Use of Mercuric Acetate Addition to Prepare Unsaturated Fatty Ester Concentrates[1]

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Mercuric acetate in methanol was reacted with unsaturated fatty acid esters in mixtures prepared from linseed, safflower and menhaden oils and pig liver and beef adrenal lipids. Methanol solutions retained predominantly the adducts of the more highly unsaturated esters when extracted with either n-pentane or commercial pentane (Skellysolve F). The free esters were recoverable from the adducts.

Linoleate of 95% purity and linolenate of 90% purity were obtained directly from natural oils by this procedure. Concentrates of more highly unsaturated acids were obtained similarly.

THE REACTION between mercuric acetate and olefinic bonds in methanol giving acetoxymercurimethoxy derivatives and the regeneration of original double bonds with retention of configuration by treatment with 5% HCl was described in 1951 (1). Since *cis* bonds react with the reagent faster than *trans* bonds (2), Jantzen and Andreas (3) were able to use the reaction in the separation of oleate from elaidate as well as from stearate. Inouye *et al.* (4) successfully separated the mercuric acetate derivatives of mixed unsaturated fatty esters by paper chromatography. The reaction of mercuric acetate with conjugated unsaturation gives an unstable product with a mercury content less than theoretical (5).

This communication deals with a rapid solvent fractionation of methyl esters as mercuric acetate addition products, which eliminates saturated esters and lowers the concentration of those of low unsaturation, leaving a product of high unsaturation which may be further fractionated by other means.

Experimental

Lipids. Raw linseed oil and edible safflower oil were used as received. Crude menhaden oil and pig liver lipid were vacuum-treated to remove residual solvents. Frozen beef adrenal glands (900 g.) were thawed and extracted three times with 1-liter portions of ethanol in a Waring blendor. Reduced pressure was used to remove alcohol from the resulting aqueous extract which was then thoroughly re-extracted with ether. The combined ether extracts, ca. 1 liter, were dehydrated by passing through a column which contained 150 g. of cellulose powder (Whatman, standard grade) and the solvent was then removed.

Ester Preparation. The linseed and safflower oils were treated to a base-catalysed methanolysis, as described by Kurz (6), using methanol which had been purified by distilling over zinc dust and KOH. The resulting esters in ether were washed successively with water, 2% potassium carbonate and water, and dried by passing through a cellulose column.

Liver, menhaden, and adrenal esters were prepared by refluxing the lipid in twice its weight of dry, purified methanol containing 2% hydrogen chloride for 24 hr. (7). The cooled reaction mixture in an equal volume of ether was washed thoroughly with water, dried by passing through a cellulose column and the solvent then removed.

Gas-Liquid Chromatography. Analyses of compositions were by gas-liquid chromatography (GLC), using an Aerograph A-100-C instrument, (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.), in conjunction with a Sargent Model SR recorder with Disc integrator, (E. H. Sargent & Co., Chicago) at 1 millivolt and 1 in. per min. chart speed. Linseed and safflower ester samples and the C_{18} series of liver lipid were separated on a 10-ft. column containing 15% diethylene glycol succinate (DEGS) on 60–80 mesh firebrick at 215–16°C., (injector at 300°C.), with a helium flow rate of 65 ml. per min. Adrenal, menhaden, and liver samples were separated on a 2.5 ft. column containing 20% 1,4-butanediol succinate polyester (BDS) on 60–80 mesh firebrick at 214–15°C., (injector at 320°C.), with a helium flow rate of 167 ml. per min.

Identifications of peaks were by comparison with standards (Hormel Institute, Austin, Minn.), where possible. Highly unsaturated esters in liver lipids were collected and, for each, chain length was determined by complete hydrogenation and rechromatography, while the degree of unsaturation was found from the maximum wavelength of peak absorption after isomerization in 21% KOH in ethylene glycol at 180°C. for 15 min. (8). Menhaden oil esters were identified by comparison with chromatographic charts (BDS columns) published by the supplier, Archer-Daniels-Midland.

Preparation of Adducts. After determination of the composition of ester mixtures by gas chromatography, the theoretical amounts of mercuric acetate were calculated and in each case a 20% excess was used. The weighed sample with the mercuric acetate in purified methanol (1–2 ml. per g. of mercuric acetate) was refluxed for 30 min. (4). After cooling to room temperature a volume of ether 2.6-fold that of the previously added methanol was added and the solution was filtered through glass wool to remove most of the unreacted mercuric acetate. Portions of the viscous adduct which remained after solvent removal were used in subsequent fractionation.

Solvent Fractionation of Adduct Mixtures. A 25 g. portion of adduct in an equal amount (w/v) of methanol was mixed thoroughly with 30 volumes of either n-pentane or commercial pentane (Skelly-solve F). The oily hypophase was withdrawn with a pipet and after removal of the solvent, it was weighed and repartitioned in 1 volume of methanol and 30 volumes of pentane (based on the new weight of material). After three extractions with 30 volume portions of pentane the hypophase was concentrated to remove solvents. The three epiphases for linseed oil were also concentrated.

Adduct Destruction. On completion of fractionation steps, the adducts were destroyed to regenerate the unsaturated esters: Ten g. of the adduct in 20 ml. of methanol was mixed with 50 ml. of HCl, and a stream of hydrogen chloride gas was bubbled into the solution for a few moments. The solution was then extracted twice with 20 ml. of commercial hexane (Skellysolve B), and the hexane extracts were combined and washed thoroughly with water. If a

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subsequent test with a dilute methanolic solution of diphenyl-carbazone (4) revealed mercury to be still present, a single repetition of the acidification treatment was sufficient to remove the metal. Finally, the solution was washed with water to remove all acid and alcohol, then dried over anhydrous sodium sulfate and the solvent removed. The product was weighed and analyzed by GLC.

Results and Discussion

Linseed Esters. Stearate was eliminated from the linseed ester-adduct mixture in the first partitioning while removal of the more abundant palmitate required two fractionations; the adduct of oleate was not entirely eliminated after three fractionations (Table I). Methyl linolenate in the final concentrate

TABLE I
Partition of Linseed Adducts Between Methanol and Pentane

Fraction	Approximate Yield	Composition [a]				
		P	S	O	Le	Len
	%	%	%	%	%	%
Original esters	100	7	3	22	17	51
Esters from:						
epiphase 1	48	15	7	34	19	26
epiphase 2	22	<1	–	20	27	52
epiphase 3	11	–	–	4	21	75
hypophase 3	25	–	–	<1	8	91

[a] P, S, O, Le and Len refer to palmitate + palmitoleate, stearate, oleate, linoleate and linolenate, respectively.

represented 46% recovery of that initially present, with a purity of at least 90%, the major contaminant being linoleate.

Safflower Esters. Cooling was necessary to obtain two phases. Hypophase 1 (Table II) was obtained slightly below room temperature, and hypophase 2 at ice-bath temperature. The total linoleate contained in the two hypophases represents 26% of the linoleate

TABLE II
Partition of Safflower Adducts Between Methanol and Pentane

Fraction	Approximate Yield	Composition [a]			
		P	S	O	Le
	%	%	%	%	%
Original esters	100	8	3	10	79
Esters from:					
hypophase 1	14	–	–	4	96
hypophase 2	8	–	–	6	94

[a] P, S, O and Le refer to palmitate + palmitoleate, stearate, oleate and linoleate, respectively.

initially present, purity at least 95%, oleate being the only contaminant observed on gas chromatograms.

Liver, Adrenal, and Menhaden Esters. Methanol-pentane partition was used on adducts from all these materials. Since emphasis with these materials was placed on the longer-chained highly unsaturated esters, all C_{18} esters are grouped together (Table III).

Gas analysis of the C_{18} fraction from Hypophase 3 of pig liver lipid showed that the ratio of stearate:

TABLE III
Partition of Adducts from Liver, Adrenal, and Menhaden Esters Between Methanol and Pentane

Source of Esters	Fraction	Approximate Yield	Components of Fractions [a, b]				
			<18	18	20:4	20:5	22:5
		%	%	%	%	%	%
Pig Liver	Original	100	15	56	7	9	13
Pig Liver	Hypophase 3	27	<1	11	12	25	52
Adrenal	Original	100	37	57	6		–
Adrenal	Hypophase 3	8	3	5	91		–
Menhaden	Original	100	45	30	–	17	9
Menhaden	Hypophase 3	27	5	9	–	27	59

[a] 20:4 refers to an ester of a C_{20} acid with 4 double bonds.
[b] Serious tailing of more highly unsaturated esters on the chromatograms may have exaggerated their proportions in the mixtures.

oleate:linoleate:linolenate was approximately 0:1:2:8. Esters of chain length shorter than C_{18} were not completely eliminated from the final hypophase; these esters were undoubtedly unsaturates rather than saturates.

Several variations in procedure were attempted using the liver adduct-mixture without much success in increasing the unsaturation. When five partitions were made, the C_{22}-pentaene component was increased to 62% of the final fraction (yield 15%) with little change in the remaining components from those found in the usual three (Table III). Changing the methanol-pentane ratio from 1:30 to 1:10 (v/v) resulted in little change in the amount of C_{22} pentaene obtainable. Holding the methanol-pentane ratio at 1:30 (v/v) but increasing the total solvent to adduct-ester mixture ratio three-fold increased the C_{22}-pentaene from 51 to 61% in the final fraction (yield, 17%). The C_{18} esters persisted in all experiments.

It is evident that high yields of the desired unsaturates from the more complex natural fats cannot be expected with the described procedures; however, it does permit a very rapid concentration of these esters with complete elimination of the saturates.

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