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CHIROSELECTIVE COMPLEXATION BASED ON HYDROGEN BONDS FOR THE RESOLUTION OF D- AND L-AMINO ACID DERIVATIVES BY SILICA GEL LIQUID CHROMATOGRAPHY

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SUMMARY

The addition of chiral N-acetyl-L-valine-*tert.*-butylamide to the mobile phase using a silica gel column resulted in the optical resolution of D- and L-amino acid derivatives of N-acetyl-O-*tert.*-butyl esters. This is the first instance of enantioselectivity generated by diastereomeric chelate-like solvates based on intermolecular hydrogen bonds between the chiral additive and amino acid enantiomers. The degree of enantioselection was found to depend markedly on the composition of the chloroform–*n*-hexane mobile phase containing the chiral additive. The chromatographic process responsible for the recognition of the enantiomers is discussed on the basis of the equilibrium relationship in the column, involving the chiral additive and amino acid derivatives to be resolved.

INTRODUCTION

Chiral recognition of enantiomers in chromatography is ascribed to differences in the affinity of chiral molecules towards those to be resolved[1,2]. Thus, the molecular association leading to chiral recognition can be made on the basis of the aggregation of two amino acid derivatives forming intermolecular hydrogen bonds. Some linear peptides are known to exhibit pleated sheet structures in which NH $\cdots$ O=C hydrogen bonds are formed between adjacent molecules[3]. It seems reasonable to expect that similar interactions making up the associated structure are imitated in this aggregation. We report in this paper that the mode of association of amino acid derivatives consisting of NH $\cdots$ O=C hydrogen bonds functions to bring about chiroselective complexation in the resolution of the D- and L-enantiomers of amino acid derivatives through application of highly efficient chromatographic technology. It should be mentioned that hydrogen-bond association is one of the fundamental building blocks involved in enzymatic chiral recognition in spite of its facile and flexible interactions and, most important, its weak association energy.

The chiroselective complexations so far examined in solution have involved combinations of various types of interaction in fashioning a rigid complex environment[4–7]. On the other hand, the potential of molecular associations depending en-

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tirely on hydrogen bonding has been recognized in the resolution of amino acid enantiomers offered by (N-formyl-L-valylamino)propyl (FVA) silica gel[8,9]. Recently, we have found that the addition of chiral N-acetyl-L-valine-*tert.*-butylamide to the mobile phase using a silica gel column results in the optical resolution of amino acid derivatives[10]. While passing through the column, this chiral additive can form diastereomeric solvates with the D- and L-enantiomers of N-acetylamino acid *tert.*-butyl esters. The present paper reports for the first time the use of chiral additives for the non-aqueous phase resolution of the enantiomers of amino acid derivatives.

EXPERIMENTAL

Apparatus

The high-performance liquid chromatographic (HPLC) system used was a home-made system equipped with a Jasco Model Tri Rotar-II pump (Japan Spectroscopic Co., Tokyo, Japan), a Rheodyne Model 7125 injector with a 20-μl loop (Rheodyne, CA, U.S.A.), a Yamato low-temperature bath Model BL-21 and a Jasco Uvidec-II variable-wavelength UV detector. A pre-packed column (Hibar, 25 × 0.4 cm I.D.) (E. Merck) containing silica gel (LiChroSorb Si 60, 5 μm) was used for all chromatographic runs.

The appearance of amino acid solutes in the column eluate was detected by UV absorption at 265 nm owing to the transparency of the chiral additive at this wavelength. The chromatographic runs were made at a constant flow-rate of 1 ml/min and a constant temperature of 40°C. The temperature was maintained using a constant-temperature bath.

Preparation of N-acetyl-L-valine-tert.-butylamide and N-acetylamino acid tert.-butyl esters as solutes

The chiral additive, N-acetyl-L-valine-*tert.*-butylamide, was prepared by treating L-valine-*tert.*-butylamide[11] with a mixture of acetic anhydride and triethylamine. Recrystallization from chloroform–diisopropyl ether gave a pure product, subliming at 200°C: Anal. ($C_{11}H_{22}O_2N_2$) C, H, N; $[\alpha]_D^{23}$ −40.26°C (*c* 1.088, ethanol). This product had an enantiomeric purity of more than 99.9%, as determined with HPLC using FVA-silica gel. The chromatography was carried out using 4% (v/v) of 2-propanol in *n*-hexane as the mobile phase. Detection was carried out by UV absorption at 230 nm.

N-Acetyl-O-*tert.*-butyl ester derivatives of DL-α-phenylglycine, S-benzyl-L-cysteine and a D- and L-tyrosine mixture containing a 2-fold excess of the L-enantiomer were prepared from their corresponding *tert.*-butyl amino acid esters. The *tert.*-butyl esters were prepared by the procedure of Roeske[12]. N-Acetyl derivatives of L-tryptophan and its racemate, DL-phenylalanine and S-benzyl-DL-cysteine were of commercial origin (Nakarai Chemicals, Kyoto, Japan). *tert.*-Butyl esters of these derivatives were prepared by reacting their corresponding N-acetylamino acids with isobutene in a mixture of dichloromethane and a catalytic amount of concentrated sulphuric acid[13]. This procedure was also used for the preparation of *tert.*-butyl esters from N-benzyloxycarbonyl derivatives of L-phenylalanine and D-α-phenylglycine. After removal of the benzyloxycarbonyl group by catalytic hydrogenation[13], the resulting *tert.*-butylamino acid esters were acetylated by using a mixture of acetic anhydride and triethylamine. Characterization of the solutes is described elsewhere.

Chromatographic studies

All solvents for chromatography were of analytical-reagent grade. Chloroform was distilled after standing over calcium chloride for 24 h. The chromatographic peaks were identified by separating the enantiomeric pair consisting of an enriched concentration of the L-enantiomer for the tyrosine derivative and co-injecting the corresponding single enantiomers for the other derivatives.

RESULTS AND DISCUSSION

We have previously shown[9] that nearly all the amino acid derivatives of N-acetyl-O-*tert.*-butyl esters can be resolved into their enantiomers by using the chiral diamide-bonded stationary phase FVA-silica gel. This technique depends entirely on hydrogen-bond association between the solutes and the chiral stationary phase so that recognition and resolution of enantiomers are achieved at the same time on the chiral stationary surface. In contrast, in the present method the chiral resolving agent is present in the mobile phase. Thus, the recognition and resolution steps involve two categories of equilibrium relationships: a mobile phase and a stationary phase.

The chiral additive, N-acetyl-L-valine-*tert.*-butylamide, is a single enantiomer of the solute resolvable on the FVA-silica gel surface and its racemate provides one of the most effective resolutions[14]. Prior chromatographic studies on FVA-silica gel demonstrated that the degree of enantioselection of the amino acid derivatives be-

TABLE I

CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) FOR THE ENANTIOMERS OF N-ACETYLAMINO ACID *tert.*-BUTYL ESTERS AS A FUNCTION OF THE CONCENTRATION OF THE CHIRAL ADDITIVE IN THE MOBILE PHASE

The packing was silica gel [Merck, LiChrosorb Si 60 (5 μm)], with a 25 × 0.4 cm I.D. column. The chromatography was carried out under the following conditions: flow-rate, 1 ml/min; column temperature, 40°C; detection, UV at 265 nm. The mobile phase was chloroform–*n*-hexane (40:60, v/v) containing various concentration of chiral additive, N-acetyl-L-valine-*tert.*-butylamide, for all solutes except the tyrosine derivative, for which chloroform–*n*-hexane (50:50) was used. N^1 indicates the NH function in the indole ring of tryptophan.

Solute	*Enantiomer*	*4.67 mM*		*9.35 mM*		*14.02 mM*	
		k'	α^*	k'	α^*	k'	α^*
N-Ac-Trp(N^1-Bu$^+$)-OBu$^+$	D	4.82	1.10	3.16	1.16	2.93	1.25
	L	5.31		3.66		3.65	
N-Ac-Cys(S-Bzl)-OBu$^+$	D	4.31	1.10	3.00	1.14	3.12	1.20
	L	4.73		3.41		3.75	
N-Ac-α-Phe-Gly-OBu$^+$	D	4.98	1.10	3.47	1.15	3.36	1.22
	L	5.48		3.98		4.09	
N-Ac-Phe-OBu$^+$	D	5.09	1.14	3.55	1.19	3.51	1.28
	L	5.78		4.24		4.49	
N-Ac-Tyr(O-Ac)-OBu$^+$	D	–**		–**		10.29	1.17
	L	–		–		12.09	

* $\alpha = k'_L/k'_D$.

** The solute was not eluted within a reasonable time.

came greater as the bulkiness of the alkyl substituent in the C-terminal groups increased[9]. Thus, both the additive and the solutes had *tert.*-butyl substituents in the C-terminal function. Our reasons for choosing chloroform–*n*-hexane as the mobile phase are the solubility of the chiral additive, the suitable retention behaviour of the solute and the promotion of effective hydrogen-bond association in the mobile phase, which cannot be achieved by using protic solvents such as 2-propanol because of the high enantioselectivity requirement.

The chiral recognition of amino acid enantiomers was found to depend markedly on the concentration of the chiral additive and that of chloroform as a stronger solvent component in the mobile phase. Table I demonstrate that changes in the additive concentration influence the separation factors between the enantiomers. Increasing the concentration of the chiral additive afforded greater separation factors, *i.e.*, degree of enantioselection, for all solutes, the most effective values being obtained at 14.02 m*M* with solvent mixtures of three different proportions. Further, there was a tendency for a decrease in the capacity ratios of the enantiomers as the additive concentration increased. This demonstrates that the chiral additive serves as a stronger solvent component for the elution of the solute as well as acting as a resolving agent. Typical resolution of the tryptophan derivative is illustrated in Fig. 1. For this derivative, the D-enantiomer emerged before the L-enantiomer.

Table II shows the influence of the amount of chloroform in the mobile phase on the separation factors between the enantiomers when using 14.02 m*M* of the chiral additive. The separation factors became larger as the amount of chloroform decreased, and a similar response was observed for the mobile phase containing other additive concentrations. However, more than 30 parts of chloroform had to be added to the mobile phase to elute the solutes within a reasonable time. The tyrosine derivative required at least 50 parts of chloroform in the mobile phase for proper retention. It should be noted that there was a clear tendency for the separation factors to increase with increasing concentration of the additive as the concentration of

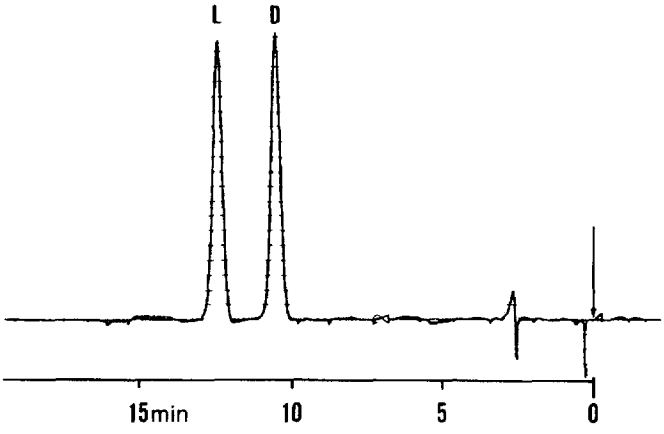

Fig. 1. Optical resolution of N-acetyl-N^{ind}-*tert.*-butyltryptophan *tert.*-butyl ester (N^{ind} indicates the NH function in the indole ring of tryptophan). The chromatographic conditions are described in the legend of Table I, except for the mobile phase: chloroform–*n*-hexane (40:60, v/v) containing 14.02 m*M* of the chiral additive, N-acetyl-L-valine-*tert.*-butylamide.

TABLE II

k' AND α VALUES FOR THE ENANTIOMERS OF N-ACETYLAMINO ACID *tert.*-BUTYL ESTERS AS A FUNCTION OF THE CONCENTRATION OF CHLOROFORM IN THE MOBILE PHASE

The mobile phase contained 14.02 mM of the chiral additive. Column and operating details as in Table I.

Solute	*Enantiomer*	*Chloroform–n-hexane*					
		50:50		*60:40*		*80:20*	
		k'	α	k'	α	k'	α
N-Ac-Trp(N^i-Bu$^+$)-OBu$^+$	D	3.07	1.18	1.64	1.12	1.21	1.00
	L	3.62		1.83		–*	
N-Ac-Cys(S-Bzl)-OBu$^+$	D	3.33	1.15	1.60	1.12	1.30	1.00
	L	3.84		1.79		–*	
N-Ac-α-Phe-Gly-OBu$^+$	D	3.55	1.17	1.97	1.12	1.60	1.00
	L	4.16		2.20		–*	
N-Ac-Phe-OBu$^+$	D	3.76	1.22	2.12	1.15	1.53	1.08
	L	4.59		2.44		1.66	
N-Ac-Tyr(O-Ac)-OBu$^+$	D	10.29	1.17	4.33	1.10	1.50	1.00
	L	12.09		4.76		–*	

* No solute was resolved.

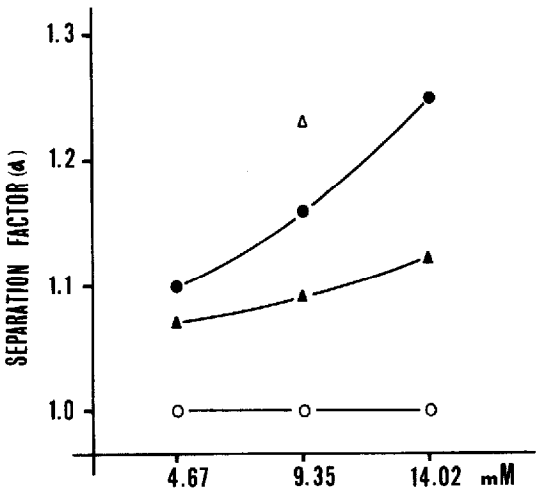

Fig. 2. Influence of the amount of chloroform in the mobile phase on the separation factors between the enantiomers of the tryptophan derivative at various concentrations of the chiral additive. Ratio of chloroform to *n*-hexane in the solvent mixtures: △, 30:70; ●, 40:60; ▲, 60:40; ○, 80:20 (v/v). The separation factor could not be determined when using chloroform–*n*-hexane (30:70) containing 4.67 mM of the additive as the chromatographic efficiency was poor and the peaks of the solute could not be definitely detected. Furthermore, chromatographic runs using 14.02 mM of the additive could not be carried out because this additive amount was not completely dissolved in the solvent mixture of this proportion at room temperature.

chloroform decreased. Fig. 2 is a good illustration of this behaviour for the tryptophan derivative. When chromatography was carried out with solvent mixtures containing 80 parts of chloroform, no solutes were resolved (except the phenylalanine derivative) at any concentration of chiral additive examined.

The chiral additive can interact with the D- and L-enantiomers of the solute to afford diastereomeric solvates in equilibrium as these chemical species pass through the column. The equilibrium relationships in the column provide an important key to understanding the resolution mechanism:

$$CA + E_L \rightleftharpoons CA{=}E_L$$

$$CA + E_D \rightleftharpoons CA{=}E_D$$

where CA is a chiral additive, E an enantiomer and CA = E a diastereomeric solvate.

Recognition of the amino acid enantiomers is, of course, attributed to the difference in stability of the diastereomeric solvates consisting of intermolecular NH $\cdots$ O=C hydrogen bonds. An interesting aspect of this resolution is that these associations are in competition with the self-association of the amino acid derivatives to be resolved as well as that of the chiral additive based on hydrogen bonds. The data suggest that the chiral recognition is greater when the concentration of the chiral additive is higher and when that of the chloroform is lower in the mobile phase. Hence we can conclude that the degree of recognition depends on the content of the diastereomeric species in equilibrium; that is, increasing the additive concentration can cause a shift in the equilibrium position toward the association side in the column, and therefore the resulting chiral recognition becomes larger. On the other hand, when there is an increase in the concentration of chloroform, the position of the equilibrium is forced to shift to the dissociation side owing to the increased solvation of the enantiomers and chiral additives; thus, the resulting recognition becomes smaller. This explanation is supported, at least in part, by a prior quantum chemical study, which indicated that chloroform containing an acidic hydrogen can favour the dissociation of NH $\cdots$ O=C hydrogen bonds[15].

In the following, chromatographic processes that allow the actual observation of the stability difference between the diastereomeric solvates are considered on the basis of the adsorption–desorption relationship between the chemical species shown in the above equations and the silica gel surface. If the diastereomeric solvates have weaker interactions than the dissociated enantiomers with the surface, the amount of chiral additive in the mobile phase controls both the elution and the enantioselectivity of the enantiomers. Indeed, the chiral additive showed itself to be a stronger solvent component for the elution.

Our results demonstrate that chelate-like solvates of amino acid derivatives consisting of intermolecular hydrogen bonds can function to bring about chiroselective complexation for resolution of D- and L-amino acid derivatives. The diastereomeric solvates resulting from the chiral additive and the enantiomers show only slight conformational differences according to a space-filling model study. These solvates provided, however, a sufficient difference in stability to permit the resolution of the enantiomers of the amino acid derivatives by silica gel chromatography. The relative orientation of the side-chains attached to the asymmetric carbon atoms in

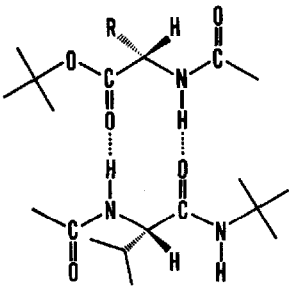

Fig. 3. Association model of the chiral additive and the L-enantiomer of N-acetylamino acid *tert.*-butyl ester as the solute. R represents the amino acid side-chains.

two amino acid derivatives assembled together determines the difference in stability of the corresponding diastereomeric solvates when dimer models, in which bidentate NH · · · · O=C hydrogen bonds are formed between the chiral additive and the solute, are assumed for the hydrogen-bond association as illustrated in Fig. 3[16].

Our method is readily capable of resolving the amino acid enantiomers simply through use of a highly efficient silica gel column and a chiral additive. We are now looking for ways to improve the chemical modification of amino acids to be resolved in the routine analysis and highly sensitive resolution of almost all members of the amino acid family. Our method should also make possible the design of a novel chiral additive from various kinds of naturally occurring chiral products containing proton-releasing or proton-accepting groups.

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