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CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS

SELECTIVE REVIEW

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1. INTRODUCTION AND SCOPE OF REVIEW

Optical isomers occur widely in nature and are utilized for pharmaceutical purposes, for experimental biochemical studies, and as intermediates in the syntheses of biologically active products. The physical property of optical rotation is itself used in studies of chemical kinetic mechanisms or metabolic pathways which produce chirality or which involve inversion or racemization in a key step. The importance of methods which distinguish or resolve enantiomers (molecules with a single chiral center) is very great, especially in pharmaceutical applications, where it is well established that different antipodes can have different pharmacological effects[1].

The conventional methods for measuring optical activity are indeed optical, *e.g.* polarimetry, optical rotatory dispersion and circular dichroism. Almost from the first, it was recognized that chromatographic methods could offer distinct advantages in the analysis and separation of optical isomers —providing that such methods could be developed. Among these advantages are: small sample size, independence from the magnitude of specific rotation, and, most important, independence from other optically active species initially present. Chromatographic methods show promise for moderate-scale separations of synthetic intermediates and final products. For large-scale separations and in consideration of the cost of plant-scale resolution processes, extraction and sorption methods offer substantial increases in efficiency over recrystal-

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lization techniques[2]. The latter are much more common because of the limited extent of present knowledge about stereospecific reactions required to tailor such separations.

In recent years, a significant number of advances have been made in chromatographic separation techniques for the resolution of enantiomers, particularly in gas chromatography. In general the resolution of enantiomers by chromatographic means has been achieved by either conversion of the racemate to a mixture of diastereomers by a suitable chemical reaction with a chiral reagent or through the use of a chiral stationary phase. The major emphasis of this review deals with the latter type of separation although the overwhelming majority of successes have been achieved with the former.

2. CONCEPTUAL MODELS FOR RESOLUTION MECHANISMS

The direct chromatographic resolution of enantiomers is complicated by the fact that individual antipodes differ only in their chirality and not in their vapor pressures, solubilities (in achiral solvents), or ionization constants. In gas chromatography, for example, one may write a simplified expression for net retention volume, V'_R, such that $V'_R = p^0\gamma$, where p^0 is the saturation vapor pressure of a solute and γ is the infinite dilution activity coefficient for the solute in a given solvent. Relative retention, α, is then given by $\alpha = (p^0\gamma)_1/(p^0\gamma)_2$ where the subscripts 1 and 2 refer to the two solutes of vapor pressures p_1^0 and p_2^0 and to the activity coefficients γ_1 and γ_2 in a given solvent.

In order to effect resolution, either the vapor pressures or the activity coefficients must be different ($\alpha > 1$). In liquid chromatography a loose analogy can be made between vapor pressure as described and solubility. The vapor pressure or solubility can be altered by derivatization of the enantiomers to form diastereomers. In this case it is not unlikely that p^0 (or solubility, in liquid chromatography) as well as γ are changed, but the only practical requirement is that $\alpha > 1$. The more elegant (and the analytically more sound) approach is the use of chiral solvents or sorbents where a diastereomeric complex is formed by solute–solvent interactions not involving the formation of a covalent bond. Here the relative stability difference (or the difference in γ values) is the controlling separation factor.

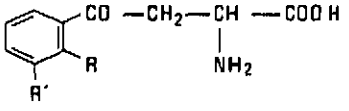
The most commonly used physical picture for this latter type of interactions is one which has its origin in early enzymology and which was adopted by Dalgliesh[3] to explain the retention behavior of amino acid antipodes in cellulose paper chromatography. This model is often termed the "three-point" model because it requires three significant points of attachment between the solute and the sorbent (hydrogen bonds, directed, complexation, etc.). Heavy emphasis has been made in the literature on the need for "attachment" and many molecular parameters have been strained to achieve such bonding schemes. The more adventurous authors suggest a steric interaction as one of the three and use only two points of "attachment". The point missed here is that the statistical requirement of this model is a minimum of three stereochemically significant interactions. For example, three significant finger contacts are required to distinguish a right or left human hand from the "handedness" of ones own probe hand. As the number of interactions multiply, distinction becomes easier and

easier (*e.g.*, entire hand clasp). It is perfectly reasonable then to predict that an environmental chirality would distinguish enantiomers, that is, a solvent or sorbent in (or on) which no "attachments" occur and all of the interactions are steric.

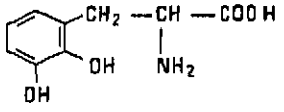
The practical consideration is the magnitude of the difference in the interaction. This is conveniently described as the difference in the thermodynamic standard free energy of formation of the complexes, $\Delta(\Delta G^0)$. If the plate requirement for separation is to remain below 10^5, $\Delta(\Delta G^0)$ must be greater than about 10 cal (equivalent to $\alpha = 1.01$ at 398°K). For preparative-scale, packed column experiments, $\Delta(\Delta G^0)$ values of about 300 cal ($\alpha = 1.5$ at 398°K) are desirable. Therefore, while there is no reason to believe that a difference in interaction energy does not exist for any antipodal racemate in any chiral solvent, only certain solvents (sorbents) will yield practical, useful results.

3. LIQUID MOBILE PHASE–CHIRAL STATIONARY PHASE METHODS FOR DIRECT RESOLUTION

The resolution of racemic amino acids was first reported by Kotake *et al.*[4]. Dalgliesh[5] subsequently studied the structural features necessary for resolution to occur in aromatic amino acids. Three basic structural types were investigated (I–III).



I



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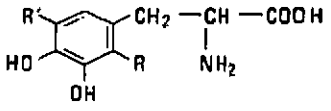


III

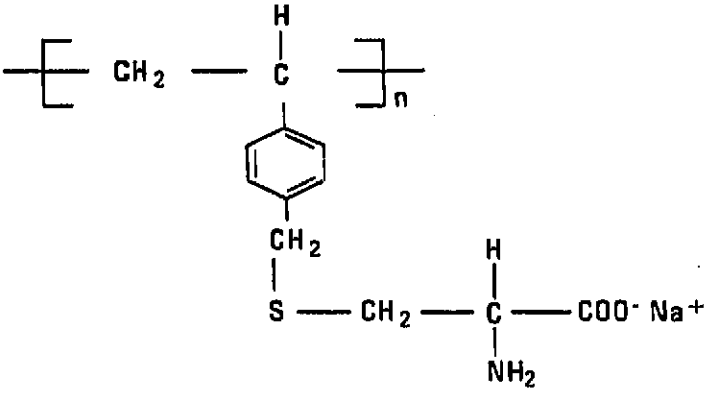
Resolution was observed with (II) and with (III; R = CH_3, R′ = H) but not with (III; R = R′ = H) or with (III; R = H, R′ = CH_3).

Dalgliesh[5] attributed the separation to a differential adsorption phenomenon with "flat surface" molecules (*e.g.*, aromatic) and to hydrogen-bonding molecules being strongly sorbed to the "flat architecture" of cellulose. He proposed that the amino group and possibly the carboxyl group should be "intact". If both these groups were unable to become simultaneously attached to the cellulose surface, presumably by hydrogen bonding, resolution would be unlikely as a three-point attachment of the molecule is required for stereochemical specificity. To give this "three-point attachment", Dalgliesh reasoned that the molecule must contain some other portion, such as an aromatic ring, which is adsorbed on the cellulose surface. In addition, the ring must carry one or more substituents allowing a closer "fit" with the cellulose surface, and hence greater adsorption with one of the optical isomers than with the other. From the work already done, it was postulated that any phenylalanine containing a small substituent in the *ortho* position may be resolvable due to preferential steric effects.

Resolution of the pharmacologically significant *d,l*-DOPA (III; R = R′ = H) was reported by Baczuk *et al.*[6]. The separation was accomplished by using an asymmetric adsorbent (bonded liquid stationary phase) obtained by linking L-arginine via a cyanuric linkage to a commercial polydextran medium conventionally used for exclusion chromatography. An α value of 1.6 was obtained for DOPA and a partial

resolution of tyrosine was observed. Phenylalanine was not resolved. The choice of arginine was based on the need for a three-point contact and for a molecule which could be separated by a bonding link from the surface in such a way that the support backbone did not interfere with the desired "three-point" contact. Interestingly, Bradley *et al.*[7] had postulated in 1954 that the arginine residues in natural wool were the active functions in the optical resolution of mandelic acids which occurs when wool is immersed in an aqueous solution of the racemate salt. In addition, the dextran gel itself used in the former study was reported by Leitch *et al.*[8] to give partial resolution of racemic mandelic acid. (Mandelic acid is used in many studies because of its general solubility and its high specific rotation, $[\alpha]_D^{20} = 158°$, in water.)

There followed a long series of attempts to synthesize polymer resins, wherein an optically active group was introduced into the polymer backbone and which could serve as adsorbents for the resolution of racemic mixtures. Worthy of note is the synthesis by Roberts and Haigh[9] of a crosslinked (1.5% divinylbenzene) poly-[S-(*ar*-vinylbenzyl)-l-cysteine] resin:



R,S-methionine was resolved to a maximum of 44% with this resin using water eluent. Suda *et al.*[10] have reported a new acrylic ion-exchange resin. The resin was prepared by polymerization of ethyl-N-acryloyl-*l*-pyroglutamate and divinylbenzene. This acid-type resin is capable of up to 90% resolution of basic amino acids such as lysine and ornithine. Neutral amino acids are only partially resolved.

Davankov *et al.* have recently reported a technique which they term "ligand-exchange chromatography" (ref. 11 and references therein). The ligand-exchange method differs from adsorption or ion-exchange in that the stationary phase–sorbate interaction is due to the formation of a coordination bond(s) inside the coordination sphere radius of a complexing metal ion. This process, they claim, enhances any sorbent–sorbate interaction which occurs. The complexing metal ion can be bound to mobile ligands and in this case separation is based on the chromatography of several ligand–metal complexes. If the metal ion is bound to the non-mobile ligands of a polymeric stationary phase, then separation arises from chromatography of mobile phase-borne ligands. Rogozhin and Davankov[12] reported separations of a number of racemates (proline for instance) on a chloromethylated styrene–*p*-divinylbenzene copolymer with L-proline.

Humbel *et al.*[13] partially resolved some amino acid derivatives into their stereo-

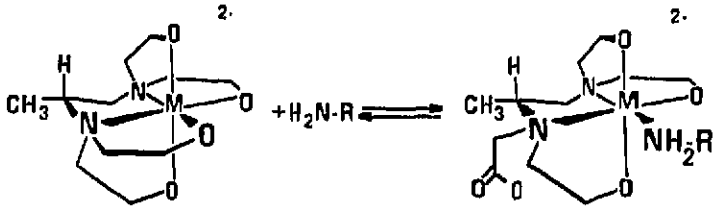
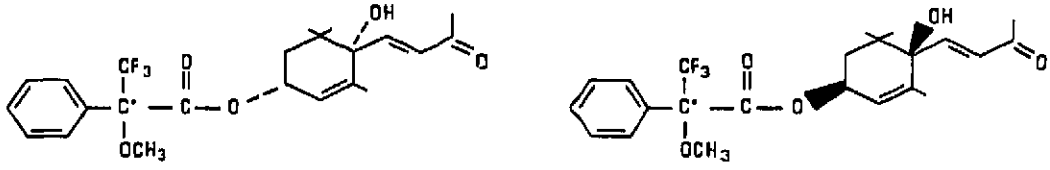


Fig. 1. Replacement of a carboxyl group by an exchange ligand, 1-phenylethylamine, in metal ion propylenediaminetetraacetates.

somers by using an ion-exchange resin containing a chiral anionic iron chelate. Two prerequisites noted for this chromatographic method were: (1) the asymmetric ligand must be labile to allow for ligand exchange and (2) the asymmetric ligand must maintain its asymmetric structure around the metal ion. Bernauer *et al.*[14] examined selectivity in formation of mixed complexes of optically active nickel(II), copper(II), and zinc(II) propylenediaminetetraacetates (PDTA) with 1-phenylethylamine. In the metal ion–PDTA complex, the exchange ligand (1-phenylethylamine) probably replaces a carboxyl group as shown in Fig. 1. Approximately 60% resolution of 1-phenylethylamine was achieved with a short column of Dowex 1-X2 saturated with $[Cu\text{-}D\text{-}PDTA]^{2-}$.

4. LIQUID MOBILE PHASE–ACHIRAL STATIONARY PHASE METHODS FOR INDIRECT RESOLUTION

With proper derivatization enantiomers can be resolved in many cases via diastereomers. A reagent for assessment of optical purity by nuclear magnetic resonance[15] has found use for derivatization for liquid chromatography: (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride. Koreeda *et al.*[16] demonstrated that preparative-scale separation of the enantiomers of abscisic acid was feasible by high-speed liquid chromatography of the acetate esters made from the latter acid and the chiral acid chloride, (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTP ester):



The MTP ester was used for separation of diastereomers by Nakanishi *et al.*[17] in connection with studies of insect juvenile hormone.

Helmchen and Strubert[18] showed that diastereomeric amides formed from reactions of racemic amines with optically pure O-methylmandelyl chloride were generally separable by high-pressure liquid chromatography. This method appeared particularly well suited for detection of trace amounts of optical impurities.

5. INDIRECT RESOLUTION BY GAS CHROMATOGRAPHIC METHODS

Observations which dealt with gas chromatographic resolution of diastereomers began to appear in the literature around 1960. Gil-Av and Nurok[19] completely resolved a series of racemic secondary alcohols as the lactic acid derivatives on a 150-ft. capillary column coated with polypropylene glycol. A similar approach was later used by Gil-Av *et al.*[20] to resolve racemic amino acids. The amino acids were chromatographed as the N-trifluoroacetyl (N-TFA) esters of 2-*n*-alkanols. The latter technique was applied to the problem of the determination of the configuration of the amino acids in two antibiotics of the vernamycin B group[21]. Gil-Av and Nurok[22] have written an extensive review of optical isomer resolution by gas chromatography of diastereomers describing procedures, classes of compounds resolved, applications, and also what is known about the mechanism of resolution.

Considerable advances toward understanding the separation mechanism(s) for diastereomers have been made by Karger and co-workers. In one study[23], diastereomeric esters of α-acetoxypropionic acid were chromatographed at the same temperature on polar and non-polar columns. Greater free energy differences on the polar column indicated larger differences in interaction between the diastereomers and the stationary phase. Also, with increasing size of the substituent at the ester asymmetric carbon, resolution of the diastereomers improved, indicating an assist in asymmetric environment at the central ester linkage due to increased conformational immobility caused by the increased steric bulk. Further experiments indicated that the distance between optical centers in the esters was critical to the separations since resolution was lost as the number of methylene groups between those centers increased.

The concept of conformational immobility previously mentioned (and the consequent nonequivalent accessibility of the ester linkage for interactions of the diastereomers with the stationary phase) was expanded in a further study by Karger *et al.*[24]. A number of racemic cyclic amines were resolved as the N-TFA L-prolyl derivatives. In all cases, both asymmetric carbon atoms in the derivatized compounds were part of cyclic systems. Because free rotations about bonds on the ring are impossible, groups attached to the asymmetric centers are immobile. A very large difference in interaction with the stationary phase was noted for the N-TFA L-prolyl derivatives of 2-methylindoline, due probably to the especially rigid planar arrangement created by the five-membered ring. This greater rigidity leads to increased chromatographic resolution.

N-TFA L-prolyl chloride is a highly versatile reagent for gas chromatographic optical purity analysis of primary and secondary (including cyclic) amines[25–28] and amino acids as their esters[27,29]. However, the proline reagent will not always be significantly more effective than other reagents for resolution of amines. In recent work by Souter[30], several new amino acid chloride resolving agents were compared to the prolyl reagent for resolution of amphetamines and related amines. As shown in Fig. 2, 1-methyl-3-phenylpropylamine was better resolved with N-TFA L-leucyl chloride than with N-TFA L-prolyl chloride. Substituent changes at the chiral centers of the amines, in close proximity to the peptide amide linkage, were shown to affect the resolution. N-Pentafluoropropionyl (N-PFP) and N-heptafluorobutyryl (N-HFB) derivatives were shown in many cases to give nearly the same degree of resolution as in the cases of the N-TFA analogs, but in much less time.

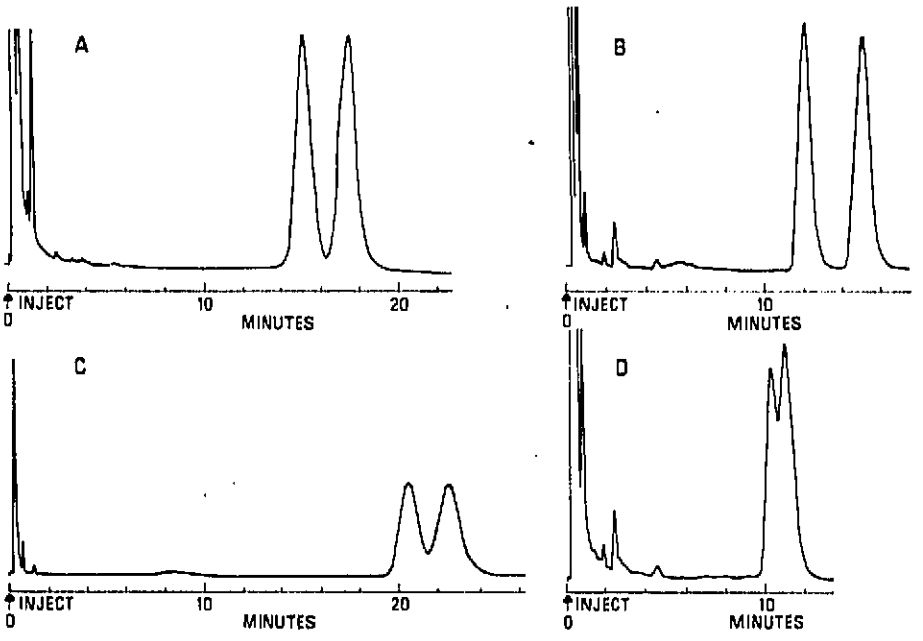


Fig. 2. Comparison of separations of N-TFA L-prolyl and N-TFA L-leucyl amine diastereomers at 200°C on a 6-ft. glass column packed with 5% DEGS on 70–80 mesh Anakrom AB with a flow-rate of 60 ml/min of helium. (A) N-TFA L-leucyl-*d,l*-1-methyl-3-phenylpropylamine, α = 1.153. (B) N-TFA L-prolyl-*d,l*-α-ethylphenethylamine, α = 1.243. (C) N-TFA L-prolyl-*d,l*-1-methyl-3-phenylpropylamine, α = 1.096. (D) N-TFA L-leucyl-*d,l*-α-ethylphenethylamine, α = 1.078.

6. DIRECT RESOLUTION BY GAS CHROMATOGRAPHIC METHODS

In early work a mixture of success and failure is apparent and a conflict of results exists[31–33]. The partial resolution of octahedral chromium complexes by gas–solid chromatography using purified helium and powdered "dextro" quartz has been reported but peaks were observed to tail for hours and the extent of resolution was marginal[34].

At the Sixth International Symposium on gas chromatography in Rome, Gil-Av *et al.*[35] reported some preliminary results of optical isomer separation experiments. Eighteen pairs of N-TFA α-amino acid esters were resolved on glass capillary columns coated with N-TFA D- or L-isoleucine lauryl ester and with N-TFA L-phenylalanine cyclohexyl ester. The separation of antipodes was theorized as involving readily reversible association between the enantiomers and the asymmetric solvent molecules. The transient diastereomeric association complexes could have different steric and polar interactions between substituents on asymmetric centers. Up to a limit the increase in bulk of the substituents on the α-carbon and on the ester group was found to enhance resolution. There followed a series of papers which stimulated investigation by several other groups[36–39].

The three basic stationary phase structural types devised by Gil-Av and Feibush are shown in Fig. 3. The amino acid ester-amides (A) and the dipeptide ester-amides have been more extensively studied because of the substantially larger relative

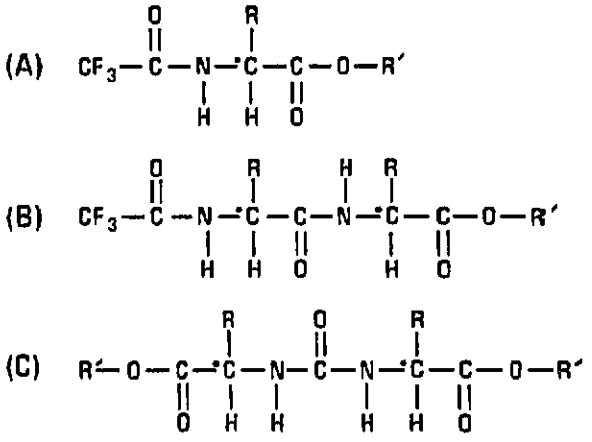


Fig. 3. Basic structural types of optically active stationary phases for gas chromatography.

retention values (α) observed when they are used as liquid phases. The third type (C) is frequently (but erroneously) referred to as a "ureide", but ureides are diacyl ureas and the proper nomenclature here is carbonyl-bis-(amino acid ester). Initial studies of this latter stationary phase type (as liquids) resulted in appreciably smaller α values than A or B, which appeared to limit the utility.

The first dipeptide phase, N-TFA L-valyl-L-valine cyclohexyl ester, produced relative retention values so large as to permit resolution of amino acid enantiomers on a 2-m packed column[40]. This latter result showed that the potential for practical separation of enantiomers by gas chromatography existed. Subsequently, phases like N-TFA L-phenylalanyl-L-leucine cyclohexyl ester were synthesized, which overcame the temperature lability of the first phase[41].

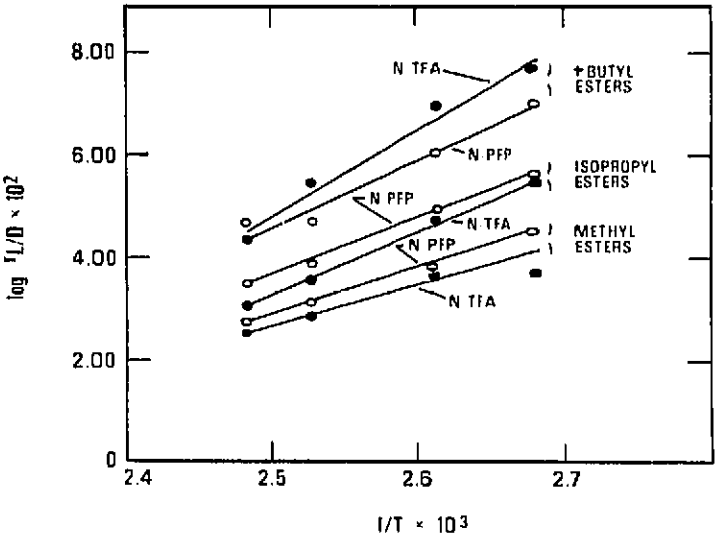


Fig. 4. Plot of the logarithm of the resolution factor $r_{(L/D)}$ *versus* the inverse of the absolute temperature for N-TFA (●) and N-PFP (○) esters of norleucine.

Comparisons of the latter two dipeptide phases showed that resolution improved as the alkyl residue of the solute ester function changed from primary to secondary to tertiary[42]. In addition, the alkyl radical at the solute asymmetric carbon decreased resolution when substituted in the β position, while the reverse was true of substitution in the γ position. Under similar conditions, N-PFP derivatives had about 25% shorter retention times than the corresponding N-TFA esters, with similar relative retentions. Some of these observations are summarized in Fig. 4 for norleucine[42].

A tripeptide phase, N-TFA L-valyl-L-valyl-L-valine isopropyl ester was synthesized by Feibush and Gil-Av[38]. Utility for resolution of optical isomers was good but relative retention values for a variety of solutes were smaller than the analogous dipeptide results.

N-Lauroyl L-valyl-*tert.*-butylamide was reported by Feibush as an asymmetric phase for enantiomer separations[39]. Compared to previously discussed dipeptides the new phase showed increased selectivity and thermal stability, and resolution factors for a number of N-TFA α-amino acid methyl esters were reported to be the largest published for such derivatives on asymmetric phases. These values are shown in Table 1.

Feibush and Gil-Av proposed a theoretical explanation for the resolving power of the dipeptide phases[38]. The fundamental basis is, they state, the formation of transient diastereomeric complexes. These authors argued that such association imposed a particular conformation on the solutes, with the acceptor and donor forming part of a spiral turn. The chirality in each case was determined by the configuration of the amino acids in the dipeptide. The resulting distortion, occurring in the same direction for both enantiomers on the particular stationary phase, had the effect of introducing a conformational element of chirality. In the conformation in which they bonded to the solvent (dipeptide), the enantiomers thus ceased to be mirror images and became "conformational diastereomers". Interactions of such diastereomers with the solvent

TABLE 1

RESOLUTION FACTORS r(L/D) OF N-TFA α-AMINO ACID METHYL ESTERS

Column: 150 ft. × 0.02 in. I.D., capillary, coated with N-lauroyl L-valyl-*tert.*-butylamide at 130° (ref. 39).

Amino acid	r(L/D)
Alanine	1.188
Valine	1.170
O-TFA threonine	1.117
tert.-Leucine	1.084
Alloisoleucine	1.186
Isoleucine	1.159
Leucine	1.280
Proline	1.057
O-TFA serine	1.101
Aspartic acid	1.078
Glutamic acid	1.170
Methionine	1.215
Phenylalanine	1.198
O-TFA tyrosine	1.262

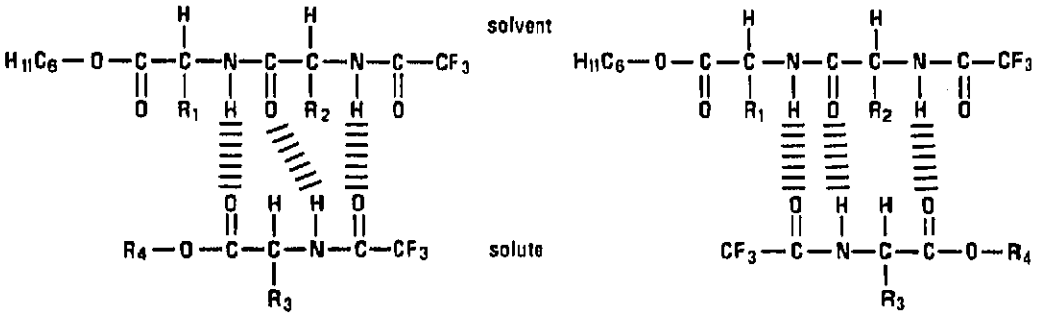


Fig. 5. Possible hydrogen bonding formation of diastereomeric association complexes between N-TFA amino acid ester (solute) and N-TFA dipeptide cyclohexyl ester stationary phase (solvent).

were different and allowed resolution to occur[38]. The asymmetric center substituents of the dipeptide and of the solutes, and the ester substituents of the dipeptides were found to influence the separations. Further understanding of the molecular basis for enantiomer separations is provided by the work of Parr and Howard[43–47] and of Corbin *et al.*[48].

Structural effects in a variety of peptide phases were examined by Parr and Howard, who contend that the amide portion of the stationary phase molecules is the primary contributor to the separations[47]. For association of N-TFA amino acid esters with N-TFA dipeptide cyclohexyl ester phases they have pictured the diastereomeric hydrogen-bonded association complexes similar to Fig. 5. Table 2 presents structures of four dipeptide phases studied recently by Parr and Howard[47]. Separations of a number of N-TFA amino acid isopropyl esters were accomplished using the phases in Table 2. The butyric acid cyclohexyl ester phase was found to effect complete resolution of the amino acids in the mixture tested as well as their respective enantiomers. Increases in the size of alkyl substituents on the asymmetric centers of the dipeptide (solvent) produced greater solute–solvent interactions while similar modifications of the side-chain on the solute α-carbon caused decreased interactions. For dipeptide phases, Parr and Howard found the amide portion of the solvent molecule to be the segment most important in complex formation. All separations were accomplished using extremely long capillary columns.

TABLE 2

HOMOLOGOUS DIPEPTIDE STATIONARY PHASES

$CF_3CO\text{-}NH\text{-}\overset{*}{C}H(R_1)\text{-}CO\text{-}NH\text{-}\overset{*}{C}H(R_2)\text{-}COOC_6H_{11}$

Phase	R_1, R_2
N-TFA L-alanyl-L-alanine cyclohexyl ester	$-CH_3$
N-TFA L-α-amino-*n*-butyryl-L-α-amino-*n*-butyric acid cyclohexyl ester	$-CH_2CH_3$
N-TFA L-norvalyl-L-norvaline cyclohexyl ester	$-CH_2-CH_2-CH_3$
N-TFA L-norleucyl-L-norleucine cyclohexyl ester	$-CH_2-CH_2-CH_2-CH_3$

Corbin *et al.*[48] have also examined effects on gas chromatographic separations due to systematic structural variations in the peptide phases. Their study also indicated that the amide end of the dipeptide appeared to make the primary contribution to the separations, although resolution was sensitive to changes at the ester end. Steadily poorer resolution resulted from increasing the bulkiness of the peptide side groups. These authors also examined a tripeptide phase and found essentially the same relative retention values as yielded by the dipeptide. This latter observation was significant in that it indicated that the ester and amide portions need not be close to each other for resolution to occur. In addition, advantages of N-PFP stationary phases over N-TFA phases were apparent.

The other major class of optically active stationary phase useful for direct gas chromatographic enantiomer separations is the carbonyl-bis-(amino acid ester), the first example of which was reported by Feibush and Gil-Av[36]. The fundamental nature of solvent–solute interactions for this phase type was studied by Corbin and Rogers[49] and to a larger extent by Lochmüller *et al.*[50–54].

The initial impetus for the preparation of carbonyl-bis-(L-valine isopropyl ester) was to allow more equivalent hydrogen-bonded association interactions in close proximity to asymmetric carbons. This phase was demonstrated to have a significant resolving power for chiral primary amines as N-TFA derivatives[36].

Corbin and Rogers conducted a systematic investigation of the chromatographic properties of this phase[49]. Successful resolution of secondary amine derivatives on the liquid phase was reported using capillary columns, and factors affecting separations such as temperature, per cent loading of the column, sample size, and flow-rate were examined. It was found that not only would the phase as a liquid yield enantiomer separations, but that when operated below its melting point the phase gave greatly enhanced separations. The authors suggested that the enhanced activity results from the increased structural rigidity of the solid phase which causes a more fixed interaction geometry than is available with the liquid phase[49]. Peak shapes were anomalous in that the leading peak was sharp while the peak for the longer-retained enantiomer was very broad; on the liquid phase, both peaks for any particular separation were essentially identical.

Lochmüller *et al.* initially reported NMR studies of the sites of hydrogen bond formation with two carbonyl-bis-(amino acid esters) and various solutes[50]. Association chemical shifts with carbon tetrachloride as the solvent medium showed (by ^{13}C and by ^{1}H NMR) that the only significant hydrogen-bonding interaction occurred between the N–H portion of the amide solute and the ester carbonyl of the carbonyl-bis-(amino acid ester). This spectroscopic evidence was interpreted to indicate that only one significant point of "attachment" is involved in formation of the diastereomeric association complexes.

The effect of substituent changes on the donor or acceptor strength of functional groups is also important in determination of the strength of hydrogen bonding, and in the case of the carbonyl-bis-(amino acid esters) the effect on the ester carbonyl of changing the ester substituent from methyl to ethyl to isopropyl and finally to *tert.*-butyl was spectroscopically shown to be inductive in nature[55]. Fig. 6 shows the structures of the carbonyl-bis-(amino acid esters) examined by Lochmüller and Souter[53–55] (spectroscopically and chromatographically). The effect of the bond polarity change on the α-values is complex, with the α-values rising from methyl to ethyl, remaining

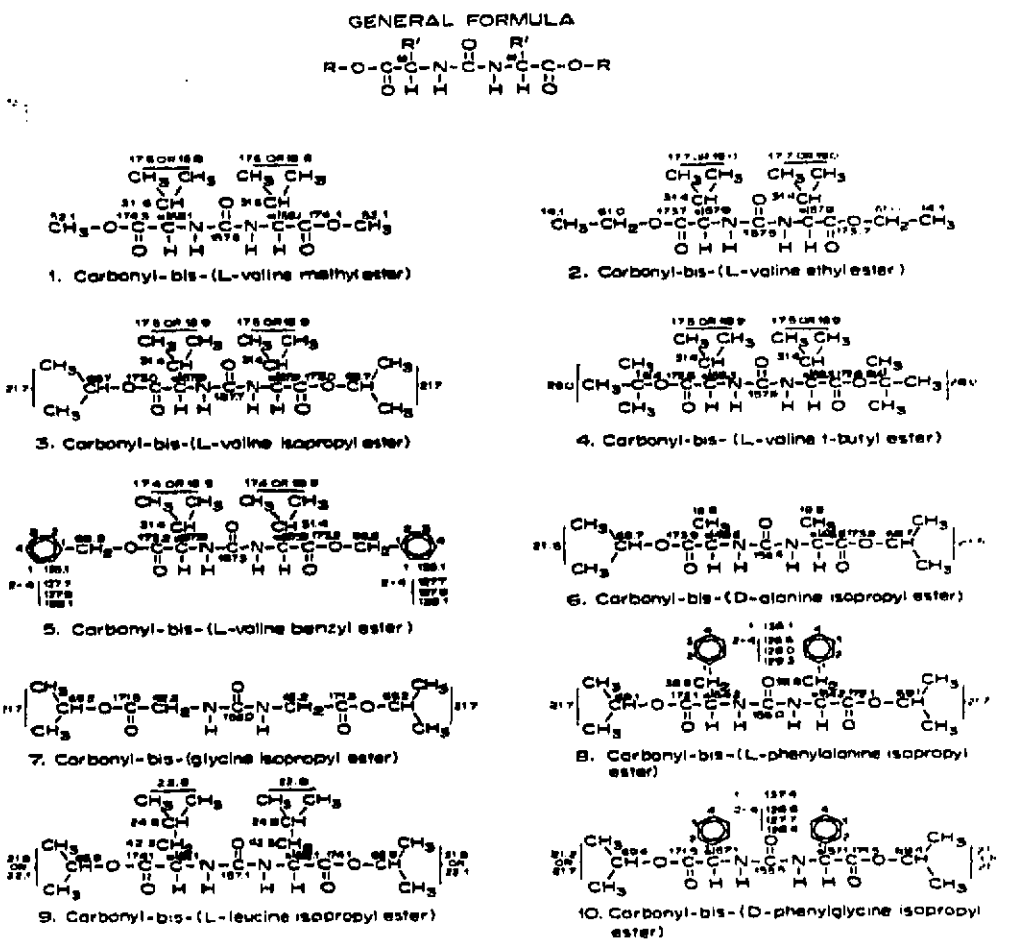


Fig. 6. Structures of chiral (except 7) carbonyl-bis-(amino acid esters). The numerical values are ^{13}C NMR chemical shifts in ppm *vs.* internal tetramethylsilane.

nearly constant at isopropyl, and then decreasing at *tert.*-butyl, as shown in Table 3. The decrease at *tert.*-butyl was attributed to steric hindrance of the large ester group in limiting the approach of hydrogen-bonding donor solutes to the ester carbonyl.

Although it has been suggested that optically active liquid crystal phases should be capable of resolving optical isomers by gas chromatography, such separations were not reported. Kelker and Winterscheidt[56] and Kelker and Von Schivizhoffen[57] have reviewed the use of liquid crystals as stationary phases in gas chromatography and have discussed the physical properties which affect their suitability for such use. Results of numerous gas chromatography experiments with nematic and smectic liquid crystal stationary phases to separate benzene positional isomers have been presented[58,59] 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 gas chromatographic stationary

TABLE 3

VARIATION OF α WITH ESTER SUBSTITUENT STRUCTURE OF STATIONARY PHASE FOR ACYLATED α-METHYLBENZYLAMINE SOLUTES

Stationary phases as isotropic liquids.

Compound	α
Carbonyl-bis-(L-valine methyl ester)	
N-TFA α-methylbenzylamine	1.065
N-PFP α-methylbenzylamine	1.060
N-HFB α-methylbenzylamine	1.066
Carbonyl-bis-(L-valine ethyl ester)	
N-TFA α-methylbenzylamine	1.096
N-PFP α-methylbenzylamine	1.106
N-HFB α-methylbenzylamine	1.105
Carbonyl-bis-(L-valine isopropyl ester)	
N-TFA α-methylbenzylamine	1.099
N-PFP α-methylbenzylamine	1.100
N-HFB α-methylbenzylamine	1.108
Carbonyl-bis-(L-valine *tert.*-butyl ester)	
N-TFA α-methylbenzylamine	1.017

phases[60] and plots of log retention time as a function of inverse absolute temperature exhibited sharp breaks or slope changes at or near mesophase transitions.

The discovery that enantiomers could be resolved with very large α values on a smectic liquid crystal phase of carbonyl-bis-(D-leucine isopropyl ester) was recently reported by Lochmüller and Souter[53]. In addition, those authors showed that several carbonyl-bis-(L-valine esters) displayed liquid crystalline behavior, and were useful as optically active liquid crystalline gas chromatographic phases[54].

That these chiral phases were indeed liquid crystalline was demonstrated by

TABLE 4

THERMAL TRANSITION PROPERTIES OF CARBONYL-BIS-(AMINO ACID ESTERS)

Compound	*Transition temperature (°K)**	*Transition heat, q (kcal/mole)***	*Transition entropy, ΔS (cal/mole·°K)*	*Total ΔS for $S \rightarrow I$ (%)*
Carbonyl-bis-(L-valine methyl ester)	382 C → S	0.73	1.91	69
	415 S → I	1.73	4.17	
Carbonyl-bis-(L-valine ethyl ester)	361 C → S	0.44	1.21	81
	388 S → I	2.00	5.15	
Carbonyl-bis-(L-valine isopropyl ester)	364 C → S	2.25	6.18	
	372 S → S′	0.10	0.27	38
	382 S′ → I	1.55	4.06	
Carbonyl-bis-(L-valine *tert.*-butyl ester)***	398 S → C	1.54	3.87	68
	402 I → S	3.27	8.13	
Carbonyl-bis-(D-leucine isopropyl ester)	328 C → S	5.38	16.4	7.9
	383 S → I	0.54	1.40	

* C = Crystal, S = smectic, I = isotropic.

** Heats of transition calculated based on the H_f of indium, 780 cal/mole.

*** Results based on cooling curves: heating produced only one broad transition at 427°K. The reported temperatures are transformation temperatures.

differential scanning calorimetry measurements for which data are shown in Table 4. Per cent of total transition entropy due to the transition to isotropic liquid was calculated since it offers a criterion for deciding whether a transition is cholesteric→isotropic, nematic→isotropic, or smectic→isotropic[61]. A very large percentage is characteristic of a smectic→isotropic transition. The temperature range over which mesomorphic behavior was observed decreased as the size of the ester group increased in the L-valine ester series. Also, the change at the chiral centers from the isopropyl function of valine to the isobutyl of leucine radically changed the observed thermal behavior.

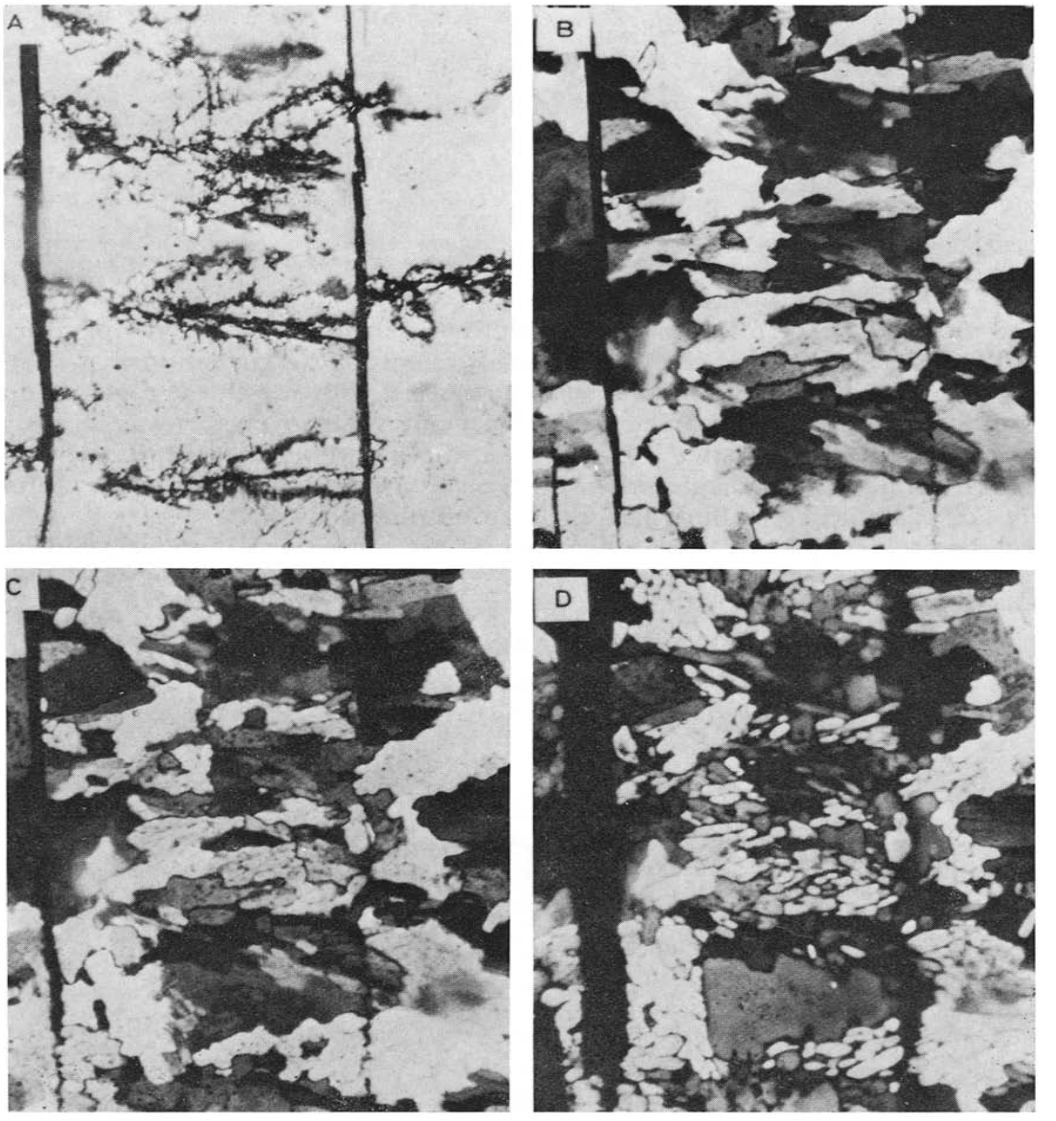


Fig. 7. Polarized photomicrographs of carbonyl-bis-(L-valine isopropyl ester). (A) Crystalline solid; (B) first smectic phase; (C) second smectic phase; (D) changing to isotropic liquid.

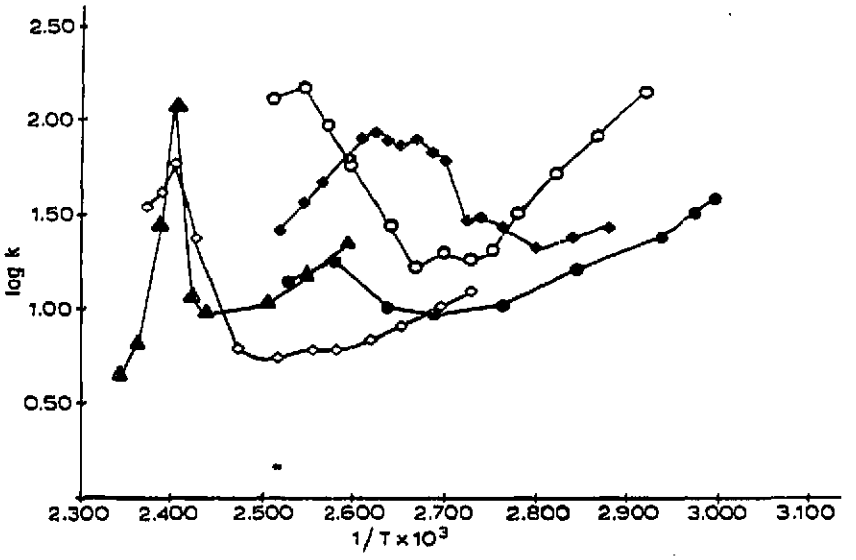


Fig. 8. Plots of log capacity ratio (k) *vs.* inverse absolute temperature for solutes on optically active stationary phases. ▲, N-TFA *d,l*-2-aminopropylbenzene on a 5.0% carbonyl-bis-(L-valine *tert.*-butyl ester) column; ○, N-TFA *d,l*-2-aminooctane on a 5.0% carbonyl-bis-(L-valine ethyl ester) column; ◇, N-TFA *d,l*-2-aminooctane on a 5.0% carbonyl-bis-(L-valine methyl ester) column; ●, N-TFA *d,l*-2-aminooctane on a 5.0% carbonyl-bis-(D-leucine isopropyl ester) column; ◆, N-TFA *d,l*-2-aminooctane on a 1.0% 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.

Mesomorphic behavior can also be characterized by microscopy, as was shown by Lochmüller and Souter[51] for phase transitions in carbonyl-bis-(L-valine isopropyl ester), as shown in Fig. 7. Textural and color changes were observable under polarized light as the sample was heated through its phase changes to isotropic liquid.

Phase transitions for the carbonyl-bis-(amino acid esters) could also be determined from plots of the log of the capacity ratio (k) for various solutes *versus* inverse absolute temperature (see Fig. 8). 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.

Carbonyl-bis-(L-valine isopropyl ester), the compound studied by Corbin and Rogers[19] as a solid stationary phase, exhibits two stable smectic states prior to melting. Lochmüller and Souter studied the chromatographic behavior of this and other closely related stationary phases[53,54]. Resolution was achieved in all cases on short packed columns, and the resolving power of the smectic phases was found generally superior to that of the "liquid" state. The largest relative retentions (α) thus far observed for direct gas chromatographic enantiomer separations were reported[54]. Typical results are shown in Tables 5–9. These constitute the first deliberate suc-

TABLE 5

GC SEPARATIONS OF VARIOUS SOLUTES ON A 6-ft. × 1/8-in.-I.D. COLUMN PACKED WITH 5.0% CARBONYL-BIS-(L-VALINE METHYL ESTER) ON 100–120 MESH CHROMOSORB G AW DMCS

Racemic solute	α	$\Delta(\Delta G°)$ *(cal/mole)*
Temperature = 390.19°K (smectic phase)		
N-TFA 2-aminooctane	unresolved	—
N-TFA α-methylbenzylamine	1.062	−46
N-PFP α-methylbenzylamine	1.067	−50
N-HFB α-methylbenzylamine	1.063	−47
Temperature = 415.70°K (isotropic liquid)		
N-TFA 2-aminooctane	unresolved	—
N-TFA α-methylbenzylamine	1.067	−50
N-PFP α-methylbenzylamine	1.072	−58
N-HFB α-methylbenzylamine	1.067	−50

TABLE 6

GC SEPARATIONS OF VARIOUS SOLUTES ON A 6-ft. × 1/8-in.-I.D. COLUMN PACKED WITH 5.0% CARBONYL-BIS-(L-VALINE ETHYL ESTER) ON 100–120 MESH CHROMOSORB G AW DMCS

Racemic solute	α	$\Delta(\Delta G°)$ *(cal/mole)*
Temperature = 385.02°K (smectic phase)		
N-TFA α-methylbenzylamine	1.173	−122
N-PFP α-methylbenzylamine	1.257	−175
N-HFB α-methylbenzylamine	1.336	−222
N-TFA 2-amino-3-phenylpropane	unresolved	—
N-TFA 2-amino-4-phenylbutane	1.108	− 78
Temperature = 398.20°K (isotropic liquid)		
N-TFA α-methylbenzylamine	1.092	− 69
N-PFP α-methylbenzylamine	1.096	− 73
N-HFB α-methylbenzylamine	1.098	− 74
N-TFA 2-amino-3-phenylpropane	unresolved	—
N-TFA 3-amino-4-phenylbutane	unresolved	—

TABLE 7

GC RESULTS OF SEPARATIONS OF SOME PERFLUOROACYL α-METHYLBENZYLAMINES ON A 6-ft. × 1/8-in.-I.D. COLUMN PACKED WITH 5.0% CARBONYL-BIS-(L-VALINE ISOPROPYL ESTER) ON 100–120 MESH CHROMOSORB G AW DMCS (AS A FUNCTION OF TEMPERATURE)

Temperature (°K)		*N-TFA*		*N-PFP*		*N-HFB*	
		α	$\Delta(\Delta G°)$ *(cal/mole)*	α	$\Delta(\Delta G°)$ *(cal/mole)*	α	$\Delta(\Delta G°)$ *(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 8

GC SEPARATIONS OF VARIOUS SOLUTES ON A 6-ft. × 1/8-in.-I.D. COLUMN PACKED WITH 5.0% CARBONYL-BIS-(L-VALINE *tert.*-BUTYL ESTER) ON 100–120 MESH CHROMOSORB G AW DMCS AT 415.61°K

Racemic solute	α	$\Delta(\Delta G°)$ *(cal/mole)*
N-TFA α-methylbenzylamine	1.102	−81
N-PFP α-methylbenzylamine	1.118	−92
N-HFB α-methylbenzylamine	1.099	−78

cessful uses of chiral mesophases for this purpose. Table 10 presents a comparison of separations of N-PFP α-methylbenzylamine enantiomers on columns coated with two different percentage loadings of the valine isopropyl ester phase. It appears that there is a strong dependence of α on the per cent stationary phase coating when the compound is in a smectic state, and a much smaller dependence when it is an isotropic liquid[54].

Retention times of solutes were found to have a definite dependence on sample size for separations on the smectic mesophases. Large samples tended to significantly increase retention time, although the effect on relative retention (α) was small.

The texture of the smectic phases studied is affected by sudden pressure changes since a sudden change in inlet pressure (to achieve higher flow-rates) was seen to reduce α to essentially zero. A substantial time was required before α was restored to its original value at the higher flow-rate.

The most anomalous feature of these separations is the relation of theoretical plate heights in the zones of R and S solutes. The anomalous peak shapes first observed by Corbin and Rogers[49] for enantiomer separations on carbonyl-bis-(L-valine isopropyl ester) as a "solid" phase were also observed by Lochmüller and Souter for separations on that phase as a smectic liquid crystal[54]. A typical chromatogram is shown in Fig. 9. In all cases the second enantiomer to elute showed a significant increase in plate height (fewer plates, larger variance). In general, one would expect that two so closely related species, eluting with relatively large k values and a small α, would have essentially the same plate height.

TABLE 9

GC SEPARATIONS OF VARIOUS SOLUTES ON A 6-ft. × 1/8-in.-I.D. COLUMN PACKED WITH 5.0% CARBONYL-BIS-(D-LEUCINE ISOPROPYL ESTER) ON 100–120 MESH CHROMOSORB G AW DMCS (AS A FUNCTION OF TEMPERATURE)

Racemic solute	*Temperature (°K)*	α	$\Delta(\Delta G°)$ *(cal/mole)*
N-TFA α-methylbenzylamine	361.67	1.119	− 81
N-PFP α-methylbenzylamine	(smectic)	1.658	−363
N-HFB α-methylbenzylamine		1.399	−241
N-TFA α-methylbenzylamine	395.63	1.073	− 55
N-PFP α-methylbenzylamine	(isotropic)	1.080	− 61
N-HFB α-methylbenzylamine		1.079	− 60
N-TFA 2-amino-3-phenylpropane		unresolved	—
N-TFA 3-amino-4-phenylbutane		1.050	− 38

TABLE 10

EFFECT OF PERCENTAGE STATIONARY PHASE LOADING ON SEPARATIONS USING CARBONYL-BIS-(L-VALINE ISOPROPYL ESTER) COATED ON 100–120 MESH CHROMOSORB G AW DMCS, PACKED INTO 6-ft. × 1/8-in.-I.D. COLUMNS

The solute is N-PFP *d,l*-α-methylbenzylamine.

1.0% coating		*5.0% coating*	
Temperature (°K)	*α*	*Temperature (°K)*	*α*
380.82 (smectic)	1.116	380.96 (smectic)	1.355
385.28 (isotropic)	1.103	385.28 (isotropic)	1.125
389.42 (isotropic)	1.101	396.96 (isotropic)	1.117

Two possible explanations for this behavior are: (1) mixed isotherm retention mechanisms (liquid surface adsorption, bulk solution and support sorption) or (2) differences in diffusion coefficients for R and S solutes in an ordered chiral liquid crystal solvent. The diffusion rate of a right-handed species in a left-handed matrix could conceivably be different from that of a left-handed species in the same matrix[54].

A major observation to be made based on these results is that the smectic mesophase forms of the optically active stationary phases have a generally greater selectivity towards 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 (besides 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[54]. 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[50].

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 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.

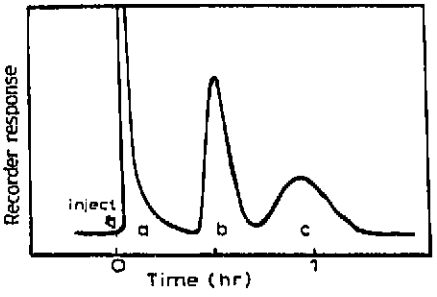


Fig. 9. Anomalous peak shapes observed for N-PFP α-methylbenzylamine enantiomers on a 5.0% carbonyl-bis-(L-valine isopropyl ester) column at 96.89°C (first smectic phase). a = Solvent peak; b = peak for the S solute; c = peak for the R solute.

7. SUMMARY

Recent research has produced a notable increase in knowledge useful for chromatographic enantiomer resolution. Some major advances have emerged in the understanding of asymmetric solute–solvent interactions, and many successful separations by gas and by liquid chromatography have been reported. This review presents a selective discussion of the major advances, with primary emphasis on the use of chiral stationary phases. The latter offer advantages (over indirect techniques) such as generally easier sample preparation, decreased analysis time, and simultaneous chemical as well as optical purity analysis.

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