

CHROMSYMP. 262

GAS CHROMATOGRAPHIC CHARACTERIZATION OF FREQUENTLY OCCURRING MONOTERPENES IN ESSENTIAL OILS

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SUMMARY

Relative retention factors on stationary phases of three different polarities were determined for the gas chromatographic characterization of frequently occurring monoterpenes in essential oils. Relative retention factors obtained with monoterpene standards are in good agreement with those of the corresponding components of various essential oils examined under identical conditions.

INTRODUCTION

Several monoterpenes (*e.g.*, α - and β -pinene, camphene, limonene, 1,8-cineole and terpinene) are found in almost every essential oil¹. The aim of this work was to determine the relative retention factors for these common monoterpenes in order to facilitate their identification in essential oils.

EXPERIMENTAL

We used a Jeol JGC 1100 gas chromatograph with a flame-ionization detector. The flow-rate of the carrier gas (nitrogen) was 30–40 cm³/min. Column 1 was a glass spiral (3 m × 2.3 mm I.D.), coated with 3% OV-17 on 100–120-mesh Gas-Chrom Q and programmed from 60 to 165°C at 8°C/min. Its efficiency referred to linalool was 1825 plates/m with McReynolds' constants of 119, 158, 162, 243 and 202². Column 2 was a glass spiral (3 m × 2.3 mm I.D.) coated with 3% PEG 20M on 60–80-mesh Chromosorb W AW and programmed from 60 to 160°C at 8°C/min. Its efficiency referred to linalool was 1642 plates/m with McReynolds' constants of 322, 536, 368, 572 and 510². Column 3 was a glass spiral (3 m × 3.4 mm I.D.) coated with 1.5% Sp-2250 + 0.95% Sp-2401 on 100–120-mesh Supelcoport and programmed from 60 to 170°C at 8°C/min. Its efficiency referred to linalool was 1776 plates/m with McReynolds' constants of 129, 189, 238, 330 and 244. (The constants of the mixed phase were calculated from the data for its pure components².)

The concentration of the investigated solution was 1–2 mg/ml of standard in chloroform and 10–20 mg/ml of essential oil in chloroform. Samples of 0.1–0.5 μ l were injected. The temperatures of the injector and detector were both 200°C. The electrometer sensitivity range was $8 \cdot 10^{-10}$ A/mV. A Jeol IR-251 A recorder and a Digint 21 integrator (Chinoin) were used.

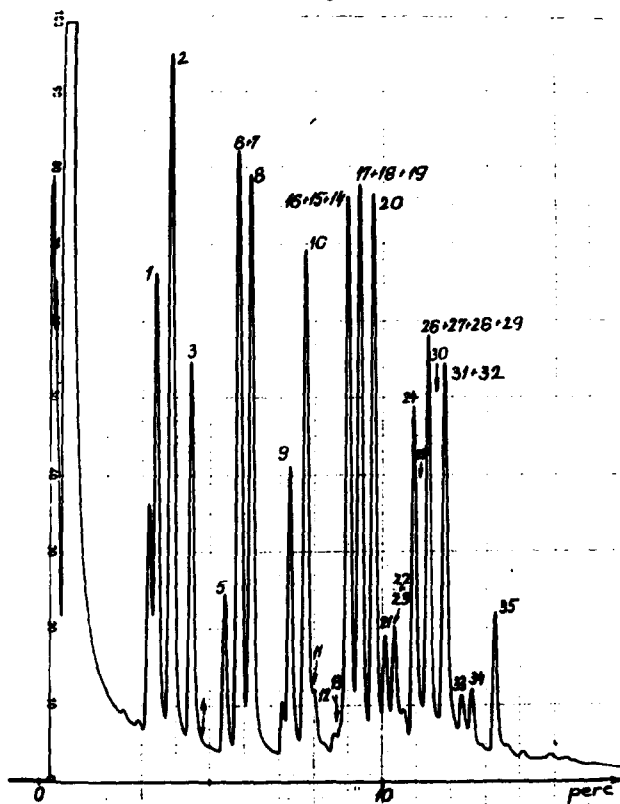


Fig. 1. Separation of monoterpenes on a 3% OV-17 stationary phase. Column, 3 m \times 2.3 mm I.D., programmed from 60 to 165°C at 8°C/min; nitrogen flow-rate, 35 ml/min. For identification of the peaks, see Table I.

Measurement of retention times was carried out with an accuracy of 0.1 sec; relative retention factors were determined from 3–5 parallel measurements and the accuracy of the elution temperatures was $\pm 1^\circ\text{C}$.

The standard series to be investigated on the three types of stationary phases consisted of 10–20 components prepared from 35 monoterpenes.

Samples of dill, sage, rosemary, lavender, *Eucalyptus citriodora* and *Cymbopogon citratus* essential oil were also analysed. Identification of the components of the essential oils was carried out by the standard additions method on two or three of the different stationary phases.

RESULTS AND DISCUSSION

The order of elution of monoterpenes on 3% OV-17 is, with few exceptions, characteristic of the type of monoterpene (Fig. 1). Hydrocarbons are eluted in the temperature range 75–110°C, ketones, aldehydes and alcohols at 110–145°C and esters and some aldehydes at 145–165°C. Linalyl acetate was the only exception among the esters investigated, appearing at an elution temperature of 144°C, similar to that of

TABLE I

RETENTION FACTORS OF MONOTERPENES RELATIVE TO DIFFERENT REFERENCE TERPENES ON 3% OV-17 STATIONARY PHASE

| No. | Monoterpene | Retention factors relative to selected standards | | | |
|-----|---------------------|--|--|---|--|
| | | <i>γ</i> -Terpinene ($T_s = 110^\circ\text{C}$) | Menthol ($T_s = 132^\circ\text{C}$) | Piperitone ($T_s = 152^\circ\text{C}$) | Geranyl acetate ($T_s = 165^\circ\text{C}$) |
| 1 | α -Pinene | 0.50 | 0.33 | 0.25 | 0.22 |
| 2 | Camphene | 0.58 | 0.38 | 0.29 | 0.25 |
| 3 | β -Pinene | 0.69 | 0.45 | 0.34 | 0.30 |
| 4 | Myrcene | 0.72 | 0.47 | 0.36 | 0.32 |
| 5 | Limonene | 0.87 | 0.58 | 0.43 | 0.38 |
| 6 | 1,8-Cineole | 0.94 | 0.61 | 0.47 | 0.41 |
| 7 | <i>p</i> -Cymene | 0.94 | 0.61 | 0.47 | 0.41 |
| 8 | <i>γ</i> -Terpinene | 1.00 | 0.66 | 0.50 | 0.44 |
| 9 | Linalool | 1.21 | 0.80 | 0.60 | 0.53 |
| 10 | Fenchone | 1.28 | 0.85 | 0.64 | 0.56 |
| 11 | α -Thujone | 1.31 | 0.87 | 0.65 | 0.57 |
| 12 | Citronellal | 1.45 | 0.96 | 0.71 | 0.63 |
| 13 | Isopulegol | 1.45 | 0.96 | 0.71 | 0.63 |
| 14 | Menthol | 1.50 | 1.00 | 0.75 | 0.66 |
| 15 | Menthone | 1.50 | 1.00 | 0.75 | 0.66 |
| 16 | Isoborneol | 1.50 | 1.00 | 0.75 | 0.66 |
| 17 | Camphor | 1.57 | 1.04 | 0.78 | 0.69 |
| 18 | Borneol | 1.57 | 1.04 | 0.78 | 0.69 |
| 19 | Isomenthone | 1.57 | 1.04 | 0.78 | 0.69 |
| 20 | α -Terpinol | 1.64 | 1.09 | 0.81 | 0.72 |
| 21 | Citronellol | 1.71 | 1.13 | 0.85 | 0.75 |
| 22 | Nerol | 1.75 | 1.17 | 0.87 | 0.77 |
| 23 | Linalyl acetate | 1.75 | 1.17 | 0.87 | 0.77 |
| 24 | Geraniol | 1.85 | 1.23 | 0.92 | 0.81 |
| 25 | Neral | 1.89 | 1.25 | 0.94 | 0.83 |
| 26 | Pulegone | 1.93 | 1.27 | 0.96 | 0.84 |
| 27 | Menthyl acetate | 1.93 | 1.27 | 0.96 | 0.84 |
| 28 | Bornyl acetate | 1.93 | 1.27 | 0.96 | 0.84 |
| 29 | Isobornyl acetate | 1.93 | 1.27 | 0.96 | 0.84 |
| 30 | Carvone | 2.00 | 1.32 | 0.98 | 0.86 |
| 31 | Piperitone | 2.03 | 1.34 | 1.00 | 0.88 |
| 32 | Geranial | 2.03 | 1.34 | 1.00 | 0.88 |
| 33 | Neryl acetate | 2.10 | 1.40 | 1.05 | 0.92 |
| 34 | Terpinyl acetate | 2.16 | 1.43 | 1.07 | 0.94 |
| 35 | Geranyl acetate | 2.29 | 1.52 | 1.14 | 1.00 |

alcohols. As a general rule, the elution temperature of an ester is always higher than that of the corresponding alcohol.

An order of elution similar to that on OV-17 was observed on the mixed stationary phase of 1.5% Sp-2250 + 0.95% 2401. However, the mixed phase, owing due to its slightly higher polarity, proved more advantageous for the separation of certain pairs such as borneol and camphor, and menthol and menthone. (Fig. 2).

The high polarity of the 3% PEG 20M stationary phase caused changes in the order of elution of numerous monoterpenes (Fig. 3). Hydrocarbons, several ketones

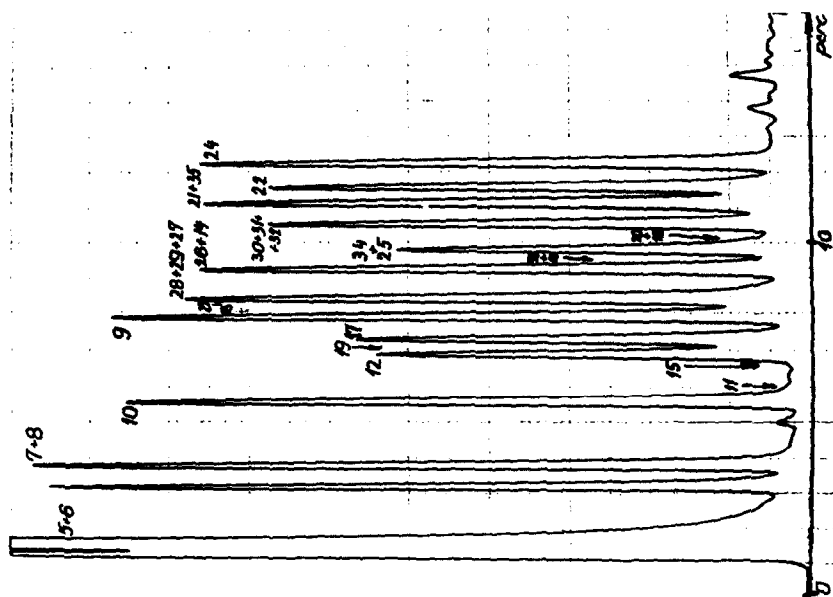


Fig. 2. Separation of monoterpenes on 1.5% Sp-2250 + 0.95% Sp-2401 mixed stationary phase. Column, 3 m \times 3.4 mm I.D., programmed from 60 to 170°C at 8°C/min; nitrogen flow-rate, 35 ml/min. For identification of the peaks, see Table II.

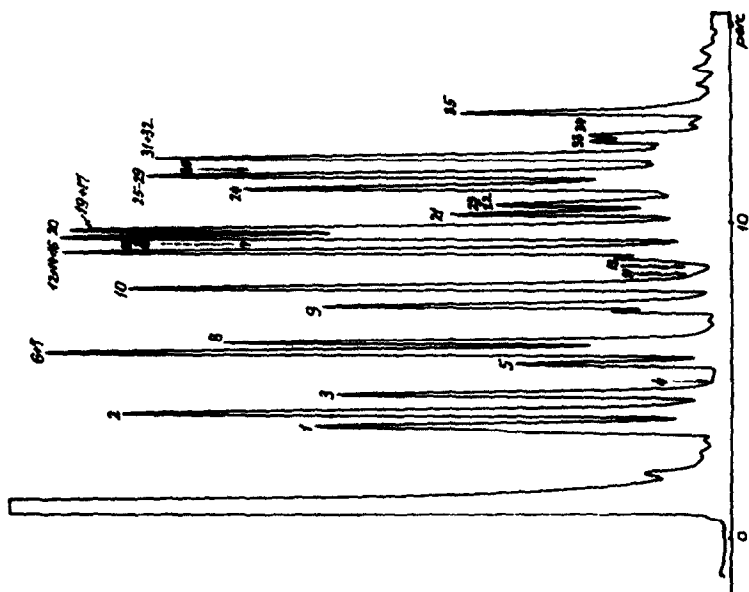


Fig. 3. Separation of monoterpenes on a 3% PEG 20M stationary phase. Column, 3 m \times 2.3 mm I.D., programmed from 60 to 160°C at 8°C/min; nitrogen flow-rate, 35 ml/min. For identification of the peaks, see Table III.

TABLE II
RETENTION FACTORS OF MONOTERPENES RELATIVE TO DIFFERENT REFERENCE TERPENES ON 1.5% SP-2250 + 0.95% SP-2401

| No. | Monoterpene | Retention factors relative to selected standards | | | | |
|-----|---------------------|--|---|--|---|---|
| | | γ -Terpinene ($T_e = 110^\circ\text{C}$) | Fenchone ($T_e = 127^\circ\text{C}$) | Menthol ($T_e = 134^\circ\text{C}$) | Piperitone ($T_e = 160^\circ\text{C}$) | Geranyl acetate ($T_e = 168^\circ\text{C}$) |
| 1 | α -Pinene | 0.55 | 0.41 | 0.37 | 0.28 | 0.26 |
| 2 | Camphene | 0.63 | 0.46 | 0.42 | 0.31 | 0.29 |
| 3 | β -Pinene | 0.73 | 0.54 | 0.49 | 0.37 | 0.34 |
| 4 | Myrcene | 0.78 | 0.58 | 0.53 | 0.39 | 0.36 |
| 5 | Limonene | 0.88 | 0.65 | 0.59 | 0.44 | 0.41 |
| 6 | 1,8-Cineole | 0.96 | 0.71 | 0.65 | 0.48 | 0.44 |
| 7 | <i>p</i> -Cymene | 0.96 | 0.71 | 0.65 | 0.48 | 0.44 |
| 8 | γ -Terpinene | 1.00 | 0.74 | 0.67 | 0.50 | 0.46 |
| 9 | Linalool | 1.20 | 0.89 | 0.81 | 0.60 | 0.56 |
| 10 | Fenchone | 1.35 | 1.00 | 0.91 | 0.68 | 0.62 |
| 11 | α -Thujone | 1.40 | 1.03 | 0.94 | 0.70 | 0.64 |
| 12 | Citronellal | 1.49 | 1.10 | 1.00 | 0.75 | 0.69 |
| 13 | Isopulegol | 1.42 | 1.05 | 0.96 | 0.71 | 0.66 |
| 14 | Menthol | 1.49 | 1.10 | 1.00 | 0.75 | 0.69 |
| 15 | Menthone | 1.53 | 1.13 | 1.03 | 0.77 | 0.71 |
| 16 | Isoborneol | 1.49 | 1.10 | 1.00 | 0.75 | 0.69 |
| 17 | Camphor | 1.62 | 1.20 | 1.09 | 0.81 | 0.75 |
| 18 | Borneol | 1.53 | 1.13 | 1.03 | 0.77 | 0.71 |
| 19 | Isomenthone | 1.62 | 1.20 | 1.09 | 0.81 | 0.75 |
| 20 | α -Terpineol | 1.60 | 1.18 | 1.07 | 0.80 | 0.74 |
| 21 | Citronellol | 1.66 | 1.23 | 1.12 | 0.83 | 0.77 |
| 22 | Nerol | 1.71 | 1.27 | 1.15 | 0.86 | 0.79 |
| 23 | Linalyl acetate | 1.71 | 1.27 | 1.15 | 0.86 | 0.79 |
| 24 | Geraniol | 1.79 | 1.33 | 1.20 | 0.90 | 0.83 |
| 25 | Neral | 1.92 | 1.42 | 1.29 | 0.96 | 0.89 |
| 26 | Pulegone | 1.92 | 1.42 | 1.29 | 0.96 | 0.89 |
| 27 | Menthyl acetate | 1.92 | 1.42 | 1.29 | 0.96 | 0.89 |
| 28 | Bornyl acetate | 1.92 | 1.42 | 1.29 | 0.96 | 0.89 |
| 29 | Isobornyl acetate | 1.92 | 1.42 | 1.29 | 0.96 | 0.89 |
| 30 | Carvone | 1.95 | 1.44 | 1.31 | 0.98 | 0.90 |
| 31 | Piperitone | 2.00 | 1.48 | 1.34 | 1.00 | 0.92 |
| 32 | Geranial | 2.00 | 1.48 | 1.34 | 1.00 | 0.92 |
| 33 | Neryl acetate | 2.03 | 1.50 | 1.37 | 1.02 | 0.94 |
| 34 | Terpinyl acetate | 2.06 | 1.53 | 1.39 | 1.04 | 0.95 |
| 35 | Geranyl acetate | 2.17 | 1.60 | 1.46 | 1.09 | 1.00 |

TABLE III

RETENTION FACTORS OF MONOTERPENES RELATIVE TO DIFFERENT REFERENCE TERPENES, ON 3% PEG 20M STATIONARY PHASE

| No. | Monoterpene | Retention factors relative to selected standards | | |
|-----|---------------------|--|---|---|
| | | Linalool ($T_e = 127^\circ\text{C}$) | Pulegone ($T_e = 136^\circ\text{C}$) | Geranyl acetate ($T_e = 150^\circ\text{C}$) |
| 1 | α -Pinene | — | — | — |
| 2 | Camphene | — | — | — |
| 3 | β -Pinene | — | — | — |
| 4 | Myrcene | — | — | — |
| 5 | Limonene | 0.32 | 0.27 | 0.21 |
| 6 | 1,8-Cineole | 0.32 | 0.27 | 0.21 |
| 7 | <i>p</i> -Cymene | 0.41 | 0.34 | 0.28 |
| 8 | γ -Terpinene | 0.41 | 0.34 | 0.28 |
| 9 | Linalool | 1.00 | 0.84 | 0.68 |
| 10 | Fenchone | 0.67 | 0.56 | 0.46 |
| 11 | α -Thujone | 0.73 | 0.61 | 0.49 |
| 12 | Citronellal | 0.85 | 0.71 | 0.50 |
| 13 | Isopulegol | 1.04 | 0.87 | 0.71 |
| 14 | Menthol | 1.19 | 1.00 | 0.81 |
| 15 | Menthone | 0.83 | 0.69 | 0.56 |
| 16 | Isoborneol | 1.24 | 1.04 | 0.84 |
| 17 | Camphor | 0.91 | 0.76 | 0.62 |
| 18 | Borneol | 1.33 | 1.12 | 0.90 |
| 19 | Isomenthone | 0.89 | 0.74 | 0.60 |
| 20 | α -Terpineol | 1.33 | 1.12 | 0.90 |
| 21 | Citronellol | 1.47 | 1.23 | 1.00 |
| 22 | Nerol | 1.53 | 1.28 | 1.04 |
| 23 | Linalyl acetate | 1.06 | 0.89 | 0.72 |
| 24 | Geraniol | 1.63 | 1.36 | 1.11 |
| 25 | Neral | 1.28 | 1.07 | 0.87 |
| 26 | Pulegone | 1.19 | 1.00 | 0.81 |
| 27 | Menthyl acetate | 1.08 | 0.90 | 0.73 |
| 28 | Bornyl acetate | 1.08 | 0.90 | 0.73 |
| 29 | Isobornyl acetate | 1.08 | 0.90 | 0.73 |
| 30 | Carvone | 1.38 | 1.16 | 0.94 |
| 31 | Piperitone | 1.38 | 1.16 | 0.94 |
| 32 | Geranjal | 1.38 | 1.16 | 0.94 |
| 33 | Neryl acetate | 1.24 | 1.04 | 0.84 |
| 34 | Terpinyl acetate | 1.28 | 1.07 | 0.87 |
| 35 | Geranyl acetate | 1.47 | 1.23 | 1.00 |

and aldehydes eluted at considerably lower temperatures (100–125°C), then esters and alcohols alternated with the remaining aldehydes and ketones (125–160°C). In contrast to the previous observations, the elution temperature of alcohols is higher in general than that of the corresponding esters. An exception was observed only with linalool/linalyl acetate.

The relative retention factors of the monoterpene components of the standard series were determined by reference to suitably selected monoterpenes (Tables I–III).

To confirm the reproducibility of the data, the retentions of the reference

TABLE IV
RETENTION FACTORS OF REFERENCE MONOTERPENE STANDARDS RELATIVE TO *n*-ALKANE SERIES (C₁₁-C₁₆) ON THE THREE STATIONARY PHASES

Relative retention factor = t'_k (monoterpene)/ t'_k (*n*-alkane), where t'_k = reduced retention time.

| <i>n</i> -Alkane reference series | 3% OV-17 | | | | 3% PEG 20M | | | | 1.5% Sp-2250 + 0.95% Sp-2401 | | | |
|-----------------------------------|---------------------|---------|------------|-----------------|------------|----------|-----------------|---------------------|------------------------------|---------|------------|-----------------|
| | γ -Terpinene | Menthol | Piperitone | Geranyl acetate | Linolool | Pulegone | Geranyl acetate | γ -Terpinene | Fenchone | Menthol | Piperitone | Geranyl acetate |
| C ₁₁ H ₂₄ | 1.13 | 1.69 | 2.26 | 2.56 | — | — | — | 1.05 | 1.41 | 1.55 | 2.06 | 2.29 |
| C ₁₂ H ₂₆ | 0.82 | 1.22 | 1.62 | 1.84 | — | — | — | 0.83 | 1.13 | 1.24 | 1.66 | 1.82 |
| C ₁₃ H ₂₈ | 0.63 | 0.95 | 1.26 | 1.43 | — | — | — | 0.64 | 0.85 | 0.95 | 1.27 | 1.39 |
| C ₁₄ H ₃₀ | 0.52 | 0.79 | 1.05 | 1.19 | 1.16 | 1.39 | 1.73 | 0.52 | 0.70 | 0.78 | 1.05 | 1.15 |
| C ₁₅ H ₃₂ | 0.45 | 0.67 | 0.89 | 1.01 | 0.90 | 1.08 | 1.34 | 0.46 | 0.62 | 0.69 | 0.92 | 1.01 |
| C ₁₆ H ₃₄ | 0.39 | 0.59 | 0.79 | 0.89 | 0.74 | 0.88 | 1.09 | 0.40 | 0.54 | 0.60 | 0.80 | 0.88 |

monoterpenes were calibrated against a suitably hydrocarbon series (Table IV).

To examine the reliability and reproducibility of the collection of relative retention factors, the standard series were compared with those of the corresponding monoterpenes of six different essential oils. The retention data obtained showed fair agreement (standard deviation <0.01).

CONCLUSION

The collection of retention factors of monoterpenes obtained facilitates the combined application of two or three types of stationary phases for the qualitative investigation of essential oils, provides an aid for the selection of a suitable stationary phase for the separation of known monoterpenes and affords a starting point for the analysis of essential oils of unknown origin.

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