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Note

High-performance liquid chromatography of potential insect sex attractants and other geometrical isomers on a silver-loaded ion exchanger

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The interest in separation methods for unsaturated compounds has increased since many sex attractants used for monitoring insects were found to be acetates of geometrical isomers of long-chain alcohols. Most analytical and semi-preparative separations are performed on silver nitrate coated silica gel on thin-layer plates or in columns. A reliable system for 0.5-g scale preparative work was developed in this laboratory[1]. A few thousand chromatograms have already been made on the columns described earlier without any deterioration during two and a half years of intensive use.

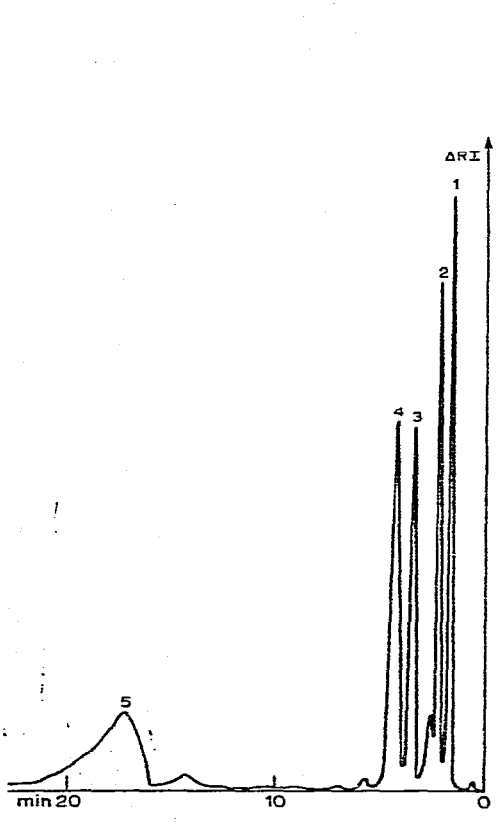
Recently, the high-performance liquid chromatography (HPLC) of olefinic pheromones on silver nitrate coated silica gel with benzene as mobile phase was described[2]. Good separations were obtained; however, it was necessary to wash the eluent with sodium chloride in order to remove the silver that bled from the column. High pressures were used to pump 50–150 ml of solvent per chromatogram to separate isomeric monoenes or dienes in 10–25 min. Another approach was the use of silver nitrate containing aqueous isopropanol as mobile phase and Lichrosorb RP8 as reverse stationary phase[3]. In this elegant analytical method, fast separations of a homologous series of geometrical isomers of 2-alkenes and of four geometrical isomers of 1,5,9-cyclododecatriene were obtained. A disadvantage, however, is that the mobile phase contains 1–2½% of silver nitrate which is corrosive.

In our search for a fast analytical check on the isomeric purity of large series of positional isomers we tried normal gas–liquid chromatography (GLC) on a number of polar stationary phases. However, no phase allowed the separation of all of our positional isomers in the *cis* and *trans* configuration. HPLC on several ion-exchange resins loaded with silver was also disappointing.

We now report the HPLC of acetates of olefinic long-chain alcohols and of methyl esters of unsaturated fatty acids. At moderate pressures (20–50 atm), fast analyses have been achieved on a silica gel-based, strongly acidic, ion exchanger loaded with silver ions and using methanol as mobile phase. Over 1000 chromatograms have been made without deterioration of the column since no bleeding of silver ions occurs.

MATERIALS

The stainless-steel (250 × 4.6 mm I.D.) column was custom-packed with silver-loaded Nucleosil® 10 SA (Macherey, Nagel & Co., Düren, G.F.R.) by Mr. M. Laane, Chrompack Nederland, Middelburg, The Netherlands. The pulses of a plunger-type pump (F. A. Hughes, Epsom, Great Britain) were damped by means of a home-made device with a membrane pressurized by nitrogen gas. A septum injector was used with a stop-flow technique. The Waters 401 differential refractometer and the column were kept at the same constant temperature by use of a precision thermostat. Glass-distilled methanol was the mobile phase. The unsaturated acetates were synthesized in our laboratory; the methyl esters of fatty acids were donated by Mr. H. J. Pabon, Unilever Research, Vlaardingen, The Netherlands.



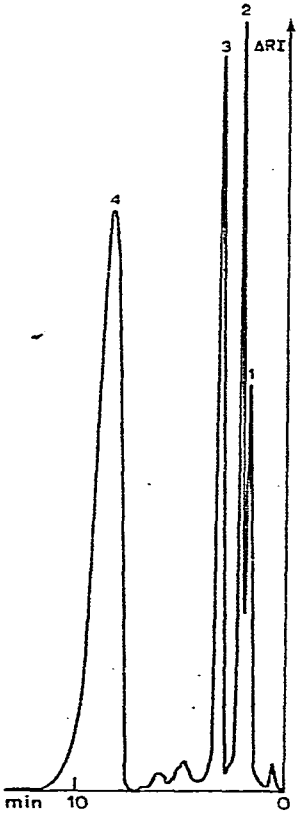


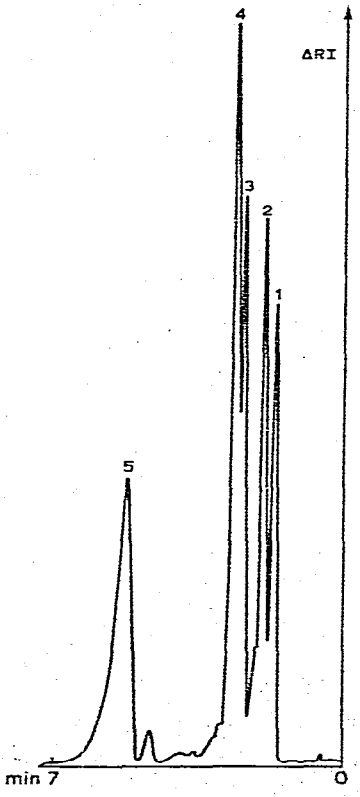
Fig. 1. Separation of test mixture A on 10-μm Nucleosil 10 SA (Ag^+). Conditions: column, 250 × 4.6 mm I.D.; solvent, methanol; flow-rate, 2 ml/min; pressure, 50 kg/cm^2; temperature, 7°; attenuator, ×4; sample size, 2 μl of methanolic solution. 1 = Tetradecan-1-ol acetate; 2 = *trans*-9-tetradecen-1-ol acetate; 3 = *cis*-11-tetradecen-1-ol acetate; 4 = *trans*-4,*cis*-7-tridecadien-1-ol acetate; 5 = *cis*-4,*trans*-7,*cis*-10-tridecatrien-1-ol acetate.

Fig. 2. Separation of testmixture B on Nucleosil 10 SA (Ag^+). Conditions as in Fig. 1. Sample size, 1.8 μl of methanolic solution. 1 = Unknown; 2 = methyl *trans*-9-octadecenoate; 3 = methyl *cis*-9-octadecenoate; 4 = methyl *cis*-9,*cis*-12-octadecadienoate; 5 = methyl *cis*-6,*cis*-9,*cis*-12-octadecatrienoate (retained on the column).

RESULTS

The best resolutions of the geometrical isomers of mono- and diunsaturated acetates or fatty acid methyl esters were obtained at temperatures below 10° as shown in Figs. 1 and 2. The optimum flow-rate for these separations was *ca.* 0.7 ml/min; however, 2 ml/min is an acceptable compromise between speed and resolution. Typical theoretical plate heights were 0.3–0.5 mm (*i.e.*, 850–500 plates per 25-cm column length) at flow-rates of from 0.7 to 2.4 ml/min at 7°. The resolution between *cis*-11-tetradecen-1-ol acetate and tetradecan-1-ol acetate ranged from 5 to 3.8 under these circumstances.

The π-bonds of triene–silver complexes are very strong at this temperature. These compounds had very large retention volumes and either gave broad peaks (Fig. 1, compound 5: *cis,trans,cis*) or were not eluted at all in 30 min (Fig. 2, compound 5: *cis,cis,cis*). It was not possible to decrease the retention volumes in other solvent systems, *e.g.*, hexane–acetone mixtures, acetone, ethyl methyl ketone or ethyl acetate. Acceptable retention times and good resolutions for the triene and diene compounds were obtained at 40° and at flow-rates of 2 ml/min as is shown in Figs. 3 and 4.



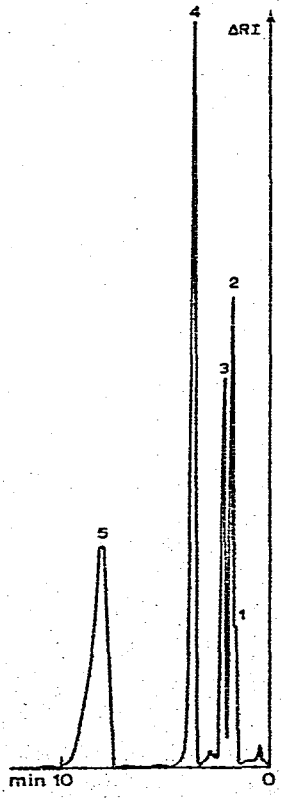


Fig. 3. Separation of test mixture A on Nucleosil 10 SA (Ag^+). Conditions and sample as in Fig. 1, except the temperature, 40°, and sample size, 1.3 μl of methanolic solution.

Fig. 4. Separation of test mixture B on Nucleosil 10 SA (Ag^+). Conditions as in Fig. 3; sample size, 0.9 μl of methanolic solution.

The larger the molecules in a homologous series, the smaller was the retention volume, *e.g.*, methyl *cis*-9-octadecenoate (6.5 ml), methyl *cis*-9-hexadecenoate (6.7 ml) and methyl *cis*-8-tetradecenoate (7.3 ml). Besides the chain length, the distance from the double bond to the end of the chain may also be important. It was difficult to determine the retention volume of an unretained compound since ethanol eluted after tetradecan-1-ol acetate.

Samples from 10 μg to 2 mg can be analysed with excellent sensitivity because of the large differences in refractive index between the solutes and the solvent which is free from silver nitrate. This fast fingerprinting of samples is an excellent guide for the optimum conditions for preparative runs on our Lewatit columns, which operate in a parallel set-up.

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