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Note

Improved gas chromatographic analysis of naturally occurring monoterpene hydrocarbons following pre-fractionation by liquid–solid chromatography

J. J. C. SCHEFFER and A. BAERHEIM SVENDSEN

Department of Pharmacognosy, State University of Leyden, Gorlaeus Laboratories, P.O. Box 75, Leyden (The Netherlands)

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Few groups of naturally occurring substances contain as many compounds as that of the lower terpenes, and a number of attempts have been made to optimize their separation and identification. Gas chromatographic (GC) analysis of a total essential oil does not usually give a complete separation of all of the components present, peaks of the hydrocarbon constituents often overlapping with those of oxygen-containing compounds. The most common method of fractionation used is separation of the hydrocarbons from the oxygenated terpenoids according to Kirchner and Miller[1]. The hydrocarbons are separated from the total oil by column chromatography on silica gel by elution with hexane. However, because of the large variations in the amounts of the different components present in such mixtures, problems may arise in their GC separation and identification. In order to be able to detect compounds which are present in small amounts, it is necessary to use relatively large amounts of the sample in the analysis. This often results in overlapping of the peaks of the main components with those of the other components, even if the retention times are quite different. Under such circumstances, special measures have to be taken in order to obtain a satisfactory separation and identification of all of the components present.

In this paper we describe an attempt to solve this problem by means of a pre-fractionation of the monoterpene hydrocarbon mixture of essential oils on a silica gel column by elution with pentane, collection of a number of small fractions and subsequent GC analysis of each fraction.

EXPERIMENTAL

Material

The oil tested was the essential oil of the needles of *Abies alba* Miller (Edeltannennadelöl; Dragoco, Holzminden, G.F.R.).

Liquid–solid chromatography

A chromatographic tube (30 cm × 18 mm I.D.) with a cooling cap was used. The temperature was kept at *ca.* 10°. The column was packed with a slurry of 35 g of Kieselgel (particle size, 0.05–0.2 mm; E. Merck, Darmstadt, G.F.R.) and pentane

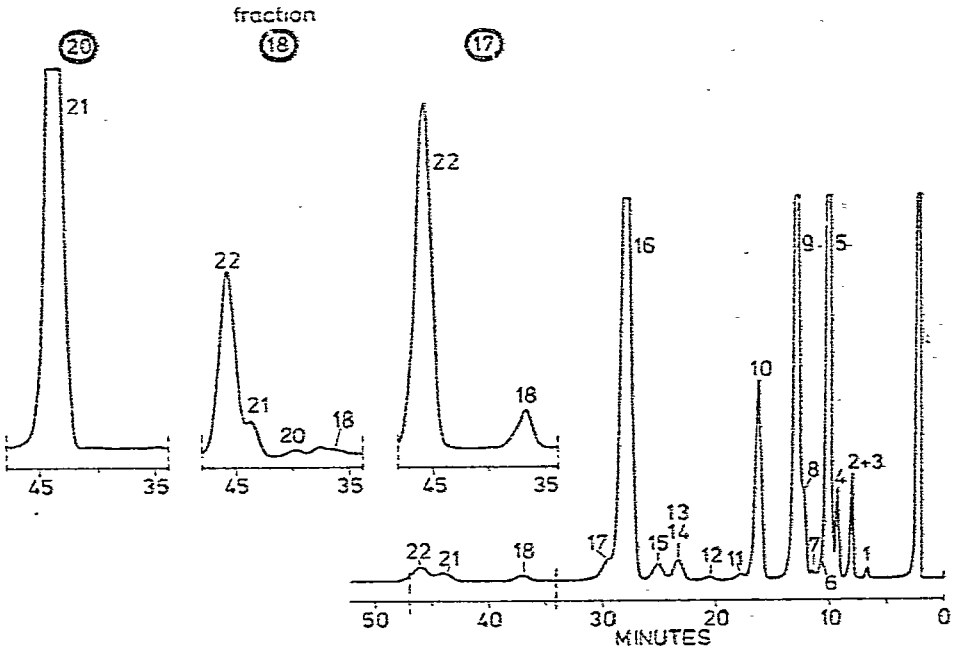

Fig. 1. Gas chromatograms obtained with the PEG 20M column at 60° for the total monoterpene hydrocarbon mixture and fractions 17, 18 and 20. For conditions see under Experimental. For numbering of peaks, see Table I.

(Analyzed Reagent; J. T. Baker, Phillipsburgh, N.J., U.S.A.). 0.25 ml of the essential oil were placed on the top of the column and the elution started. The eluate (235 ml) was collected as follows: 1 fraction of 50 ml; 15 fractions each of 3 ml; 4 fractions each of 10 ml and 2 fractions each of 50 ml. Each fraction was concentrated under reduced pressure in a rotary evaporator at *ca.* 1° and 1 μl of each fraction was used for the GC analysis.

TABLE I

MONOTERPENE HYDROCARBONS FOUND IN THE ESSENTIAL OIL FROM THE NEEDLES OF *ABIES ALBA* MILLER

Peak number	*Compound*	*Peak number*	*Compound*
1	*	12	Δ^3-Carene
2	*	13	Myrcene
3	Santene	14	α-Phellandrene
4	Tricyclene	15	α-Terpinene
5	α-Pinene	16	Limonene
6	α-Thujene	17	β-Phellandrene
7	β-Fenchene	18	γ-Terpinene
8	α-Fenchene	20	*trans*-β-Ocimene
9	Camphene	21	*p*-Cymene
10	β-Pinene	22	Terpinolene
11	Sabinene		

* Unknown.

Gas–liquid chromatography

Becker gas chromatographs (Models 409 and 1452 D; Becker, Delft, The Netherlands) equipped with flame-ionization detectors (FID) and copper columns (8 m × 1.5 mm I.D.) were used. The stationary phases used were 10% polyethylene glycol (PEG) 20M and 5% β,β'-oxydipropionitrile on Chromosorb W AW (60–80 mesh). Other conditions were: injector and detector temperature, 200°, column temperature, 60° (PEG), 20° and 36° (β,β'-oxydipropionitrile); carrier gas (nitrogen) flow-rate, 30 ml/min (PEG), 22 and 23 ml/min (β,β'-oxydipropionitrile, 36° and 20°, respectively). In order to obtain constant column temperatures of 36° and 20°, the column was placed in a thermostatted water-bath. With the thermally labile β,β'-oxydiproprionitrile, no bleeding of the stationary phase and no changes in the retention times of the compounds were observed during several weeks.

RESULTS AND DISCUSSION

Fig. 1 shows the gas chromatograms obtained with the PEG 20M column for the total monoterpene hydrocarbon fraction of the essential oil and for fractions 17, 18 and 20, eluted by liquid–solid chromatography. The peaks of γ-terpinene, *p*-cymene and terpinolene (numbered as in Table I) can be seen as small peaks on the chromatogram of the total hydrocarbon fraction. These three compounds were enriched in some of the fractions and those fractions are therefore valuable for further investigations using IR, UV and mass spectroscopy. *trans*-β-Ocimene, which is not seen on the chromatogram of the total monoterpene fraction, appears clearly in fraction 18.

Fig. 2 shows similar chromatograms for the total monoterpene hydrocarbon

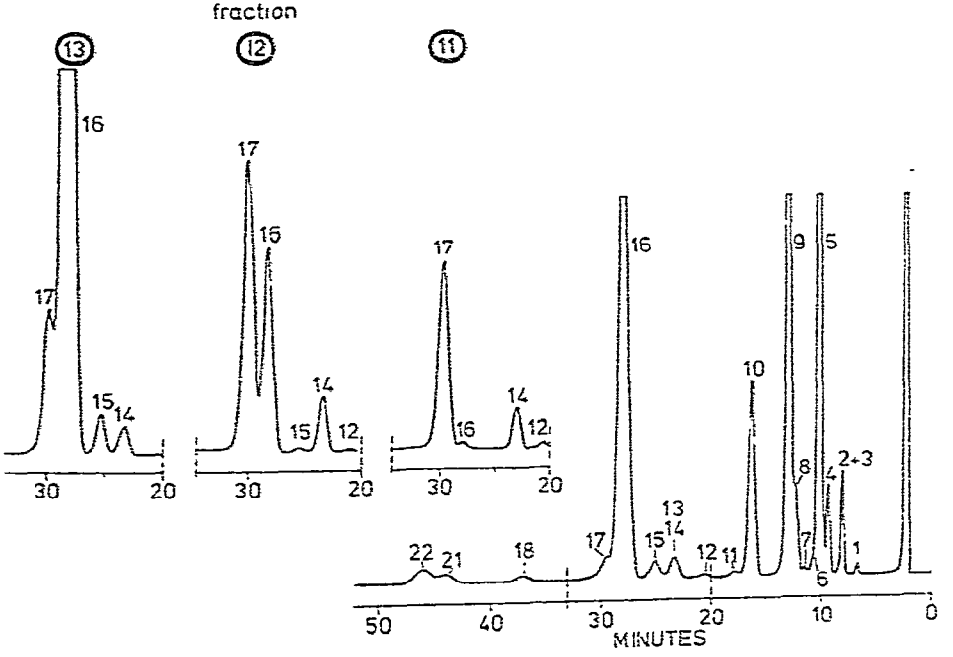

Fig. 2. Gas chromatograms obtained with the PEG 20M column for the total monoterpene hydrocarbon mixture and fractions 11, 12 and 13. For conditions see under Experimental.

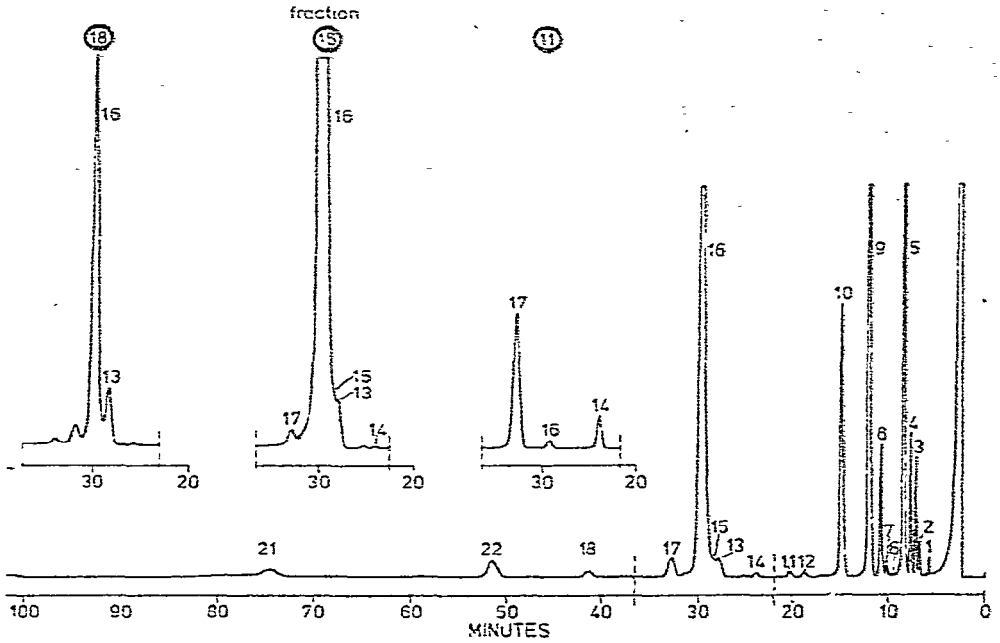

Fig. 3. Gas chromatograms obtained with the β,β'-oxydipropionitrile column at 36° for the total monoterpene hydrocarbon mixture and fractions 11, 15 and 18. For conditions see under Experimental.

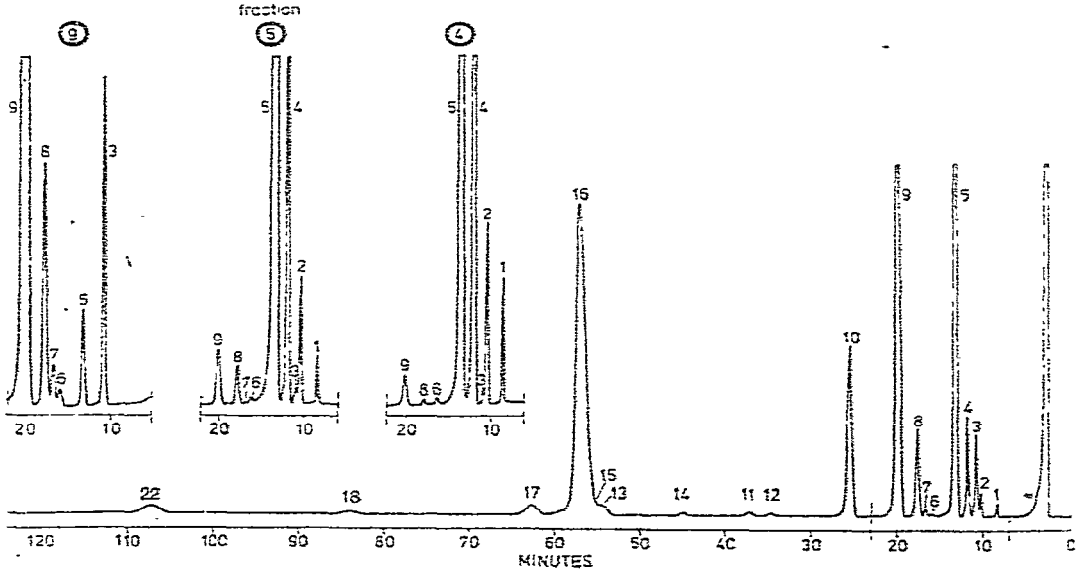

Fig. 4. Gas chromatograms obtained with the β,β'-oxydipropionitrile column at 20° for the tota monoterpene hydrocarbon mixture and fractions 4, 5 and 9. For conditions see under Experimental.

fraction and for fractions 11, 12 and 13. β-Phellandrene was enriched in fractions 11 and 12. Myrcene and α-phellandrene, which could not be separated on the PEG 20M column, were separated by liquid–solid chromatography and appeared in different fractions, as can be seen by comparing the chromatograms in Figs. 2 and 3. The chromatograms in Fig. 3 were obtained with the β,β'-oxydipropionitrile column at 36°.

Fig. 4 shows the chromatograms obtained with the β,β'-oxydipropionitrile column at 20° for the total monoterpene hydrocarbon fraction and fractions 4, 5 and 9. Peak 2 (an unknown compound) is slightly separated from santene on the chromatogram of the total hydrocarbon mixture. However, the chromatograms of the fractions show that a satisfactory separation of the two compounds is achieved by liquid–solid column chromatography, so that further investigations of the unknown compound can be carried out more easily with fractions 4 and 5.

CONCLUSION

Pre-fractionation of a naturally occurring mixture of monoterpene hydrocarbons of essential oils by means of liquid–solid column chromatography leads to a better GC separation and, therefore, to a better identification of the various compounds present.

REFERENCE

1 J. G. Kirchner and J. M. Miller, *Ind. Eng. Chem.*, 44 (1952) 318.