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Note

Separation of essential oils by liquid chromatography

HISASHI KOMAE and NANAO HAYASHI
Faculty of Integrated Arts and Sciences, Hiroshima University, Hiroshima 730 (Japan)
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Essential oils consist of mixtures of volatile compounds that are often complex, and almost any type of organic compound may be found. In general, the analysis of essential oils is performed by means of gas chromatography, as they contain volatile compounds. Liquid chromatography, however, is the preferred method for evaluating such materials, as they may be sensitive to changes in temperature.

Gel permeation chromatography (GPC) is a technique used for the separation of polymeric materials on the basis of molecular size. The use of μStyragel column packing material (μ indicates it is of low diameter, 10 μ) developed by Waters Associates[1], permits the separation of compounds of small molecular size by high-performance liquid chromatography. This study was aimed at developing a GPC method that is applicable to essential oils.

Little work has been reported on the separation of essential oils or terpenic compounds. Waters Associates[2] reported the analysis of the essential oils anise, cojeput, cedarwood, rose and wormwood using a Porasil column with chloroform. Recently, Nakayama *et al.*[3] reported the separation of mono- and sesquiterpene compounds by liquid chromatography using a Hitachi 3010 gel column with methanol, *n*-hexane and methanol–water.

The purpose of this paper is to indicate the possibility of using liquid chromatography for the analysis of essential oils.

EXPERIMENTAL

Apparatus

A Waters Ass. Model ALC202 liquid chromatograph was used, with an M6000 pumping system. Injections were made with a Waters Ass. Model U6K universal injector and detection was carried out with a UV monitor at 254 nm or with a Waters Ass. Model R401 refractive index detector. A μBondapak C_{18} column (300 × 4 mm) was used for analysis. For GPC analysis, a 300 mm × 9.52 mm O.D. × 7.6 mm I.D. μStyragel column, made of seamless stainless-steel tubing, was used.

Essential Oils

The essential oils of *Lindera umbellata* and *L. sericea* were obtained by steam distillation of the fresh leaves. The physical properties of two essential oils were as follows: *L. umbellata*, d_4^{25} 0.8925, n_D^{25} 1.4658, α_D^{25} −17°; *L. sericea*, d_4^{25} 0.9736, n_D^{25} 1.4955, α_D^{25} +34.1°.

RESULTS

Fig. 1 shows the liquid chromatograms of the essential oils of the two *Lauracea* species obtained by using a μBondapak C_{18} column with methanol–water (1 :1). The essential oils were separated completely into more than 20 peaks.

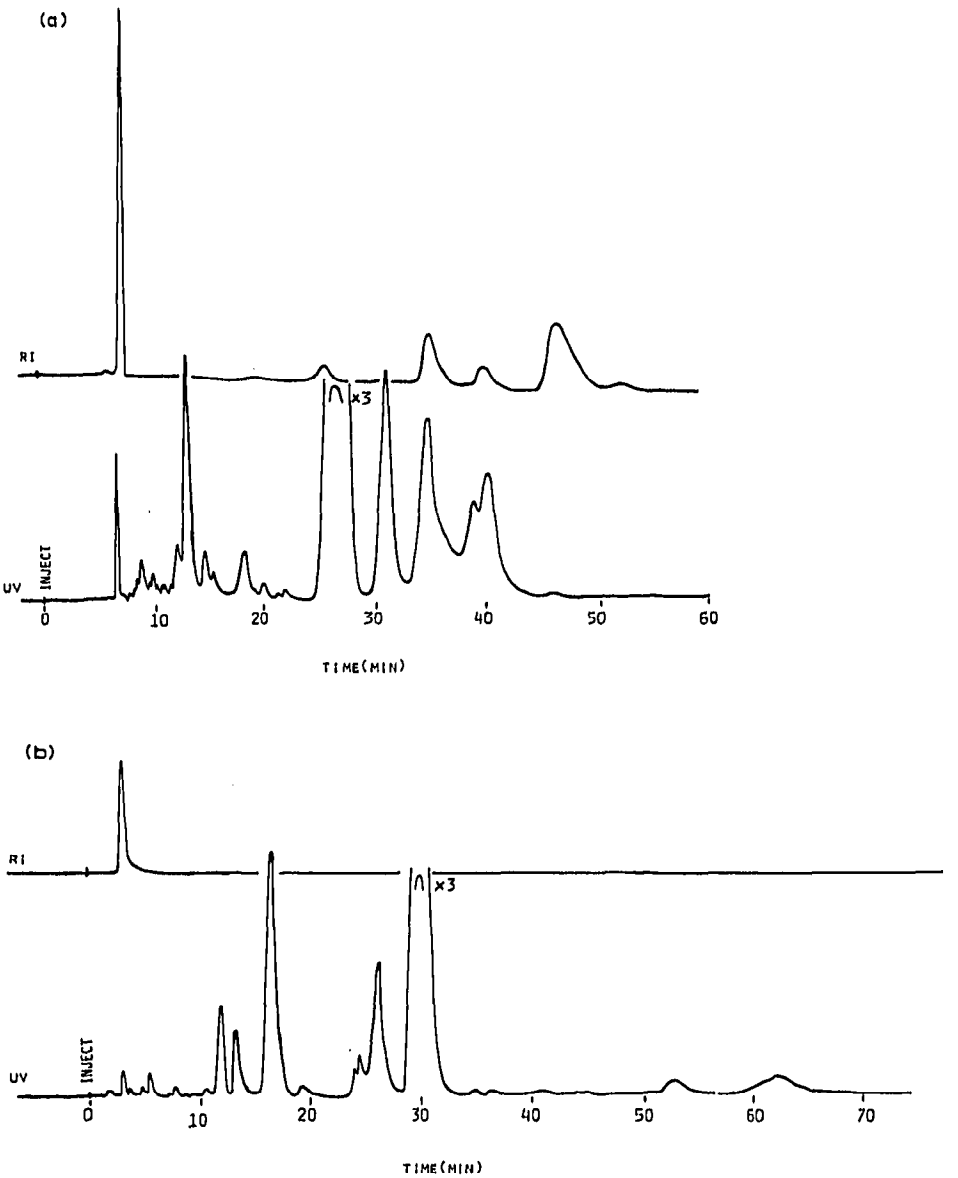

Fig. 1. Separation of essential oils on a μBondapak C_{18} column (300 × 4 mm). Mobile phase, methanol–water (1 :1); flow-rate, 1 ml/min. (a) *L. umbellata* Thunb.; (b) *L. sericea* var. *glabrata* Blume.

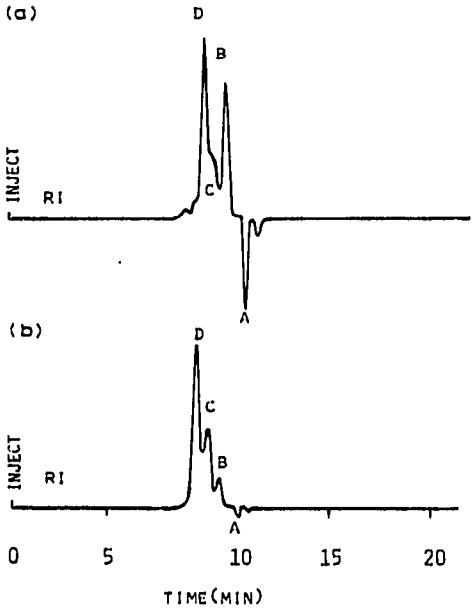

Fig. 2. GPC analysis of essential oils on a μStyragel column of dimensions 300 × 9.52 mm O.D (1 × 10^3 Å, 1 × 500 Å, 2 × 100 Å). Mobile phase, tetrahydrofuran; flow-rate, 4 ml/min. (a) *L. umbellata* Thunb.; (b) *L. sericea* var. *glabrata* Blume. Peaks: A, monoterpene hydrocarbon (mol. wt. 136); B, monoterpene alcohol (mol. wt. 154); C, monoterpene acetate (mol. wt. 196) and sesquiterpene hydrocarbon (mol. wt. 204); D, sesquiterpene alcohol and ketone (mol. wts. 220 and 218).

Gel permeation chromatograms of the essential oils are shown in Fig. 2, indicating a separation into at least five peaks. These separations were obtained in less than 15 min using a μStyragel column (packing consisting of one 10^3-Å column, one 500-Å column and two 100-Å columns).

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