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Note

Separation of enantiomers by high-performance liquid chromatography on triacetylcellulose*

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Techniques for detecting enantiomers by gas chromatography on optically active stationary phases have advanced considerably in recent years, although only a few semipreparative attempts^{2,3} to trap the products after separation on packed columns have been published. Liquid chromatography (LC), on the other hand, offers distinct advantages for the preparative scale and uses lower temperatures to reduce the rate of possible decompositions or racemizations. Although examples for baseline separations are known (e.g., ref. 4), LC of enantiomers (for a review, see ref. 5) frequently results in an overlap of the two peaks which necessitates time-consuming recyclings. Time consumption can be reduced by applying pressure, which has been achieved with some synthetic sorbents (refs. 6-10 and references cited therein), none of which, however, is easily accessible. Therefore, further optically active LC sorbents are of interest if they are obtained more easily and can be shown to separate enantiomers at high pressures.

Microcrystalline triacetylcellulose swells in organic solvents, e.g. in ethanol-water (95:5), by ca. 40% of its volume¹¹. The swollen sorbent successfully separates enantiomers at pressures up to 2 bar¹¹⁻¹³ and a 111 bar^{1,14-16}. We have shown that this readily obtained material does not, at higher pressures, lose its LC properties (e.g. by shrinkage of the crystal lattice). Apparently, the only published fact related to our results is concerned with acetylcelluloses^{17,18} differing from our material. These have been used at 15-103 bar for the separation of achiral aromatic hydrocarbons^{17,18,***}.

EXPERIMENTAL

Triacetylcellulose, prepared and milled as previously described⁷, was air-separated (Zickzacksichter A 100 MZR, Alpine AG). 7.9 g of the fraction with a particle size of 5-10 μm (measured by the air jet sieve 200 LS, Alpine AG, and by a

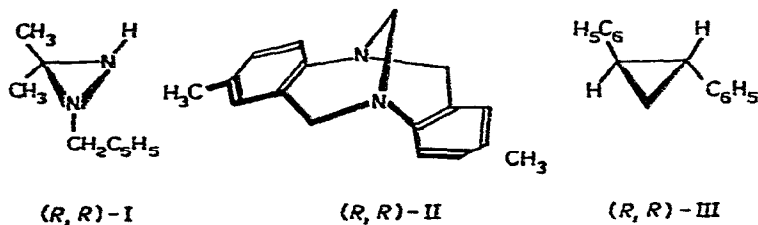
* Liquid Chromatography on Triacetylcellulose, Part II. For Part I, see ref. 1.

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*** Note added in proof. The enantiomers of a cyclic allene hydrocarbon have been partially separated on triacetylcellulose¹¹ at 48 bar¹⁹.

microscope) were swollen during 15 min by boiling in absolute ethanol. At *ca.* 40° the ethanol suspension was slurry-packed into a steel column (250 × 8 mm I.D.) at 195 bar. The sample injector 71-20 (Rheodyne) and the pneumatic amplifier pump 830 (DuPont) were used for the separations, with absolute ethanol as the eluent.

RESULTS AND DISCUSSION



The first experiments were carried out with racemates (\pm) -I¹, (\pm) -II¹¹ and (\pm) -III¹⁶, for which good separations had been achieved at low pressures and for which the order of appearance of the enantiomers was known. Although the chromatogram of (\pm) -I (70 bar, ambient temperature) showed no splitting, the trapped halves of the eluate resulted in negative and positive rotation angles (365 nm), respectively, as expected. The same is true for (\pm) -II at ambient temperature (104 bar, flow-rate 2 ml/min) which, however, showed a separation close to baseline at 60° (49 bar, 1.5

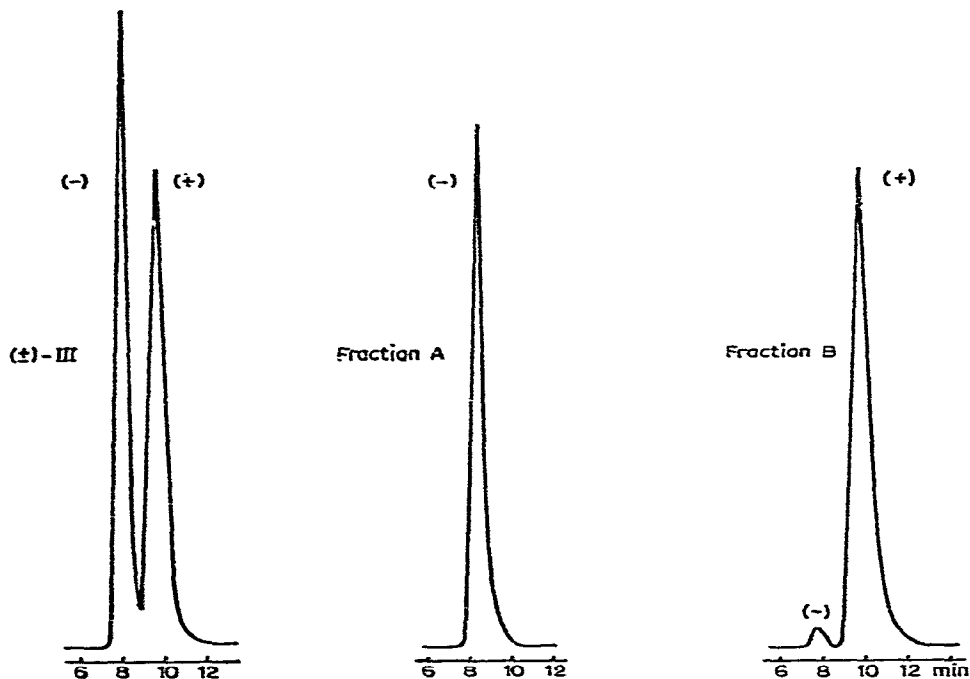


Fig. 1. Analytical chromatograms of compound III in absolute ethanol on triacetylcellulose (see text). Numbers represent time *t* (min) after injection. Absorbance at 254 nm was monitored. Ambient temperature; flow-rate, 2 ml/min. Fractions A and B were collected from 20 injections, each consisting of 1 mg of (\pm) -III.

ml/min). The times between injection and complete elution were 16 min at ambient temperature and 13 min at 60°. The chromatogram of 0.06 mg of (\pm)-III (Fig. 1, left) at ambient temperature exhibits satisfactory separation. Twenty injections, each consisting of 1 mg of (\pm)-III, allowed the semipreparative collection of fractions A (6.5 mg of (-)-III, $P = 99 \pm 1\%$, $[\alpha]_{565}^{22} = -1825^\circ$, C^2HCl_3 , $c = 0.320$) and B (7.9 mg of (+)-III, $P = 94 \pm 2\%$, $[\alpha]_{565}^{22} = +1748^\circ$, C^2HCl_3 , $c = 0.397$). The enantiomeric purities, P , were determined from analytical chromatograms* (Fig. 1) which showed no further impurities. Calculation of P from the above $[\alpha]$ -values and those of the pure enantiomers¹⁶ gives practically identical results. The ¹H nuclear magnetic resonance spectra in C^2HCl_3 showed a small impurity peak at $\delta = 1.6$ (probably water).

Further development of this technique seems to be promising, because experience^{1,11-16} at low pressures has shown that triacetylcellulose is a relatively versatile sorbent for many types of enantiomers (and other compounds). The main advantage of using high pressure with a small particle size sorbent is the much shorter time needed for a separation. Possible improvements include detection by polarimetry, recycling of the eluate, use of other eluents, measurement of column loadability, and a narrow range of small particle sizes of the stationary phase.

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* Fraction A does not show any (+)-III (Fig. 1), i.e. $P = 100\%$. The estimate that 2% of (+)-III might be invisible results in $P = 99 \pm 1\%$.