Note

Semipreparative high-performance liquid chromatographic separation of a characterized flavor mixture of monoterpenes

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The use of high-performance liquid chromatographic (HPLC) techniques for the separation of volatile flavors and essential oils has already been tried on several occasions and has been recently discussed by Ross[1]. Although the resolution afforded by gas–liquid chromatography (GLC) for the separation of volatile flavor constituents (especially monoterpenes) remains unsurpassed, it is conceivable that HPLC could speed up considerably any necessary prefractionation required for optimal semipreparative GLC separations of complex mixtures having varied functionality. For example, the separation of mixtures of monoterpenes by classes consisting of hydrocarbons, ethers, ketones, monoalcohols and diols is usually done by column LC on silica gel[2]. In addition, a second chromatography is usually necessary to resolve further those fractions containing refractory mixtures of ketones and monoalcohols, frequently present in oxidized citrus oils. This requires the use of gradient elution procedures, which are slow and laborious.

In the work reported here, we have found that a completely identified model mixture consisting of (+)-limonene (1), (+)-carvone (2), *trans-p*-mentha-1(7),8-dien-2-ol (3), *cis-p*-mentha-1(7),8-dien-2-ol (4), *trans*-carveol (5), *cis*-carveol (6),
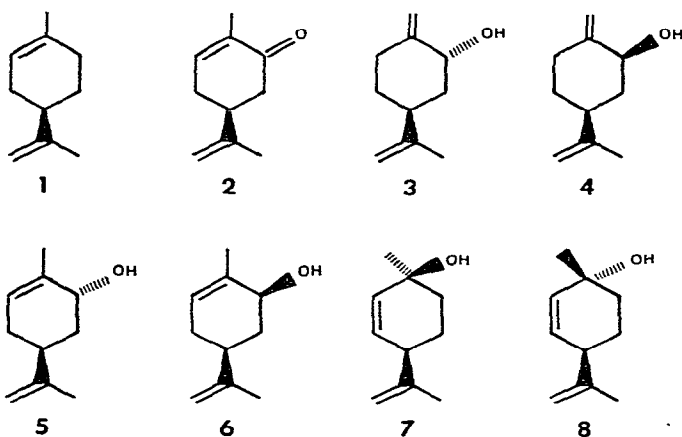
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trans-p-mentha-2,8-dien-1-ol (7) and *cis-p*-mentha-2,8-dien-1-ol (8) could be pre-fractionated satisfactorily in 15 min on a Partisil 10 column. The HPLC system was operated on a semipreparative scale with resolutions much superior to the ordinary column silica gel prefractionations that required 48 h. In work now in progress, a similar method is being applied to the analysis of a more complex mixture of mono-terpenes derived from the autoxidation of limonene.

EXPERIMENTAL

Chemicals
        (+)-Limonene (1) was from cold-pressed orange oil and was purified to 99.8 % by distillation followed by percolation through a column of basic alumina (activity I).
        The alcohols (3–8) were prepared from (+)-limonene by the rose bengal-sensitized singlet oxygen reaction of Schenck *et al.*[3].
        (+)-Carvone from oil of caraway was from Fritzsche D and O (New York, N.Y., U.S.A.) and was used without further purification.
        The model chromatographic mixture consisted of the above crude oxidation mixture plus carvone added at a ratio of 1:7 (w/w). The total area percentage of each compound was determined by analytical GLC (Fig. 1).
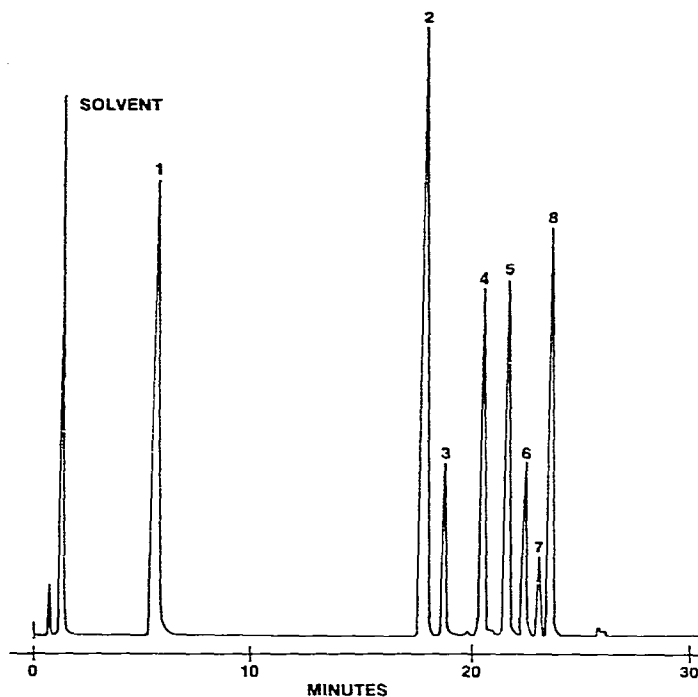

Fig. 1. Analytical GLC separation of the model chromatographic mixture. 0.05 μl on a 12-ft. glass column; 5 % Triton X-305; programmed 5 /min, 70–170 °C, 5 min initial time; area percent corrected for RWR. 1 = Limonene, 20 %; 2 = *trans-p*-mentha-2,8-dien-1-ol, 24 %; 3 = *cis-p*-mentha-2,8-dien-1-ol, 6 %; 4 = carvone, 14 %; 5 = *trans-p*-mentha-1(7),8-dien-2-ol, 12 %; 6 = *trans*-carveol, 6 %; 7 = *cis*-carveol, 3 %; 8 = *cis-p*-mentha-1(7),8-dien-2-ol, 14 %.
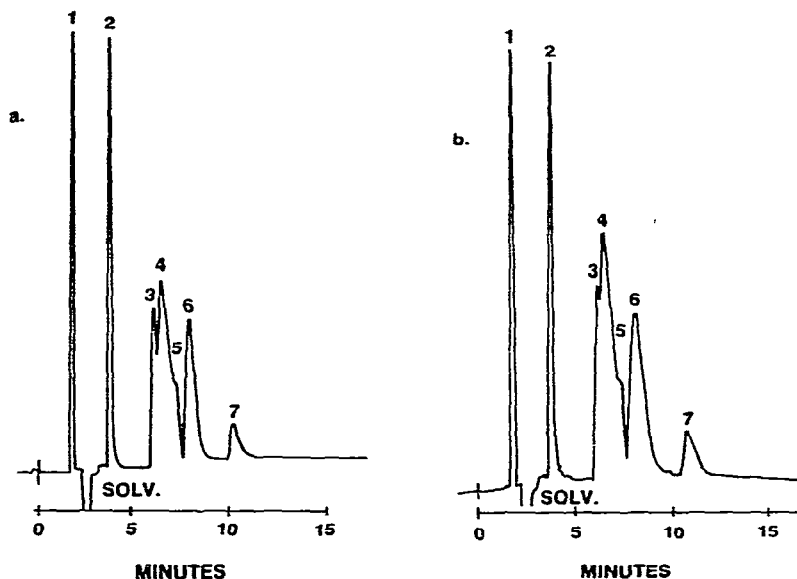
Fig. 2. HPLC separation of the terpene mixture with methylene chloride–ethyl acetate. 1 = Limonene; 2 = carvone; 3 = trans-p-mentha-1(7),8-dien-2-ol; 4 = cis-p-mentha-1(7),8-dien-2-ol; 5 = trans- and cis-carveol; 6 = trans-p-mentha-2,8-dien-1-ol; 7 = cis-p-mentha-2,8-dien-1-ol. a, 4 µl of neat solution; b, 7 µl.
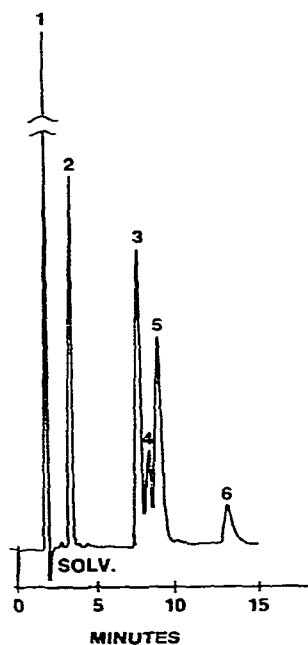

Fig. 3. HPLC separation of 2 µl mixture with ethyl acetate–hexane (10:90). 1 = Limonene; 2 = carvone; 3 = trans- and cis-p-mentha-1(7),8-dien-2-ol; 4 = trans- and cis-carveol; 5 = trans-p-mentha-2,8-dien-1-ol; 6 = cis-p-mentha-2,8-dien-1-ol.

Equipment

A Waters Model ALC/GPC 201 HPLC system was used and included a M-6000 pumping system, a M-U6K universal injector and a M-R 401 differential refractometer. A Whatman prepacked Partisil 10-PXS column (25 cm × 4.6 mm I.D. stainless steel) packed with 10-μm silica gel was used in series with a guard column (7 cm × 2.1 mm I.D. stainless steel) packed with Whatman HC-Pellosil, a pellicular silica. The flow-rate was approximately 2 ml/min and the solvent was degassed under aspirator vacuum for 1 min.

RESULTS AND DISCUSSION

Fig. 2a shows the separation obtained on Partisil 10 with ethyl acetate–methylene chloride (2.5:97.5, v/v). Methylene chloride–hexane mixtures and 100% methylene chloride were not as effective. The order of elution on HPLC differs from the separation observed on GLC (Fig. 1). A preparative-scale run (7 μl) gave a respectable separation of most of the constituents with proper collection (Fig. 2b). Another useful HPLC separation of the mixture was obtained with ethyl acetate–hexane (10:90, v/v) (Fig. 3). Fractions were collected from each HPLC run and the peak composition confirmed by GLC. The separations were surprisingly good considering the complexity of the alcohol fraction.

REFERENCES

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