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## Note

# Direct resolution of enantiomers by high-performance liquid chromatography on a bonded chiral stationary phase

C. H. LOCHMÜLLER* and RALPH R. RYALL

*Paul M. Gross Chemical Laboratory, Duke University, Durham, N.C. 27706 (U.S.A.)*

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As a continuation of our studies on chemically bonded $\pi$-complexing phases for high-performance liquid chromatography (HPLC)[1], we wish to report the successful synthesis and use of a chiral charge-transfer complexing phase. Of particular interest is the enantiomeric resolution obtained for the racemic solutes 1-aza[hexa]-helicene and heptahelicene using the bonded chiral phase.

Newman *et al.*[2] first showed that hexahelicene could be resolved by fractional crystallization from a solution containing optically active $\alpha$-(2,4,5,7-tetranitro-9-fluorenylideneaminooxy)propionic acid (TAPA), but a later attempt[3] at enantiomeric resolution on a column of optically active polymer, derived from TAPA, was unsuccessful. Klemm and Reed[4] obtained optical resolution on a chromatographic column of silicic acid coated with optically active TAPA. Recently, Mikeš *et al.*[5] have reported the successful optical resolution, by HPLC, of ten racemic helicenes and two double helicenes on a stationary phase support coated with chiral TAPA. Some work on a chemically bonded chiral TAPA phase was reported in the same study as well.

The bonded chiral phase discussed here incorporates a much simpler charge-transfer agent than the TAPA reagent used in earlier studies of this nature and, in addition, the synthetic route to the desired chiral phase is more straightforward.

## EXPERIMENTAL

The liquid chromatographic equipment has been described earlier[1]. Packing of HPLC columns was achieved by the balanced-density slurry method[6]. The existence of optical activity in chromatographically resolved samples was confirmed by recording the circular dichroic absorption spectra from 300 to 210 nm for collected fractions on a JASCO ORD-CD spectropolarimeter.

After drying at 160°, 10-$\mu$m LiChrosorb SI-60 (E.M. Ls., Elmsford, N.Y., U.S.A.) was reacted with a 10% solution of 3-aminopropyltriethoxysilane in toluene. The terminal amino groups of the modified silica were then coupled with N-*tert.*-butyloxycarbonyl-L-alanine using 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (Sigma, St. Louis, Mo., U.S.A.) as a dehydrating agent. When 90% of the available

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[*] To whom correspondence should be addressed.

amino groups had been coupled (as determined by ninhydrin reagent), the *tert.*-butyloxycarbonyl group was cleaved with trifluoroacetic acid. The amino acid-bonded phase was then reacted with 1-fluoro-2,4-dinitrobenzene (Sigma) to give the bonded $\pi$-complexing chiral phase.

Spectrograde isooctane and acetonitrile were used for the liquid chromatographic experiments.

## RESULTS AND DISCUSSION

Chromatograms of racemic 1-aza[6]helicene and heptahelicene are shown in Figs. 1 and 2, respectively. Two 24-cm (2.1 mm I.D.) columns, connected in series, were used to obtain the resolution shown. It was also possible to effect partial resolution with only one column. Chromatographic data are presented in Table I. Values of $\alpha$, the selectivity factor, were calculated from the relation $\alpha = k'_2/k'_1$ where $k'_2$ and $k'_1$ are the capacity factors of the most-retained and least-retained species, respectively. Benzene was chosen as the unretained species in these experiments.

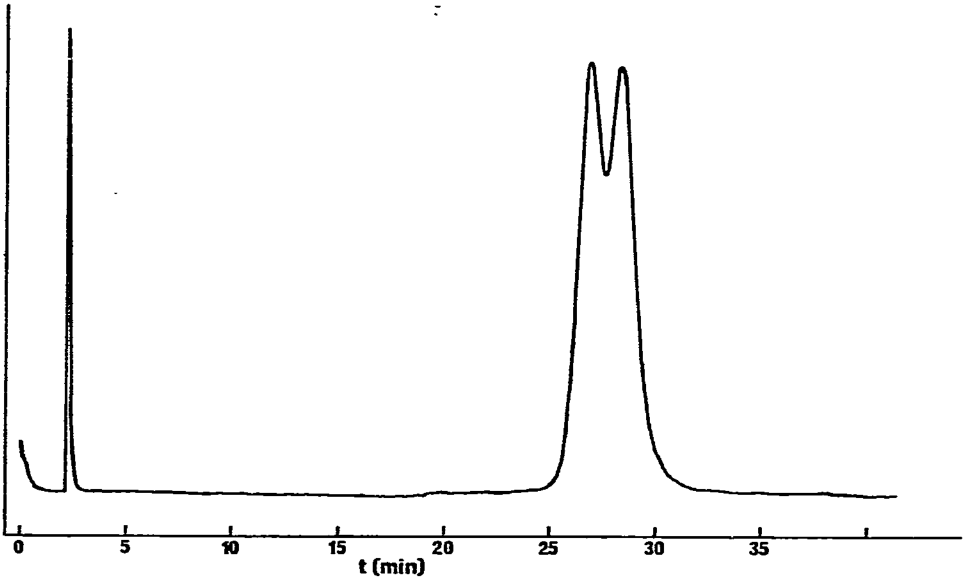

Fig. 1. Resolution of 1-aza[6]helicene. Mobile phase: 1.5% acetonitrile in isooctane; flow-rate: 0.90 ml/min; detector: UV at 254 nm, 0.02 a.u.f.s.

The fact that optical resolution of racemic helicenes can be achieved on this simple bonded phase indicates that the toposelective-interaction model suggested by other workers[5,7,8] may not reflect an absolute requirement for optical resolution. The charge-transfer agent used here could hardly participate in the type of orbital overlap with the helicene solutes that has been proposed to occur with TAPA. By analogy to the previous work of Lochmüller *et al.*[9], it appears that enantiomeric resolution may require only one significantly strong interaction (in this case a site of charge-transfer)

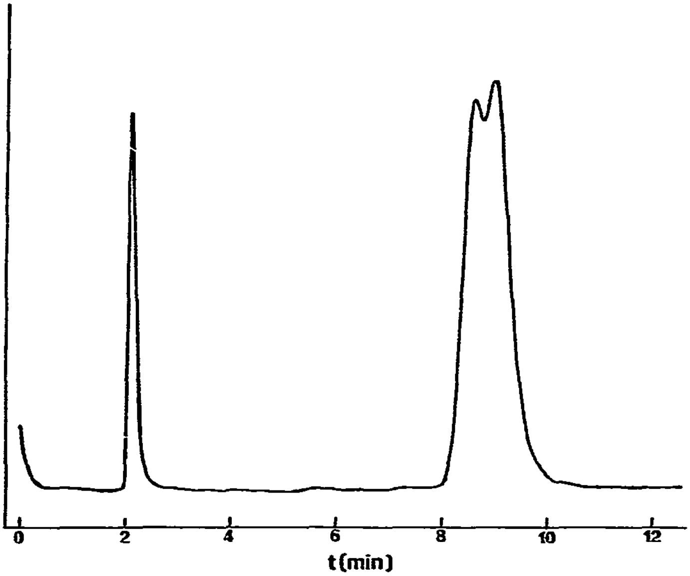

Fig. 2. Resolution of heptahelicene. Mobile phase: 1.5% acetonitrile in isooctane; flow-rate: 0.90 ml/min; detector: UV at 254 nm, 0.08 a.u.f.s.

TABLE I

RESOLUTION OF RACEMIC HELICENES BY HPLC USING A BONDED CHIRAL $\pi$-COMPLEXING PHASE

| *Racemate* | $k'_1$ | $k'_2$ | $\alpha$ | $R_s$ *(resolution)* |
|---|---|---|---|---|
| Heptahelicene | 2.94 | 3.16 | 1.06 | 0.47 |
| 1-Aza[6]helicene | 10.47 | 11.14 | 1.06 | 0.57 |

near a center of asymmetry. It should be noted that attempts to resolve the enantiomers of 1- or 2-methylcholanthrene failed under the same conditions which yielded resolution of the helicenes.

Work in progress involves the effect of alpha substituents on the amino side chain and the degree of nitration of the charge-transfer acceptor on the extent of resolution. The practical utility of bonded, charge-transfer agents for HPLC is actively under investigation.

## ACKNOWLEDGEMENT

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