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Note

Microcolumn chromatography as an aid to the gas chromatographic analysis of volatile oils

M. S. F. ROSS

Pharmacognosy Section, School of Pharmacy, U.W.I.S.T., King Edward VII Avenue, Cardiff, Glamorgan, Wales (Great Britain)

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The important constituents of most volatile oils fall into three main categories, *viz.* monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes.

The best gas chromatographic (GC) separations of these compounds are routinely carried out on Carbowax-type stationary phases and frequently interpretations of the analyses are complicated by overlap of the oxygenated monoterpene and sesquiterpene components. Separation of the oxygenated from the non-oxygenated compounds greatly aids interpretation. This separation is simply carried out by column chromatography using silica, Florosil[1], pretreated silica[2], and alumina[3]. These separations have all been carried out using normal columns which require relatively large amounts of oil. When examining volatile oil containing plants in the laboratory, these large amounts of oil are frequently not available, especially where the oil from a specific part of a plant is required[4].

Microcolumn chromatography has been previously used as a clean-up procedure before analysis, particularly of pesticides[5], and techniques have been described using columns as small as 4.2 mm I.D.[6]. Use of this type of column for fractionation of volatile oils was found to require 0.2–0.5 ml of neat oil.

A microcolumn separation using capillary tubes of 1 mm I.D. in combination with a GC automatic sampler using metal capsules allows the separation and GC analysis of the monoterpene and sesquiterpene hydrocarbons from as little as 2 μl of oil. By comparison of the chromatograms obtained before and after column separation the oxygenated terpenes may also be identified.

Because the time spent on the column is short (approximately 2 min) there is little danger of the types of rearrangement that may occur on larger columns and should such a rearrangement occur it will be easily detected by a comparison of the chromatograms of the fractionated and the neat oil.

EXPERIMENTAL

Microcolumn fractionation

A capillary tube (100 × 1 mm I.D.) with a cotton or glasswool plug was dry packed to a height of 50 mm with silica gel for column chromatography (Merck Kieselgel 60) and petrol (b.p. 40–60°) allowed to run through until all the air was

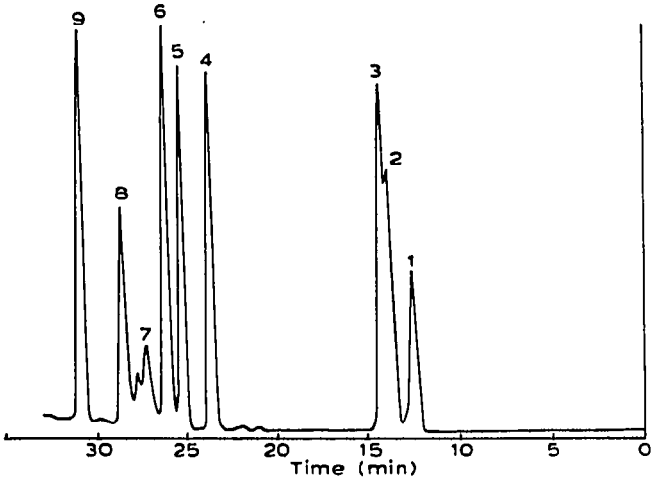

Fig. 1. GC analysis of volatile oil components. 1 = α-Phellandrene; 2 = limonene; 3 = cineole; 4 = linalol; 5 = bornyl acetate; 6 = caryophyllene; 7 = citral a; 8 = citral b; 9 = geraniol.

displaced. The neat oil (2 μl) was injected on to the top of the column under a 5-mm head of petrol using a standard GC syringe. The column was topped up with petrol and the eluent collected in a capsule for use in the Perkin-Elmer AS 41 automatic injection system.

GC analysis

GC analyses were performed using a Perkin-Elmer F30 gas chromatograph with an AS 41 injection system and a flame ionization detector. Separation was on 6-ft. stainless-steel columns with 15% Carbowax 20M stationary phase on Chromo-

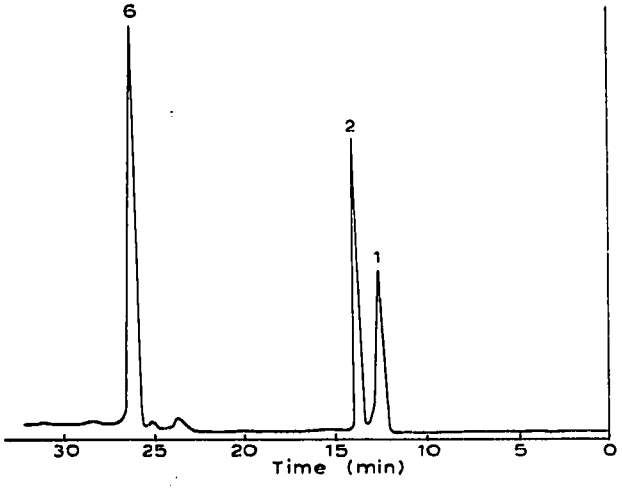

Fig. 2. GC analysis of volatile oil components after microcolumn fractionation. 1 = α-Phellandrene; 2 = limonene; 6 = caryophyllene.

sorb W 80–100 mesh. Nitrogen at a flow-rate of 40 ml/min was the carrier gas and the temperature was 60° for 5 min, then rising at 5°/min to 220°.

RESULTS AND DISCUSSION

The GC separation of a mixture of typical volatile oil constituents is shown in Fig. 1.

Fig. 2 shows the GC analysis of the non-oxygenated fraction obtained by microcolumn fractionation of 2 μl of the mixture. Only peaks 1, 2 and 6 are obtained, corresponding to the hydrocarbon components of the oil. Comparison of the two chromatograms shows how easily the sesquiterpene caryophyllene may be distinguished from the oxygenated monoterpenes and cineole (an oxygenated monoterpene) separated from limonene.

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