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# DIRECT GAS CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS ON OPTICALLY ACTIVE MESOPHASES

## II. EFFECTS OF STATIONARY PHASE STRUCTURE ON SELECTIVITY

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## SUMMARY

The direct gas chromatographic resolution of enantiomers on optically active mesophases has been achieved using mesomorphic chiral carbonyl-bis-(amino acid esters). The resolving power of the smectic phases was generally superior to that of the corresponding isotropic liquids and plots of the log of the capacity ratio as a function of inverse absolute temperature for suitable solutes show breaks or slope changes at temperatures closely corresponding to phase transition temperatures determined by differential scanning calorimetry. Resolution on phases was achieved using short glass columns packed with Chromosorb G AW DMCS which had been coated with an optically active carbonyl-bis-(amino acid ester) phase. The largest relative retentions ($\alpha$) and corresponding $\Delta(\Delta G^\circ)$ values thus far observed for gas chromatographic enantiomer separations are reported. Structural variations in the stationary phases were observed to have marked effects on selectivity and on the temperature ranges over which the smectic phases existed.

## INTRODUCTION

The first successful direct separation of enantiomers by gas chromatography (GC) was reported in 1966 by Gil-Av and co-workers[1]. Since that time, a number of GC studies have evaluated various optically-active phases[2–13]. These results have improved the working knowledge of the asymmetric solute–solvent interactions involved, an important accomplishment, because both improvements in separation efficiencies and development of stationary phases of practical value for preparative-scale separations depend on such knowledge.

Two major classes of optically active phases are known to be useful for direct GC enantiomer separations. One of these is the dipeptides, for which most GC work exists[2–9], and the other is the carbonyl-bis-(amino acid esters)[10–13]. The postulated separation mechanism in both cases is based on formation of transient diastereomeric hydrogen-bonded association complexes between the stationary phase (solvent) and the enantiomeric pair (solutes). For the dipeptide phases, all conclusions concerning

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the separation mechanism are based solely on inferences drawn from GC work. In the case of the carbonyl-bis-(amino acid esters), spectroscopic and other data correlate well with chromatographic results, and provide a basis for the reported interactions[10,14].

Carbonyl-bis-(L-valine isopropyl ester) was shown by Feibush and Gil-Av[11] to be a very useful stationary phase for the GC resolution of various secondary amine derivatives. Corbin and Rogers[12] significantly extended the work with that compound and showed that separations on the solid phase as well as on the liquid could easily be achieved. Solid phase separations were reported to give significant improvement over liquid phase separations (relative retentions, $\alpha$) for all enantiomeric solutes studied. It was suggested that increased structural rigidity of the solid phase could result in a more fixed interaction geometry than is available with the liquid phase. Corbin and Rogers observed peak shapes for separations on the solid phase that were anomalous in that the first peak was sharp and the second was much broader. Separations on the liquid phase required much more time but peaks were sharp and essentially identical.

Although it has been suggested that optically active liquid crystal phases should be capable of resolving optical isomers by GC[15,16], such separations have never been reported. Kelker and Von Schivizhoffen[17] have reviewed the use of ilquid crystals as stationary phases in GC and have discussed the physical properties which affect their suitability for such use. Results of numerous GC experiments with nematic and smectic liquid crystal stationary phases to separate benzene positional isomers have been presented[16,18] with arguments that both the relatively high viscosity and the high degree of order of smectic phases may influence the separations. Cholesteryl ester liquid crystalline phases have also received attention as GC stationary phases, and plots of log retention time as a function of inverse absolute temperature exhibited sharp breaks or slope changes at or near mesophase transitions[19].

The discovery that enantiomers could be resolved with very large $\alpha$ values on a smectic liquid crystal phase was recently reported[13]. Results of separations on smectic and on isotropic liquid phases of a series of optically active carbonyl-bis-(amino acid esters) are presented here and the effects of structural changes in the stationary phases on the separations are examined.

## EXPERIMENTAL

### *Reagents*

Preparation, purification and structural verification methods of the carbonyl-bis-(amino acid ester) stationary phases and the fluorinated amides have been described previously[10,20].

An adaptation of a condensation by Richer and Perelman[21] was used to synthesize 2-amino-4-phenylbutane. One equivalent of benzylacetone was dissolved in benzene and one equivalent of distilled benzylamine was added slowly while stirring. The mixture was refluxed overnight under nitrogen while removing water by means of a Dean–Stark trap. Removal of solvent left a bright yellow imine which was mixed with a catalytic amount of 10% palladium on charcoal in absolute ethanol, and shaken in a Paar apparatus under 55 p.s.i. hydrogen pressure for 24–36 h.

Hydrogenation of the double bond and cleavage was achieved, after which the free, distilled and colorless amine (bp. 140–145°/0.2 mm) was trifluoroacetylated.

### *Gas chromatography*

The GC apparatus used here has been previously described[10], with the addition of an all-glass flash vaporization inlet (No. 86800; Hamilton, Reno, Nev., U.S.A.), which was mounted in the oven lid. Column head pressure was read from a Bourdon Type 8½-in. pressure gauge, 0–45 p.s.i. in two revolutions (Heise Bourdon Tube, Newton, Conn., U.S.A.). Flow controllers were used for all gases.

Columns were all-glass, 6 ft. in length. Acid-washed, dimethylchlorosilanized (AW DMCS) Chromosorb G, 100–120 mesh, was coated in each case by the pan method to give the reported percent liquid loading. The carrier gas in all experiments was helium.

A Perkin-Elmer Model DSC-1 differential scanning calorimeter (Perkin-Elmer, Norwalk, Conn., U.S.A.) was used for the calorimetric measurements presented.

## RESULTS

The utility of each optically active stationary phase was investigated over a

TABLE I

TRANSITION TEMPERATURES FOR CARBONYL-BIS-(AMINO ACID ESTER) STATIONARY PHASES

C= Crystal; S= smectic; I= isotropic.

| *Stationary phase* | *Phase transition temperature (°K)* * |
|---|---|
| Carbonyl-bis-(L-valine methyl ester) | $C \xrightarrow{382} S$ |
| | $S \xrightarrow{415} I$ |
| Carbonyl-bis-(L-valine ethyl ester) | $C \xrightarrow{361} S$ |
| | $S \xrightarrow{388} I$ |
| Carbonyl-bis-(L-valine isopropyl ester) | $C \xrightarrow{364} S$ |
| | $S \xrightarrow{372} S'$ |
| | $S' \xrightarrow{382} I$ |
| Carbonyl-bis-(L-valine *tert.*-butyl ester) ** | $C \xrightarrow{98} S$ |
| | $S \xrightarrow{402} I$ |
| Carbonyl-bis-(D-leucine isopropyl ester) | $C \xrightarrow{328} S$ |
| | $S \xrightarrow{383} I$ |

* Determined calorimetrically.

** Calorimetric data based on cooling curves; heating produced only one transition at 427°.

temperature range chosen to include all phase transitions which had been previously determined calorimetrically. The transition temperatures are given in Table I. The mesophases preceding the isotropic liquid were determined in each case to be smectic, as described previously[13,22].

Structures of the stationary phases studied and of the enantiomeric solutes examined are shown in Fig. 1. The structural modification in the L-valine ester series of stationary phases is the change of ester group from methyl and ethyl through isopropyl and *tert.*-butyl. The 2-aminoethylbenzene solute derivatives were examined in detail as the presence of a benzene ring in immediate proximity to the chiral center was expected to enhance interactions. Various perfluoroacyl derivatives of 2-aminoethylbenzene were studied, and by use of 2-amino-3-phenylpropane and

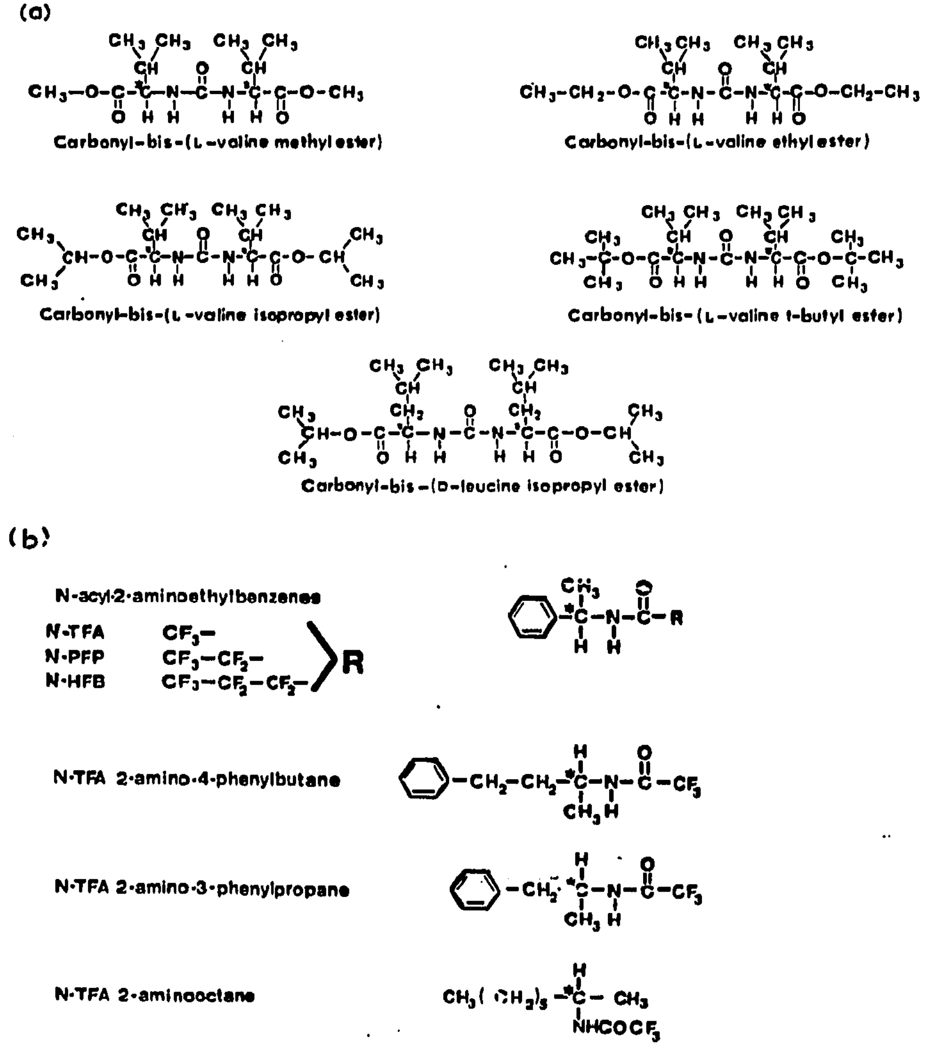


Fig. 1. Structures of (a) stationary phases and (b) enantiomeric solutes studied.

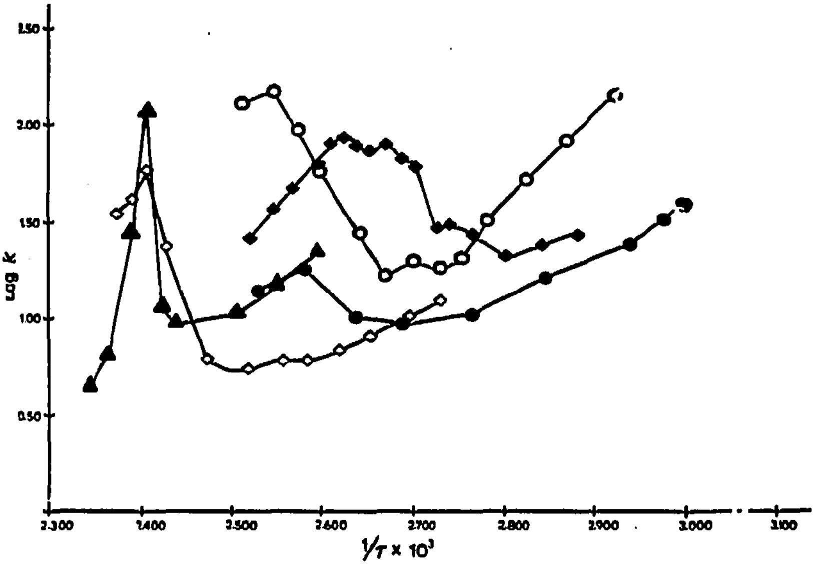


Fig. 2. Plots of log *k vs.* inverse absolute temperature for solutes on optically active stationary phases. ▲, N-TFA (*d,l*) 2-aminopropylbenzene, on the 5.00% carbonyl-bis-(L-valine *tert.*-butyl ester) column; ○, N-TFA (*d,l*) 2-aminooctane on the 5.00% carbonyl-bis-(L-valine ethyl ester) column; □, N-TFA (*d,l*) 2-aminooctane on the 5.00% carbonyl bis-(L-valine methyl ester) column; ●, N-TFA (*d,l*) 2-aminooctane on the 5.00% carbonyl-bis-(D-leucine isopropyl ester) column; ■, N-TFA (*d,l*) 2-aminooctane on the 1.00% carbonyl-bis-(L-valine isopropyl ester) column. On the carbonyl-bis-(L-valine ethyl ester) phase, N-TFA (*d,l*) 2-aminooctane showed resolution at some of the temperatures studied; the retention behavior of the leading peak was studied in all cases.

2-amino-4-phenylbutane derivatives, the effects of the proximity of the benzene ring to the asymmetric center were examined.

Phase transition temperatures for the stationary phases were determined from plots of the log of the capacity ratio (*k*), for various solutes, *versus* inverse absolute temperature (see Fig. 2). This type of plot has been shown to exhibit breaks and/or slope changes at or near phase transition temperatures[19]. Largest increases in capacity ratios occurred at temperatures where the stationary phases changed to isotropic liquids. When the temperature was increased beyond the isotropic transition point, a simple boiling point relationship was observed in all cases. Relative retentions (α) on the isotropic liquid phases showed significant decreases compared with their values on the smectic phases. Transition temperatures determined by this method agreed closely with those calorimetrically determined.

Chromatographic behavior of the carbonyl-bis-(L-valine methyl ester) phase was examined over the temperature range 366–421°K, and values of α and $\Delta(\Delta G°)$ for separations on the phase as a solid (mesophase) and as a liquid are presented in Table II. The 2-aminoethylbenzene solutes yielded separations with low α and $\Delta(\Delta G°)$ values in all cases, and the N-TFA 2-amino-3-phenylpropane (amphetamine) and 2-amino-4-phenylbutane compounds were unresolved even after very long retention times. Values of relative retention (α) were very close at the two temperatures,

TABLE II

RESULTS OF SEPARATIONS OF DIFFERENT SOLUTES ON CARBONYL-BIS-(L-VALINE METHYL ESTER)

5.00% coating.

| *Solute* | *Temperature (°K)* | $\alpha$ | $\Delta(\Delta G°)$(*cal/mole*) |
|---|---|---|---|
| N-TFA (*d,l*) 2-aminoethylbenzene | 390.19 (smectic phase) | 1.062 | −46 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.067 | −50 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.063 | −47 |
| N-TFA (*d,l*) 2-aminoethylbenzene | 415.70 (isotropic liquid phase) | 1.067 | −53 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.072 | −58 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.067 | −54 |

indicating relatively little difference in selectivity on the smectic phase as compared to the isotropic liquid.

Much better separations in terms of $\alpha$ and $\Delta(\Delta G°)$ were obtained for a variety of solutes on the carbonyl-bis-(L-valine ethyl ester) stationary phase on both the smectic and the isotropic liquid phases. Typical results may be seen in Table III. For the structural change of ester group from methyl (to ethyl), it is seen that the selectivity of the stationary phase, especially as a smectic phase, is significantly increased. It has been shown previously[10] that the ester carbonyls of the carbonyl-bis-(amino acid ester) stationary phases are an important site of interaction for the hydrogen bonding. It is also interesting to note that, on the smectic phase, antipodes of 2-amino-3-phenylpropane could not be resolved, while the 2-aminoethylbenzene and 2-amino-4-phenylbutane solutes were separated.

TABLE III

RESULTS OF SEPARATIONS OF VARIOUS SOLUTES ON CARBONYL-BIS-(L-VALINE ETHYL ESTER)

5.00% coating.

| *Solute* | *Temperature (°K)* | $\alpha$ | $\Delta(\Delta G°)$ (*cal/mole*) |
|---|---|---|---|
| N-TFA (*d,l*) 2-aminooctane | 385.02 (smectic phase) | 1.036 | − 27 |
| N-TFA (*d,l*) 2-aminoethylbenzene | | 1.173 | −122 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.257 | −175 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.336 | −222 |
| N-TFA (*d,l*) 2-amino-3-phenylpropane | | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | | 1.108 | −78 |
| N-TFA (*d,l*) 2-aminooctane | 398.04 (isotropic liquid phase) | no resolution | — |
| N-TFA (*d,l*) 2-aminoethylbenzene | | 1.092 | −69 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.097 | −74 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.098 | −74 |
| N-TFA (*d,l*) 2-amino-3-phenylpropane | | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | | no resolution | — |

Carbonyl-bis-(L-valine isopropyl ester) —the compound studied by Corbin and Rogers[12] as a solid— exhibits two stable smectic states prior to melting. The largest interactions, in terms of $\alpha$ and $\Delta(\Delta G^\circ)$, were observed for the solutes studied with the smectic phases of this stationary phase, and the values measured on the isotropic liquid agreed well with those of previous measurements involving open tubular columns[10]. Included in the chromatographic results presented in Table IV is the largest GC value of $\alpha$ ever reported for an enantiomer resolution on an optically active phase ($\alpha$=2.205; N-HFB (*d,l*) 2-aminoethylbenzene). Table V presents a comparison of separations of N-PFP (*d,l*) 2-aminoethylbenzene on smectic and isotropic liquid phases of carbonyl-bis-(L-valine isopropyl ester). It appears that there is a strong dependence of $\alpha$ on the per cent stationary phase coating when the compound is a smectic phase, and a much smaller such dependence when it is an isotropic liquid. Corbin and Rogers[12] observed similar results on the "solid phase".

Separations with the carbonyl-bis-(L-valine *tert.*-butyl ester) stationary phase were observed only near the change to isotropic liquid (see Table I). The compound exhibits monotropic behaviour, *i.e.*, it shows both crystal→smectic and smectic→isotropic transitions only when cooled from the liquid, and the smectic mesophase

TABLE IVA

RESULTS OF SEPARATIONS OF VARIOUS PERFLUOROACYL 2-AMINOETHYLBENZENES ON CARBONYL-BIS-(L-VALINE ISOPROPYL ESTER) AS A FUNCTION OF TEMPERATURE

5.00% coating.

| | *Temperature (°K)* | *$\alpha$ N-TFA* | *$\Delta(\Delta G^\circ)$ (cal/mole)* | *$\alpha$ N-PFP* | *$\Delta(\Delta G^\circ)$ (cal/mole)* | *$\alpha$ N-HFB* | *$\Delta(\Delta G^\circ)$ (cal/mole)* |
|---|---|---|---|---|---|---|---|
| Smectic | 366.17 | 1.171 | −115 | 1.366 | −227 | 1.580 | −333 |
| | 370.05 | 1.420 | −258 | 1.861 | −457 | 2.205 | −581 |
| Smectic′ | 373.34 | 1.317 | −205 | 1.708 | −397 | 2.116 | −556 |
| | 377.28 | 1.242 | −162 | 1.555 | −331 | 1.830 | −453 |
| | 380.96 | 1.161 | −113 | 1.340 | −221 | 1.456 | −285 |
| Isotropic | 396.96 | 1.112 | −84 | 1.117 | −87 | 1.131 | −97 |

TABLE IVB

OTHER CHROMATOGRAPHIC RESULTS OF SEPARATIONS ON CARBONYL-BIS-(L-VALINE ISOPROPYL ESTER)

5.00% coating.

| *Solute* | *Temperature (°K)* | *$\alpha$* | *$\Delta(\Delta G^\circ)$ (cal/mole)* |
|---|---|---|---|
| N-TFA (*d,l*) 2-amino-3-phenylpropane | 377.28 (smectic) | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | | 1.192 | −131 |
| N-TFA (*d,l*) 2-amino-3-phenylpropane | 396.96 (isotropic) | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | , | no resolution | — |

temperature range spans only four degrees. Chromatographic data are presented in Table VI.

A detailed study with carbonyl-bis-(D-leucine isopropyl ester), which was previously reported to give enantiomer resolution as a smectic phase[13], has been completed and results are given in Table VII. In comparison to smectic phase separations on the ethyl- and isopropylvaline stationary phases, the resolution (α) is poorer.

Retention times of various solutes were found to have a definite dependence

TABLE V

COMPARISON OF CHROMATOGRAPHIC RESULTS FOR N-PFP(*d,l*)2-AMINOETHYLBENZENE AS A FUNCTION OF PER CENT STATIONARY PHASE LOADING

Separations on smectic and on isotropic liquid phases of carbonyl-bis-(L-valine isopropyl ester).

| *1.00% coating* | | *5.00% coating* | |
|---|---|---|---|
| *Temperature (°K)* | α | *Temperature (°K)* | α |
| 380.82 (smectic) | 1.116 | 380.96 (smectic) | 1.355 |
| 385.28 (isotropic) | 1.103 | 385.82 (isotropic) | 1.125 |
| 389.42 (isotropic) | 1.101 | 396.96 (isotropic) | 1.117 |

TABLE VI

RESULTS OF SEPARATIONS OF DIFFERENT SOLUTES ON CARBONYL-BIS-(L-VALINE *tert.*-BUTYL ESTER) AT 415.61 °K

5.00% coating.

| *Solute* | α | $\Delta(\Delta G^\circ)$ *(cal/mole)* |
|---|---|---|
| N-TFA (*d,l*) 2-aminoethylbenzene | 1.102 | −81 |
| N-PFP (*d,l*) 2-aminoethylbenzene | 1.118 | −92 |
| N-HFB (*d,l*) 2-aminoethylbenzene | 1.099 | −78 |
| N-TFA (*d,l*) 2-amino-3-phenylpropane | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | no resolution | — |

TABLE VII

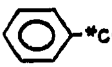
RESULTS OF SEPARATIONS ON CARBONYL-BIS-(D-LEUCINE ISOPROPYL ESTER)

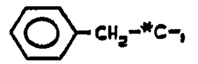
5.00% coating.

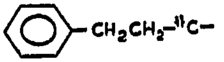
| *Solute* | *Temperature (°K)* | α | $\Delta(\Delta G^\circ)$ *(cal/mole)* |
|---|---|---|---|
| N-TFA (*d,l*) 2-aminoethylbenzene | 361.67 (smectic) | 1.119 | −81 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.658 | −363 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.399 | −241 |
| N-TFA (*d,l*) 2-aminoethylbenzene | 395.63 (isotropic) | 1.073 | −55 |
| N-PFP (*d,l*) 2-aminoethylbenzene | | 1.080 | −61 |
| N-HFB (*d,l*) 2-aminoethylbenzene | | 1.079 | −60 |
| N-TFA (*d,l*) 2-amino-3-phenylpropane | | no resolution | — |
| N-TFA (*d,l*) 2-amino-4-phenylbutane | | 1.050 | −38 |

on sample size for separations on the smectic mesophases. Large samples tended to significantly increase retention time although the effect on relative retention was small. Similarly, sudden changes in carrier gas pressure were found to cause temporary, but large changes in retention time on some of the smectic phases. For example, a sudden increase of downstream carrier gas pressure from 15 p.s.i. to 17 p.s.i. approximately doubled the $k$ values measured on carbonyl-bis-(L-valine isopropyl ester). After a period of 1–2 h, capacity ratios decreased to the previous value (at the higher pressure and flow-rate). Apparently, sudden pressure changes have a temporary influence on the mesophase structure.

Capacity ratios for the N-TFA series 2-aminoethylbenzene, 2-amino-3-phenylpropane and 2-amino-4-phenylbutane with the carbon skeletons:







always followed the same order. The 2-aminoethylbenzene derivative eluted in the shortest time, 2-amino-3-phenylpropane was next, and 2-amino-4-phenylbutane was last. Capacity ratios were very high, especially for the latter case. The first and last compounds were nearly always resolved, while the 2-amino-3-phenylpropane solute never resolved.

The anomalous peak shape (width or variance) observed in the mesophase experiments (Fig. 3) for solutes led to a comparison of $N$ (number of theoretical plates) for those solutes on the four phases studied and an extensive temperature study of $N_R$ *vs.* $N_S$ ($R$ and $S$ refer to the absolute configuration of the solute molecules) in the case of carbonyl-bis-(L-valine isopropyl ester). Table VIII presents the comparison of $N$ among the carbonyl-bis-(L-valine ester) phases. Except for the monotropic *tert.*-butyl phase, $N$ was greater on the isotropic liquids than on the smectic mesophases. In fact, the smectic mesophases, which yielded such high relative retentions for racemic mixtures, had relatively small values of $N$ compared to values calculated for the isotropic liquids. Results of a temperature-dependence study of

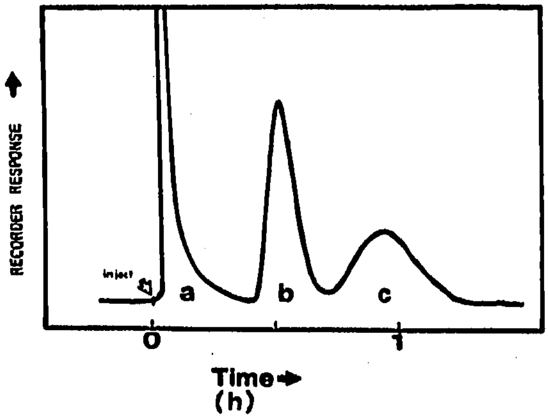


Fig. 3. Anomalous peak shapes observed for separation of N-PFP (*d,l*) 2-aminoethylbenzene on 5.00% carbonyl-bis-(L-valine isopropyl ester) column at 96.89 °C (on the first smectic phase). a= Solvent peak; b= peak for $S$, the D(+) solute; c= peak for $R$, the L(−) solute.

TABLE VIII

COMPARISON OF *N* FOR CARBONYL-BIS-(L-VALINE ESTER) STATIONARY PHASES AS SMECTIC MESOPHASES AND ISOTROPIC LIQUIDS

5.00% coating.

| *Carbonyl-bis-(L-valine ester) phase* | *Solute derivative* | *Isotropic liquid temperature (°K)* | *N* | *(Solid) mesophase temperature (°K)* | *N* |
|---|---|---|---|---|---|
| Methyl | N-TFA (*d,l*) 2-amino-octane * | 421.34 | 617 | 390.19 | 397 |
| Ethyl | Dodecane | 398.20 | 613 | 381.47 | 270 |
| Isopropyl | N-HFB [D(+)] 2-amino-ethylbenzene | 397.01 | 971 | 366.15 **<br>377.28 *** | 73.0<br>10.4 |
| *tert.*-Butyl | Dodecane | 426.81 | 286 | 409.60 | 700 |

* Unresolved on this column.

** First smectic phase.

*** Second smectic phase.

*N* on the carbonyl-bis-(L-valine isopropyl ester) phase are shown in Fig. 4. The very large α values reported on the smectic phases were achieved with very low values of *N*.

For the optical antipodes, $N_R$ and $N_S$ are observed to be not equal on the smectic mesophases. The *S* [D(+)] N-HFB 2-aminoethylbenzene on the L(−) stationary phase (which has the sharper peak shape, as Fig. 1) yields the larger values of *N* over the smectic mesophase regions. The *R* [L(−)] N-HFB enantiomer (the longer retained, with the much broader peak shape) gives quite low *N* values until near the isotropic transition. The broader second peak of the pair slowly sharpens as temperature is raised over the two smectic regions until, at the isotropic transition, *N* increases sharply and both peaks for the separation are nearly identical, as would be expected.

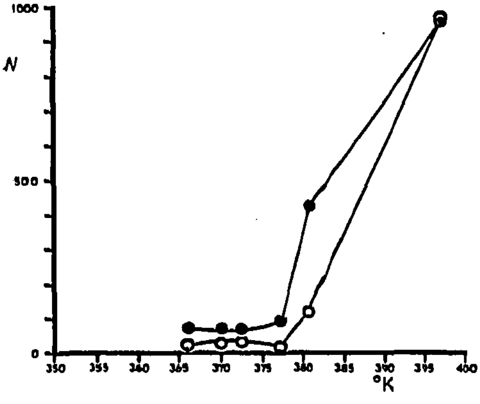


Fig. 4. Temperature dependence of *N* (number of theoretical plates) for N-HFB (*d,l*) 2-aminoethylbenzene on 5.00% carbonyl-bis-(L-valine isopropyl ester) column. ○, L(−); ●, D(+).

## DISCUSSION

A major observation to be made based on the results presented here is that the smectic mesophase forms of the optically active stationary phases have a generally greater selectivity towards the solutes studied than do the corresponding isotropic liquids. Selectivity increased as the size of the L-valine ester group was increased in that series until *tert.*-butyl. Comparison of results from separations on the L-valine isopropyl ester and the D-leucine isopropyl ester phases shows (aside from opposite retention orders due to opposite configurations of chiral centers) that the leucine compound, which bears isobutyl groups at the asymmetric centers as opposed to isopropyl groups in the valine cases, is a qualitatively poorer separator. Selectivity has been shown to be dependent on the asymmetric center substituents, which are in close proximity to the hydrogen bonds to the ester carbonyls[10].

Relative retentions and the differences in standard free energies of association of enantiomers separated on the valine ethyl and isopropyl ester smectic mesophases were very large, whereas in the methyl and *tert.*-butyl cases the measured $\alpha$ and $\Delta(\Delta G^\circ)$ values were low due perhaps in the former case to the small size of the methyl group and in the latter case to so great a bulk as to hinder hydrogen bonding. Calculations of "swept volumes" of ethyl and isopropyl groups show that they are very similar[14].

Of liquid crystalline forms, smectic mesophases are the most ordered which exist. Consistent with this higher order, smectic phases always occur over lower temperature ranges than those of nematic or cholesteric phases which may occur in a compound[23]. In addition to a high degree of order, smectic phases have been shown to be very viscous[23–25]. Both the high degrees of order and the high viscosities are believed to have effects on separations on smectic liquid crystals, the order having its effect on the degree of difficulty of foreign molecules to insert themselves into the liquid crystalline "lattice" and the viscosity having its effect on the rate of diffusion of molecules through the smectic mesophases[16,18]. For a normal stationary liquid phase (*e.g.* Carbowax) one would expect $N_S$ to be equal to $N_R$ since the physical properties (except, of course, chirality) of enantiomeric pairs are identical. Even in the case where selective association occurs (and $\alpha$ is small) in a normal liquid one would expect $N_R \approx N_S$. Since both peaks (*R* and *S*) are fairly symmetrical, it may be that the observed difference has a kinetic (or dynamic) rather than a thermodynamic basis. The latter might involve a situation analogous to the type of peak profile arising in gas–solid chromatography where interactions occur between the adsorbate molecules and a heterogeneous distribution of surface active sites. This leads to a distribution of retention times and peak tailing. On the other hand, a simple increase or decrease in peak variance, $\sigma_x^2$, can result from a change or difference in the resistance-mass-transfer solute-mobile or solute-stationary phase.

It can be shown that *N*, the number of theoretical plates, is related to the diffusion coefficients of the substance in the gas and liquid phases. One may apply eqn. 1 to a treatment of the contribution to peak variance due to slow diffusion of the solute molecule in the stationary phase[26].

$$\sigma_x^2 = \frac{8}{\pi^2} \cdot d_f^2 \cdot \frac{1}{D_L} \cdot \frac{kx}{(1+k)^2} \cdot \mu \qquad (1)$$

where the following symbols are defined:

$\sigma_x^2$ = variance of a Gaussian function
$\mu$ = linear flow-rate
$d_f$ = thickness of the stationary phase
$D_L$ = diffusion coefficient of a substance in the stationary phase
$x$ = position of the mean of the curve (peak)
$k$ = capacity ratio

If one writes such an equation for each enantiomer at a particular temperature and eliminates the constants (including $d_f$ and $\mu$, which were constant at any particular temperature), the ratio becomes

$$\frac{\sigma_R^2}{\sigma_S^2} = \frac{A \cdot \dfrac{1}{D_L^R} \cdot \dfrac{k^R x}{(1+k^R)^2}}{A \cdot \dfrac{1}{D_L^S} \cdot \dfrac{k^S x}{(1+k^S)^2}} \tag{1a}$$

since $\mu$, $d_f$ and $8\pi^{-2}$ are constants. For $k$ very large, $k^R \approx k^S$ and so

$$\frac{\sigma_R^2}{\sigma_S^2} = \frac{A^1 \cdot \dfrac{1}{D_L^R}}{A^1 \cdot \dfrac{1}{D_L^S}} \tag{2}$$

Now $\sigma_x^2 \propto H$ (height equivalent to theoretical plate) $\propto l/N$, where $l$ is the column length (and a constant) and one may write:

$$\frac{\sigma_R^2}{\sigma_S^2} \propto \frac{\dfrac{1}{D_L^R}}{\dfrac{1}{D_L^S}} \propto \frac{\dfrac{1}{N_R}}{\dfrac{1}{N_S}} \tag{3}$$

or finally

$$\frac{N_S}{N_R} \propto \frac{D_L^S}{D_L^R} \tag{4}$$

Inspection of Fig. 4 indicates that for both smectic regions the ratio of $N_S/N_R$ is always greater than one. As might be expected from the high intrinsic viscosity of smectic phases the total number of theoretical plates for either solute is small in the regions of smectic behavior compared to the isotropic phase. In the case of chiral liquid "crystal lattice" systems, particularly a very highly-ordered smectic type, the chirality of the solute must be considered as well[27]. The rate of diffusion of a "right-handed" species in a "left-handed" matrix could conceivably be different from that of a "left-handed" species. At this time, this is an admittedly speculative hypothesis. However, if one accepts this premise, and eqn. 4 as a rough approximation, then at 383° (Fig. 4) $N_S/N_R \approx 4$ or $D_L{}^S/D_L{}^R \approx 4$. This is not a large difference in diffusion rate but does account for the anomalous peak width differences.

The effects of pressure jump and of sample size on the capacity ratio observed in this work as well as the effect of film thickness on $\alpha$ bear a striking similarity to those observed independently for a solid phase and reported elsewhere[12]. Interpretation of these effects is more straightforward in the case of a smectogenic liquid phase. Texture of the phase or its liquid crystal lattice character will be influenced by support effects, impurities (in this case large excesses of hydrogen-bond donating solvents) and sudden temperature and pressure shocks. Re-equilibration times can be quite long in these phases; the compound carbonyl-bis-(D-leucine isopropyl ester) requires up to five days to recrystallize and give a reproducible thermogram.

The significance of increasing the size of the fluoroacyl group in the amide solutes appears to be greater, in terms of $\Delta(\Delta G°)$, in the mesophase region than in the isotropic liquid. This is reasonable if one accepts a "goodness-of-fit" model for liquid crystal phase separation interactions. The effect is lost in the less ordered isotropic liquid and the increase in the inductive effect $CF_3$–, $CF_3$–$CF_2$–, $CF_3$–$CF_2$–$CF_2$–, in terms of enhancement of N–H donor capacity, has been shown to be small[14].

## CONCLUSION

Smectic mesophases of a number of carbonyl-bis-(amino acid esters) have effected excellent separations of enantiomers by GC. The relative retentions, $\alpha$, vary markedly with changes of ester substituent. The effect of increased numbers of fluorines in the perfluoroacyl 2-amino-ethylbenzenes was to give widely separated $\alpha$ values on the smectic phases, but the $\alpha$ values for the same set of solutes on the isotropic liquid phases were nearly identical. The result of removing the asymmetric center in the solutes from close proximity to the benzene rings was to decrease the differences in retention.

Differences in $N$ calculated from peaks for an $(R,S)$ pair separation on smectic phases may be related to differences in diffusion coefficients of the solutes in the stationary phase. Further work is required to establish the actual mechanism responsible for this difference. Results indicate that smectic, optically-active liquid crystals of various structural designs may be quite useful as GC stationary phases for rapid, efficient, preparative-scale enantiomer separations and experiments with optically active liquid crystal stationary phases of new designs are currently in progress in this laboratory.

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