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Direct enantiomeric resolution of monoterpene hydrocarbons via reversed-phase high-performance liquid chromatography with an α-cyclodextrin bonded stationary phase

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ABSTRACT

Chiral recognition in liquid chromatography (LC) generally requires relatively strong interactions between the analyte and the chiral stationary phase, often in combination with steric interactions. Hence most LC enantiomeric separations favor compounds that have hydrogen bonding groups, aromatic rings, strong dipoles, possibilities for $\pi-\pi$ or charge transfer interactions, etc. Compounds with little or no functionality are usually difficult to resolve by LC methods. Conversely gas chromatography (GC) has been very useful in resolving compounds with limited functionality, including hydrocarbons. In this sense GC has been very complementary to LC methodologies. In this work the enantiomeric separation of hydrocarbons by reversed-phase LC is described. It appears that chiral recognition results largely from "shape-selectivity" (*i.e.*, the tight fit of a hydrophobic moiety into a hydrophobic cavity) with few other substantial contributing interactions. Small amounts of methyl *tert.*-butyl ether greatly enhanced the separation efficiency. All commercial samples contain significant quantities of enantiomeric impurities.

INTRODUCTION

The resolution of enantiomeric hydrocarbons is one of the more difficult problems in separation science. They have few or no functional groups which limits both derivatization techniques and direct separation via enantioselective association. Many hydrocarbon enantiomers have not been resolved. In many cases the optical rotations of these compounds is small or unknown.

Far and away the most successful technique for resolving chiral hydrocarbons seems to be direct resolution on cyclodextrin-based gas chromatographic (GC) stationary phases. Unlike HPLC methods, GC techniques are able to directly resolve a variety of molecules that have limited functionality. Smolkova-Keulemansova and co-workers [1,2] first separated structural isomers of hydrocarbons by packed column GC using native and methylated and acylated cyclodextrins. Koscielski and co-workers [3,4] used a similar system to resolve enantiomers of α- and β-pinene. Schurig *et al.* [5] used capillary GC with permethylated β-cyclodextrin dissolved in silicone oil to resolve several unfunctionalized cycloalkanes. König and co-workers [6,7] resolved several enantiomeric olefins on neat alkylated cyclodextrin-based capillary columns. Armstrong *et al.* [8] resolved aromatic and aliphatic hydrocarbon biomarkers on capillary GC columns coated with alkylacylcyclodextrin derivatives and hydroxypropylated cyclodextrins. It appears that the facile resolution of chiral hydrocarbons was dependent on the advent of cyclodextrin-based capillary GC columns.

There has been very little success in resolving enantiomeric hydrocarbons by liquid chromatog-

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raphy (LC). Both the analyte and the stationary phase are solvated by mobile phase components in LC. The stereoselective interactions of the analyte with the stationary phase must be sufficiently energetic that they can compete with those of the solvating molecules. Hence LC enantiomeric separations often are characterized by relatively strong interactions between the analyte and the chiral stationary phase (*i.e.*, strong dipolar, hydrogen bonding, $\pi-\pi$, charge transfer, etc.). Steric considerations also are important but they are almost always accompanied by one or more strong interactions.

There were two early reports on the indirect HPLC separation of olefinic compounds. These were done as diastereoisomeric Pt(II) complexes [9,10]. Recently the direct HPLC separation of (±)-α-pinene was reported using α-cyclodextrin as a mobile phase additive [11]. In this work we describe the enantiomeric resolution of (±)-α-pinene, (±)-β-pinene (±)-camphene on a native α-cyclodextrin bonded phase. The effect of analyte concentration and mobile phase additives on selectivity and efficiency are considered as well.

EXPERIMENTAL

Materials

Racemic α-pinene was purchased from Aldrich (Milwaukee, WI, USA). All other enantiomeric compounds were from different sources listed in Table I. Methanol and water were of OmniSolve grade and supplied from EM Science (Gibbstown, NJ, USA). Dichloromethane, isopropylether and *tert.*-butyl methyl ether were HPLC grade from Aldrich.

Methods

The HPLC system consisted of a pump (LC-6A, Shimadzu, Kyoto, Japan), a system controller (SCL-6B, Shimadzu), Chromatopac (CR 601, Shimadzu), UV detector (SPD-6A, Shimadzu) and 5-μl loop injector valve (Rheodyne, Cotati, CA, USA). The columns were 250 × 4.6 mm and were packed with α-cyclodextrin bonded to 5-μm spherical silica gel (Astec, Whippany, NJ, USA). UV detection at 210 nm was used. All separations were carried out at room temperature (22°C). Prior to injection all analytes were dissolved in a mixture of methanol–water (80:20, v/v). Volumes of 5 μl of sample were injected with analyte concentration between 5 and 20 μg/ml.

TABLE I

SUMMARY OF THE AMOUNT OF ENANTIOMERIC IMPURITIES FOUND IN VARIOUS COMMERCIAL SAMPLES OF MONOTERPENE HYDROCARBONS

Compound, enantiomeric	Producer and catalog number	Content (%)	
		(+)	(−)
(+)-α-Pinene	Aldrich 26,807-0	99.3	0.7
(+)-α-Pinene	Aldrich P4568-8	95.6	4.4
(−)-α-Pinene	Aldrich 30,571-5	1.3	98.7
(−)-α-Pinene	Aldrich 27,439-9	5.4	94.6
(−)-α-Pinene	Aldrich P4,570-2	10.3	89.7
(+)-β-Pinene	Fluka 80607	94.9	5.1
(−)-β-Pinene	Fluka 80609	3.7	96.3
(−)-β-Pinene	Aldrich 11,208-9	4.6	95.4
(−)-β-Pinene	ICN	2.9	97.1
(+)-Camphene	Aldrich C301	57.2	42.8
(−)-Camphene	Aldrich 31,042-5	40.0	60.0
(−)-Camphene	Fluka 21290	39.3	60.7

RESULTS AND DISCUSSION

Chiral recognition between native α-cyclodextrin and the bicyclic monoterpene hydrocarbons (±)-α-pinene, (±)-β-pinene and (±)-camphene can be significant in methanol–water solvent mixtures. Using indirect detection, the saturated analogue (±)-α-pinane also produced two peaks. However, no pure enantiomeric standards were available for confirmation. Chiral recognition does not seem to occur with some other hydro–organic solvents such as acetonitrile–water. Solute retention results from inclusion complex formation in this chromatographic mode [12]. However, chiral recognition must be due largely to the strong shape selectivity of the α-cyclodextrin cavity for these compounds. Hydrogen bonding to the mouth of the cyclodextrin cavity usually plays an important role in solution-based chiral recognition [12]. However, there are no good hydrogen bonding groups on these compact hydrocarbons. Hence, in these cases the inclusion complex is formed as the result of the hydrophobic effect and chiral recognition most

likely results from steric interactions and Van der Waals forces.

Both the chromatographic enantioselectivity and efficiency of these separations is dependent on analyte concentration and the presence of additional mobile phase additives. Fig. 1 shows that increasing analyte concentration not only decreases retention but rapidly inhibits the enantiomeric separation. Baseline separation is lost if more than *ca.* 1 μg of sample is injected. This is one of the more pronounced effects of analyte concentration on α-value that we have seen to date.

Fig. 2 shows the effect of small amounts (0.5%) of neutral mobile phase additives on the chromatographic resolution of (±)-α-pinene. Entirely analogous results were obtained with (±)-β-pinene and (±)-camphene. The mobile phase consisted of 60% water and either 40% or 39.5% methanol (depending on whether or not 0.5% of additive was present). As seen in Fig. 2A, the enantioselectivity of α-cyclodextrin for (±)-α-pinene is fairly high ($\alpha > 2.0$). The efficiency, however, is poor. The addition of neutral modifiers (Fig. 2B–D) tends to decrease both retention and enantioselectivity ($\alpha \approx 1.6$–1.7) somewhat. However some modifiers greatly improve peak shape and efficiency. For the analytes resolved in this study, methyl *tert.*-butyl ether seemed to the most effective additive (see Fig. 2D).

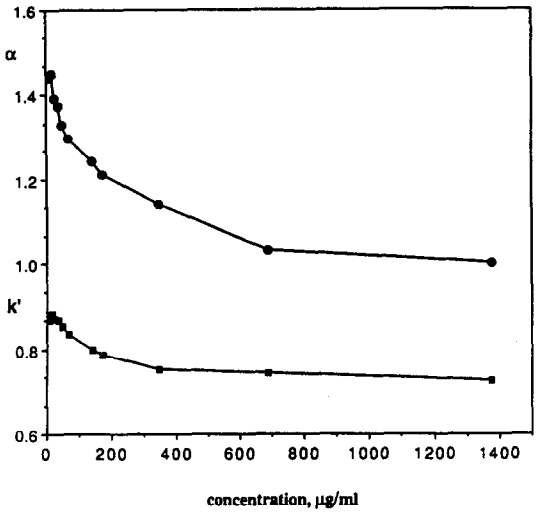

Fig. 1. Plot showing that both the retention ($k' = \blacksquare$) and enantioselectivity ($\alpha = \bullet$) decreases when the concentration of injected analyte (±)-α-pinene is increased. Note that at concentrations above 400 μg/ml enantioselectivity is lost. The same behavior was found for all chiral compounds in this study. The column was a 25 × 0.46 cm I.D. Cyclobond III (α-CD) operated at 0.8 ml/min. In all cases 5 μl of sample were injected (see Experimental). The mobile phase was water–methanol–methyl *tert.*-butyl ether (60:39.5:0.5, v/v/v). UV detection at 210 nm was used.

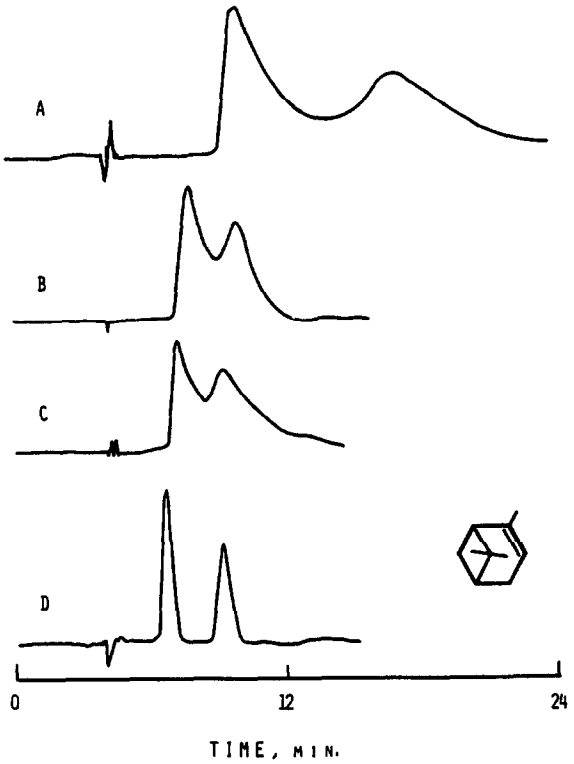

Fig. 2. Four chromatograms showing the effect of small amounts of mobile phase additives on the reversed phase HPLC separation of (±)-α-pinene on a 25 × 0.46 cm Cyclobond III column at 0.8 ml/min flow-rate. Nearly identical results were obtained for (±)-β-pinene and (±) camphene. The mobile phases were as follows: (A) water–methanol (60:40, v/v), (B) water–methanol–diisopropyl ether (60:39.5:0.5, v/v/v), (C) water–methanol–methylene chloride (60:39.5:0.5, v/v/v) and (D) water–methanol–methyl *tert.*-butyl ether (60:39.5:0.5, v/v/v).

Using the optimized HPLC technique several commercial standard compounds were analyzed for enantiomeric purity. The results are shown in Table I and in representative chromatograms in Fig. 3. Clearly enantiomeric impurities exist in all commercial samples. The camphene analyses were particularly revealing (Table I and Fig. 3)

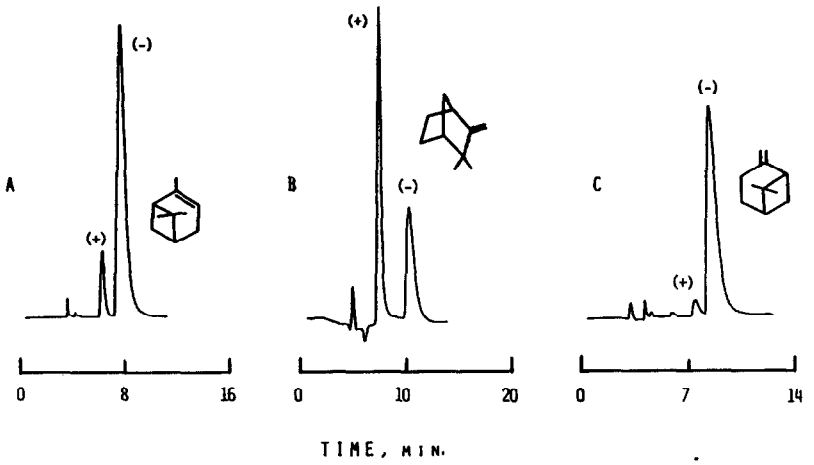

Fig. 3. Representative chromatograms showing the retention order and enantiomeric purity of commercial samples of (A) (−)-α-pinene, (B) (+)-camphene and (C) (−)-β-pinene. Experimental conditions as for Fig. 2D.

in terms of their small enantiomeric excesses. It was found that (+)-camphene contained almost 43% of the opposite enantiomer while (−)-camphene contained approximately 40% of its antipode.

Although the enantioselective separation of compounds with limited functionalities currently are more rare in LC than in GC, they are possible under certain circumstances. In this case the relevant factors seem to include a strong association of the analyte with a chiral selector that can discriminate largely on the basis of steric considerations.

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