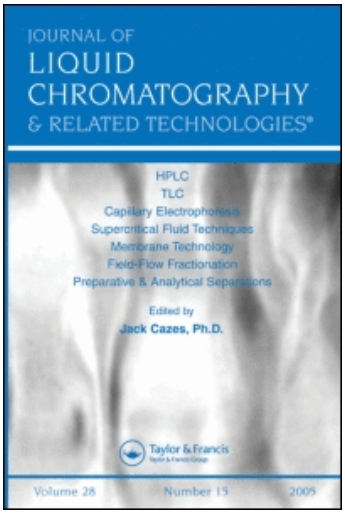

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PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF NATURAL FARNESOL ISOMERS (1)

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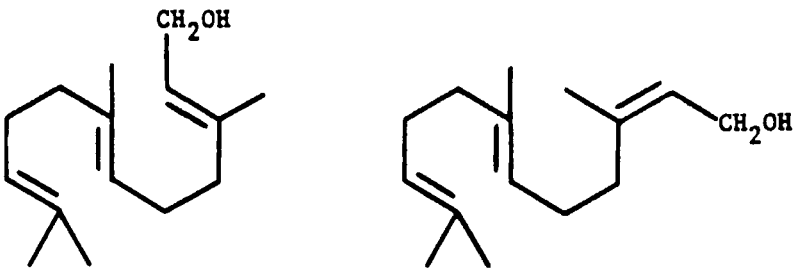
ABSTRACT

Farnesol, 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol, is a naturally occurring sesquiterpenoid that is used in perfumery and synthesis. In synthesis, it is used as a starting material for other terpenoids and insect juvenile hormone derivatives. A preparative high-performance liquid chromatographic separation procedure was developed whereby 2.1 g of 2E,6E-farnesol could be readily isolated from 4.8 g of a 1:1 mixture of the 2E,6E- and 2Z,6E-isomers of farnesol. This rapid one-step procedure simplifies the separation of the mixed isomers on a preparative scale.

INTRODUCTION

Farnesol ($C_{15}H_{26}O$), 3,7,11-trimethyl-2,6,10-dodecatrien-1-ol, is a sesquiterpenoid that occurs in small amounts in many plants (2). It occurs in nature (Fig.1) mainly as the 2E,6E-isomer (3,4), but the 2Z,6E-isomer also occurs in petitgrain oil and several other oils (5). Farnesol, consisting of the two naturally occurring geometric isomers (6,7), can be prepared commercially by isomerization (2) of the more abundant natural (E)-nerolidol.



(E,E)-3,7,11-Trimethyl-2,6,10-dodecatrien-

(Z,E)-3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol

FIGURE 1

Natural farnesol isomers.

The use of farnesol isomers in perfumery (5), in juvenile hormone bioassays (8), and in synthesis (9,10) often requires the separation of the two isomers for a specific purpose. The 2E,6E-isomer is particularly desirable as a starting material in synthetic design.

Although the four isomers of farnesol have been isolated in small amounts by gas-liquid chromatography (GLC) (11), this procedure would be too cumbersome and lengthy to utilize for separation of large quantities of the pure isomers. Bates et al. (2) reported that the 2E,6E-isomer has the longest retention time of the four by GLC and the highest boiling point. Since Corasil II (12) was used effectively to separate 2E,6E- and 2Z,6E-methyl farnesates, it seemed likely that a preparative separation procedure could be developed with the use of a silica column. Therefore, such a rapid preparative high-performance liquid chromatographic (HPLC) procedure was developed for the separation of the two natural stereo-

isomers of farnesol as opposed to the utilization of the difficult stereospecific synthetic route to the 2E,6E-isomer (4).

MATERIALS AND METHODS

Apparatus

A Waters Associates (Milford, Mass.) Model ALC-100 Liquid Chromatograph equipped with a Model 6000A pump, a U6K closed-loop injector, a Kratos Inc. Schoeffel Instrument Division (Westwood, N.J.) SF 770 multiwavelength UV detector at 210 nm, and an R-400 differential refractometer was used for analytical HPLC. The column used was μ Porasil (30 X 0.39 cm I.D., Waters Associates).

A Waters Associates Prep LC/System 500 Liquid Chromatograph equipped with two Prep PAK-500/SILICA cartridges was used for preparative HPLC.

Chemicals

Solvents used for HPLC were 2,2,4-trimethylpentane (LiChrosolv) from E. Merck (Darmstadt, Germany) and diethyl ether (A.C.S. reagent) from Fisher Scientific Co. (Fair Lawn, N.J.). Farnesol (natural stereoisomers) was obtained from International Flavors and Fragrances (IFF) (Union Beach, N.J.); samples of farnesol (mixed stereoisomers) were obtained from Aldrich Chemical Co. (Milwaukee, Wisc.) and Fluka AG (Buchs, Switzerland).

GLC

A Packard (Downers Grove, Ill.) Model 417 Gas Chromatograph equipped with a column (91.4 X 0.2 cm I.D.) packed with 2% OV-101 on Chromosorb W-HP 100/120 mesh (Alltech Associates, Arlington Heights, Ill.) with 30 cc/min N_2 flow rate at 100° C was used for

GLC analyses with flame ionization detection. Injections (1 µl) of farnesol (natural stereoisomers) and the isolated 2E,6E-isomer at a concentration of 1 µl/ml in 2,2,4-trimethylpentane were made to determine isomer content.

Analytical HPLC

Farnesol (natural stereoisomers) and the isolated 2E,6E-isomer (30 µg/ml in 2,2,4-trimethylpentane) were injected (10 µl) into a µ-Porasil column (30 X 0.39 cm I.D.) with a flow rate of 1.8 ml/min of 90/10 2,2,4-trimethylpentane/diethyl ether and detection at 210 nm.

A maximum of 12 mg farnesol could be injected into the analytical column (RI detection); this allowed enough separation of the isomers for preparative HPLC.

Preparative HPLC

An injection of 2.4 g (200 X the analytical amount) of farnesol in sufficient 2,2,4-trimethylpentane to make 5 ml of solution was made into a single Prep PAK-500/SILICA cartridge with a flow rate of 350 ml/min (≈200 X the analytical rate) of 90/10 2,2,4-trimethylpentane/diethyl ether and with RI detection. The R_V was noted and then 4.8 g of farnesol in sufficient 2,2,4-trimethylpentane to make 10 ml of solution was injected into two Prep PAK-500/SILICA cartridges with the same flow rate, elution solvent, and detection method.

RESULTS AND DISCUSSION

GLC analyses of farnesyl acetate, prepared in this laboratory from farnesol (mixed stereoisomers), had revealed the presence of

three peaks, assumed to be the 2Z,6Z-, mixed 2Z,6E- and 2E,6Z, and the 2E,6E-isomers, respectively. Since reversed-phase HPLC analysis of the mixed acetates showed only one peak, it was examined on μ Porasil. Two peaks were observed, and collections of each peak were analyzed by GLC. According to GLC, each HPLC peak contained two isomers: the first contained the mixed 2Z,6Z- and 2Z,6E-isomers, and the second contained the mixed 2E,6Z- and 2E,6E-isomers. Therefore, the alcohols (two samples of farnesol mixed stereoisomers) were analyzed by GLC. The presence of four isomers was observed in each sample. HPLC analysis showed only two peaks in each sample as with the farnesyl acetate, but separation was better than with the acetate isomers.

Since it was not possible to separate the 2E,6Z- and 2E,6E-isomers, only farnesol containing the natural stereoisomers offered any hope of successful isolation of the pure 2E,6E-isomer if a tedious stereospecific synthesis (4) was to be avoided. Figure 2 shows the HPLC analyses of the IFF farnesol and the isolated 2E,6E-isomer. The R_v for the 2E,6E-isomer is 39.6 (22 min), and that for the 2Z,6E-isomer is 32.4 (18 min). On the Prep 500 with two silica cartridges, the corresponding R_v values are 9450 and 7700 respectively.

One recycle of the 2E,6E-isomer 0.5 min after the trough between the two isomers also aided in purification. The 80-min procedure requires only 15 L of eluant as opposed to the theoretically required 28 L. Recycle and the return of eluate to the reservoir for the first 12 min of the run allows for the reuse of 13 L of solvent. Waste and collected eluate were flash evaporated

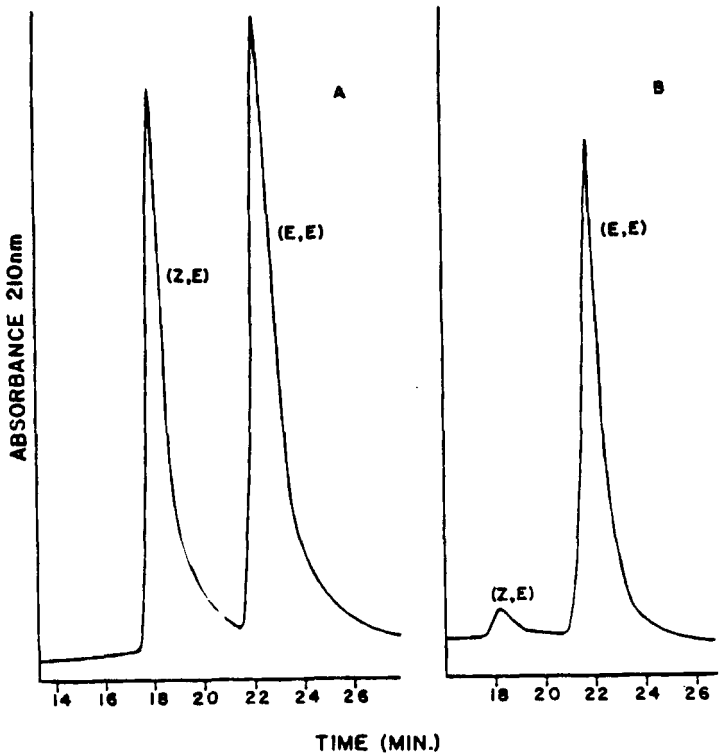


FIGURE 2

HPLC analytical analyses of (A) IFF farnesol and (B) (E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol on μ Porasil (30 x 0.39 cm I.D.) at 1.8 ml/min of 90/10 2,2,4-trimethylpentane/diethyl ether.

under reduced pressure. The recovered solvent was then fractionally distilled to recover the 2,2,4-trimethylpentane for reuse.

Figure 3 shows the GLC analyses of the IFF farnesol and the isolated 2E,6E-isomer. A total of 2.1 g 2E,6E-farnesol of high isomeric purity can be obtained from 4.8 g of farnesol (containing roughly 1:1 natural stereoisomers). The separation in 80 min of

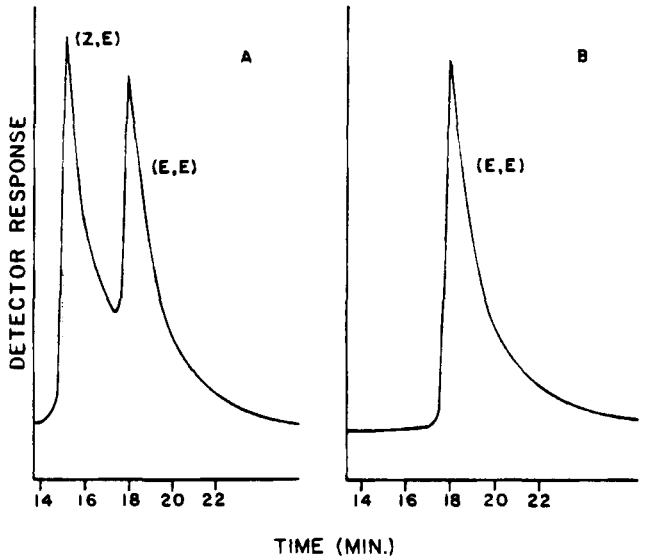


FIGURE 3

GLC analyses of (A) 1FF farnesol and (B) (E,E)-3,7,11-trimethyl-2,6,10-dodecatrien-1-ol on 2% OV-101 (91.4 x 0.2 cm I.D.) with 30 cc/min N_2 at 100°C.

gram amounts of two compounds differing only in their geometry around one double bond is of great utility.

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