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Note

Gram-scale preparative separation of *exo*- and *endo*-isomers by high-performance liquid chromatography using a partial recycle technique

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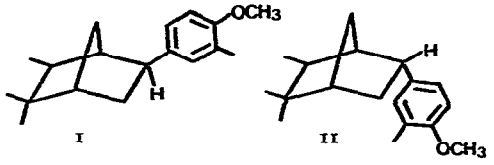
In the last 5 years, high-performance liquid chromstography (HPLC) has become an increasingly effective technique for the analytical and preparative separation of structurally similar organic compounds[1–3]. However, most of these separations have been limited to milligram amounts at most. We report here an effective large-scale separation of *exo*- and *endo*-isomers using a partial recycling technique with HPLC.

In the multi-stage synthesis of chiral norbornylcyclohexanones in our laboratories, it became necessary to separate, at an intermediate stage, the *exo*- and *endo*-isomers of *d*- and *l*-2-methyl-4-[5,5,6(*exo*-)trimethyl-2-norbornyl]anisole (I and II), which had been prepared as a 3:2 mixture. This separation is not difficult by gas–liquid chromatography, but only very small amounts can be handled and there is the usual significant loss of material. Using a 25 ft. × 3/8 in. stainless-steel column packed with 20% Carbowax 20M on Chromosorb W at 150° (isothermal), the sample loading capacity on such a column was limited to 100 mg for complete separation. As several grams of each stereoisomer were needed, HPLC was investigated as a means of effective preparative separation.



EXPERIMENTAL AND RESULTS

Preliminary runs using a 1 ft. × 1/5 in. I.D. stainless-steel column (total column volume, 3.5 ml) pre-packed with μPorasil (Waters Assoc., Milford, Mass., U.S.A.) and equipped with a pump (Model C-903, Water Assoc.) and a UV detector operating at 254 nm (Varian Aerograph, Walnut Creek, Calif., U.S.A.) demonstrated the feasibility of the separation, as shown in Fig. 1. Samples were injected through

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a septum and *n*-hexane (spectrophotometric grade) was used as the mobile phase. However, this analytical system, although rapid, is limited to very small samples (<2 mg). It became obvious that for larger samples, a larger column and a recycling technique[4,5] are essential. Especially for a recycling system, a high ratio of column volume to dead-space volume is the most important factor for success.

These requirements were fulfilled by using a 3.3 ft. × 3/8 in. I.D. stainless-steel column dry-packed with Porasil T (particle size, 37 μm) (Waters Assoc.). This column had an effective volume of 275 ml and was connected to a pump (Water Assoc. Model 6000) with a small internal volume (200 μl) and a loop valve injector (Disc Instruments, Santa Ana, Calif., U.S.A.). As the compounds of interest contained an aromatic ring, they were easily monitored with a UV-254 detector (Varian Aerograph). However, a variable-wavelength detector would be much more useful in order to avoid saturation of the detector with large samples. On this system, simple

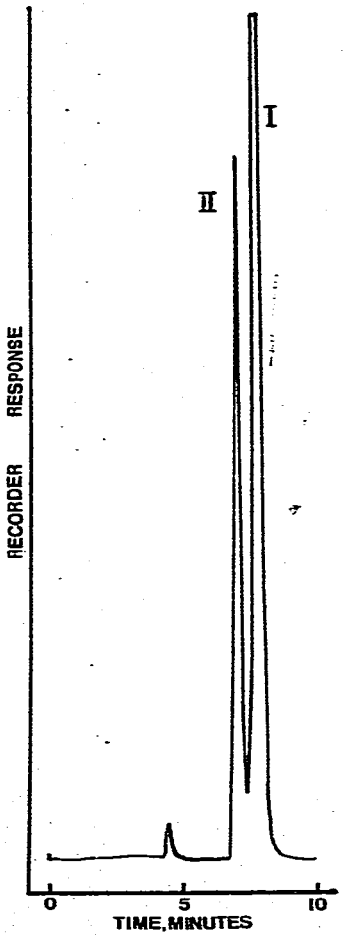

Fig. 1. Analytical separations of *exo*- and *endo*-isomers. Column: μporasil, 1 ft. × 1/4 in. O.D. Mobile phase: *n*-hexane, Flow-rate: 1 ml/min. Detector: UV (254 nm).

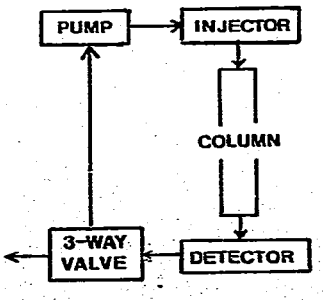

Fig. 2. Recycle system.

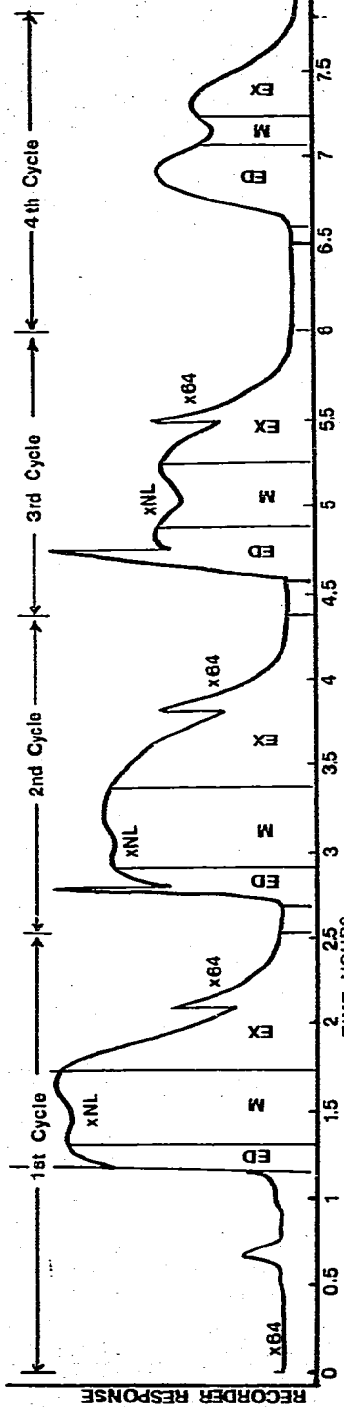

Fig. 3. Preparative separations of *exo*- and *endo*-isomers using the recycle technique. Column: Porasil T. Mobile phase: *n*-hexane. Flow-rate: 9.9 ml/min. Detector: UV (254 nm). ED, withdrawn from the system, pure *endo*-isomer portion; M, recycled, mixture portion; EX, withdrawn from the system, pure *exo*-isomer portion; ×NL, non-linear attenuation.

recycling caused overlapping between the second peak and the first peak of the second cycle. This effect was avoided simply by using a three-way valve (Valco Instruments, Model CV-3HP) connected after the detector. A diagram of this system is shown in Fig. 2.

With this system, 2 g of the mixture of *exo*- and *endo*-isomers could be separated effectively as follows. *n*-Hexane was used as the mobile phase and the pump used had a maximum flow-rate of 9.9 ml/min, which resulted in a 2-h cycle. In the first cycle, the detector was saturated because of the concentrations involved, but sampling showed that only the middle portion of the eluate required recycling. As the front and back portions were withdrawn in each cycle, the separation of the middle portion became easier. After four cycles of 2 h each, 95% of the original 2-g sample had been resolved into the *endo*- and *exo*-isomers of purity $\geqslant$99%. Fig. 3 shows a typical chromatogfam obtained with the partial recycle system.

The technique described presents no unusual problems, although the specific conditions may vary with each mixture. We feel confident that the method will prove useful for many different gram-scale separations.

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