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SEPARATION OF THE ISOMERS OF SOME DISUBSTITUTED BENZENES ON LIQUID-CRYSTALLINE STATIONARY PHASES IN SMALL-BORE PACKED MICRO-COLUMNS

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SUMMARY

Liquid-crystalline azoxy-compounds of the type 4-[(4-alkoxyphenyl)azoxy]-benzonitrile, where the alkyl group is pentyl, octyl or dodecyl, were used as stationary phases in separating isomeric disubstituted benzenes. The liquid crystals were deposited on Polsorb B NAW and Polsorb B AW and placed in columns (0.8 mm I.D.). Lower HETP values were obtained with Polsorb B NAW as support. Separations of dichlorobenzenes, bromotoluenes and chlorotoluenes were achieved on a 5-m column containing 5% of the nematic pentyl derivative of the azoxy-compound concerned; xylenes were separated on an 18-m column containing the same liquid crystal. The separations of chlorotoluenes and xylenes were obtained with the liquid crystal in the super-cooled state. Diethylbenzenes were separated on an 18-m column containing the smectic octyl derivative of the azoxy-compound concerned.

INTRODUCTION

Liquid-crystalline stationary phases are particularly useful in separating close-boiling isomers, such isomers being very difficult or even impossible to separate on conventional stationary phases. The reason that these compounds are well separated on liquid crystals is that the latter exhibit selective interaction with isomers of different molecular shapes. Liquid crystals were employed for the first time in gas chromatography by Kelker[1,2]. Recently, liquid crystals have been successfully employed in the separation of naphthalene homologues[3], polycyclic aromatic hydrocarbons[4-6], steroids[7] and isomers of benoxaprofen[8], and particularly difficult separations of

isomers have been achieved by using capillary columns[9,10]. In this work, we have combined the separating properties of liquid crystals with the advantages of small-bore packed micro-columns.

EXPERIMENTAL

We used four liquid-crystalline phases having the following general formula:
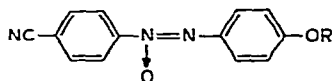
where R is pentyl, octyl or dodecyl; the preparation and purification of these compounds has been described[11]. Each of the crystals used was a pure isomer (one of the two possible forms differing by the position of the oxygen atom in the azoxy-group). With the pentyl derivative, a mixture of the two isomers was also used. The characteristics of these phases are shown in Table I.

TABLE I

PARAMETERS OF STATIONARY PHASES USED

Phase No.	Alkyl radical (R in the formula above)	Temperature of transition to:		
		smectic state, °C	liquid-nematic state, °C	isotropic state, °C
I	C_5H_{11} (pure isomer)	—	124	153
Ia	C_5H_{11} (mixed isomers)	—	94	141.5
II	C_8H_{17} (pure isomer)	101.5	137	151.5
III	$C_{12}H_{25}$ (pure isomer)	106	—	147

The columns used were of our own design, 5 or 18 m in length and of I.D. 0.8 mm. Polsorb B AW and Polsorb B NAW (particle size 0.15–0.20 mm) were used as supports, and were coated with the liquid-crystalline stationary phase and packed into columns as described previously[12]. We used a GCHF 18.3 gas chromatograph, equipped with a small-volume flame ionization detector and a splitter at the column inlet (our own design).

We separated the *ortho-, meta-* and *para*-isomers of the following disubstituted benzenes: xylene, diethylbenzene, chlorotoluene, bromotoluene, dichlorobenzene, chloroaniline, cresol and toluidine. The isomers, in approximately equal proportions, were dissolved in *n*-hexane (1 part of total isomers to 33 parts of *n*-hexane), and 2 μl of this solution were injected into each column through the 1:100 splitter so as to obtain 0.02 μl per column.

RESULTS AND DISCUSSION

The calculated dependence of the relative retention time, $\alpha = t_r'\,p\text{-xylene}/t_r'\,m\text{-xylene}$, on column temperature shows that α is highest at the temperatures of highest retention; the efficiency of each column was therefore determined at this

temperature. For each 5-m column, the dependence of HETP on the flow-rate of carrier gas (nitrogen) was determined. The curves in Fig. 1 indicate this dependence for stationary phase I on Polsorb B (AW and NAW), and show that Polsorb B NAW is characterized by a lower .HETP than Polsorb B AW. The optimum amount of liquid crystal on Polsorb B NAW is 5%. On Polsorb.B AW, lower HETP values are obtained by using smaller amounts of liquid crystal. As Polsorb B NAW was the better of the two supports, we decided to use it in our further studies.
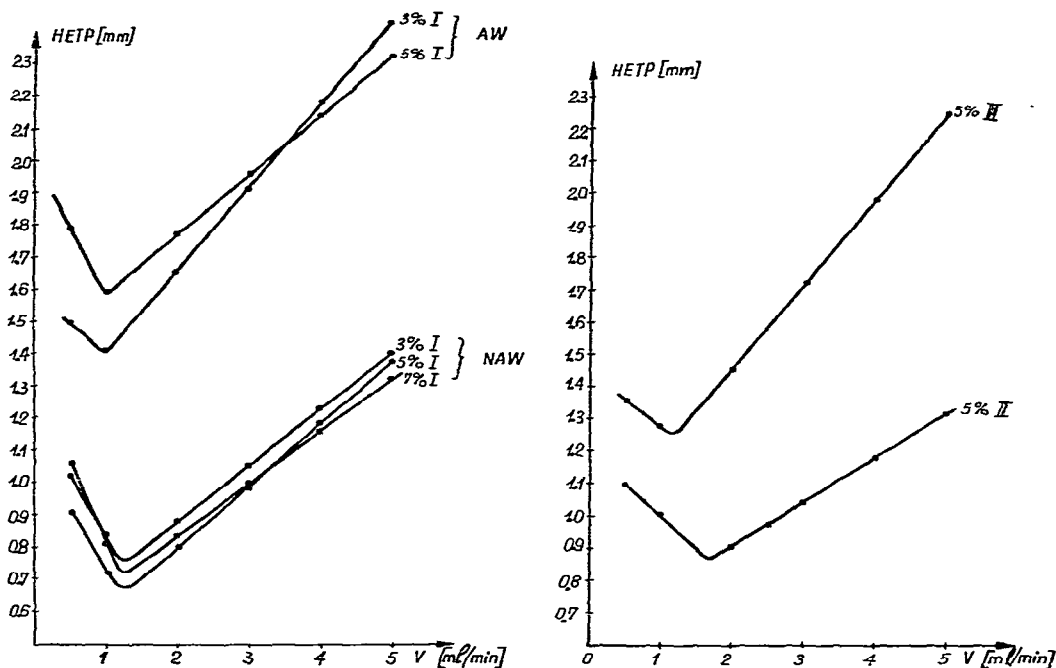

Fig. 1. Curves of HETP $= f(V)$ for different amounts of liquid crystal I deposited on Polsorb B (NAW and AW). Column temperature, 126°; test substance, m-bromotoluene.

Fig. 2. Curves of HETP $= f(V)$ for 5% of liquid crystals II and III on Polsorb B NAW. Column temperatures, 100° for compound II and 105° for compound III; test substance, m-bromotoluene.

Fig. 2 shows the dependence of HETP on $f(V)$ for phases II and III deposited at the level of 5% on Polsorb B NAW. The increase in HETP in the series of phases I, II and III is in agreement with their increasing viscosities. For all phases and both supports, the lowest HETP values were obtained at flow-rates in the range 1 to 2 ml/min. The curves in Figs. 1 and 2 were obtained at temperatures corresponding to the highest retention values at the transition from solid to nematic liquid (phase I) or solid to smectic liquid (phases II and III).

Phase III was of little value in the separation of the isomer mixtures investigated. Separations achieved with this phase were worse than those with the other phases; this is in agreement with observations (e.g., those of Dewar and Schroeder[13]) that smectic liquid crystals are of limited value as stationary phases. The best separations were achieved on phase I, especially phase Ia (a mixture of two isomers). The better results achieved with this mixture were probably due to the higher differ-

entiation of the liquid-crystalline structure, resulting in enhanced differentiation of interactions with the molecules being separated.

With phase I, good separations were achieved even in 5-m columns, the best results being obtained with 5% of the phase on the support. At 126°, complete separation of dichlorobenzene (Fig. 3) or bromotoluene (Fig. 4) isomers was achieved; complete separation of chlorotoluene isomers (Fig. 5) was obtained after the column had been supercooled to 87°. A partial separation of *m*- and *p*-xylenes was also achieved at this temperature.
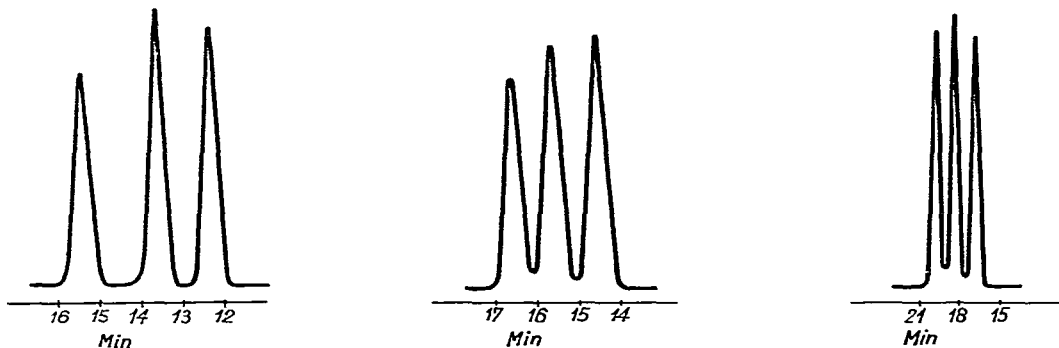

Fig. 3. Separation of dichlorobenzenes, on a 5-m column containing 5% of liquid crystal I on Polsorb B NAW and operated at 126°, with nitrogen (1.3 ml/min) as carrier gas. Isomer order: *meta, para, ortho*.

Fig. 4. Separation of bromotoluenes. Column and conditions as in Fig. 3. Isomer order: *ortho, meta, para*.

Fig. 5. Separation of chlorotoluenes. Column as in Fig. 3, but at 87° and with nitrogen flow-rate of 1.4 ml/min. Isomer order: *ortho, meta, para*.

Somewhat poorer results were obtained with dichlorobenzenes and bromotoluenes at 101° on a 5-m column containing 5% of phase II. On this phase, *m*- and *p*-xylenes could not be separated, and *p*- and *o*-diethylbenzenes were only partially separated. An interesting fact not reported in the literature available to us is that retention of *ortho*-isomers is relatively longer on this phase. The relative retention times for dichlorobenzenes (DCB) are as follows: $\alpha = t'_r$ *o*-DCB/t'_r *m*-DCB = 1.25 for phase I and 1.26 for phase II; $\alpha = t'_r$ *o*-DCB/t'_r *p*-DCB = 1.13 for phase I and 1.17 for phase II. The relatively longer retention time for the *ortho*-isomer is connected with the lower shorter relative retention time $\alpha = t'_r$ *p*-DCB/t'_r *m*-DCB, which is 1.10 for phase I and 1.07 for phase II. These relationships are also true for the remaining isomers.

If one considers the calculations presented above, as well as the fact that phase II does not yield a supercooled mesophase, it becomes clear why *m*- and *p*-xylenes are not separable on this phase, whereas *o*- and *p*-diethylbenzenes (DEB) are separable. On this liquid crystal, the DEB isomers leave the column in the order *meta, para* and *ortho*. The respective boiling-points of these isomers are 181.10°, 183.75° and 183.42°. The relatively longer retention of *ortho*-isomers by phase II throws fresh light on the mechanism of the separating action of liquid crystals. Bearing in mind the fact[14] that molecules having a length-to-width ratio greater than 1 remain in the column for a longer time, one must seek other reasons for the longer retention of *ortho*-isomers as compared with *para*-isomers. One such reason is,

doubtless, that the structure of the liquid-crystalline molecule is such that its inter-action with *ortho*-isomers is stronger. Another, perhaps the decisive, factor is that phase II has a smectic structure, whereas phase I is nematic. However, we have not yet established which particular properties of the liquid-crystalline structure are responsible for the characteristic behaviour of *ortho*-isomers on liquid crystals.

- We utilised the above-mentioned properties of phase II to separate DEB isomers on an 18-m column (see Fig. 6). DEB isomers are normally very difficult to separate, and the most commonly used system for separating them (and one that has hitherto been regarded as the best for isomer separation) is Bentone 34 mixed with, *e.g.*, silicones. With such a mixed phase and an analysis time of *ca.* 44 min, it is im-possible to completely separate DEB isomers[15].

Although we achieved good separation of DEB isomers on an 18-m column with phase II, we were unable to separate *m*- from *p*-xylene with this phase. However, complete separation of these isomers was achieved on an 18-m column containing 5% of phase Ia on Polsorb B NAW (see Fig. 7); separations obtained on a similar column, but with phase I, were worse. The relatively long analysis time (47 min) was, however, shorter than the 72 min needed with squalane as stationary phase on a 50-m capillary column[16]. On columns with squalane, the xylene isomers leave the column in the order *para*, *meta* and *ortho*, that is, in accordance with their boiling-points (138.5, 139.5 and 144.5°, respectively). For xylene isomers on a liquid-crystalline column, the order of separation is *meta*, *para* and *ortho*. This confirms the finding that the order in which the individual components of a mixture leave the liquid-crystalline column is determined by the shape of the molecules concerned. For this reason, the selectivity of columns containing liquid crystals does not always correspond to their efficiency. By decreasing the temperature of the nematic liquid (particularly to achieve super-cooling) increasingly better separations can be attained, with a decreasing number of theoretical plates. For example, for a 5-m column containing phase I, the number of theoretical plates for *o*-xylene was 4400, 4250 and 4000 at 126, 97 and 87°, respec-tively; at 87°, the supercooled state of this phase was permanent. In our opinion, it is a true supercooled state, despite the fact that some workers question the existence
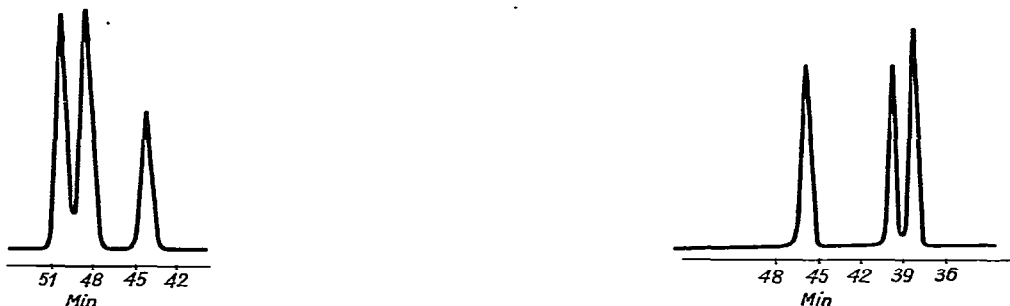

Fig. 6. Separation of diethylbenzenes on an 18-m column containing 5% of liquid crystal II on Polsorb B NAW and operated at 99°, with nitrogen (1.8 ml/min) as carrier gas. Isomer order: *meta, para, ortho*.

Fig. 7. Separation of xylenes on an 18-m column containing 5% of liquid crystal Ia on Polsorb B NAW and operated at 96°, with nitrogen (1.9 ml/min) as carrier gas. Isomer order: *meta, para, ortho*.

of liquid crystals in a supercooled state on a support[17,18]. It may be that this difference of opinion is simply calling the same phenomenon by a different name. At 87°, we obtained reproducible retention times for particular isomers over a period of several hours. After switching off the apparatus and cooling the column to room temperature, it was then heated to *ca.* 5° above the transition to nematic liquid and then cooled gradually (in 5°-steps every 10 min) to the required temperature of supercooling. In this way, retention times similar to those obtained before the apparatus had been switched off were measured. The practical use of a supercooled nematic liquid has also been reported[3]. No supercooled state was obtained with smectic phase III, or phase II (which has a smectic phase up to 35° above the melting-point, after which it changes into a nematic liquid with a narrower temperature range).

CONCLUSIONS

Of the phases examined, the dodecyl derivative (III) was the least effective in the separation of disubstituted-benzene isomers; the best results were achieved with phase Ia. For analytical application, it is unprofitable to separate this liquid crystal into the individual isomers. Phase Ia, however, does not separate DEB isomers, but these can be successfully separated on the octyl derivative (phase II). On the stationary phases investigated, operated at feasible column temperatures, the isomeric toluidines, cresols and chloroanilines leave the column only after several hours and with considerable broadening of the peaks.

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