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# SMECTIC LIQUID CRYSTAL FOR THE GAS–LIQUID CHROMATOGRAPHIC SEPARATION OF LEPIDOPTEROUS SEX PHEROMONES AND RELATED ISOMERIC OLEFINS

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## SUMMARY

Precise knowledge of isomeric purity is essential if insect sex pheromones are to be of use in pest management. Unique gas–liquid chromatographic separations are presented as evidence that diethyl 4,4'-azoxydicinnamate, a smectic liquid crystal, can be utilised to provide this knowledge quickly and efficiently on packed columns[*].

## INTRODUCTION

Many of the insect sex pheromones that have been identified are linear aliphatic alcohols, aldehydes or acetates, with a chain length of between twelve and eighteen carbon atoms and one or two olefinic linkages. Species specificity is conferred in part by variation of pheromone structure, and this variation can be achieved through differences in functional group, in chain length, or by changes in position or geometry of the double bond(s). Research has shown that rigorously defined mixtures of isomers are often vital for maximum activity of synthetic pheromones, and so an analytical technique capable of providing precise geometrical definition is required if pheromones are to be effectively identified and utilised.

The analytical separation of geometric and positional isomers of insect sex pheromones has been attempted by many workers using a variety of techniques, notably gas–liquid chromatography (GLC) and high-performance liquid chromatography (HPLC). Of these two techniques, HPLC currently suffers from a lack of detector sensitivity which precludes its use at submicrogram levels in identification of natural pheromones, and with GLC it has generally been necessary to resort to high-efficiency open tubular columns in order to obtain satisfactory resolution of the geometric isomers of many known pheromones. This paper will present a smectic liquid crystal, diethyl 4,4'-azoxydicinnamate, as a gas chromatographic (GC) stationary phase for the fast and efficient analysis of mono- and di-unsaturated insect sex pheromones and of geometric and positional isomers of related fatty acid derivatives. In particular, reference will be made to the separation of some pheromone

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[*] A preliminary account of this work was given in ref. 1.

isomers which have previously only been separated on open tubular GC columns, and also to some pheromone isomers which have not as yet been separated even by this method.

## EXPERIMENTAL

The apparatus used was a Varian Model 2100 gas chromatograph equipped with flame ionisation detectors. The columns were 2 m × 2 mm I.D. × 6 mm O.D. glass U-tubes, and support materials were 100–120 mesh Chromosorb G AW DMCS, 100–120 mesh Gas-Chrom Q, and 100–120 mesh Gas-Chrom RZ.

Diethyl 4,4'-azoxydicinnamate, the liquid crystal stationary phase, was purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.). The specification of the mesophase quoted by the manufacturer was confirmed in the laboratory using a Kofler melting-point apparatus as being 136–260°C. The supports were coated by deposition of the stationary phase from a dichloromethane solution using the slurry technique. The packed columns were conditioned for 2 h before use with a nitrogen carrier gas flow-rate of 10 ml/min at a temperature of 180°C.

For all columns used the following parameters were standard: nitrogen carrier gas flow-rate 10 ml/min; injector temperature 120°C; detector temperature 185°C.

With the exception of dodecyl acetate and n-hexadecane which were purchased from Ralph Emanuel, London, Great Britain (now Aldrich Chemical Co.), all the compounds used in this study were prepared in our laboratory. Their structures were deduced from their mode of synthesis, their spectral and chromatographic characteristics, and by suitable microchemical reactions where appropriate[2].

## RESULTS AND DISCUSSION

For the initial investigation of the dicinnamate as a stationary phase, a standard synthetic mixture was used, containing 1 mg/ml each of n-hexadecane, dodecyl acetate and the Z- and E-isomers of 9,11-dodecadienyl acetate, in n-pentane. 9,11-Dodecadienyl acetate is the major component of the sex pheromone complex of the red bollworm moth, *Diparopsis castanea* Hmps.[3,4], and was chosen to evaluate the ability of the dicinnamate to separate isomers because, to date, the Z- and E-isomers of this particular diene have not been resolved using open tubular GC columns of up to 100 m in length. In the identification of these isomers in the pheromone of the red bollworm, resort was made to indirect methods of analysis such as preparation of the mono-epoxides, selective reduction of the double bonds with diimide and selective removal of the E-isomer by a Diels–Alder reaction[4].

The test mixture was first examined on a column packed with 2.4% (w/w) of the dicinnamate on 100–120 mesh Chromosorb G AW DMCS. Fig. 1 shows plots of the logarithm of the corrected retention time (log. corr. RT) against reciprocal temperature (°K) for the four components of the test mixture, and demonstrates the way in which the state of the liquid crystal influences retention times. There is a decrease in retention time of all four components with increasing column temperature until the crystal–smectic melting point is approached. There then follows an increase in retention time due to intercalation of the solute molecules within the liquid crystal lattice[5]. With further increase in temperature the retention times again de-
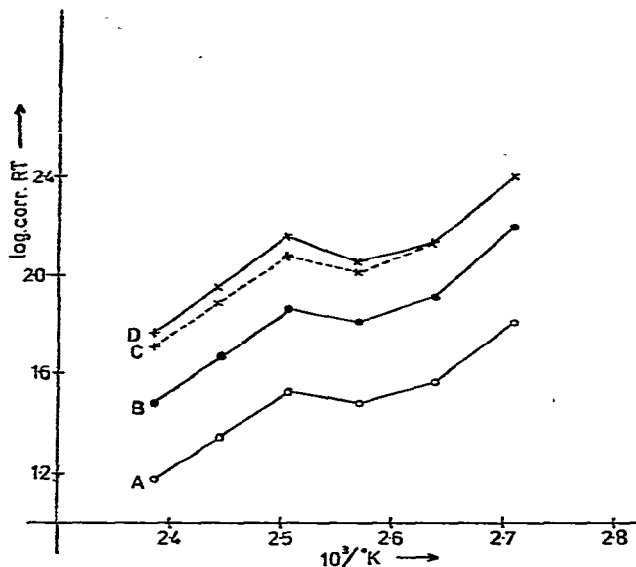
Fig. 1. Relationship between the logarithm of the corrected retention time (log. corr. RT) and the reciprocal absolute temperature (1/°K) for *n*-hexadecane (A), dodecyl acetate (B), Z-9,11-dodeca-dienyl acetate (C) and E-9,11-dodecadienyl acetate (D) on a column packed with 2.4% (w/w) diethyl 4,4'-azoxydicinnamate on Chromosorb G.

crease, due to a combination of increased solute volatility and a progression towards isotropic character of the liquid crystalline solvent.

The change of the state of the liquid crystal with temperature is further illustrated in Fig. 2 which shows measurements of the column efficiency ($N$) for each
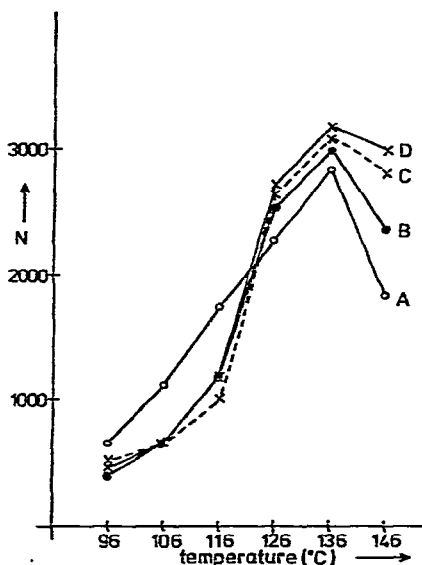


Fig. 2. Relationship between the number of theoretical plates ($N$) and the column temperature (°C) for compounds A, B, C and D. Column and test compounds as in Fig. 1.

of the test compounds plotted against column temperature (°C). As the column temperature is increased and the crystalline stationary phase progresses towards the liquid crystalline (smectic) region, its solvating power increases and this gives rise to increased column efficiency. Also, the column efficiency for the acetate test compounds improves relative to that for the n-hexadecane. This could be a result of the expansion of the solvent lattice permitting penetration of the more bulky acetate molecules as well as the n-hexadecane molecules into the lattice. After the column temperature rises above the crystal–smectic transition temperature, the crystalline stationary phase melts and column efficiency starts to decrease as the phase begins to lose its liquid crystalline properties.

The effect of the state of the liquid crystal on the resolution of the Z- and E-isomers of the diene test compound is illustrated in Fig. 3, which is a plot of the resolution (R) of these two isomers against column temperature (°C). For incremental increases in column temperature no detectable resolution of the isomers is observed below 106°C. Above this temperature the resolution increases to a maximum of $R = 1.87$ at 126°C, with baseline resolution ($R = 1.5$) being achieved at 123°C. When the liquid crystalline phase is subjected to incremental decreases in temperature from a point within the mesophase, the temperature range for which resolution of these isomers is observed is extended downwards to 96°C. These observations are consistent with the liquid crystal stationary phase displaying a super-cooled region which is physically identical with the smectic region[6].

Different support materials for the liquid crystal stationary phase were compared with respect to the resolution obtained for the Z- and E-isomers of 9,11-
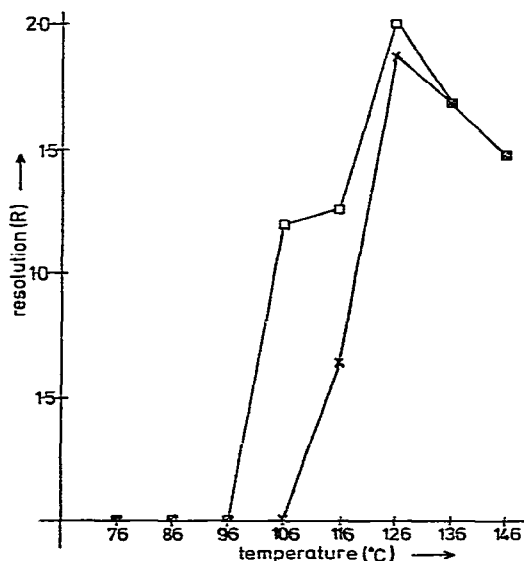


Fig. 3. Dependence of the resolution factor (R) for the Z- and E-isomers of 9,11-dodecadienyl acetate on the column temperature (°C) in the liquid crystal smectic and super-cooled phases. Column as for Fig. 1. ×, heating; □, cooling.

dodecadienyl acetate. The results, summarised in Table I, show that the flux-calcined support materials (Gas-Chrom Q and Chromosorb G) provide a superior vehicle for the liquid crystalline stationary phase compared with the fire-brick support (Gas-Chrom RZ). The resolution is also improved by increasing the w/w percentage of liquid phase used.

TABLE I

RESOLUTION OF Z- AND E-9,11-DODECADIENYL ACETATES ON COLUMNS PACKED WITH DIETHYL 4,4'-AZOXYDICINNAMATE ON DIFFERENT SUPPORT MATERIALS AND AT DIFFERENT LOADINGS

| Support material | Support type | Stationary phase (%, w/w) | | Resolution at 126 °C |
| --- | --- | --- | --- | --- |
| | | Actual | Equivalent on Gas-Chrom Q | |
| Gas-Chrom Q | Flux-calcined diatomite | 10.2 | 10.2 | 2.48 |
| Gas-Chrom Q | Flux-calcined diatomite | 4.5 | 4.5 | 1.97 |
| Gas-Chrom RZ | Fire-brick | 7.0 | 10.2 | 0.84 |
| Chromosorb G | Flux-calcined diatomite | 2.4 | 4.5 | 1.87 |

On the basis of the above results, the 10.2% (w/w) loading of stationary phase on Gas-Chrom Q was used in an examination of the GC behaviour of various mono- and di-unsaturated pheromones and related compounds on the liquid crystal. Fig. 4 shows plots of equivalent chain length (E.C.L.; value for tetradecyl acetate = 14.0) of all the internally mono-unsaturated tetradecenyl acetate isomers with respect to the position of unsaturation on the liquid crystal phase and, for comparison, on Apolar 10C which is a polar isotropic liquid stationary phase often used for analysis of isomeric olefins[7].

These plots illustrate the fundamental difference between isotropic and meso-morphic liquid phases. The traditional isotropic liquid phases separate isomeric solutes essentially on the basis of differences in their electronic properties, hence the trend towards use of the more polar liquid phases. Mesomorphic liquid phases, on the other hand, differentiate isomeric solutes on the basis of their varying abilities to penetrate the liquid crystalline lattice which is a result of their steric properties, and can be conveniently expressed in terms of their molecular length-to-breadth ratio[8].

Incorporation of a double bond in an alkyl chain imparts some distortion to the time-averaged linear nature of the saturated molecule, decreasing the length-to-breadth ratio and the ability to penetrate a liquid crystal lattice. For a given pair of geometric isomers this distortion is greater for the Z- than for the E-isomer, and the overall change in shape is greatest when the double bond is in the middle of the molecule[9].

Fig. 4 shows that on the Apolar 10C column all the mono-ene isomers are eluted after the saturated compound, and for most of the Z–E pairs the Z-isomer is eluted after the E-isomer. On the liquid crystal column, however, the picture is completely reversed. Nearly all the mono-unsaturated compounds are eluted before the saturated compound, and for each pair of geometric isomers the Z-isomer is eluted before the E-isomer, the Z–E separation being greatest when the double bond is towards the middle of the molecule. These results are easily rationalised in terms
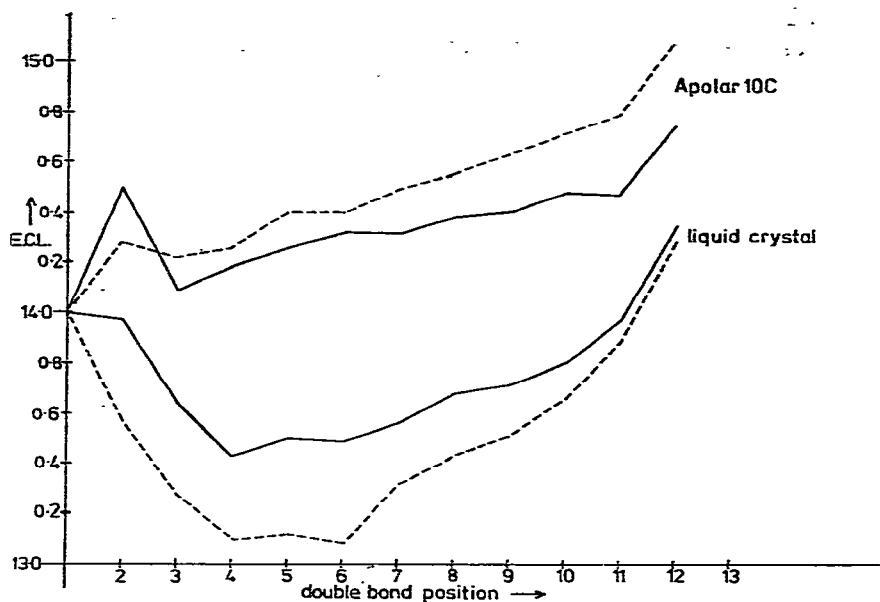
Fig. 4. Variation in equivalent chain length (E.C.L., tetradecyl acetate = 14.0) with position of the double bond for mono-unsaturated tetradecenyl acetates, on Apolar 10C (1.5% on Chromosorb G at 180°C) and diethyl 4,4'-azoxydicinnamate (10.2% on Gas-Chrom Q, temperature programmed from 100 to 180 °C at 2 °C/min). ——— E, ------ Z.

of the changes in length-to-breadth ratio and the corresponding ability to penetrate the liquid crystal lattice described above.

The complementary nature of this data is in itself a valuable aid in the identification of unknown compounds, but the use of columns packed with the liquid crystal phase does offer certain advantages over columns packed with isotropic stationary phases. The liquid crystal is readily available as a pure compound so there is no risk of different batches having different chromatographic properties, as has been reported for Apolar 10C[7]. Along with Apolar 10C, OV-275 and SP 2340 represent the most polar stationary phases used for the gas chromatographic analysis of unsaturated aliphatic compounds[10–12]. These phases can provide excellent separations of isomeric mono-enes, but necessitate the use of long columns, high column temperatures and stationary phase loadings of 15–20% (w/w) on fire-brick type supports[10].

These isotropic phases are also effective for analysis of di- and tri-unsaturated compounds, but they fail to give complete resolution of the isomers of conjugated dienes[1]. However, several insect pheromones involve conjugated dienes, and complete separation of the geometric isomers has hitherto required the efficiency of open tubular columns[13]. With the dicinnamate liquid crystal stationary phase, complete separation of diene isomers is possible on a 2-m packed column with a correspondingly much reduced analysis time, as shown in Fig. 5.

Fig. 5A and B show the separations of the geometric isomers of the internal conjugated dienes, 8,10-dodecadienyl acetate and 9,11-tetradecadienyl acetate respectively, obtained using the 2-m column packed with the liquid crystal stationary phase
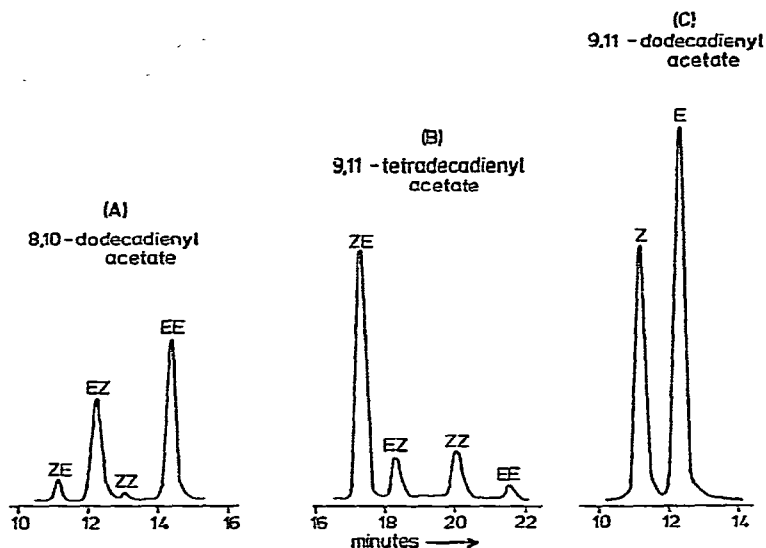
Fig. 5. Chromatograms of conjugated diene acetates on diethyl 4,4'-azoxydicinnamate (10% (w/w) on Gas-Chrom Q, temperature programmed from 100 to 180 °C at 2 °C/min).

on Gas-Chrom Q referred to above. E,E-8,10-Dodecadienyl acetate is a powerful attractant for the male pea moth, *Cydia nigricana* Steph.[14] and has been identified as a component of the female sex pheromone of the Nantucket pine tip moth, *Rhyacionia frustrana* Comstock[15]. Z,E-9,11-Tetradecadienyl acetate is the attractant component of the female sex pheromone of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisd.[3].

In Fig. 5C a chromatogram is shown illustrating the separation of the two geometric isomers of the terminal conjugated diene, 9,11-dodecadienyl acetate. An E–Z mixture (80:20) of these isomers constitutes the major component of the sex pheromone of the red bollworm moth, *Diparopsis castanea* Hmps.[4], and this ratio is important in achieving maximal attractiveness with synthetic pheromone sources[16]. As indicated above, no separation whatsoever has been reported previously for these isomers, even when high-resolution open tubular columns have been employed.

Apart from the fact that columns packed with the dicinnamate liquid crystal phase can separate isomers which have not yet been separated on open tubular columns, the use of packed columns in general still offers a number of advantages. Packed columns are typically shorter than open tubular columns and analyses can be performed more quickly. Glass open tubular columns are still expensive and fragile, and they require sophisticated injector and detector units which limit the sample size and so prohibit their use in a preparative mode. In contrast, packed columns are cheap and robust, the packings are easily replaced when contaminated and the columns can accommodate large samples for analytical or preparative separations.

The test compounds reported in this study are all $C_{12}$ and $C_{14}$ acetates, but the liquid crystal packed column has also been used for analysis of unsaturated $C_{16}$ and $C_{18}$ acetates, as well as representative unsaturated aldehydes, ketones and fatty

acid methyl esters, and isomeric epoxides [including Z-7,8-epoxy-2-methylocta decane, the sex pheromone of the gypsy moth, *Porthetria dispar* L[17]][18]. Alcohols cannot, in general, be chromatographed on this column, although this limitation can be circumvented by suitable derivatisation, *e.g.*, the corresponding acetates can be prepared even at the nanogram level[19].

CONCLUSIONS

Analysis of geometric isomers of insect pheromones can be accomplished quickly and efficiently by gas chromatography on a column packed with diethyl 4,4'-azoxydicinnamate, a liquid crystal with a smectic mesomorphic state ranging from 136 to 260°C. This paper is the first report of use of a liquid crystal stationary phase in the analysis of pheromone-type compounds, and the results indicate that resolution of geometric isomers on this phase in packed columns is in some cases superior to the resolution obtained on open tubular columns prepared using conventional isotropic liquid phases.

It is probable that this particular liquid crystal phase will be generally useful in the analysis of fatty acid derivatives and that the development of other liquid crystals, with wider mesophases and improved thermal stability, will extend the use of liquid crystal stationary phases into other areas of analysis.

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