Preparation of Methyl Malvalate from *Sterculia foetida* Seed Oil

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Abstract

Methyl malvalate has been prepared from mixed methyl esters of *Sterculia foetida* seed oil fatty acids by reversed-phase liquid-liquid partition chromatography, and confirmatory evidence adduced for its structure.

Introduction

THE NATURALLY OCCURRING cyclopropenoid fatty acids, sterculic acid and malvalic acid, have been studied extensively over the last decade and their chemistry and biological effects have been recently reviewed (2,12). Malvalic acid was first isolated by Shenstone and Vickery (14–17) from leaf and seed oils of plants of the order Malvales. From its equivalent weight (14) and from a comparison of IR spectra of malvalic, sterculic, dihydromalvalic, dihydrosterculic, and lactobacillic acids Macfarlane, Shenstone and Vickery (9) established it as a C_{18} homolog of sterculic acid. Jeffrey et al. (3,6) by crystal structure analysis of dihydromalvalic acid, later located the ring at the 8,9 position in the chain. Although unable to isolate the pure compound, Wilson et al. (19) obtained further chemical evidence for the structure of malvalic acid by identifying pelargonic and suberic acids among oxidation products of *Hibiscus syriacus* oil, a known source of malvalic acid.

For further investigations in this field relatively large quantities of methyl malvalate were required, and it was decided to use *Sterculia foetida* seed oil as the source. Sterculic acid (11) and methyl sterculate (7) have been prepared from this oil, but, although the quantity of malvalate obtainable from it is at least one-tenth that of the sterculate, the oil does not appear to have been utilized previously for the preparation of methyl malvalate in quantity. In our work reversed-phase liquid-liquid partition chromatography, based on the method of Privett and Nickell (13), was found to be a satisfactory method for separating methyl malvalate from the residual esters left after isolation of sterculate from *Sterculia foetida* oil.

Experimental

Sterculia foetida seeds were donated by the Department of Forestry, Republic of the Philippines, and the oil extracted by homogenizing the seeds with hexane.

Methyl esters were analyzed by gas chromatography (GLC) using a Packard Model 7508 Gas Chromatograph with two argon ionization detectors and two 6 ft × 4 mm I.D. coiled glass columns, packed with Chromosorb G containing either 5% diethylene glycol adipate or 1% Apiezon M. Simultaneous use of columns of both phases in the one oven was achieved by using the low Apiezon loading; under these conditions the emergence times for both columns were similar when the oven was maintained at the optimum temperature (170–190C) for the adipate column. Operating temperature was usually 175C for mixtures of methyl esters from C_8 to C_{16}, and 185C for methyl esters beyond C_{16}. Methyl *n*-alkyl ketones above C_7 could be analyzed by GLC with the same columns at 130C. Standard methyl ester mixtures obtained from the National Institutes of Health, Bethesda, Maryland, were used to calibrate the instrument. Peak areas were determined by ball and disc integrators coupled to the recorders.

The composition of the methyl esters at each stage of the separation procedure outlined below was followed by GLC. Samples of mixtures containing methyl malvalate and methyl sterculate were hydrogenated using palladium (10% on calcium carbonate) in methanol and re-examined by GLC, to overcome problems in calculating the methyl ester composition due to peak overlap from cyclopropene ring rearrangement during GLC of the unsaturated esters.

Preparation of Malvalate Concentrate

Sterculia foetida seed oil (210 g) was transesterified with sodium methoxide in dry methanol and the solution of methyl esters was neutralized with glacial acetic acid, and diluted with methanol and treated with urea as described by Kircher (7). The solution was cooled to −16C, filtered, and the filtrate cooled successively to −30C and −50C. The adducts that crystallized at −30C and −50C were combined, added to water, and the esters extracted therefrom with hexane. The evaporated residue (95 g) was taken up in methanol (2) and at −50C yielded crystals of methyl sterculate (60 g).

The methanolic solution containing methyl esters not precipitated as urea adducts at −50C was concentrated under vacuum to one-fifth of its original volume. After adding water and extracting as before the free esters were obtained as an orange oil (30 g) containing mainly methyl malvalate, sterculate, linoleate, and oleate. This oil was dissolved in acetone (150 ml) and the crystals (21 g) deposited at −70C were filtered and recrystallized at −70C from acetone. The product (15 g) contained methyl malvalate (about 60%) and methyl sterculate (about 30%), with methyl oleate and linoleate as minor components. This malvalate methyl ester concentrate was chromatographed on Florisil (1) to free it from degradation products and non-ester compounds.

Liquid-Liquid Partition Chromatography of Malvalate Concentrate

Separation of methyl malvalate from the malvalate concentrate was obtained by using a reversed-phase liquid-liquid chromatography technique based on that of Privett and Nickell (13). The solvent used as the mobile phase was acetonitrile-methanol (85:15 v/v) saturated with *n*-heptane, and *n*-heptane saturated with acetonitrile-methanol was used as the stationary phase. The support for the stationary phase was 100-mesh Hyflo Supercel, made repellent to polar solvents with dichlorodimethyl silane, as described by Howard and Martin (5), or (for large quantities of support) by treatment with a petroleum solution of the silane.

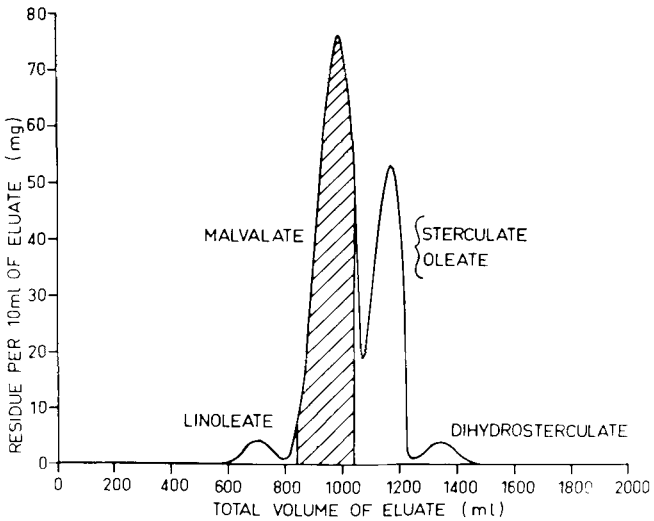

FIG. 1. Separation of methyl malvalate from malvalate fraction of *S. foetida* methyl ester mixture by reversed-phase liquid-liquid chromatography on a 22 mm by 900 mm column of Hyflo Supercel treated with silane. Mobile phase, acetonitrile-methanol (85:15 v/v); stationary phase, *n*-heptane. Column load 1.62 g.

The support material (100 g) was added to a mixture of stationary phase (120 ml) and mobile phase (500 ml) in a filter flask. The mixture was vigorously shaken and stirred to break up lumps, and air bubbles were removed by applying a low vacuum to the flask, which was then set aside at 25C for several days to ensure uniform distribution of the stationary phase on the support.

A 22 mm I.D. column was packed by running this slurry into it, a perforated plunger being used to disperse the support uniformly. The support was allowed to settle under gravity, with only very light packing pressure from the plunger, to give a packed length of about 900 mm. The column was maintained at 25C by a water jacket. To test the column performance, a mixture (1.4 g) comprising equal parts of methyl oleate and stearate, with minor amounts of palmitate, linoleate, and other esters, was transferred to the column with a minimum of mobile phase. Elution with equilibrated mobile phase was commenced at a flow rate of 0.3 to 0.4 ml per minute. Fractions (10 ml) were collected in tared tubes and evaporated. The residues were weighed, and the compositions determined by GLC analysis. On satisfactory completion of the test separation, the malvalate concentrate (1.5–2 g sample) was chromatographed in a similar manner.

The gravimetric and GLC results indicated that it was possible to combine a group of fractions for recovery of methyl malvalate. The infra-red spectrum of the material was consistent with that of a cyclopropene methyl ester. Purity of the final product was confirmed by GLC (before and after hydrogenation), thin layer chromatography (TLC) and Kieselgel G plates with hexane—diethyl ether—glacial acetic acid (90:10:1) as solvents, and by mass spectrometry (Atlas CH-4 instrument).

A sample of the methyl malvalate was oxidized with periodate-permanganate reagent (8,18) and the resultant β-diketoester was divided into two portions, one of which was hydrolyzed as described by Faure and Smith (4), while the other was oxidized further with peracetic acid as described by Nunn (11). The oxidation and hydrolysis products were grouped into nonacidic and acidic fractions. The non-acidic products were analyzed by GLC, with methyl *n*-alkyl ketones as reference standards. The acids were separated further by steam distillation into steam-volatile and nonvolatile acids and analyzed by GLC after conversion into methyl esters with borontrifluoride-methanol reagent. (10)

Results and Discussion

A preliminary treatment of the *Sterculia foetida* methyl esters was necessary to obtain material suitable for the preparation of methyl malvalate by liquid-liquid chromatography. Saturated esters were mostly precipitated in the −16C urea adduct, and the main component, methyl sterculate, appeared to be preferentially precipitated in the −30C and −50C adducts, most of the methyl malvalate remaining in solution. Crystallization of the mother liquor residue from acetone at −70C was undertaken to reduce the content of very soluble unsaturated fatty acid esters, such as methyl linoleate, most of these remaining in the discarded filtrates.

The separation of methyl malvalate from the malvalate concentrate by liquid-liquid partition chromatography is illustrated in Figure 1.

The shaded area indicates the fractions which were combined for recovery of methyl malvalate. In two consecutive runs 1.62 g and 1.95 g of concentrate yielded 0.94 g and 1.11 g of methyl malvalate, mp −18C to −17C, 57% of the original concentrate. GLC and TLC analysis indicated that the methyl malvalate was at least 98% pure. Figure 1 indicates that the main impurities likely were methyl sterculate and oleate. A study of the mass spectra of the methyl malvalate at various energy levels revealed that methyl sterculate was present to the extent of 0.6% (molecular ion peak at m/e 308 being 0.5 to 0.6% of the malvalate molecular ion peak at m/e 294), and the m/e peak at 296 suggested that methyl oleate was also present (about 1%).

Oxidation of a sample of the methyl malvalate yielded a mixture containing a β-diketoester, analogous to the compound obtained from sterculic acid by Nunn (11) and Faure and Smith (4). Reaction with ethanolic ferric chloride solution gave a characteristic intense red coloration, and reaction with copper acetate in methanol yielded a copper chelate compound which crystallized from methanol as a pale blue solid, mp 92C–93C.

Hydrolysis of the diketoester (4) yielded a small quantity of non-acidic material as a fragrant oil containing methyl *n*-octyl ketone but the main products were acids, and oxidation of the diketoester with peracetic acid (11) also yielded only acids. In both reactions the steam-volatile acids consisted largely of pelargonic acid (over 60% of the mixture), but also contained *n*-octanoic and *n*-heptanoic acids and traces of other monocarboxylic acids; the nonvolatile acids contained suberic acid (80%) and several monocarboxylic acids considered to be products of over-oxidation by periodate-permanganate (18). The isolation and identification of pelargonic acid, suberic acid, and methyl *n*-octyl ketone from the degradation of the diketoester shows that the latter is methyl 8,10-dioxooctadecanoate (I):

$$CH_3(CH_2)_7\,\underset{\underset{O}{\|}}{C}\,CH_2\,\underset{\underset{O}{\|}}{C}\,(CH_2)_6\,COOCH_3 \qquad (I)$$

as would be expected from the structure (II), established for malvalic acid by previous workers (3,6,9, 19), which corresponds to 8,9-methyleneheptadec-8-enoic acid:

$$CH_3\ (CH_2)_7\ \underset{\diagdown\ \diagup}{\overset{CH_2}{C = C}}\ (CH_2)_6\ COOH \qquad (II)$$

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Phospholipids of Menhaden Muscle

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Abstract

The phospholipids of menhaden fish muscle represent about 0.5% of the wet weight. They were separated by fractionation on a silica gel column. Choline glycerophosphatides were the major components, about 60%. Ethanolamine and serine glycerophosphatides accounted for about 20%, and sphingomyelin and less well-characterized components for the rest. The fatty acid composition of the CGP,[1] EGP[1] + SGP,[1] and triglyceride preparations were compared. The EGP + SGP fraction contained 20% stearic acid whereas the neutral or CGP fractions had only 2–4%. The phospholipids contained about 30% of docosa-hexaenoic acids compared to about 10% in the neutral fraction.

Introduction

THE MENHADEN (*Brevoortia tyrranus*) is the most important United States fishery in terms of tonnage. Over a million tons are caught each year, essentially all of which are used for oil and meal manufacture (1). Information is available concerning the fatty acid composition of pressed menhaden oil (2) but the nature of the phospholipid components in menhaden muscle has not previously been described. This paper presents the results of such a study. The methods were those used in a parallel study of the phospholipids of tuna muscle (3).

Experimental

Raw Material

Menhaden fish weighing about 500 gm each were obtained as follows: They were caught Aug. 7, 1961, in Delaware Bay, eviscerated while still alive, and placed on dry ice within an hour after having been caught. They were then shipped with dry ice packaging and held at −18C.

TABLE I

Analyses of Menhaden Samples (Wet Weight)

Batch No.	No. fish	Wgt. muscle tissue gm.	Total lipid %	Phospholipid %	Residue %
1	15	372	3.53	0.55	16.0
2	18	433	3.56	0.45	16.2

[1] CGP refers to total choline diacylglycerophosphatides plus choline plasmalogens, EGP to ethanolamine glycerophosphatides and SGP to serine glycerophosphatides.

Preparation and Handling of Lipid Extracts

The fish were allowed to thaw, filleted, and the muscle tissue was separated and extracted with chloroform:methanol by the Bligh and Dyer (4) procedure as previously described (3). Yields are shown in Table I. The total lipids were stored in chloroform at −18C under nitrogen.

Fractionation and Analysis Procedures

A preliminary separation of phospholipids was achieved by passing solutions of the total lipids in chloroform through a silica gel column and washing with chloroform. The silicic acid had been carefully prepared by washing, deoxygenation and dehydration according to Rouser et al. (5). The portion that was not retained contained fatty acids, triglycerides, cholesterol and cholesterol esters. The phospholipids remaining on the column were eluted with chloroform: methanol (1:1), concentrated, and fractionated on another silica gel column. By this method, the amount of material to be fractionated was reduced; separations of the individual phospholipids were then more readily reproducible. Fractions were obtained by gradually increasing the amount of methanol in chloroform.

TABLE II

Tentative Identification of Menhaden Muscle Lipid Fractions

Eluant[a]	Identity[b]	Yield[c] %
0.5% CH_3OH	Pigment, oxid. glycerides, unknown	4
7% CH_3OH	White flaky solid, unknown	2
12% CH_3OH	EGP + SGP	17
17% CH_3OH	Same plus unknown white solid	3
23% CH_3OH	CGP	63
27% CH_3OH	Sphingomyelin	3
above 27% CH_3OH	Lysolecithin plus unknown	8

[a] Chloroform plus the amounts of methanol noted.
[b] Based on infrared absorption and analytical data. See footnote 1.
[c] Separations in duplicate runs occurred in the same order but were not always equally sharp or at the same methanol level. The data are from a single run.