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Note

Liquid crystals for the gas chromatographic determination of the stereochemistry of insect sex pheromones[a]

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Many of the insect sex pheromones that have been identified are linear aliphatic alcohols, aldehydes or acetates, with a chain length of 12–18 carbon atoms and one or two olefinic linkages. If synthetic pheromones are to be used in pest management, a precise knowledge of their isomeric purity is required because their biological activity depends on the stereochemistry of the product synthesized.

Various techniques may be used for the determination of the geometric isomers of insect sex pheromones. The analytical and semi-preparative separations were performed on silver nitrate-coated silica gel on thin-layer plates or in columns [1]. However, none of these methods was convenient or accurate enough for the detection of isomeric impurity.

There have been several reports on the use of high-performance liquid chromatographic (HPLC) columns with silver nitrate-coated silica. HPLC of the olefinic pheromones on silver nitrate-coated silica gel with benzene as mobile phase has been described [2]. Another approach was the use of silver nitrate-containing isopropanol as the mobile phase and LiChrosorb RP-8 reversed stationary phase for the HPLC separation of aliphatic unsaturated pheromones [3]. Houx and Voerman [4] reported the HPLC of acetates of olefinic long-chain alcohols at moderate pressures on a silicagel-based, strongly acidic ion exchanger loaded with silver ions and using methanol as mobile phase. A practical, reproducible procedure developed to prepare analytical and preparative silver nitrate-coated silica columns for the separation of geometric isomers of pheromones with one and two double bonds was described by Heath *et al.* [5]. Heath and Sonnet [6] developed a method for the *in situ* coating of silver nitrate onto silica gel in HPLC columns, and effectively separated a series of

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geometric isomers. However, HPLC currently suffers from a lack of detection sensitivity, which precludes its use to submicrogram levels for the identification of natural pheromones.

The development of high-polarity stationary phases has made possible the analysis of isomeric pheromones on packed gas chromatographic (GC) columns [7]. However, the resolution of the isomers of conjugated dienes on packed columns is not good. Many investigators have made use of capillary columns with diethylene glycol succinate [8–12], 1,2,3-tris(β-cyanethoxy)propane [13] or cyanopropylsiloxane [14] for the determination of the geometric and positional isomers of mono- and diunsaturated alcohols and their derivatives. However, it is difficult to obtain high-efficiency capillary columns with polar stationary phases, which are necessary to obtain satisfactory resolution of the geometric isomers of many known pheromones.

Recently many workers have used liquid crystals as GC stationary phases which exhibit unique selectivity towards geometric isomers. A smectic liquid crystal, diethyl 4,4′-azoxydicinnamate, was reported [15] as a GC stationary phase for the rapid and efficient analysis of mono- and diunsaturated conjugated long-chain acetates and aldehydes, which have previously only been separated on capillary GC columns, and also some pheromone isomers which have not yet been separated even by this method. A packed GC column was used. However, this liquid crystalline phase was unsuitable for the direct determination of the corresponding alcohols. The nematic liquid crystal 4-(*p*-methoxycinnamyloxy)-4′-methoxyazobenzene has been found [16] to exhibit similar chromatographic properties to the above smectic liquid crystal, but it allowed the direct chromatography of alcohols.

Several reports have been published [12,17,18] concerning the use of cholesteric liquid crystals that showed potential as stationary phases in the separation of aliphatic insect pheromone geometric isomers by capillary GC. The unique resolving power of the liquid crystalline stationary phase was coupled with the high efficiency of the capillary column.

Interest in liquid crystals is increasing because of their unique abilities in the separation and analysis of many mixtures. In this work, an attempt was made to apply liquid crystals containing benzene rings as stationary phases for the separation of *trans* and *cis* isomers of aliphatic acetates with 10–13 carbon atoms and one and two unsaturated bonds and to establish their chromatographic properties in detail.

EXPERIMENTAL

The liquid crystalline stationary phases tested have the following formulae:

I C_4H_9COO–*p*-C_6H_4–N=N–*p*-C_6H_4–CH_2CH_2–*p*-C_6H_4–N=N–*p*-C_6H_4–$COOC_4H_9$
165°C(S)–184°C(N)–303°C(I)

II C_7H_{15}–*p*-C_6H_4–*p*-C_6H_4–COO–*p*-C_6H_4–*p*-C_6H_4–CN
122°C(S)–143°C(N)–300°C (I)

III C_2H_5O–*p*-C_6H_4–CH=CHCOO–*p*-C_6H_4–N=N–*p*-C_6H_4–C_4H_9
134°C(N)–285°C (I)

IV $C_7H_{15}O$–*p*-C_6H_4–COO–*p*-C_6H_4–OOC–*p*-C_6H_4–OC_7H_{15}
120°C(N)–195°C (I)

The liquid crystals I, II and III were obtained from the Institute of Chemistry, Military Technical Academy (Warsaw, Poland) and IV from Reakhim (U.S.S.R.). Stationary phase I melts at 165°C and is converted into the smectic phase; the transition to the nematic phase takes place at 184°C and to the isotropic liquid at 303°C. Liquid crystals III and IV have only a nematic mesophase, ranging from 134 to 285°C and from 120 to 195°C, respectively.

The liquid crystalline stationary phases I, II and III were deposited on Chromosorb W AW DMCS (80–100 mesh) (Applied Science Labs., State College, PA, U.S.A.) and IV on Chromaton N-Super (0.125–0.16 mm) (Chemapol, Prague, Czechoslovakia) from chloroform solution by evaporation of the solvent in a rotary vacuum evaporator. The packings were then dried and screened. Glass columns of 3 mm I.D. filled with packings prepared in this way were placed in the thermostat of a Tswet Model 100 gas chromatograph (U.S.S.R.) equipped with a flame ionization detector. The temperature of the column was increased at 2°C/min and the carrier gas (nitrogen) flow-rate was 20 ml/min. The columns were conditioned at 200°C for 7 h. The characteristics of the prepared columns are given in Table I.

The stationary phases were tested at temperatures ranging from 200 to 100°C during cooling of the columns. The highest temperature was limited by the thermal stability of the liquid crystals.

Efficiency, selectivity and retention data tests were carried out using a mixture of *trans,cis*- and *cis,cis*-7,9-dodecadienyl acetate. The retention times of the *n*-alkanes were used for calculating the dead time and the retention indices of the geometric isomers. The Kováts retention indices and efficiencies were calculated from generally known equations.

The selectivities (r) of the liquid crystalline stationary phases were determined from the dependence of the relative retention time and the Kováts retention indices of *trans,cis*- and *cis,cis*-7,9-dodecadienyl acetate on temperature. The selectivity is equal to the ratio of the adjusted retention time of *cis,cis*-7,9-dodecadienyl acetate to that of *trans,cis*-7,9-dodecadienyl acetate ($r = t'_{ZZ}/t'_{EZ}$).

The efficiency of the column was expressed in terms of the height equivalent to a real plate (HERP).

TABLE I

CHARACTERISTICS OF THE COLUMNS

Column	Stationary phase	Amount of phase on the support (%)	Column length (m)
1	I	10.0	3
2	II	7.5	3
3	III	7.5	2
4	IV	7.5	3

RESULTS AND DISCUSSION

Fig. 1. shows the dependence of the efficiency on temperature for columns 1–4. Higher efficiencies are obtained for columns containing stationary phases IV, II and III (0.51, 1.1 and 1.6 mm, respectively). It can be seen that the efficiencies are greatest at the crystallization points of the liquid crystals and in the range of their transitions from the mesophase to the solid state. The lowest efficiency is shown by the column 1, and is distinctly lower than those of the other columns. Its efficiency is optimum over the temperature range 190–200°C, which corresponds to the nematic phase of the liquid crystal I.

The dependence of the Kováts retention indices for *trans,cis-* and *cis,cis*-7,9-dodecadienyl acetate on temperature for stationary phases I–IV is shown in Fig. 2. These dependences for stationary phases I and IV are linear, corresponding to the equation $I = at + b$. In these instances all the phase transitions are seen distinctly and affect the values of the coefficients in the equation. The Kováts retention indices of *trans,cis* and *cis,cis* isomers increase with increasing temperature and the difference between the retention indices reached a maximum value of 33 units at 200°C for liquid crystal I. The Kováts retention indices of *trans,cis-* and *cis,cis*-7,9-dodecadienyl acetate decrease with decreasing temperature in the nematic state of liquid crystal IV and rise distinctly after its crystallization (120°C). The difference between the retention indices of isomers is 19 units on supercooling and transition of the stationary phase to the solid state. The Kováts retention indices of the isomers decrease insignificantly with a decrease in temperature from 200 to 120°C in the nematic and smectic ranges of liquid crystal II and decrease distinctly when the stationary phase adopts the solid state. The maximum difference between the Kováts retention indices of *cis,cis-* and *trans,cis*-7,9-dodecadienyl acetate (38 units) is observed with stationary phase III. In this instance the transition from the nematic phase to the solid state is not observed. However, the greatest difference between the Kováts retention indices of the *cis,cis* and *trans,cis* isomers (59 units) is achieved for this stationary phase at 200°C.

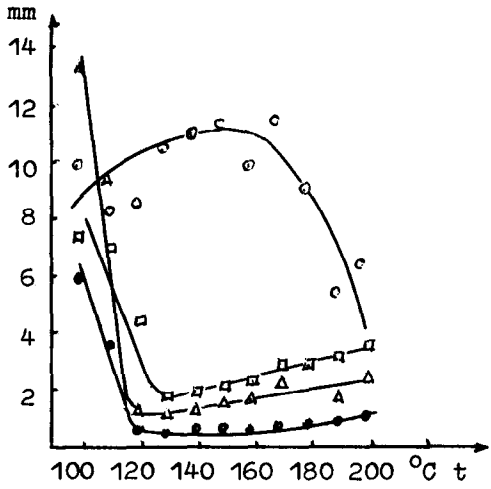

Fig. 1. Temperature dependence of the real plate height for phases (○) I, (△) II, (□) III and (●) IV.

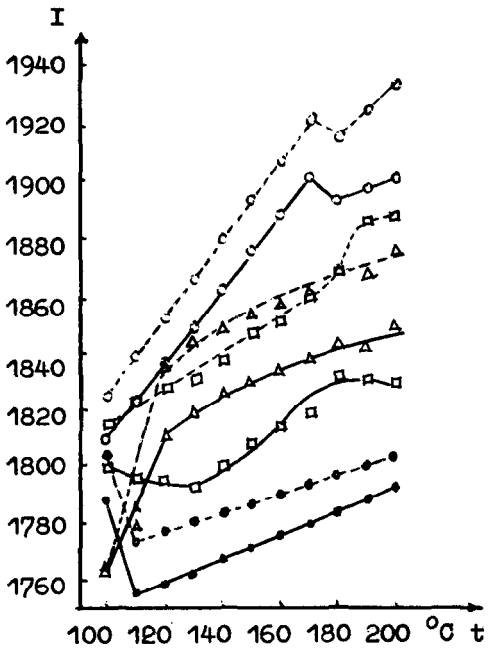

Fig. 2. Temperature dependence of the Kováts retention indices of *trans,cis*-(solid lines) and *cis,cis*-7,9-dodecadienyl acetate (dashed lines) on phases (○) I, (△) II, (□) III and (●) IV.

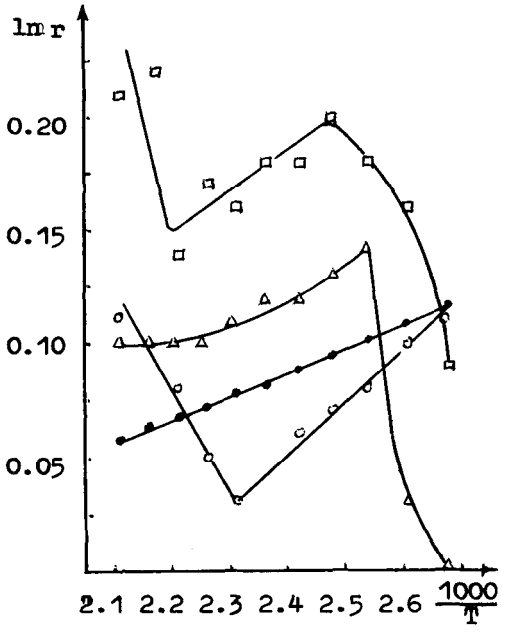

Fig. 3. Temperature dependence of the relative retention times of *cis,cis*- and *trans,cis*-7,9-dodecadienyl acetate on phases (○) I, (△) II, (□) III and (●) IV.

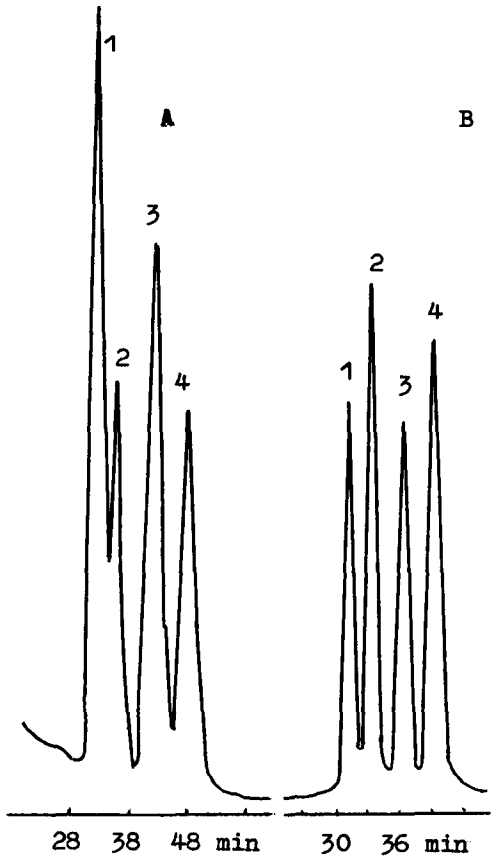

Fig. 4. Separation of the geometric isomers of the European grape vine moth pheromone on the liquid crystalline stationary phases: (1) *cis,trans*-, (2) *trans,cis*, (3) *cis,cis*- and (4) *trans,trans*-7,9-dodecadienyl acetate. (A) Column III, temperature 130°C; (B) column IV, temperature 160°C.

Fig. 3. shows variation of selectivity expressed as the logarithm of the relative retention times of *cis,cis*- and *trans,cis*-7,9-dodecadienyl acetate with temperature. The highest selectivity is obtained for the liquid crystalline stationary phase III. Phases I, II and IV have lower selectivities than III. The plots differ considerably. The transition is not observed for the liquid crystals III and IV.

The knowledge of the temperature dependence of the selectivity and efficiency gave the possibility of establishing the optimum temperature conditions where the column has the best separating properties with respect to the geometric isomers of the compounds studied. Fig. 4 shows examples of such separations.

CONCLUSIONS

The results of these investigations show that the phases tested may find practical application for the separation of the geometric isomers of aliphatic diunsaturated conjugated acetates. Liquid crystal IV exhibits the best separation properties because

a column containing this stationary phase has the highest efficiency. Attempts to separate the geometric isomers of acetates with one olefinic bond and with two olefinic bonds separated by methylene groups were made on all the columns, but they failed.

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