Journal of Chromatography, 131 (1977) 185–190

CHROM. 9487

PREPARATIVE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF HIGH-MOLECULAR-WEIGHT ISOMERS

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(Received June 28th, 1976)

SUMMARY

The three positional methoxy isomers, 3,4-di(*p*-bromophenyl)-1,2,5-triphenylcyclo-2,4-pentadien-1-methyl ether, 2,3-di(*p*-bromophenyl)-1,4,5-triphenylcyclo-2,4-pentadien-1-methyl ether, and 1,2-di(*p*-bromophenyl)-3,4,5-triphenylcyclo-2,4-pentadien-1-methyl ether (mol. wt. 634) were obtained during the course of synthesizing a compound suitable for obtaining a chemically bonded phase for high-performance liquid chromatography (HPLC). Attempts to separate these three isomers by open-column liquid chromatography failed and only partial separation is obtained with multiple-development thin-layer chromatography. A preparative column, 20 × 9.9 cm I.D., packed with 10-μm Lichrosorb SI-100 was successfully used. Structures were determined by mass spectrometry, nuclear magnetic resonance and elemental analysis. Equipment and technique for slurry packing preparative HPLC columns is described.

INTRODUCTION

In an attempt to synthesize 3,4-di(*p*-bromophenyl-3,4,5-triphenylcyclo-2,4-pentadien-1-methyl ether from the corresponding 1-bromo compound, three components were obtained as illustrated in the chromatogram (Fig. 1). There is a reaction mechanism involving an intermediate carbonium ion which could lead to three positional isomers, as shown in Fig. 2.

This problem was of sufficient interest to warrant the preparative scale separation of these three postulated isomers so that mass spectrometry (MS), nuclear magnetic resonance (NMR), UV and visible photometry and elemental analysis data could be obtained[1]. Their structures were verified and a reaction mechanism proposed. This paper describes the preparation of an efficient preparative-scale high-performance liquid chromatography (HPLC) column which successfully separated these three positional isomers of mol. wt. 634.

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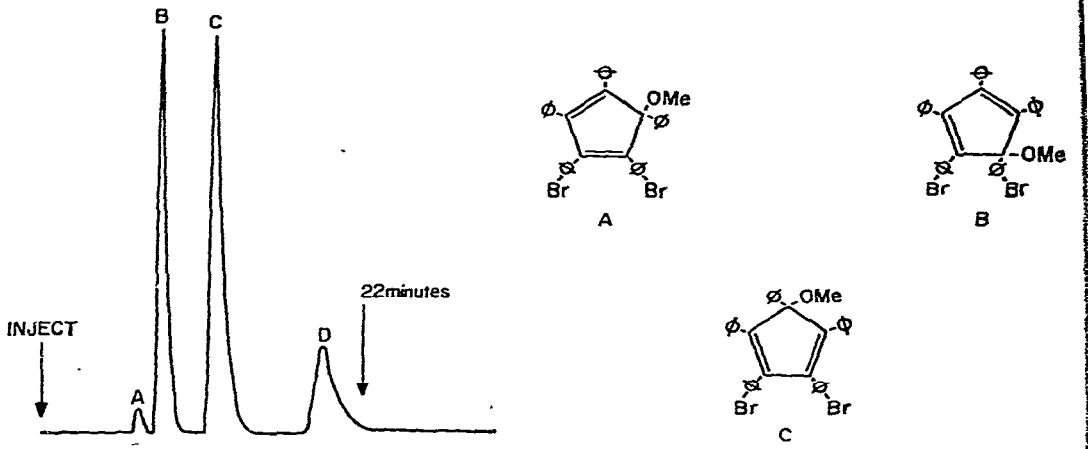

Fig. 1. HPLC separation of isomeric methoxy derivatives B, C, D (see Fig. 2). Column, 50 cm × 2.1 mm I.D. × 1/4 in. O.D., 10-μm Lichrosorb SI-100. Mobile phase, 0.1 % ethyl acetate in *n*-hexane at a flow-rate of 1.1 ml/min. Detector, UV at 254 nm and 0.02 a.u.f.s. sensitivity. Sample size, 10 μl. A = Starting product.

Fig. 2. Structural formulas. A = 2,3-di(*p*-bromophenyl)-1,4,5-triphenylcyclo-2,4-pentadien-1-methyl ether; B = 1,2-di(*p*-bromophenyl)-3,4,5-triphenylcyclo-2,4-pentadien-1-methyl ether; C = 3,4-di(*p*-bromophenyl)-1,2,5-triphenylcyclo-2,4-pentadien-1-methyl ether.

EXPERIMENTAL

High-pressure slurry packing apparatus

The columns used were packed using the high-pressure slurry packing apparatus shown schematically in Fig. 3.

A Model DST-122 pneumatic amplifier pump (Haskel Engineering and Supply, Burbank, Calif., U.S.A.) B is capable of delivering the solvent contained in the reservoir A at constant pressure up to 19,000 p.s.i.g. The pump head has a volume of 4 ml. The air pressure D applied to the pump inlet is amplified 122 times at the pump outlet. The balanced density slurry is fed into the 80-ml slurry reservoir G through the valve F, Model SS-4354 (Whitey Research Tool, Emeryville, Calif.,

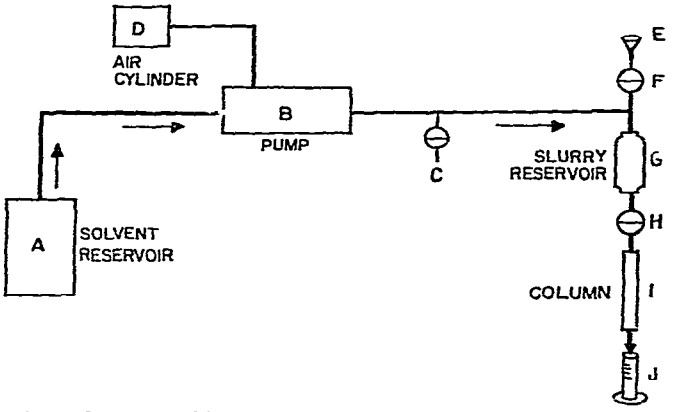

Fig. 3. Slurry packing apparatus.

U.S.A.), with the help of a special narrow-bore glass funnel E. Air can escape through the valve C. The pressurized liquid forces the slurry through valve H into the chromatographic column I. A cylinder J collects and measures the effluent from the column during the packing procedure. All the tubing and Swagelok® fittings connecting the pump to the chromatographic column are 1/4 in. O.D. The fittings connecting valve F to column I are specially drilled out to give a larger I.D. to allow air to escape while the slurry is being loaded.

Chromatographic columns

Stainless-steel tubing (Handy and Harman Tube, Norristown, Pa., U.S.A.) were machine cut to obtain clean and flat ends. Columns were then washed with detergent, rinsed with acetone, and dried. The Swagelok® end fittings (Dibert Valve and Fitting, Richmond, Va., U.S.A.) were machined out to a flat surface to allow for a "zero" dead volume with the flat tube end. The holes of the end fittings were drilled out to allow for the 1/16-in. inlet and outlet tubings to touch the surface of the 2-μm stainless-steel frits (Mott Metalurgical, Farmington, Conn., U.S.A.) which were placed between the column ends and the end fittings.

The HPLC column for the analytical separation was 20 cm $\times$ 3.9 mm I.D. $\times$ 1/4 in. O.D. stainless steel. For the preparative separations, the column dimensions were 20 cm $\times$ 9.9 mm I.D. $\times$ 1/2 in. O.D. with the same packing material.

Chemicals

Tetrabromoethane and tetrachloroethane were reagent grade (Fisher Scientific, Fair Lawn, N.J., U.S.A.). Prior to use, the halogenated solvents were purified by passing them through silica gel, 60–200 mesh (Grace and Davidson Chemical, Baltimore, Md., U.S.A.). A yellow impurity was removed by this treatment. The silica had been activated by heating for 4 h at 200°.

Distilled water was used as an immiscible layer between the balanced density slurry and the pressurizing liquid, technical grade *n*-hexane (Fisher Scientific). Reagent grade methanol, methylene chloride and *n*-hexane (Fisher Scientific) were used as activation solvents. Ethyl acetate and *n*-hexane (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.) were used as the mobile phase.

Packing material

E. Merck Lichrosorb SI-100 (EM Laboratories, Elmsford, N.Y., U.S.A.), totally porous, non-spherical silica gel with an average particle diameter of 10 μm was used (particle size analysis: $d_{10} = 8\,\mu$m; $d_{50} = 10\,\mu$m; and $d_{90} = 13\,\mu$m, where $d_{90} = 13\,\mu$m means that 90% of the particles will pass through a 13-μm screen).

Balanced density slurry packing procedure

For each column size, different volumes of balanced density solvent and weight of packing were used. For a 20 cm $\times$ 9.9 mm I.D. $\times$ 1/2 in. O.D. column, the procedure was as follows. Into a 125-ml erlenmeyer was placed 8.3 g of silica, previously dried at 200° for 4 h, and 70 ml of the balanced density solvent. This solvent was made up of 60% tetrabromoethane and 40% tetrachloroethane. Adjustment of the final density was performed by trial and error when necessary. If the silica floated, tetrachloroethane (less dense liquid) was added; if the silica had the tendency

to precipitate, more of the denser tetrabromoethane was added. Experiments with these two solvents were carried out with good ventilation due to their moderate toxicity. If the silica was not adequately dry, the particles would stick together in the hydrophobic media. No ultrasonic degassing was utilized. Using a long-stem funnel (E in Fig. 3) the stable suspension was introduced to the slurry reservoir G. Air would escape through valve C. Column I had been previously filled with the balanced density solvent and valve H closed. At this point 10 ml of water was carefully added to the top of the slurry, care being taken not to disturb the slurry since this would transfer silica to the water layer and the volume completed with *n*-hexane. Valves F and C were then closed and pump B was pressurized to 5000 p.s.i.g. with *n*-hexane from solvent reservoir A. Valve H was opened and the slurry rapidly transferred to column I. When 65 ml of solvent had been collected in the graduated cylinder J, the pump was shut off and the pressure allowed to slowly decrease to ambient. Valve H was then closed, column I disassembled, and the top and fittings placed on the chromatographic column. The slurry reservoir was then rinsed with water and acetone.

Adsorbent activation

Activation of the silica packing was performed by pumping through the column 400 ml of methanol followed by 400 ml of methylene chloride and 400 ml of *n*-hexane.

HPLC equipment

The liquid chromatograph used (Fig. 4) was a MSI Model B-500 (Molecular Separations, Champion, Pa., U.S.A.) equipped with a pneumatic amplifier pump

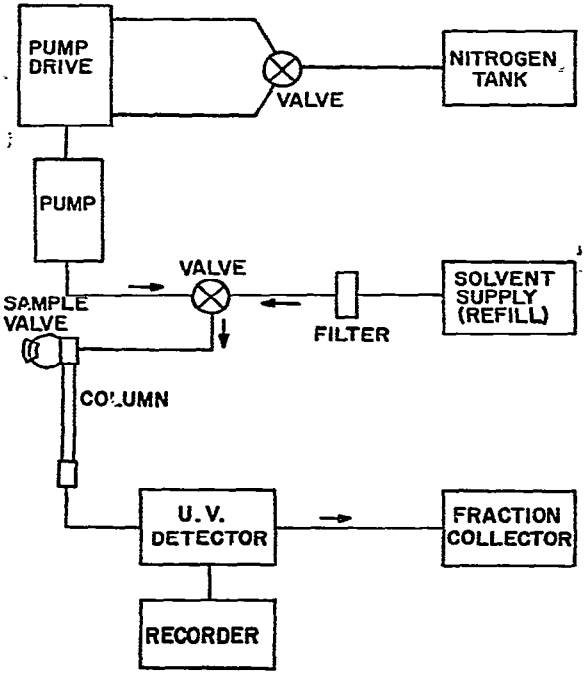

Fig. 4. Schematic diagram of the preparative HPLC system used.

with a 230-ml capacity and 2000 p.s.i.g. maximum operating pressure, adapted with an injection valve Model HPSV (Spectra Physics, Santa Clara, Calif., U.S.A.) and a dual-cell UV detector operating at 254 nm and having a cell path of 10 mm.

Sample loops used were 20 μl for the analytical and 334 μl for the preparative separations.

RESULTS AND DISCUSSION

The analytical separation shown in Fig. 1 was obtained by adjusting the polarity of the mobile phase (*n*-hexane–chloroform (90:10)) that had been previously used in a multiple-development thin-layer chromatographic separation.

The analytical separation indicated the initial mobile phase to be used in the preparative column. Resolution was then increased by decreasing the solvent strength by using 0.08% ethyl acetate in *n*-hexane. Separation was considered optimized with this mobile phase since a further decrease in ethyl acetate would decrease resolution and the peaks would tail badly.

Sample volume was gradually increased until the limit of 1.5 mg could be injected in the column. This is less material than typically expected from a 9.9 mm I.D. column[2]. This small sample size can be attributed to the three positional isomers of high molecular weight (634) in which the only differentiating feature is the position of a methoxy group that is sterically hindered by bulky phenyl groups. It is known that high molecular weight solutes and steric hindrance are factors that decrease the selectivity of separation by adsorption chromatography. The yield of collected sample is further decreased by not collecting 100% of the peak as shown in Fig. 5. The purity of the collected fraction was determined by HPLC and the results for fraction 1 are shown in Fig. 6. Results for fractions 2 and 3 were similar. The squared-off peaks obtained from the preparative separations result from the overload of the UV absorption detector.

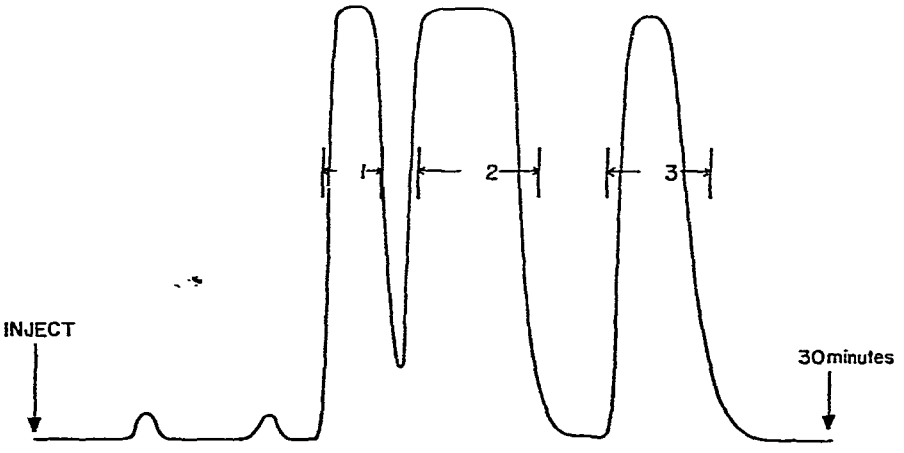

Fig. 5. Preparative separation of isomeric methoxy derivatives. Column, 20 cm × 9.9 mm I.D. × 0.5 in. O.D., 10-μm Lichrosorb SI-100. Mobile phase, 0.08% ethyl acetate in *n*-hexane at a flow-rate of 4.9 ml/min. Detector, UV at 254 nm and 0.64 a.u.f.s. sensitivity. Sample size, 1.5 mg in 334 ml.

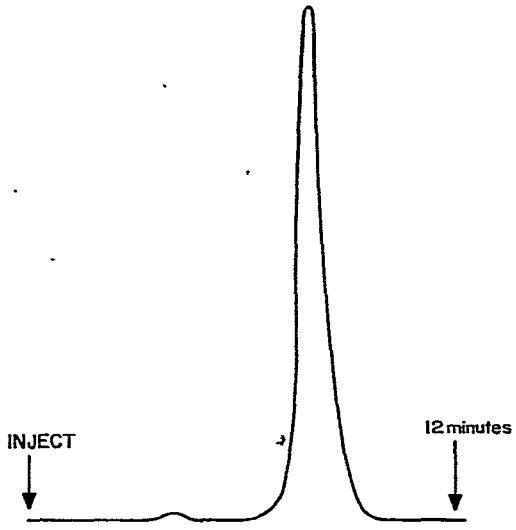

Fig. 6. Analytical chromatogram of preparative fraction 1 shown in Fig. 5. Column, 20 cm × 3.9 mm I.D. × 1/4 in. O.D., 10-μm Lichrosorb SI-100. Mobile phase, 0.08% ethyl acetate in *n*-hexane at a flow-rate of 1.5 ml/min. Detector, UV at 254 nm and 0.01 a.u.f.s. sensitivity. Sample size, 20 μl.

An important aspect in preparative HPLC is the polarity of the sample solvent. Methylene chloride is a much better solvent for these three methoxy isomers than *n*-hexane. However, when injecting several hundred microliters of a more polar solvent (methylene chloride) into the column having a less polar mobile phase (*n*-hexane) the separation process is disturbed momentarily, and, as a result, the separation is diminished. When only a few microliters are injected this effect is not seen. Recommended practice is to use the mobile phase to dissolve the sample. However, in this case, the sample was only sparingly soluble in the mobile phase.

The three fractions were concentrated by evaporation and transferred to a 1-ml glass vial. By gentle heating in a water bath and applying vacuum to the vial, a solid precipitate is left behind. Each of the pure fractions was submitted for MS and NMR indicating that three high-molecular-weight positional isomers had been obtained and could be successfully separated by a large-diameter HPLC column.

ACKNOWLEDGEMENT

R. G. Berg acknowledges the financial assistance of CNPq, Brazil, to conduct this research.

REFERENCES

1 R. G. Berg, *Ph. D. Dissertation*, Virginia Polytechnic Institute and State University, Blacksburg, Va., 1976.
2 C. D. Carr, *Preparative Liquid Chromatography*, Varian, Palo Alto, Calif., 1975.