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Use of a liquid crystal stationary phase at temperatures below its melting point for the gas chromatographic study of some volatile oil constituents

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ABSTRACT

The liquid crystal bismethoxybenzilidine-bitoluidine (BMBT), used as the stationary phase for the gas chromatographic study of some aromatics and a monoterpenoid constituent of volatile oils, gave best results used below its melting point of about 180°C. At these temperatures (120–175°C), relative retention times to linalol were dependent on whether the column was heated from ambient conditions, or cooled from above the melting point. Changes in the sequence of retentions (terpineol–estragole and anethole–thymol "shifts") suggested this liquid crystal may operate by three different mechanisms, dependent on the column treatment. Results are given for fennel and tea-tree oils.

INTRODUCTION

Betts [1] has recorded introductory studies of bismethoxybenzilidineanil-chloroaniline $(MBCA)_2$ liquid crystal stationary phase for the gas chromatographic (GC) examination of aromatic constituents of essential oils. The observed melting point of the commercial $(MBCA)_2$ used was 142°C, and he logically used it after heating to 190°C (the maximum before decomposition became likely) and cooling it down to operational conditions. However, Betts also recorded "normal" results for three aromatic oil constituents and two mono-terpenes at 135°C, below the liquid crystal melting point when it should, theoretically, no longer function as a liquid stationary phase. He remarked [1] "it is amazing that $(MBCA)_2$ ... functions as a stationary phase ... even as low as 50°C". This penomenon was noticed originally by Dewar and Schroeder [2], who were jointly the first in 1964 to publish on liquid crystal GC. Their liquid crystal had a melting point of 120°C, but still resolved methyl isomers of methyl benzoate at 115°C, and they suggested that the "stationary liquid phase had supercooled and was still in the nematic (liquid crystal) form". Two years later Barrall *et al.* [3] obtained good separations of benzene derivatives and paraffins operating liquid crystals at well below their melting points (*e.g.*, at 49°C using a liquid crystal melting at 88°C) and explained this by the mixed liquid crystal and support "substrate results in a general lowering of all transition temperatures" (*i.e.*, melting point of the liquid

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crystal). This phenomenon was confirmed by others [4,5], and Lester and Hall [6] went so far as to say "it is clear that the liquid crystal stationary phase must be used in the supercooled state ... (which) dramatically improves the resolution" for dodecadienyl acetates. Their liquid crystal had a melting point of 167.5°C, but was used down to 100°C after being "conditioned" just above this at 180°C.

"The properties of the supercooled region" were studied by Wasik and Chesler [7] using as liquid crystal a non-chlorinated dimer of a di-aromatic anil BMBT or $(MBT)_2$ with a higher melting point than that used by Betts [1]. Plotting retention data against temperature they observed a hysteresis loop for dimethylnaphthalenes in which retention values at a particular temperature (below the liquid crystal melting point of 181°C) were higher if the column was cooled to this point rather than heated to it. This applied down to 120°C, which they commented "essentially extends the temperature range of the liquid crystal column (downwards, with) no abrupt change in (the separation factor) α (values) at or near 181°C." They gave results at 130°C. Haky and Muschik [8] recorded that $(MBT)_2$ could be used down to 115°C for resolving dimethylnaphthalenes and commented that "interactions of the support with the liquid crystal ... and the existence of supercooled nematic thermal regions ... lower the minimum operating temperatures (so that) the versatility of such columns is increased."

In the course of our GC studies of essential oils it seemed of interest to see how this 'supercooled' dimer $(MBT)_2$ behaved with them. Witkiewicz and Waclawczyk [9] classify this as a "high-temperature" liquid crystal and found that similar liquid crystal dimers gave retention times for dimethylnaphthalenes which were "longer during cooling of the column than during heating". Plots of retention time versus increasing column temperature revealed, in some cases, transition temperatures by changing in direction. With about 2.5% liquid crystal column loading "the relative retention times are highest in the solid-state temperature region... In some instances they are similar to those observed with conventional stationary phases".

EXPERIMENTAL

Apparatus

A Pye Unicam GCD gas chromatograph fitted with flame-ionisation detector and wide-range amplifier with a Hewlett-Packard 3380A recorder/integrator were used. Three glass columns (1.5 m × 4 or 2 mm I.D.) were packed with Supelco Chromasorb WAW 80/100 mesh coated with 3% $(MBT)_2$ which was purchased as N,N′-bis(*p*-methoxy-benzylidine)-α,α′-bi-*p*-toluidine (BMBT) from TCI (Tokyo, Japan) (melting point observed 179°C). This is the di-(anisole-anil) derivative $(CH_3O–C_6H_4–CH=N–C_6H_4–CH_2–)_2$. No preliminary 'conditioning' heating was applied to obtain the values given in the lowest portion of Table I (see Results below). Nitrogen was used as mobile phase at a slow flow of about 7.5–10 ml min^{-1}.

A Technoterm 7300 probe was used to observe oven temperatures with a reading to 0.1°C if below 200°C, and to 1°C if above.

Materials and methods

Anethole, estragole (4-allylanisole), linalol and thymol were obtained from Sigma. Also used were α-terpineol (TCI), safrole (Fritzsche), and cuminal (*p*-isopropyl-

benzaldehyde, Eastman). These were injected from a micro-syringe which had been filled and then "emptied" of the substances. The solid thymol was injected in strong solution in ethanol. Retention times were determined, after deducting hold-up time. Various isothermal conditions were used, as detailed in Table I.

About 0.1 μl was injected of a Western Australian fennel oil, which is an old specimen donated by a Perth pharmacy, of unknown origin. The same volume was injected of the Australian antiseptic 'Thurday Plantation' tea-tree oil (distilled from *Melaleuca alternifolia*).

RESULTS AND DISCUSSION

Average results are presented in Table I and Figs. 1 and 2. They are calculated as retentions relative to linalol, which is preferable to using simple retention times or more complex presentations. Linalol once again proved an ideal GC standard, as it has in the past for conventional and liquid crystal phase work [1,10]. Linalol has a reasonably short retention time, giving sharp, sensitive peaks, and is easy to fill and empty from the micro-syringe, for it is not viscous.

These results reflect trends rather than absolute values, as some variation is found from one run to another, particularly in the region of the liquid crystal melting

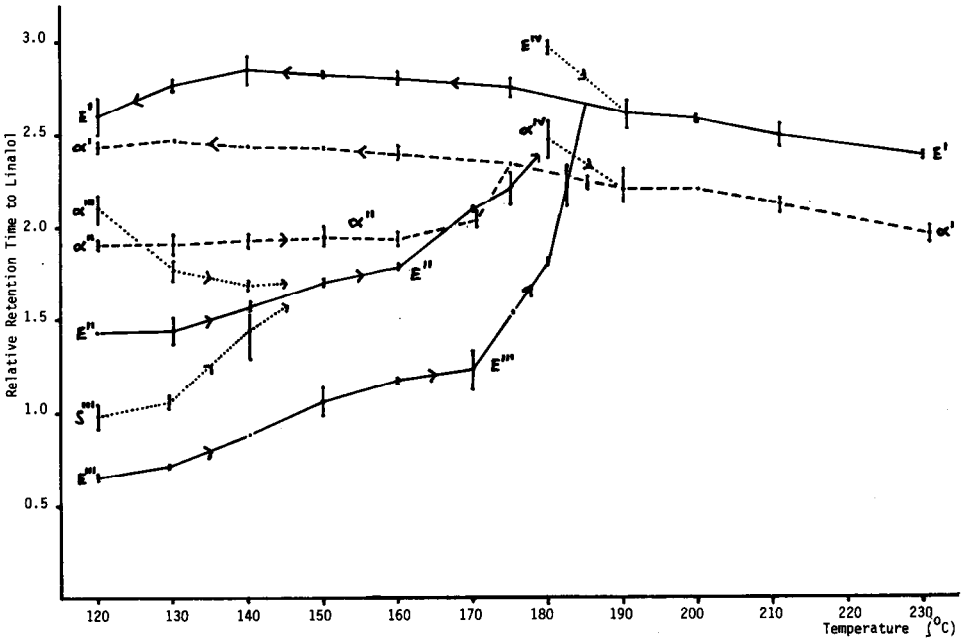

Fig. 1. Average relative retention times to linalol for some test solutes over a range of isothermal column temperatures in °C. Vertical bars depict range of observations. α = α-Terpineol; E = estragole; S = safrole. Suffix [i]: results after the liquid crystal was heated above 190°C; [ii]: results on heating from cold the liquid crystal which had been previously heated above 190°C; [iii]: results on a 'naive' liquid crystal which had not been previously taken above 190°C; [iv]: results are unusually high and sometimes obtained on heating the liquid crystal.

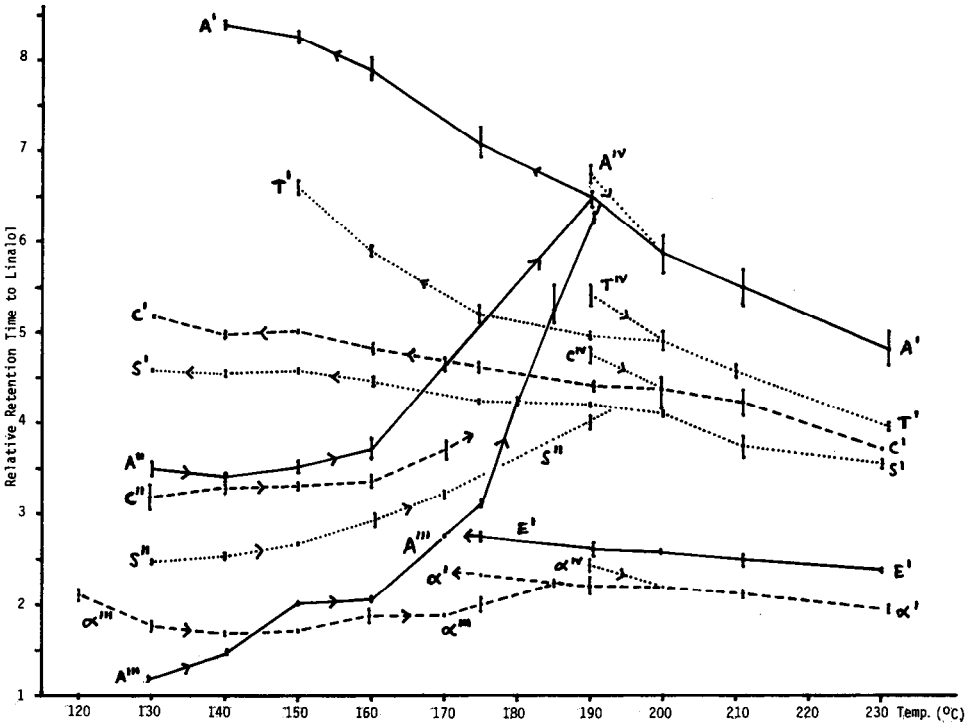

Fig. 2. As for Fig. 1. A = Anethole; C = cuminal; T = thymol. For clarity, the full results from Table I are not shown.

point. However, close agreement was observed in some cases, *e.g.*, estragole at 160°C. Celsius rather than absolute temperatures were used as being more practical for chromatographers.

In Table I, some aromatic substances always emerge in the same sequence: estragole (quickest)–safrole–cuminal–thymol (slowest). The positions of α-terpineol and anethole change in relation to this sequence, and are noteworthy.

Fig. 1 displays average results for the linear aromatic substance estragole, which typify the characteristics of this $(MBT)_2$ liquid crystal column. A newly prepared, never-before heated column behaves in a different way from one which has been used above the melting point of the liquid crystal. At temperatures up to about 150°C with the new column, estragole shows shorter retention time than linalol, slowly increasing to about 170°C, after which retention quickly increases to almost three times that of linalol by 185°C. It then settles down with further temperature increase to about 2.6–2.4 times the retention of linalol. If the liquid crystal column is now cooled, estragole retention time gradually increases to about 2.8 times linalol from 175 down to 140°C, below which temperature estragole retention decreases towards that of linalol at about 85°C. Either this procedure, or just cooling overnight, gives a return to initial lower relative retention times, but not to the 'naive' values below unity seen with the freshly prepared column. These very low values cannot be obtained again, even though the column is kept in a freezer for 16 h. Retention times

in the 'mature' used column are more than 1.4 times linalol from 120°C up, rising to more than two times at 170°C before climbing to the levels given above. This suggests that the effect of estragole on the melting point of this liquid crystal on the GC support is apparent from 170°C or less, and below its melting-point-apparatus value (179°C). If the liquid crystal column is pre-heated at 210°C, and the relative retention time of estragole observed with gradual increase of temperature from 170°C, there is a slight increase in value at 180°C, confirming this as the melting point. Near this temperature, estragole, like most of the solutes studied, sometimes gave relative retention times above the normal. They are shown in Table I and Figs. 1 and 2. With estragole and α-terpineol these occurred at the liquid crystal melting point, but above this with other solutes, at 190°C.

The propenyl isomer of estragole, anethole, shows a more extreme form of this cycle of behaviour (Fig. 2). The naive column at 130–160°C shows retention times for anethole just over one to two times linalol, rising quite sharply then to over six times linalol at 190°C. Retention now drops away, with further temperature increase, to less than five times that of linalol at 230°C. Cooling this hot column gives an increase in relative retention times to over eight, below 155°C. With the 'mature' column left to cool overnight, the 'unmelted' liquid crystal now gives initial relative retention times increased to about three-and-a-half from 130 to 160°C, before climbing rapidly to joint the cooling plot.

The monocyclic monoterpenoid α-terpineol (Table I and Fig. 1) shows these changes apply to this non-aromatic too, although with a shallower behaviour cycle than estragole. There is an interesting 'cross-over' response, in that with the column heated up to only 160°C or less, α-terpineol has higher retention times than estragole, as with conventional GC phases [10], but that above this temperature estragole shifts to show higher retention times which are maintained during subsequent heating and supercooling. This 'terpineol–estragole shift' is reversed if the column is cooled below 85°C, and so indicates the 'condition' of true or pseudo liquid crystal. Results in Table I show that with a 'naive' column, as well as estragole, some other aromatics like safrole and anethole, also with unbranched side chains, have shorter retention times than α-terpineol up to about 145°C, but these cannot be reobtained if the liquid crystal has been melted once.

As was previously observed with the $(MBCA)_2$ column [1], the sequence of certain aromatics above the liquid crystal melting point is estragole (always first), then safrole–thymol–anethole (last). On a polyethylene glycol column anethole precedes safrole [10]. If the liquid crystal column is not heated above 165°C, thymol is always 'conventionally' behind anethole (Table I) although safrole does not shift. This 'anethole–thymol shift' was previously detected on $(MBCA)_2$ [1], although this liquid crystal has a lower melting point than $(MBT)_2$. Only above the melting point does the matching anisole character of the liquid crystal preferentially retain anethole. This 'shift' provides a second check on the liquid crystal condition. Cuminal has a branched isopropyl side chain, like thymol, but does not show any 'shift' in the mature column. Cuminal behaves like safrole (Fig. 2), which has an allyl side chain. Oddly, this GC system seems less sensitive to injections of cuminal than of other aromatics.

An interesting observation is that thymol behaves differently to cuminal and to the other aromatic solutes studied, which must reflect not so much its molecular

TABLE I

RELATIVE RETENTION TIMES (LINALOL = 1.00)[a] ON A PACKED COLUMN OF 3% $(MBT)_2$

Mobile phase, nitrogen at a flow-rate of 7.5 to 10.0 ml min^{-1} at the flame ionisation detector outlet. Averages of two observations, or in parentheses, the number of observations made.

Solute	Nominal column temperature (°C)												
	120	130	140	150	160	170	175	180	185	190	200	210	230
After heating of liquid crystal above 190°C													
Anethole[b]			8.39	8.26	7.88 (3)		7.06 (3)			6.49 (3)	5.87 (3)	5.49 (4)	4.81 (3)
Thymol				6.60	5.88		5.19			4.95 (3)	4.90	4.57	3.96
Cuminal		5.08	4.98	5.01	4.82		4.62			4.41 (3)	4.38 (4)	4.22	3.71
Safrole		4.59	4.55	4.58	4.46		4.24			4.20	4.10	3.74 (5)	3.55
Estragole	2.60	2.77	2.85	2.83	2.80 (5)		2.75 (3)			2.61 (7)	2.58	2.49 (4)	2.38
α-Terpineol[b]	2.43	2.47	2.44	2.43 (3)	2.39 (3)		2.34			2.20 (6)	2.20	2.12 (3)	1.96
"Mature" liquid crystal heated from cold with a history of heating above 190°C													
Thymol		5.49	5.02	4.71	4.53 (3)	4.58				5.40[c]			
Anethole[b]		3.50	3.41	3.53	3.72	4.65				6.74[c]			
Cuminal		3.18	3.28 (3)	3.30	3.35	3.70				4.72[c]			
Safrole		2.47	2.53	2.67	2.93	3.21				4.00			
α-Terpineol[b]	1.90	1.90	1.92	1.94 (4)	1.94	2.03 (3)		2.47[c]					
Estragole	1.43	1.44	1.57 (3)	1.70	1.79	2.09 (3)	2.21	2.97[c]					
"Naive" liquid crystal never heated to 190°C													
Thymol				3.21	3.17 (3)	3.41	3.73						
Cuminal					2.76	2.98							
α-Terpineol[b]	2.10	1.77	1.69	1.71	1.87	1.88	2.00		2.23	2.43[c] (3)			
Anethole[b]		1.18	1.46	2.02 (3)	2.07	2.75	3.10	4.25	5.25 (3)	6.25 (4)			
Safrole	0.97	1.05	1.44	1.70	1.80	2.06	2.38	2.81					
Estragole	0.65	0.71	0.88	1.05	1.16	1.23	1.54	1.81	2.22				

[a] After subtraction of holdup time, retention times for linalol were approx. 1.0 min at 120°C (from cold), 0.25 min at 190°C, 0.1 min at 230°C; then on cooling 0.4 min at 160°C, and 1.3 min at 120°C.

[b] Anethole and α-terpineol shift in retention position in relation to the other constant solutes.

[c] These unusually high values are sometimes seen.

shape, which is like cuminal, but its polar phenolic nature. With a mature column heated from 130°C upwards there is a drop from initial high relative retention times (Table I) to a minimum at about 160°C. This is unlike the continuous slow initial increase shown by the others. Cooling from above the liquid crystal melting point gives only a limited usable temperature range for thymol down to 150°C, below which a rapid increase in relative retention time occurs. Other substances studied level off in such values at this temperature, and estragole then shows a decline at lower temperatures, as does safrole.

This $(MTB)_2$ column could certainly be used at 120°C or lower for some solutes, but gave best results for the aromatic substances studied at 130°C or above, as noted by Haky and Muschik [8]. Use above the melting point of the liquid crystal (*i.e.*, above 180°C) gave retention times that were too short, and generally best results were found on cooling down to 140–175°C after preliminary heating at 210°C. As safrole is always ahead of anethole, this indicates that the liquid crystal is not like a conventional GC stationary phase whether melted or not. This is confirmed by the terpineol–estragole and anethol–thymol shifts. Another solute shift, of anethole–terpineol, is seen only in the naive column (Fig. 2) at 145°C, and may indicate some change in the liquid crystal condition then. These pairs of substances do not occur together in natural volatile oils, and peaks from such oils can be anticipated to emerge in the sequence seen from a conventional phase. Thus, the bicyclic monoterpenoid fenchone in fennel oil appears after the monoterpene hydrocarbons present and before estragole, as usual (Fig. 3). This is an atypical oil, yielded by an unusual chemovar [11], rich in estragole.

The $(MBT)_2$ column did not yield peaks of cinnamal, eugenol or vanillin after their injection, even at 290°C, so it firmly retains some aromatics, and would be no use for analysis of cinnamon, clove or pimento oils. However, it does handle some monoterpenoids well such as the fenchone in fennel oil (Fig. 3), and it resolves α-terpineol from terpinen-4-ol (α value 1.25 or more) in tea-tree oil (Fig. 4), which is not well achieved by a programmed methylpolysiloxane capillary (α value found only 1.04). Best resolution of fenchone is obtained if the liquid crystal column is directly heated to 140°C from cold, and not melted. Similarly, the *ca.* 3% α-terpineol in tea-tree oil is better resolved from the *ca.* 40% terpinen-4-ol just preceding it on the unmelted liquid crystal at 120°C.

The liquid crystal column was evaluated for its polarity by the method in use in these laboratories [12], utilising the average of retention indices for linalol, estragol and carvone against both *n*-alkanes and *n*-alcohols. Against the standard phenylmethylpolysiloxane-packed column the liquid crystal gave a value of −41 when heated (as a 'mature' column) from ambient conditions to 160°C; then only −24 when cooled to 160°C from 230°C. These values are between those of a fully methylpolysiloxane column and the standard column, so this liquid crystal behaves as a non-polar GC phase which becomes less non-polar after heating above its melting point. This agrees with the observations of Isenberg *et al.* [13], who found that some di-ester aromatic liquid crystals "correspond to silicone phases of low polarity" using Rohrschneider solute probes.

The $(MBT)_2$ was clearly a better liquid crystal column than the previously used $(MBCA)_2$ [1] for the GC analysis of many volatile oils, due to its higher melting point allowing the use of a range of temperatures below this, both cooled from above it, or

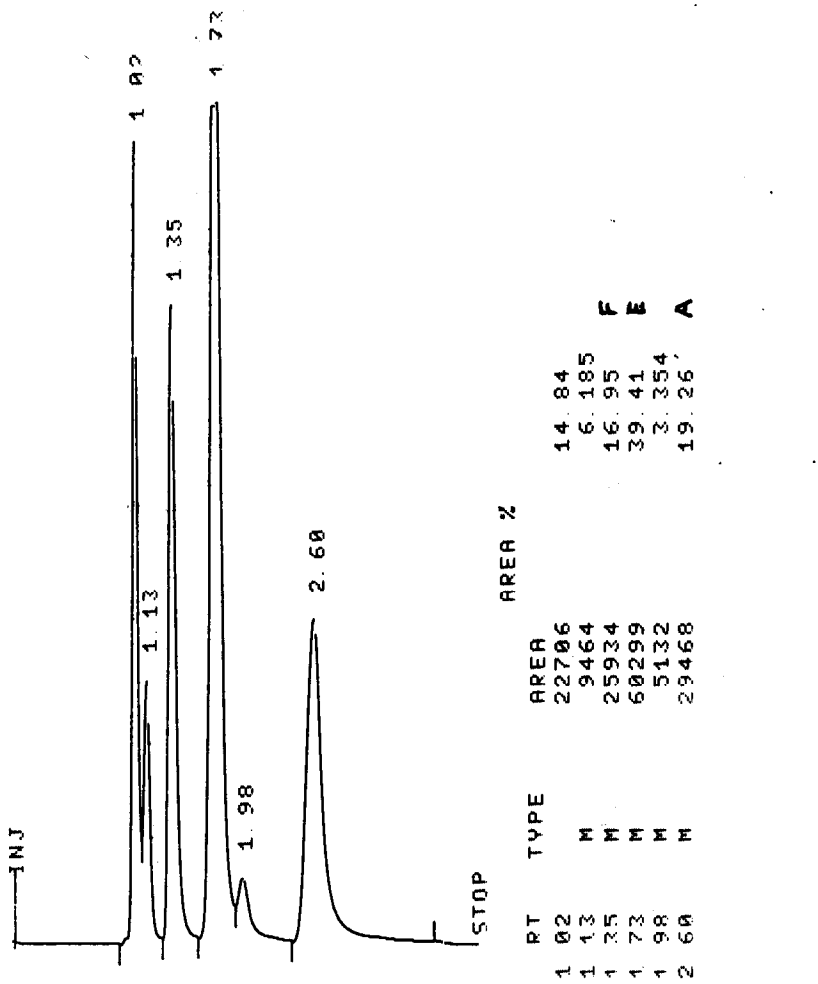

Fig. 3. Gas chromatogram of Western Australian fennel oil on $(MTB)_2$ at 140°C on a mature liquid crystal heated from cold. A = Anethole; E = estragole; F = fenchone. Early peaks are monoterpene hydrocarbons. RT = Retention time in minutes; type = peak merged (M) with previous one and tangent (T) skimmed baseline; area% given at right.

heated from ambient conditions. From the different sequences of the substances indicated in Table I, three different separation mechanisms may be involved, depending on the liquid crystal column temperature and its history. After the liquid crystal has melted, it is able to 'supercool' to a considerable extent due to interaction with the column support [3,8]. The lower temperature use of this column is restricted more by excessively long retention times producing broad peaks than by any sudden change in physical condition by the liquid crystal. Before melting, it is still a useful GC stationary phase, and we suggest that the moving band of solute traversing the column is able to form a temporary eutectic liquid mixture with the unmelted liquid crystal. If it has been previously heated above its melting point, the liquid crystal has left a more

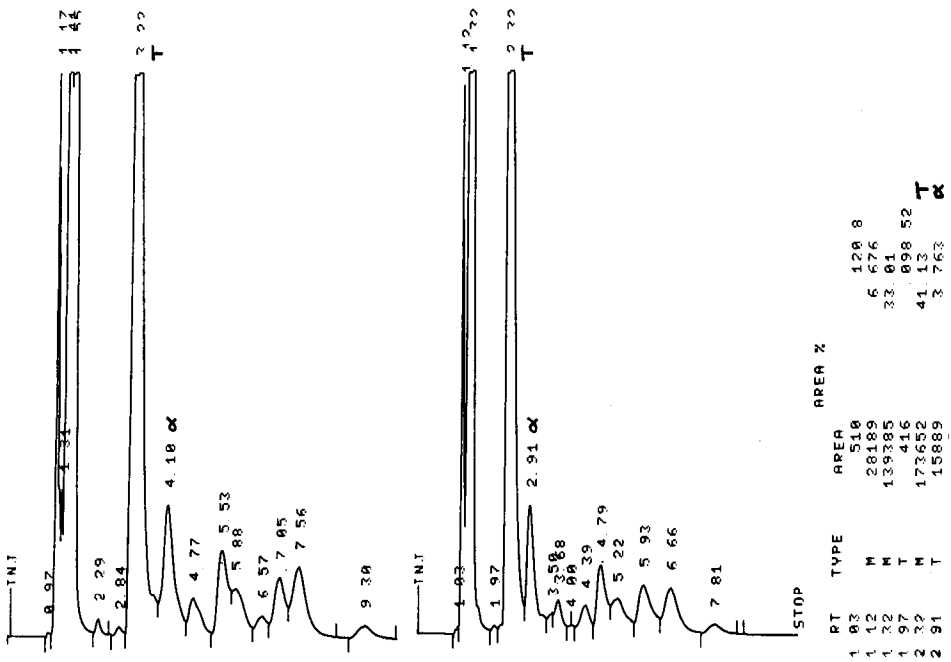

Fig. 4. Gas chromatograms of Australian tea-tree oil on $(MBT)_2$. Left: 120°C after 'supercooling' from 210°C (no printout). Right: 120°C on a mature liquid crystal heated from cold. α = α-Terpineol; T = terpinen-4-ol. Early peaks are monoterpene hydrocarbons, particularly terpinenes. See Fig. 3 for abbreviations. The complete area% printout is not shown, and at the temperature used *ca.* 5% of slow-moving oil constituents are retained on the liquid crystal.

organised stationary phase than that in a never-melted column, and this resembles the true liquid crystal condition during the transient eutectic state with passing bands of estragole, safrole, cuminal and thymol, although not for α-terpineol or anethole. If an 'overload' of a solute is injected it usually gives a delay in retention, as seen on conventional GC phases, when the liquid crystal has not been heated to its melting point. If this occurs after melting, the 'overload' causes a decrease in retention, typical of liquid crystals. This was noted by Betts [1] on another liquid crystal.

A general conclusion from this is that, for GC work, the nematic temperature range of a liquid crystal (from its melting point, to when it becomes a normal isotropic liquid) is irrelevant, as it functions well at lower temperatures. More important to the analyst is knowledge of the particular behaviour of each test solute on the liquid crystal, and details of 'solute shifts' which indicate a change of liquid crystal condition. There should be no hesitation in using a liquid crystal column below its melting point, although temperatures approaching the melting point in a column heated from cold should be avoided, as relative retention times can undergo rapid increase.

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