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Note

Separation of farnesol isomers by liquid chromatography

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Farnesol (3,7,11-trimethyldodeca-2,6,10-triene-1-ol) is a linear sesquiterpene having four geometric isomers due to the double bonds at the C-2 and C-6 positions: 2-*cis*,6-*cis* (2c-6c), 2-*cis*,6-*trans* (2c-6t), 2-*trans*,6-*cis* (2t-6c) and 2-*trans*,6-*trans* (2t-6t) isomers. The widespread occurrence of the 2t-6t isomer has been recognized in many higher plants, and the presence of the 2c-6t isomer accompanied by the 2t-6t isomer has been reported in petigrain and some other plant oils[1]. On the other hand, a commercially obtained synthetic farnesol contained all of four isomers.

The separation of farnesol isomers has been investigated by gas and liquid chromatography. Using gas chromatography only small amounts of the pure isomers are collected[2]. The liquid chromatographic separation of farnesol isomers has been carried out using silica gel as the stationary phase; the four isomers have been separated into two fractions consisting of 2c-6c + 2c-6t and 2t-6c + 2t-6t isomers[3].

In this work a farnesol mixture was separated into the four isomers by liquid chromatography using styrene–divinylbenzene copolymer gel as the stationary phase. The separation mechanism is discussed in terms of the solvent polarity.

EXPERIMENTAL

Materials

Farnesol containing all four isomers was obtained commercially (Aldrich, Milwaukee, WI, U.S.A.) and was used without further purification. All the eluents were obtained commercially and were used without further purification.

Gas chromatography

Gas chromatography was carried out using a Hitachi 163 gas chromatograph equipped with a 30-m glass capillary column coated with free fatty acid polyester (FFAP).

Liquid chromatography

Liquid chromatography was carried out using a JASCO Trirotar II as a high-pressure pump and a Waters R 401 differential refractometer as a detector. For analytical purposes a 500 × 10 mm I.D. stainless-steel column was used and for preparative purposes a 600 × 21 mm I.D. column. The columns were packed with

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styrene–divinylbenzene copolymer gel having an exclusion limit of 3000 in gel permeation chromatography, which was prepared by conventional suspension polymerization[4]. The flow-rate was controlled at 1 and 6 ml/min in analytical and preparative work, respectively.

RESULTS AND DISCUSSION

Elution behaviour of farnesol

The separation of farnesol was examined with an analytical column using seven eluents. The elution volumes of farnesol are given in Table I together with the solvent strengths on alumina and the solbility parameter for each eluent[5]. Using chloroform as the eluent farnesol was eluted at an elution volume of 16 ml, earlier than with any other eluent. This finding indicates the absence of adsorption of farnesol on the gel in chloroform. Acetone and diisopropyl ether eluted farnesol at an elution volume of *ca.* 33 ml, indicating weak adsorption of farnesol on the gel. With these three eluents separation due to geometric isomerism was not observed.

TABLE I

ELUTION VOLUMES OF FARNESOL

Measured with a 500 × 10 mm I.D. column at a flow-rate of 1.0 ml/min.

Eluent	*Solvent strength on alumina*[5], ε^0	*Solubility parameter*[5], δ	*Elution volume of farnesol (ml)*
2,2,4-Trimethylpentane	0.01	7.1	78.0, 86.5
Cyclohexane	0.04	8.2	55.1, 58.2
Diisopropyl ether	0.20	7.3	33.6
Chloroform	0.40	9.1	15.3
Acetone	0.56	9.4	32.6
Acetonitrile	0.65	11.8	53.2, 54.0, 55.2
Methanol	0.95	12.9	61.3, 65.2, 69.9

Cyclohexane, 2,2,4-trimethylpentane, acetonitrile and methanol eluted farnesol at elution volumes of 50–90 ml, indicating strong adsorption. With these eluents farnesol was separated into two or three peaks, as shown in Fig. 1. In methanol and acetonitrile farnesol was separated into three peaks, which were identified by gas chromatography of the collected fractions as 2c-6c, 2t-6c + 2c-6t and 2t-6t isomers, in order of increasing elution volume. On the other hand, cyclohexane and 2,2,4-trimethylpentane provided only two peaks; 2c-6c and 2c-6t isomers eluted earlier than 2t-6c and 2t-6t isomers.

The different separation patterns of these eluents can be explained as follows. In methanol and acetonitrile farnesol was adsorbed on the gel by the alkenyl part of the molecule, because these eluents are more polar than the gel, as can be seen from their solubility parameters. Therefore, the separation was governed by the total number of *cis* or *trans* double bonds in the isomers, as illustrated in Fig. 2a. On the other hand, in cyclohexane and 2,2,4-trimethylpentane, which are less polar than the gel, farnesol was adsorbed on the gel by the terminal hydroxyl group. This means that

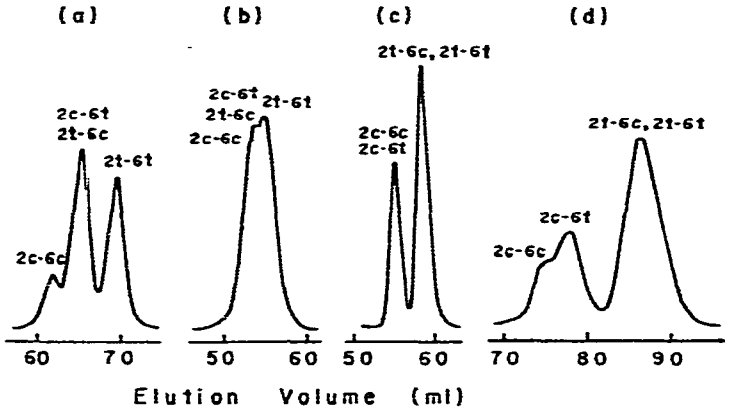

Fig. 1. Liquid chromatograms of farnesol. Eluent: (a) methanol; (b) acetonitrile; (c) cyclohexane; (d) 2,2,4-trimethylpentane.

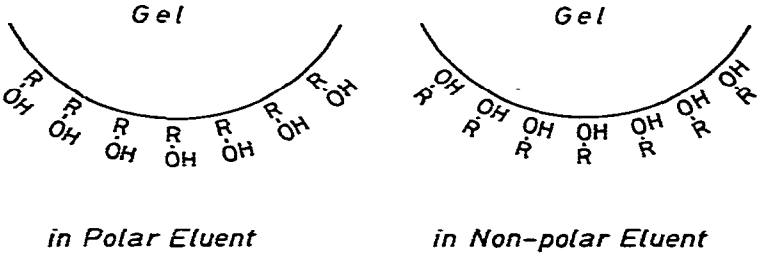

Fig. 2. Schematic representation of the adsorption of farnesol on the polymer gel.

the separation was carried out mainly according to the *cis* or *trans* configuration of the double bond at the C-2 position, as illustrated in Fig. 2b.

From these findings the eluents can be classified into three groups: (1) polar eluents, providing three peaks; (2) eluents with medium polarity, providing no separation due to geometric isomerism; and (3) non-polar eluents, providing two peaks. With styrene oligomers the solubility parameter was demonstrated to be a good indicator of the polarity of eluents[6]. However, with farnesol the solubility parameter is not a good indicator, because diisopropyl ether is classified as an eluent of medium polarity, although it has a small solubility parameter. This can be explained by the presence of a hydrogen bond between the hydroxyl group in farnesol and the ether oxygen atom in the eluent. In such a case the solvent strength on alumina is a better indicator of the polarity of the eluents, as shown in Table I.

Preparative separation of farnesol

The preparative separation of farnesol was carried out using methanol and cyclohexane as eluents, because these eluents provided columns with a high number of theoretical plates and good resolution for farnesol isomers. With a sample size of 120 mg in 1 ml almost pure 2c-6c and 2t-6t isomers were obtained after six recyclings (*ca.* 4 h) with methanol as the eluent (Fig. 3a). The 2c-6t and 2t-6c isomers overlapped with each other and displayed only one peak, even after seventeen recyclings. How-

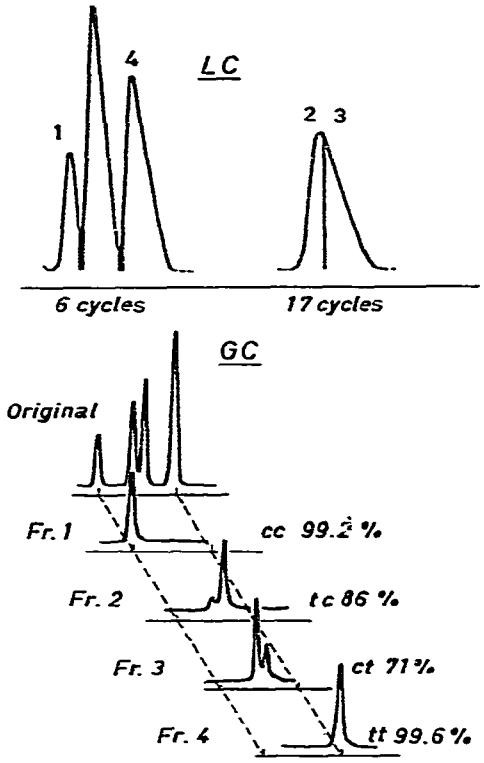

Fig. 3. Preparative-scale fractionation of fornesol isomers using methanol as eluent, and the composition of each fraction analysed by gas chromatography.

ever, the first half of the peak contained 86 % of the 2t-6c isomer and in the latter half the 2c-6t isomer was enriched up to 71 %, which was detected by gas chromatography (Fig. 3b).

The sample size was increased from 120 mg to 200, 400 and 600 mg in 1 ml. With a 200-mg injection the separation was almost the same as with a sample size of 120 mg. With further increases in sample size each peak became broader. By removing a portion of the first and the last peak several times in the course of recycling, which is called the "shaving" method, almost pure 2c-6c and 2t-6t fractions and a mixture of 2t-6c and 2c-6t isomers were collected after eight or nine recyclings (Fig. 4).

The second fraction obtained by fractionation with methanol as the eluent, which contained 2c-6t and 2t-6c isomers, was separated into almost pure isomers with cyclohexane as the eluent after eight recyclings, as shown in Fig. 5.

The purities of the four fractions obtained above were shown by gas chromatography to be more than 98 %. Thus, by combination of the two eluents, methanol and cyclohexane, synthetic farnesol was separated into four almost pure isomers.

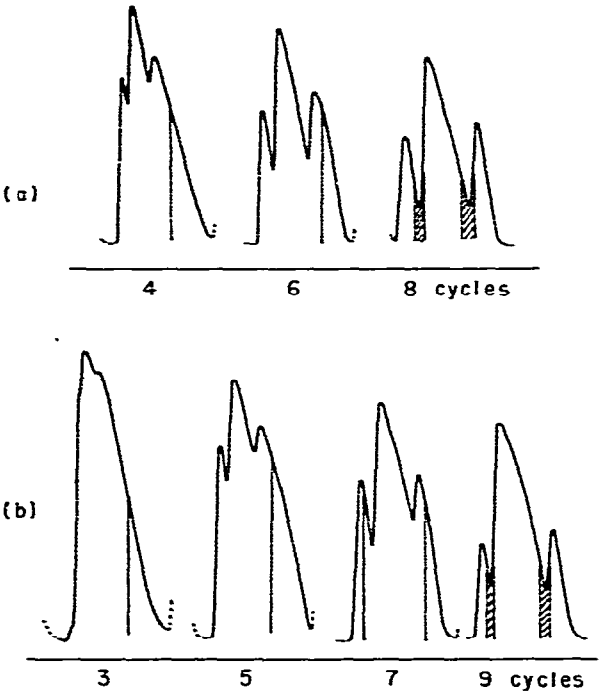

Fig. 4. Preparative-scale fractionation of farnesol isomers using methanol as eluent with sample sizes of (a) 400 mg and (b) 600 mg.

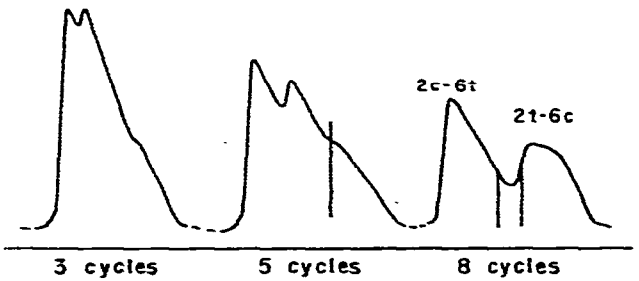

Fig. 5. Separation of the 2c-6t and 2t-6c isomers with cyclohexane as eluent with a sample size of 450 mg.

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