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Short Communication

Cholesteryl acetate as a stationary phase for the gas chromatography of some volatile oil constituents

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ABSTRACT

Cholesteryl acetate provides a useful low-polarity stationary phase in packed columns for the gas chromatography of some volatile oil constituents like terpene hydrocarbons, certain terpenoids and some aromatics. With a high mobile phase flow-rate, it is best used above its melting point as a normal liquid (115°C and more) although it has a narrow mesomeric temperature range below this as a chiral nematic liquid crystal. It can be used to resolve racemic linalol, but not carvone.

INTRODUCTION

We have previously reported on the gas chromatographic behaviour of some volatile oil constituents when chromatographed on packed columns of three nematic liquid crystals [1–4]. These stationary phases were all multi-aromatic linear ether molecules, and showed appropriate affinity for the volatile oil linear aromatics whilst melted to the nematic state, and at temperatures both below this (supercooled or "unmelted") and above it when a normal isotropic liquid {for azoxydiphenetole (ADP) only [3]}. They were not well suited to studying most oxygenated terpenoids.

It seemed useful now to examine a differently shaped liquid crystal to see how general was the previously observed behaviour. Cholesteryl esters are interesting as being bulky rigid polycyclic non-aromatic molecules of terpenoid character with an unsaturated ring, also having a C_8 flexible terpene hydrocarbon branched side chain. Their liquid crystal condition however, is more complex than the simple roughly parallel oriented molecular nematic state of those previously used. Cholesteryl esters form chiral nematic (cholesteric) liquid crystals, with the molecules again arranged parallel, but here in layers, with each layer oriented at an angle to those adjacent to it. These liquid crystals therefore offer solutes a stratified three-dimensional terpenoid rather than an aromatic linear molecule to retain them, suggesting they may have special value for terpenoid gas chromatography.

Barrall *et al.* [5], who first used cholesteryl esters, observed gas chromatographic slope changes (plotting log retention time against column temperature) at 88°C and 105–113°C for the acetate with some *n*-alkanes, benzene and alkylbenzenes. They considered these indicated, under gas chromatographic conditions, the melting point of the cholesteryl acetate and its transition temperature (range, depending on the solute) to a normal liquid. Differential thermal analysis [6] had confirmed the melting point as 88.3°C but given a higher transition point of 118.6°C, the widest mesomeric range of the three

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aliphatic esters they used. A slightly lower transition was indicated by an earlier optical study of a cooling melt as 116.5°C [7], but this is still above the range of Barrall *et al.* They considered this discrepancy was due to the influence of the gas chromatographic support, as first suggested by Dewar and Schroeder [8], yet claimed "the gas chromatographic method appears to be an excellent and general determination for liquid crystal transition temperatures", noting "the elution times increase sharply in the transition to the isotropic liquid". Cholesteryl esters were also used independently as stationary phases in 1966 by the originator of liquid crystals for gas chromatography, Kelker, and Winterscheidt [9]. However, the results were confused because they mixed the bulky benzoate with a linear aromatic liquid crystal. Benzoates were later used by Kirk and Shaw [10] at their melting points, resolving α- and β-androstanes and -enes, but failing with racemic mixtures. They noted "the cholesteric liquid (phase) shows a strong tendency to become supercooled". Subsequently, other cholesteryl esters have been used in capillaries in their supercooled condition for olefinics using the cinnamate [11] and butyrate [12]. Thus after initial use, the acetate, which offers minimal size non-aromatic ester component, has been neglected for twenty-five years.

EXPERIMENTAL

Apparatus

A Pye Unicam GCD gas chromatograph fitted with flame ionisation detector and a wide range amplifier were used with a Hewlett-Packard 3390A recorder/integrator. Oven temperatures were observed with a Technoterm 7300 probe.

Two glass columns were used, 1.5 m × 2 mm I.D., packed with 10% cholesteryl acetate (Sigma) on Chromosorb W AW, 80–100 mesh. The weighed materials were mixed in ethanol and taken to dryness in a rotary evaporator for packing the column. No preheating was used before initial results were obtained, but subsequent behaviour of the columns depended on their history of heating. A high nitrogen mobile phase flow-rate was required, about 40 ml/min, and the detector thus needed a higher flow-rate of hydrogen than usual.

A Reichert hot stage microscope with polarisers was used to observe transition temperatures.

Materials and methods

Sources of some solutes used were as before [3,4] plus citronellal from Sigma, linalyl acetate (TCI, Tokyo), (−)-menthol (Plaimar) used in strong alcohol solution, and pulegone as the main peak from pennyroyal oil (D.G.F., Granada, Spain).

RESULTS AND DISCUSSION

On the hot stage, after an initial melting and cooling, the slowly heated cholesteryl acetate melted at about 112°C to give a birefringent fluid with the typical loop texture of a chiral nematic liquid crystal. These loops disappeared at 116°C when a normal liquid formed. This cholesteryl acetate thus had a short 4°C mesomeric range, and only observations at 115°C could be claimed to be truly made on a liquid crystal. However, previous studies [2–4] gave anticipation of successful use outside this limited range.

Linalol was taken as the relative retention standard, as before [1–4], but caused problems by being resolved more or less into two peaks on the cholesteryl acetate. Under optimum conditions of full resolution (around 125°C) these had almost identical peak areas, as would be expected from a racemic mixture. Comparison with lavender oil indicated that the slower of the two peaks was (−)-linalol, and this was used to calculate relative retention times. No other substance with optical isomers gave double peaks, not even a mixture of (+)-and (−)-carvones.

Gas chromatographic results are presented in Figs. 1 and 2, and show relative retention times against (−)-linalol.

As was found for the multi-aromatic liquid crystals [2,3] cholesteryl acetate exhibits a naive behaviour if it is not taken above a particular temperature, in this case 105°C. Previously, the naive response seen was that relative retention times were less than those obtained on the supercooled liquid crystal at the same temperature. Such naive lower values were only observed now with menthol (Fig. 2), whilst four other solutes (terpenoids included) showed an extension of the melted plot which was not greater than the naive state. On the naive column, (−)-linalol retention time was just less than 0.5 min at 105°C, rising to about 0.9 min at 110°C, and then about 2.9 min at 115°C, with the same

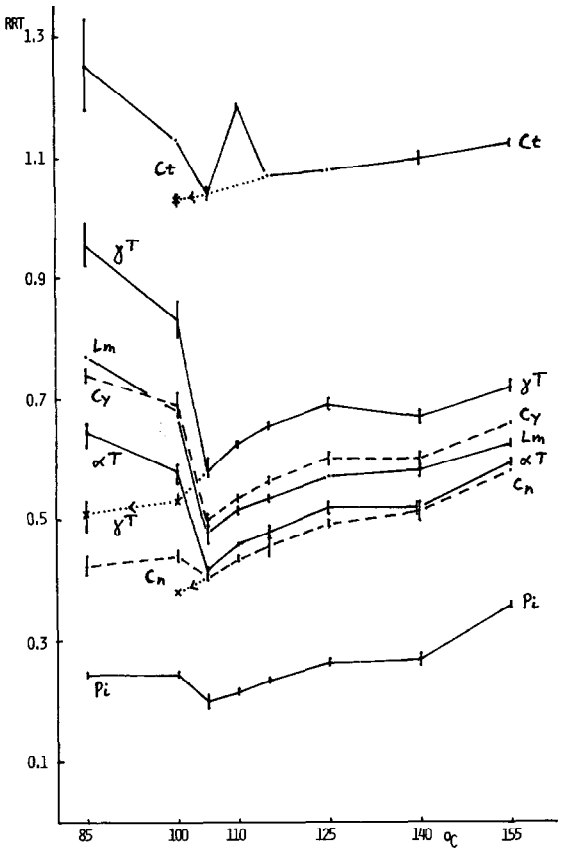

Fig. 1. Plots (connected points) at various temperatures of relative retention times (RRT) against (−)-linalol (1.00) for some volatile oil constituents on a packed column of 10% cholesteryl acatate. Cn = dashes connecting cineole; Ct = citronellal; Cy = dashes connecting *p*-cymene; Lm = limonene; Pi = α-pinene; αT = α-terpinene; γT = γ-terpinene. Dotted lines show supercooled results. Vertical bars depict range of results used for average value, and points show perfect confirmation of first observation.

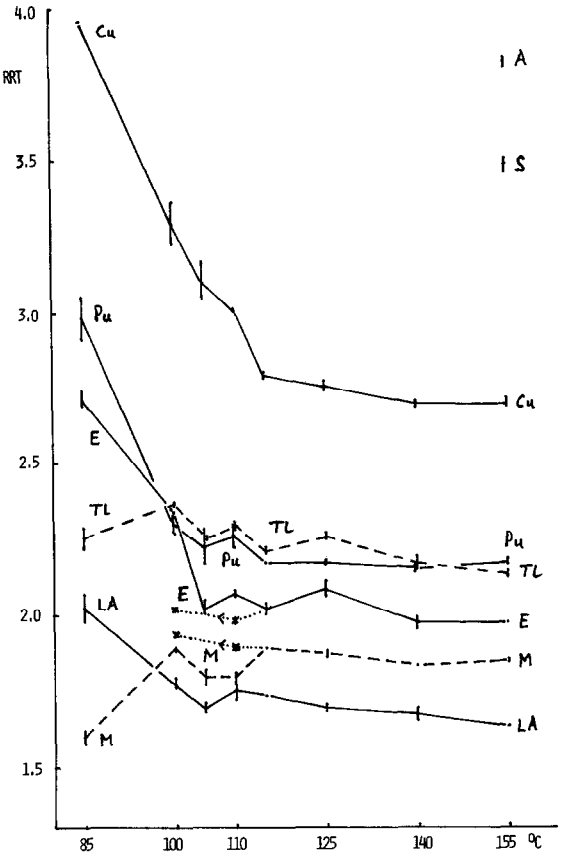

Fig. 2. Plots (connected points) at various temperatures of relative retention times (RRT) against (−)-linalol (1.00) for some volatile oil constituents on a packed column of 10% cholesteryl acetate. Cu = cuminal; E = estragole; LA = linalyl acetate; M = dashes connecting menthol; Pu = pulegone; Tl = dashes connecting α-terpineol. Dotted lines show supercooled results. A = anethole and S = safrole results at 155°C only. Vertical bars depict range of results used for average value, and points show perfect confirmation of first observation.

nitrogen flowrate. After heating to 125°C (linalol about 2.0 min retention) and cooling, values at 105°C were about 3.6 min.

Safrole preceded anethole in elution sequence on cholesteryl acetate, confirming the value of this "test" for liquid crystal phases [3], and this was observed both on a naive column at 100°C (before melting) and at 140°C and 155°C when the phase would be a normal isotropic liquid. At 155°C, the elution sequence of six standard probe solutes previously used [3] was estragole-α-terpineol-cuminal-safrole-anethole-thymol (last), which resembled melted ADP and mature unmelted bis(methoxybenzylidineanil-bi-toluidine) [$(MBT)_2$] apart from transposing their safrole-cuminal sequence. The polysiloxane polyaromatic liquid crystal "MPMS", used in a capillary below its melting point, also yielded safrole after cuminal, [3] like cholesteryl acetate, to which it otherwise seems unrelated.

Volatile oil constituents with short retention times such as the terpinenes, etc., show a rapid fall in relative retention time from 100°C to 105°C (Fig. 1), suggesting a gas chromatographic phase transition of the cholesteryl acetate then, agreeing with

Barrall *et al.* who observed a "break" in their plots [5]. Further heating does not suggest any obvious further transition with these solutes. The 100–105°C change is echoed by the plots for citronellal and estragole (Fig. 2), but these solutes show another fall from 110 to 115°C, agreeing with the microscopical observation of transitions for cholesteryl acetate. Other solutes studied show an angularity in their plots at 115°C, further supporting this, as does a sharp increase in actual retention times. Melted cholesteryl acetate can be used reliably for chromatography, with fairly high gas flow-rate, from 115°C upwards, and gives only slightly changing relative retention times in this its isotropic normal liquid condition. It is probably unwise to use it at lower temperatures, so it is not being used as a true liquid crystal.

Some solute positional shifts in sequence of their retention times were observed here, as previously [2,3] when these were thought to be indicators of changes in condition of liquid crystals. Between 95–100°C on the naive column these are *p*-cymene–limonene, menthol–linalyl acetate and α-terpineol–estragole–pulegone; and they provide some support for the literature melting point of about 90°C for cholesteryl acetate. α-Terpineol–pulegone provide a novel two shifts. The lower temperature one at about 100°C is the consequence of the distinctive initial upward slope seen for the two alcohol plots (menthol and α-terpineol). The higher temperature shift at about 143°C may be caused by partial decomposition of the liquid crystal, as some plots, eg. terpene hydrocarbons, rise from 140°C {as on decomposing $(MBCA)_2$ from 180°C [3]}.

There is hardly any difference, other than quantitative, between the plots of the aromatic cuminal and the terpenoid pulegone, both being smoothly continuous, apart from the angularity at 110°C. Similarly, plots for the aromatic estragole, and α-terpineol, linalyl acetate and citronellal are modified by the inflexions peaking at 110°C. Although linalyl acetate is an ester, like the liquid crystal used, it gave a plot no different to some other solutes tested.

The polarity of the cholesteryl acetate phase was evaluated as before [13], both methods being applied when it was a normal liquid. At 120°C, 2-octyne emerged last after *n*-butanol and pyridine, indicating a low-polar phase. Average retention indices observed were butanol 742, pyridine 831 and octyne 884. This was confirmed at 160°C when the indices for the three probe solutes were 1067 for cineole, 1165 for linalol and 1289 for estragole; their average differed from that of the phenylmethylpolysiloxane base by −59, this "P rating" being similar to $(MBT)_2$ and approaching that of a fully methyl polysiloxane. Such low polarity suggests that cholesteryl acetate is suited to the gas chromatography of terpene hydrocarbons and similar substances, as it is. The "standard" sequence [4] of α-pinene, α-phellandrene–limonene–γ-terpinene was seen whether melted or unmelted, with cineole about level with α-phellandrene (well ahead on naive phase), *p*-cymene just behind limonene (ahead if naive at 85°C), and α-terpinene just behind α-phellandrene. This is like the sequence on melted ADP and $(MBT)_2$, except that the aromatic *p*-cymene is not the last to emerge, reflecting the absence of aromatic structure from cholesteryl acetate. It should be used above its melting point, and with a high gas flowrate could be used to assay the constituents of oils of citronella, *Eucalyptus citriodora*, lavender, coriander, peppermint, basil, tarragon, pennyroyal, cumin and perhaps others, as a supplement to conventional phases, although it resolves no chiral solute pairs other than linalol.

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