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UTILITY OF HIGH-TEMPERATURE THERMOTROPIC LIQUID CRYSTALS AS STATIONARY PHASES FOR NOVEL GAS-LIQUID CHROMATOGRAPHIC SEPARATIONS

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SUMMARY

In this paper, we have attempted to highlight the capabilities of high-temperature thermotropic nematic liquid crystal phases for providing enhanced separations of important solute isomers that have been traditionally difficult to resolve by other chromatographic methods. The most notable of these is represented by the broad class of polycyclic aromatic hydrocarbons (PAHs). It is shown that such liquid crystals may be successfully used as wall coatings in wide-bore open-tubular columns, and it is suggested that the preparation of high-efficiency 0.25-mm I.D. capillary columns should be feasible. Further, it is pointed out that numerous applications can be made using liquid crystals for improving the separation of solute isomers of diverse chemical classes, other than PAH. Finally, it is hoped that the use of high-temperature liquid crystals can provide a powerful supplemental parameter for the separation of compounds (in addition to solute vapor pressure differences and the degree of polar interactions between solutes and the stationary phase), namely separations based on subtle differences in solute shape.

INTRODUCTION

The development of optimal chromatographic procedures for the separation and quantification of closely related structural isomers present in wide varieties of samples generally involves a considerable time dedication. While much excellent work has been carried out to approach the absolute limit of various chromatographic techniques for the maximum attainable resolution of isomers of diverse chemical classes, complete isomer resolution continues to present one of the more difficult problems that confront researchers. In gas-liquid chromatography (GLC) much of the effort related to this problem has been directed towards the enhancement of chromatographic efficiencies using capillary columns. In high-performance liquid chromatography (HPLC), the use of reversed-phase column packings and mobile

phase gradients has assisted isomer separations in a number of cases. The use of conventional adsorbent column chromatography or thin-layer chromatography (TLC) for initial separations, followed by HPLC or capillary GLC analysis of isolated sample fractions has also provided useful schemes for the separation of certain types of isomers.

This present paper addresses our experiences in the development and GLC evaluation of high-temperature thermotropic liquid crystals as novel stationary phases for chemical isomer separations. While it had been recognized for some time that liquid crystals could be used as GLC stationary phases to provide solute shape-selective separations of rigid solute isomers¹⁻³, essentially all of these earlier studies employed low-molecular-weight liquid crystals for the separation of low-molecular-weight solutes, such as disubstituted benzene isomers. The focus of our work has been aimed towards the development of high-molecular-weight liquid crystals and their use in separations of solutes of practical importance having substantially higher molecular weight. While this work has centered on 3-6 ring polycyclic aromatic hydrocarbon (PAH) isomers⁴⁻¹¹, we have also demonstrated the utility of these liquid crystals for the separation of PAH metabolites¹², steroid epimers¹³, and bile acids¹⁴. In addition, other workers have employed high-temperature liquid crystals we reported on for improved separations of isomers of polychlorinated biphenyls (PCBs)¹⁵, aromatic marine pollutants¹⁶, pharmaceuticals¹⁷, polynuclear azaheterocyclic air pollutants¹⁸; as well as synthetic PAH metabolites¹⁹, and PAH associated with combustion effluents²⁰ and in coal-tar pitch²¹.

EXPERIMENTAL

All high-temperature liquid crystals studied were homologs or analogs of the general formula, N,N'-bis(R-benzylidene)- α,α' -bi-*p*-toluidine, and were synthesized at the NCI Frederick Cancer Research Center (Frederick, Md., U.S.A.) under direction of G. M. Muschik; with the exception of R = methoxy, which was obtained from Eastman Kodak (Rochester, N.Y., U.S.A.). The liquid crystals were synthesized by condensation reaction of the appropriate benzaldehyde with α,α' -bi-*p*-toluidine. The starting materials were purified prior to use, and transition temperatures of the recrystallized liquid crystal products were determined by differential scanning calorimetry (Table I). All GLC solutes studied were obtained from standard commercial sources, and solvents used were all glass-distilled. GLC analyses were run on commercial systems containing a dual flame ionization detector, and retention data were obtained from a multi-input on-line data system. GLC carrier gas flow-rates and column oven temperatures were independently calibrated. GLC-mass spectrometry (MS) was run using a quadrupole mass spectrometer linked to a dedicated data system.

RESULTS AND DISCUSSION

Liquid crystal characteristics

Mesomorphic liquid crystal transition temperatures as determined by differential scanning calorimetry (*e.g.*, of BBBT, Fig. 1), are listed in Table I. A number of additional high-temperature liquid crystals not listed have also been synthesized

TABLE I

TRANSITION TEMPERATURES OF LIQUID CRYSTALS STUDIED

Data determined by differential scanning calorimetry.

Liquid crystal*	Transition temperatures ($^{\circ}\text{C}$)**				
	T_N	T_S	$T_{S_1-S_2}$	T_{S-N}	T_{N-I}
BMBT	181				337
BEBT	173				341
BPrBT		169		176	311
BBBT		159		188	303
BPeBT		139	201	208	283
BHxBT		127	203	229	274
BHpBT		119	203	238	262
BOBT		118	202	244	255
BPhBT	257				403
BABT	253				>370

* Second letter in liquid crystal abbreviation stands for R as methoxy, ethoxy, *n*-propoxy, *n*-butoxy, *n*-pentoxy, *n*-hexoxy, *n*-heptoxy, *n*-octoxy, phenyl, and anisyl derivatives of the series, *N,N'*-bis(*R*-benzylidene)- α,α' -bi-*p*-toluidine.

** T_N , solid-nematic transition; T_S , solid-smectic; $T_{S_1-S_2}$, smectic I-smectic II; T_{S-N} , smectic-nematic; T_{N-I} , nematic-isotropic.

at the NCI Frederick Cancer Research Center. The more interesting of those unlisted, being alkoxy analogs of BPhBT, will be the subject of future reports. For the first eight materials in Table I, several interesting trends can be pointed out. For example, while an increase in the terminal alkoxy chains translates to increasing thermal stability as column substrates, this trend also relates to a lessening of the useful nematic temperature range. It has been well established that the nematic mesophase offers the greatest utility for isomer separations in GLC. Solutes can more readily enter a nematic than the more densely packed smectic or cholesteric mesophases; thus nematics offer the highest solubility for solute isomers⁵. For this reason, the alkoxy homologs below BHpBT have been of particular interest, and detailed GLC evaluations of BMBT⁴, BBBT⁵, and BHxBT⁶ have been reported. A second homologous series can be initiated from the bis(*R*-phenylbenzylidene) structure, starting with BPhBT; however, R as methoxy (BABT), or as higher alkoxy chains are more difficult to prepare, owing to the unavailability of the appropriate *p*-substituted phenylbenzaldehydes.

To be sure, these liquid crystals have substantially lower molecular weights than conventional polymeric GLC liquid phases. Nonetheless, GLC columns containing a liquid crystal phase can have useful lifetimes in the order of several months without prohibitive deterioration, provided proper operating care is taken. Recommendations for maximizing the life of such columns have been enumerated elsewhere⁸.

Nematic liquid crystals possess a rod-like molecular structure. It is the alignment of liquid crystal molecules in the nematic temperature range that results in high solute-shape specificities. In essence, the more rod-like solute isomer sacrifices more translational and rotational freedom, but in turn, its favorable geometry permits it to interact more strongly with the aligned nematic liquid crystal phase. Thus,

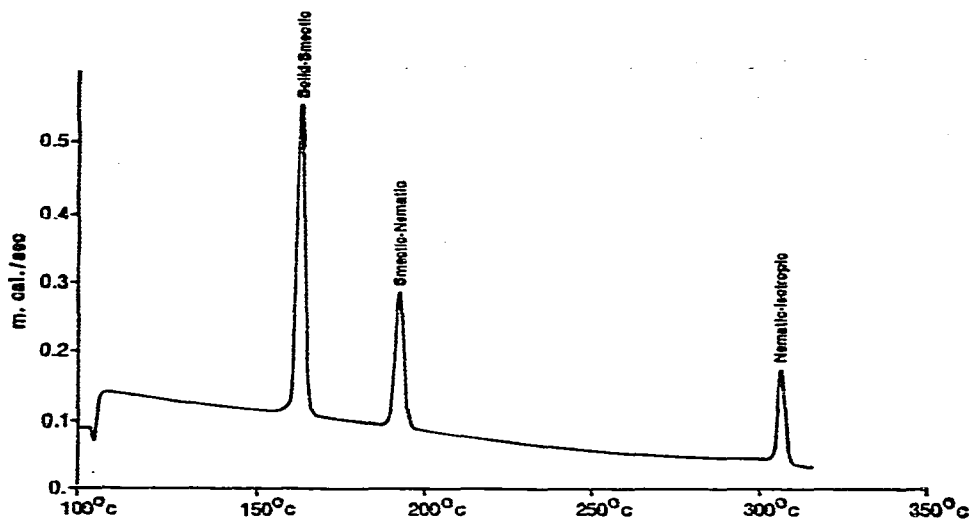


Fig. 1. Differential scanning calorimetry of BBT liquid crystal m. cal. = millicalories.

its entropy loss is more than compensated by its enthalpy gain, making it more soluble than a less rod-like solute. To illustrate, *p*-disubstituted benzenes elute by a time factor of 1.2–1.3 longer on a nematic phase than corresponding *m*-disubstituted benzenes, due to the longer length-to-breadth ratio of the former³.

Separation of PAH isomers

Owing to the established carcinogenicity and mutagenicity of a number of PAH compounds (*e.g.*, refs. 22–24), this chemical class has become one of the most intensely studied in recent years. While numerous analytical techniques have been developed in an attempt to resolve and quantitate isomeric PAH components present in a wide variety of samples (*e.g.*, cigarette smoke condensate, coal tar pitch, air particulate matter, oils, polluted waters, etc.), no single chromatographic substrate has been reported to be as useful as a high-temperature nematic liquid crystal for providing general separations of 3–6 ring PAH isomers⁴.

HPLC systems have been reported for such separations, but lack the higher column efficiencies of high-resolution GLC systems. Further, while the most successful HPLC separations have been obtained on C₁₈ reversed-phase columns^{25–27} the complete separation of 4-ring isomers (triphenylene, chrysene and benz[*a*]anthracene) often presents a problem, and a wide variability has been observed in the degree of resolution of such isomers using different commercially available C₁₈ columns²⁷.

The most successful GLC separations of PAH isomers have been carried out using glass capillary columns coated with conventional stationary phases. Such columns offer substantially higher efficiencies than have been obtained by HPLC, yet the separation factors for important isomer pairs are very much lower than those attainable by the best HPLC conditions²⁷, or those readily attainable using GLC packed columns of liquid crystals^{4–11}. To illustrate, data taken from the literature using glass capillary columns^{28,29} or HPLC^{25–27} are compared to packed liquid crystal column data in Table II.

TABLE II
CHROMATOGRAPHIC SEPARATION FACTORS OF PAH ISOMER PAIRS

Isomer pair [*]	HPLC ^{**}	Capillary GLC ^{***}	Packed liquid crystal column N ^{††}
C/BaA	1.0-1.2	1.004	1.26
C/T	1.0-1.7	1.000	1.69
N/C	—	1.021	1.49
P/BaP	1.0-1.1	1.012	1.23
BaP/BeP	1.2-1.7	1.006, 1.043 [†]	1.57

* C = chrysene; BaA = benz[*a*]anthracene; T = triphenylene; N = naphthacene; P = perylene; BeP = benzo[*e*]pyrene; BaP = benzo[*a*]pyrene.

** Data from refs. 25-27.

*** Data from ref. 28.

† Data from ref. 29.

†† Data from ref. 4.

As mentioned earlier, GLC separations of rigid isomers on liquid crystal columns follow the general elution order of increasing retention for increased length-to-breadth ratio of solute isomer structures. This can be illustrated for PAH isomers having a fixed number of fused rings (Table III). It is also of interest to note that the same PAH isomer elution order and magnitude of selective separation has been obtained in the nematic phase of all high-temperature liquid crystals we have studied^{4-6,9}. The principal advantage of the higher molecular weight members of the N,N'-bis(*R*-benzylidene)- α,α' -bi-*p*-toluidine series (Table I) we have studied, has been the enhancement of column stability at the elevated temperatures required to elute 5- and higher-ring PAH isomers.

Furthermore, the primary motive in examining various nematogenic liquid crystals using packed GLC columns was to explore the potential of such materials for providing a general improvement in the separation of chemical isomers. It was originally felt⁴ that the large separation factors obtained for PAH isomers offered general utility to moderate efficiency packed columns; and indeed, a number of successful applications have been reported using packed liquid crystal columns¹⁵⁻²¹.

TABLE III
CORRELATION OF SOLUTE ISOMER SHAPE VS. RETENTION ON A LIQUID CRYSTAL

PAH isomer	Ring size	Relative L/B ratio [*]	Relative GLC retention ^{**}
Phenanthrene	3	1.00	1.00
Anthracene	3	1.35	1.36
Triphenylene	4	1.00	1.00
Benz[<i>a</i>]anthracene	4	1.94	1.35
Chrysene	4	2.61	1.69
Naphthacene	4	3.56	2.51
Benzo[<i>e</i>]pyrene	5	1.00	1.00
Perylene	5	1.34	1.23
Benzo[<i>a</i>]pyrene	5	1.77	1.57

* Relative length-to-breadth ratio, estimated from the planar molecular structure of the PAH isomers.

** Calculated from data in ref. 4, using BMBT.

Nonetheless, it is clear that higher column efficiencies are needed for very complex PAH samples containing up to several hundred components²⁹.

In order to evaluate the degree of candidacy of high-temperature nematogenic liquid crystals as wall coating substrates in open-tubular columns (WCOT), we undertook preliminary studies using wide-bore (0.75 mm I.D.) stainless-steel capillary columns coated with either BBT or BPhBT¹¹. The separation of 3-ring PAH isomers on a WCOT BBT column in the nematic temperature range at 195° is shown in Fig. 2. While the column efficiencies noted here are understandably much less than those attainable on conventional 0.25-mm I.D. columns, they are a factor of 10–20 greater than for packed columns^{4–5}. Further, the selectivity reported for PAH isomer separations on packed columns^{4–6} remains intact.

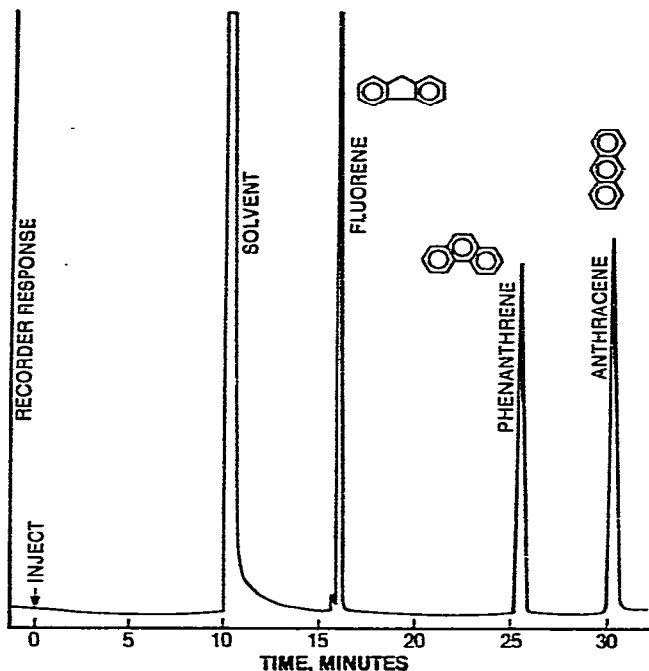


Fig. 2. Separation of 3-ring PAH isomers.

This latter point is highly significant. Since high-temperature liquid crystals in their nematic temperature range continue to exhibit solute shape selectivity when coated on column walls, there is no reason that these substrates could not be used to prepare 0.25-mm I.D. capillary columns having very high efficiencies. This technological step could substantially enhance the limited degree of separations of PAH isomers presently attainable on high efficiency glass capillaries (Table II), and thus provide notable improvements in the analysis of complex PAH samples derived from natural sources. It should be noted that the separation achieved between phenanthrene and anthracene (Fig. 2) is superior to that reported for any other chromatographic technique.

Separations of selected 3–6 ring PAH isomers is shown in Fig. 3, using a

0.75-mm I.D. WCOT BPhBT column. The separation of triphenylene, benz[*a*]anthracene, and chrysene in chromatogram A (265°) is far superior to that achievable by either HPLC or capillary GLC²⁵⁻²⁹. Further, since benz[*a*]anthracene and chrysene are suspected carcinogens²⁴, the accuracy of their quantitation in environmental samples can be improved using liquid crystal capillary columns. Finally, since these peaks are so widely separable, the identification (using available standards and GLC-MS) and measurement of other components present in complex PAH samples may be more tractable. Chromatogram B in Fig. 3 (280°) demonstrates that 5-ring PAH isomers of the so-called "benzopyrene fraction" can be easily resolved; while chromatogram C (300°) shows the separation of several PAH isomers that elute after benzo[*a*]pyrene.

In summary then, high-temperature nematic liquid crystals can offer improved separations of PAH isomers relative to other chromatographic techniques. These liquid crystals, used as wall coatings on capillary columns, mimic the large separation factors for PAH isomers reported previously on packed columns of the same materials⁴⁻⁶. Clearly, additional work will be needed to extend the use of these coatings to very high efficiency (0.25 mm I.D.) capillary columns, but our preliminary work indicated this to be an attainable objective.

Application to other separations problems

High-temperature nematic liquid crystals can provide improved GLC separations of rigid solute isomers other than neutral PAH. Reports have been issued on the separation of steroid epimers¹³, bile acids¹⁴, polychlorinated biphenyls¹⁵, pharmaceuticals¹⁷, and aromatic pollutants^{16,28}. Chromatograms in Fig. 4 were obtained on a nematic liquid crystal WCOT wide-bore capillary column, to illustrate separations of PCBs (A), pesticide isomers (B), and steroid epimers (C). Chromatogram A represents in excess of 40 peaks. No attempt was made to optimize the chromatographic conditions. The use of column temperature programming coupled to GLC-MS should provide a useful complement to analyses of PCBs conducted by workers using capillary columns coated with commercial polymeric phases. Essentially no work has been reported on the separation of pesticides using high-temperature liquid crystals. This represents an interesting area of application, since a number of aromatic pesticides contain isomeric impurities or form isomeric metabolites *in vivo*. The distinct advantage represented by the separation of the 3-hydroxy isomers of 5 α -androstanol (chromatogram C) and elaborated previously¹³, is that the preparation of steroid derivatives prior to GLC analysis is unnecessary. One highly exciting area for high-temperature liquid crystals application lies in metabolism studies of carcinogenic PAH. A considerable amount of work has been conducted in recent years concerning the metabolism of benzo[*a*]pyrene. This potent carcinogen forms numerous *in vitro* metabolites when incubated with liver microsomes. One major problem in these types of studies has been the chromatographic resolution, identification and measurement of the monophenol metabolites. While HPLC has been used to identify up to four of the 12 possible phenols of the parent carcinogen³⁰, the use of conventional GLC phases and earlier HPLC work lacked the chromatographic resolution needed for separation of the phenol isomers. In contrast, we recently reported a GLC procedure using a BPhBT packed column in which 10 of the 12 possible phenols could be resolved and identified¹². Further, since all 12

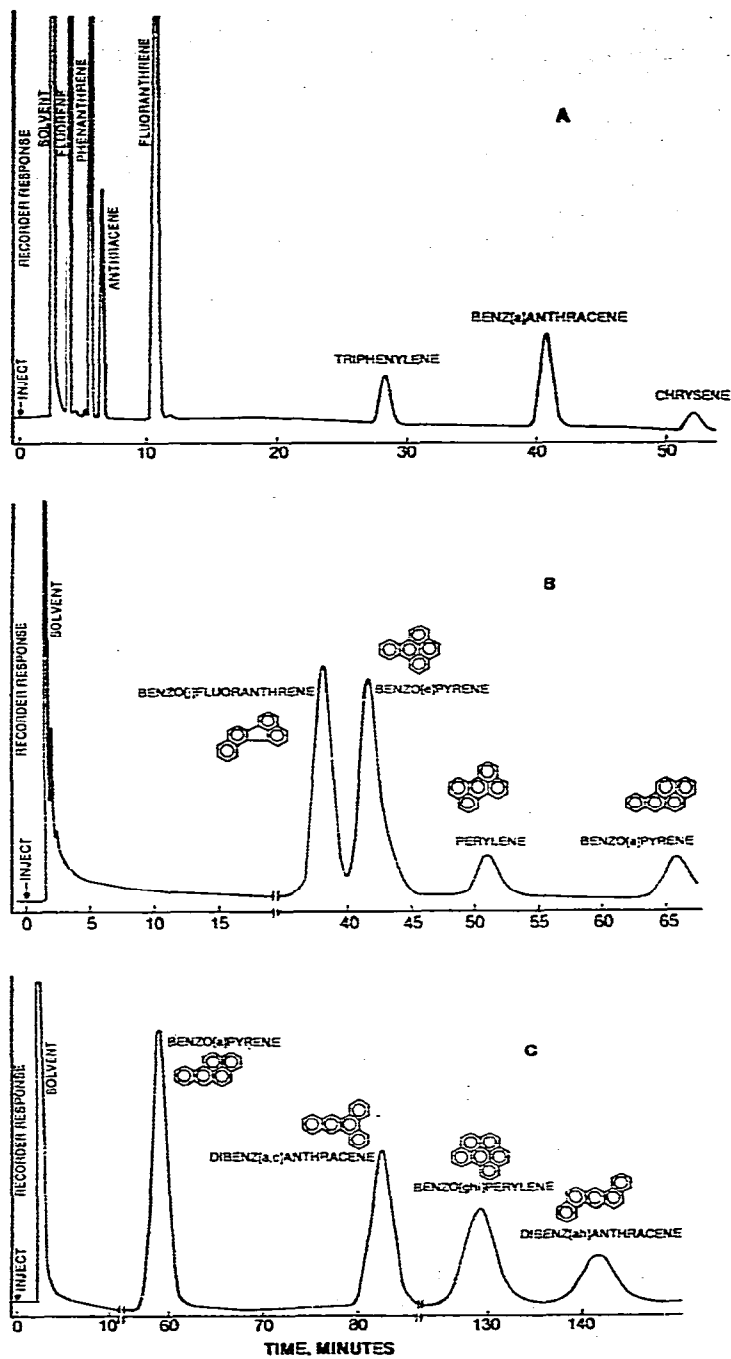


Fig. 3. Separation of selected 3-6 ring PAH isomers. Temperature: A, 265°; B, 280°; C, 300°.

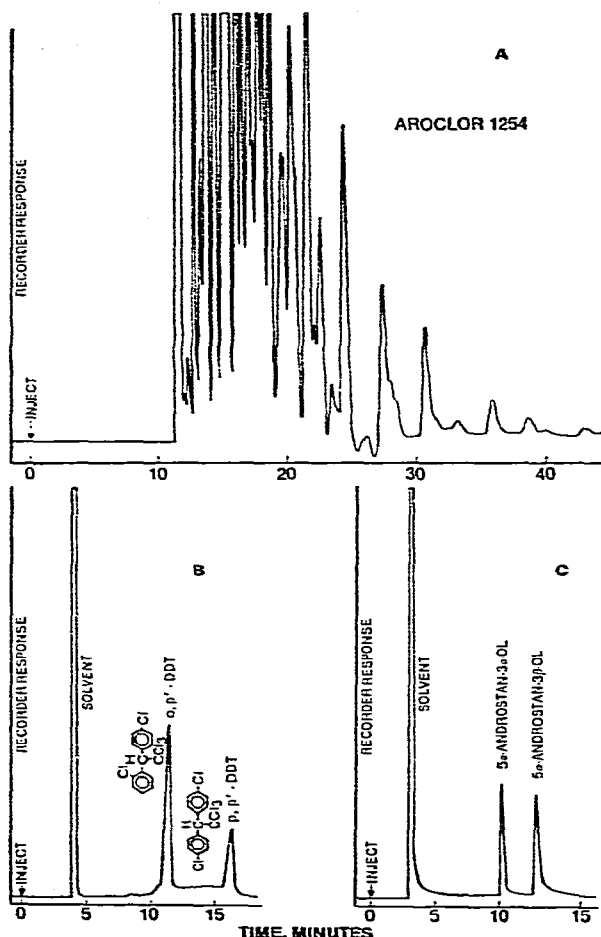


Fig. 4. Separation of various chemical isomer classes. A, PCBs; B, pesticides; C, steroids.

phenol isomers produce the same molecular ion (analyzed as trimethylsilyl (TMS) ethers) as the base peak in mass spectrometry, GLC-MS selective ion monitoring has been found to be useful and sufficiently sensitive to monitor the BPhBT column effluent. Using this procedure, we were able to ascertain additional information regarding the phenol metabolites that has not been observed using other separation methods³¹. To illustrate further the use of liquid crystals for carcinogenic PAH metabolism studies, a considerable level of research has been conducted in recent years concerning the metabolism of the highly carcinogenic PAH derivative, 7,12-dimethylbenz[*a*]anthracene (DMBA). The separation of structurally related hydroxymethyl isomers (as their TMS derivatives) from each other and from DMBA itself using a WCOT liquid crystal column is illustrated in Fig. 5. Hence, liquid crystals may offer some considerable assistance for the resolution and subsequent identification of metabolites of carcinogenic PAH.

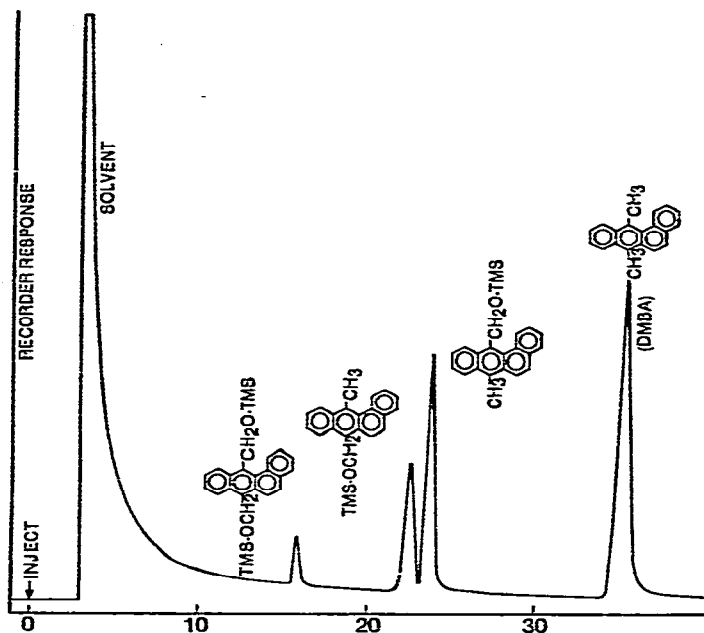


Fig. 5. Separation of DMBA hydroxy isomers.

Future options

In addition to the potential extension of liquid crystals to the development of high efficiency 0.25-mm I.D. WCOT columns, there are two significant areas that can be explored, and these tend to be intertwined to some degree. These are principally, the evaluation of additional members of the *N,N'*-bis(*p*-alkoxyphenylbenzylidene)- α,α' -bi-*p*-toluidine series to provide (a) further enhancement of GLC column stability and (b) an extended capability for useful temperature programming analysis. It should be noted that several nematic liquid crystals we have examined can be substantially super-cooled below their normal solid-nematic transition temperature, and remain in the ordered liquid state. Wasik and Chesler¹⁶ used a BMBT packed column to obtain selective separation of disubstituted naphthalene isomers at column temperatures as low as 120° (solid-nematic transition of BMBT is 181°). BMBT is stable in the super-cooled nematic state for at least 8 h, hence programmed temperature analysis over a BMBT range of 120° to *ca.* 230° is perfectly feasible. Further, a liquid crystal of substantially higher thermal stability, BABT (Table I), can be operated in its super-cooled state at 230°⁹; thus, column temperature programming from 230–280° can be carried out. This aspect of employing column temperature programming for liquid crystals has not been fully taken advantage of, but should be quite useful for separating wide-boiling complex mixtures (*e.g.*, 3–6 ring PAH).

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