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Note

Preparative-scale separation of alkene geometric isomers by liquid chromatography

R. P. EVERSHED, E. D. MORGAN* and LORNA D. THOMPSON
Department of Chemistry, University of Keele, Keele, Staffordshire ST5 5BG (Great Britain)
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The separation of mixtures of geometrical isomers is often difficult to achieve on a preparative scale, though the acquisition of a single pure isomer may be important. In the field of insect pheromones in particular, isomer purity can be of highest concern and small amounts of contamination by an unnatural isomer may strongly modify the insect response.

A number of techniques have been applied to the separation of geometric isomers on a preparative scale or for the quantitative analysis of mixtures. Silver-loaded thin-layer chromatography (TLC) plates have been widely used and a discussion of this and other types of argentation chromatography is presented in a review by Guha and Janák[1]. More recently the use of silver-loaded supports for high-performance liquid chromatography (HPLC) have become popular. Heath and co-workers[2,3] separated isomers of olefinic insect attractants by HPLC on silica impregnated with silver nitrate. Silver-loaded HPLC supports have also been prepared by loading of silver ions on a strong cation exchanger and insect sex attractants[4–6] and prostaglandins[7] have been separated using these supports. Lam and Grushka[8] separated isomers of C_{16} and C_{18} fatty acids and permethrin (a synthetic pyrethrin) using silver loaded aluminosilicate as a support for HPLC. Silica gel was treated with sodium aluminate to form a polyanionic surface and the counter ions were then exchanged for silver ions. Conversely, silver ions in the mobile phase were used by Vonach and Schomburg[9] to separate olefinic isomers by HPLC on a reversed-phase support.

Gas chromatography (GC) has also been employed for the separation of geometrical isomers. Warthen and Green[10] analysed *cis* and *trans* isomers of fatty alcohol acetates using diethylene glycol succinate (DEGS) in a 300-ft. capillary column. Other liquid phases have been used by Litchfield *et al.*[11,12], Kaufman and Lee[13] and Lipsky *et al.*[14,15] for similar separations.

It is also possible to separate geometrical isomers of some olefins by a non-chromatographic method. Leadbetter and Plimmer[16] have recently used the preferential formation of urea inclusion complexes by *E* isomers to separate alkenes prepared by Wittig condensations. The *Z* isomers did not form inclusion compounds, and the method can be used on a large scale.

In view of the present high cost of silver, the simplest efficient method of separation of geometric isomers: silver nitrate-loaded TLC plates, is unattractive. The silver can be recovered but recovery is neither simple nor efficient. Ideally a

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system is required where the silver salt remains on the support, the column or thin layer can be used repeatedly, and the separation is as efficient as can be obtained by TLC.

We describe here a silver nitrate-loaded silica column which we have found both cheap and efficient for the preparative separation of geometric isomers. By using particles of 50 μm diameter and medium pressure to force the solvent through, efficiencies comparable to TLC are obtained. The method may be described as argentation medium-pressure liquid chromatography (Ag-MPLC).

EXPERIMENTAL

Apparatus

An MPL series II micropump (Metering Pumps, London, Great Britain) with a PTFE diaphragm pumphead (Maximum pressure ≈100 p.s.i.) was used for solvent delivery. Samples were injected (Altex, Berkeley, CA, U.S.A.) onto the column through a 4-way Tefzel slider valve using a 10-ml Hamilton 1010 gas-tight syringe. Fractions were collected with an LKB 2112 Redirac fraction collector and individually analysed on a Pye series 104 gas chromatograph with flame ionisation detector using either a 3 m 5% DEGS on supersorb (100–120 mesh) column or a 1.5 m 3% OV-101 on Chromosorb W (100–120 mesh) column.

Reagents

Diethyl ether and ethyl acetate were dried over Type 4A molecular sieves. Light petroleum (b.p. 40–60° and b.p. 60–80°) was used without further purification.

Preparation of silver-loaded support

Kieselgel 60 (100 g Merck, Darmstadt, G.F.R.) of particle size 40–63 μm (230–300 mesh, ASTM) was suspended in a solution of silver nitrate (20 g) dissolved in acetonitrile. The solvent was then evaporated under vacuum, slowly and carefully in a rotary evaporator, to avoid the formation of finer particles. The silver-loaded silica was finally dried on a fluid bed drier with a stream of nitrogen. Silica gel loaded with 40% of its weight of silver nitrate was prepared similarly.

Column packing

The silver-loaded silica was dry-packed by the tap-and-fill method into a 100 cm × 15 mm glass column (Whatman, Maidstone, Great Britain), and the ends capped with Whatman barrel sleeve assemblies and pistons. The column was wrapped in a covering of thick card, to exclude light, and connected to the pump and valve with 1/16 in O.D. PTFE capillary tubing (Alltech, Arlington Heights, IL, U.S.A.). Adaptor connections were necessary to connect to the 1/16 in. I.D. PTFE tubing at the ends of the Whatman column.

Use of the column

A 1-g amount of a mixture of (*Z*) and (*E*)-8-heptadecene, approximately 80:20 was injected into the capillary line leading into the 20% loaded column and eluted with light petroleum (b.p. 40–60°) at a flow-rate of 1 ml min^{-1}. Fractions of 1 ml were collected and analysed by GC on a 5% DEGS column at 100°C with nitrogen carrier gas at 60 ml min^{-1}. Results are shown in Fig. 1.

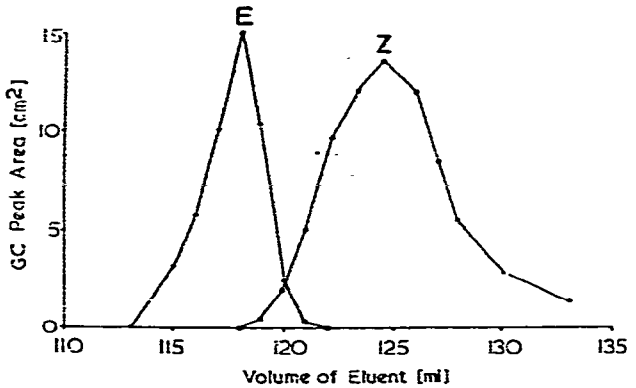

Fig. 1. Separation of (*E*) and (*Z*)-8-heptadecene on a 20% silver nitrate loaded silica gel column. Fraction composition was determined by gas chromatography.

From a mixture of (*Z*,*E*)- and (*Z*,*Z*)-α-farnesene, 1 g was similarly chromatographed on the 20% loaded column, eluting with 10% diethyl ether in light petroleum (b.p. 40–60°C) at a flow-rate of 6.5 ml min^{-1}. Fractions of 15 ml were collected and analysed by GC on a 3% OV-101 column at 120°C and nitrogen carrier gas at 60 ml min^{-1}. Results are shown in Fig. 2.

From a mixture of (*Z*)- and (*E*)-7-methyl-6-nonen-3-one, 1.1 g was similarly chromatographed on the 20% loaded column, eluting with light petroleum (b.p. 60–80°C) containing 2% ethyl acetate, flow-rate 1.6 ml min^{-1}, and also on the 40% loaded column, eluting with light petroleum containing 5% diethyl ether.

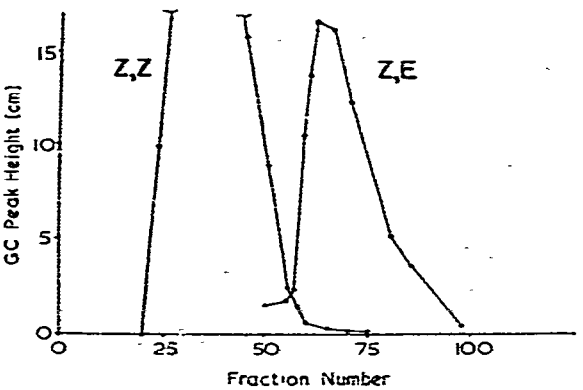

Fig. 2. Separation of (*Z*,*Z*) and (*Z*,*E*)-α-farnesene on a 20% silver nitrate loaded silica gel column.

The columns were prepared for re-use by backflushing with diethyl ether. When not needed for an extended period, the solvent was blown out and the column stored dry.

Pure *Z* and *E* isomers of 8-heptadecene and 9-nonadecene were required for comparison with substances obtained from the Dufour glands of various ant

species[17,18]. A mixture of the two isomers in each case was prepared by Wittig reaction from octanal or decanal, respectively, and triphenylnonylphosphonium bromide. The separation achieved on the silver-loaded silica column is shown in Fig. 1, for 8-heptadecane; 9-nonadecene behaved similarly.

The farnesenes contain four double bonds; in the case of the α-farnesenes which contain all the four bonds in the same positions, 4 isomers exist, namely (*E*,*E*), (*E*,*Z*), (*Z*,*E*) and (*Z*,*Z*)[19,20]. These can be separated by GC only by the most efficient columns. A mixture of (*Z*,*E*) and (*Z*,*Z*)-α-farnesene (Fig. 3) was obtained by a partially stereo-selective synthesis, the final stage of which gave two isomers. These were efficiently separated as shown in Fig. 2. The presence of other double bonds did not greatly complicate the separation.

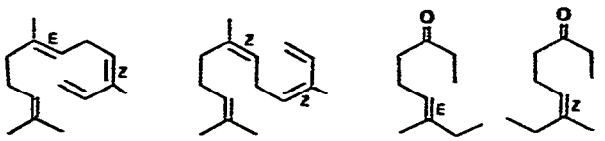

Fig. 3. Structures of farnesene and methylnonenone isomers.

The mixture of (*Z*) and (*E*)-7-methyl-6-nonen-3-one (Fig. 3) was not completely separated on any of our packed GC columns, it was shown by ^{13}C nuclear magnetic resonance (NMR) spectroscopy to consist of a 66:34 mixture of *E*:*Z* isomers. The C-7 carbon of the *E* isomer resonated at 122.5 ppm from tetramethylsilane (TMS) and in the *Z* form at 121.3 ppm. Chromatography on the 20% silver nitrate column gave only partial separation of the isomers. The *E* isomer eluted first, but some contamination with the *Z* isomer was soon detected. The first 16 fractions of 5 ml each, when combined, were found to consist of a 78:22 *E*:*Z* mixture (by ^{13}C NMR spectroscopy). The total material was eluted in 36 fractions. Chromatography on the 40% column did not achieve a significantly improved separation.

DISCUSSION

A silver nitrate-loaded silica column has been used for separations of alkene isomers, on a gram scale. For larger quantities the column size can be scaled up proportionally. By choice of the right particle size, separations can be as efficient as those obtained at much greater cost in time and chemicals by TLC, and without using high pressure pumping. The polyunsaturated farnesenes are rapidly oxidized in air and the loss in handling them on TLC plates and recovering the separated isomers is considerable. By the liquid chromatography method they remain out of contact with air and losses are considerably reduced.

The column can be used repeatedly without any apparent deterioration in performance. The loading of 20% of silver nitrate was chosen by comparison with what can be conveniently used in argentation-TLC. We have not seen any reports of columns or thin layers with loadings as high as 40%. When we failed to separate the ketone isomers on the 20% column we tried a column with 40% loading, but did not achieve any significant improvement. Not too much should be concluded from this failure, for the separation of this particular isomer pair may be inherently difficult.

since it could not be achieved completely on a GC column either. The two isomers differ only slightly in structure.

It is noteworthy that for heptadecene, nonadecene and methylnonenone, the *E* isomer eluted before the *Z* on both the column and TLC. For the farnesenes, the *Z,Z* isomer eluted in both cases before the *Z,E* isomer. This is probably a function of the co-ordination of the somewhat crowded farnesene bonds around the silver ion.

Our own interest has concentrated on the separation of alkenes encountered in our study of ant pheromones, but the method should be equally applicable to other types of compounds, provided the isomers differ sufficiently in their spatial arrangements.

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