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Short Communication

Study of the response of three liquid crystals as stationary phases for the gas chromatography of some cyclic monoterpene volatile oil constituents with short retention times

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ABSTRACT

Eight cyclic monoterpene volatile oil constituents of short retention time were studied using three liquid crystals as gas chromatographic stationary phases in packed columns. Two phases (below) exhibited a different solute elution sequence after melting and supercooling. The third, bis-(methoxy-benzylidineanil-chloroaniline), did not, but still showed fairly good resolution. However, azoxydiphenetole offered the identification advantage of considerable changes in solute relative retention times, with melted *vs.* unmelted ratios of × 1.5 to × 3.0. Cineole and terpinolene were distinctive in giving for this ratio only × 0.9 on bis-(methoxy-benzylidineanil-bitoluidine). The results suggest that various parts of the melted or unmelted liquid crystal molecules participate in retaining the various solutes.

INTRODUCTION

In a previous study [1] of three liquid crystal stationary phases in packed columns used for gas chromatography it was noted that only on one of them, bis-(methoxy-benzylidineanil-bitoluidine), $(MBT)_2$, see Fig. 1, did cineole and caryophyllene give lower relative retention times to linalol on the melted, supercooled phase than on the unmelted liquid crystal at the same temperature. Most solutes were found to give higher relative retention times on melting the liquid crystals [1,2], including limonene on $(MBT)_2$. This author has previously used a liquid crystal phase to study some volatile oil constituents with short retention times such as cineole and limonene, although this was a mixed lyotropic phase [3]. The three packed columns of thermotropic liquid crystals (see Fig. 1) allowed a study of eight such quickly-emerging oil constituents on unmelted and melted supercooled phases to lead to deductions on the retention mechanisms of these liquid crystals and to see if there was any other case of the anomalous cineole-behaviour. The ability of the phases to resolve these eight cyclic substances, five with very similar molecular structure and boiling points, was also of interest.

EXPERIMENTAL

Apparatus

A Pye Unicam GCD gas chromatograph fitted with a flame ionization detector and a wide-range

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Acronym of liquid crystal and m.p. °C		Chemical formula	Chemical feature
$(MBCA)_2$	154	$(CH_3O—C_6H_4—CH{=}N—C_6H_3Cl—)_2$ bis-(methoxy-benzylidineanil-chloroaniline)	Anil dimer without spacing atoms between the central aromatic rings
$(MBT)_2$	181	$(CH_3O—C_6H_4—CH{=}N—C_6H_4—CH_2—)_2$ bis-(methoxy-benzylidineanil-bitoluidine)	Anil dimer with two central spacing methylene groups between aromatic rings
ADP	138	$(C_2H_5O—C_6H_4—N{=})_2{\to}O$ azoxy-diphenetole	Azoxy compound with two central spacing nitrogen atoms between aromatic rings

Fig. 1. Details of liquid crystal stationary phases used. Note all are terminal di-ethers.

amplifier with a Hewlett-Packard 3380A recorder/integrator were used.

A packed glass column of 3% azoxy-diphenetole (ADP) was used, 1.5 m × 4 mm I.D, prepared as described previously [1]. A 20 ml min^{-1} flow of nitrogen was used as mobile phase. A similar column of 3% bis-(methoxy-benzylidineanil-chloroaniline), $(MBCA)_2$, was also studied using a slower flow of nitrogen, 8 ml min^{-1}. The 3% $(MBT)_2$ column was 1.5 × 2 mm I.D., and also had a nitrogen flow of 8 ml min^{-1}. All three columns had received previous careful use: none was “naive”.

A Technoterm 7300 probe was used to observe oven temperatures to ± 0.1°C. Conditions used are given in the tables.

Materials and methods

The liquid crystals were from TCI, Tokyo.

p-Cymene, α-phellandrene*, α-terpinene, and γ-terpinene were from TCI. 1,8-Cineole (Faulding), limonene and α-pinene (BDH) and terpinolene* (Shanghai Essential Oils) were also used. See Fig. 2 for formulae. Materials marked * were impure, and the main peak was used. Injections were made from a microsyringe which had been filled, then “emptied”.

Terpinolene (185°^{a}) has a 4-8 double bond.
γ-Terpinene (183°) has a 4-5 double bond.
Limonene (176°) has an 8-9 double bond.
α-Terpinene (174°) has a 3-4 double bond.
α-Phellandrene (175°^{a}) has a 5-6 double bond.
α-Pinene (156°) has a 6-8 bond.
Cineole (176°) has a 1-8 *ether* bond and is fully saturated.
p-Cymene (177°) has a fully *aromatic* ring.

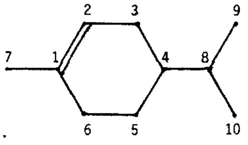

Fig. 2. Formulae of solutes used, with boiling points taken from ref. 5, except those marked *a* which come from ref. 6.

Holdup times were determined by extrapolating to methane the retention times of *n*-heptane and *n*-hexane plotted on logarithmic graph paper.

RESULTS AND DISCUSSION

Average results are given in Tables I and II. Due to the various melting points of the liquid crystals, different temperatures were used for each. α-Pinene, α-phellandrene, limonene and γ-terpinene retain this elution sequence in both tables, with the other four solutes changing positions.

Unlike the other two phases studied here, $(MBCA)_2$ after melting gave no change in the retention times for the eight solutes compared to its “unmelted” state. Their sequence was like that from the other two unmelted phases (Table I) with cineole last. The other di-anil liquid crystal $(MBT)_2$ on melting showed only slight, but definite, changes in relative retention times (from just over × 0.9 to × 1.2), and a different solute sequence, with cineole not last (Table II). With a similarly changed solute sequence, ADP on melting gave a considerable increase in relative retention times from × 1.5 to × 3.0. Thus ADP offers the greater chance for solute identification using the melted *vs.* unmelted phase observations: cineole distinctively changes only × 1.5; three solutes change about × 1.9; another three about × 2.6; and × 3.0 indicates *p*-cymene. Only terpinolene was found to behave like cineole and show a lower relative retention time to linalol on $(MBT)_2$ after melting. Cineole gave the lowest increase on melted ADP, but here behaves differently to terpinolene.

On the three “unmelted” phases (Table I), if the distinctively fastest α-pinene is ignored, $(MBT)_2$ clearly gives the poorest resolution with the longest

TABLE I

RELATIVE RETENTION TIMES (LINALOL = 1.00) ON COLUMNS OF 3% UNMELTED LIQUID CRYSTALS

Solute	ADP at 125°C	$(MBCA)_2$ at 120°C[a]	$(MBT)_2$ at 150°C
Cineole	0.19	0.32	0.44
Terpinolene	0.15	–[b]	0.44
γ-Terpinene	0.14	0.25	0.44
p-Cymene	0.12	0.24	0.43
Limonene	0.12	0.21	0.40
α-Phellandrene	0.13	0.19	0.39
α-Terpinene	0.11	0.20	0.38
α-Pinene	0.06	0.09	0.23

[a] $(MBCA)_2$ gave the same results after heating above its melting point and slowly supercooling to this temperature.

[b] No main peak discernable from impure solute.

time (0.44) being only about 115% of the shortest (α-terpinene). The other two phases show a time range of 160% or more, although $(MBCA)_2$ exhibits a better distribution of solute peaks. But ADP has the advantage of its two conditions, melted and unmelted.

To a considerable extent, the solutes studied emerge from the packed columns in the sequence of their boiling points (Fig. 2), particularly from the unmelted liquid crystals. Thus α-pinene is always quickest, as it is on conventional phases [4]. The ether cineole, however, is last from unmelted liquid crystals, reflecting its affinity for their ether structures (see Fig. 1) although this is not so apparent on $(MBT)_2$. On melted, supercooled liquid crystals the rigid structure of cineole probably becomes dominant, so it is one of the quickest solutes to emerge after the similarly box-like α-pinene, these two being unable to penetrate the liquid phase well. Terpinolene is similarly rigid due to its 4–8 double bond (see Fig. 2) and this sees it pass through these melted liquid crystals just after cineole, in contrast to being about last on the unmelted phases. Terpinolene, with the highest boiling point, is the last from conventional non-polar and polar phases [4]. The branched three-carbon side chains of the other solutes (see Fig. 2) can rotate allowing various molecular conformations, some of which presumably fit more readily into the melted liquid crystals. *p*-Cymene has the extra affinity of being an aromatic substance like the liquid crystals, and this is revealed by the melted phases retaining it longest (Table II). However, γ-terpinene, with its two unconjugated unsaturated groups on opposite sides of the ring, behaves very similarly to *p*-cymene. On the melted, supercooled liquid crystals, the least strongly retained solutes are, together with the three "rigid" molecules, those with conjugated double-bonds in their ring: α-phellandrene and α-terpinene. Thus the unconjugated double bond systems of limonene and γ-terpinene may allow them to be more strongly retained.

Why do the two similar anil phases behave differently? $(MCBA)_2$ behaves only like an unmelted liquid crystal here, although it has shown a "super-

TABLE II

RELATIVE RETENTION TIMES (LINALOL = 1.00) ON COLUMNS OF 3% MELTED, SLOWLY SUPERCOOLED LIQUID CRYSTALS, AND RATIOS TO THE UNMELTED VALUES

Solute[a]	ADP at 125°C		$(MBT)_2$ at 150°C	
	Time	Ratio	Time	Ratio
p-*Cymene*	0.36	× 3.0	0.53	× 1.2
γ-Terpinene	0.35	× 2.5	0.53	× 1.2
Limonene	0.32	× 2.7	0.49	× 1.2
α-Terpinene	0.29	× 2.6	0.43	× 1.1
Terpinolene	0.29	× 1.9	0.42	× 0.9
Cineole	0.28	× 1.5	0.41	× 0.9
α-Phellandrene	0.25	× 1.9	0.41	× 1.0
α-Pinene	0.12	× 2.0	0.23	× 1.0

[a] Solutes named in italics are in a different sequence to the unmelted phase (Table I).

cooled melted" response with other solutes [1]. It is the only one of the three liquid crystals used here without a central "spacer" of atoms between the aromatic rings. $(MBT)_2$ has $-Ar-CH_2-CH_2-Ar-$ present, and ADP has $-Ar-N=N-Ar-$ which may account for the two types of behaviour of solutes on these phases: there is no central space in $(MBCA)_2$ for them to fit into. Therefore it seems that various parts of the liquid crystal molecules participate in retaining different solutes: the terminal ether groups, and the central aromatic rings with or without spacing atoms between them. Some parts may be more effective in either the melted or the unmelted condition.

In other work by this author [1] a commercially available polysiloxane liquid crystal capillary seemed unsuitable for the analysis of these monoterpenes. It is unlikely that the three liquid crystals used here can be used for capillaries. But in packed columns they could supplement work with conventional phase capillaries. A column of ADP can b used for two different analyses without change (before and after melting) to provide an "extra dimension" in identification of these compounds.

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