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Short Communication

Preparative enantiomer separation with modified cyclodextrins as chiral stationary phases

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ABSTRACT

Preparative gas chromatographic enantiomer separation was achieved by using a packed column with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin (2,6-Me-3-Pe-β-CD) as a chiral stationary phase. Depending on the selectivity factor up to milligram amounts of almost pure enantiomers or at least enantiomeric mixtures, sufficiently enriched to determine the sign of optical rotation, could be obtained with only one injection of a racemate. This technique may be very useful for stereochemical assignments, for attaining pure enantiomers for bioassays, pharmacokinetic and metabolic studies and investigations of fragrance and flavour compounds.

INTRODUCTION

After the introduction of selectively substituted cyclodextrins as a new type of chiral stationary phases an immense progress in analytical enantiomer separation was achieved [1,2]. This is documented by many applications in different research fields and an increasing number of publications. However, only few publications deal with preparative separations of enantiomers, due to the very low sample capacity of open-tubular capillary columns [3,4]. Preparative-scale separations were only possible on a microgram scale with expensive, fully automatic gas chromatographic equipment and with repetitive operation [5,6]. Packed gas chromatographic columns, though having comparatively low separation efficiency, have a much larger sample capacity due to the larger amount of stationary phase used. We have compared the separation efficiency of capillary columns with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin (2,6-Me-3-Pe-β-CD) as a chiral stationary phase [7–9] with a packed column of only 1.8 m length and 4 mm inner diameter with a 2.5% coating of the same cyclodextrin derivative on Chromosorb W.

EXPERIMENTAL

Analytical capillary gas chromatography was performed with fused-silica capillaries coated by the static procedure [10] with 1:1 (w/w) or 1:4 (w/w) mixtures of 2,6-Me-3-Pe-β-CD with polysiloxane OV-1701, after careful deactivation of the inner column surface according to Grob [11]. Carlo Erba Model 2150 and 4160 gas chromatographs with flame ionization detectors, split injectors and hydrogen as a carrier gas were used.

Stainless-steel packed columns were prepared by coating Chromosorb W HP (Merck, 100–120

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mesh) with 5% (w/w) of a 1:1 (w/w) mixture of 2,6-Me-3-Pe-β-CD and OV-1701. A Varian 1400 Model gas chromatograph was used. Injector and detector temperatures were kept at 250 and 275°C, respectively. The column effluent can be split between flame ionization detector and exit port by a needle valve (SGE, MCVT/100). A make-up gas device is attached at the detector side. For analytical use of the packed column the exit port is closed and no make-up gas is added. Samples of approximately 0.5 μg were injected at a flow-rate of maximal 130 ml/min, which allows the smooth operation of the flame ionization detector.

For preparative use the column effluent is split between detector and exit port at a 1:400 ratio. A carrier gas flow of 400 ml/min (!) helium is adjusted by an inlet pressure of 4.0 bar. Under these conditions of operation the addition of make-up gas is necessary to support fractional sample transfer to the detector. The fractions to be collected were trapped in a bath of liquid nitrogen by sleeving pieces of PTFE tubing (Reichelt, 40 cm × 1.5 mm I.D.) over the column outlet inside the oven through a guideway made out of a Swagelok fitting. By this arrangement it is warranted that the first part of the PTFE tubing has the same temperature as the column end and sample trapping at the outlet is eliminated. This trapping device also permits fast manual exchange of tubing traps during a chromatographic run. The recovery for volatile compounds like linalool varies between 40 and 80% depending on the carrier gas velocity.

Polarimetric measurements were carried out on Perkin-Elmer 243 and 241 polarimeters in $CHCl_3$. Due to the low sample concentrations only qualitative values of positive or negative optical rotations were measured.

RESULTS AND DISCUSSION

2,6-Me-3-Pe-β-CD was selected as a chiral stationary phase because it exhibits unique enantioselectivity towards a great variety of compounds with biological or pharmaceutical activity [7,9,12]. In many cases separation factors larger than 1.1 were observed (27% out of 178 separated enantiomeric pairs [13]). This is an important prerequisite for preparative applications. The relatively high amount of stationary phase in packed columns causes an increase in temperature for sample elution. The great dependence of selectivity from operation temperature, however, suggested to use the lowest possible column temperature [14]]. We therefore worked at unusually high carrier gas velocity to reduce retention times. The Van Deemter plot of plate height *versus* carrier gas velocity (Fig. 1) shows a flat branch towards high carrier gas flow. Thus, the loss in separation efficiency associated with such high flow-rates is overcompensated by the gain in selectivity.

This is demonstrated in Fig. 2 for the mucoregulatory drug *trans*-sobrerol. At 135°C the enantiomers of this compound are resolved with very similar selectivity factors and with almost identical retention times from a 25-m capillary column and from the 1.8-m packed column.

Very good results are achieved in the preparative separation of hexobarbital (Fig. 3). The injection of *ca.* 1 mg of racemic sample yielded both enantiomers in high optical purity. In such a case it would be possible without much effort to isolate enough material of both enantiomers for pharmacokinetic and metabolic studies.

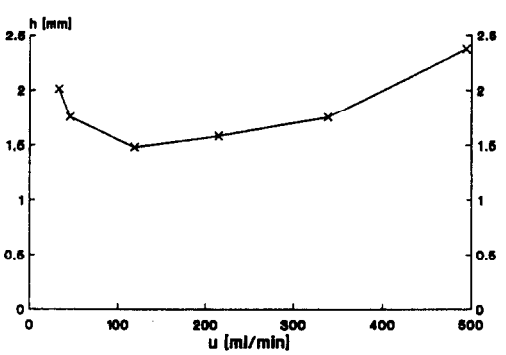

Fig. 1. Plot of plate height *vs.* linear carrier gas velocity (Van Deemter plot) for linalool. A 1-mg amount of sample was injected at 120°C on a 1.8-m packed column with 5% 2,6-Me-3-Pe-β-CD (50%, w/w, in OV-1701) on Chromosorb W; helium as carrier gas.

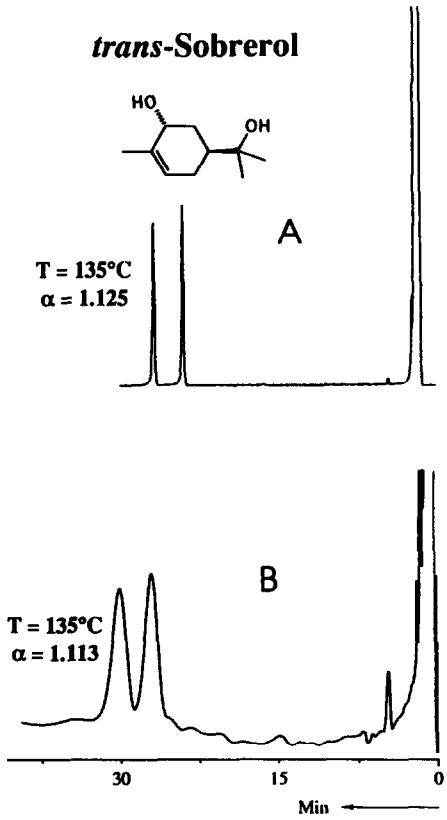

Fig. 2. (A) Analytical enantiomer separation of *trans*-sobrerol on a 25-m capillary column with 2,6-Me-3-Pe-β-CD (50%, w/w, in OV-1701) at 135°C. (B) Analytical enantiomer separation of the same sample on 1.8-m packed column with the same chiral phase. A 0.5-μg amount of sample at 135°C and 2.2 bar helium as carrier gas was injected.

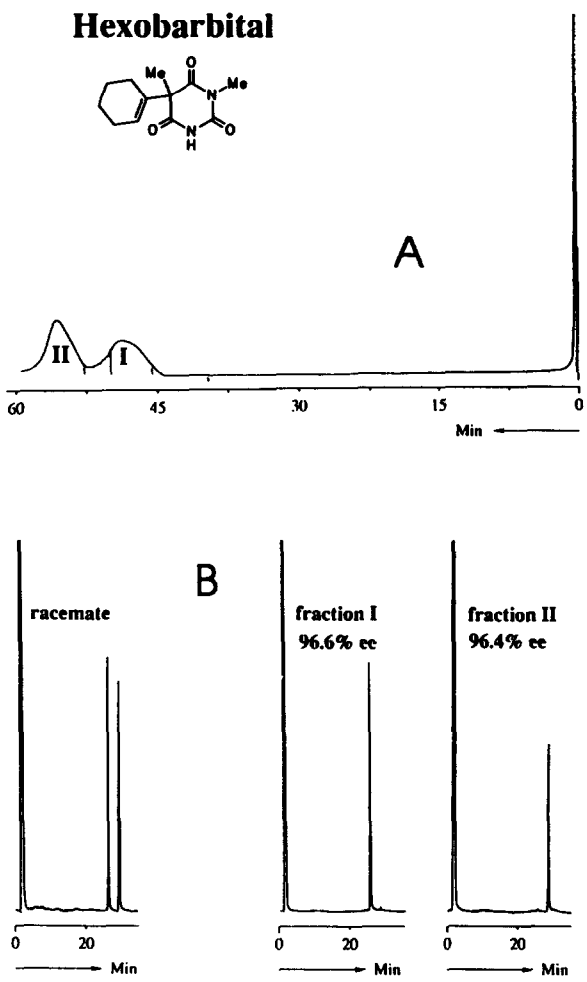

Fig. 3. (A) Preparative enantiomer separation of hexobarbital. *Ca.* 1 mg of the racemate was injected at 165°C and an inlet pressure of 4.0 bar helium. (B) Analysis of the collected fractions. Same capillary column as in Fig. 2, column temperature 180°C. ee = Enantiomeric excess.

The efficiency of the trapping device is demonstrated with the fragrance compound methyl jasmonate. In Fig. 4 *ca.* 2 mg of a mixture of racemic methyl jasmonate and methyl *epi*-jasmonate were injected. The analysis of the collected fractions shows high enantiomeric purity for the (+)- and (−)-isomer and the assignment of the direction of the optical rotation is possible by polarimetric measurements without further sample enrichment. The diastereoisomeric methyl *epi*-jasmonate enantiomers are not resolved with this phase.

In the examples discussed so far relatively large separation factors for the enantiomers were observed. Baseline separation of the fragrance compound muscone was possible for the first time on capillaries with 2,6-Me-3-Pe-β-CD [7] (separation factor $\alpha = 1.025$ at 140°C, 25-m capillary). In the preparative run 5 mg of racemic sample were injected (Fig. 5). No resolution is indicated in the chromatogram; however, by collecting fractions at the flanks of the peak it was possible to achieve sufficient enantiomeric enrichment for optical rotation measurements and peak assignment, as shown in the subsequent analysis of the collected fractions. In

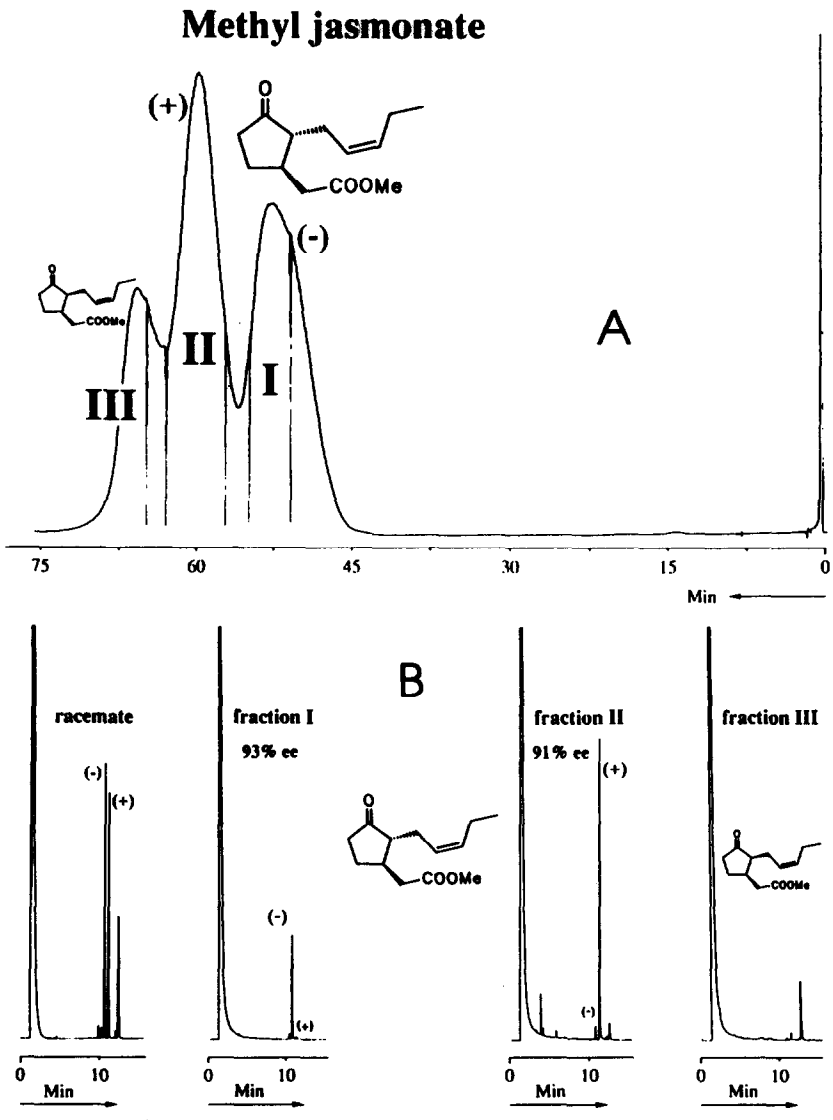

Fig. 4. (A) Preparative enantiomer separation of methyl jasmonate. *Ca.* 2 mg of mixture of four stereoisomers were injected at 120°C at an inlet pressure of 4.0 bar helium. (B) Analytical investigation of collected fractions. Same capillary column as in Fig. 2, column temperature 160°C.

such a case the enantioselective synthesis of optically active material is a laborious task [15].

CONCLUSIONS

A simple, non-expensive and efficient procedure for preparative enantiomer separation by packed column gas chromatography is described. With only few injections of milligram amounts of racemic material optically active fractions can be collected. The described method may be useful for assignment of the order of elution of enantiomers in enantioselective chromatography, for bioassays and pharmacokinetic investigations. It is shown that the enantiomeric enrichment is highly depending on the separation factors. Separation factors larger than 1.1 may be suffi-

cient for gaining enantiomeric enrichment exceeding 90% enantiomeric excess.

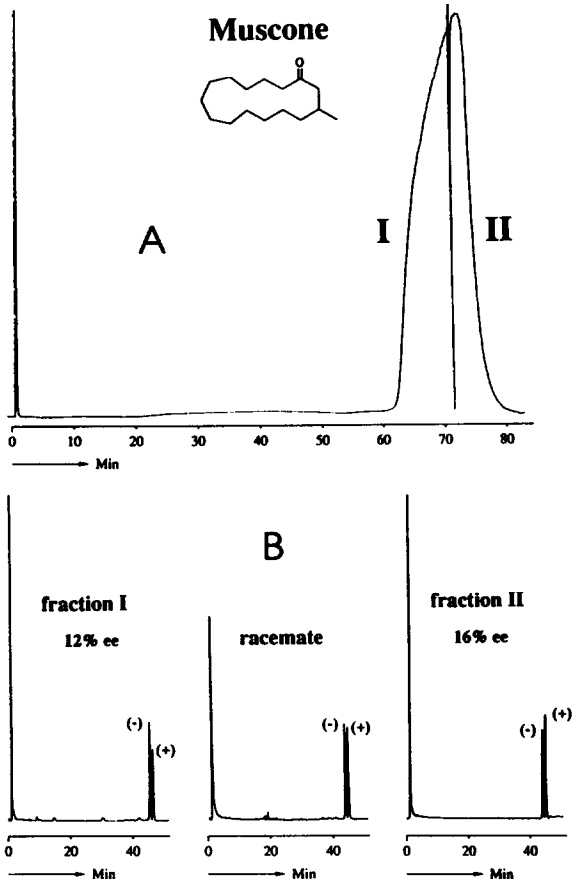

Fig. 5. (A) Preparative enantiomer separation of muscone. *Ca.* 5 mg of racemate were injected at 130°C at an inlet pressure of 4.0 bar helium. (B) Analysis of the collected fractions on a 25-m capillary column with 2,6-Me-3-Pe-β-CD (20%, w/w, in OV-1701) at 135°C.

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