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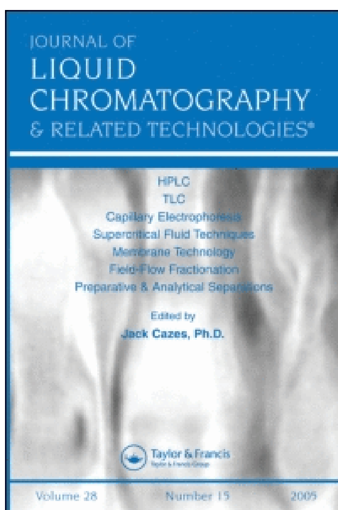

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Liquid Crystals as Stationary Phases for High Performance Liquid Chromatography

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LIQUID CRYSTALS AS STATIONARY PHASES FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Liquid crystals have not yet been used as stationary phases in High Performance Liquid Chromatography. This is surprising since Gas Chromatography has demonstrated some remarkable separations, many of which are not possible with normal stationary phases, that have been achieved where liquid crystals have been employed as the stationary phase. The objective of the work reported here was to evaluate the chromatographic properties of several cholesteric liquid crystals as stationary phases in HPLC. Included in this study was an investigation of the feasibility of bonding a cholesteric moiety to a solid support for use in HPLC. The columns showed a dramatic increase in capacity factor for steroid molecules as the temperature of the column was increased.

INTRODUCTION

Liquid crystals were first observed by an Austrian botanist, Friedrich Reinitzer in 1883 (1). The unique properties of liquid crystals have resulted, since that time, in their use in a wide number of applications. Within analytical chemistry, liquid crystals have been used as stationary phases in gas chromatography where their primary application has been in separating isomeric organic compounds (2-15), polynuclear aromatic hydrocarbons (16,17) and optical isomers (18-20). Gas Chromatography has also been used

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to evaluate the thermodynamic parameters of liquid crystals (10, 21-30). Other studies have been directed more toward the chromatographic properties of the liquid crystals (31-33) and the effect of electric fields on the chromatographic properties (34, 35,36). Several reviews deal with the use of liquid crystals in gas chromatography (37-39). Other analytical applications of liquid crystals include their use of molecular ordering solvents for NMR (40), electron spin resonance (41) and UV spectroscopy (42).

Numerous stationary phases have been used in High Performance Liquid Chromatography to effect separations. Especially popular are stationary phases which are chemically bonded to solid supports (43) thus preventing column bleed.

Liquid crystals, however, have not been used as stationary phases in High Performance Liquid Chromatography. This is surprising since Gas Chromatography has demonstrated some remarkable separations, many of which are not possible with classical stationary phases, that have been achieved where liquid crystals have been employed as the stationary phase.

The objective of the work reported here was to evaluate the chromatographic properties of several cholesteric liquid crystals as stationary phases in High Performance Liquid Chromatography. Included in this study was an investigation of the feasibility of bonding a cholesteric moiety to a solid support surface for use in High Performance Liquid Chromatography. Selection of the proper linkage for the bonding process should give a bonded phase with the liquid-crystalline properties.

EXPERIMENTAL

Materials. Solvents used for the mobile phases were purchased from Burdick and Jackson, Muskegon, Michigan. Corasil-II was obtained from Waters Associates. The liquid crystals were obtained from Eastman Organic Chemicals (Rochester, New York 14650), Shawnee Chemicals (Springfield, Ohio 45501), or Matheson Coleman and Bell (Norwood, Ohio 45212).

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Apparatus. Most separations were performed with a Waters Associates Model 202 High Performance Liquid Chromatograph employing a U6K septumless injector. Some of the steroid separations were performed with a Micromeritics Model 7115-24 High Performance Liquid Chromatograph.

Temperature studies were performed using a Milton Roy minipump capable of 5,000 PSI and employing pump dampeners, a Waters Associates U6K injector and a Hewlett-Packard Model 5711 Gas Chromatograph to house the columns and provide heating and temperature control.

A Waters Associates fixed wavelength UV detector or a Micromeritics Model 780 or Model 785 variable wavelength UV detector was used to monitor the eluent. A Perkin-Elmer Model 137 Infrared Spectrophotometer was used to measure IR and ATR spectra. A DuPont DSC-1 was used for differential scanning calorimetry and F&M Model 185 was used for CH&N analysis.

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The columns were 2.1 mm x 550 to 680 mm stainless steel, with glass wool plugs or 5μ frits on both ends.

Column Construction. Cholesteryl Corasil direct reaction. Five grams of Corasil II were added to a 100 ml round-bottom flask containing 50 ml toluene and 1 g of cholesteryl chloroformate. Triethylamine, 0.5 g, was added and the mixture heated under reflux for 1 to 48 hours while stirring with a teflon clad stirring bar. After cooling, the reaction mixture was filtered through a Whatman 50 filter, washed with methylene chloride and dried in a vacuum oven at 740 mm of mercury at 100° C for 18 hours. Approximately 3 to 3.5 g of the packing was used to pack a column. The product was analyzed *via* IR, ATR, DSC, CHN and optical microscopy to determine the extent of reaction.

Cholesteryl-Corasil "Toasting". Five grams of Corasil II and 1 g of cholesteryl chloroformate were dry-mixed in a vial on a Vortex mixer. The resulting mixture was placed in a 6-inch long x 0.5-inch I.D. glass tube. The tube was placed in a tube furnace and heated to 150° C with argon gas passing through the tube at 10 ml/min. Heating was continued until acidic gas was no longer

evolved as evidenced by absence of color change of a strip of moist litmus paper held near the exit end of the column. The tube was allowed to cool under an argon flow. The resulting reddish brown powder was washed, vacuum dried and analyzed as described above.

Cholesteryl-Corasil II, via Propyl Silane Group. Five grams of Corasil II with 4% active surface hydroxyl groups (equal to 0.2 g or 0.012 moles of OH) were reacted with 2.3 g (0.012 moles) of dimethyl (3-acetoxypropyl) chlorosilane in 25 ml of silylation grade pyridine under a nitrogen blanket with stirring for 18 hours. The product was filtered using Whatman 50 filter paper, washed with three 25 ml portions of reagent grade diethyl ether and dried in a vacuum oven for 24 hours. Yield was 6.523 g.

The product isolated above was slurried with 500 ml of distilled water containing 0.4705 g (0.01176 moles) of sodium hydroxide and shaken on a flat-bed shaker for 1 hour. The dimethyl (3-hydroxypropyl) silane bonded Corasil hydrolysis product was filtered, washed with methanol, and vacuum dried for 24 hours. Yield 6.5 g.

The hydrolysis product (6.15 g) was slurried with 5.2816 g (0.01176 moles) of cholesteryl chloroformate and 2 ml of triethylamine in 50 ml of toluene in a 100 ml round bottom flask. A reflux condenser was attached and the slurry refluxed for 24 hours. The product was filtered with Whatman 50 filter paper, washed with diethylether and dried in a vacuum oven for 24 hours. Yield 6.5 g (54%).

Mobile Phase Composition. Unless otherwise noted the mobile phase used was 95% cyclohexane, 4.2% chloroform, and 0.8% methanol. When liquid crystals were used in the mobile phase they were present at a concentration of 0.2%. Thus, the solvent composition was 94.8% cyclohexane, 4.2% chloroform, 0.8% methanol and 0.2% liquid crystal. To equilibrate the column this solvent was pumped through the column at 1.0 ml/min for 16 hours. The flow rate for all chromatographic studies was 2.0 ml/min.

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Temperature Studies. When temperature changes were made, a 5 min. equilibration was found to be satisfactory.

RESULTS AND DISCUSSION

Cholesteric esters are, for the most part, liquid crystals in the range 20 to 100° C. Cholesteryl chloroformate was chosen to react with Corasil II because the esterified product was expected, even though it was chemically bonded to the support surface, to retain its liquid crystalline properties.

Direct Reaction. The cholesteryl-corasil direct reaction procedure was similar to the method used by Elser (44) to form a series of cholesteryl carbonate esters via the reaction of cholesteryl chloroformate and n-alkyl alcohols. However, analysis of the reaction product using IR, ATR, DSC, CHN and optical microscopy, indicated that cholesteryl chloroformate did not bond to the corasil surface. This was confirmed by High Performance Liquid Chromatography when columns packed with the reaction product had, within experimental error, identical chromatographic properties to columns containing uncoated Corasil II.

Toasted Corasil. After failure to achieve reaction between the cholesteryl chloroformate and the silanol groups on the Corasil II, a second reaction scheme was purposed and evaluated. Corasil II and cholesteryl chloroformate were dry mixed and the mixture was heated in a stream of inert gas until hydrochloric acid was no longer evolved. The remaining solid was washed with solvent, dried and then used to pack a column as described above. This approach is very similar to one used by Vermont (45) to prepare a "toasted" stationary phase for liquid chromatography. This procedure was successful as evidenced by an increased capacity factor, k', when compared with a column packed with Corasil II alone. Table 1 lists the capacity factor ratios for six androgens on this column at 26 and 53° C.

When a series of estrogens and corticoid steroids was chromatographed on the same columns, an opposite relationship was found when the ratios of the capacity factors were examined. The

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TABLE 1

The Ratio of k' Values on Cholesteryl Corasil Column to the k' Values on Corasil II for Selected Androgens and Estrogens at 26 and 53° C.

Compound	Ratios	
Androgens[a]	26° C	53° C
Androstenedione	1.59	1.37
$\Delta^{1,4}$ Androstdienedione	1.54	1.38
Methyltestosterone	1.45	1.38
Testosterone	1.44	1.30
19-Nortestosterone	1.78	1.29
Dehydrotestosterone	1.45	1.30
Estrogens[b]		
Esterone	0.33	
Estradiol	0.72	
Estratriol	0.72	

[a]Mobile phase 95.4% cyclohexane, 4.2% chloroform and 0.4% methanol

[b]Mobile phase 79% cyclohexane, 19% chloroform and 2% methanol

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estrogen and corticoid results are listed in Table 1 and in Table 2 column 2, respectively.

Subsequent analysis of capacity factor ratios for the two columns at a series of temperatures for both corticoids (Table 2) and androgens (Table 3) indicated that a combination of the more polar solvent mixture used to chromatograph the corticoids and heating the column had apparently stripped the column of whatever coating there had been on the support.

Cholesteryl-Corasil Via Propyl Silane Group. As discussed above, the formation of a cholesteryl carbonate ester-bonded stationary phase based on the direct reaction did not occur, while the toasting procedure, although initially marginally successful, produced unstable columns. The Si-O-C bond is known to be hydro-

TABLE 2

The Ratio of k' Values on Cholesteryl Corasil Column to the k' Values on Corasil II for Five Corticoids at Various Temperatures

	Ratio					
Compound	25°C*	-20°C	0°C	20°C	40°C	60°C
11-Desoxycorticosterone	0.71	0.95	0.90	0.93	0.94	0.96
11-Dehydrocorticosterone	0.84	0.95	0.94	0.93	0.98	0.94
Corticosterone	0.86	1.00	0.99	0.99	1.01	0.97
Dehydrocortisone	0.85	0.97	0.95	0.99	1.01	0.97
Cortisol	0.82	0.97	0.96	0.96	1.00	0.97

*initial values

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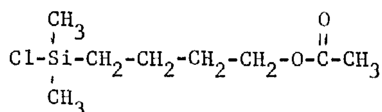
TABLE 3

The Ratio of k' Values on Cholesteryl Corasil Column to the k' Values on Corasil II for Four Androgens at Various Temperatures

	Ratio				
Compound	0°C	25°C	40°C	60°C	80°C
Androstenedione	1.06	1.09	0.99	0.83	0.84
$\Delta^{1,4}$Androstdienedione	1.03	1.08	0.98	0.81	0.80
Testosterone	1.02	1.03	0.99	0.86	0.82
Dehydrotestosterone	1.01	1.03	0.95	0.83	0.83

lytically unstable. The bond stability of a Si-O-C-O linkage might be even more unstable, and this bond may be very thermally unstable as well. An alternative method of preparing a cholesteryl carbonate ester bonded liquid phase was, therefore, evaluated. The preparation involved the silylation of the surface silanol groups with a substituted chlorosilane. A substituent group on the chlorosilane must then either react with, or be easily connected to, a reactive group which would react with cholesteryl chloroformate to form a cholesteryl carbonate ester.

The substituted silane used was dimethyl(3-acetoxypropyl) chlorosilane, I.



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There are several reasons for selecting this reagent. The three-carbon chain, rather than a one or two carbon chain, extends the cholesteryl group further from the surface and the $Si-CH_2$ bond would be expected to be stronger. Two methylene groups attached to a silicon atom result in a particularly unstable situation.

Hydrolysis of $Si-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$ would result in the formation of $Si-OH$, $HO-\overset{O}{\overset{\|}{C}}-CH_3$ and $CH_2=CH_2$. This reaction is peculiar to silanes and occurs with all $Si-CH_2-CH_2-R$ compounds. While dimethyl(3-acetoxypropyl) chlorosilane is not as reactive as a trimethylchlorosilane or a dimethyldichlorosilane, it is still very reactive with hydroxyl groups. Corasil II, containing active surface Si-OH groups, should react with dimethyl(3-acetoxypropyl) chlorosilane in pyridine to yield $Si-O-\underset{CH_3}{\overset{CH_3}{Si}}-CH_2-CH_2-CH_2-O-\overset{O}{\overset{\|}{C}}-CH_3$ and pyridinium chloride. Hydrolysis with dilute sodium hydroxide should yield $Si-O-\underset{CH_3}{\overset{CH_3}{Si}}-CH_2-CH_2-CH_2-OH$. Reaction of this material with cholesteryl chloroformate in toluene, with triethylamine as a catalyst, should form $Si-O-Si(CH_3)_2[(CH_2)_3-O-\overset{O}{\overset{\|}{C}}-O\text{-cholesteryl})]$.

Infrared spectra of the product from the above reactions indicated that little or no cholesteryl chloroformate was bonded to the Corasil.

The failure of the reaction between cholesteryl chloroformate and dimethyl(3-hydroxypropyl) silane-bonded Corasil to go to com-

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pletion may be due to the large size of the cholesteryl moiety. This large size could make placement of cholesteryl moieties close together on the Corasil surface difficult.

Liquid-liquid Chromatography. The above procedures were unsuccessful in achieving a useful bonded liquid crystal-corasil system. The next approach, and the simplest, was to merely deposit the liquid crystal as a film on the surface of the corasil.

In gas chromatography a difference in the capacity factor is possible depending upon whether cholesteric liquid crystals are coated on inert or active supports. When cholesteryl liquid crystals are coated on inert solid supports such as Teflon (46), the capacity factor generally decreases with increasing temperature until the mesophase transition temperature is reached. This is the temperature at which the material passed from a solid to an intermediate state between a liquid and crystalline. At this temperature, the capacity factor sharply increases until the temperature at which transition to an isotropic liquid phase occurs. Upon entering the isotropic phase the capacity factor again decreases with increasing temperature. When the cholesteric liquid crystal is coated on an active support such as Silochrome (46) there is a steady decrease in the capacity factor with increasing temperature for the solutes examined, but the resolution of isomeric compounds (i.e., m-xylene and p-xylene) remains constant over a broader temperature range than observed for cholesterics coated on an inert support.

Liquid crystals may not behave the same way in liquid-liquid chromatography for several reasons. First, the mobile phase used in gas chromatography is an inert gas. Second, the solute molecules in gas chromatography are at essentially infinite dilution. Therefore, neither the mobile phase nor the solute molecules disrupt the molecular ordering of the liquid crystal. In contrast, when liquid crystals are used as a stationary phase for High Performance Liquid Chromatography, both the mobile phase and the solute, with its associated sphere

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of mobile phase molecules, can interact with the stationary phase. Whether this interaction results in disruption of the stationary phase ordering is not known. However, the support used in this study, Corasil II, is very active and possibly, as in gas chromatography with an active substrate, the liquid crystal stationary phase may retain some of its order.

The stationary phases selected for this study were liquid-crystalline at or near room temperature. The first cholesteric liquid crystal studied was cholesteryl 2-ethylhexyl carbonate. Its phase transition temperatures are 25° C (solid-smectic), 32° C (smectic-cholesteric) and 35° C (cholesteric-isotropic).

The chromatographic characteristics of the column were evaluated using a series of androgenic steroids at a series of temperatures between 22 and 38° C. The results are shown in Figure 1.

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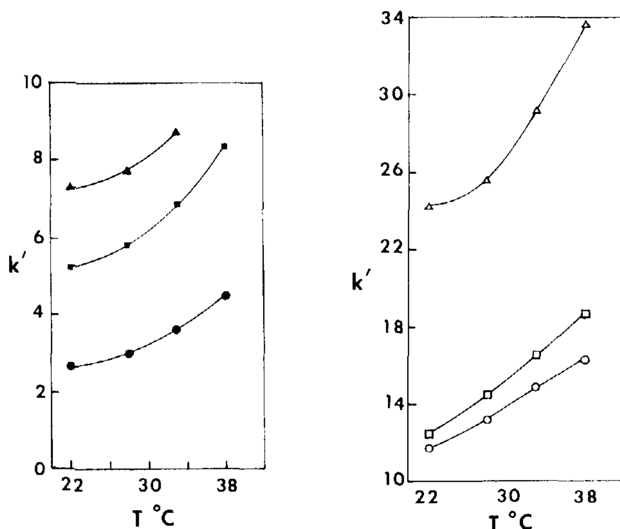

FIGURE 1

Effect of temperature on capacity ratios, k', for androstenedione (●), $\Delta^{1,4}$androstdienedione (■), methyl testosterone (▲), testosterone (o), 19-nortestosterone (□), and dehydrotestosterone (Δ) when chromatographed on a cholesteryl 2-ethylhexyl carbonate coated corasil (II) stationary phase.

Significantly, as the temperature is increased the capacity factors (k') for all of the compounds increase. For comparison the same compounds were chromatographed on the same Corasil II column with the same mobile phase, but without the cholesteryl 2-ethylhexyl carbonate. Without the liquid crystal in the mobile phase the percentage increase for k' from 25 to 40° C was only between 1 and 12%, while with the liquid crystal over the temperature range of 22 to 38° C, the percentage increase was between 38 to 68%. Evidently the liquid crystal-coated the support and influenced the retention of the solutes.

To determine the full extent of increasing the temperature on the cholesteryl 2-ethylhexyl carbonate liquid crystal stationary phase and its ability to influence capacity factors, another series of experiments was performed with the androgens. A mixture of three androgens, androstenedione, $\Delta^{1,4}$ androstdienedione, and testosterone was then chromatographed at a larger series of temperatures ranging from 10 to 52° C. The next day the same mixture was chromatographed with the same column and mobile phase in the range of 40 to 90° C. The capacity factors for each compound at each temperature are listed in Table 4. The data are plotted in Figure 2. Again, as was seen in the previous experiment, the capacity factor increases dramatically with temperature. The three steroids were also chromatographed on the same column with the same mobile phase minus the liquid crystal. The results are shown graphically in Figure 2. The capacity factor increases between 521 and 434% for androstenedione and $\Delta^{1,4}$androstdienedione respectively, using the liquid crystal column over the temperature range of 10 to 80° C. While in the same temperature range the capacity factors for androstenedione and $\Delta^{1,4}$androstdienedione increase only between 176 and 164%, respectively, on the Corasil II column. On the liquid crystal coated column testosterone's k' increase 79% from 10 to 60° C, while on the Corasil column k' increased only 12.6%. The testosterone capacity factor was not

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TABLE 4

The Effects of Temperatures Between 10-90° C on the Capacity Factor for Selected Androgens Chromatographed on a Column with a Liquid Crystal, Cholesteryl 2-Ethylhexyl Carbonate Stationary Phase

	Capacity Factors, k'		
Temperature, T^{o}C	Androstenedione	$\Delta^{1,4}$Androstdienedione	Testosterone
10	2.13	4.22	9.88
16	2.23	4.34	9.99
22	2.34	4.46	9.96
28	2.57	4.88	10.54
34	3.06	5.80	11.85
40	3.56	6.47	12.42
(40)	(3.77)	(6.77)	(12.91)
(40)	(3.86)	(6.95)	(13.38)
46	4.04	7.51	13.54
52	5.19	9.68	15.81
(52)	(5.15)	(9.60)	(15.40)
60	6.69	12.38	17.65
70	9.74	18.18	20.32
80	13.22	22.54	---
90	15.65	27.81	---

( ) data repeated on second day

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measurable at 80 and 90° C on the cholesteryl 2-ethylhexyl carbonate coated column or at 80° C on the Corasil II column, because its peak is under the $\Delta^{1,4}$androstdienedione at these temperatures. Figure 3 shows the chromatographic traces recorded at 10, 46, and 80° C on the cholesteryl 2-ethylhexyl carbonate-coated column.

Another factor which is sometimes affected by changes in the liquid phase is the height equivalent to a theoretical plate, H.E.T.P. The data for the three compounds on the cholesteryl 2-ethylhexyl carbonate coated column are listed in Table 5. Also

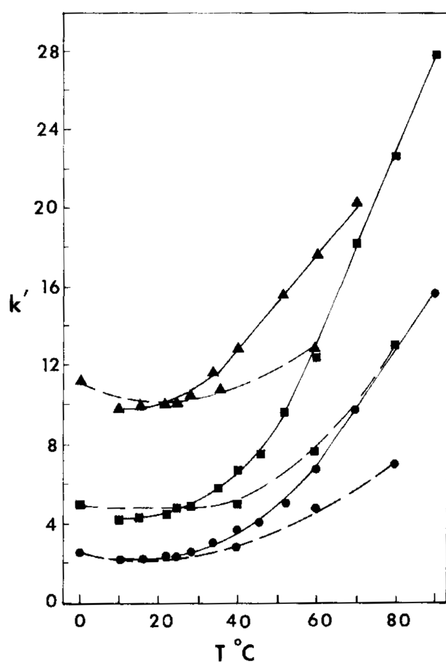

FIGURE 2

Effect of temperature on capacity ratios, k', for androstenedione (●), $\Delta^{1,4}$androstdienedione (■), and testosterone (▲) when chromatographed on Corasil (II) columns with (——) and without (---) 2-ethylhexyl carbonate as stationary phase.

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listed in the table are the retention times, t_r, and peak widths, w, for these three compounds. Variability of the H values for these compounds is fairly small and could be primarily due to inaccuracies in measuring the retention times or peak widths for the peaks. Since there is no definite trend detectable for changes in H during this study, the primary factors causing the increased capacity factors are changes in the relative solubilities of the compounds in the liquid crystal stationary phase as compared with their solubilities in the mobile phase, or changes in the structure of the liquid crystal stationary phase with changes in temperature.

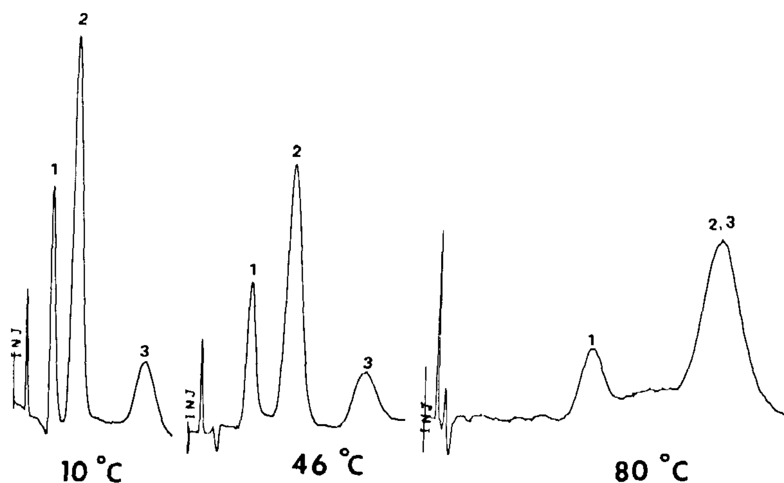

FIGURE 3

Chromatograms at selected temperatures of androstenedione (1), $\Delta^{1,4}$androstdienedione (2) and testosterone (3) on cholesteryl-2-ethylhexyl carbonate coated Corasil (II).

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TABLE 5

The Effects of Temperature on the Retention Time, Peak Width and H.E.T.P. for Selected Androgens when Chromatographed on a Cholesteryl 2-Ethylhexyl Carbonate Coated Corasil (II) Stationary Phase

Temperature °C	Andro-stene-dione			$\Delta^{1,4}$Androst-dienedione			Testo-sterone		
	t_r (mm)	w (mm)	HETP (mm)	t_r (mm)	w (mm)	HETP (mm)	t_r (mm)	w (mm)	HETP (mm)
10	13.2	4.0	3.2	22.1	6.8	3.3	46.3	12.4	2.5
22	14.4	4.0	2.7	23.5	6.5	2.6	47.5	12.1	2.2
28	15.6	4.8	3.3	25.5	7.3	2.8	49.8	13.8	2.6
40	19.2	5.1	2.3	31.8	9.0	2.8	51.2	14.0	2.6
60	33.0	9.0	2.6	58.0	15.5	2.5	81.0	19.0	1.9
70	44.8	11.6	2.6	79.0	20.5	2.6	93.5	26.0	2.8
80	58.0	14.0	2.0	102.0	25.1	2.1	--	--	--
90	96.6	17.8	2.2	120.8	36.3	3.1	--	--	--

To evaluate whether the increase in capacity factors with increasing temperatures was peculiar to steroid compounds, two solutes, androstenedione and benzyl alcohol, were chromatographed. The chromatograms are shown in Figure 4 and the results are shown in Figure 5. The left chromatogram in each pair is for benzyl alcohol and the right chromatogram is for androstenedione.

There were two possible explanations for the observed results. First, as the temperature increases the surface of the Corasil II coated with cholesteryl 2-ethylhexyl carbonate becomes less polar. Therefore, the larger, less polar androstenedione is retained longer by the stationary phase and the smaller, more polar benzyl alcohol is retained less. A second explanation would be as theorized by Helfrich (47) that cholesterics may be effective as separating (permeation) media for large molecules while having almost no affinity for small molecules.

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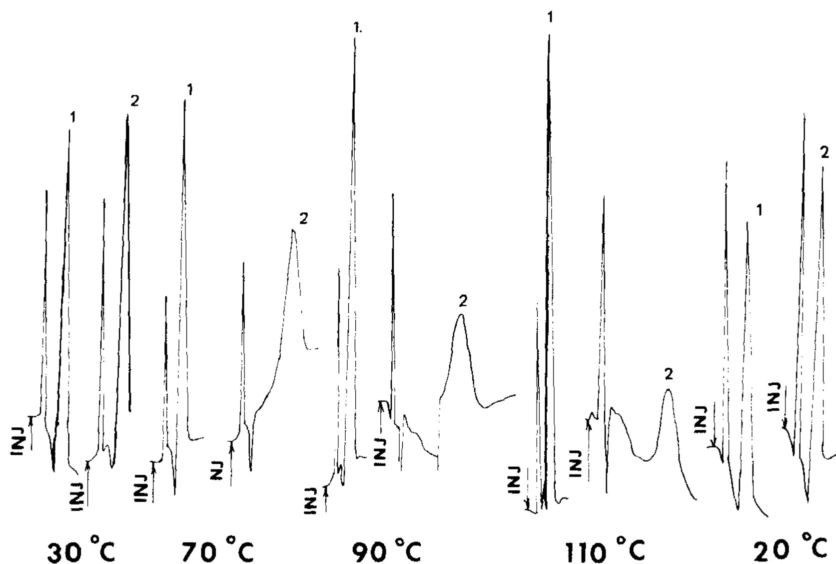

FIGURE 4

Chromatograms at selected temperatures of benzyl alcohol (1) and androstenedione (2) on cholesteryl-2-ethylhexyl carbonate coated Corasil (II).

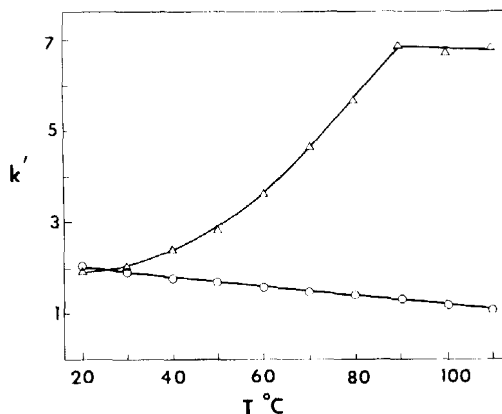

FIGURE 5

Effect of temperature on capacity ratios, k', for benzyl alcohol (o) androstenedione (Δ) when chromatographed on a cholesteryl-2-ethylhexyl carbonate coated Corasil (II) stationary phase.

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Interestingly, while the compounds have almost identical capacity factors at 20° C, they differ by a factor of 6.12 at 90° C, and when the temperature was returned to 20° C from 110° C, the capacity factors for both compounds were almost identical to what they were before heating the column.

To determine whether the observed changes in k' were unique to cholesteryl 2-ethylhexyl carbonate or associated with liquid crystals in general, another liquid crystal, cholesteryl 2-ethylhexanoate, which exhibits mesomorphic behavior over the temperature range indicated, was evaluated. This compound has a melting point range of 48 to 50° C and a monotropic cholesteric transition at 30° C when cooled (48).

A mixture of the three androgens was chromatographed on Corasil II column coated with cholesteryl 2-ethylhexanoate at temperatures between 0 and 80° C. Figure 6 shows the results in a graph of the capacity factor _vs._ temperature. The capacity factor increased 559% and 489% for androstenedione and $\Delta^{1,4}$androstdienedione, respectively, using this column between 0 and 70° C.

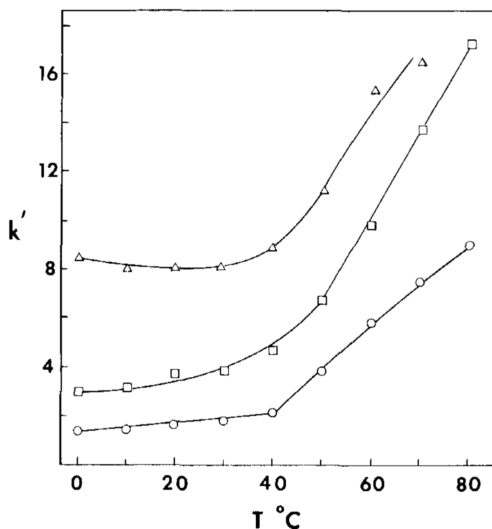

FIGURE 6

Effect of temperature on capacity ratios, k', for androstenedione (o), $\Delta^{1,4}$androstdienedione (□) and testosterone (Δ) when chromatographed on a cholesteryl-2-ethylhexanoate coated Corasil (II) stationary phase.

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It appears, therefore, that the dramatic changes in capacity ratio with temperature are not only associated with 2-ethylhexyl carbonate but a general property of liquid crystals or, at least, with these two types of liquid crystals studied. Other liquid crystals, both cholesteric and non-cholesteric, are currently under study and will be reported later.

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