Journal of Chromatography, 178 (1979) 411–417

CHROM. 12,161

DIRECT GAS CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERS ON OPTICALLY ACTIVE MESOPHASES

IV. EFFECT OF STRUCTURE ON SELECTIVITY AND LIQUID CRYSTALLINE BEHAVIOR

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(Received June 5th, 1979)

SUMMARY

The liquid-crystalline thermal properties of a variety of carbonyl-(α-amino esters) are found to depend strongly upon the substitution of the amino ester side-chains, and to a lesser degree upon the presence of appropriate central carbonyl moieties. Further evidence for a mechanism of the enantiomeric separation of perfluoroacyl amides of optically active amines on carbonyl-bis-((*S*)-valine isopropyl ester) involving fewer than three hydrogen bonding interactions is presented, along with a new synthesis of this class of mesomorphic stationary phases.

INTRODUCTION

The direct chromatographic resolution of enantiomeric mixtures on chiral stationary phases has long been a topic of active interest[1]. Problems of column bleed at high temperatures and of low separation factors (α) have been overcome to the point that it is now feasible, for example, to resolve most amino acid enantiomorphs in a single gas chromatographic (GC) run[2] as well as a variety of chiral drugs such as ephidrines and epinephrines[3]. More knowledge as to a specific model for the mechanism of these separations, however, would allow the design of new phases with yet superior resolving power.

Enantiomers differ in none of their normal physical properties, that is, properties measured in a non-chiral matrix. Thus, the boiling points, melting points and solubilities of enantiomeric pairs are identical. Since GC separations depend upon differences in either the standard vapor pressures (P_0) of solutes or in their activities in a stationary phase (γ), enantiomers are not separated by achiral media (eqn. 1).

$$\alpha = (\gamma_1 P_{0,1})/(\gamma_2 P_{0,2}) \quad (1)$$

$$\Delta(\Delta G^0) = RT \ln \alpha \quad (2)$$

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A stationary phase capable of separating enantiomers must then exhibit different activities for an enantiomeric pair dissolved in that stationary phase. This requirement is met in a stationary phase that is itself chiral, and is the basis for all direct enantiomeric GC separations. A convenient measure of the selectivity of a stationary phase for a pair of solutes is expressed in eqn. 2 as the difference in the standard free energy of solution between them ($\Delta(\Delta G^0)$).

The great majority of stationary phases for the GC separation of enantiomers reported to date may be classified in two categories: (1) the di- and tripeptides and related materials (type A), and (2) the carbonyl-(α-amino esters) (type B) (Fig. 1). Both of these classes of chiral substances separate enantiomers such as perfluoroamides of secondary amines and of α-amino esters. The type B materials generally exhibit somewhat smaller values of $\alpha_{S/R}$ in the liquid state than type A phases, and a larger number of type A phases have been reported. At reduced temperatures, however, type B phases exist in a liquid-crystalline state and show greatly enhanced separations[4]. Proposed mechanisms for enantiomeric separations on both types of phases have involved dynamic, hydrogen-bond formation between solutes and sta-

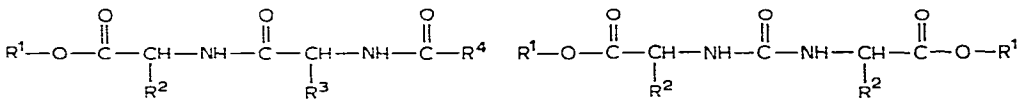

Fig. 1. Principal classes of chiral stationary phases for the direct separation of enantiomers.

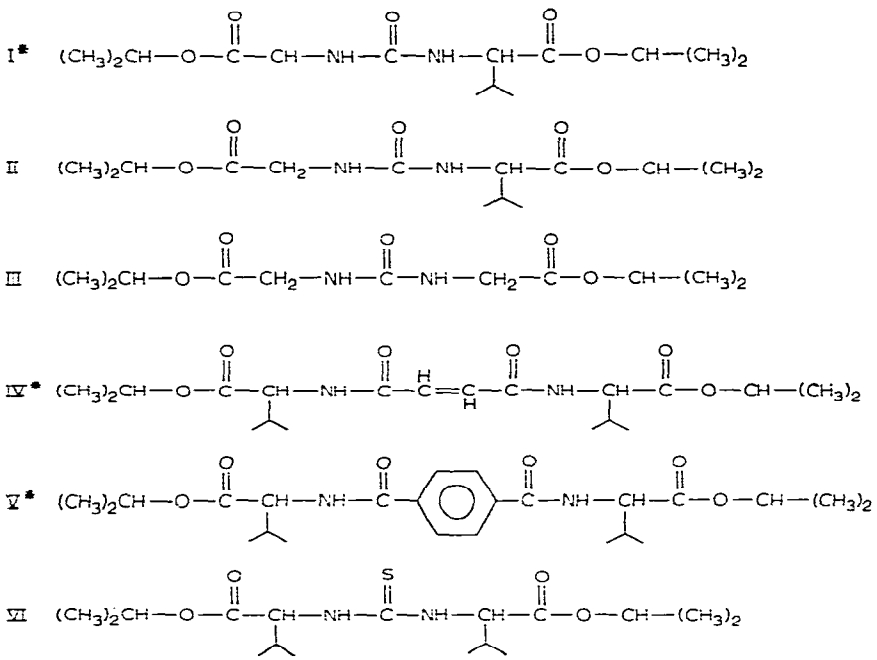

Fig. 2. Structures of chiral stationary phases (* = mesomorphic). All chiral centers are (*S*).

tionary phases, resulting in the creation of transient diastereomeric complexes. Originally involving three strong hydrogen bonding interactions[5], this model has been modified to include only one strong hydrogen bond plus one chirotopical steric factor. The basis for this modification is found in the solute-induced ^{13}C and ^{1}H nuclear magnetic resonance (NMR) chemical shift changes ($\Delta\delta$) that were observed for type B phases. Only the ester carbonyl of the stationary phase and the amide N–H proton of the solute show significant hydrogen bonding effects[6].

Since type B molecules possess two chiral centers, however, it is not clear whether the two centers exert equal and independent effects upon enantiomers, or if in fact they act in a concerted fashion to give the observed selectivity. The series of stationary phases I, II, III, and VI (Fig. 2) have been synthesized for this study, and their GC, thermal and hydrogen bond association properties were assessed.

In order to synthesize II, a new route to this class of compounds was developed. This route, although involving more steps than previously followed, gives products of improved purity that crystallize easily, and also allows the synthesis of a large variety of other "mixed" compounds like II.

EXPERIMENTAL

Reagents

Carbonyl-1-((*S*)-valine isopropyl ester)-1'-(glycine isopropyl ester) (II) was synthesized as follows. Both (*S*)-valine isopropyl ester and glycine isopropyl ester hydrochlorides were obtained by esterification of the free amino acid in HCl-saturated isopropanol (30 g of amino acid in 500 ml alcohol) for 2–3 h at reflux, followed by removal of the solvent by rotary evaporation and washing of the resultant semi-crystalline mass with aliquots of benzene and diethyl ether. The glycine isopropyl ester hydrochloride was then recrystallized from acetone. The (*S*)-valine isopropyl ester hydrochloride was not further purified.

The free ester of the glycine salt was obtained by the method of Greenstein[7]. The hydrochloride (30 g) was dissolved in warm isopropanol (30 ml), and cooled to 5°. Then triethylamine (20 g, excess) was added dropwise over a 2-min interval. Diethyl ether was then added to bring the volume of the resulting suspension to 500 ml, and it was cooled in an ice bath for 1 h. Filtration followed by rotary evaporation of the solvent and fractional distillation yielded the free glycine isopropyl ester (13.4 g, b.p. 61–62° at 20 torr).

The preparation of the isopropyl ester of 2-isocyanatoacetic acid was carried out after a procedure outlined in two reviews[8,9]. A 500-ml round-bottomed flask, equipped with a mechanical stirrer, a reflux condenser, and an addition funnel was continuously flushed with dry nitrogen and was charged with an ice-cold solution of phosgene in benzene (12%, w/w, 181 ml). The addition funnel was filled with a solution of glycine isopropyl ester (13.4 g) in benzene (25 ml), and addition carried out with ice-bath cooling over a 45-min period. The solution was allowed to slowly warm to room temperature over a 1-h period and then was carefully warmed on a heating mantle to 40°, during which time large quantities of gas were evolved. After the gas evolution subsided, the solution was heated at reflux for 18 h, overnight. Most of the benzene and remaining phosgene were removed by simple distillation in the hood under a nitrogen atmosphere, up to a pot temperature of 95°. The yellow

residue was then vacuum distilled in a Kugelrohr (bulb-to-bulb) distillation device to yield 2-isocyanatoacetic acid, isopropyl ester (10.5 g, b.p. 65–70° at 20 torr).

The condensation of the isocyanate with the free amino ester was performed according to Morel[10]. Thus, free (*S*)-valine isopropyl ester was obtained from the hydrochloride in the manner previously described for glycine isopropyl ester. The free amino ester (5.6 g) was dissolved in dry diethyl ether (10 ml) and added dropwise over 3 min to a solution of 2-isocyanatoacetic acid, isopropyl ester (5.0 g) in dry diethyl ether (35 ml). The solution warmed and boiled slightly. After the reaction subsided, the solution was cooled to 5°, and the resultant suspension of white crystals was filtered; the nature of these crystals was not further investigated. Removal of solvent followed by recrystallization from boiling cyclohexane gave II (7.8 g, m.p. 107–109°). The structure and purity were confirmed by ^{1}H and ^{13}C NMR and by thin-layer chromatography (TLC) on activated silica gel (20% diethyl ether in methylene chloride, ninhydrin detection).

Carbonyl-bis-((*S*)-valine isopropyl ester) (I) and carbonyl-bis-(glycine isopropyl ester) (III) were prepared after the method of Humphlett and Wilson[11], by self-condensation of the appropriate isocyanato esters, obtained as described above, in aqueous tetrahydrofuran (5% water). In both cases, recrystallization from boiling cyclohexane gave the desired product, confirmed by ^{1}H and ^{13}C NMR and by thin-layer chromatography. Thermal properties were measured on a Perkin-Elmer DSC 1A.

Thiocarbonyl-bis-((*S*)-valine isopropyl ester) (IV) was prepared in a manner analogous to that used for I. (*S*)-Valine isopropyl ester isothiocyanate was prepared from (*S*)-valine isopropyl ester hydrochloride, using the method of Halpern *et al.*[12]. A solution of thiophosgene (10 g) in toluene (200 ml) was added over a 30-min period to a refluxing solution of (*S*)-valine isopropyl ester hydrochloride (10 g) in toluene (200 ml), and was kept at reflux overnight. The bulk of the solvent was removed by simple distillation, and the last traces of toluene were removed by rotary evaporation at reduced pressure. The dark red oily residue was distilled on a bulb-to-bulb distillation device, yielding the product as a yellow oil (9.8 g, b.p. 65–67° at 0.1 torr). When an attempt was made to self-condense the isothiocyanate, however, only a small amount of product was obtained. Instead, the isothiocyanate was condensed with (*S*)-valine isopropyl ester in dry diethyl ether. The reaction mixture did not boil, and it was heated at reflux overnight. Removal of solvent and recrystallization from boiling cyclohexane gave IV as white crystals (m.p. 118–120°).

The solutes were commercially obtained as resolved secondary amines which were perfluoroacylated with trifluoroacetic anhydride as has been previously reported[13], with the exception of 2-aminooctane which was obtained as the racemate, and then perfluoroacylated. (*R*)- and (*S*)-2-aminoethylcyclohexane were prepared by hydrogenation of (*R*)- and (*S*)-2-aminoethylbenzene over a rhodium catalyst as described by Deutsch[14].

Gas chromatography

A Varian 3700 gas chromatograph equipped with glass-lined injectors and a flame-ionization detector was used with 91 cm × 3.2 mm I.D. borosilicate glass columns. The columns were packed with Chromasorb 750 coated with 3.2% (w/w) of stationary phase. 1-μl injections of a 1 mg/ml solution of solutes in pentane were made. The oven temperature was 115.6°, and helium was used as the carrier gas.

^{13}C NMR spectroscopy

The ^{13}C NMR spectra were obtained with a JEOL FX-60 pulse Fourier transform spectrometer operating at 15.00 MHz. The NMR lock was the deuterium resonance of hexadeuteroacetone contained in a coaxial insert in the 10-mm sample tube.

RESULTS AND DISCUSSION

The thermal properties of I, II, and III as determined by differential scanning calorimetry are summarized in Table I. Unexpectedly, removing one of the valine isopropyl side-chains from the Val-Val (I) material, giving rise to II, eliminated the liquid-crystalline state found in I. Whereas the Val-Val material undergoes smectic transitions both upon heating and cooling, the Val-Gly (II) material does not do so, nor does the Gly-Gly phase (III). Lochmüller and Souter[6] have shown that small changes in the ester substituents or in the substitution on the chiral sites of type B materials also affect the range of the smectic region, but none of these modifications completely eliminates this behavior. The melting point of all of the type B phases synthesized to date lie within a relatively narrow range. It appears, then, that the class of carbonyl-(α-amino esters) described here all are strongly associated via the central urea moiety, giving rise to the observed narrow range of melting points, and that the liquid-crystalline behavior is primarily an effect modulated by weak electrostatic associations of the side-chains, as first asserted by Lochmüller[15]. This model is supported by the data presented here for the series I, II, and III.

The smectic behavior is not entirely dependent upon side-chain substitution, however. Souter[15] found that replacement of the central carbonyl group with either fumaryl or terephthaloyl groups (IV, V) yields compounds that show smectic behavior over an increased range of temperatures. Substitution of a thiocarbonyl group for

TABLE I

THERMAL PROPERTIES OF CHIRAL STATIONARY PHASES BY DIFFERENTIAL SCANNING CALORIMETRY

crys = Crystalline; smec = smectic; isotr = isotropic.

Material	*Transition*	*Temperature (°C)*
Carbonyl-bis-((*S*)-valine isopropyl ester) (I)	crys–smec I	91.0
	smec I–smec II	99.0
	smec II–isotr	109.0
Carbonyl-1-((*S*)-valine isopropyl ester)-1′-(glycine isopropyl ester) (II)	crys–isotr	105.0
Carbonyl-bis-(glycine isopropyl ester) (III)	crys–isotr	108.0
Fumaryl-bis-((*S*)-valine isopropyl ester) (IV)	crys–smec I	157.9
	smec I–smec II	177.9
	smec II–isotr	208.9
Terephthaloyl-bis-((*S*)-valine isopropyl ester (V)	crys–smec	107.9
	smec–isotr	173.9
Thiocarbonyl-bis-((*S*)-valine isopropyl ester) (VI)	crys–isotr	110.0

the central carbonyl of I, giving rise to VI, yields a material that again shows no liquid-crystalline behavior. Since the C = S···H–N hydrogen bond strength is different than that of C = O···H–N, the liquid-crystalline properties of type B substances are also highly dependent upon the associative properties of the central moiety.

The GC properties of I, II, and III are summarized in Table II. For each solute, stationary phase selectivity is reduced in the Val-Gly (II) phase as compared to the Val-Val (I) phase. The Gly-Gly (III) phase was not studied since it shows no enantiomeric separations. In fact, the selectivity as measured by $\Delta(\Delta(G^0))$ is cut approximately in half in each case, with the exception of (*R,S*)-N-trifluoroacetyl-2-aminooctane. It is necessary to measure retention times for each antipode separately on packed columns since these columns are not efficient enough to give sufficient resolution with α less than 1.05, but 2-aminooctane was available only as the racemate. Thus one broad peak was obtained for this solute on II.

TABLE II

GAS CHROMATOGRAPHIC SEPARATIONS OF ENANTIOMERIC SOLUTES AT 115.6° ON THE VAL-VAL AND THE VAL-GLY STATIONARY PHASES

Solute	*Phase*	$\alpha_{S/R}$	$\Delta(\Delta G^0)$ *(cal/mole)*
(*R,S*)-N-Trifluoroacetyl-2-aminoheptane	I	1.04	−33.0
	II	1.02	−16.0
(*R,S*)-N-Trifluoroacetyl-2-aminooctane	I	1.05	−34.0
	II	1.00	0.0
(*R,S*)-N-Trifluoroacetyl-2-aminoethylcyclohexane	I	1.07	−52.0
	II	1.03	−20.0
(*R,S*)-N-Trifluoroacetyl-2-aminoethylbenzene	I	1.10	−75.0
	II	1.04	−33.0

The results of a ^{13}C NMR hydrogen bond induced shift study on II are shown in Table III. As was shown earlier, the only significant shift observed was for the ester carbonyls, which were conveniently separated in the ^{13}C NMR spectrum, allowing simultaneous determination of $\Delta\delta$ for both the glycyl and valyl ester carbonyls. Since the magnitudes of both of the induced shifts are nearly identical, association of the solute with the solvent (II) must be occurring with nearly equal frequencies at either the chiral valyl site or the achiral glycyl site. The bulkier, isopropyl side-chain of

TABLE III

^{13}C CARBONYL ASSOCIATION SHIFTS (δ) IN ppm RELATIVE TO UREA CARBONYL

0.17 *M* II in CCl_4 with (*S*)-N-trifluoroacetyl-2-aminoethylbenzene as solute. 15.003-MHz pulse Fourier transform NMR.

Solute concentration	*Valyl C = O*	*Glycyl C = O*
0.017 *M*	+15.41	+13.25
0.17 *M*	+15.11	+12.89
0.26 *M*	+15.05	+12.81
Overall change ($\Delta\delta$)	− 0.36	− 0.44

the valyl side may cause a slight shift to the glycyl side, but not a significantly large one. This lack of discrimination for the two ester carbonyls gives rise to the observed loss of one half of the selectivity of I compared to II since in II half the transient hydrogen-bond complexes formed will be achiral and will not differentiate enantiomers.

CONCLUSIONS

The liquid-crystalline thermal properties of carbonyl-(α-amino esters) were found to be strongly dependent upon side-chain substitution and to lesser extent upon the presence of appropriately constructed central moieties. Large deviations in either case caused loss of liquid-crystalline behavior.

Further evidence for a mechanism of enantiomeric separation based upon formation of transient complexes involving one hydrogen bond and one steric interaction was presented. The two sides of carbonyl-(α-amino esters) exert their influence on enantiomeric solutes independently and only one chiral center per solute molecule is required for a separation to occur.

Work is underway in this laboratory utilizing these concepts in the design of more efficient stationary phases for enantiomeric resolution.

ACKNOWLEDGEMENTS

This work was supported under a grant from the National Science Foundation CHE-78-17087.

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