

Note

Possible value of a liquid crystal stationary phase for the gas chromatographic study of aromatic constituents of essential oils

T. J. BETTS

School of Pharmacy, Curtin University of Technology, GPO Box U1987, Perth, Western Australia 6001 (Australia)

(First received January 8th, 1990; revised manuscript received April 5th, 1990)

This author has previously recorded¹ the use of a mixed liquid crystal as a gas chromatographic (GC) stationary phase for resolving the most volatile constituents of essential oils such as limonene, cineole, etc. The potential of liquid crystal phases for studying the other less volatile constituents of essential oils has not been studied, and an initial evaluation is given here. These oils are often complex mixtures of aromatics and terpenoids, and results on liquid crystals could usefully supplement those obtained using conventional isotropic phases with their randomly distributed molecules.

In 1965, Dewar and Schroeder² described the use of eight liquid crystals for the GC of aromatic solutes and commented that “the effectiveness of II (a chlorinated tetra-aromatic di-anil) as a stationary phase agreed with our expectations, since II has a very long nematic (existing as a liquid crystal) range (180°) and a high transition temperature” at which it changes to a normal isotropic liquid. Although they record that “II decomposed slowly even at temperatures just above its melting point” they used it at 200°C as well as 158°C.

As this liquid crystal “II” is relatively cheap and commercially available under various names, it was selected for an initial study, despite its possible decomposition hazard, but only used up to 190°C. It is $(\text{CH}_3\text{O}-\text{C}_6\text{H}_4-\text{CH}=\text{N}-\text{C}_6\text{H}_3\text{Cl})_2$ and is bis-4-(4-methoxybenzylideneanil-2-chloroaniline) (MBCA)₂.

EXPERIMENTAL

Apparatus

A Pye GCD gas chromatograph fitted with flame ionisation detector and a Hewlett-Packard 3380A recorder/integrator were used. A glass column (1.5 m × 4 mm I.D.) was packed with 2.5% (MBCA)₂ [*p*-dianisal-3,3'-dichlorobenzidine (TCI, Tokyo, Japan), melting point observed 142°C]. The weighed (MBCA)₂ was dissolved in dichloromethane and taken to dryness in a rotary evaporator with weighed Chromosorb W AW, 80–100 mesh. The column, after packing, was heated at 190°C

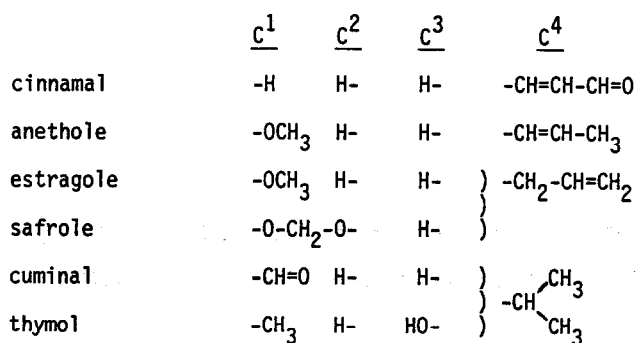


Fig. 1. Formulae of some benzene derivatives studied.

TABLE I

RELATIVE RETENTION TIMES (LINALOL = 1.00 AFTER SUBTRACTION OF HOLD-UP TIME)^a ON PACKED COLUMN OF 2.5% (MBCA)₂

Mobile phase, nitrogen at a flow-rate of 3.75 to 5.00 ml min⁻¹ at the flame ionization detector outlet. Averages (in italics) and ranges of values shown.

<i>Essential oil constituent (and source)</i>	<i>Relative retention time</i>				
	<i>Column temperature (°C)</i>				
	<i>134.7</i>	<i>149.8</i>	<i>160.3</i>	<i>175.4</i>	<i>189.3</i>
	134.2-135.2	148.0-151.6	159.0-161.3	174.7-176.3	186.8-191.1
<i>α</i> -Pinene (BDH)	<i>0.15</i>	<i>0.18</i> 0.16-0.19	<i>0.20</i> 0.18-0.22	<i>0.19</i> 0.18-0.20	<i>0.25</i> 0.21-0.27
1,8-Cineole (Faulding)	<i>0.37</i>	<i>0.38</i>	<i>0.46</i> 0.44-0.48	<i>0.46</i> 0.44-0.50	<i>0.52</i> 0.47-0.55
Estragole (Sigma)	<i>1.58</i> 1.53-1.63	<i>1.58</i> 1.52-1.63	<i>1.71</i> 1.63-1.76	<i>1.58</i> 1.52-1.65	<i>1.81</i> 1.69-1.91
Safrole (Fritzsche)	<i>2.67</i> 2.61-2.70	<i>2.61</i> 2.45-2.77	<i>2.55</i> 2.41-2.73	<i>2.51</i> 2.45-2.59	<i>2.63</i> 2.53-2.79
Thymol (Sigma)		<i>3.92</i> 3.87-3.98	<i>3.40</i> 3.24-3.61	<i>3.27</i> 3.15-3.35	<i>3.06</i> 2.87-3.19
Cuminal (Eastman)			<i>3.90</i> 3.66-4.24	<i>3.45</i> 3.30-3.59	<i>3.61</i> 3.21-3.91
Anethole (<i>trans</i>) (Sigma)	<i>3.74</i> 3.64-3.82	<i>3.73</i> 3.62-3.85	<i>3.78</i> 3.50-4.07	<i>3.48</i> 3.25-3.64	<i>3.64</i> 3.42-3.83
Perillal ^b (Koch-Light)			<i>4.95</i> 4.46-5.26	<i>4.50</i> 4.40-4.55	<i>4.84</i> 4.74-5.00
Cinnamal (Ajax)				<i>9.02</i> 8.70-9.44	<i>9.45</i> 9.00-9.63

^a Average retention times for linalol (Sigma) at each of the above temperatures were 0.79, 0.44, 0.32, 0.25 and 0.19 min, respectively.

^b Perillal is not an aromatic substance but an unsaturated cyclic mono-terpenoid structurally like cuminal.

for a few hours with a flow of mobile phase (nitrogen) before analytical use. It could be used repeatedly.

A Technoterm 7300 probe was used to observe oven temperatures to 0.1°C.

Materials

The materials used are indicated in Table I and Fig. 1.

Method

Repeated injections were made from a microsyringe which had been filled then "emptied" of materials. Relative retention times were calculated against linalol, a "standard" used previously³, after deducting hold-up time. Various column temperatures (isothermal) were used, indicated in Table I.

RESULTS AND DISCUSSION

Results are given in Table I. An unusually low mobile phase flow-rate of 5 ml min^{-1} or less was needed, and then sharp symmetrical peaks were only obtained with aromatic substances and with compounds having short retention times. Terpenoids such as carvone, citral and geraniol exhibited considerable "tailing" and were subject to excessively varying relative retention times, thus appearing unsuited to analysis on $(\text{MBCA})_2$. The terpenoid perillal was, however, satisfactory. The variations often involved an atypical increase in retention time as the quantity of solute injected was reduced. Dewar and Schroeder² recommended using small samples, and this was found necessary in this study to approach consistent results. The short retention times involved also increased the chance of variation.

Six of Dewar and Schroeder's liquid crystals² were di-, tri- or tetra-aromatics, and it is likely that the use of such phases for GC will be best for aromatic solutes, as found here. Ignoring estragole, which always emerges first, results for some aromatics on $(\text{MBCA})_2$ are safrole (first) — thymol — anethole — cinnamal (last). This is interestingly different from results on polyethylene glycol³ where anethole precedes safrole, and thymol is a long last. It seems that the double bond conjugated with the aromatic ring present in anethole and cinnamal (see Fig. 1) is preferentially retained by $(\text{MBCA})_2$. On polyethylene glycol it is the polar nature of the phenol (thymol) and aldehyde (cinnamal) groups which show greatest affinity, so deciding the elution sequence.

Liquid crystals have been used for the GC resolution of geometric isomers since 1977, when Vetrova *et al.*⁴ separated *cis*- from *trans*-decalin. It was thus disappointing that $(\text{MBCA})_2$ used here failed to resolve *cis*- from *trans*-citral (neral from geraniol). Nor did it resolve the *dextro*- and *laevo*-isomers of carvone and linalol.

At 80°C $(\text{MBCA})_2$ resolved cineole from α -pinene (separation factor, $\alpha = 3.15$) almost as efficiently as the previously used¹ mixed liquid crystal ($\alpha = 3.45$). It is amazing that $(\text{MBCA})_2$ with a melting point above 140°C functions as a stationary phase at a temperature well below this, even as low as 50°C, where it resolves β - from α -pinene. However, Witkiewicz⁵ records, in his recent review, that "several examples are known of the use of liquid crystals in the solid state". $(\text{MBCA})_2$ gave "normal" results at 135°C, just below its melting point (given as 154°C by Dewar and Schroeder² for their synthesised product), fitting into the pattern given at higher temperatures

when it had reliably become a liquid crystal. The melt, observed on a microscope slide between crossed polarisers, showed a striated birefringence of liquid crystallinity, with spherulites of solid growing in it as it cooled.

A change in behaviour was observed for (MBCA)₂ at temperature above 175°C, when relative retention times increased instead of decreasing as usual, apart from thymol. This has been observed by Janini *et al.*⁶ using another di-anil liquid crystal GC phase for which "retention increases due to the enhanced retention property of the nematic state".

Substances with short retention times gave relative (linalol) retention times on (MBCA)₂ falling between those previously observed³ on packed columns of conventional phases. The low-polarity solute cineole shows the highest relative retention time on the low-polarity methylpolysiloxane, whilst "polar" aromatic solutes like estragole and safrole show the highest relative retention times on the polar polyethylene glycol. Other aromatic solutes with longer retention times give greater relative retention times on (MBCA)₂ than on either conventional phase, although these are still fairly brief retentions.

(MBCA)₂ thus may have potential for the analysis of essential oils which are rich in aromatic constituents such as aniseed, basil, cinnamon, cumin, fennel, sassafras, tarragon and thyme, where it could supplement conventional phase work. It does not seem suited to the GC of oils containing terpenoids apart from coriander and lavender, which contain linalol.

ACKNOWLEDGEMENT

Thanks are due to Mr. B. MacKinnon for preparing the liquid crystal column.

REFERENCES

- 1 T. J. Betts, *J. Chromatogr.*, 467 (1989) 272.
- 2 M. J. S. Dewar and J. P. Schroeder, *J. Org. Chem.*, 30 (1965) 3485.
- 3 P. N. Breckler and T. J. Betts, *J. Chromatogr.*, 53 (1970) 163.
- 4 Z. P. Vetrova, N. T. Karabanov and I. A. Jashin, *Chromatographia*, 10 (1977) 341.
- 5 Z. Witkiewicz, *J. Chromatogr.*, 466 (1989) 37.
- 6 G. M. Janini, G. M. Muschik and W. L. Zielinski, *Anal. Chem.*, 48 (1976) 809.