Isolation of Methyl *cis*, *cis*-5,13-Docosadienoate from *Limnanthes douglasii* Oil

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ABSTRACT

Methyl *cis,cis*-5,13-docosadienoate has been isolated from the mixed methyl esters of the fatty acid moiety of *Limnanthes douglasii* oil by a combination of low temperature fractional crystallization and fractionation of mercuric acetate adducts. The methyl ester and its free acid have been characterized.

INTRODUCTION

The recent publication (1) of the synthesis of *cis,cis*-5,13-docosadienoic acid prompts us to publish our work on the isolation in pure form of the methyl ester of this acid from the mixed methyl esters derived from *Limnanthes douglasii* oil, and characterization of the methyl ester and its free acid. Bagby et al. (2) reported isolation of the acid in impure form and proof of structure by degradation of the tetrahydroxy compound derived from the acid by epoxidation followed by hydrolysis. No method for isolating moderate quantities of either *cis,cis*-5,13-docosadienoic acid or its methyl ester from *Limnanthes douglasii* oil has been reported. Since linoleic acid has been successfully purified either by low temperature fractional crystallization (3) or by urea complex fractionation (4,5), the possibility of using these techniques for purifying docosadienoic acid or its methyl ester was explored. Preliminary experiments indicated that while either technique was useful for concentrating the diene, neither appeared very promising as a means of purifying it in reasonable yield. The separation of methyl esters having different degrees of unsaturation by chromatography on a silver nitrate impregnated column has been reported by De Vries (6). While we were able to prepare methyl docosadienoate of approximately 99% purity, by gas-liquid chromatographic (GLC) analysis, by this method, the large quantities of the highly inflammable solvents, diethyl ether and petroleum ether, which would be required to prepare moderate quantities of material by this method made it unattractive. Stearns et al. (7) prepared 95% methyl linoleate by partitioning the mercuric acetate adducts of safflower methyl esters between methanol and petroleum ether. Our preliminary experiments indicated that the methyl docosadienoate could be more effectively purified by extraction of impurities from the methanol-free adduct with petroleum ether and that this technique could be combined with low temperature crystallization of the methyl esters to give a reasonably good yield of essentially pure methyl *cis,cis*-5,13-docosadienoate.

EXPERIMENTAL PROCEDURE AND DATA

Materials

Limnanthes douglasii oil was obtained by hexane extraction of the ground seed. The oil was converted to the methyl esters by alcoholysis with methanol employing sodium methoxide as catalyst. The composition of the methyl esters, as determined by GLC, was: $C_{16:0}$, 0.4; $C_{16:1}$, 0.2; $C_{18:0}$, 0.2; $C_{18:1}$, 1.9; $C_{18:2}$, 0.8; $C_{20:0}$ 0.6; $C_{20:1}$, 63; $C_{20:2}$, 0.6; $C_{20\text{-unknown}}$, 0.2; $C_{22:1}$, 20; $C_{22:2}$, 11; $C_{22\text{-unknown}}$, 0.4; $C_{22\text{-unknown}}$, 0.7%.

Unsaturated methyl esters and cyclic olefins used to prepare aldehyde standards were obtained from Hormel Institute and K& K Laboratories, Inc., respectively. ECNSS-S organosilicone polymer was obtained from Applied Science Laboratories, stabilized DEGS from Analabs and other GLC column packing materials from Wilkins Instrument Co.

Analytical Methods

GLC analyses were made on an Aerograph A-700 gas chromatograph equipped with a thermal conductivity detector, and employing ¼ in. O.D. aluminum columns. In all cases helium was used as flow gas and 80–100 mesh regular chromosorb W as stationary phase. All quantitative GLC analyses of methyl esters were carried out on a 10 ft stabilized-DEGS (20%) column operated at 245C and at a flow rate of 50 or 75 ml/min. Peak areas were determined by triangulation and corrected by multiplication by the square root of the molecular weight of the compound (8). Other columns and conditions were used for other special applications and these are described at the appropriate place in the paper.

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Iodine values were determined by the AOCS Official Method (9) and hydrogen-iodine values by the method of Pack and Planck (10). Elemental analyses were made by Midwest Microlabs, Inc.

Purification of Methyl cis,cis-5,13-Docosadienoate

Mixed methyl esters derived from *Limnanthes douglasii* oil, 1000 g, was dissolved in 9 liters of acetone and crystallized in a step-wise manner, first at −56C and then at −75C. The −75C precipitate fraction, 107.5 g, contained 70% C-22 diene by GLC.

A mixture of 50.0 g of the 70% methyl docosadienoate, 97.0 g of mercuric acetate and 100 ml of methanol was refluxed for 15 min, then cooled to room temperature and mixed with 500 ml of water. After the layers separated, the aqueous methanol was decanted, and then the residue was washed two additional times with water. The wet residue was transferred to a liquid-liquid extractor and extracted with boiling petroleum ether (bp, 30–60C). The progress of the extraction was followed by GLC analysis of esters regenerated by treatment with HCl from aliquots taken from the residue at intervals. Extraction was stopped after 5½ hr at which time the methyl esters regenerated from an aliquot of the residue contained 95% of methyl docosadienoate and no further purification appeared to be being effected. Adduct which oiled out of the petroleum ether extract collected during the last 2 hr of extraction had the same methyl docosadienoate content as the residue and was combined with it. Adduct recovered from the supernatant liquid from the last 2 hr of extraction was combined with adduct which oiled out of the remainder of the extract at −10C and reextracted with boiling petroleum ether. The two residues were combined, dissolved in ether, and first washed twice with concentrated HCl, then 3 times with water. The amber-colored residue, 18.5 g, remaining after drying over sodium sulfate and removal of the ether was distilled rapidly (mostly at 153–156C/50 μ) through a 3 in. Vigreaux column to yield 16.2 g of colorless liquid having the same methyl docosadienoate content, 95%, as the undistilled material.

Two recrystallizations from 10 ml of petroleum ether (bp 30–60C)/1 g of ester at −75C yielded 12.43 g of ester, which had mp −23.5 to −21.5C (uncorr) and $n_D^{25.0}$, 1.4617. Analysis on both the stabilized DEGS column and a 20% Apiezon L column, 4 ft in length, operated at 295C and a flow rate of 100 ml/min, indicated that the sample was pure methyl docosadienoate.

Anal. Calc. for $C_{23}H_{42}O_2$: C, 78.79; H, 12.07; O, 9.12; I.V. and hydrogen - I.V., 144.8. Found: C, 78.58; H, 12.10; O, 9.51; I.V., 145.8; hydrogen - I.V., 143.0.

No evidence of the presence of *trans* bonds was discernible when the sample was examined as a liquid film between salt plates on an "Infracord" infrared spectrophotometer.

Methyl docosanoate recovered from the hydrogen-I.V. determinations had the following constants: mp 51.6–52.7C (corr) (lit. (11) 53.3); $n_D^{59.2}$, 1.4357 (lit. (12) 1.4344).

Anal. Calc. for $C_{23}H_{46}O_2$: C, 77.90; H, 13.08; O, 9.02. Found: C, 77.90; H, 13.00; O, 9.02.

Location of Ethylenic Bonds in Methyl Docosadienoate

Methyl docosadienoate was converted to aldehyde fragments by reductive ozonolysis employing the method of Privett and Nickell (13). The aldehydes were analyzed by GLC employing columns of three different polarities: 10% EGNSS-S, 5 ft in length, programmed at 100–200C, gas flow, 75 ml/min; 15% QF-1, 4 ft in length, programmed at 80–200C, gas flow, 25 ml/min; and 20% Apiezon L, 4 ft in length, programmed at 100–225C, gas flow, 50 ml/min. In order to test the suitability of the GLC method for identification of aldehydes of the type expected from the methyl docosadienoate, a standard mixture consisting of pentanal, hexanal, octanal, nonanal, decanal, dodecanal, heptanedial, octanedial, methyl formylbutanoate, methyl formylpentanoate, and methyl formyloctanoate was prepared by addition of pentanal, octanal, and decanal to the products of reductive ozonolysis of methyl arachidonate, methyl oleate, methyl petroselinate, cycloheptene and cyclooctene. All components of the mixture were resolved except dodecanal and methyl formylbutanoate on the EGNSS-S column, nonanal and methyl aldehydopentanoate, and heptanedial and methyl formylpentanoate on the QF-1 column, and nonanal and heptanedial on the Apiezon L column; and the order of elution for the various aldehydes was different on each column. The aldehydes derived from methyl docosadienoate were shown to be methyl formylbutanoate, octanedial and nonanal by chromatographing on all three columns, both alone and mixed with known samples of these three aldehydes. Thus the ester is methyl 5,13-docosadienoate.

5,13-Docosadienoic Acid

A mixture of 0.18 g sodium hydroxide, 0.25 ml water, 6 ml ethanol and 1.00 g of methyl 5,13-docosadienoate was refluxed for 1 hr, cooled, diluted with 100 ml of water and extracted 3 times with 50-ml portions of diethyl ether. The soap solution was then acidified with 6 ml of 0.6 *N* HCl and extracted 3 times with 50-ml portions of petroleum ether. The petroleum ether solution was washed with water and dried over sodium sulfate, after which solvent was removed by stripping under vacuum with nitrogen gas. The acid, 0.76 g, had the following constants: mp −0.5 to 1.5C (uncorr); $n_D^{25.0}$, 1.4684 (lit. (1) mp −4C; n_D^{22}, 1.4697).

Anal. Calc. for $C_{22}H_{40}O_2$: C, 78.51; H, 11.98; O, 9.51. Found: C, 78.55; H, 12.17; O, 9.43.

DISCUSSION

Although attempts to purify methyl *cis,cis*-5,13-docosadienoate or its derived acid by several methods which have been used successfully with another *cis,cis*-diene, linoleic acid, were unsuccessful, methyl *cis,cis*-5,13-docosadienoate was isolated from the mixed methyl esters derived from *Limnanthes douglasii* oil in a yield of 24% of that present in the original esters by a combination of low temperature crystallization and mercuric acetate adduct fractionation techniques. The various analyses performed on the methyl ester indicate that it is essentially pure *cis,cis*-5,13-docosadienoate. The compound possesses some unusual infrared and nuclear magnetic resonance (NMR) characteristics which are being explored further and will be the subject of a future publication.

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