

Note

Use of an inexpensive low-temperature liquid crystal stationary phase for the study of volatile oil constituents with short gas chromatographic retention times

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I have previously reported on the use of gas chromatographic (GC) relative retention times to characterise the various constituents of volatile oils: long retention time (t_R) constituents such as aromatics and sesquiterpenoids compared to geraniol¹; and medium t_R constituents such as monoterpenoids against linolol^{2,3}. Whilst the relatively shorter t_R aromatic anethole or the relatively longer t_R monoterpeneoid citronellol can be examined against either geraniol or linolol, the ability of the latter to characterise monoterpene hydrocarbons with their very short t_R is limited by the discriminatory ability of the two usual stationary phases. These stationary phases are substituted polysiloxanes and polyethyleneglycols, and they function as normal liquids, which are isotropic in that the molecules of their fluids are randomly arranged, and they reveal inability to distinguish between substances such as limonene and cineole, despite their different molecular shapes. Such shapes, however, should provide discrimination for liquid phases with an ordered molecular arrangement, as is provided by liquid crystals.

The use of liquid crystals for GC stationary phases dates back to the early 1960s. Both the early studies^{4,5} used azoxy-di-(aromatic ethers). Polysiloxanes with aryl, triaromatic side chains are the modern form of liquid crystal phases⁶ but these all only change from the glassy to the desired smectic state, with layers of parallel molecules, at about 120°C or more. Witkiewicz⁷ reviewed the situation in 1982 pointing out that “so far only thermotropic liquid crystals have been used (where) the liquid crystalline state is established after the solid is melted. The resulting liquid preserves, in a certain temperature range, properties intermediate between those of the solid and the liquid”. This molecularly ordered liquid crystal is “well suited for the separation (of) many substances (*e.g.* isomers) that have identical or very similar boiling temperatures (while) their separation on ... most conventional (especially non-polar) stationary phases ... is very difficult or impossible”. He commented “that certain lyotropic systems could also find application in GC, for instance in the form of mixed phases. (These) show liquid crystalline properties over a wide range of concentrations in a suitable solvent”. They should be useful at temperatures below 100°C, which are appropriate for examining the monoterpene hydrocarbons that occur in volatile oils.

Friberg *et al.*⁸ have studied the solubilization of hydrocarbons in lyotropic solutions of lecithin in ethylene glycol. Recently, Belmajdoub *et al.*⁹ have recorded the phase behaviour of lyotropic solutions of cetyl-trimethylammonium bromide (CTAB) in formamide, and a 50% (w/v) solution of CTAB is examined here for its GC potential as stationary phase for resolving monoterpene hydrocarbons and other short t_R constituents of volatile oils. Byrd and Acree¹⁰ were the first to use such a phase and found in 1988 that *p*- and *o*-xylenes were resolved on this stationary phase. They also found that nitropropane and butanone have shorter t_R than xylenes on such a column¹¹, which is the reverse of what occurs with conventional isotropic liquid phases.

EXPERIMENTAL

Apparatus

A Pye GCD gas chromatograph fitted with flame ionisation detector and Hewlett-Packard 3390A and 3380A recorder integrators were used. Glass columns (1.5 m × 4 mm I.D.) were packed as indicated in Table I. The weighed CTAB and formamide were dissolved in acetone and dried with the weighed support in a rotary evaporator. This column needs to be freshly prepared and not subjected to high temperature pretreatment. In contrast, the two polysiloxane columns have had extensive previous use.

Materials

The materials used are indicated in Table I.

Method

Repeated injections were made from a microsyringe which had been filled and "emptied" of materials, after rinsing with *n*-heptane. This gave a "standard" peak for the alkane along with the test solute.

TABLE I

RELATIVE RETENTION TIMES (α -PINENE = 1.00) AT 80°C ON PACKED COLUMNS

Mobile phase, nitrogen at a flow-rate of ca. 40 ml min⁻¹ at the flame ionization detector exit.

Volatile oil constituent (and source)	t_R vs. α -pinene (BDH)		
	Fresh 15% CTAB-formamide (1:1) ^a	Fully methyl polysiloxanes (old) ^b	
		2% SP-2100	2% OV-1
<i>n</i> -Heptane ^c (Mallinckrodt)	0.31 – 0.32 – 0.33	0.37	0.49
β -Pinene (turpentine oil)	1.47 – 1.48 – 1.50	1.25	1.19
Limonene (BDH)	2.21 – 2.27 – 2.40	1.69	1.59
<i>p</i> -Cymene (TCI)	2.90 – 3.04 – 3.21	1.60	1.54
1,8-Cineole (Faulding)	3.17 – 3.45 – 3.72	1.69	1.58

^a Average and range of six results on different columns with t_R for α -pinene 1.61 – 2.18 min. Support was Chromosorb WAW 80–100.

^b Supports were Chromosorb WAW DCMS 80–100 for SP-2100, and Diatomite CQ 120–150 for OV-1.

^c *n*-Heptane was used as standard.

RESULTS AND DISCUSSION

Results are presented in Table I. About 80°C was found to be the most satisfactory operating temperature for the CTAB-formamide liquid crystal phase. Formamide is presumably lost during use as the column packings needed replacing periodically to restore performance. Any such "bleed" of formamide will not affect the detector, as it does not respond to substances like formic acid. Byrd and Acree¹¹ indicate that a relative decrease in the formamide present may convert the mixed liquid crystal to a lamellar mesophase from the initial hexagonal one, although they found that "retention times were reproducible over a two-week period".

The non-polar fully methyl polysiloxanes should show the greatest possible discrimination between the very low polarity constituents of volatile oils examined that can be achieved with conventional liquid stationary phases. This is very poor despite their different molecular shapes and chemical natures. Limonene is a fairly flat monocyclic hydrocarbon, cymene is a flat aromatic hydrocarbon and cineole is a box-like dicyclic ether. Table I indicates that the inexpensive liquid crystal phase improves the resolution between such substances, and it was found that mixtures of these three could be resolved on a packed column of CTAB-formamide with the recorder returning nearly to baseline between the three peaks, something unachievable on the methyl polysiloxane columns.

In turpentine oil, β -pinene is clearly better resolved from α -pinene on the liquid crystal column than on methyl polysiloxane packed columns. Pumilio pine oil revealed its α - and β -pinenes and limonene amongst other early peaks on the liquid crystal. Lime oil clearly revealed its *p*-cymene content in the presence of over 50% limonene. Small amounts of *p*-cymene and limonene could also be detected in various eucalyptus oils containing 70% or more cineole, so confirming the efficiency of the CTAB-formamide liquid crystal phase. If such oils contain long t_R constituents, they will remain in the column, the packing of which should be replaced after a few days use.

For characterising the various constituents of volatile oils my procedures are currently as below, considering them in three groups, which have some overlap. (A) Short t_R , e.g. hydrocarbons including monoterpenes vs. α -pinene at 80°C, packed column of CTAB-formamide; (B) medium t_R , e.g. oxygenated monoterpenoids vs. linalol; and (C) long t_R , e.g. aromatics and sesquiterpenoids vs. geraniol. Both B and C at 135°C, capillaries of methyl-polysiloxane and polyethylene glycol 20M used.

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