; the spectra showed a characteristic absorption for the C–O stretching of ethers at 9.1 μ. The methyl ether probably was formed when the acids were esterified with diazomethane and concentrated in the nonhydroxy esters during chromatography on silicic acid.

[4] In early experiments recoveries were high, presumably due to oxidation of the samples. Samples should be protected by addition of antioxidant.

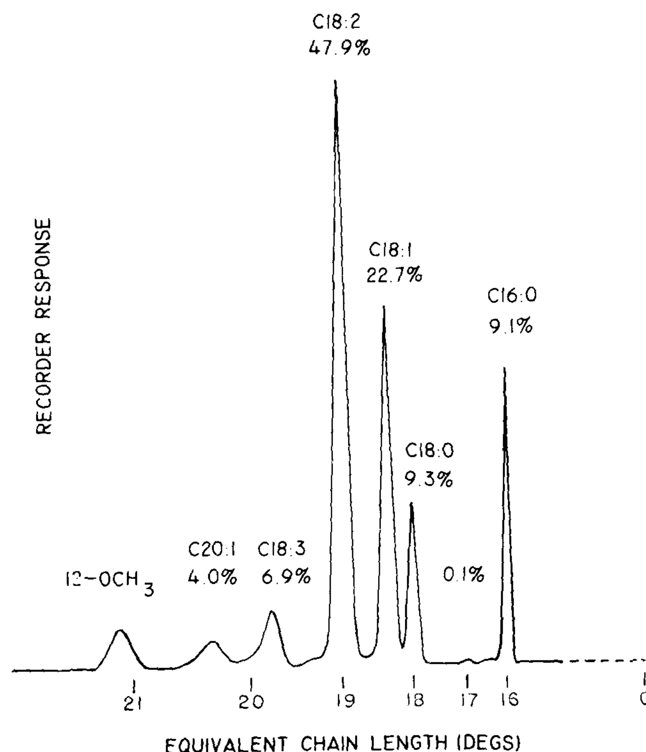

FIG. 2. Gas chromatogram of the nonhydroxy esters from a domestic castor oil (No. 9).

The nonhydroxy esters were also chromatographed using an Apiezon L column, which can readily separate arachidate from linolenate. The presence of both arachidate and 12-methoxyoleate was substantiated.

The response of the thermal conductivity detector was calibrated by means of a GLC standard mixture of equal parts by wt of methyl palmitate, stearate, oleate, linoleate, and linolenate obtained from the Hormel Institute. The response factor used for methyl eicosenoate was not determined experimentally but could be calculated according to the method of Horrocks et al. (23). The percentages indicated in Figure 2 are response-corrected wt percentages based on the total nonhydroxy esters exclusive of the ether of methyl ricinoleate. The estimated composition of a sample of nonhydroxy esters before and after hydrogenation is given in Table II.

The possible presence of hydroxystearic acid in castor oil has been the subject of considerable speculation and indeed a published abstract (24) reports its presence. The abstract appears to be in error, because the original article of Panjutin and Rapoport (5) reports an identical amt of dihydroxystearic acid with no mention of hydroxystearic acid. To determine whether hydroxystearic acid could be determined by

TABLE II

Estimated Composition of Nonhydroxy Esters

Compound	Before hydrogenation, %		After hydrogenation, %
C16:0	8.8	Σ = 9.0	9.0
C16:1	0.2		
C17:0	0.2		0.2
C18:0	9.1	Σ = 87.4	87.5
C18:1	22.3		
C18:2	49.3		
C18:3	6.7		
C19:0	......		0.3
C20:0	0.3	Σ = 3.4	3.0
C20:1	3.1		

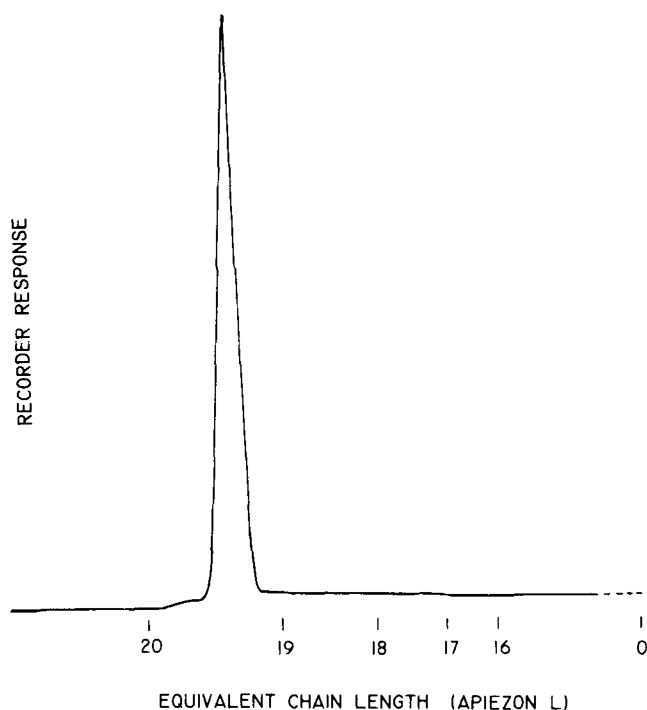

FIG. 3. Gas chromatogram of methyl ricinoleate and added (1%) methyl 12-hydroxystearate.

GLC, 1% of methyl 12-hydroxystearate in methyl ricinoleate was chromatographed on an Apiezon L column. The added hydroxystearate is clearly evident just beyond the methyl ricinoleate peak (Fig. 3). However, none of the chromatograms of the monohydroxy esters of the castor acids showed any evidence of the presence of hydroxystearate.

Ultraviolet Analysis. Ultraviolet spectra were determined for some of the castor oils and the methyl esters before and after isomerization with potassium tertiary butoxide (25). Spectra were taken in methanol solution by use of a DK-2 spectrophotometer. As representative examples, the curves for the isomerized nonhydroxy esters from Brazil No. 1 oil (sample No. 4) and for the isomerized oil itself are shown in Figure 4.

Discussion

The fatty acid composition of the castor oils was calculated from the results of the silicic acid and gas-liquid chromatograms. The proportion of each nonhydroxy ester as a wt percentage of the total nonhydroxy esters was determined by gas-liquid chromatography. From this information, an average mol wt of the nonhydroxy acids was obtained. Then the mole percentages of the nonhydroxy acid, monohydroxy acid (ricinoleic), and dihydroxy acid (dihydroxystearic) were converted to wt percentages. The results obtained for eleven castor oils are given in Table III.

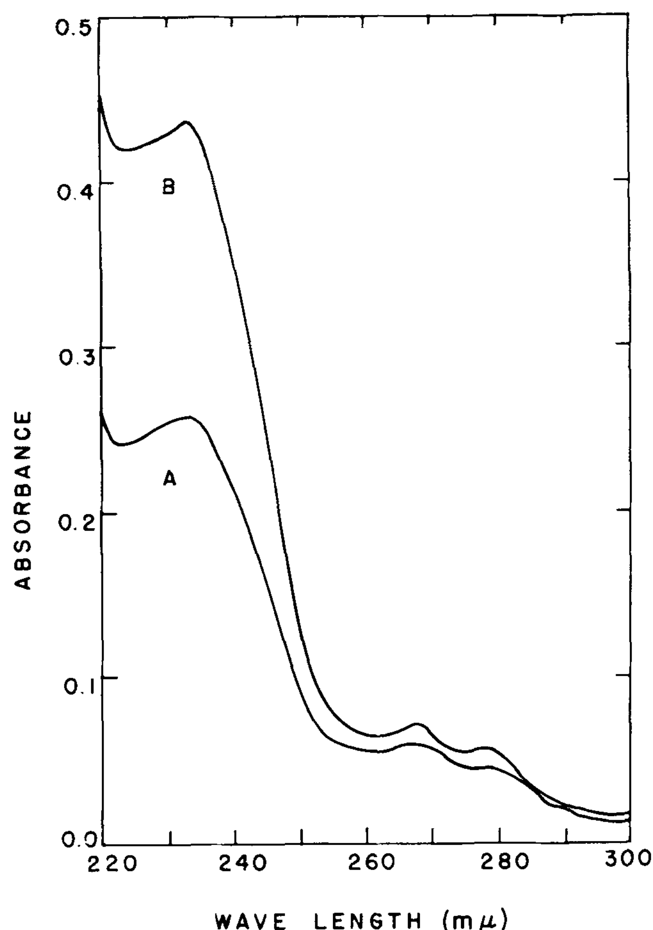

FIG. 4. Ultraviolet spectra (methanol solvent, 0.1-cm cell) of base isomerized: (A) Brazil No. 1 oil (0.5 mg/ml), (B) nonhydroxy esters of Brazil No. 1 oil (0.1 mg/ml).

Although these oils differ widely in origin, there is relatively little difference in their fatty acid composition. With the exception of eicosenoic acid, all of these acids have been reported previously. A peak was assigned to eicosenoate by comparing its ECL's on the DEGS and Apiezon L columns and observing the shift to an ECL of 20 following hydrogenation. Myddleton (3), who did not mention linoleic acid, reported the presence of 7% linolenic acid [Sreenivasan et al. (1) tabulated this as linoleic acid]. However, Heiduschka and Kirsten (4) found no evidence for the presence of linolenic acid in their castor oil on determination of the hexabromide number. In our investigation, the presence of linolenic acid was substantiated by isomerizing both the nonhydroxy esters isolated by chromatography and the parent castor oil sample with potassium tertiary butoxide (25). In Figure 4 the presence of both conjugated diene and triene is clearly indicated, although the third max due to triene is obscured by the large absorbance of

TABLE III
Fatty Acid Composition of Castor Oils[a]

Sample No.	Source	C16:0	C18:0	C18:1	C18:2	C18:3	C20	Ricin.	Di(OH)
1	Angola	1.0	0.7	2.9	4.3	0.6	0.5	89.4	0.6
2	Angola	1.0	0.7	2.7	4.2	0.7	0.8	89.0	0.9
3	Brazil	1.0	0.8	3.3	4.2	0.5	0.4	88.9	0.9
4	Brazil	1.0	0.8	2.8	4.4	0.5	0.4	89.4	0.7
5	Brazil	1.0	0.8	3.2	4.5	0.5	0.4	88.6	1.0
6	Brazil	0.9	0.8	3.0	4.4	0.5	0.5	88.9	0.9
7	India	1.1	1.0	3.3	4.6	0.7	0.5	87.7	1.1
8	India	1.1	1.0	3.1	4.7	0.5	0.5	88.4	0.7
9	U.S.	0.8	0.8	2.0	4.4	0.6	0.4	90.0	0.9
10	U.S.	0.8	0.8	2.2	4.1	0.5	0.4	90.4	0.8
11	U.S.	0.9	0.8	2.0	4.3	0.6	0.3	90.3	0.8
	Range	.8–1.1	.7–1.0	2.0–3.3	4.1–4.7	.5–.7	.3–.8	87.7–90.4	.6–1.1

[a] Wt % of total fatty acids.

the diene. Control experiments with an oil containing about 5% linolenate and 50% linoleate showed similar curves. The proportions of diene and triene estimated from the ultraviolet absorption data are in good agreement with those obtained by GLC. The spectra of unisomerized castor oils and methyl esters indicated only traces of conjugated diene and triene.

In addition to the components listed in Table III, traces of myristic, palmitoleic, heptadecanoic, and nonadecanoic acids were found. Altogether, these minor components constitute only about 0.1% of the total fatty acids.

The combination of chromatographic techniques used offers several advantages over the usual analytical methods. The analyses are relatively rapid and simple. Only a small sample is needed, most of which can be recovered. The incompleteness of reaction and the side reactions in previously used procedures (iodine and thiocyanogen absorption, alkali isomerization, acetylation and oxidation) were avoided. Here alkali isomerization, thin layer chromatography, and ultraviolet and infrared spectrophotometry were used to provide qualitative substantiating evidence for the results obtained by quantitative chromatographic procedures.

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REFERENCES

1. Sreenivasan, B., N. R. Kamath, and J. G. Kane, JAOCS *33*, 61–66 (1956).
2. Eibner, A., and E. Münzing, Chem. Umschau Gebiete Fette, Öle, Wachse, u. Harze *32*, 166–176 (1925).
3. Myddleton, W. W., R. G. Berchem, and A. W. Barrett, J. Am. Chem. Soc. *49*, 2264–2269 (1927).
4. Heiduschka, A., and G. Kirsten, Pharm. Zentralhalle *71*, 81–91 (1930).
5. Panjutin, P., and M. Rapoport, Chem. Umschau Gebiete Fette, Öle, Wachse. u. Harze *37*, 130–135 (1930).
6. Steger, A., J. Van Loon, and C. Smelt, J. Soc. Chem. Ind. *55*, 41T–42T (1936).
7. Kaufmann, H. P., and H. Bornhardt, Fette u. Seifen *46*, 444–446 (1939).
8. Riley, J. P., Analyst *76*, 40–44 (1951).
9. Gupta, S. S., T. P. Hilditch, and J. P. Riley, J. Sci. Food Agr. 2, 245–251 (1951).
10. Achaya, K. T., and S. A. Saletore, Analyst *77*, 375–380 (1952),
11. Bolley, D. S., JAOCS *30*, 396–398 (1953).
12. Bergier, A., Industrie chim. belge, *20*, Spec. No. 681–4 (1954).
13. Narayan, K. A., and B. S. Kulkarni, J. Indian Chem. Soc., Ind. & News Ed., *17*, 79–86 (1954).
14. Vézinet, P., and M. Naudet, Rev. franc. corps gras *7*, 85–88 (1960).
15. Frankel, E. N., C. D. Evans, H. A. Moser, D. G. McConnell, and J. C. Cowan, JAOCS *38*, 130–134 (1961).
16. De Boer, Th. J., and H. J. Backer, Org. Syntheses *36*, 16–19 (1956).
17. Applewhite, T. H., M. J. Diamond, and L. A. Goldblatt, JAOCS *38*, 609–614 (1961).
18. Smith, E. D., Anal. Chem. *32*, 1301–1304 (1960).
19. King, G., J. Chem. Soc., 387–391 (1942).
20. Toyama, Y., and T. Ishikawa, Bull. Chem. Soc. Japan *11*, 735–741 (1936).
21. Miwa, T. W., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal. Chem. *32*, 1739–1742 (1960).
22. Woodford, F. P., and C. M. van Gent, J. Lipid Research *1*, 188–191 (1960).
23. Horrocks, L. A., D. G. Cornwell, and J. B. Brown, J. Lipid Research *2*, 92–94 (1961).
24. Panjutin, P., and M. Rapoport, Chem. Umschau Gebiete Fette, öle, Wachse, u. Harze *37*, 130–135 (1930); Chem. Abs. *24*, 3665 (1930).
25. Sreenivasan, B., and J. B. Brown, JAOCS *33*, 521–526 (1956).

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Isolation of Pure Linolenate as Its Mercuric Acetate Adduct[1]

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Abstract

After addition of mercuric acetate to the unsaturated methyl esters obtained from the methanolysis of linseed oil, linolenate of 99% minimum purity was isolated by means of a liquid-liquid continuous extraction technique and subsequent decomposition of the mercurial adduct. The methyl linolenate-mercuric acetate addition compound was extracted with 10% methanol in water from an ether solution of the reaction mixture. Infrared analysis of the regenerated methyl linolenate showed the complete absence of *trans*-linkages. The yield, at least 60% of the linolenic acid present in linseed oil, was considerably higher than that obtained by bromination-debromination procedures.

The solubility in aqueous solutions of fatty acid derivatives having 3 or more acetoxymercuri-groups/molecule provides an approach to the fractionation of highly unsaturated oils. For example, a fraction having an iodine value of 395 was isolated readily from the methyl esters of pilchard oil by this technique.

Introduction

THE ISOLATION of highly pure polyunsaturated fatty acids from natural oils always has been a difficult task. A number of techniques have been developed to meet the problem, some of which are effective whereas others yield only concentrates of the desired product.

Although the purest isolates probably are realized from the use of chromatographic techniques, a serious defect usually encountered is the small amount of sample that can be charged on the column. Isolation with this technique of pure linoleate and linolenate (1) as well as fatty esters with 5 or 6 double bonds (2,3) has been described.

The urea segregation and the low temp crystallization procedures (4,5) are of much value in the recovery of linoleate or the free acid, respectively, on a preparative scale. Neither of these methods, however, has proved successful in the isolation in pure form of fatty acid components having more than two double bonds.

Countercurrent distribution is an inherently mild procedure by which a good yield of pure linoleate and linolenate (6) can be obtained. However, its use as a preparative method is limited to the capacity of the complex instrument.

Modification of the fatty acid components by addition to the double bonds, especially of bromine, is used extensively in large scale preparations (7). However, bromination-debromination linoleic and linolenic acids contain an appreciable quantity of isomeric acids that differ from their natural form in vegetable oils (8). This difficulty is not observed with the use of mercuric acetate addition in methanol.

The present investigation describes a procedure for the isolation of 99+% pure methyl linolenate as its

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