tion of the lactic acid. Sample size is adjusted by diluting the titrated aqueous phase to known volume with distilled water and removing an aliquot portion for periodate oxidation. Lactic acid does not interfere with the periodic acid reaction if the reaction is carried out at room temp. Fatty acid and glycerine contents determined in this manner agree well with the values obtained using the standard AOCS Methods G-4-40 and Ca-14-56, respectively.

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Separation of Fatty Ester-Mercuric Acetate Adducts of Alumina[1,2]

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Abstract

A chromatographic procedure is described for the elution separation on alumina for 100-mg samples of methyl palmitate and fatty ester-mercuric acetate adducts. Oleate and linoleate were obtainable in pure form; however, the linolenate adduct did not separate completely from the linoleate adduct. For quantitative results the use of alumina of proper activity and basicity is essential. Ether with small amounts of acetic acid is a suitable eluant. Since the original esters can be regenerated easily the ability to isolate individually the oleate and the linoleate adducts in high yield can be useful in radiobiochemical work.

Introduction

ATTEMPTED separations of the fatty acids from small samples (ca. 100 mg) have employed three general methods: (a) direct separation of the acids or their esters by physical means (1,2,3,4); (b) controlled oxidation of unsaturates, with subsequent fractionation (5,6,7,8); and (c) the formation of derivatives or adducts to magnify differences in properties before fractionation. Disadvantages of direct separation and oxidation procedures are that the former generally are incomplete and the latter do not permit recovery of the intact unsaturated acids. Some derivatives may promote separation but fail in the requirement for fatty acid regeneration (9). Mercuric acetate adducts are potentially suitable in both respects. They have been used to separate saturated from unsaturated fatty acids on Florisil (10) and on silica gel (11) columns. Recently, partial separations of the adducts of individual unsaturated components have been reported on silica gel (12).

The present report concerns the adaptability of alumina as an adsorbent in separation procedures which employ mercuric acetate adducts.

Materials and Methods

Samples of methyl palmitate and oleate of high purity were obtained from the Hormel Institute. Linoleate of 98% minimum purity with oleate as the contaminant was isolated by urea adduct fractionation from the mixed methyl esters of safflower oil (13). Pure linolenate was extracted as its mercuric acetate adduct from linseed oil methyl esters (14).

Basic and neutral aluminas of different degrees of activity (adsorbing capacity) were prepared by supplying known amounts of deionized water to the anhydrous material. The degree of deactivation was proportional to the amount of water added. Activities I, II, III, and IV refer to the different grades of alumina established for standardization purposes by Brockmann and Schodder using a number of azo dyes (15). These grades were obtained through the addition of 0%, 3%, 6%, and 10% water, respectively, on a w/w basis to the dry metal oxide. No attempt was made to remove moisture that may have been present on the alumina before the deactivation treatment.

A sample of methyl ester weighing about 100 mg and of known composition was dissolved in 1 ml of methanol and heated at 80C for 30 min in the presence of mercuric acetate, 20% in excess of the theoretical amount needed to react with the double bonds. The reaction mixture was cooled to room temp and transferred in 10 ml of ethyl ether to a previously prepared column of 15 g of alumina, 11.5 cm high by 1.3 cm wide, covered with ether. A water jacket at 15C surrounded the column. Eluting solvents with increasing proportions of acetic acid in ether and corresponding increasing eluting power were passed through the column in stepwise manner. The flow rate was between four and five ml/min.

Solvent was removed from the eluates with a rotary evaporator until a residual vol of approx 10–20 ml remained. Following decomposition of the adducts by a 10-min treatment at room temp with 100 ml of methanol-ether (1:1, v/v) and 10 ml of concentrated hydrochloric acid, the homogeneous solution was transferred to a separatory funnel with 100 ml of hexane. After the epiphase was extracted with water and dried on a cellulose column, the solvent was removed and the residue transferred with methanol to a 50-ml volumetric flask and made to vol.

A simplified spectrophotometric determination for ester groups (16) was used on an aliquot of the methanolic solution. A further aliquot was analyzed for ester composition by gas liquid partition chromatography under conditions described previously (17).

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TABLE I

Comparison of Aluminas for the Separation of Mercuric Acetate Adducts and Methyl Palmitate

Experiment	Alumina		Esters[b]	Eluant		Amount of Ester		
	Kind	Activity grade[a]		Vol	Acetic[c]	Weighed	Recovered	Difference
				ml	%	*mg*	*mg*	*mg*
1	Merck (old)[d] basic	I	P	100	.....	12.8	13.2	+0.4
			O	200	0.6	63.0	62.3	−0.7
2	basic	I	P	100	.....	12.5	13.7	+1.2
			O, Le	300	4.0	83.0	80.9	−2.1
3	Merck (new)[d] basic	I	P	100	.....	12.6	12.1	−0.5
			O, Le	300	4.0	72.0	56.5	−15.5
4	Woelm basic	I	P	100	.....	15.6	2.7	−12.9
			O	200	0.6	36.4	37.3	+0.9
5	basic	III	P	100	.....	15.9	15.9	0.0
			O	200	0.6	42.4	43.2	+0.8
6	basic	III	P	100	.....	18.4	17.0	−1.4
			O, Le	300	4.0	91.2	90.3	−0.9
7	basic	III	P	100	.....	11.4	11.6	+0.2
			O, Le, Len	300	10.0	89.4	80.1	−9.3
8	basic	IV	P	100	.....	12.5	12.7	+0.2
			O, Le, Len	300	10.0	91.3	83.3	−8.0
9	neutral	II	P	100	.....	14.4	13.5	−0.9
			O, Le, Len	300	10.0	83.1	75.9	−7.2
10	neutral	III	P	100	.....	14.4	14.3	−0.1
				100	.....	.....	.....	.....
			O, Le, Len	300	10.0	85.1	86.4	+1.3
11	neutral	III	P	100	.....	9.9	10.2	+0.3
				100	.....	.....	.....	.....
			O, Le, Len	300	10.0	90.6	90.5	−0.1

[a] I, II, III, and IV refer to the addition of 0%, 3%, 6%, and 10% water, respectively, to the anhydrous alumina on a w/w basis (15).
[b] P, O, Le, and Len refer to palmitate, oleate, linoleate, and linolenate, respectively.
[c] Percentages of acetic acid in ethyl ether on a v/v basis.
[d] The old Merck alumina was purchased at least 3 yr earlier than the new.

Results and Discussion

Separation of Saturated from Unsaturated Methyl Esters. Mercuration of fatty ester mixtures and subsequent adsorption chromatography on alumina provided a means for separating the saturates from the unsaturates. Palmitate and the mixed oleate, linoleate, and linolenate mercurials were quantitatively separated on neutral alumina in activity III (Table I).

The basicity and degree of activity of the alumina were important in obtaining a satisfactory separation. Complete recovery of the palmitate was not possible with all basic aluminas of activity I under the chosen conditions (Exp. 4). Basic aluminas of activities III and IV permitted recovery of the saturated esters, but the unsaturated groups could not be regained completely (Exps. 5,6,7,8). Neutral alumina of activity III (Exps. 10 and 11) permitted separation of saturated from unsaturated and recovery of all four components. The data suggest that the basic adsorbent effected a partial ester hydrolysis on the column. That the desired separation was not a particularly close one was shown by the fact that ethyl ether in 100 ml excess of the amount usually employed to eluate the saturates did not elute any unsaturated material (Exps. 10 and 11).

Separation of Oleate and Linoleate. Under suitable conditions palmitate, oleate, and linoleate could be isolated each from the others in yields of 90% or better (Table II). The eluant for the oleate fraction, 0.4% acetic acid in ether insured that all oleate was removed from the column. The small proportion of contaminating linoleate (3%) was removed by rapid passage of the eluant through a second column of only 5 g of alumina. Both columns employ the neutral product of activity II. Most of the linoleate was eluted from the first column with 300 ml of 2.5% acetic acid in ether.

Separation of Oleate, Linoleate, and Linolenate. Although the majority (93%) of oleate was removed from the other unsaturates in pure form, the isolation of pure linoleate and linolenate was not achieved on a single column (Table III). However, concentrates of 98% linoleate and 82% linolenate were obtained with recoveries of 86% and 56%, respectively.

Tailing of the unsaturated adducts was a serious problem in obtaining absolute separations. Before all

TABLE II

Elution Separation of Mercurated Mixture of Palmitate, Oleate, and Linoleate[a]

Column[c]	Eluant		Eluate				
	Vol	% Acetic[b]	Weight	% Composition[d]			% Recovery
				P	O	LE	
	(ml)		*(g)*				
I	100	0.0	12.0	100	.....	.....	97
I	300	0.4	33.2	.....	97	3	97
II	100	0.4	30.2	.....	100	.....	89
I	300	2.5	44.5	.....	.....	100	90

[a] Mixture prepared from 12.4 mg palmitate, 34.1 mg oleate, and 49.4 mg linoleate.
[b] % acetic acid in ether (v/v basis).
[c] Woelm neutral alumina activity grade II. Column I contained 15 g alumina; Column II, 5 g, was used to rechromatograph the second from Column I.
[d] Determined by gas chromatography.

TABLE III

Elution Separation of Mixture Containing Linolenic Ester Adduct[a]

Eluant		Eluate					% Recovered
Volume	% Acetic	Weight	% Composition				
			P	O	LE	LEN	
(ml)		*(g)*					
100	0.0	12.6	100	.....	.....	.....	94
250	0.3	27.4	.....	100	.....	.....	93
250	0.4	2.7	.....	46	54	.....	.....
250	2.0	38.3	.....	.....	98	2	.....
250	2.0	5.3	.....	.....	40	60	.....
200	10.0	6.9	<1	<1	18	82	.....
Total		93.2					97

[a] Mixture prepared from 13.4 mg palmitate, 29.4 mg oleate, 43.8 mg linoleate, and 10.2 mg linolenate. Column contained 15 g of alumina II.

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.

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

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 %	Dihydroxystearic %
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.