Journal of Chromatography, 508 (1990) 341–348
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 324

Use of two-dimensional gas chromatography in the direct enantiomer separation of chiral essential oil components

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(First received October 17th, 1989; revised manuscript received February 2nd, 1990)

SUMMARY

The enantiomeric excess of a component of an essential oil can be determined on-line with normal gas chromatographic analysis by applying two-dimensional gas chromatography with a second column coated with a chiral stationary phase. The enantiomeric excess for the examples reported was evaluated by complexation gas chromatography, which was demonstrated to give successful enantiomer separations without derivatization of several monoterpenoids and compounds peculiar to the essential oil field.

INTRODUCTION

The enantiomer resolution of a component in a complex mixture, such as an essential oil, is of importance both from a quality control point of view, where it can contribute to the evaluation of the quality and origin of the oil itself, and in scientific studies to evaluate, for instance, the biosynthetic pathway of a component, the geographic origin of an essential oil or the production of artefacts. A two-dimensional gas chromatographic (TDGC) system, provided with a second column coated with a chiral stationary phase, allows us to determine the enantiomeric excess of a component of an essential oil on-line with normal GC analysis, while avoiding the isolation of the component itself. Wang *et al.*[1] first used a two-column GC system provided with valveless switching for the determination of amino acid enantiomeric excess in 1983. In 1984, Schomburg *et al.*[2] described the chiral resolution of the four racemic menthol isomers and the two α-ionol isomers in the form of their isopropylurethane derivatives by TDGC using XE-60–(*S*)-valines–(*S*)-α-phenylethylamide as the stationary phase in the second column. Unfortunately, when isopropylurethane or other derivatives are used, the information on the total oil normally obtained from the first column is lost; in other words, the gas chromatographic pattern of the total oil is no longer available simultaneously with that of the enantiomeric resolution of the essential oil component in question.

In the last few years, new chiral stationary phases have been developed which permit racemate analysis without requiring derivatization. In particular, two different

0021-9673/90/$03.50

types of stationary phases were shown to be successful in the GC separation of several enantiomeric compounds of natural origin as such: the variously modified α-,β- and γ-cyclodextrins (used as such or diluted in normal GC stationary phases), which were introduced among others by Koscielsky and co-workers[3,4], Juvancz *et al.*[5], Venema and Tolsma[6], Koenig *et al.*[7] and Nowotny *et al.*[8], and the Ni^{II}, Co^{II} and Mn^{II} derivatives of bis-3-heptafluorobutanoyl camphorate, which operate on the better known principle of complexation chromatography developed by Schurig and co-workers[9,10].

Recently, Mosandl[11] applied TDGC to the direct enantiomer separation of underivatized chiral γ-lactones from food and beverages by using a heptakis(3-O-acetyl-2,6-di-O-pentyl)-β-cyclodextrin as a chiral stationary phase for the second column.

The aim of this paper is to show the possibilities of TDGC provided with a column for complexation chromatography as a second column in the enantiomer separation of essential oil components. The reliability and selectivity of the proposed technique are demonstrated by assessing the enantiomeric purity of menthol and menthones from peppermint essential oil and of linalyl acetate from lavender essential oil.

EXPERIMENTAL

Reagents

The order of elution of the compounds under analysis from the Chira-Metal (1–4) column was established analysing optically pure compounds. Racemic menthol, menthones and linalyl acetate and (−)-menthol, (−)-menthone, (−)-isomenthone and (−)-linalyl acetate were supplied by Aldrich (Milan, Italy).

Essential oil preparation

Peppermint and lavender essential oils were obtained by submitting 10 g of dried plant material to steam distillation in the modified Marcusson micro-apparatus developed in the authors' laboratory[12]. Commercially available peppermint and lavender oils were also analysed.

TDGC system

TDGC analyses were carried out by applying the MUSIC system (Chrompack, Middelburg, The Netherlands) to a two-oven GC system obtained by coupling a Carlo Erba 2900 with a Carlo Erba 4160 GC unit. The transfer line between the first and second ovens was constructed in the authors' laboratory; it was heated and temperature controlled through the second oven injector heating system. MUSIC is a two-dimensional chromatographic system based on the principle of flow switching proposed about 20 years ago by Deans[13] and provided with a cold trap between the first and second column (*i.e.*, the analytical column). The working principle of MUSIC can be briefly summarized as follows. During the heart-cut step, the effluent from the first column is directed into a 1 m × 220 μm I.D. fused-silica capillary and trapped by expanding liquid carbon dioxide (or liquid nitrogen). When the transfer is complete, the cold trap is ballistically heated in a few seconds to a temperature high enough to vaporize the sample instantaneously. The trapped fraction is then directed into a 220-μm analytical column. Further details of the operation of MUSIC have

been reported elsewhere[14,15]. The operating conditions for MUSIC were trap-base temperature 220°C, trap temperature on reinjection 220°C and pressure at the switching point 0.92 atm.

TDGC analysis

A 20 m × 530 μm I.D. fused-silica open tubular (FSOT) column coated with OV-1 (film thickness 1 μm) and a 12 m × 220 μm I.D. SE-54 FSOT column containing nickel(II) bis[3-heptafluorobutanoyl)-(1*R*)-camphorate] [Chira-Metal (1–4), CC&CC, Kirchentellinsfurt, F.R.G.] were installed in the first and second ovens, respectively. The column temperature in the first oven was programmed from 50 to 220°C at 3°C/min. The following conditions were used: injector temperature, 240°C; detector temperature, 250°C; transfer-line temperature, 230°C; carrier gas, helium; and flow-rate at the head of the first column, 10 ml/min.

Peppermint oil. A 1-μl volume of peppermint essential oil solution diluted 1:15 000 with hexane was injected into the first column. The heart-cut temperatures were *ca.* 95°C for menthone and 98°C for menthol. The analysis on the second column was carried out isothermally at 110°C.

Lavender oil. A 1-μl volume of lavender essential oil solution diluted 1:15 000 with hexane was injected into the first column. The heart-cut temperature was 105°C. The analysis on the second column was carried out isothermally at 100°C.

RESULTS AND DISCUSSION

The GC resolution of a racemate cannot easily be planned theoretically because, unfortunately, a stationary phase of general use for GC enantiomer analysis has not yet been developed and a suitable stationary phase for the separation of a particular racemate must therefore be found experimentally. Complexation chromatography was demonstrated to be very effective in enantiomer analysis without derivatization for several monoterpenoids and compounds peculiar to the essential oil field[16]. The fundamental principles of complexation chromatography were recently reviewed by Schurig[17]. The low maximum allowable operating temperature (110°C, and only for short periods) of complexation chromatographic stationary phases currently available limits their applicability to the essential oil field as it makes the enantiomeric analysis of several monoterpenoids and of most sesquiterpenoids generally impossible[16]. For this reason, a double-oven GC system was assembled in order to operate with the two columns independently heated, and to protect the column coated with the thermolabile complexation chromatographic stationary phase, although MUSIC could successfully operate with a single-oven GC unit (of course provided with two flame ionization detectors).

The optical activity of a compound of natural origin is generally peculiar and related to its biogenetic pathway. Therefore, an unadulterated essential oil is characterized by a definite and plant-specific distribution of its chiral components. Peppermint essential oil, for instance, is characterized by the presence of (−)-menthone and (−)-menthol[18] and lavender essential oil by (−)-linalyl acetate. The enantiomeric excess of menthone and menthol was investigated in a freshly distilled peppermint essential oil and in some commercially available oils; the gas chromatographic pattern of the freshly distilled oil is shown in Fig. 1. Menthol and methone/isomenthone

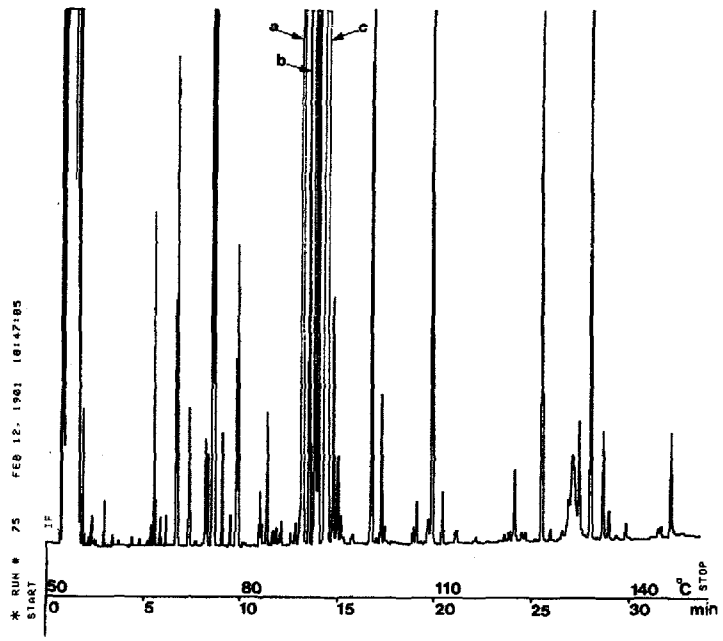

Fig. 1. Gas chromatographic pattern of a freshly distilled peppermint oil: (a) menthone; (b) isomenthone; (c) menthol.

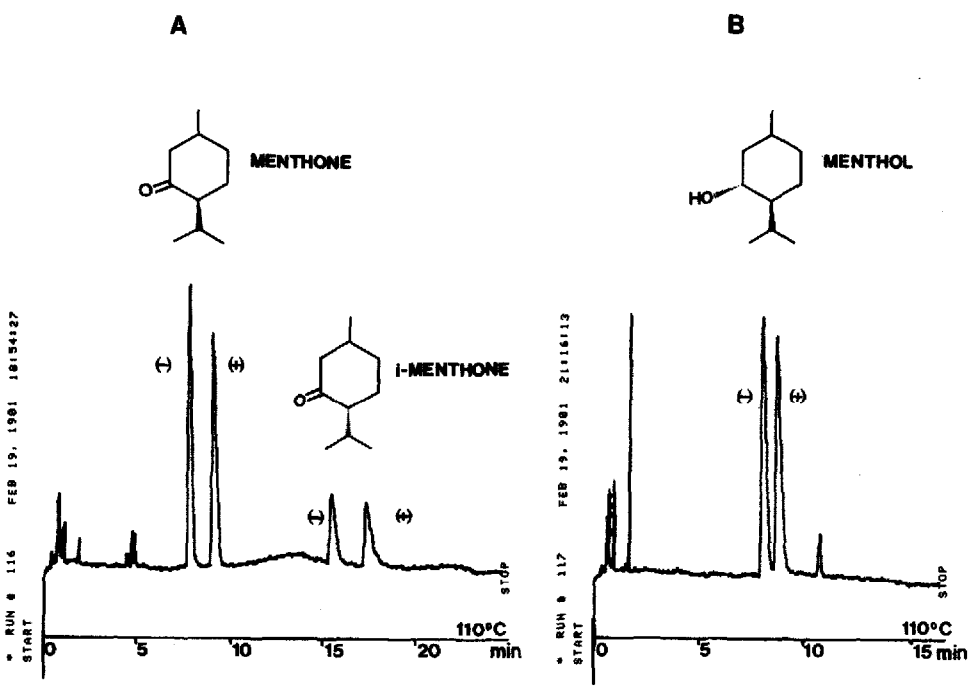

Fig. 2. Enantiomer analyses by TDGC of the menthone/isomenthone (A) and menthol (B) racemates heart-cut from standard solutions.

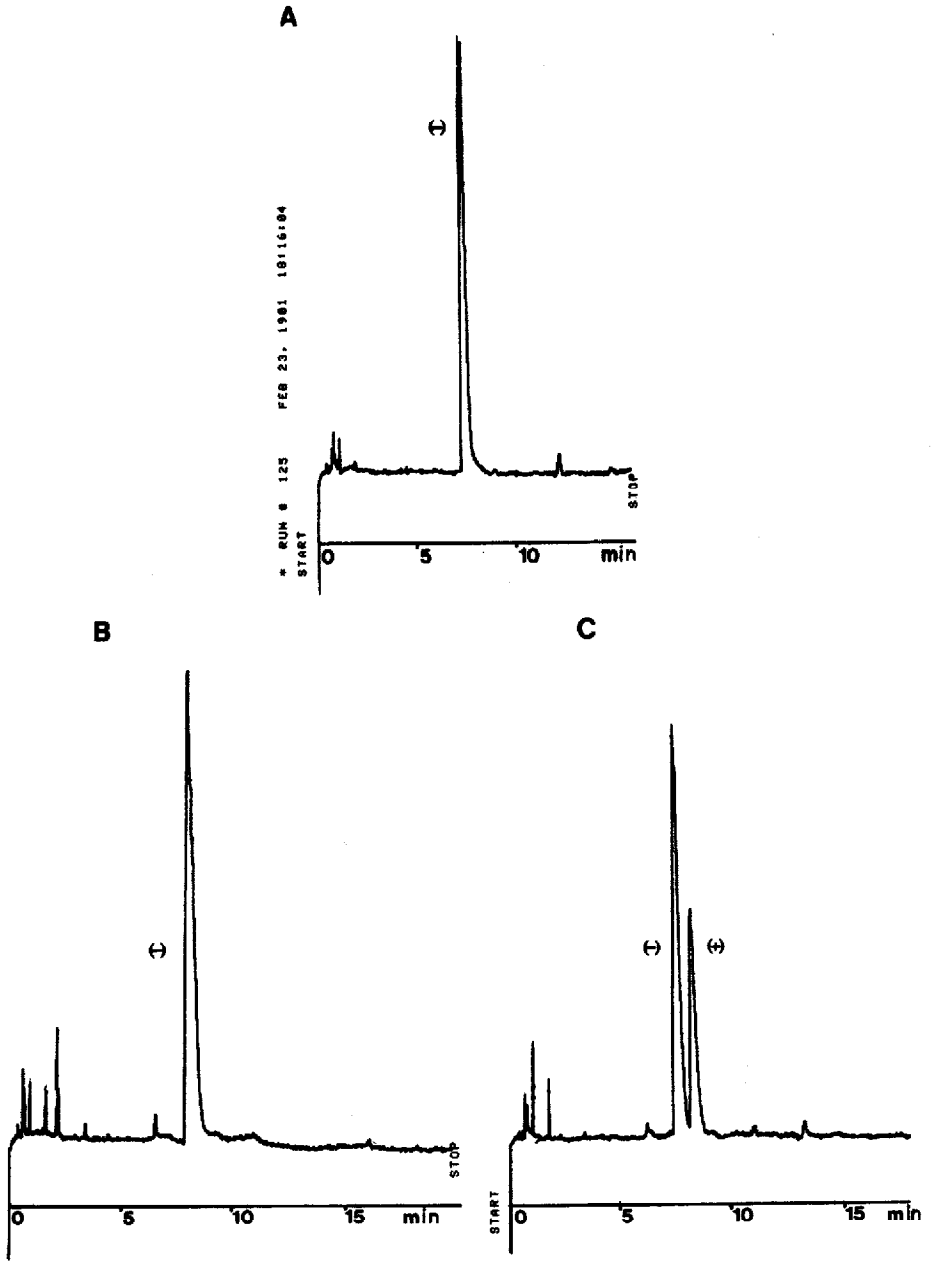

Fig. 3. Analysis by TDGC of menthone (A) and menthol (B) enantiomeric excess in the peppermint oil in Fig. 1 and (C) menthol in a commercially available oil.

racemates were first heart-cut from the standard solutions (Fig. 2); these analyses were each repeated five times to control their qualitative and quantitative reproducibility. The menthol and menthone stereoisomer ratios in the above oil and in one of the commercial oils, probably spiked with menthol racemate, are reported in Fig. 3.

Similar results were obtained in the enantiomer recognition of linalyl acetate from a freshly distilled lavender oil (Fig. 4) and from some commercially available oils. Fig. 5 shows the heart-cut of the standard solution of linalyl acetate racemate, the linalyl acetate stereoisomer ratio in the lavender oil in Fig. 4 and that in a commercial oil probably spiked with linalyl acetate racemate.

The results reported here are clearly encouraging and demonstrate how the direct enantiomer analysis by TDGC of a component in an essential oil is rapid, easy and effective. Further studies of the use of direct TDGC enantiomer analysis for characterizing optically active components of essential oils using various stationary phases are in progress.

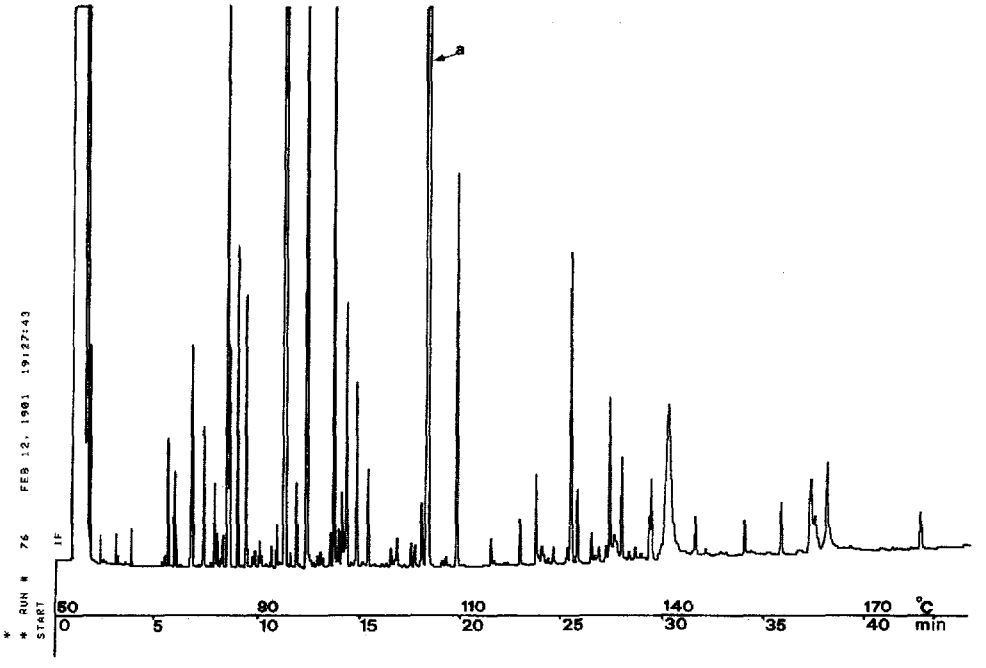

Fig. 4. Gas chromatographic pattern of a freshly distilled lavender oil: (a) linalyl acetate.

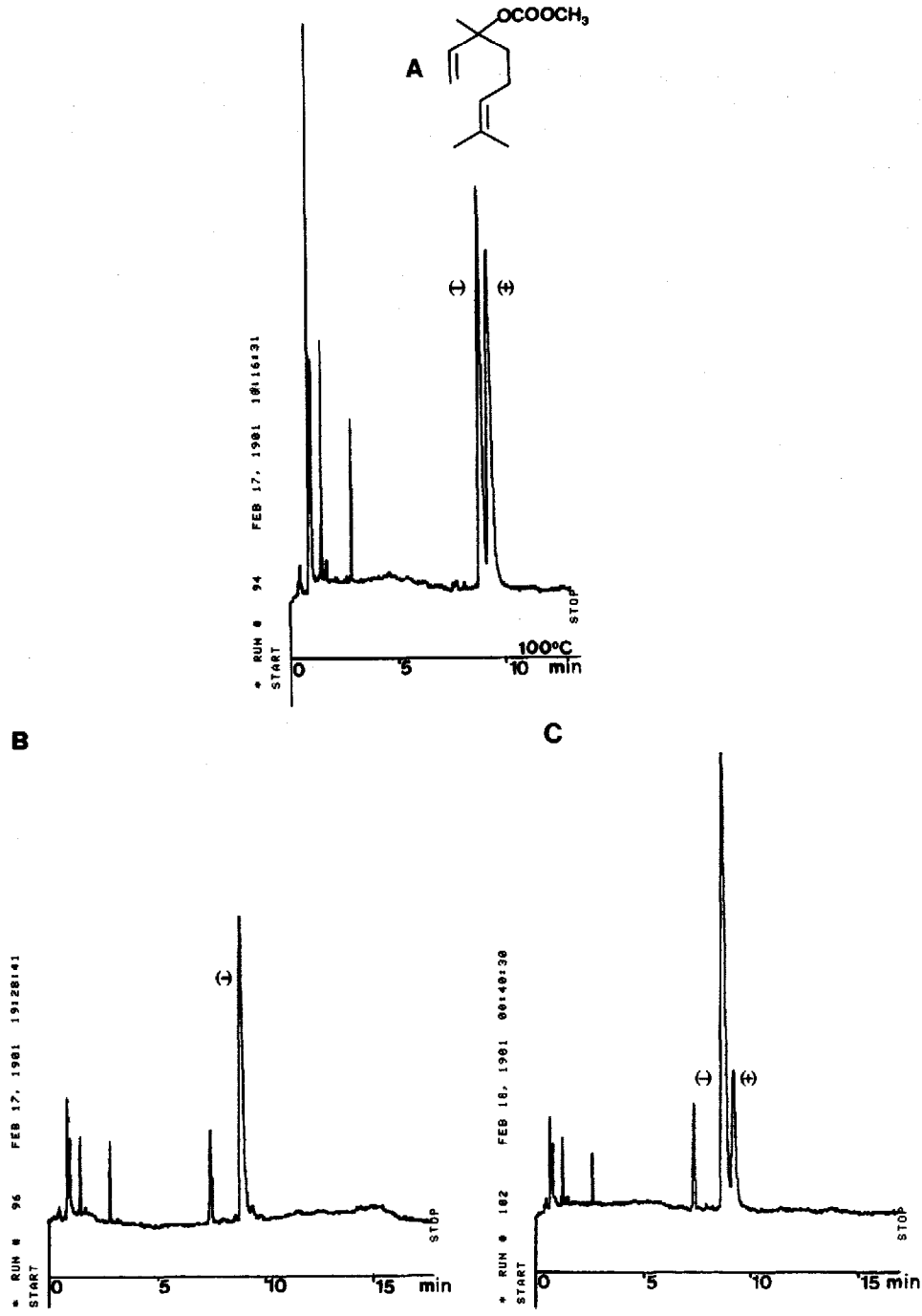

Fig. 5. Analyses by TDGC of the enantiomeric excess of linalyl acetate (A) in a racemate standard solution, (B) in the lavender oil in Fig. 4 and (C) in a commercially available oil.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministero della Pubblica Istruzione (60% and 40% research funds) and the Assessorato Agricoltura Foreste e Ambiente Naturale della Regione Autonoma Valle d'Aosta for financial support to the laboratory. The authors thanks Professor F. V. Schurig for helpful discussions and advice.

REFERENCES

1 C. Wang, H. Frank, G. Wang, L. Zhou, E. Bayer and P. Lu, *J. Chromatogr.*, 262 (1983) 352.
2 G. Schomburg, H. Husmann, E. Hubinger and W. A. Konig, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 404.
3 T. Koscielski, D. Sybilska, S. Belniak and J. Jurczak, *Chromatographia*, 21 (1986) 413.
4 T. Koscielski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 364 (1986) 299.
5 Z. Juvancz, G. Alexander and J. Szejtli, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 105.
6 A. Venema and P. J. Tolsma, *J. High Resolut. Chromatogr.*, 12 (1989) 32.
7 W. A. Koenig, S. Lutz, G. Wenz and E. von der Bey, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 506; and references cited therein.
8 H.-P. Nowotny, D. Schmalzing, D. Wistuba and V. Schurig, *J. High Resolut. Chromatogr.*, 12 (1989) 383; and references cited therein.
9 V. Schurig and W. Burkle, *J. Am. Chem. Soc.*, 104 (1982) 7573.
10 V. Schurig and R. Weber, *J. Chromatogr.*, 289 (1984) 321.
11 A. Mosandl, U. Hener, U. Hagenauer-Hener and A. Kustermann, *J. High Resolut. Chromatogr.*, 12 (1989) 532.
12 C. Bicchi, A. D'Amato, G. M. Nano and C. Frattini, *J. Chromatogr.*, 279 (1983) 409.
13 D. R. Deans, *Chromatographia*, 1 (1986) 18.
14 C. Bicchi, A. D'Amato, F. David and P. Sandra, *Flavour Fragr. J.*, 2 (1987) 49.
15 C. Bicchi, A. D'Amato, F. David and P. Sandra, *Flavour Fragr. J.*, 3 (1988) 143.
16 P. Werkoff and R. Hopp, in E.-J. Brunke (Editor), *Progress in Essential Oil Research*, Walter de Gruyter, Berlin, New York, 1986, p. 529.
17 V. Schurig, *J. Chromatogr.*, 441 (1988) 135.
18 R. Croteau, in J. W. Porter and S. L. Spungeon (Editors), *Biosynthesis of Isoprenoids Compounds*, Vol. 1, Wiley, New York, 1981, p. 227.