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Note

Structural requirements for enantioselectivity in gas chromatography of chiral α-hydroxy acids

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Enantiomer separation on chiral stationary phases has been widely used in configurational studies of low molecular weight compounds[1]. As to the separation mechanism, numerous publications have appeared in the literature[2–4]. For the separation of the enantiomers of amino acids[5–8], amino alcohols[9], amines[10–12] and α-substituted carboxylic acid amides[13,14], (–NH....CO–)–hydrogen bond interactions between solute (volatile derivative) and solvent (liquid phase) may contribute considerably to the enantioselectivity as demonstrated by model considerations in a recent article by Feibush *et al.*[9]. Additional forces, different from hydrogen bonding, definitely contribute to enantiomer separation as shown by the enantioselective solvent–solute interaction of compounds, where hydrogen bond formation is not possible[15,16]. In this work several new optically active stationary phases were used to investigate the structural requirements for enantioselectivity.

EXPERIMENTAL

Synthesis of compounds I–XIII

The compounds used as liquid phases on glass capillary columns are represented in Table I.

The *Z*-derivative of *S*(*R*)-mandelic acid was prepared according to Thamm[17] and coupled with α-phenylethylamine after formation of the mixed anhydride with chloro-carbonic acid ethyl ester. Analogously, *Z*-mandelic acid was coupled to *tert.*-butylamine, L-valine cyclohexyl ester, L-valine *tert.*-butylamide and *R*-phenylglycinol. Compound XI was prepared by esterification of *S*-mandelic acid with cyclohexanol and *p*-toluenesulfonic acid as a catalyst[18]. The *Z*-derivative XII was obtained as indicated above. VII was obtained from *S*-mandelic acid by reaction with dodecanoyl chloride and coupling with *tert.*-butylamine. X was prepared according to Staab[19] from *Z*-*S*-mandelic acid and XI with N,N'-carbonyldiimidazole. Racemic 2-phenylbutyric acid could be separated by fractional crystallization from diisopropyl ether of the diastereoisomeric *S*-2-phenylethylamides, obtained according to the mixed anhydride method described above.

Preparation of glass capillary columns

Glass capillaries were drawn from Pyrex glass tubes with a Hupe and Busch

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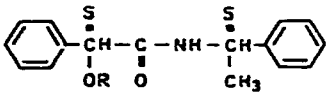
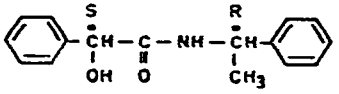
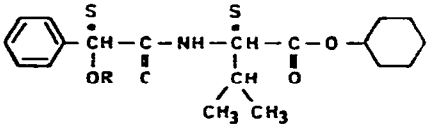
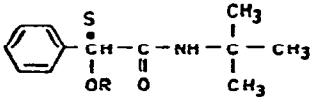
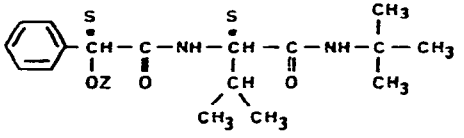
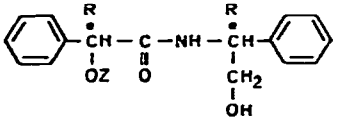
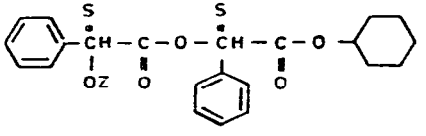
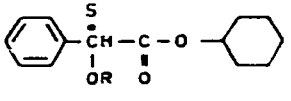
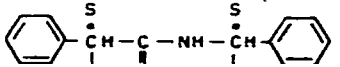
TABLE I

STRUCTURE, CONFIGURATION AND MELTING POINT OF COMPOUNDS USED AS STATIONARY PHASES FOR ENANTIOMER SEPARATION

Structure/configuration	*Melting point (°C)*
	I:oil II: 93
I : R = H , II : R = Z	
	III:oil
	IV:oil V: 70
IV : R = H , V : R = Z	
	VI: 78 VII: 47
VI R = Z , VII : R = n - $C_{11}H_{23}$ - CO -	
	VIII:145
	IX:139
	X:oil
	XI: 27 XII: 57
XI R = H , XII R = Z	
	XIII:116

capillary drawing machine and coated as described in a previous publication[16]. Gas chromatography was performed with hydrogen as carrier gas in Carlo Erba model 2101 gas chromatographs.

Formation of derivatives

Isopropyl esters of amino acids and α-hydroxy acids were prepared by reaction of 100–200 μg of sample with isopropanol–HCl gas (1.5 *N*) at 100°C for 30 min. After removing the excess of reagent in a stream of dry nitrogen the samples were trifluoroacetylated at room temperature (30 min) in a mixture of 200 μl of methylene chloride and 50 μl of trifluoroacetic anhydride. The excess of reagent was evaporated in a smooth stream of dry nitrogen and the samples were dissolved in 100 μl of methylene chloride and used for gas chromatography. The esters of higher alcohols and pentafluoropropionyl and heptafluorobutyryl derivatives were prepared analogously.

RESULTS AND DISCUSSION

Structures I–XII contain *S*- or *R*-mandelic acid as a chiral constituent. In compound XIII *S*-α-phenylbutyric acid was used, which was prepared by fractional crystallization of the *R,S*-α-phenylbutyric acid *S*-α-phenylethylamides. In several cases a benzyloxycarbonyl residue was connected to the hydroxy group of mandelic acid. In order to investigate the influence of a second chiral constituent, mandelic acid or O-benzyloxycarbonylmandelic acid were combined with *S*- and *R*-α-phenylethylamine, *S*-valine cyclohexyl ester, *S*-valine *tert.*-butylamide, *S*-mandelic acid cyclohexyl ester and *R*-phenylglycinol. *S*-Mandelic acid cyclohexyl ester and *S*-mandelic acid *tert.*-butylamide seemed to be adequate models to investigate the minimal requirements for "chiral recognition". Racemic mixtures of N-trifluoroacetylamino acid isopropyl esters, N-trifluoroacetylamines, N,O-bistrifluoroacetyl-α-amino alcohols, O-trifluoroacetylated secondary alcohols and the O-trifluoroacetyl derivatives of α-hydroxycarboxylic acid isopropyl esters were chromatographed on glass capillary columns coated with compounds I–XIII.

The results obtained with compounds I–IV have been reported already[20]. All four compounds show enantioselectivity; they all separate the enantiomers of O-trifluoroacetyl-(TFA)-mandelic acid isopropyl ester. However, phase I shows much higher separation values (α) than III, not only for mandelic acid, but also for secondary amines and for amino acids. This indicates that the configuration of a second chiral constituent in the stationary phase effects the enantioselectivity. Also a surprising influence of the O-benzyloxycarbonyl (*Z*) group in II can be observed. The additional polar group enhances the enantioselective interaction with aliphatic α-hydroxy acids. This results in chiral recognition of these compounds without a nitrogen atom being present in the solute. In the case of compound IV a particularly high enantioselectivity for secondary amines is observed[20]. In the corresponding *Z*-derivative V, however, the enantioselectivity is drastically reduced: *R,S*-alanine and *R,S*-2-aminoheptane show only marginal separation (*R,S*-Ala: $\alpha = 1.009$; *R,S*-2-aminoheptane: $\alpha = 1.008$).

N-Acyl-L-valine *tert.*-butylamides[7] proved to be excellent chiral phases for the separation of amino acid enantiomers but have no enantioselectivity for α-

hydroxy acid derivatives. In order to obtain enantioselectivity for α-hydroxy acids we synthesized compounds VI and VII. Both stationary phases have only one asymmetric centre. Table II gives the results for VI and VII. Compound VI partly separates the enantiomers of O-TFA-α-hydroxy acid isopropyl esters (Fig. 1) and N-TFA derivatives of secondary amines and amino acid esters. For the α-hydroxy acids we tested, in addition to the trifluoroacetyl derivative, the pentafluoropropionyl and heptafluorobutyryl derivatives and, in addition to the isopropyl esters, *n*-propyl, isobutyl, *n*-butyl, isopentyl and *n*-pentyl esters. No significant influence was noted by any of these alterations. The order of elution is consistent: on the *S*-mandelic acid phases the *S*-enantiomers are retarded and have longer retention times (*S*-(+)-mandelic acid, *S*-(+)-2-aminopentane, *S*-(−)-α-phenylethylamine, *S*(L)-alanine).

TABLE II

SEPARATION FACTORS (α) AND OPERATING TEMPERATURES FOR ENANTIOMER SEPARATION ON GLASS CAPILLARY COLUMNS COATED WITH STATIONARY PHASES VI, VII AND XIII

Racemate	*Phase VI on 44.5-m glass capillary*	*Phase VII on 26.5-m glass capillary*	*Phase XIII on 39-m glass capillary*
R,S-Alanine	1.02/90	1.037/80	1.038/120
R,S-Valine	1.02/90	1.031/80	1.044/120
R,S-Alaninol	shoulder/110	1.016/100	1.018/120
R,S-Valinol	1.005/110	—	1.018/120
R,S-2-Aminopentane	1.01/100	1.012/100	—
R,S-2-Aminohexane	1.012/100	1.013/100	not tested
R,S-2-Amino-5-methylhexane	1.015/100	1.013/100	not tested
R,S-2-Aminoheptane	1.015/100	1.013/100	1.023/120
R,S-2-Amino-6-methylheptane	1.015/100	1.014/100	not tested
R,S-2-Amino-octane	1.014/100	1.015/100	not tested
R,S-α-Phenylethylamine	1.033/110	1.039/100	1.107/120
R,S-2-Hydroxybutyric acid	1.007/80	—	not tested
R,S-2-Hydroxyisopentanoic acid	1.009/80	—	not tested
R,S-2-Hydroxyisohexanoic acid	1.014/80	shoulder/60	not tested
R,S-2-Hydroxyoctanoic acid	1.015/80	—	—
R,S-2-Hydroxydodecanoic acid	not tested	not tested	1.010/120
R,S-Mandelic acid	1.018/110	1.012/100	1.029/120
R,S-3-Phenyllactic acid	1.008/110	—	—

In compound VII, having an *n*-dodecanoyl residue instead of a *Z*-residue connected to the α-hydroxy group, the enantioselectivity for α-hydroxy acids is strongly decreased; only the enantiomers of mandelic acid are separated ($\alpha = 1.012$; 100°C; 26.5-m glass capillary). The separation of 2-aminoalkanes and of amino acid derivatives is not effected, however.

By replacing the nitrogen-containing functional group in VI by a cyclohexyl ester group, enantioselectivity is further decreased. In compound XI (*S*-mandelic acid cyclohexyl ester) enantioselectivity is completely lost. Again, substitution of the hydroxyl group by the *Z*-residue improves the situation: on a 39-m glass capillary the enantiomers of mandelic acid ($\alpha = 1.010$; 110°C) and α-phenylethylamine ($\alpha = 1.015$; 110°C) are partly separated.

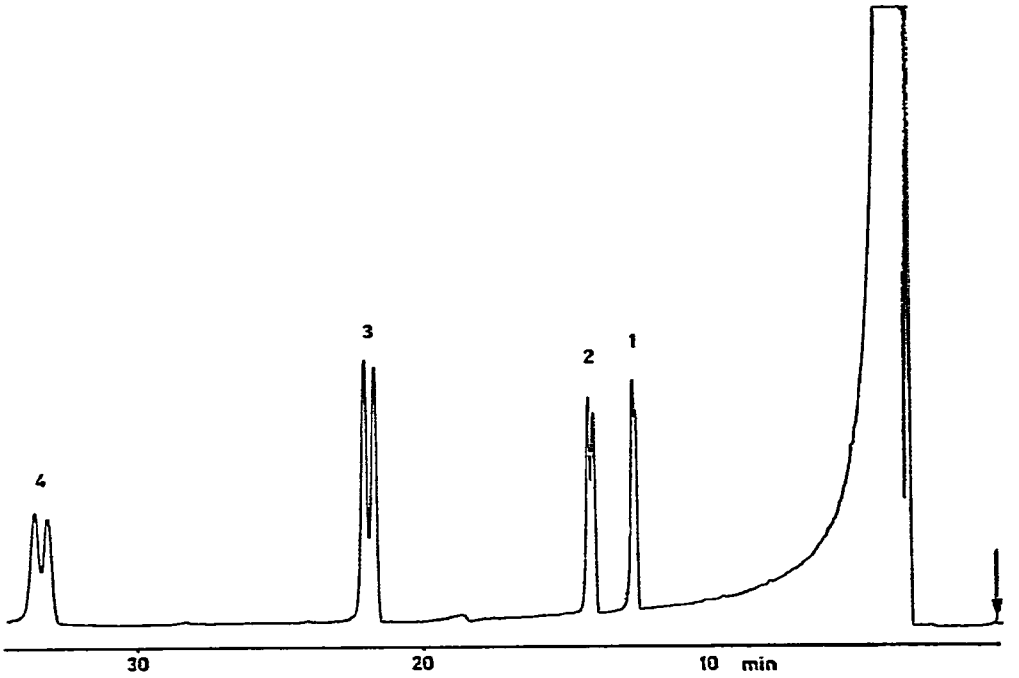


Fig. 1. Separation of *R,S*-O-TFA-α-hydroxybutyric acid isopropyl ester (1), *R,S*-O-TFA-α-hydroxyisopentanoic acid isopropyl ester (2), *R,S*-O-TFA-α-hydroxyisohexanoic acid isopropyl ester (3), and *R,S*-O-TFA-α-hydroxyhexanoic acid (4) on a 44.5-m glass capillary column, coated with compound VI. Column temperature, 76°C; carrier gas, 0.2 bar hydrogen.

The separation of mandelic acid on compound XII is a very interesting case. Neither solvent nor solute contains nitrogen functions. Enantioselectivity may be due to the formation of energetically different diastereomeric complexes based on the interaction of the aromatic residues of the molecules or on dipole–dipole interaction.

The introduction of a second chiral centre as in X does not improve enantioselectivity. On this liquid phase only the derivatives of the enantiomers of mandelic acid and α-phenylethylamine are separated with exactly the same separation values as for XII.

The compounds VIII and IX have relatively high melting points and seem to be thermally labile at column operating temperatures above the melting point, as can be concluded from the short lifetime of the columns. No separation of *R,S*-mandelic acid was observed on VIII and IX.

By replacing the *S*-mandeloyl residue in I by *S*-2-phenylbutyryl one obtains compound XIII, which shows an enantioselectivity similar to I. The results for this liquid phase are also given in Table II. Again, the separation value for *R,S*-mandelic acid is remarkably high ($\alpha = 1.029$; 120°C; 39-m glass capillary).

CONCLUSIONS

One chiral centre is sufficient for enantioselectivity (compounds VI, VII, XII). A second chiral constituent in the stationary phase does not necessarily improve but may influence the separation (compound I *vs.* III).

Mandelic acid derivatives as chiral stationary phases show enantioselectivity for volatile derivatives of the enantiomers of mandelic acid. The enantioselectivity is extended to aliphatic α-hydroxycarboxylic acids by benzyloxycarbonyl substitution of the hydroxy group (compound I *vs.* II, VI *vs.* VII and XI *vs.* XII).

Amino or amide functions as sites for hydrogen bonding in the solvent or solute seem to support enantiomer separation (compound VI, VII *vs.* XII), but are not essential for enantioselectivity (compounds X, XII). Aromatic residues also seem to facilitate enantiomer separation.

The enantiomers of trifluoroacetylated secondary alcohols were not separated on any of the described stationary phases.

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