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PURIFICATION AND ANALYSIS OF SYNTHETIC INSECT SEX ATTRACTANTS BY LIQUID CHROMATOGRAPHY ON A SILVER-LOADED RESIN

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SUMMARY

A description is given of a reliable low-pressure liquid chromatographic system for the analytical and preparative separation of geometrical isomers of alken-1-ol acetates, which are used as insect sex attractants. Baseline separations of 1–600 mg in 1–6 h on columns packed with a macroporous cation-exchange resin treated with silver nitrate are reported. These reusable columns are eluted with methanol and continuously monitored by a differential refractometer, which also allows quantitative analysis.

INTRODUCTION

Female lepidopterous insects usually attract males of their own species by emitting very small amounts of one or more typical chemicals[1–3] and, in the last decade, several of these attractants have been isolated and identified. They often consist of monoenoic or dienoic alcohols with a straight chain of 12, 14 or 16 carbon atoms, or their acetates. For example, male *Adoxophyes orana** moths are attracted by a mixture of *cis*-9-tetradecen-1-ol acetate (*cis*-9-TDA) and *cis*-11-tetradecen-1-ol acetate (*cis*-11-TDA) in a ratio of 9:1 (refs. 4 and 5). The same components but in a ratio of 1:9 are optimum for catches of *Clepsis spectrana** males[6]. Small amounts of the geometrical isomers either inhibit the attractancy of the active isomer[7,8] or, on the contrary, enhance in other species the attractancy and are even necessary for optimum catches[9,10]. A 2:3 mixture of *cis*-11-TDA and *trans*-11-tetradecen-1-ol acetate (*trans*-11-TDA) is attractive for male *Archips podana** moths[11].

Compounds are obtained as *cis*-isomers by reducing alkynes in cold *n*-hexane with hydrogen using Lindlar's catalyst[2,12], while *trans*-isomers can be made by reduction with sodium in liquid ammonia[13,14]. The reaction products nearly always contain small amounts of both the undesired isomer and the completely saturated compound.

Synthetic products can be analysed by infrared spectrometry and by (capil-

* Lepidoptera: Tortricidae.

lary) gas chromatography[9,15–17]. Small amounts of pure isomers can be collected by gas chromatography[4] or thin-layer chromatography on silver nitrate-impregnated silica gel[5]. Slow spinning band distillation has been reported as a method for the purification of *trans*-11-TDA[9].

For the large-scale purification of synthetic products, we used columns packed with silicic acid or acid-washed Florisil impregnated with silver nitrate according to generally accepted methods[18,19].

Fractions were analysed by gas–liquid chromatography. Although compounds of acceptable purity could be obtained, this method had several drawbacks:

(1) The total elution time was long (12–18 h) and the volume per fraction was large, as excessive tailing occurred.

(2) Monitoring of the eluate with a refractometer was difficult, if not impossible, as gradient elution was necessary.

(3) After each run, the column had to be regenerated by passing a large volume of the initial less-polar solvent mixture through it.

(4) The columns could be used for only a few runs, as the resolution decreased gradually and pressure build-up was observed, probably due to changes in the water content of the adsorbent.

Emken *et al.*[20] described a preparative chromatographic procedure in which a silver-saturated ion-exchange resin was used with methanol as the mobile phase. However, their cation-exchange resin was not commercially available, and it is perhaps for this reason that their method has been neglected for the purification of (potential) sex attractants. We investigated a silver-saturated commercial cation-exchange resin for the analysis and purification by liquid chromatography of acetate esters of unsaturated alcohols. This system does not have any of the drawbacks of the impregnated silicic acid columns and is suitable for amounts of 1–600 mg of material.

EXPERIMENTAL

Apparatus

The liquid chromatographic system consisted of a 210 × 1.2 cm polypropylene tube or 150 × 0.6 cm glass tubes with PTFE wool to contain the packing and silicone rubber stoppers as column terminators, a plunger-type pump (F. A. Hughes, Epsom, Great Britain) and a differential refractometer (Waters Ass., Framingham, U.S.A., Model 401). A 500-ml stainless-steel spiral in the solvent line before the column, the column itself and the detector were maintained at the same, constant temperature by a precision thermostat. The water jackets surrounding the columns and the tubing were insulated with a 2.5-cm layer of polyether padding. The injection system, with a blue silicone rubber septum (Applied Science Labs., State College, Pa., U.S.A.), was home-made.

Chemicals

All of the compounds used in this study were synthesized in our laboratory. In addition, *cis*-7-and *trans*-7-dodecen-1-ol acetate, *cis*-9-tetradecen-1-ol acetate (*cis*-9-TDA) and *cis*-11-tetradecen-1-ol acetate (*cis*-11-TDA) were obtained from Farchan Div. (Story Chem. Corp., Willoughby, Ohio, U.S.A.). The strongly acidic macroporous cation-exchanger Lewatit SP 1080, particle size 0.07–0.15 mm (Catalogue

No. 5253) and particle size 0.1–0.2 mm (Catalogue No. 5245) were purchased from E. Merck (Darmstadt, G.F.R.). The solvents were freshly distilled.

Preparation of the column packing

As the wet resin of the commercial product could not be sieved, the adhering liquid was removed by suspending the resin in methanol and washing it twice with 0.7 l of methanol per litre of resin. The resin was washed a further twice with chloroform–methanol (2:3), and once more with methanol. The material was sucked dry on a Büchner funnel and dried overnight at 80°. The dry material was sieved and three fractions were used in this study: 0.075–0.090, 0.105–0.125 and 0.175–0.250 mm. After swelling in deionized water, the slurry was packed in a glass column of 2.5 cm I.D. and a 0.4 mole/l solution of silver nitrate was passed through the bed until the first silver ions were detectable in the effluent. Deionized water was then passed through the bed until no more silver ions could be detected, and finally 3–5 bed volumes of methanol were pumped through the bed. Gas bubbles developed during passage of the methanol front and the bed volume decreased to about 85% of the bed volume in water. A capacity of 1.8 mequiv. of Ag^+ per millilitre of swollen resin was found. The resin slurries prepared in this manner were degassed and packed in the 150 × 0.6 cm glass columns, except for the 0.105–0.125-mm fraction, which was packed in the 210 × 1.2 cm polypropylene column.

RESULTS

Experiments with a 150 × 0.6 cm column packed with a 0.175–0.250 mm fraction of Lewatit SP 1080 (Ag^+) gave good baseline separations of a 20:35:45 test mixture of tetradecan-1-ol acetate, *trans*-11-TDA and *cis*-11-TDA. Plate heights and resolutions were slightly better for the test compounds at 13° than at 9° or 20° under the conditions used (Table I). Consequently, all further work was undertaken with a system regulated at a water-bath temperature of 13°.

In order to optimize these separations, a 150 × 0.6 cm column was packed

TABLE I

INFLUENCE OF TEMPERATURE ON PLATE HEIGHT AND RESOLUTION OF TETRADECAN-1-OL ACETATE, *trans*-11-TDA, AND *cis*-11-TDA

Column (150 cm × 0.6 cm I.D.) packed with Lewatit SP 1080 (Ag^+), 0.175–0.250 mm. Solvent, methanol; flow-rate, 0.73 ml/min; sample size, 4 μl.

$$N = 16\left(\frac{V_R}{4\sigma}\right)^2; H = \frac{L}{N}; R = \frac{V_{R2} - V_{R1}}{2\sigma_1 - 2\sigma_2},$$

where N is number of plates, V_R retention volume, σ standard deviation, H plate height, L column length and R resolution.

Temperature (°C)	*V_R (ml)*			*H (mm)*			*R*	
	Saturated	*Trans*	*Cis*	*Saturated*	*Trans*	*Cis*	*Saturated–trans*	*Trans–cis*
9	27.7	48.3	96.6	12.7	16.6	19.5	1.35	1.50
13	27.3	45.9	88.8	10.2	14.4	14.4	1.38	1.65
20	26.7	40.7	73.3	14.3	20.3	20.3	0.99	1.29

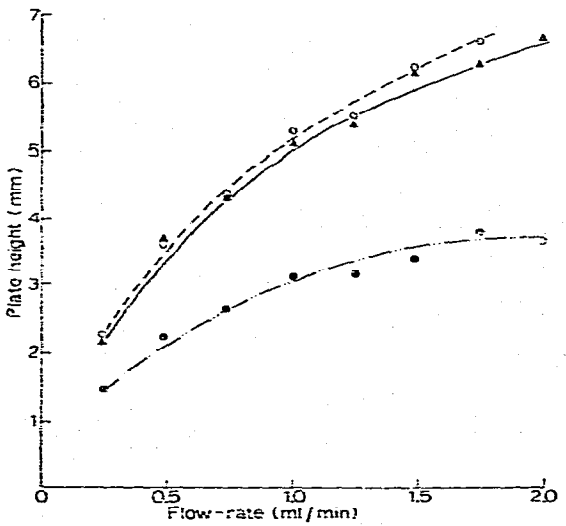

Fig. 1. Relation of plate heights to flow-rates. Conditions as in Fig. 2, except for the flow-rates. ●—·—●, Tetradecan-1-ol acetate; ▲—▲, *trans*-11-TDA; ---, *cis*-11-TDA.

with 0.075–0.090 mm particles of Lewatit SP 1080. The resolution was 1.6–3.7 for the components of the test mixture at flow-rates of 2.0–0.25 ml/min. Hence the three substances of the test mixture were completely separated at all flow-rates. Typically, plate heights of 4.3 mm for both *trans*-11-TDA and *cis*-11-TDA, and resolutions of 2.0 and 2.7, respectively, for separations of the saturated–*trans* and the *trans*–*cis*

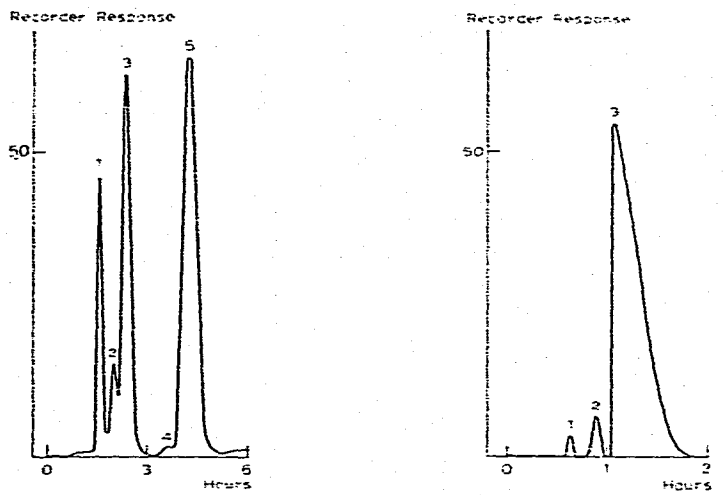

Fig. 2. Separation of a test mixture at low flow-rate. Column (150 cm × 0.6 cm I.D.) packed with Lewatit SP 1080 (Ag^+), 0.075–0.090 mm. Solvent, methanol; flow-rate, 0.29 ml/min; temperature, 13°; attenuator, 4 ×; sample size, 4 μl. 1 = Tetradecan-1-ol acetate; 2 = unknown; 3 = *trans*-11-tetradecen-1-ol acetate; 4 = 11-tetradecyn-1-ol acetate; 5 = *cis*-11-tetradecen-1-ol acetate.

Fig. 3. Separation of a large sample of *cis*-11-TDA indicating column capacity. Sample size, 150 μl; flow-rate, 0.74 ml/min; attenuator, 128 ×; other conditions as in Fig. 2. 1 = Tetradecan-1-ol acetate; 2 = *trans*-11-TDA; 3 = *cis*-11-TDA.

pairs, were found at 13° at a flow-rate of 0.74 ml/min (Fig. 1). Although the components of interest were well separated in 50 min at a flow-rate of 2 ml/min, flow-rates of about 0.75 ml/min were preferred because of the impurities in the synthetic material. The composition of the synthetic material used is clearly shown in a chromatogram at low flow-rate (Fig. 2). The first peak of tetradecan-1-ol acetate is unretained, like all the other saturated compounds used in the study (see Table II), as it was co-chromatographed with ethanol, chloroform and other unretained compounds. The retention volume of these unretained compounds is almost unaffected by variations in the temperature used. However, a slight increase in retention volumes of the *trans*-compounds and a considerable increase in those of the *cis*-compounds with decreasing temperature were found. This might be expected, as the silver complexes involved are more stable at lower temperatures (Table I).

TABLE II

RETENTION VOLUMES OF A HOMOLOGOUS SERIES OF ACETATE ESTERS OF SATURATED AND 9-MONOENOIC STRAIGHT-CHAIN ALCOHOLS

Column (150 cm × 0.6 cm I.D.) packed with Lewatit SP 1080 (Ag^+), 0.075–0.090 mm. Solvent, methanol; flow-rate, 0.70 ml/min; temperature, 13°; sample size, 4 μl; detection, differential refractive index.

Compound	*Retention volume* (ml)*	*Relative retention volume***
Decan-1-ol acetate	28.0	1.00
9-Decen-1-ol acetate	135.2	4.83
Undecan-1-ol acetate	28.0	1.00
trans-9-Undecen-1-ol acetate	46.2	1.65
cis-9-Undecen-1-ol acetate	88.2	3.15
Dodecan-1-ol acetate	28.0	1.00
trans-9-Dodecen-1-ol acetate	46.2	1.65
cis-9-Dodecen-1-ol acetate	85.4	3.05
Tridecan-1-ol acetate	28.0	1.00
trans-9-Tridecen-1-ol acetate	43.4	1.55
cis-9-Tridecen-1-ol acetate	72.1	2.58
Tetradecan-1-ol acetate	28.0	1.00
trans-9-Tetradecen-1-ol acetate	40.6	1.45
cis-9-Tetradecen-1-ol acetate	67.9	2.43
Pentadecan-1-ol acetate	28.0	1.00
trans-9-Pentadecen-1-ol acetate	39.9	1.43
cis-9-Pentadecen-1-ol acetate	63.7	2.28

* Total retention volume from injection.

** Retention volume relative to ethanol or saturated alcohols (unretained compounds).

Sample sizes up to 150 μl of ester (density 0.84 g/ml) could be chromatographed in 2 h on the 150 × 0.6 cm column and five times as much on the 210 × 1.2 cm column in 3.5 h without overlap of the components of interest. The sample used in Fig. 3 did not contain the unknown impurity nor the 11-tetradecyn-1-ol acetate.

The influence of chain length on the retention volumes of a homologous

TABLE III

RETENTION VOLUMES OF POSITIONAL ISOMERS OF *cis*- AND *trans*-TETRADECEN-1-OL ACETATES

Flow-rate, 0.72 ml/min; other conditions as in Table II. Retention volume of unretained compounds, 28.2 ml.

Position of double bond in tetradecen-1-ol acetate	*Retention volume (ml)*	
	Trans	*Cis*
2	29.8	33.8
6	40.6	68.5
7	41.5	68.3
9	40.6	69.1
10	41.8	68.4
11	41.8	75.2

series of acetate esters of saturated and 9-monoenoic straight-chain alcohols is clearly demonstrated in Table II. There was no clear correlation between retention volumes of the positional isomers of *trans*-TDA or *cis*-TDA and the position of the double bond (Table III). On two different columns, we found similar retention volumes for the compounds with the double bond in positions 6 to 10, and the retention volume of *cis*-11-TDA was much greater than for any other of this series.

DISCUSSION

We feel that the excessive tailing that was found in our former work with silver nitrate-impregnated silica gels, even if particles of 0.032–0.063 mm were used, occurred because chromatographic processes other than that of the silver complex principle (such as adsorption or partition) are involved if silica gel is the support material[21]. The chromatographic process on the ion-exchange resin with silver ions as counter-ions behaves as if only one principle is involved, namely formation of a silver complex with the olefin. Even with overloaded columns, sharp peaks were obtained in a peak volume of not more than 30 ml and no excessive tailing occurred. The use of narrower sized fractions of smaller particles must be the greatest contribution to the better separations obtained in this study than by Emken *et al.*[20].

The increase in peak width and, accordingly, the slight loss in resolution at the lower temperature that occurred in the experiment with varying temperatures (Table I) is probably due to the impairment of mass transfer by the slower diffusion processes in the larger particles used in that experiment. By merely decreasing the temperature, the retention volumes of the *trans*-compounds, and to a much greater extent, those of the *cis*-compounds, can be increased, whereas those of the saturated compounds are almost unaffected. With smaller packing material, much may be gained by exploiting these temperature-dependent increases in retention volume. In conventional argentation chromatography with silica gel as support material, a similar effect can also be achieved by decreasing the polarity of the solvent mixture. This affects both the saturated and unsaturated components and always causes excessive tailing, especially if continuous gradients are used.

In accordance with the results obtained by thin-layer chromatography of

homologous isomeric fatty acid esters on silica gel impregnated with silver nitrate[22,23], we also found that the components with the longer aliphatic chain eluted earlier (Table II). The large difference between the mobilities of the *trans*-complex and the *cis*-complex indicates that in the present system the individual *cis*-compounds are always separated from each of the *trans*-compounds, without overlap.

The separation pattern of the positional isomers on the column used shows less clearly the sinusoidal type of curve described as the chromatographic pattern of positionally isomeric *cis*- and *trans*-octadecenoates on thin-layer plates impregnated with silver nitrate[23]. If plotted as in ref. 21, Fig. 2, a relatively high mobility is found with our results for *cis*- and *trans*-2-TDA. However, the compounds with double bonds in positions 6–10 show the same relative mobilities and *cis*-11-TDA has the lowest mobility (Table III).

There are also some similarities with the chromatographic pattern of epoxyoctadecenoates on a gas chromatographic system as described by Emken[17]. In his system, the *cis*-6,7-, *cis*-9,10-, and *cis*-12,13-epoxyoctadecenoates have the same retention times, whereas the (*n*-3)-substituted component, the *cis*-15,16-epoxyoctadecenoate, has a longer retention time. Likewise in our system, the component with three carbon atoms after the double bond, *cis*-11-TDA, has the longest retention time.

CONCLUSION

The high resolution for the geometrical isomers of alkenyl esters, the relatively short time needed to obtain a chromatogram, and the direct measurement with the recording differential refractometer offer great advantages over gas chromatographic techniques[16,17] and thin-layer or liquid chromatography with the unpleasant silver nitrate-impregnated silica gel.

As already mentioned[20], the stability and reliability of this "clean" system are outstanding: we have run the columns for 4 months with more than 100 injections on each without any sign of deterioration.

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