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4-(*p*-METHOXYCINNAMYLOXY)-4'-METHOXYAZOBENZENE: A NEMATIC LIQUID CRYSTAL FOR THE GAS-LIQUID CHROMATOGRAPHIC ANALYSIS OF THE STEREOCHEMISTRY OF LEPIDOPTEROUS SEX PHEROMONES AND RELATED UNSATURATED FATTY ALCOHOLS AND DERIVATIVES

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SUMMARY

A study of the gas-liquid chromatographic behaviour of unsaturated fatty alcohols and acetates on a nematic liquid crystal, 4-(*p*-methoxycinnamyloxy)-4'-methoxyazobenzene, as stationary phase has shown that excellent separations of isomers are possible within the nematic and supercooled temperature limits of this compound. Separations of isomers of some mono- and di-unsaturated insect pheromones are given to illustrate the selectivity of this liquid crystal towards olefinic isomers.

INTRODUCTION

The development of high-polarity, cyanopropylsiloxane stationary phases has made possible the analysis of isomeric, unsaturated fatty acid derivatives on packed gas-liquid chromatographic (GLC) columns¹⁻⁵. However, these isotropic phases have several disadvantages: high column temperatures and long analysis times are often required^{5,6}, resolution of the isomers of conjugated dienes is generally not particularly good, and, with some of these phases, *e.g.* OV-275, direct analysis of fatty alcohols is not possible⁷.

We recently reported⁸ the use of a smectic liquid crystal, diethyl 4,4'-azoxydicinnamate, as a stationary phase for the separation of isomers of mono-unsaturated and conjugated, di-unsaturated long-chain acetates, aldehydes and methyl esters on packed GLC columns. However, this liquid crystal phase was unsuitable for direct analysis of the corresponding alcohols. It had also been withdrawn from sale by the suppliers, Eastman Kodak Company, although, following the appearance of our paper⁸, the compound is now on the market again⁹.

Continuing investigations in this laboratory on the use of liquid crystals as

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stationary phases for GLC separation of various stereoisomers have lead to a further study of the nematic liquid crystal, 4-(*p*-methoxycinnamyloxy)-4'-methoxyazobenzene originally reported by Kelker¹⁰. This compound has been found to exhibit similar chromatographic properties to the above smectic liquid crystal, but it allows direct chromatography of alcohols, possesses superior thermal stability and has a wider mesomorphic temperature range. The compound is no longer commercially available, but is easily prepared by standard laboratory procedures.

EXPERIMENTAL

A Varian Model 2100 gas chromatograph was used, equipped with flame ionisation detectors, linear temperature programmer and standard 2 m × 2 mm I.D. × 6 mm O.D. glass U-columns. Carrier gas was nitrogen at 25 ml/min. The nematic liquid crystal was slurry coated in chloroform onto 100–120 mesh Gas-Chrom Q to give a 10% (w/w) packing. The column was gravity packed and conditioned at 180° for 2 h before use. Subsequent to this initial conditioning procedure, the column was maintained at 150° when not in use and then reconditioned for use by raising the column temperature to 180° for 10–15 min.

Most of the work described here was carried out using a sample of 4-(*p*-methoxycinnamyloxy)-4'-methoxyazobenzene generously provided by Professor H. Kelker. The compound is no longer available from the suppliers (Riedel-de Haën, Seelze-Hannover, G.F.R.) but is readily prepared as outlined in Fig. 1. After three recrystallisations from chloroform, the product had a melting point of 167.5°, compared with values of 175° (ref. 10) and 163° (ref. 11) quoted in the literature.

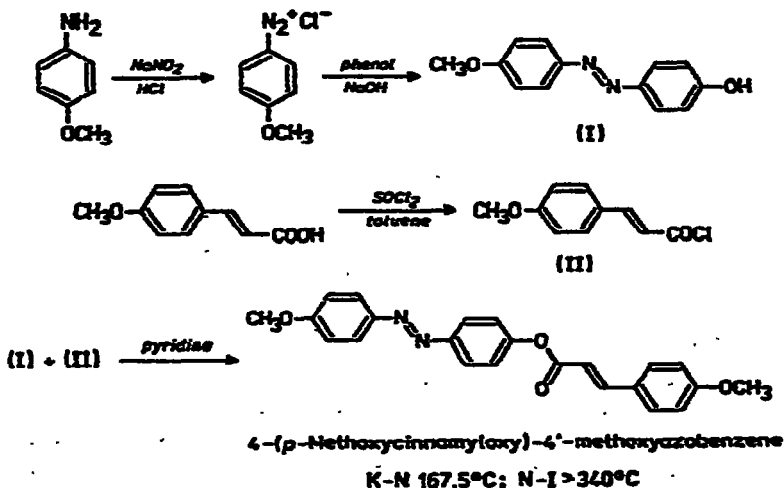


Fig. 1. Synthesis of 4-(*p*-methoxycinnamyloxy)-4'-methoxyazobenzene.

All the test compounds 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.

RESULTS AND DISCUSSION

The chromatographic behaviour of the *Z* and *E* isomers of 9,11-dodecadienyl acetate was used as an indicator of the resolving power of the nematic liquid crystal stationary phase. These isomers constitute part of the sex pheromone of the female red bollworm moth, *Diparopsis castanea*¹², and our previous paper⁸ on the smectic liquid crystal stationary phase, diethyl 4,4'-azoxydicinnamate, was the first record of direct separation of these isomers on any GLC column.

In Fig. 2, the number of theoretical plates (N) with respect to the diene isomers is plotted against the column temperature ($^{\circ}\text{C}$), and this illustrates the way in which the state of the liquid crystal influences the efficiency of the GLC column. Starting from a point at which the liquid crystal is in the crystalline state, the column efficiency starts to increase as the temperature is raised and the stationary phase approaches the mesomorphic state. The efficiency passes through a maximum and then declines as further increases in temperature tend to dissipate the mesomorphic character of the liquid crystalline state. However, starting from a temperature within the mesomorphic region, decreasing the column temperature then leads to a steady increase in column efficiency as the mesomorphic state supercools^{13,14} before it reverts sharply to the crystalline form below 90° .

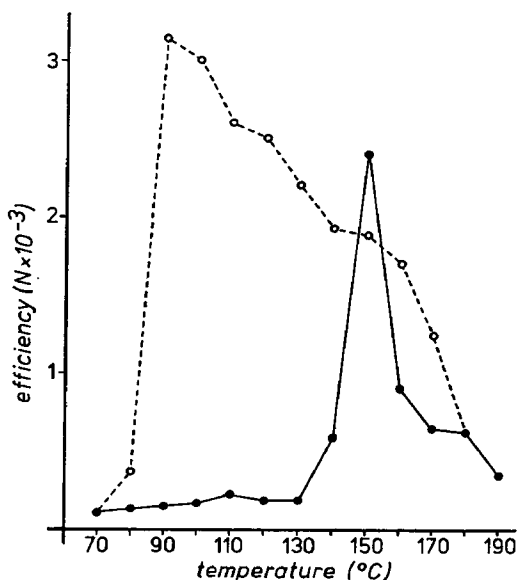


Fig. 2. Relationship between column efficiency as expressed by the number of theoretical plates (N) and column temperature ($^{\circ}\text{C}$) for (*Z*)- and (*E*)-9,11-dodecadienyl acetate. ●—●, Heating; ○—○, cooling.

The chromatograms used to obtain the results shown in Fig. 2 were also used to produce Fig. 3 which shows the variation in the resolution (R) of the two isomers of 9,11-dodecadienyl acetate with changing column temperature. With increasing column temperature, no resolution of the isomers is observed below 120° . Above this temperature the resolution passes through a maximum corresponding to the change

in efficiency (Fig. 2), but at no point is baseline separation ($R > 1.5$) achieved. Decreasing the temperature from a point within the mesomorphic region dramatically improves the resolution, with baseline separation of the two isomers being achieved between 85 and 137°. This resolution is maximal in the supercooled region, and diminishes rapidly when the stationary phase reverts to the crystalline state.

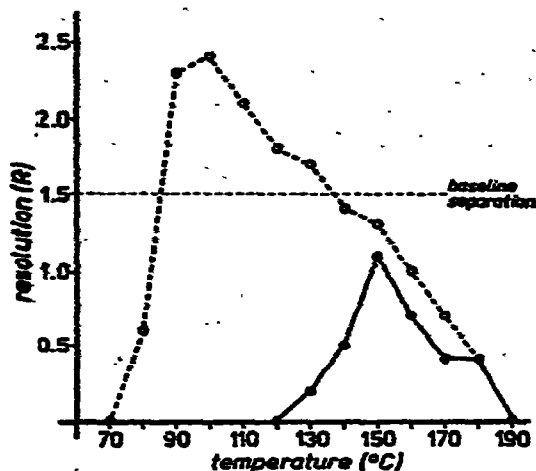


Fig. 3. Relationship between resolution (R) and column temperature ($^{\circ}\text{C}$) for (Z)- and (E)-9,11-dodecadienyl acetate. ●—●, Heating; ○- - -○, cooling.

From these results it is clear that the liquid crystal stationary phase must be used in the supercooled state, and reproducible results are only obtained when this state is maintained. It has been found that, if the column is conditioned at 180° for 10–15 min before use, it can then be cooled to not less than 100° and used isothermally or with an appropriate temperature programme within this range. With the 2 mm I.D. \times 6 mm O.D. columns used in the Varian 2100 instruments, cooling by setting the temperature control to 100° and opening the oven door in the normal manner was found to be satisfactory, as the thick-walled columns retain sufficient heat to ensure that the stationary phase does not revert to the crystalline state. With a Varian 2700 instrument fitted with thinner 2 mm I.D. \times 4 mm O.D. columns, such a procedure allowed the stationary phase to solidify, and cooling must be carried out more carefully with the oven lid closed or only slightly open. A similar effect was observed with a Pye 104 instrument fitted with 4 mm I.D. \times 6 mm O.D. columns.

Some typical results using a temperature programme at 1°/min starting from 120° are represented in Figs. 4–6. Fig. 4 shows the variation in equivalent chain length (ECL, tetradecyl acetate = 14.0) with double bond position and geometry for all the isomers of tetradecenyl acetate. For all positions of the double bond the Z isomer is eluted before the E isomer, and, apart from the (E)-2-, (E)-11-, (E)-12-, (Z)-12- and 13-tetradecenyl acetates, all isomers are eluted before the parent, saturated tetradecyl acetate. These phenomena are the result of changes in the length-to-breadth ratio of the molecules, and are qualitatively similar to those observed with the smectic liquid crystal stationary phase, diethyl 4,4'-azoxydicinnamate⁸, although the Z/E separations here are better for most positional isomers.

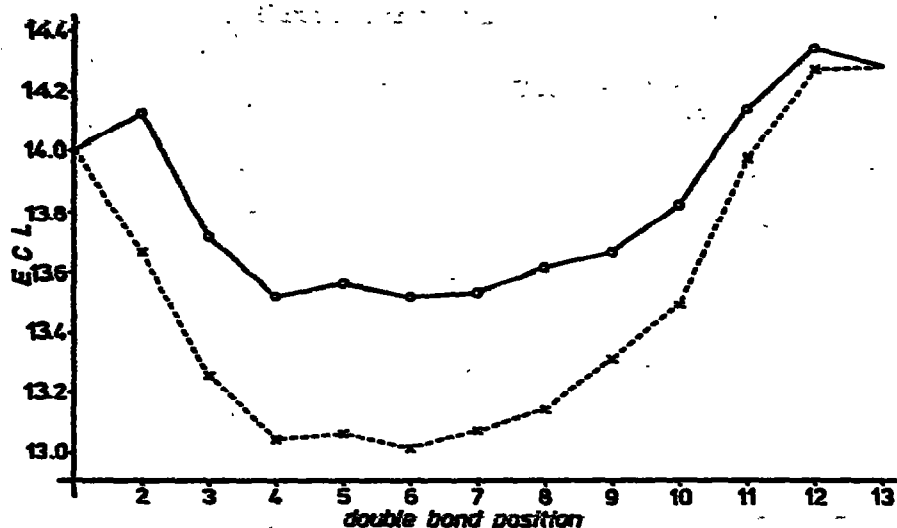


Fig. 4. Variation in equivalent chain length (ECL, tetradecyl acetate = 14.0) with position and geometry of the double bond for mono-unsaturated tetradecenyl acetates. O—O, *E*; x----x, *Z*.

Fig. 5 shows a chromatogram of some of these tetradecenyl acetates. Although it is not feasible to separate all 23 isomers on a single chromatogram, it is possible to distinguish between any *Z/E* pair in a single run.

Alcohols cannot be chromatographed directly on the smectic liquid crystal stationary phase, diethyl 4,4'-azoxydicinnamate⁸, or on the highly polar, isotropic phase, OV-275⁷. Fig. 6 shows the separations of the isomers of some conjugated dienes chromatographed on 4-(*p*-methoxycinnamyloxy)-4'-methoxyazobenzene. These include the isomers of 8,10-dodecadien-1-ol which chromatograph as well as the di-unsaturated acetates. (*E,E*)-8,10-Dodecadien-1-ol is the sex pheromone of the female

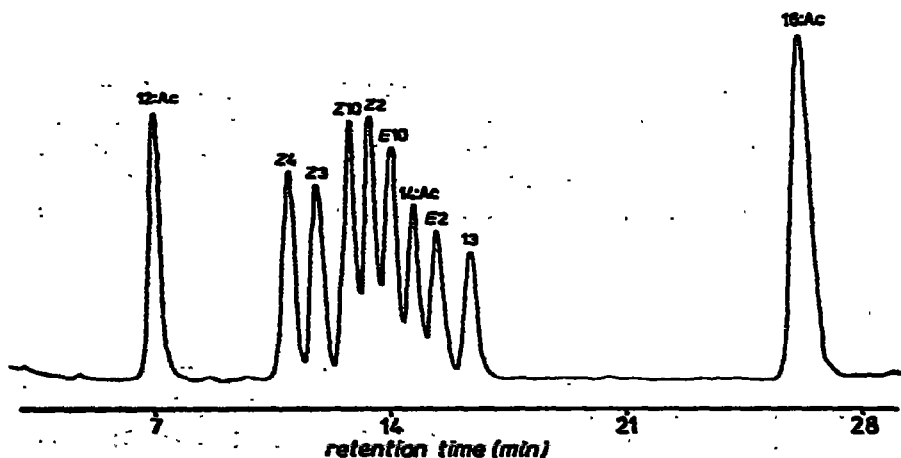


Fig. 5. Chromatogram of some tetradecenyl acetate isomers with dodecyl acetate (12:Ac), tetradecyl acetate (14:Ac) and hexadecyl acetate (16:Ac).

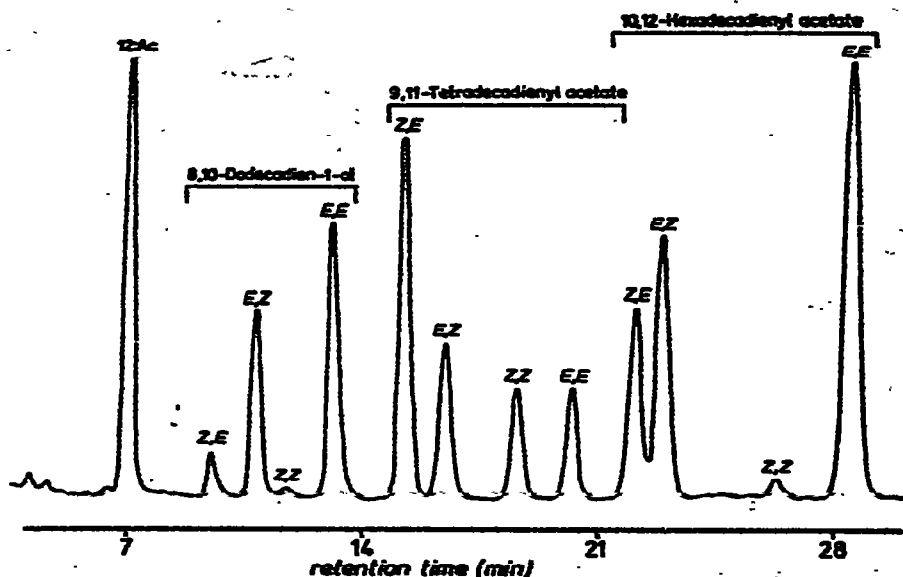


Fig. 6. Chromatogram of isomers of 8,10-dodecadien-1-ol, 9,11-tetradecadienyl acetate and 10,12-hexadecadienyl acetate with dodecyl acetate (12:Ac).

codling moth, *Laspeyresia pomonella*¹⁵, and (*Z,E*)-9,11-tetradecadienyl acetate is the attractant component of the sex pheromone of the female Egyptian cotton leafworm moth, *Spodoptera littoralis*¹⁶. The 10,12-hexadecadienyl acetates include derivatives of (*E,Z*)-10,12-hexadecadien-1-ol and (*E,Z*)-10,12-hexadecadienal, components of the sex pheromone of the female silkworm moth, *Bombyx mori*^{17,18}, and (*E,E*)-10,12-hexadecadienal, a component of the sex pheromone of the female spiny bollworm moth, *Earias insulana*¹⁹.

CONCLUSIONS

4-(*p*-Methoxycinnamyloxy)-4'-methoxyazobenzene is a readily-available, nematic liquid crystal which can be utilised as a stationary phase in gas-liquid chromatography. It provides a rapid and powerful means of separating isomeric olefins, including fatty alcohols and acetates, on packed GLC columns without recourse to the capillary columns often otherwise required for the analysis of insect pheromones and fats and oils.

ACKNOWLEDGEMENTS

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