GAS CHROMATOGRAPHY USING CHOLESTERYL ESTER LIQUID PHASES*

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The term liquid crystal was first suggested by LEHMANN[1] for those substances which, within defined temperature intervals, are liquid in mobility and crystalline in optical properties. These materials do not pass directly from the solid to the normal or isotropic liquid but go through one or more discreet phase transformations involving liquid crystal intermediate phases (mesophases). The liquid crystal forming compounds have been covered in detail in several reviews[2–5]. These compounds are divided into categories depending on the nature of the liquid crystal phases formed, *viz.*, smectic, nematic, and cholesteric.

The smectic phase exhibits stratification of molecular units. In each stratum the molecules are arranged side by side, the thickness of the stratum being approximately the length of the molecule (or some low multiple). These sheets are flexible and represent highly ordered structures capable of free movement over one another[3].

The nematic mesophase can be represented by molecules aligned in a parallel fashion but not held rigidly in sheets. The molecules are free to move within the limits of the parallel configuration in bundles[3].

Some measure of stratification exists in the cholesteric mesophase, as in the smectic state; but rotation in the plane of the sheet is possible[2]. Uniqueness lies in the possible ordered forms resulting from the alignment in the plates of the cholesterol rings. Some or all of these three liquid crystal phases may be exhibited by a given compound. In excess of 500 organic compounds are known to exhibit liquid crystal phases under some conditions of temperature or solution. The general physical properties and classifications have been compiled in detail[2].

Recently, liquid crystal compounds have received attention as possible gas chromatographic liquid phases[6,7]. The materials studied to date have been principally of the nematic type which exhibit a relatively large caloric event at the solid-nematic transition and a calorically small nematic-isotropic transition[8–10]. Heretofore, the cholesteric phase, which exhibits quite different thermal behavior, has not been studied as a liquid phase for gas chromatography.

The unique structure of the cholesteric phase suggests its potential usefulness as a separatory phase for chromatography. A series of cholesteryl esters has been studied as the liquid phase in gas–liquid chromatography at temperatures which span the true liquid or isotropic phase and the mesophases characteristic of the particular cholesteryl ester. Evaluations were performed in order to reveal unique analytical applications and to provide an independent technique for defining mesophase transitions.

* Part VI of a series on "Order and Flow of Liquid Crystals".

EXPERIMENTAL

Purification of esters

Samples of cholesteryl acetate, *n*-valerate, and *n*-nonanoate were obtained from Columbia Organic Chemicals, Columbia, South Carolina, and Applied Science Laboratories, Inc., P.O. Box 140, State College, Pennsylvania. Each ester was recrystallized three times from ethanol. The carbon–hydrogen analyses for purified esters are shown in Table I.

TABLE I

CARBON–HYDROGEN ANALYSES OF PURIFIED ESTERS

Cholesteryl ester	*Calculated*		*Found*	
	% *C*	% *H*	% *C*	% *H*
Acetate	81.3	11.2	81.2	11.2
n-Valerate	81.7	11.5	81.7	11.5
n-Nonanoate	82.1	11.75	82.1	11.8

The closeness of the elemental analytical percentages to the theoretical values indicates the materials are likely as pure as those used by GRAY[11]. Infrared and ultraviolet analyses indicated no free cholesterol hydroxy or unreacted carbonyl hydroxy. The high extinction coefficient of the cholesterol hydroxy permits as little as 0.07 % unreacted material to be detected by infrared. Gas chromatographic analysis indicated no free acid or cholesterol down to 100 p.p.m. These materials were all identified in chromatograms run initially on the crude materials. The ethanol recrystallization appears to be an excellent and generally applicable purification step in the isolation of the esters of cholesterol from crude reaction mixtures.

Preparation of columns

Each of the purified esters was deposited from 100 ml of 10 % solution on to 80–100 mesh Chromosorb W by evaporation of the solvent (Baker reagent-grade) benzene from the slurry. A weighed portion of the coated packing was extracted in a reflux extractor with boiling benzene in order to determine packing composition. An equal weight of uncoated packing was extracted as a blank. Extraction analysis indicated the individual packings contained 10.5 %, 11.0 %, and 9.8 % by weight cholesteryl acetate, *n*-valerate, and *n*-nonanoate, respectively. The packings were dried at 50°C and 10 torr nitrogen pressure for 24 h. The dried packings were loaded into 7 ft. by $^1/_8$-in. O.D. (0.05 in. wall thickness) stainless steel columns with a vibrator packer. Each column contained 4 g of packing. The columns were spiralled to fit the chromatograph, and copper–constantan thermocouples were attached with epoxy-glass tape at the 1, 3, 5, and 6-ft. lengths. Two columns of each type were prepared and tested. Averaged results are presented.

Instrumentation

A Perkin-Elmer dual column 800 gas chromatograph with dual flame detectors was used. Thermocouple potentials were measured against an ice reference with a

potentiometer. A 2°C thermal gradient was noted between the 1-ft. and 3.5-ft. thermocouples. This was due to the heated inlet which was maintained at 150°C. The gradient remained relatively constant throughout the temperature range studied. The flow rate was 60 cc/min of helium measured at 25°C.

Standard samples

Two standards for chromatographing on cholesteryl esters were prepared from Phillips Petroleum 99 mole % hydrocarbons. The paraffinic standard contained *n*-heptane, *n*-octane, and *n*-decane in equal volumes. The aromatic standard contained equal volumes of benzene, toluene, and ethylbenzene. The sample size was 0.9 μl injected without a splitter. Each curve presented for the elution time–temperature plots consisted of at least 75 points with clusters around inflexes.

RESULTS

The results of the gas chromatographic analyses are summarized with other thermal data from the literature in Table II. Because of the complex nature of the data, each ester liquid phase is discussed in detail below.

TABLE II

COMPARISON OF THERMAL DATA ON CHOLESTERYL ESTERS STUDIED BY GAS CHROMATOGRAPHY

Compound	*Optical methods*[11], *cooling, °C*			*DTA*[12], *heating, °C*			*Gas chromatographic temperatures of slope changes*	
	*S**	*C**	*I**	*1*	*2*	*3*	*Paraffins*	*Aromatics*
Cholesteryl acetate	—	(94.5)	116.5	43.2	87.3	118.6	63, 88, 105–113	Same
Cholesteryl valerate	—	93	101.5	87.8	91.9		49, 56, 81–90	49, 83, 89
Cholesteryl nonanoate	(77.5)	80.5	92	74.1	80.8	93.2	84–89, 91.7	87.7, 90, 96–105.3

* S = solid–smectic; C = solid–cholesteric; I = cholesteric–isotropic liquid.

Cholesteryl acetate

Figs. 1a–b show the corrected retention time–temperature characteristics of representative paraffin and aromatic compounds on cholesteryl acetate columns. Corrected retention times are elution times measured from the maximum of the air peak. The *n*-heptane curve was omitted from Fig. 1a for the sake of clarity in presentation. Breaks in the retention time curve are noted at 63°C, 88°C, and 105–113°C. The two lower temperature breaks are changes in the slope of the curve. The 63°C slope change is sharper for aromatics than paraffins and the 88°C change sharper for paraffins than aromatics. The 105–113°C break is a shift to longer retention times for all materials amounting to a 4- to 5-fold increase. This sharp shift resembles the elution characteristics noted by KELKER[7] for a *p,p'*-azoxyphenetole liquid phase and by DEWAR AND SCHROEDER[6] for 4,4-di-*n*-hexyloxy-azoxybenzene at the nematic–isotropic transition of those compounds.

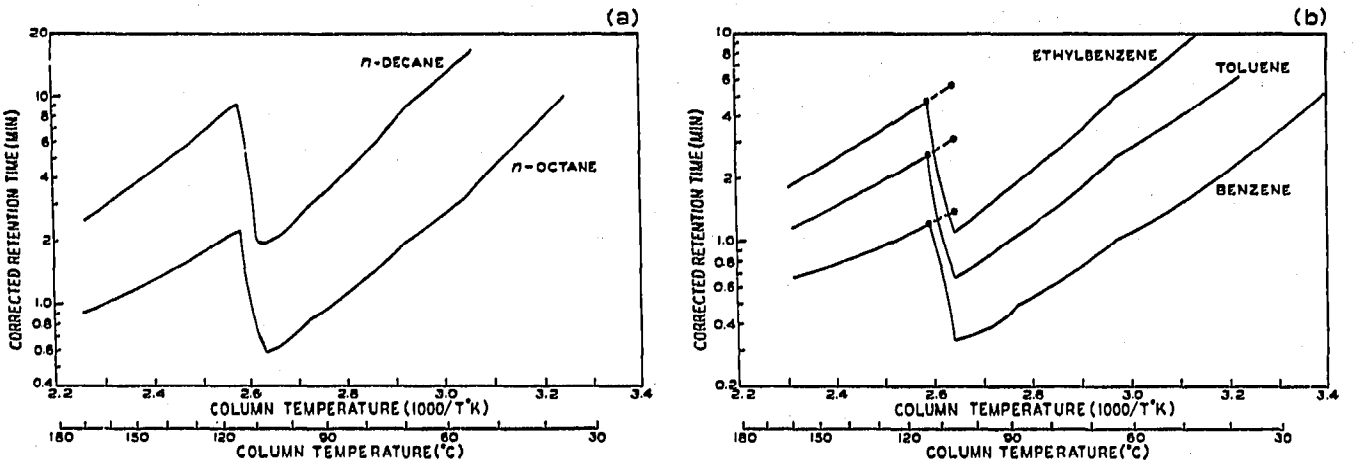

Fig. 1. Temperature *versus* retention time for cholesteryl acetate column.

Cholesteryl acetate has been reported to undergo a solid–cholesteric transition at 94.5°C from optical observations and a cholesteric–isotropic liquid transition at 116.5°C[11]. The solid–cholesteric phase transition was reported by GRAY to be monotropic with the liquid phase; that is, it forms only on cooling from the melt. Differential thermal analysis (DTA) has shown the acetate ester transitions to be more complex on heating. Transitions at 43°C, 87°C, and 118°C have been reported[12]. The lowest transition was movable by DTA, depending on the thermal history.

Only one acetate transition, the most prominent in gas chromatography, can be identified positively with transitions derived from cooling data reported in the literature. That is, the cholesteric–isotropic liquid transition at near 110°C. As DEWAR AND SCHROEDER[6] have pointed out, the gas chromatographic support can probably play a role in the exact location of mesophase transitions. When the acetate gas

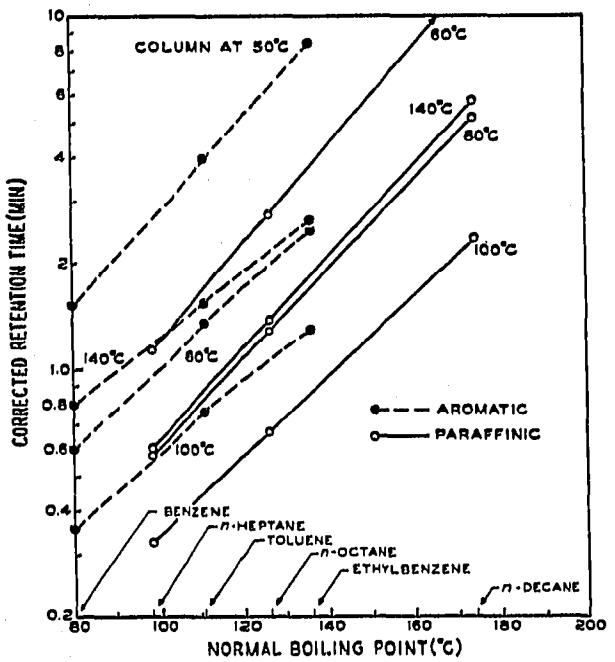

Fig. 2. Retention time–boiling point relationship for cholesteryl acetate liquid phase.

chromatographic column was cooled below 118°C, the isotropic liquid phase persisted into the cholesteric phase range by supercooling. This effect is shown in Fig. 1b as a dotted extension of the retention time curve. The isotropic liquid phase supercooled to 80°C. As long as the phase existed, the retention time–temperature plot followed a smooth extension of the curve obtained above 118°C. Processes favoring crystallization of a supercooled liquid, *e.g.*, shaking the column, caused the retention time to drop to the level noted on the heating cycle.

Fig. 2 shows the retention time–normal boiling point relationships for the acetate ester column at four temperatures for aromatics and paraffins. The aromatics and paraffins display an eluting order expected from their boiling points. Aromatics were eluted later than paraffins of equal boiling point. Extrapolation indicates that the retention time of aromatic compounds may become shorter than the paraffins for aromatics boiling higher than propylbenzene.

Within an homologous series, the retention time–normal boiling point relationships are shown to be nearly linear for both aromatics and paraffins. Secondary interactions, such as polarity and selective solubilities, apparently are minimized on the acetate liquid phase. Paraffins and aromatics interact with the acetate ester phases in the same manner at all temperatures.

Cholesteryl n-valerate

The corrected retention time–temperature curves for the *n*-valerate ester are shown in Fig. 3a and b. Differences between paraffin and aromatic retention times are apparent. Paraffins show a change in slope at 49°C. An intermediate stable range follows to 56°C. At 56°C a second slope change occurs which is followed by a nearly linear decrease in retention time with increasing temperature to 81°C. Between 81°C and 90°C, a sharp increase in retention time occurs which is followed by a fourth linear range which persists to the upper experimental limit at 172°C.

Aromatic materials show a change in slope at 49°C. From 49–83°C, a curving retention time–temperature range exists which shows a normally decreasing retention time with increasing temperature. Between 83°C and 89°C a fivefold increase in retention time occurs. Thereafter, the retention time decreases in a nearly linear fashion to the experimental limit.

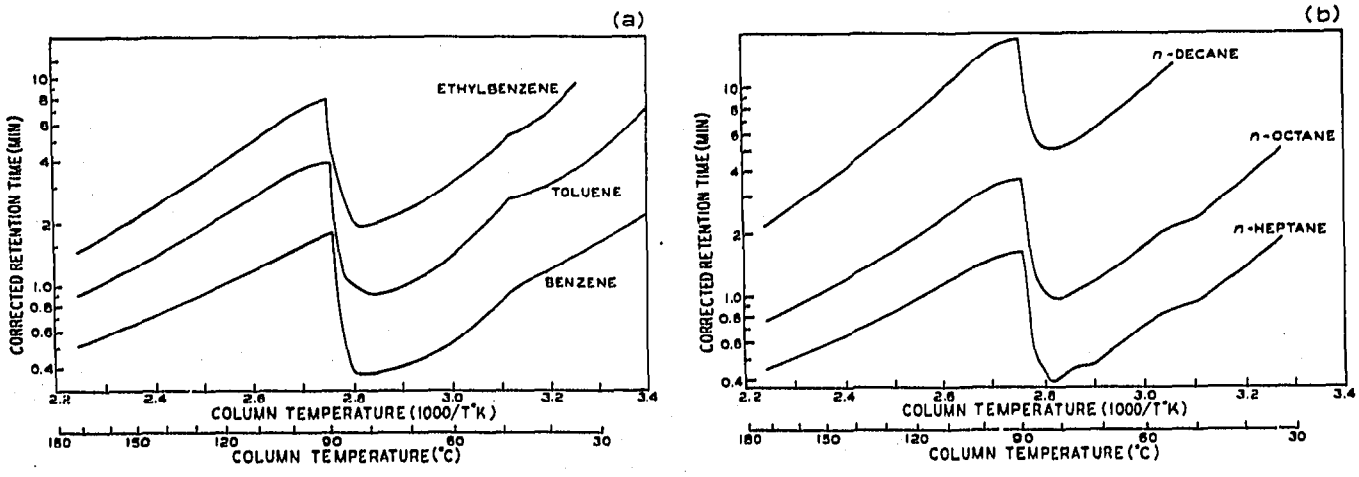

Fig. 3. Temperature *versus* retention time for cholesteryl valerate column.

GRAY[11] has reported two transitions for cholesteryl valerate, solid–cholesteric at 93°C and cholesteric–isotropic liquid at 101.5°C. These data were obtained from cooling curves. In contrast, differential thermal analysis (DTA) of this purified compound shows transitions at 87.8°C and at 91.9°C[12]. Visually the upper transition occurs at 91.5°C. DTA of the compound on the support showed a transition at 90.6°C, indicating that the phase change is not significantly altered by the support. These concordant values, which are also consistent with gas chromatographic results, are recognized as differing from the transitions published by GRAY. An explanation for the difference cannot as yet be offered. Consistent data indicate that in the temperature interval from 49°C to 83°C, the ester is in the solid phase, from 83°C to 89°C the cholesteric phase, and above 89°C the compound is an isotropic liquid. The slope change at 49°C may be due to some modification of the solid phase.

The boiling point–retention time data for the *n*-valerate ester are shown in Fig. 4. The general distribution of the boiling isotherms is the same as that for the acetate, with the important exception that some of the *n*-paraffin boiling point isotherms are curved, whereas all of the aromatic boiling point isotherms are straight lines. In particular, the column in the solid phase shows curved isothermal boiling point lines for paraffins, *i.e.*, not a regular boiling point behavior.

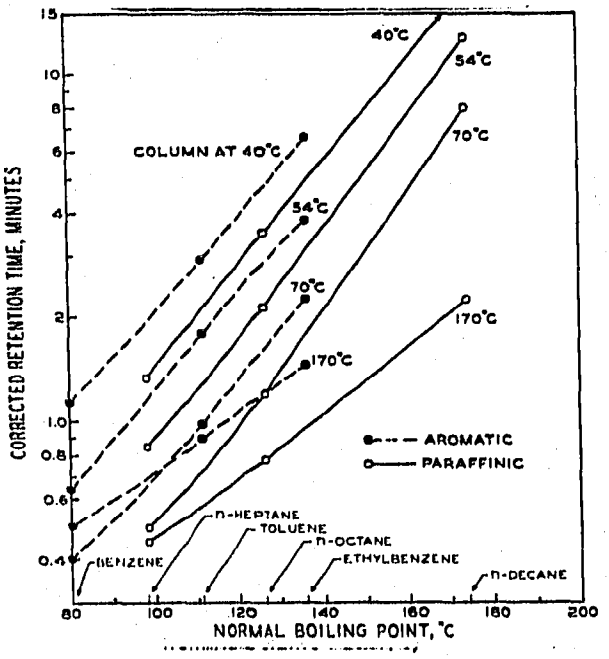

Fig. 4. Retention time–boiling point relationship for cholesteryl valerate liquid phase.

Cholesteryl n-nonanoate

The corrected retention time–column temperature curves for the *n*-nonanoate ester are shown in Figs. 5a and b. The general shape of the curves for *n*-paraffins is roughly similar to those obtained for the other esters. A nearly linear range exists from 70°C to 84°C for *n*-heptane, to 88°C for *n*-octane, and to 89°C for *n*-decane. The *n*-heptane retention time increases to 89°C and then establishes a new linearly decreasing range to the limit of the experiment. The *n*-octane retention time decreases sharply from 88°C to 90°C and then increases sharply to 91.7°C. Thereafter, a linearly

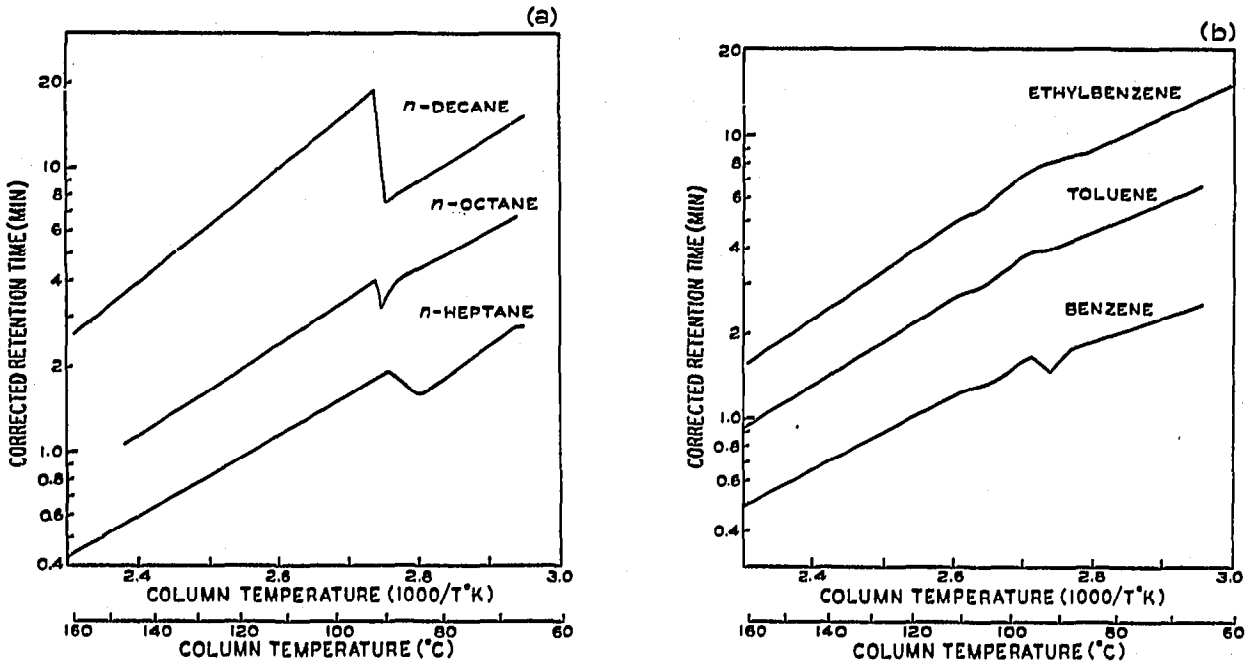

Fig. 5. Temperature *versus* retention time for cholesteryl nonanoate column.

decreasing range is established. The retention time of *n*-decane increases sharply from 89.5°C to 91.7°C. A linearly decreasing retention time range is established above 91.7°C. The retention time–column temperature curves for aromatic hydrocarbons on the *n*-nonanoate ester column are relatively featureless in comparison to those obtained on *n*-valerate and acetate ester columns. The retention time for benzene decreases linearly from 65°C to 87.7°C. A small sharp decrease to 90°C followed by a small increase to 96°C occurs. A curved relationship exists between

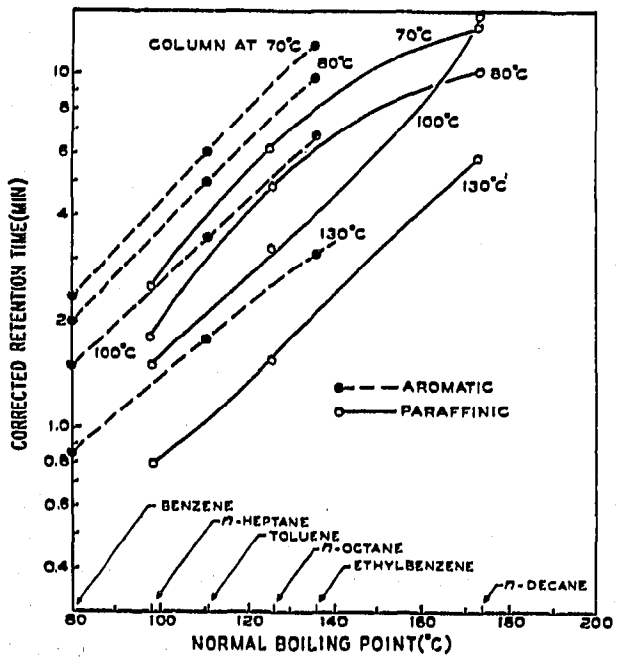

Fig. 6. Retention time–boiling point relationship for cholesteryl nonanoate liquid phase.

96°C and 104.1°C. Above 104.1°C, the retention time of benzene decreases linearly with temperature. The toluene retention time–temperature curve exhibits changes in slope at 90.5°C, 96.4°C, and 105.3°C. Ethylbenzene shows changes in slope at 85.1°C, 96.0°C, and 105.3°C.

GRAY has reported the following transitions for cholesteryl *n*-nonanoate: solid–smectic 77.5°C, smectic–cholesteric 80.5°C, and cholesteric–isotropic liquid 92°C from cooling curve data[11]. The 77.5°C transition is monotropic. DTA shows transitions at 74.1°C, 80.8°C, and 93.2°C with the transition at 80.8°C being the calorically largest[12].

The boiling point–retention time relationships for compounds on *n*-nonanoate ester exhibit sharply curved lines for paraffins (Fig. 6). The aromatic materials generally fall on a straight line. The normal hydrocarbons do not elute in a linear boiling point fashion until the liquid phase is formed above 92°C. The curved relationships for the paraffins indicate that the solid and mesophases of cholesteryl *n*-nonanoate do not form a boiling point stationary phase and interact in an anomalous manner with the paraffin chains.

DISCUSSION

The cholesteric esters when used as stationary phases in gas chromatography have several features in common with other liquid crystal materials similarly employed. The log retention time–reciprocal absolute temperature relationships exhibit slope changes and sharp discontinuities at or near mesophase transition points. The acetate and *n*-valerate ester adhere more closely to this observation than the *n*-nonanoate.

With little exception, a sharp increase in retention time is noted in going from the highest temperature mesophase to the isotropic liquid. It has been noted for *p*-azoxyanisole that the retention time–temperature relationship is curved prior to the known isotropic liquid point[6]. The explanation for this offered by DEWAR AND SCHROEDER is that the rise is caused by a decrease in the free energy of solubility of the sample on passing from nematic to normal liquid. Probably, this conclusion also applies to the cholesteric–isotropic liquid. A close correspondence between the gas chromatographically determined transition temperatures and those obtained by DTA and optical means cannot be expected since substrate effects are known to be very large for these materials[2,3]. The cholesteric–isotropic liquid transition temperature has been observed to move by as much as 40°C for cholesteryl benzoate, depending on the nature of the surface supporting the sample[2]. The gas chromatographic substrate results in a general lowering of all transition temperatures, Table II.

The cholesteryl esters have a number of unique properties as stationary phases which have not been seen in the previous studies with other liquid crystal compounds. Many of these effects can be explained by considering the shape of the cholesteryl ester molecule and its special orientation in the various mesophases. The central core of all the solid and mesophase structures is the nearly flat or saucer-shaped cholesteryl ring system. The ester groups in such a stack radiate outward following both a horizontal and a screw glide plane. When the ester groups are long enough, *viz.* *n*-nonanoate, organization probably takes place in this side-chain portion of the molecule as well. For a longer ester group, a greater degree of interaction between it

and other long-chain molecules would be expected. This is observed in the data given here. The retention time–boiling point curves for both aromatic and paraffin compounds in both the mesophase and isotropic liquid of cholesteryl acetate are linear. This indicates a simple boiling point or regular vapor pressure interaction between the ester stationary phase and aromatics and paraffins. On the other hand, the boiling point–retention time isotherms of the *n*-nonanoate ester are sharply curving for paraffins at temperatures below the isotropic liquid transition point, whereas little curving is noted for the aromatics under corresponding conditions. Specific interactions between the paraffin chains and the oriented ester tails can account for this.

When the orientation of the cholesteryl stack is destroyed at the isotropic transition, a simple boiling point relationship is observed. Further evidence for this specific interaction between paraffin and oriented ester chain is shown by the generally higher retention times of paraffins on the nonanoate column compared to the acetate and valerate ester columns below the isotropic liquid transition temperature and the nearly equal retention times over the isotropic liquids.

For example, at 120°C all of the esters are in the isotropic liquid phase; and the elution times for all paraffins and aromatics are the same irrespective of the ester on the column. Benzene elutes at 1.1, 1.1, and 1.0 min; heptane at 0.92, 0.93 and 0.93 min; ethylbenzene at 4.1, 3.8, and 4.0 min; decane at 8.0, 7.6, and 7.8 min from the cholesteryl acetate, valerate, and nonanoate columns, respectively. This demonstrates the identity of the esters in the isotropic liquid phase. On this basis the heats of solution should also be equivalent. The solid and mesophases appear to be the structural forms which are unique.

Long-chain cholesteryl esters in the mesophase range interact specifically with paraffinic hydrocarbons. The other reported liquid crystals interacted specifically with benzene hydrocarbons. Further work on the separation of position isomers of paraffins with cholesteryl ester liquid phases is in progress.

SUMMARY

The acetate, *n*-valerate, and *n*-nonanoate esters of cholesterol, when distributed in the form of pure compounds as liquid phases on a gas chromatographic packing, exhibit anomalous log elution time–temperature relationships. Sharp changes in the elution relationship for benzene, toluene, ethylbenzene, *n*-octane, and *n*-decane are noted at or near previously reported liquid crystal or mesophase transition temperatures. The elution times increase sharply, as much as 3.5 times, in the transition from the cholesteric mesophase to the isotropic liquid. This represents an anomalous elution time increase with increasing temperature. Near mesophase transitions, the relationship between elution time and eluant boiling point also exhibits an anomaly. Aromatic compounds exhibit shorter elution times than those for corresponding aliphatic materials of equal carbon number. When a given mesophase is supercooled, the solute elution characteristics lie on a curve which extrapolates from the data derived within the normal mesophase range.

The gas chromatographic method appears to be an excellent and general determination for liquid crystal transition temperatures. The unique temperature behavior of the cholesteryl esters also gives them some importance as liquid phases in analytical gas chromatography.

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