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# Cyclodextrin derivatives in the gas chromatographic separation of racemic mixtures of volatile compounds

# VII. The use of 2,6-di-O-methyl-3-O-pentyl-β-cyclodextrin diluted in phases with different polarity in the separation of racemates in complex mixtures

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ABSTRACT

In the GC separation of volatile racemates, cyclodextrin (CD) derivatives may be used either as such or diluted in polysiloxane. If diluting phases with different polarity are used, different interactions and as a consequence a different retention of each component in a complex mixture are produced. The combination of the retention indices from columns coated with a CD derivative diluted in different polysiloxane phases makes it possible to locate and to identify unambigously the enantiomers of an optically active compound in a complex mixture and hence from their areas to determine directly the enantiomeric excess (e.e.). Some examples in the fields of essential oils and aromas illustrate the abilities of 2,6-di-O-methyl-3-O-pentyl-β-CD diluted with different phases (PS-347.5, PS-086, OV-1701 and OV-1701-OH) to evaluate the e.e. of one or more optically active compounds in a complex mixture.

## INTRODUCTION

In a previous paper [1], the influence of diluting phases on the capillary gas chromatographic (cGC) separation of standard racemates of volatiles through cyclodextrin (CD) derivatives was evaluated. In particular, the performance of columns coated with 2,6-di-O-methyl-3-O-pentyl-β-cyclodextrin (2,6-DiMe-3-Pe-β-CD), diluted in OV-1701 or OV-1701-OH (*i.e.*, the most used diluting phase) was compared with that of columns prepared with diluting phases with different polarity such as PS-086, PS-347.5 and OV-225. In the separation of the racemates under investigation, none of the four diluting phases gave unequivocably better results than the other three, although PS-347.5, PS-086 and OV-1701 generally produced better separations than OV-225. In general, with the same temperature programme, the less polar diluting phases gave shorter retentions for racemates, and as a consequence lower elution temperatures; as demonstrated by Grob and co-workers [2–4], lower elution temperatures produce better resolutions. Comparable results were also reported by Dietrich *et al.* [5], who obtained better

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resolutions for trifluorophenylethanol and alkyl-branched free fatty acid racemates when SE-52 and SE-30 were used, rather than OV-1701-vi as diluting phase for 2,3-dimethyl-6-*tert.*-butyldimethylsilyl-β-CD.

Diluting phases of different polarity can be very helpful in identifying a component in complex mixtures. A different diluting phase actually produced a different retention of each component in the mixture, which is related to its different chromatographic interaction with that phase. The combination of the retention indices from different columns makes it possible to identify the component in question directly in the complex mixture in a procedure which is alternative or complementary to the more time-consuming clean-up procedures or to the expensive multi-dimensional GC techniques. At the same time, in most instances, different retentions with different columns for the same enantiomers can reveal whether co-elution has occurred, and hence afford a more correct determination of the enantiomeric excess (e.e.). Koenig and co-workers [6,7] reported a different approach for the analysis of essential oils and proposed combining the chromatographic data obtained from more than one column coated with different CD derivatives but with the same diluting phase (OV-1701).

This paper aims to illustrate the possibility of using 2,6-DiMe-3-Pe-β-CD diluted with different phases to determine the e.e. of a component in real samples, through some examples in the fields of essential oils and aromas.

## EXPERIMENTAL

### *Column preparation*

2,6-DiMe-3-Pe-β-CD was synthesized and purified as reported previously [8]. Fused-silica columns (25 m × 0.25 mm I.D.) were prepared by static coating. The columns were coated with a 0.15-μm film of 10% 2,6-DiMe-3-Pe-β-CD in PS-347.5 (polymethylsiloxane) (Petrarch Systems, Bristol, PA, USA), PS-086 (polymethylphenylsiloxane, 12% phenyl) (Petrarch Systems), OV-1701 (polymethylphenylcyanopolysiloxane, 7% cyanopropyl, 7% phenyl) (Ohio Valley Speciality Chemical, Marietta, OH, USA) or OV-1701-OH (polymethylphenylcyanopolysiloxane, 7% cyanopropyl, 7% phenyl, OH terminated) (Ohio Valley Speciality Chemical) CD diluting phases. Columns using PS-347.5 and PS-086 were deactivated with diphenyltetramethyldisilazane [9] and those coated with OV-1701 were deactivated with Carbowax 20M [10,11]. A concentration of the coating solutions (0.24% w/v) suitable to obtain a 0.15-μm film, thickness was prepared following the equation reported by Grob [9]. The complete procedures for column preparation have been described in detail in a previous paper [12].

### *Capillary GC and capillary GC–MS conditions*

Capillary gas chromatography was performed with a Carlo Erba (Milan, Italy) Model 4160 gas chromatograph equipped with a flame ionization detector. Split injection was used with a splitting ratio of 1:30. The injection temperature was 250°C and the detector temperature 260°C.

The column temperature was programmed from 50°C (held for 1 min) at 2°C/min to 200°C (held for 30 min). The volume injected was 1 μl. Sample dilutions for each example are reported in the figure captions.

A standard mixture of $C_8$–$C_{20}$ hydrocarbons (50 ng/μl) was used as a reference for retention index ($I$) determination. Retention indices were calculated following Van den Dool and Kratz [13].

The chromatographic data were processed on HP 3396 Series II computing integrators (Hewlett-Packard, Avondale, PA, USA), and then transferred on-line to an HP Vectra 386SX personal computer (Hewlett-Packard, Grenoble, France). Retention indices were calculated through an improved version of a programme developed in the authors' laboratory and reported elsewhere [14].

GC–MS analyses with electron impact ionization were carried out on an HP 5988 A GC–MS system provided with an HP 5890 GC unit (Hewlett-Packard). cGC separations were carried out with the same columns and under conditions analogous to those reported for GC analyses. The carrier gas was helium.

Each enantiomer was identified by comparing

its $I$ value with that of a pure standard that was analysed under the same GC conditions on each of the above columns. Identification was confirmed, when necessary, by GC–MS and GC–MS with selected-ion monitoring (SIM) (see Results and discussion).

*Standards, essential oils and aromas*

Linalool, terpinen-4-ol and α-terpineol were obtained from Aldrich Chimica (Milan, Italy), γ-lactone C10 from Roth (Karlsruhe, Germany) and *cis*-linalyl oxide from SGA Flavours (Turin, Italy). Lemon and tangerine essential oils were supplied by Professor G. Dugo (Dipartimento Farmaco-Chimico, Università di Messina, Messina, Italy). Apricot and mango aroma were obtained from SGA Flavours.

## RESULTS AND DISCUSSION

The retention index is a well established parameter for locating and identifying a component in the chromatogram of a complex mixture [15,16]. The technology of the instruments and the consistency of capillary column performance available today afford intra-laboratory retention index reproducibility within ±2 units. Moreover, when the retention indices obtained from two or more columns coated with stationary phases of different polarity are used in combination, the proportion of correct identification can be as high as 90%.

The technique described here can be carried out in one step with a single injection into two or more columns either by cold on-column injection through a multi-way press-fit connector or by split injection, either with the columns assembled in the injector body or by splitting the vaporized sample in the chromatographic oven through a multi-way press-fit connector [17].

A single CD derivative diluted with phases with different polarity produces a consistent enantiomer separation capacity of the selected CD derivative, and different retentions produced by diluting phases with different polarity on structurally different components. When compatible with the complexity of the mixture under analysis, suitable temperature programmes for each diluting phases can improve enantiomer separation, an demonstrated elsewhere [1].

The use of different CD derivatives with the same diluting phase can be very helpful when not all the racemates to be investigated are separated by a single CD derivative. As the diluting phase makes a constant contribution to retention, the different retention increases produced by different CD derivatives can cause sufficient variations in the retention indices to identify successfully each enantiomer of the component(s) under investigation in the total chromatogram [18]. However, in the authors' experience, the difference in retention (and as a consequence the difference in the retention index values) produced by different polarity diluting phases with a single CD derivative on every component in the mixture, *i.e.*, including those components which are not optically active, is more significant than that produced by different CD derivatives diluted with the same stationary phase. Moreover, different CD derivatives with the same diluting phase can produce inversion of the elution order of the enantiomers while a single CD derivative diluted with phases with different polarity does not produce this effect [19].

The following applications illustrate some possibilities of the proposed method. The first examples concern the characterization of lemon and tangerine essential oils through the determination of the e.e. of linalool, terpinen-4-ol and α-terpineol. The e.e. of these components can give information about the technology of preparation of the oils [20]. The analyses were carried out on three columns coated with 2,6-Di-Me-3-Pe-β-CD diluted with three different diluting phase (PS-347.5, PS-086 and OV-1701). Table I reports the retention indices on the three columns of the components investigated in the examples described here. Fig. 1 reports the cGC pattern of a lemon oil on a column coated with 2,6-Di-Me-3-Pe-β-CD with PS-347.5 as diluted phase and the location of the peaks of the three components investigated. Fig. 2 reports those parts of the chromatograms in which the three components in question eluted.

With the column coated with PS-347.5 as diluting phase, one of the linalool enantiomers ($I_I = 1155$, $I_{II} = 1162$) partially overlaps another

TABLE I

RETENTION INDICES ON THE THREE COLUMNS COATED WITH 2,6-Di-Me-3-Pe-β-CD DILUTED IN PS-347.5, PS-086 AND OV-1701 OF THE RACEMATES INVESTIGATED IN THE REPORTED EXAMPLES

$I_I$ = Retention index of the first-eluted enantiomer; $I_{II}$ = retention index of the second-eluted enantiomer.

| Compound | Diluting phase | | | | | |
|---|---|---|---|---|---|---|
| | PS 347.5 | | PS 086 | | OV-1701 | |
| | $I_I$ | $I_{II}$ | $I_I$ | $I_{II}$ | $I_I$ | $I_{II}$ |
| Linalyl oxide | 1092 | 1103 | 1129 | 1141 | 1156 | 1167 |
| Linalool | 1155 | 1162 | 1191 | 1200 | 1226 | 1236 |
| Terpinen-4-ol | 1240 | 1247 | 1288 | 1296 | 1320 | 1329 |
| α-Terpineol | 1278 | 1282 | 1327 | 1331 | 1369 | 1373 |
| γ-Lactone C10 | 1497 | 1502 | 1583 | 1588 | 1679 | 1683 |

component ($I_I$ = 1158), while terpinen-4-ol ($I_I$ = 1240, $I_{II}$ = 1247) and α-terpineol ($I_I$ = 1278, $I_{II}$ = 1282), which are trace and minor components, respectively, eluted very close to major components. With PS-086, there was an improved resolution of α-terpineol ($I_I$ = 1327, $I_{II}$ = 1331) and of that part of the chromatogram in which linalool ($I_I$ = 1191, $I_{II}$ = 1200) elutes but a major component overlapped one of the terpinen-4-ol enantiomers ($I_I$ = 1288, $I_{II}$ = 1296). With OV-1701, terpinen-4-ol ($I_I$ = 1320, $I_{II}$ = 1329) and α-terpineol ($I_I$ = 1369, $I_{II}$ = 1373) eluted in a less complex part of the chromatogram, but one of the linalool enantiomers ($I_I$ = 1226, $I_{II}$ = 1236) co-eluted with the peak at $I$ = 1158 on PS-347.5 mentioned above.

Because of the complexity of the sample, much care was devoted to correct component location and e.e. determination. The enantiomers under investigation were identified by comparison with the $I$ values of the corresponding standards. Peak purity was evaluated through the peak-area ratios of the two enantiomers on the three different columns. The possible co-elution of one or both enantiomers with other sample components was investigated, when necessary, by GC–MS and GC–SIM-MS analysis. GC–SIM-MS was very useful for confirming the e.e. and it was applied in particular when the e.e. values on all the three columns were differ-

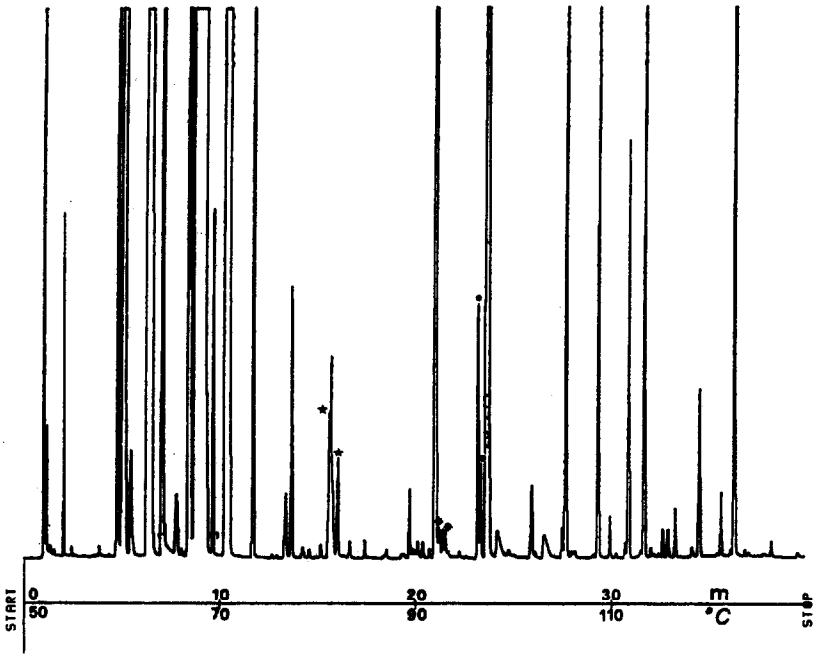


Fig. 1. Lemon oil cGC pattern. Sample dilution, 1:10. Column, 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5. ★ = Linalool; * = terpinen-4-ol; ● = α-terpineol. All figures: m = min.

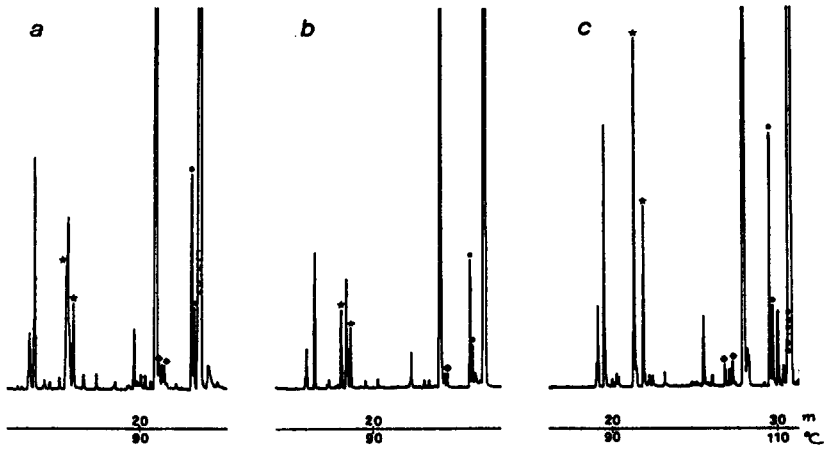


Fig. 2. Parts of lemon oil chromatograms in which linalool (★), terpinen-4-ol (*) and α-terpineol (●) elute. Columns: (a) 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5; (b) 10% 2,6-Di-Me-3-Pe-β-CD–PS-086; (c) 10% 2,6-Di-Me-3-Pe-β-CD–OV-1701.

ent. Only those e.e. values which were comparable on at least two different columns were taken as valid. Table 2 reports the e.e. calculated on the GC peak areas with flame ionization detection (FID) of the components in the examples described, determined on the three columns under investigation. The reported e.e. values are the means of three replicate analyses of each sample for each column.

In the tangerine oil, the three components in question are contained in smaller amounts than in lemon oil, and this can make the determi-

TABLE II

ENANTIOMERIC EXCESS CALCULATED ON THE GC–FID PEAK AREAS OF THE COMPONENTS INVESTIGATED IN THE LEMON AND TANGERINE OILS AND IN THE APRICOT AROMAS DETERMINED ON THE THREE COLUMNS UNDER INVESTIGATION

I = % of the first-eluted enantiomer; II = % of the second-eluted enantiomer; e.e. = enantiomeric excess. For the first column (PS-347.5), the enantiomer eluting first is indicated.

| Sample | Compounds | Diluting phase | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | PS-347.5 | | | PS-086 | | | OV-1701 | | |
| | | I | II | e.e. | I | II | e.e. | I | II | e.e. |
| Lemon oil | Linalool | 56.8(−) | 44.2(+) | 12.6 | 57.3 | 43.7 | 13.6 | 67.2 | 32.8 | 34.4 |
| | Terpinen-4-ol | (+) | (−) | N.M.[a] | | | N.M.[a] | 38.4 | 61.6 | 23.2 |
| | α-Terpineol | 75.8(−) | 24.2(+) | 51.6 | 75.8 | 24.2 | 51.6 | 76.3 | 23.7 | 52.6 |
| Tangerine oil | Linalool | 16.1 | 83.9 | 67.8 | 16.1 | 83.9 | 67.8 | 16.8 | 83.2 | 66.4 |
| | Terpinen-4-ol | 11.6 | 88.4 | 76.8 | 11.9 | 88.1 | 76.2 | 11.3 | 88.7 | 77.4 |
| | α-Terpineol | 76.7 | 23.3 | 53.4 | 74.9 | 25.1 | 49.8 | 74.8 | 25.2 | 49.6 |
| Apricot aroma | Linalyl oxide | 50.1(−) | 49.9(+) | 0.2 | 50.1 | 49.9 | 0.2 | 49.2 | 50.8 | 1.6 |
| | Linalool | 96.8 | 3.2 | 93.6 | 96.6 | 3.4 | 93.2 | 96.6 | 3.4 | 93.2 |
| | α-Terpineol | 50.4 | 49.6 | 0.8 | 47.9 | 52.1 | 4.2 | 50.6 | 49.4 | 1.2 |
| | γ-Lactone C10 | 49.8(+) | 50.2(−) | 0.4 | 50.0 | 50.0 | 0 | 54.8 | 45.2 | 9.6 |

[a] Not measured.

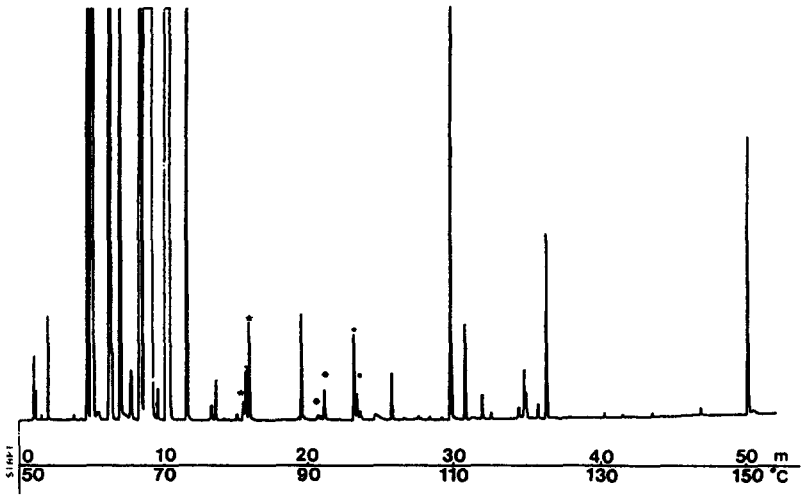


Fig. 3. cGC pattern of tangerine oil. Sample dilution, 1:10. Column, 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5. ★ = Linalool; * = terpinen-4-ol; ● = α-terpineol.

nation of the e.e. less reliable because of the difficulty of correctly integrating the area. On the other hand, the part of the chromatogram in which the components under investigation elute is "cleaner" than with lemon oil, thus facilitating their correct identification. The analyses were carried out with the same three columns as used for lemon oil. Fig. 3 shows the cGC pattern of the tangerine oil on the column coated with 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5. Fig. 4 reports those parts of the chromatograms, obtained on the three columns, in which linalool, terpinen-4-ol and α-terpineol eluted. The chromatographic behaviour of linalool and α-terpineol is similar to that already described for lemon oil, while terpinen-4-ol enantiomers are easier to determine here than in lemon oil, because the two enantiomers elute in a part of the chromatogram without interfering peaks.

The next two examples deal with the charac-

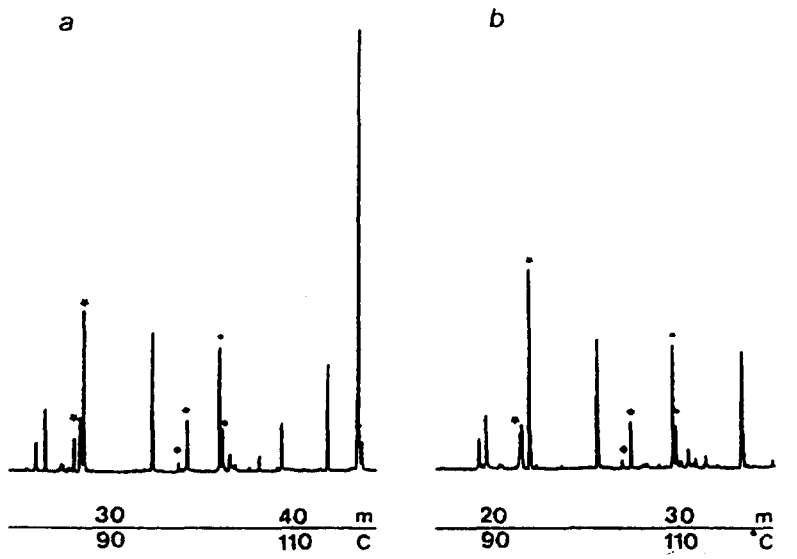


Fig. 4. Parts of tangerine oil chromatograms in which linalool (★), terpinen-4-ol (*) and α-terpineol (●) elute. Columns: (a) 10% 2,6-Di-Me-3-Pe-β-CD–PS-086; (b) 10% 2,6-Di-Me-3-Pe-β-CD–OV-1701.

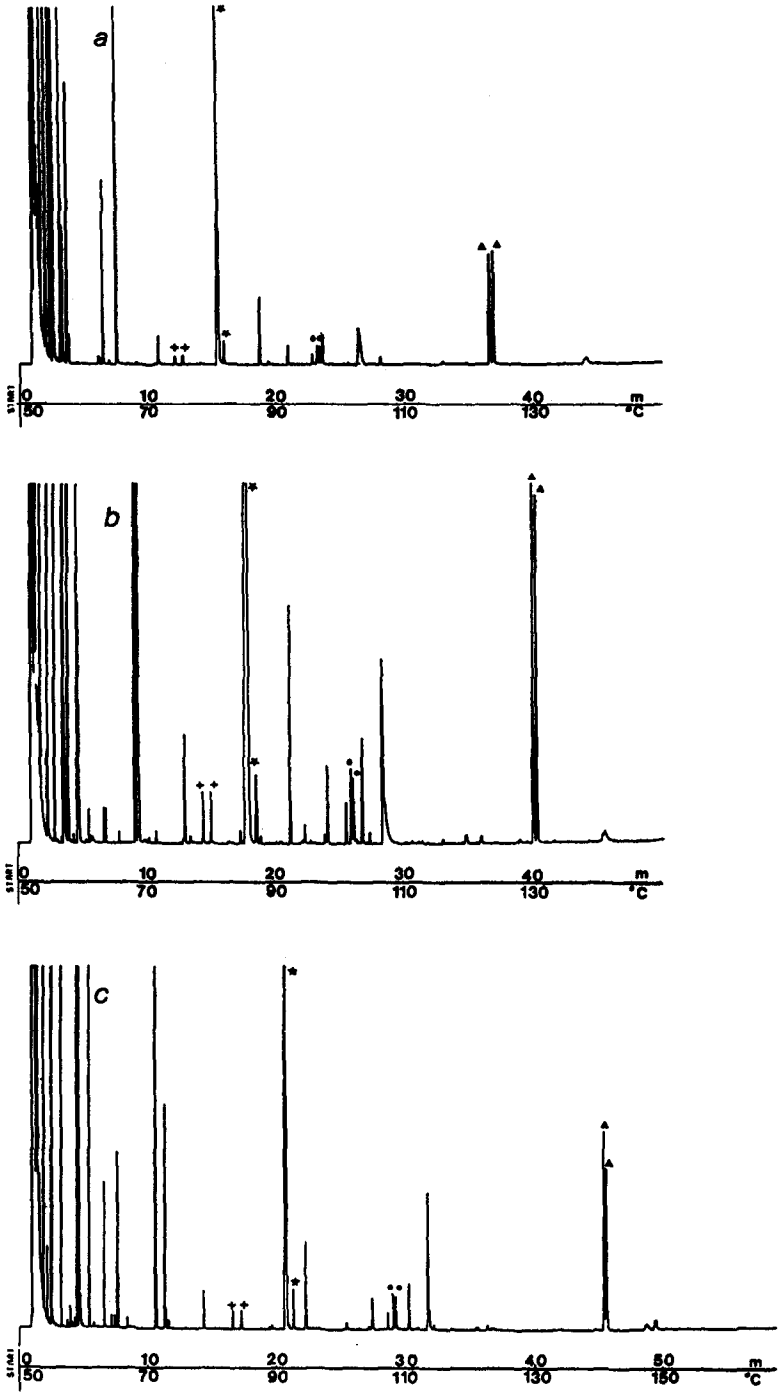


Fig. 5. cGC patterns of apricot aroma. Columns: (a) 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5, sample dilution 1:100; (b) 10% 2,6-Di-Me-3-Pe-β-CD–PS-086, sample dilution 1:30; (c) 10% 2,6-Di-Me-3-Pe-β-CD–OV-1701, sample dilution 1:70. + = *cis*-Linalyl; ★ = linalool; * = terpinen-4-ol; ▲ = γ-lactone C10.

terization of two different aromas. The first concerns a synthetic aroma of apricots, which can be characterized in particular by four components, *cis*-linalyl oxide, linalool, $\alpha$-terpineol and $\gamma$-lactone C10. Because in the formulation the components under investigation are present in a different range of concentrations, three different dilutions of the sample were injected, in order to detect both major and minor components in a single run (see caption to Fig. 5). Fig. 5 shows the cGC patterns of the aroma on the three columns investigated. Linalool was present in the aroma with a very high e.e., whereas for the other components of interest the enantiomer ratio was about 1:1. In this instance, all the components were easy to locate in the chromatogram, and the three columns were mainly used to confirm both the identification of the component under investigation through their retention indices and the reproducibility of the e.e. The e.e. for $\gamma$-lactone C10 on the 10% 2,6-Di-Me-3-Pe-$\beta$-CD–OV-1701 column was similar to that on the other two columns when analysed by GC–SIM-MS through the diagnostic peak at $m/z$ 85.

The last example concerns the discrimination between two mango aromas, one containing synthetic $\gamma$-lactone C10 and the other natural $\gamma$-lactone C10. Fig. 6 reports the chromatograms of the two aromas on a 10% 2,6-Di-Me-3-Pe-$\beta$-CD–OV-1701 column. With this column, the two aromas can be distinguished because in the part

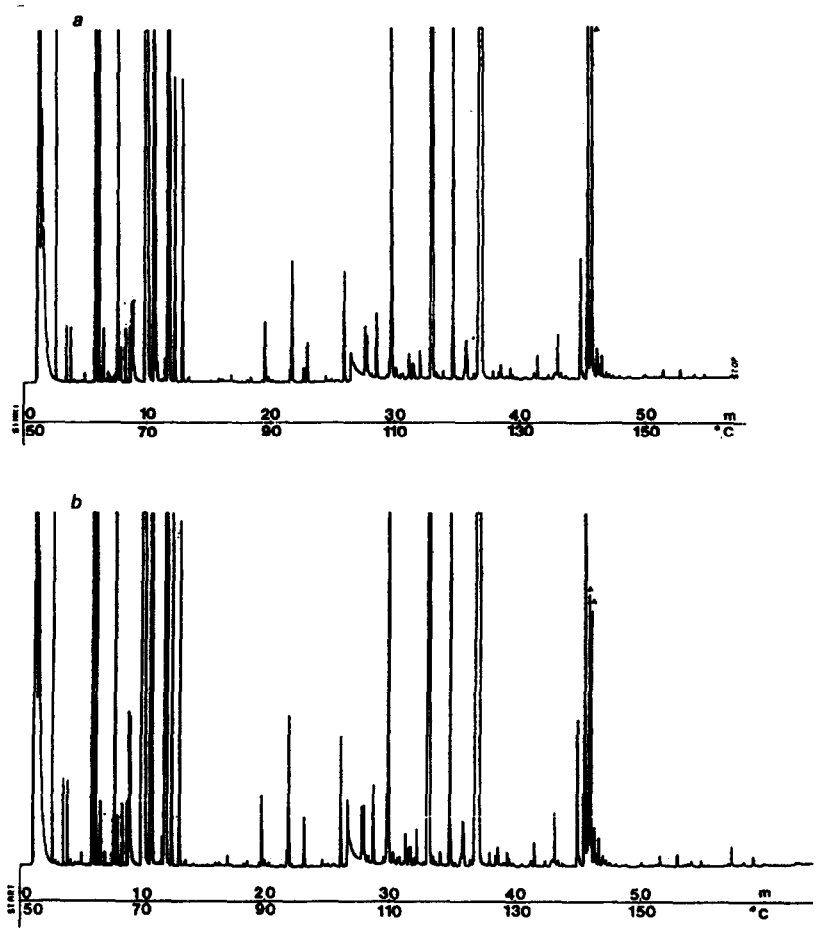


Fig. 6. cGC patterns of the two mango aromas. Sample dilution, 1:50. Column, 10% 2,6-Di-Me-3-Pe-$\beta$-CD–OV-1701. ▲ = $\nu$-Lactone C10.

of the chromatogram where the γ-lactone C10 ($I_I = 1679$, $I_{II} = 1683$) peaks elute in the aroma containing the natural γ-lactone C10 a group of three peaks was detected, whereas in the aroma containing the synthetic lactone a group of four peaks was detected. The co-elution or partial overlapping with other peaks made correct peak-area integration difficult, and the lactone still required unequivocal identification. Fig. 7 reports those parts of the chromatograms of both the aromas in which γ-lactone C10 eluted, when analysed with (a) PS-347.5 and (b) PS-086 as diluting phases. With both PS-347.5 and PS-086 as diluting phases, γ-lactone C10 (PS-347.5, $I_I = 1497$, $I_{II} = 1502$; PS-086, $I_I = 1583$, $I_{II} = 1588$) elutes almost alone in a part of the chromatogram where no other interfering peaks elute, facilitating its determination and, as a consequence, the characterization of the aromas.

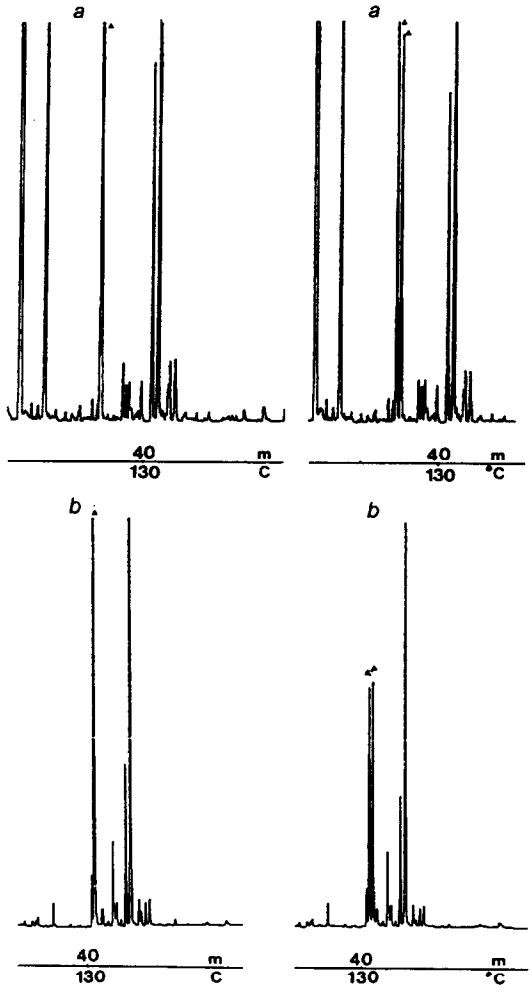


Fig. 7. Parts of the chromatograms of the two mango aromas in which ν-lactone C10 (▲) elutes. Columns: (a) 10% 2,6-Di-Me-3-Pe-β-CD–PS-347.5; (b) 10% 2,6-Di-Me-3-Pe-β-CD–PS-086.

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