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NEW LIQUID CRYSTALLINE STATIONARY PHASES FOR GAS CHROMATOGRAPHY OF POSITIONAL AND GEOMETRICAL ISOMERS HAVING SIMILAR VOLATILITIES

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SUMMARY

New liquid crystalline compounds of the type 2-methyl-4′-R-4-(*p*-methoxycinnamoyloxy)azobenzene, where R = methoxy, ethoxy, ethyl or *n*-butyl, were synthesized. Their properties as stationary phases for the gas chromatographic separation of positional isomers of xylene, dichlorobenzene, methoxynaphthalene and geometrical isomers of insect sex pheromones of similar volatilities have been investigated. The effect of temperature on the separation ability of the liquid crystalline compounds was studied. Large separation factors, α, for isomers were obtained on a column in its supercooled nematic state. The separation of insect sex pheromones was found to depend on the structure of the liquid crystalline compound used as stationary phase rather than on its nematic range.

INTRODUCTION

Liquid crystalline stationary phases have been used successfully in gas chromatography (GC) for the separation of positional isomers of mono- and disubstituted naphthalenes[1,2], disubstituted benzenes[3–5], methoxyquinone derivatives and chlorobiphenyls[6] and steroids[7]. The advantages of such phases over conventional phases in the separation of alkylbenzenes[8] and disubstituted benzenes[9] have been reported. GC with liquid crystalline stationary phases has been reviewed by Kelker and Von Schivizhoffen[10], Schroeder[11], Janini[12] and Witkiewicz[13].

It has also been shown that liquid crystalline stationary phases are promising for the separation of geometrical isomers of *n*-alkenes[14,15]. However, the separation of geometrical isomers of insect sex pheromones with *n*-alkenes by GC on conventional stationary phases has not been reported yet.

The purpose of this work is to exploit new liquid crystals as stationary phases for the separation of positional isomers of aromatic compounds and geometrical isomers of insect sex pheromones, which have similar volatilities.

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EXPERIMENTAL

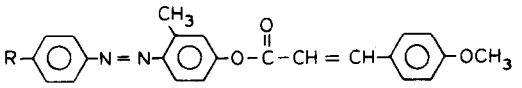
Four new liquid crystalline compounds were synthesized[16]. *para*-Substituted phenyldiazonium chloride was first condensed with *m*-cresol to give 4-(*para*-substituted phenylazo)-*m*-cresol, and then with *p*-methoxycinnamoyl chloride. The structures and properties of the resulting liquid crystals were determined by spectroscopy (IR, NMR), thermal analysis [differential thermal analysis, differential scanning calorimetry (DSC)] and polarizing microscopy (see Table I). Sharp transition points from the crystal to the nematic (C-N) and from the nematic to the isotropic (N-I) phases for each compound were observed by thermal analysis. A DSC scan of 2-methyl-4′-*n*-butyl-4-(*p*-methoxycinnamoyloxy)azobenzene (compound III) is shown in Fig. 1.

Liquid crystalline compounds were coated on Celite 545(A) (80–100 mesh) using chloroform as a solvent, followed by gradual elimination of solvent on a water-bath at 40–60°C. The coated solid support was dried in an oven at 80°C for 2 h and then packed into a 2.6 m × 2.6 mm I.D. glass column.

TABLE I

STRUCTURES, TRANSITION TEMPERATURES, COLUMNS AND PERCENTAGES OF LIQUID CRYSTALLINE COMPOUNDS

C-N = Crystal to nematic; N-I = nematic to isotropic liquid.



Compound No.	*R*	*Transition temperatures (°C)*		*Nematic range (°C)*	*Column No.*	*Amount of stationary phase (%)*
		C-N	*N-I*			
I	$-OCH_3$	149	298	149	1	5
II	$-OC_2H_5$	154	>300	>155	2	5
III	$-n-C_4H_9$	109	253	143	3	5
IV	$-C_2H_5$	126	262	136	4	10

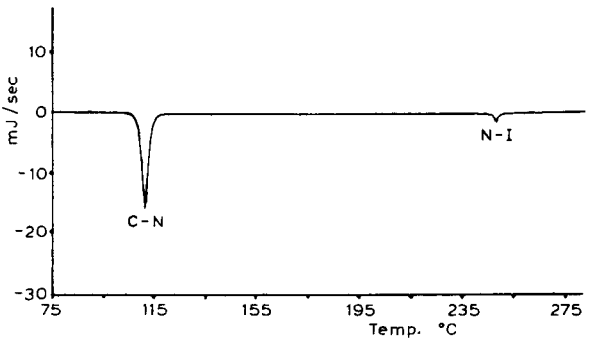


Fig. 1. DSC scan of 2-methyl-4′-*n*-butyl-4-(*p*-methoxycinnamoyloxy)azobenzene. Scanning rate: 10°C/min.

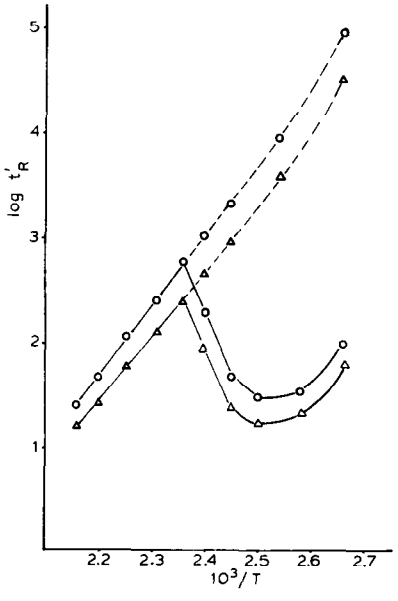
Insect sex pheromones were obtained from Shin-Etsu Chemicals (Tokyo, Japan). Celite 545(A) was obtained from Shinwa-Kako Chemicals (Kyoto, Japan). All other chemicals were purchased from Nakarai Chemicals (Kyoto, Japan).

A Shimadzu dual-column gas chromatograph GC-5A equipped with thermal conductivity detectors was employed with helium as a carrier gas. All the columns were conditioned at 100°C above the C-N transition temperatures of the liquid crystalline stationary phases. The injector and the detector temperatures were set at 200 and 250°C respectively.

RESULTS AND DISCUSSION

The separation of positional isomers by GC on liquid crystalline stationary phases depends mainly upon the nematic range and the structure of the liquid crystalline compound. A high selectivity for isomers of linear solute molecules can be obtained by using liquid crystalline compounds with wide nematic ranges which have highly ordered structures. The present compounds obtained from *p*-methoxycinnamic acid have wider (*ca.* 55°C) nematic ranges than the previously reported benzoic acid analogues[17]. These compounds can therefore be employed over a wider temperature range than previously reported compounds[1,3,8].

After packing, the columns were conditioned at 195°C for 2 h then cooled to 10°C and left overnight. The retention behaviour of *m*- and *p*-xylenes, dichlorobenzenes and α- and β-methoxynaphthalenes were investigated. It was observed that the retention times on all four columns decreased with increasing column temperature



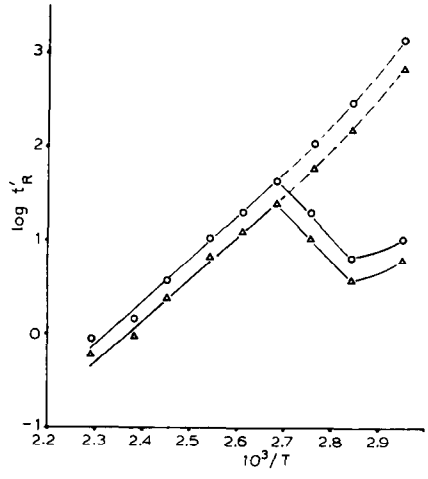


Fig. 2. Relationship between the logarithm of retention time and reciprocal column temperature for methoxynaphthalenes on column 1. △—△, α-Methoxynaphthalene; ○—○, β-methoxynaphthalene.

Fig. 3. Relationship between the logarithm of retention time and reciprocal column temperature for dichlorobenzenes on column 2. △—△, *m*-Dichlorobenzene; ○—○, *p*-dichlorobenzene.

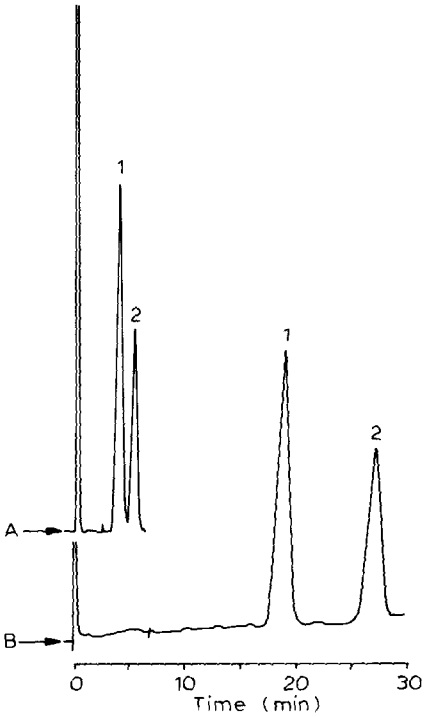


Fig. 4. Comparison of chromatograms obtained under different conditions on column 1. Temperature: 135°C. Helium flow-rate; 60 ml/min. A, Liquid crystalline stationary phase in the solid state (heating from 10°C). B, Liquid crystalline stationary phase in the supercooled nematic state (cooling from 200°C). Peaks: 1 = α-methoxynaphthalene; 2 = β-methoxynaphthalene.

up to 10–20°C below the C-N transition temperature, then increased abruptly to a maximum value at the C-N transition temperature before decreasing again with further increase in column temperature. The maximum retention and separation factor, α, for positional isomers was observed at 2–4°C above the C-N transition temperature when the column was heated from room temperature. After heating to temperatures higher than the C-N transition point, the column was gradually cooled, whereupon the retention increased until the column temperature was below the C-N transition temperature.

The retention behaviour of α- and β-methoxynaphthalenes on column 1 and of *m*- and *p*-dichlorobenzenes on column 3 is shown in Figs. 2 and 3 respectively. The solid lines indicate the logarithm of retention time obtained upon increasing the temperature from room temperature, while broken lines indicate the values obtained when the columns were cooled from temperatures above the transition points. Maxima in the solid curves were observed near the C-N transition temperature. However, after conditioning, the liquid crystal materials in their supercooled nematic state could retain an ordered structure below the C-N transition temperature and, therefore, the retention increased upon cooling as shown by the broken lines. Fig. 4 shows two chromatograms obtained at the same column temperature before and after heating above the transition temperature.

In previous work[3] it was reported that the liquid crystalline stationary phase changed completely from the supercooled nematic state to the solid crystal state upon standing at room temperature overnight. Therefore, on that column, below the C-N transition temperature, no isomers were separated without heating to temperatures higher than the transition point. Even on the present columns the separation of positional isomers was not obtained when columns were operated just after packing, at temperatures below the C-N transition point, and the retention was lower than those shown in Figs. 2–4. However, once the column had been conditioned, the retension increased below the transition temperature and the isomers could be resolved as shown in these Figures. This phenomenon can be explained as follows. The molecules of the liquid crystal compound on the solid support are rearranged upon heating to form an ordered structure which is retained after cooling to room temperature, due to the terminal groups in the molecules. It is the ordered structure which retains and separates the isomers on the column. Chromatograms of *m*- and *p*-isomers of xylene and dichlorobenzene are shown in Fig. 5, the *m*-isomers being eluted faster than the corresponding *p*-isomers.

Insect sex pheromones have recently attracted much attention as non-polluting agricultural chemicals. The presence of even very small amounts of the different

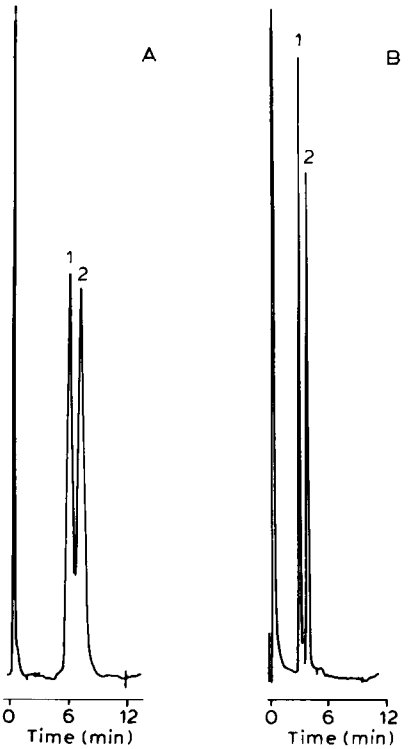


Fig. 5. Chromatograms of positional isomers on column 3. A, Temperature: 60°C. Helium flow-rate: 35 ml/min. Peaks: 1 = *m*-xylene; 2 = *p*-xylene. B, Temperature: 110°C. Helium flow-rate: 50 ml/min. Peaks: 1 = *m*-dichlorobenzene; 2 = *p*-dichlorobenzene.

geometrical isomers of the biologically active component often has a large effect on the activity of a particular insect sex pheromone, therefore, the separation and identification of all geometrical isomers is very important. Their separation on conventional packed columns is difficult and the isomers of insect sex pheromones have been separated successfully on columns using liquid crystalline stationary phases only in a few cases[18,19].

Four pairs of tetradecenyl acetate (TDA) isomers were chromatographed on two kinds of liquid crystalline columns. The retention times observed for the *Z* and *E* isomers of TDAs having double bonds at positions 7, 9, 11 and 12 respectively are shown in Table II together with the separation factor, α, calculated as the ratio of the adjusted retention times of the *E* and *Z* isomers for each compound. In all cases, the *Z* isomers were eluted faster than the corresponding *E* isomers. This elution order can be explained on the basis of the separation mechanism known for liquid crystals, where linear molecules fit tightly within the ordered arrangement of the liquid crystal molecules in the nematic state, and therefore are retained longer, while bulky molecules deform the structure of liquid crystalline compounds by intercalation, resulting in their more rapid elution. The retention times for a particular pair of *Z* and *E* isomers depend upon the position of the double bond in the solute molecule. The retention increased for all isomers when the position of the double bond was shifted towards the end of the alkyl chain, *i.e.*, from position 7 to 12, with the exception of *Z*-12-TDA which was eluted faster than *Z*-11-TDA. An excellent resolution of *Z* and *E* isomers was obtained on these columns, the separation factor for *E* to *Z* isomers ranging from 1.07 to 1.25; these values are better than those obtained on glass capillary columns containing conventional stationary phases[20].

TABLE II

RETENTION TIMES OF *Z* AND *E* ISOMERS AND RELATIVE RETENTIONS, α, OF THE *E* ISOMERS (*Z* ISOMER = 1)

$t'_R = t_{R\ (sample)} - t_{M\ (hexane)}$; $\alpha = t'_R$ (*E* isomer)/t'_R (*Z* isomer).

Compound	*Column II*		*Column IV*	
	t'_R *(min)*	α	t'_R *(min)*	α
Z-7-Tetradecenyl acetate	10.00		15.60	
E-7-Tetradecenyl acetate	10.80	1.08	16.80	1.07
Z-9-Tetradecenyl acetate	10.00		16.00	
E-9-Tetradecenyl acetate	11.20	1.12	17.40	1.07
Z-11-Tetradecenyl acetate	12.00		18.40	
E-11-Tetradecenyl acetate	13.60	1.13	20.00	1.08
Z-12-Tetradecenyl acetate	11.20		17.60	
E-12-Tetradecenyl acetate	14.00	1.25	21.20	1.20

The retention behaviour of 9,11- and 9,12-tetradecadienyl acetate (9,11- and 9,12-TDDA) was also studied on all four columns. The separation of (*Z*,*E*)-9,11-TDDA, the active component of the sex pheromone from the female Egyptian cotton-leaf worm, *S. Littoralis*[21], from the other three geometrical isomers was obtained

on column 2, and the chromatogram is shown in Fig. 6. Isomers of 9,12-TDDA were separated from each other on column 4. Kelker and von Schivizhoffen[10] and Schroeder[11] stated that a column coated with a liquid crystalline compound having wider nematic range will give better resolution for positional isomers of similar volatilities. This may explain the results obtained on column 2, where the stationary phase has the widest nematic range of the four studied here. However, the separation of 9,12-TDDA isomers achieved only on column 4, which has the narrowest nematic range, cannot be so explained. Both phases in columns 1 and 3 have wider nematic ranges than that of the phase in column 4 (Table I), but these TDDA isomers could not be separated on columns 1 and 3.

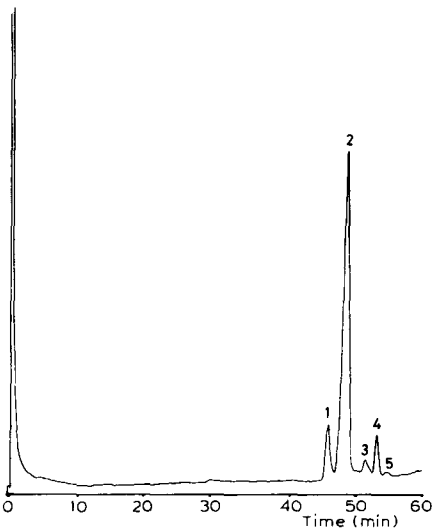


Fig. 6. Chromatogram of 9,11-tetradecadienyl acetate isomers on column 2. Temperature: 104°C for 20 min then program started at the rate of 2°C/min. Helium flow-rate: 35 ml/min. Peaks: 1 = unknown; 2 = *Z,E*-9,11-TDDA; 3 = *E,Z*-9,11-TDDA; 4 = *Z,Z*-9,11-TDDA; 5 = *E,E*-9,11-TDDA.

The present results indicate that the separation of insect sex pheromones on liquid crystalline stationary phases depends not only on the nematic range but also on the structure of the liquid crystalline compound. It is difficult to establish a correlation between the structure of the liquid crystalline compound and its effect on the separation of positional and geometrical isomers due to the limited data currently available.

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