

Comparison of four liquid crystal stationary phases used above and below their melting point temperatures for the gas chromatography of some volatile oil constituents

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ABSTRACT

All four liquid crystal stationary phases used for the gas chromatography of volatile oil constituents function below their melting points, but the azoxydiphenetole (ADP)-packed column does not exhibit reliable supercooling. Safrole always preceding anethole provides an identifying test for these liquid crystal phases. Various distinctive shifts in solute sequence were noted for each phase. An "MPMS" polysiloxane capillary gave the highest relative retention times of the four phases and is required for satisfactory peaks of monoterpenes with a terminal polar group, like geraniol. It is also good for polar aromatics like thymol, although bis(methoxybenzylideneanil-chloroaniline) in a packed column is an alternative. Linear ether aromatics like estragole can be studied on all four phases, which have this structure themselves. Whilst ADP may be best for terpene hydrocarbons, especially caryophyllene, bis(methoxybenzylideneanil-bi-toluidine) is the best general purpose liquid crystal for packed columns.

INTRODUCTION

We have reported on the use of liquid crystal stationary phases for the gas chromatography (GC) of some constituents of volatile oils. Their organised nematic fluid state may offer separation advantages by retaining molecules of particular shapes. Bis-(methoxybenzylideneanil-chloroaniline) [(MBCA)₂; Fig. 1] was chosen for initial studies [1] as it has a moderate melting point around 150°C. The long nematic range of this substance (190°C) before it changed to a true isotropic liquid was an attraction, but decomposition of the (MBCA)₂ severely limits its use to below 190°C. This first work did not reveal the complexity of the response of some liquid crystals, which was appreciated only during detailed work with another anil, bis(methoxybenzylideneanil-bi-toluidine) [(MBT)₂], which has a higher melting point around 180°C (Fig. 1). This second study [2] revealed that (MBT)₂, when heated from cold for the first time, had a "naive" condition

giving low relative retention times to linalol which sharply increased as the melting point of the (MBT)₂ was passed. With further increase of temperature, these values gradually decreased, but could be increased (or maintained) by supercooling the liquid crystal. Cooling to room temperature or below restored a lower value state, which gave higher relative retention times than the naive column, but below the supercooled results. A changing unmelted *versus* melted/supercooled condition exists.

It seemed of interest to check whether these changes of liquid crystal state as a stationary phase applied to (MBCA)₂ and to other liquid crystals, for example with a lower melting point and a short nematic span [azoxydiphenetole (ADP) with only a 30°C range (Fig. 1)] and with a polysiloxane liquid crystal commercially available in capillaries. This "MPMS" (Fig. 1) represents a modern liquid crystal, with side-chains attached to a polysiloxane backbone, whilst ADP is like the first azoxy liquid crystals employed by Dewar and Schroeder [3] for

Acronym of liquid cryst. & abbreviation	Chemical formula	Chemical feature	Melting point (L) and transition temp. °C
(MBCA) ₂	C (CH ₃ O—C ₆ H ₄ —CH=N—C ₆ H ₃ Cl—) ₂	anil dimer	154 344
(MBT) ₂	T (CH ₃ O—C ₆ H ₄ —CH=N—C ₆ H ₄ —CH ₂ —) ₂	anil dimer	181 320
ADP	D (C ₂ H ₅ O—C ₆ H ₄ —N=) ₂ →O	azoxy	138 168
'MPMS'	S (CH ₃ O—(C ₆ H ₄) ₂ -O-CO—C ₆ H ₄ -O—(CH ₂) ₃ -Si ₂ O—CH ₃) _n	ester polymer	145? 320

Fig. 1. Details of liquid crystal stationary phases used. The abbreviations are used in Figs. 2 and 3. These substances function as GC stationary phases below their melting points. The two anils decompose before they reach their transition points to give normal liquids. MPMS details are from ref. 10.

the GC of disubstituted benzenes. Finkelmann and Laub [4] first synthesised a mesogenic polymeric methyl siloxane in 1982, and MPMS has recently been used to separate polycyclic aromatics [5]. It has already been shown [2] that supercooled (MBT)₂ can be used advantageously for volatile oil studies, so this condition was investigated with the other three phases. ADP also allowed observations when it was a true liquid and no longer a liquid crystal.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 3380A integrator/recorder was used with (a) the MPMS capillary in a Hewlett-Packard 5790A gas chromatograph fitted with a flame ionisation detector, capillary control unit and splitter injection port in split mode or (b) the packed columns in a Pye GCD gas chromatograph fitted with a flame ionisation detector.

An MPMS Heliflex fused-silica capillary (Alltech Assoc., Deerfield, IL, USA), 25 m × 0.25 mm I.D., was used. Test conditions imply it had been checked at 265°C.

Two 3% ADP-packed columns were used, 1.5 m × 4 or 2 mm I.D. The weighed ADP was dissolved in dichloromethane and taken to dryness in a rotary evaporator with weighed Chromosorb W AW 80–100 mesh for packing. No preheating was used before the first results were obtained, but subsequent behaviour of the columns depended on their history of heating.

Four 3% (MBCA)₂-packed columns were used, all 1.5 × 4 mm I.D., prepared as for ADP. These showed substantial changes in behaviour with use.

One 3% (MBT)₂-packed column was used, no longer naive as it has been used previously [2].

Operating conditions are given in Tables I–IV, observing the GCD oven temperatures with a Technoterm 7300 probe.

Materials and methods

ADP was obtained from TCI (Tokyo, Japan). As were (MBCA)₂ and (MBT)₂, see refs. 1 and 2, respectively. Formulae details are shown in Fig. 1.

Anethole, cineole, estragole (4-allylanisole), geraniol, linalol and thymol were from Sigma. Also used were α-terpineol (TCI), caryophyllene (Koch-Light), limonene (BDH), safrole (Fritzsche) and cuminal (*p*-isopropylbenzaldehyde, Eastman). Repeated injections were made from a microsyringe which had been filled then "emptied" of these solutes.

RESULTS AND DISCUSSION

Results are presented in the tables and in Figs. 2 and 3. (MBCA)₂ and (MBT)₂ observations are best made on a column heated above the melting point of the liquid crystal and cooled to the desired temperature (supercooling). However, values on heating a naive (never used before) column of (MBCA)₂ and for heating a mature (MBT)₂ column are given. These supplement results previously published in refs. 1 and 2. In contrast, ADP is best used by heating from cold, as it does not supercool reliably. Owing to the design of the 5790A gas chromatograph the MPMS capillary could not be cooled slowly (less than 5°C/min is recommended) so it could only be observed in the heating mode, rising to the desired temperature at 4.5°C/min.

TABLE I

RELATIVE RETENTION TIMES (LINALOL = 1.00) ON A PACKED COLUMN OF 3% ADP HEATED TO ISOTHERMAL OPERATING CONDITIONS

Mobile phase, nitrogen at a flow-rate of 20–27 ml/min at the flame ionisation detector outlet. Values are averages of two or more observations. Values in italics are "out of sequence" (descending) at the temperature given and indicate a solute "shift" in relation to subsequent solutes.

Solute	Nominal column temperature (°C) heated from lower temperature											
	111	120	125	130	135 <i>m</i>	140	150 ^a	165	<i>t</i>	170 ^b	174 ^b	186 ^b
Anethole			<i>1.11</i>	<i>1.28</i>		6.17		5.28		4.50		
Cuminal			2.71	2.81		4.64		4.20				
Safrole	0.63	<i>0.87</i>	{ <i>3.37^c</i> <i>0.94</i>	<i>1.09</i>	2.34	4.22	4.21	3.83			3.52	3.08
α -Terpineol			2.46	2.41		2.59	2.50	<i>2.31</i>		2.27		
Caryophyllene		0.99		1.47		2.22	2.52	3.00				
Estragole	0.38	0.52	{ <i>2.15^c</i> <i>0.57</i>	0.86	1.41	2.50	2.50	2.26			2.06	1.86
Cineole		{ <i>0.30^c</i> <i>0.24</i>										
Limonene		{ <i>0.34^c</i> <i>0.18</i>										

^a Typical holdup time 0.33 min; linalol retention time 0.50 min.

^b Temperatures above the transition temperature (*t*) of ADP to a normal liquid are not recommended as column deterioration may result.

^c Values only obtained on slow cooling (4°C/min) from above the melting point of ADP (*m*).

TABLE II

RELATIVE RETENTION TIMES (LINALOL = 1.00) ON A PACKED COLUMN OF 3% (MBCA)₂ COOLED TO ISOTHERMAL OPERATING CONDITIONS

Mobile phase, nitrogen at a flow-rate of 6–10 ml/min at the flame ionisation detector outlet. Values are averages of two or more observations. Values in italics are "out of sequence" (descending) at the temperature given and indicate a solute "shift" in relation to subsequent solutes.

Solute	Nominal column temperature (°C) cooled from above the melting point (<i>m</i>)											
	122	125	130	135	140	145	150 <i>m</i>	160 ^a	170	175	180	190 ^e
Thymol								3.72 ^b	3.62	<i>3.24</i>		3.02
Cuminal		{ 3.65 3.22 ^c		3.38 ^c	3.60	3.61	3.52	3.48 ^b	3.35	3.25 ^b	3.20	3.64
Anethole	3.73	3.64	{ 3.60 2.27 ^c	3.57 ^b	{ 3.53 2.55 ^c	{ 3.51 3.04 ^c	{ 3.54 ^{b,d} 3.22 ^c	3.44 ^b		3.41	3.30	3.58
Safrole	2.76	2.69	<i>1.69^c</i>	{ 2.67 1.78 ^c	2.64		2.57	2.54	2.55	2.52	2.42	2.62
Caryophyllene						2.53		2.51		2.43		
α -Terpineol			{ 2.29 2.11 ^c	2.32	2.32	2.31	2.29	2.21	2.19		2.06	
Estragole	1.56		{ 1.53 1.00 ^c	1.57	{ 1.55 1.14 ^c	1.20 ^c	{ 1.57 1.43 ^c	1.57 ^b		1.56	1.52	1.80
Cineole			{ 0.38 0.38 ^c	0.39			0.41 ^b	0.48		0.48		0.56
Limonene			{ 0.27 0.27 ^c				0.30			0.36		

^a Typical holdup time 0.90 min; linalol retention time 0.65 min.

^b Results not within previously published ranges [1], probably the consequence of not overheating (MBCA)₂ here, see footnote *e*.

^c Values only obtained on initial heating of a naive column.

^d Unusually high value of 3.64 sometimes observed for anethole at 150°C.

^e Temperature not recommended as sharp increases in values suggest decomposition of the liquid crystal.

TABLE III

RELATIVE RETENTION TIMES (LINALOL 1.00) ON A CAPILLARY OF MPMS HEATED TO ISOTHERMAL OPERATING CONDITIONS

Mobile phase, helium at a flow-rate of about 0.75 ml/min at the flame ionisation detector outlet. Values are averages of two or more observations. Values in italics are "out of sequence" (descending) at the temperature given and indicate a solute "shift" in relation to subsequent solutes. $m^?$ = Approximate melting point of the liquid crystal.

Solute	Nominal capillary temperature ($^{\circ}$ C) heated from lower temperature					
	100	120	140 ^a	145 $m^?$	150	160
Anethole		9.83	8.61		7.60	
Thymol					6.20	
Safrole	6.44	6.32	5.64	5.48	5.21	4.86
Cuminal		5.91	5.50	5.48	5.21	4.97
Caryophyllene		3.82	3.47	3.44	3.26	3.12
Estragole	3.49	3.36	3.18	3.13	3.03	2.95
Geraniol	3.92	3.45	3.06	2.88	2.58	2.68
α -Terpineol	2.86	2.76	2.68	2.61	2.54	2.36
Limonene		0.60				0.75
Cineole		0.53				0.75

^a Typical holdup time 1.40 min; linalol retention time 0.20 min.

This discussion draws on comments made earlier when studying packed columns of (MBT)₂. The previously recorded [2] sequence of the aromatics estragole (quickest)–safrole–cuminal can be seen on all four liquid crystal phases, although only on MPMS at high temperatures (160 $^{\circ}$ C or more) (see Table III). On the MPMS capillary safrole and cuminal are close together, but with cuminal ahead of safrole below 145 $^{\circ}$ C. This safrole–cuminal shift (see Fig. 3) is not shown by the three packed columns. Thymol gives no results on ADP but it comes after cuminal on the other liquid crystals (see Tables II and III and ref. 2).

Fig. 2 (plot D¹) shows that the solute estragole, on a packed ADP column heated up to 125 $^{\circ}$ C from room temperature, gives relative retention times to linalol quite close to those on (MBT)₂, around 0.5 (plot T¹). These values then rise quite sharply as the melting point of this liquid crystal (138 $^{\circ}$ C) is approached, and are about 2.5 at 140 $^{\circ}$ C, which is almost the value on supercooled (MBT)₂ at this temperature [2]. ADP exhibits the most rapid large

TABLE IV

RELATIVE RETENTION TIMES (LINALOL = 1.00) ON A PACKED COLUMN OF 3% (MBT)₂ HEATED TO ISOTHERMAL OPERATING CONDITIONS

Mobile phase, nitrogen at a flow-rate of 7–10 ml/min at the flame ionisation detector outlet. Values are averages of two or more observations. For more results see ref. 2. Values in italics are "out of sequence" (descending) at the temperature given and indicate a solute "shift" in relation to subsequent solutes.

Solute	Nominal column temperature ($^{\circ}$ C) heating mature column from lower temperature				
	150	160 ^a	175 m	185	200
Safrole [2]	{ 4.58 ^b 2.67	{ 4.46 ^b 2.93	4.24 ^b		4.10
Geraniol	{ 4.27 ^b 3.85		3.29	3.38	
Estragole [2]	{ 2.83 ^b 1.70	{ 2.80 ^b 1.79	2.75 ^b		2.61
Caryophyllene	{ 2.18 ^{b,c} 2.87	{ 2.33 ^{b,c} 2.80	2.41	2.28	2.22
Cineole	{ 0.35 ^{b,c} 0.41	{ 0.43 ^b 0.43		0.49	
Limonene	{ 0.42 ^b 0.34	{ 0.49 ^b 0.39			

^a Typical holdup time 0.60 min; linalol retention time 0.20 min.

^b Values only obtained on cooling from above the melting point (m) of (MBT)₂.

^c Values lower than when column is heated, a phenomenon not previously observed.

increase in relative retention times seen on a packed liquid crystal column. With temperature further increasing above 150 $^{\circ}$ C (plot D) the values fall away to below 2 more rapidly than on (MBT)₂ (plot T) and without any change to the plot as ADP passes its transition point (168 $^{\circ}$ C) when it changes from a liquid crystalline state to a normal isotropic liquid. The supercooled condition, which is easily obtained on (MBT)₂, can only be seen on ADP if it is slowly cooled (at 4 $^{\circ}$ C/min) to 125 $^{\circ}$ C. Even then, supercooled ADP gives lower values than in its true liquid crystal condition [unlike (MBT)₂ or (MBCA)₂, plots T and C, respectively] and tends to revert to the naive low retention times seen on unmelted ADP. It is unwise to take this liquid crystal above its transition point, as on cooling a "gummed up" inferior column may result and there are no advantages in using it as a true liquid. Transition points

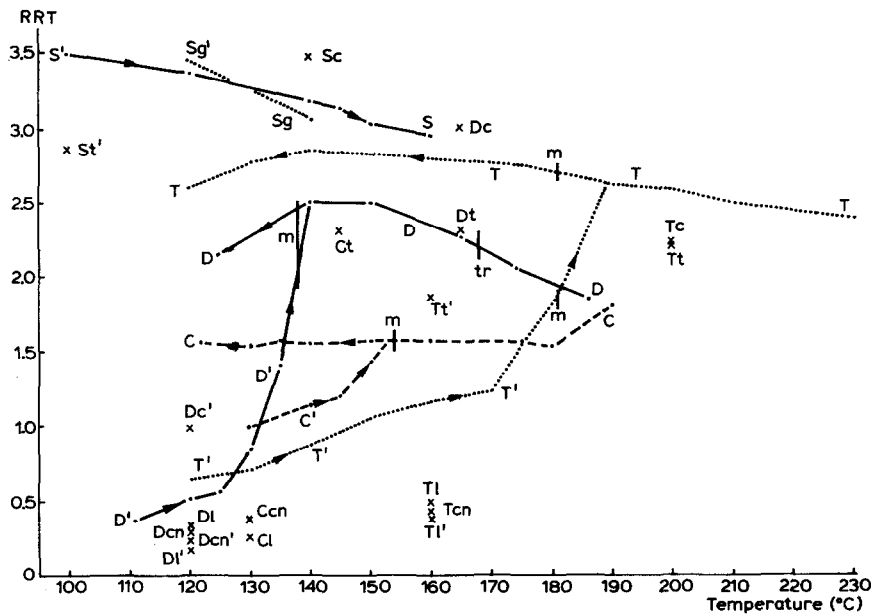


Fig. 2. Plots (connected points) at various temperatures ($^{\circ}\text{C}$) of relative retention times for estragole against linalol (1.00) on the four liquid crystal columns. Crosses (unconnected) are selected values for some other solutes against linalol. C = dashes connecting estragole results on $(\text{MBCA})_2$; D = lines connecting estragole results on ADP; S = lines connecting estragole results on MPMS capillary; T = dots connecting estragole results on $(\text{MBT})_2$ taken from ref. 2. Suffix ' = results on unmelted liquid crystal (C' and T' being naive). c = Caryophyllene; cn = cineole; g = geraniol; l = limonene; m = melting point of the liquid crystal; t = α -terpineol; tr = transition point of the liquid crystal to normal liquid (only D). Sc is thus the caryophyllene result on MPMS, etc.

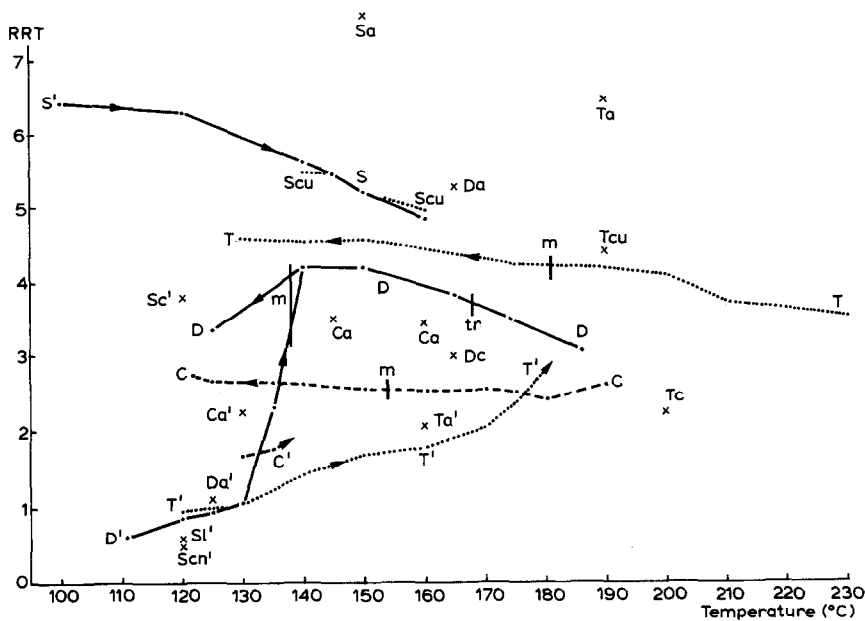


Fig. 3. Plots (connected points) at various temperatures ($^{\circ}\text{C}$) of relative retention times for safrole against linalol (1.00) on the four liquid crystal columns. C = dashes connecting safrole results on $(\text{MBCA})_2$; D = lines connecting safrole results on ADP; S = lines connecting safrole results on MPMS capillary; T = dots connecting safrole results on $(\text{MBT})_2$ taken from ref. 2. Otherwise, as for Fig. 2. a = Anethole; cu = cuminal. Ta is thus the anethole result on $(\text{MBT})_2$, and Dc' is the caryophyllene result on unmelted ADP.

were not passed with (MBT)₂, (MBCA)₂ or MPMS.

Safrole (3,4-methylenedioxyallylbenzene) gives a similar pattern (Fig. 3, plot D¹) on ADP to estragole, with larger relative retention times. These remain below 1 up to 125°C, but rapidly climb to about 4.2 by 140°C, falling away to near 3 past the transition point. Safrole, on this and the other three liquid crystals studied, whether "unmelted", melted or supercooled, is always ahead of anethole (4-propenylanisole), which is the reverse of normal GC phases, and so provides an identifying test for liquid crystals (see Fig. 3, safrole plots and anethole points).

Fig. 2 (plot C) also shows that (MBCA)₂, with the solute estragole, gives a greatly contracted version of the situation previously recorded [2] with (MBT)₂ (plot T). On naive unmelted (MBCA)₂ relative retention times to linalol (C¹) start to rise from unity and level off past the liquid crystal melting point at just under 1.6. These are maintained (plot C) on rapid supercooling, as with (MBT)₂ but unlike ADP. Also as with (MBT)₂, on cooling down, the (MBCA)₂ naive-condition low values are not fully recovered. The only reason for using (MBCA)₂ is that it is suitable for some of the more polar aromatic solutes like cinnamal [1]. It is unwise to heat (MBCA)₂ above 180°C as an increase in relative retention times (Fig. 2), seen before [1], suggests decomposition has started.

From Figs. 2 and 3, the MPMS capillary plots S show the highest relative retention times for estragole and safrole, respectively, of the four liquid crystals. This capillary gives satisfactory peaks at a lower temperature than the packed columns, and shows, as the temperature increases towards its melting point, a fairly steady decline (not rise) in values towards those given by supercooled (MBT)₂ (plots T). MPMS revealed a solute shift involving estragole, not shown by any of the other liquid crystals. Above 130°C geraniol is faster than estragole, but there is a "shift" (see Fig. 2) at lower temperatures to the normal sequence seen on other liquid crystals, even though they yield variable retentions with geraniol which are not given in some tables. Unlike the other three phases, geraniol is always ahead of the sesquiterpene caryophyllene on MPMS (Table III).

The shift terpineol-estragole previously [2] detected on (MBT)₂ and indicated by points Tt on

Fig. 2 seems exclusive to this. On (MBCA)₂ estragole is always ahead of α -terpineol (Table II), whilst the reverse holds for MPMS (Table III). These two solutes have about the same retention on melted ADP (point Dt on Fig. 2), but estragole is well ahead on unmelted ADP as the terpenoid α -terpineol keeps a reasonably "constant" retention relative to linalol of about 2.4. The anethole-thymol shift also seen before [2] on (MBT)₂ could be detected on (MBCA)₂ too (see Table II), but not on the other two liquid crystals, and occurs well above its melting point, unlike on (MBT)₂.

Four new shifts of limited occurrence were noted. (a) Limonene typically is faster than cineole on normal GC phases [6], a lyotropic (mixed) liquid crystal [7] and (MBCA)₂ (Table II); but the reverse holds after melting and supercooling ADP and (MBT)₂ (Tables I and IV and Fig. 2) and is always true for MPMS at low temperatures (Table III and Fig. 3). (b) On MPMS caryophyllene is always faster than safrole (point Sc' in Fig. 3), but this only applies to (MBT)₂ and ADP on melting (Tc and Dc in Fig. 3). Their values are very close on (MBCA)₂ (Table II). On normal polar GC phases, caryophyllene is ahead of safrole, although the reverse holds for non-polar phases [8]. (c) An anethole-cuminal shift can be seen on melting ADP and on naive (MBT)₂ (see Table I and ref. 2). (d) The fourth shift is another practically unique to (MBT)₂ if melted or supercooled, when caryophyllene (Tc, Fig. 2) is ahead of estragole, otherwise their values are close. The reverse applies (see Dc and Sc, Fig. 2) on the other liquid crystals (apart from ADP near 140°C) and on normal GC phases. Both caryophyllene and cineole anomalously show lower relative retention times on supercooling than they do on unmelted (MBT)₂ (Table IV).

Like (MBT)₂, (MBCA)₂ can be used below its melting point, either supercooled or "unmelted". It exhibits the anethole-thymol but not the anethole-terpineol shift [2]; the latter is seen on ADP. However, ADP does not give reliable supercooling and is best used just above its melting point at 140–150°C. MPMS is also best below its "recommended" lower limit of 150°C (just below which temperature it melts) except for polar aromatics like eugenol. Retentions of solutes tend to be variable on MPMS at 150°C or more. It has recently been claimed [9] that polyethyleneglycol 20M can be used

as a stationary phase at 30°C, below its melting point (about 60°C), so this phenomenon may not be peculiar to liquid crystals.

Acyclic monoterpenoids with terminal polar (oxygen function) groups like citral and geraniol do not fit very well into the three non-polymeric liquid crystals, tending to "tail", and should be studied on MPMS. Monoterpenoids with more central (concealed) polar groups like linalol and cyclic α -terpineol give a better liquid crystal "fit" and respond with narrower peaks unless a low temperature is used like 120°C or less on ADP, or 135°C on (MBCA)₂. At higher temperatures, the cyclic monoterpenoid perillal with a protruding polar group at one end gives satisfactory peaks. The non-polar, relatively large bicyclic sesquiterpene caryophyllene, however, shows an unusual rapid increase in relative retention times with temperature rise on ADP and can even fit into it at 120°C, possibly better than on MPMS at the same temperature. MPMS is also poor at resolving the pinenes (bicyclic monoterpene hydrocarbons).

All the liquid crystals are well suited to resolving some linear aromatic substances like estragole, safrole and anethole, as might be expected from their

linear multi-aromatic structures (Fig. 1). However, thymol and eugenol, with protruding polar oxygen-containing groups are not always handled well; MPMS and (MBCA)₂ are the phases of choice here.

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