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NEW CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF THE ENANTIOMERIC PURITY OF TERPENOIC HYDROCARBONS

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SUMMARY

The application of a very selective chiral stationary phase (Celite coated with α-cyclodextrin in formamide solution) to gas–liquid chromatography is described. A new convenient method for determination of the enantiomeric purity of terpenoic hydrocarbons, *e.g.*, α-pinene, β-pinene, *cis*-pinane, *trans*-pinane and 2-carene, based on complete chromatographic separation of the enantiomers, is presented.

INTRODUCTION

Terpenes are a class of compounds found in large quantities in most plants; they are of special interest because of their industrial uses and their chemical and biological properties. For example, α-pinene [readily available in the (+) and (−) isomeric forms] is of increasing importance for directed chiral synthesis[1]. This results in the need for α-pinene of high and precisely determined optical purity. Unfortunately, the commercially available (+)- and (−)-α-pinene are of variable enantiomeric purity; the best available commercial (+)-α-pinene is 91.3% enantiomerically pure, and (−)-α-pinene is only 81.3% enantiomerically pure (Aldrich). The determination of the optical purity of chiral hydrocarbons is very difficult[2] and to our knowledge no direct and satisfactory method has yet been published.

We have recently reported on the successful resolution of α- and β-pinene[3] and *cis*- and *trans*-pinane[4] into enantiomers using α-cyclodextrin (α-CD) as selective agent in gas–liquid chromatography, with formamide as a matrix. Now, we report a new and in our opinion a general, simple and very convenient method for determination of the enantiomeric purity of chiral hydrocarbons, based on chromatographic separation of enantiomers via inclusion complexes with α-CD.

EXPERIMENTAL

Reagents

α-CD was supplied by Chinoin (Budapest, Hungary), pure formamide by E. Merck (Darmstadt, F.R.G.). Celite (100–120 mesh) for gas chromatography was from BDH (Poole, U.K.). Enantiomers of α-pinene, *cis*- and *trans*-pinanes and 2-

carene were puriss. commercial products (Fluka, Buchs, Switzerland) having the following optical rotations: (+)-α-pinene (*1a*), $[\alpha]_D^{20}$ +48.0°; (−)-α-pinene (*1b*), $[\alpha]_D^{20}$ −42.0°; (+)-*cis*-pinane (*3a*), $[\alpha]_D^{20}$ +24.0°; (−)-*cis*-pinane (*3b*), $[\alpha]_D^{20}$ −24.0°; (+)-*trans*-pinane (*4a*), $[\alpha]_D^{20}$ +17.0°; (−)-*trans*-pinane (*4b*), $[\alpha]_D^{20}$ −17.0°; (+)-2-carene (*5a*), $[\alpha]_D^{20}$ +85.0°. (−)-β-Pinene (*2b*) of optical rotation $[\alpha]_D^{20}$ −16.9° was from Glidden Organics. All other materials were of analytical grade and were used without further purification.

Apparatus and procedures

Optical rotations for neat liquids were measured with a Perkin-Elmer 141 spectropolarimeter. Chromatographic studies were performed using a Hewlett-Packard 5890 gas chromatograph equipped with a flame ionization detector. The peak areas and retention times were measured by means of a Hewlett-Packard 3390A integrator. Glass columns (2 m × 4 mm I.D.) were used. The column packings, *i.e.*, Celite coated with a formamide solution of α-CD (4.5 g formamide per 20 g Celite), were prepared as described earlier[5]. Before use the columns were conditioned for 2 h at 70°C. In all experiments, special care was taken to maintain a constant inlet pressure (130 kPa) and helium flow-rate (40 ml/min). All samples (0.03 μl) were injected with SGE microsyringes. The retention time of each compound was determined as the mean value (relative error <0.5%) from each of a series of six injections. Similarly, the peak areas were established as the mean values (relative error <1.5%) of six replicate experiments. The studies were carried out in the temperature range 35–50°C.

RESULTS AND DISCUSSION

As model compounds for the chromatographic experiments we selected five representative terpenoic hydrocarbons: α-pinene (*1a, 1b*), β-pinene (*2a, 2b*), *cis*-pinane (*3a, 3b*), *trans*-pinane (*4a, 4b*), and 2-carene (*5a, 5b*).

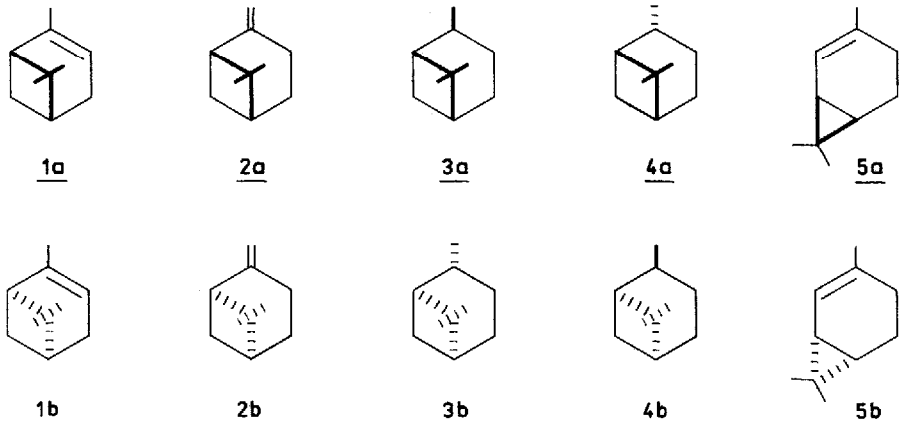


Fig. 1. shows the chromatograms of a mixture of (+)-α-pinene (*1a*) and (−)-α-pinene (*1b*). Similar results were obtained for compounds *2a*+*2b* (Fig. 2), *3a*+*3b* (Fig. 3), *4a*+*4b* (Fig. 4) and *5a*+*5b* (Fig. 5). The respective separation factors are presented in Table I.

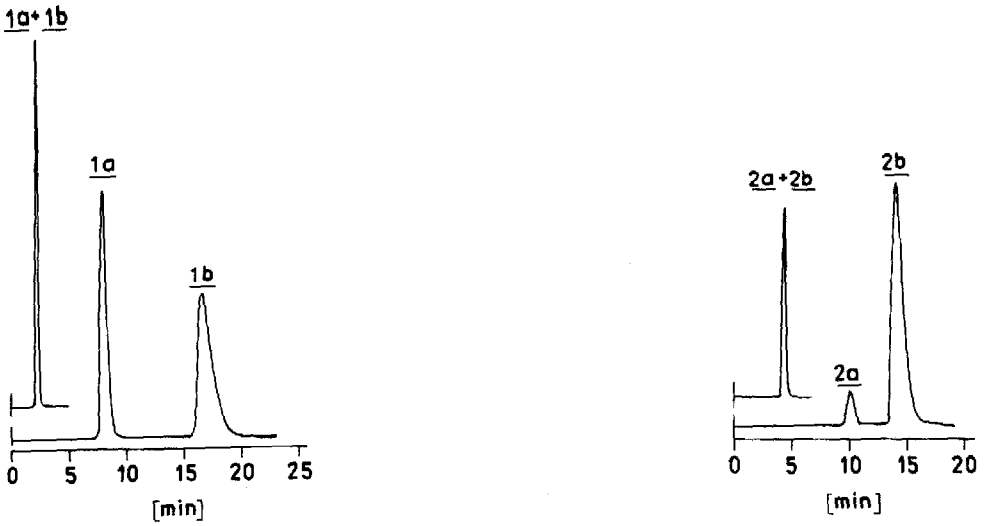


Fig. 1. Chromatograms of a mixture of (+)-α-pinene (*1a*) and (−)-α-pinene (*1b*) obtained at 35°C on columns (2 m × 4 mm I.D.) filled with Celite coated by (a) formamide (upper curve), (b) 0.65 mol % α-CD in formamide (lower curve).

Fig. 2. Chromatograms of a mixture of (+)-β-pinene (*2a*) and (−)-β-pinene (*2b*) obtained under the same conditions as in Fig. 1.

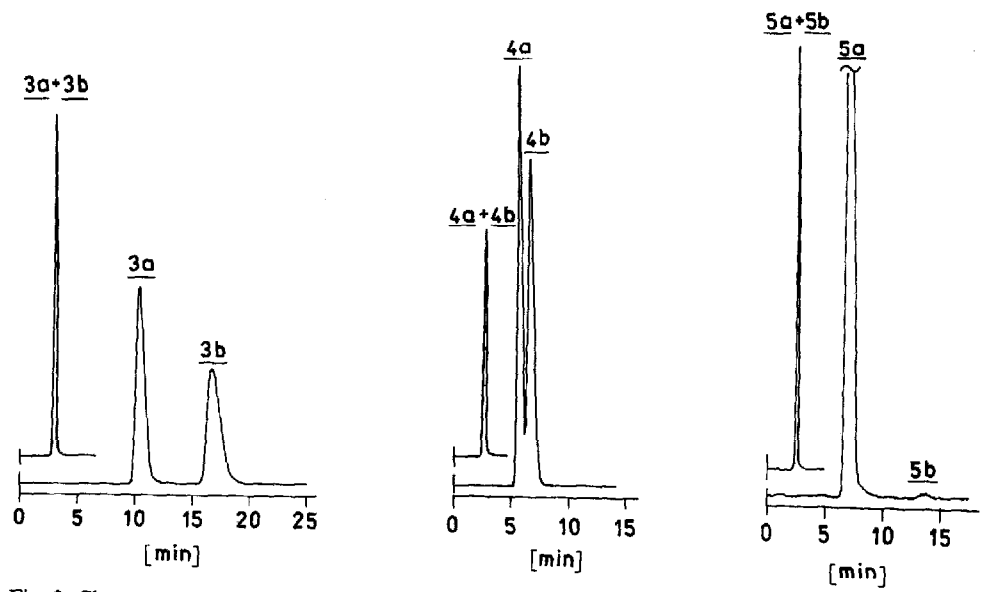


Fig. 3. Chromatograms of a mixture of (+)-*cis*-pinane (*3a*) and (−)-*cis*-pinane (*3b*) obtained under the same conditions as in Fig. 1.

Fig. 4. Chromatograms of a mixture of (+)-*trans*-pinane (*4a*) and (−)-*trans*-pinane (*4b*) obtained under the same conditions as in Fig. 1.

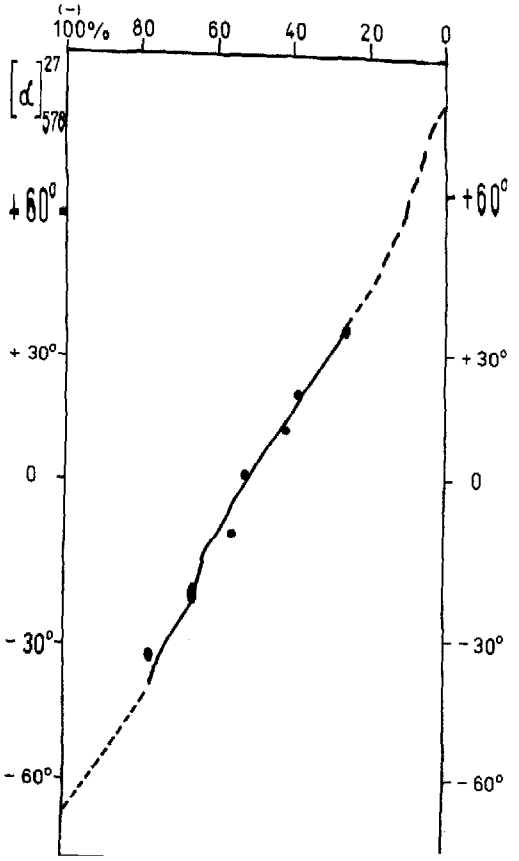
Fig. 5. Chromatograms of a mixture of (+)-2-carene (*5a*) and (−)-2-carene (*5b*) obtained at 50°C on columns as described in Fig. 1.

TABLE I

SEPARATION FACTORS, $\alpha_{-/+}$, OF ENANTIOMERS OF α-PINENE, β-PINENE, *cis*-PINANE, *trans*-PINANE AND 2-CARENE DETERMINED ON A COLUMN (2 m × 4 mm I.D.) FILLED WITH CELITE COATED BY 0.65 mol % α-CD IN FORMAMIDE

Compound	*Temperature (°C)*	$\alpha_{-/+}$
α-Pinene	35	2.00
β-Pinene	35	1.40
cis-Pinane	35	1.60
trans-Pinane	35	1.20
2-Carene	50	2.17

The chromatograms shown in Fig. 1 suggest that α-CD dissolved in formamide forms two different diastereoisomeric inclusion complexes with both enantiomers of α-pinene (*Ia* and *Ib*). This dynamic process is responsible for the separation of the enantiomers in all cases studied (Figs. 1–5). It is remarkable that the molecular inclusion in α-CD molecules exhibited enantioselectivity towards three structurally different unsaturated and two saturated hydrocarbons. The value of the separation factor obtained for the conditions given in the Experimental depended on the structural differences among the hydrocarbons (*cf.*, Table I). In all cases we observed



separate peaks originating from the enantiomers, which after integration afforded the enantiomeric composition of the initial mixture.

To verify the applicability of the above chromatographic phenomenon for quantitative treatment, we attempted to determine the enantiomeric composition of seven artificial mixtures (of precisely measured optical rotation) of (+)-α-pinene (*1a*) and (−)-α-pinene (*1b*); the results are presented in Fig. 6.

The relationship between the resulting enantiomeric ratios of the seven mixtures and their optical rotations, $[\alpha]_{578}^{20}$, is linear. Extrapolation of the plot for both pure enantiomers indicates that the absolute values of the optical rotation of *1a* and *1b* are virtually identical. This confirms that the present chromatographic method for the determination of enantiomeric purity is not only simple and convenient, but also of high accuracy. Since this method enables complete chromatographic separation of enantiomers, it ought to be particularly useful in view of the increasing importance of stereoselectivity in organic chemistry. It may also prove to be applicable to other groups of low-polarity chiral compounds.

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