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SEPARATION OF *cis/trans* ISOMERS OF 1,4-DISUBSTITUTED CYCLOHEXANES AND OF *Z/E*-MONOUNSATURATED FATTY ACID METHYL ESTERS BY GAS-LIQUID CHROMATOGRAPHY ON NOVEL LIQUID CRYSTAL PHASES

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SUMMARY

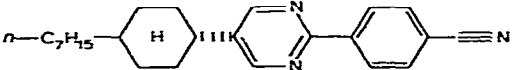
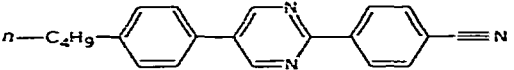
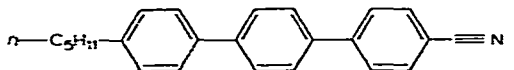
Three liquid crystals, *trans-p*-[5-(4-*n*-heptylcyclohexyl)-2-pyrimidinyl]benzotrile (RO-CP-7870), *p*-[5-(*n*-butylphenyl)-2-pyrimidinyl]benzotrile (RO-CP-7334) and 4'-*n*-pentyl-4-cyanoterphenyl (RO-CB-5515), have been applied as stationary phases for gas-liquid chromatography (GLC). These compounds have been utilized to solve several difficult separation problems, for example separation of the *cis/trans* isomers of 1,4-disubstituted cyclohexanes, the *Z/E*-isomers of monounsaturated fatty acid methyl esters and the xylene isomers. The described nematic phases can be applied as GLC phases in the range between 60°C and *ca.* 180°C.

INTRODUCTION

In 1963, Kelker^{1,2} reported the application of nematic liquids as stationary phases for gas-liquid chromatography (GLC). Such phases have since been increasingly utilized for GLC determinations. For example, liquid crystal phases have been applied to separations of isomers of disubstituted benzenes or naphthalenes³⁻⁶, to the determination of polycyclic hydrocarbons⁶⁻⁹, to separations of epimeric steroids¹⁰, aza-heterocyclic compounds¹¹ and steroids and bile acids¹². Lester and Hall¹³ utilized liquid crystals to investigate *Z/E* mixtures of fatty alcohols and their acetyl derivatives.

The Schiff base N,N'-bis(*p*-methoxybenzylidene)- α,α' -bi-*p*-toluidine (BMBT) (Eastman-Kodak, Rochester, NY, U.S.A.) and its homologues⁵⁻⁸, and a variety of azo and azoxy compounds^{1-4,14}, have most frequently been employed as stationary phases. In addition, many different types of nematic and smectic phases can be applied, *e.g.*, esters of benzoic acid or of cinnamic acid. BMBT and its homologues BBBT (butoxy), BHxBT (hexyloxy) and BPhBT (phenyl) are now commercially available (*e.g.*, from Analabs, Applied Science Labs, Chrompack, Supelco and other suppliers). Most users of azo and azoxy derivatives synthesize these compounds in their own laboratories. The high melting points ($\geq 180^\circ\text{C}$) and the corresponding temperature limits represent the main disadvantage of these nematic phases.

TABLE I
STRUCTURES, MELTING POINTS AND CLEARING POINTS OF THE LIQUID CRYSTALS

Structure		Melting point (°C)	Clearing point (°C)	Nematic range (°C)
I RO-CP-7870		80	226	80-226
II RO-CP-7334		95	244	95-224
III RO-CB-5515		129	238	129-238

In this report we describe GLC investigations of newly developed nematic compounds, the structures and some of the physical properties of which are given in Table I. Their systematic names are *trans-p*-[5-(4-*n*-heptylcyclohexyl)-2-pyrimidinyl]benzonitrile (RO-CP-7870, I), *p*-[5-(*n*-butylphenyl)-2-pyrimidinyl]benzonitrile (RO-CP-7334, II) and 4'-*n*-pentyl-4-cyanoterphenyl (RO-CB-5515, III). These compounds are currently utilized for digital displays. All three are commercially available or can be obtained on special request from Hoffmann-La Roche (Basle, Switzerland).

These liquid crystals can be applied as GLC phases between temperatures of 80°C and *ca.* 180°C. The upper temperature limit is determined by the boiling points of the compounds. In addition, RO-CP-7870 can be utilized in a supercooled condition with a lower limit of 60°C (separation properties and retention times for xylene isomers remained constant during 20 h of operation).

TABLE II
PARAMETERS OF THE COLUMNS

BMBT = N,N'-bis(*p*-methoxybenzylidene)-*z,z'*-bi-*p*-toluidine (Eastman-Kodak);
BBBT = N,N'-bis(*p*-butoxybenzylidene)-*z,z'*-bi-*p*-toluidine (Eastman-Kodak).

Column no.	Stationary phase	Percentage of stationary phase	Support material	Column length (m.)
1	I	2	Gas-Chrom Q, 120-140 mesh	3.6
2	I	2	Gas-Chrom Q, 80-100 mesh	1.8
3	II	5	Gas-Chrom Q, 80-100 mesh	1.8
4	II	2	Gas-Chrom Q, 80-100 mesh	1.8
5	III	2	Gas-Chrom Q, 80-100 mesh	1.8
6	BMBT	2	Gas-Chrom Q, 80-100 mesh	1.8
7	BBBT	2	Gas-Chrom Q, 80-100 mesh	1.8

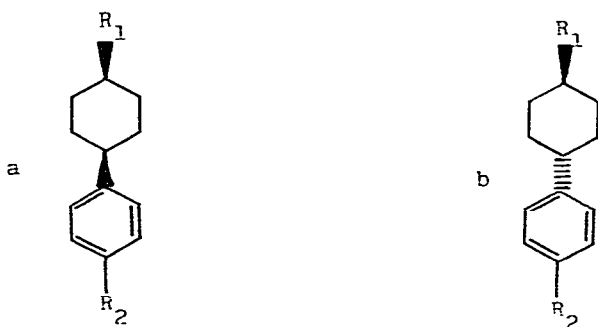
EXPERIMENTAL

Liquid crystals were coated onto the carrier Gas-Chrom Q by conventional techniques (evaporation of a methylene chloride solution under reduced pressure on a rotavapor). Analyses were carried out with glass columns (2.2 mm I.D.) and Model 3920 or Sigma 3 B chromatographs (Perkin-Elmer). Table II summarizes the types of columns utilized.

RESULTS

Separation of cis/trans-1-phenyl-4-alkylcyclohexanes

The following *cis/trans* isomers of 1-phenyl-4-alkylcyclohexanes have been separated on stationary phases prepared with liquid crystals I, II or III (Table I):



- 1a, 1b R₁ = C₃H₇, R₂ = CH₃
 2a, 2b R₁ = C₄H₉, R₂ = H
 3a, 3b R₁ = C₃H₇, R₂ = C₂H₅
 4a, 4b R₁ = C₃H₇, R₂ = C₃H₇
 5a, 5b R₁ = C₄H₉, R₂ = C₂H₅

We were not able to separate these pairs of isomers on conventional stationary phases such as Apiezon L, SE-30, Carbowax 20M, Carbowax 20M + Bentone 34, XE-60 + Bentone 34, QF-1, Silar 10C, Poly-S-179, DC-560, OV-17, OV-225, OV-275, Reoplex 400, Polyphenylether 6 R or Dexsil 300. Utilization of packed columns with RO-CP-7870, RO-CP-7334 or RO-CB-5515 coated onto Gas-Chrom Q (80–100 mesh) yielded separations of *cis*- and *trans*-isomers with a relative retention, α , of 1.6–2.0. The *cis* isomer was always eluted prior to the corresponding *trans* compound.

Fig. 1 shows a separation of 4a and 4b, carried out on column 2 (Table II) at an analysis temperature of 165°C and a nitrogen flow-rate of 30 ml/min. Relative retentions for the *cis/trans* pairs 1–5 obtained on column 3 are given in Table III.

A mixture of the *cis/trans* isomers of 1-(4'-propylphenyl)-4-propylcyclohexane has been analysed on columns 2, 4 and 5. Approximately identical retention times for the *trans* isomer 4b were achieved by adjustment of the column temperatures, while the carrier gas flow-rate was kept constant. The nematic phases I, II or III were coated at 2% onto Gas-Chrom Q (80–100 mesh), and the column length was 1.8 m. Table IV shows the relative retentions obtained for 4b (α for *cis* isomer was set at

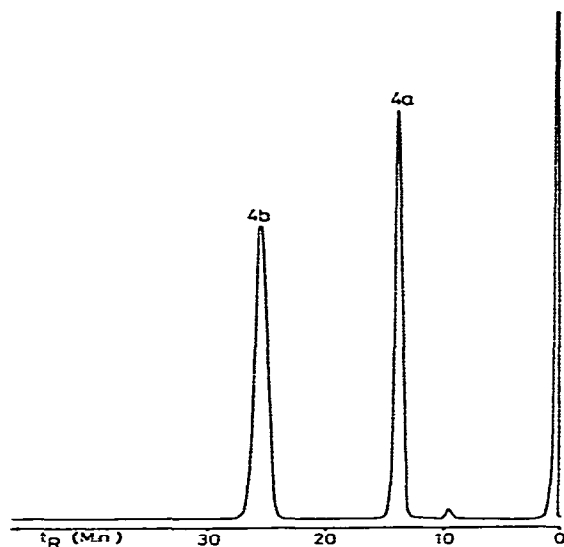


Fig. 1. Separation of *cis*- (4a) and *trans*- (4b) -1-(4'-propylphenyl)-4-propylcyclohexane on column 2 at 165°C. t_R = Retention time.

1.00). They are compared to the values obtained on similarly prepared columns of BMBT and BBBT. The BMBT column could be supercooled to 130°C (m.p. 181°C), but a similar treatment of BBBT was not possible.

Relative retentions for 4a and 4b are in the same range for four of the phases tested. BMBT behaves differently and yields a considerably higher α . However, it should be noted that BMBT was utilized in a supercooled condition, and that the GLC peaks exhibited some tailing. Determinations on BMBT columns are not possible without preliminary heating to 200°C, *i.e.*, above the melting point. In addition, prolonged application of this supercooled system may not be feasible. The retention time for the *trans* isomer on the BBBT column is very short, and consequently the corresponding α value is only an approximation. It is known that routine analyses of

TABLE III

RETENTION DATA (α) FOR *trans*-1-PHENYL-4-ALKYLCYCLOHEXANES

For *cis*-isomers $\alpha = 1.00$. Column 3 (5%, RO-CP-7334 on Gas-Chrom Q, 80-100 mesh).

Substance	α (<i>trans cis</i>)	Retention time of <i>trans</i> (min)	Analysis temperature (°C)
1b	1.9	16.5	170
2b	1.6	10.0	170
3b	1.9	18.0	170
4b	2.0	34.4	170
5b	2.0	28.6	170

TABLE IV

RELATIVE RETENTION DATA (α) FOR *trans*-1-(4'-PROPYLPHENYL)-4-PROPYLCYCLOHEXANEFor *cis*-isomer $\alpha = 1.00$.

Nematic phase	Column	Analysis temperature ($^{\circ}\text{C}$)	Retention time of <i>trans</i> (min)	α (<i>trans/cis</i>)
RO-CP-7334	4	155	22.4	2.15
RO-CB-5515	5	155	21.2	2.30
RO-CP-7870	2	165	25.4	1.87
BMBT*	6	130	22.5	3.52
BBBT	7	200	2.7	2.25

* Supercooled.

compounds with retention times of 1 or 2 min are not reproducible due to overlap with the solvent front. In conclusion, the BMBT and BBBT phases are not suitable for this particular analytical problem.

Separation of methyl esters of (Z|E)-monounsaturated fatty acids

GLC separations of the methyl esters of oleic acid (*Z*) and elaidic acid (*E*) or of palmitoleic acid (*Z*) and palmitelaidic acid (*E*) are difficult. Of a number of conventional stationary phases on packed columns, only nitrile silicones have yielded satisfactory results. Ottenstein *et al.*¹⁵ applied the cyanopropyl silicone OV-275 to separate the methyl esters of oleic acid and elaidic acid and obtained a relative retention

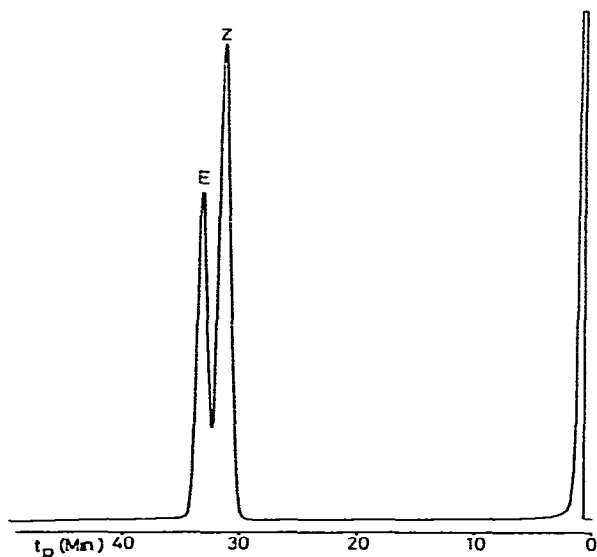


Fig. 2. Separation of methyl esters of palmitoleic acid (*Z*) and of palmitelaidic acid (*E*) on column 1 at 160°C ; flow-rate 30 ml/min.

of 1.07. The (*E*)-ester was eluted in advance of the (*Z*)-ester. Investigations in our laboratory revealed that packed columns with OV-275 or similar phases have relatively low numbers of theoretical plates. Conventional gas chromatographs can accommodate glass columns with lengths of up to 3 or 4 m; accordingly we did not achieve satisfactory separations of these methyl esters on cyanopropyl silicone columns. However, utilization of column 1 (2% RO-CP-7870 on Gas-Chrom Q 120–140 mesh, length 3.6 m, 160°C, nitrogen flow-rate 30 ml/min) resulted in a separation of the methyl esters of palmitoleic acid and palmitelaidic acid (Fig. 2). About 35 min were required to complete the analysis, which yielded a relative retention of 1.06. Separation of the methyl esters of oleic acid and elaidic acid was accomplished under the same conditions, except at an increased carrier flow (90 ml/min). The analysis time was less than 1 h (Fig. 3), and a relative retention of 1.08 was obtained. In both cases the (*E*)-ester was eluted after the (*Z*)-ester.

Separation of xylene isomers

Separation of disubstituted benzenes and naphthalenes has been the most frequent application of liquid crystal phases in GLC³⁻⁶. Generally, the separation of *meta* and *para* isomers is quite difficult, and the pair *m*-/*p*-xylene represents a particularly demanding task. Accordingly, separation of this pair is often used as an indicator of the suitability of a given nematic phase for analyses of such isomers^{2,14,16-20}.

Fig. 4 shows the results obtained with column 1 (RO-CP-7870) and injection of about 1 µg of each of the xylene isomers and 0.5 µg of ethylbenzene. The column was operated at 60°C with a nitrogen flow-rate of 7 ml/min. The relative retentions were 1.14 for *p*-xylene (α for *m*-xylene set at 1.00) and 1.12 for *o*-xylene (α for *p*-xylene set at 1.00). Analysis was completed after 15 min. Application of column 3 (RO-CP-

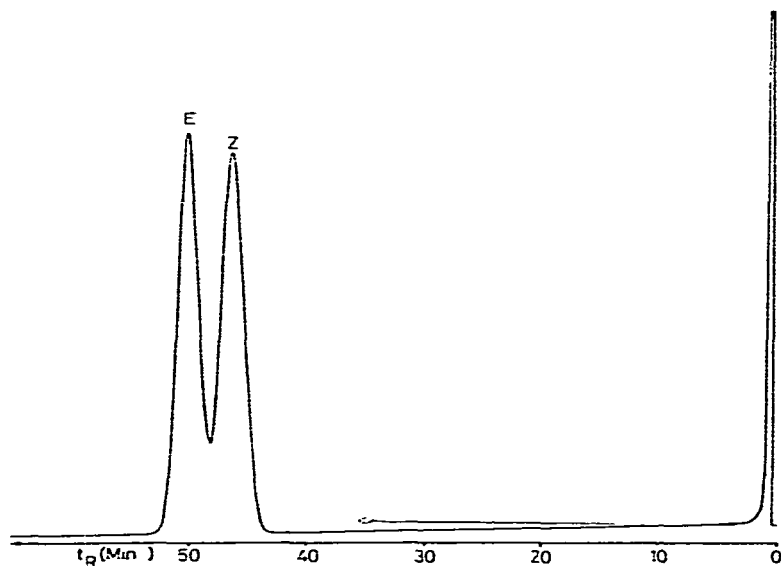


Fig. 3. Separation of methyl esters of oleic acid (*Z*) and of elaidic acid (*E*) on column 1 at 160°C; flow-rate 90 ml/min.

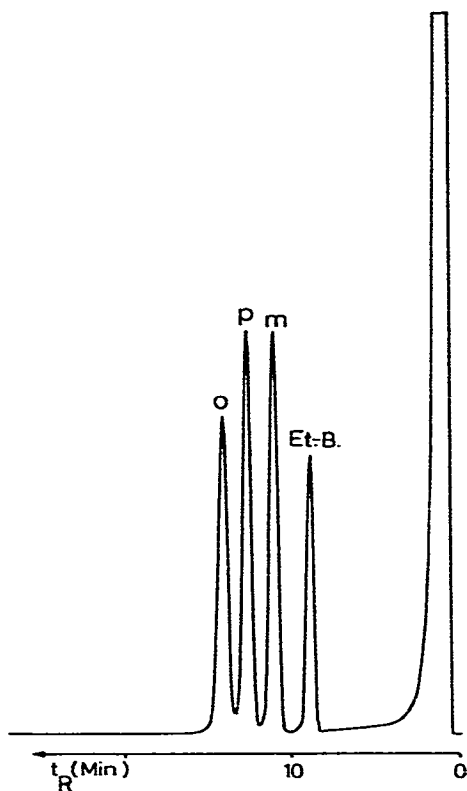


Fig. 4. Separation of *o*-, *m*- and *p*-xylene and ethylbenzene (Et.-B.) on column 1 at 60°C; flow-rate 7 ml/min.

7334, 60°C, nitrogen flow-rate 10 ml/min) yielded α values of 1.13 for *p*-xylene (α for *m*-xylene set at 1.00) and 1.18 for *o*-xylene (α for *p*-xylene set at 1.00). Since RO-CB-5515 cannot be supercooled, it is not suitable for determination of these isomers. xylene set at 1.00) and 1.18 for *o*-xylene (α for *p*-xylene set at 1.00). Since RO-CB-5515 cannot be supercooled, it is not suitable for determination of these isomers. Until now the highest α value reported for the separation of *m*-/*p*-xylenes was 1.17¹⁹.

The application of liquid crystals as stationary phases can be recommended only for separations that cannot be accomplished with conventional phases. The applicability of a given liquid crystal is restricted to a rather narrow temperature range, the lower temperature limit often being dictated by the melting point of the compound. In addition, liquid crystals have a relatively high vapour pressure—in comparison to silicone phases—which may limit their usefulness at higher temperatures.

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