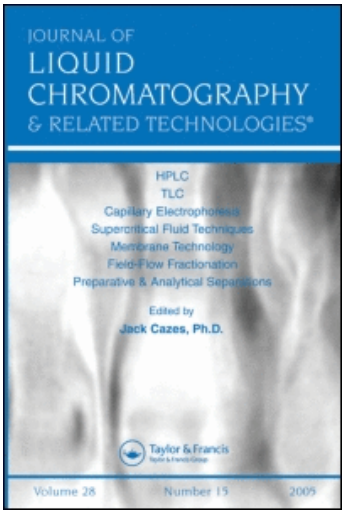

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ENANTIOSELECTIVITY OF HYDROGEN-BOND ASSOCIATION IN LIQUID-SOLID CHROMATOGRAPHY

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ABSTRACT

Liquid chromatographic resolutions of enantiomers induced by molecular associations whose main driving force is the action of weak hydrogen bonds are described. The hydrogen-bond association potential was first demonstrated through the optical resolution of racemic N-acylated amino acid esters using a chiral stationary phase (CSP) (N-acyl-L-valylamino)propyl silica gel. Following this preliminary study, application was made of the chiral mobile phase additive (CMPA) on which the fundamental structure of the chiral graft of CSP is reproduced, to the resolution of the above solute enantiomers in liquid-solid chromatography. The addition of N-acetyl-L-valine tert-butylamide to the nonaqueous mobile phase solvent of a silica gel column successfully brought about this optical resolution; by this method, a novel and more effective chiral resolving agent was found. Two types of chiral additives derived from a chiral skeleton (R,R)-tartaric acid were found capable of resolving various kinds of enantiomers, such as dialkyl tartrate and dialkyl tartramide. Of these two, the latter in particular, having an isopropyl substituent, led to a wide range of resolution of enantiomers of the following categories: α- and β-hydroxycarboxylic acid, β-hydroxy ketone, β-amino alcohol, α-amino acid, α-hydroxy ketoxime derivatives, and bi-β-naphthol. This occurred when the enantiomers, except β-hydroxy ketones, α-hydroxy ketoximes, 1,2-diols and bi-β-naphthol, were derivatized so as to respond to the hydrogen bonding sites of the additive molecules.

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INTRODUCTION

The data of the present paper demonstrate that hydrogen bonding is capable of acting as the driving force for molecular associations leading to the chiral recognition of enantiomers in liquid-solid chromatography (LSC). Also, clarification is made of the structure-resolution relationships between chiral resolving agents and solute enantiomers to be resolved. Since the guiding principle in optical resolution is introduction of a chiral environment into enantiomers either intermolecularly or intramolecularly, certain functionalities with which chiral molecules are bound to enantiomers are thus required in their structures. Optical resolution by chromatography is possible through reversible diastereomeric association between a chiral environment introduced into a column and solute enantiomers (1-3). Thus, binding forces of maximum applicability are ideal for extending the scope of direct resolution of a number of enantiomers possessing various functionalities. Hydrogen-bond formation may possibly qualify as a driving force to meet this objective. A wide range of resolution may be possible using the least highly structured association assembled with one or more of the most general binding forces although such an association as the so-called "three-point contact" model (4), proposed originally by Dalgliesh, requires at least three simultaneous interactions between a chiral resolving agent and one of an enantiomeric pair and has been used to justify direct resolution by chromatography without sufficient justification (5, 6). Stereochemical differences in diastereomeric associated enantiomers of course make enantiomer recognition possible but attractive or repulsive interactions which are stereochemically dependent may not always be involved, as schematically shown in Figure 1.

Our preliminary study was prompted by the pioneering work of Gil-Av and co-workers (7, 8), in which chiral amino acid derivatives forming intermolecular hydrogen bonds were prepared as stationary liquids of gas-liquid chromatography (GLC) and used to resolve volatile D- and L-amino acid derivatives. However, hydro-

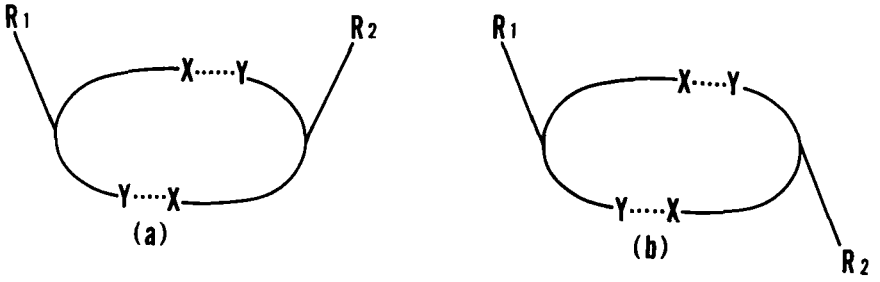


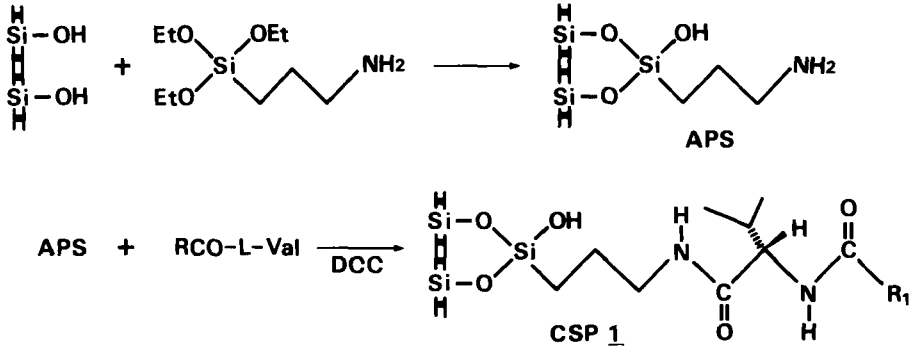
Figure 1 Diastereomeric associated structures whose hydrogen bonds are formed between functions X and Y. Even though the side-chains R_1 and R_2, connected to asymmetric carbons, are electrostatically neutral and no interaction is present between the two substituents, the structures (a) and (b) are in a diastereomeric relationship. These types of associations can also generate enantioselectivity by which optical resolution of enantiomers can be acheived.

gen bonding had been considered too weak to form diastereomeric associated complexes for generating enantioselectivity in LSC (9). The above criterion for the hydrogen-bond association was concluded unfounded from successful resolution using two different liquid chromatographic techniques, the chiral stationary phase (CSP) and chiral mobile phase additives (CMPA).

Optical Resolution by the Chiral Diamide-bonded Stationary Phase

CSPs were obtained by coupling between N-acyl-L-valine and 3-aminopropylsilanized silica gel by the dicyclohexylcarbodiimide-1-hydroxybenzotriazole procedure (10-12). The chiral diamide-bonded phases were found quite effective for the liquid chromatographic resolution of the enantiomers of N-acylated amino acid ester derivatives in a nonaqueous phase operation (10-13). The mobile phase solvent used consisted of n-hexane as the nonpolar component and 2-propanol as the stronger component.

The chiral graft onto the silica gel surface provides hydrogen bonding sites through two amide units and N-acylation and O-



Chiral diamide-bonded stationary phase (N-acyl-L-valylamino)propyl silica gel. The R_1 substituents used are as follows: hydrogen, methyl, ethyl, n-propyl, n-butyl, and tert-butyl.

alkylation of amino acids to be resolved provide functionalities which respond to these sites. The effects arising from changes in N-acyl groups on the CSP were studied to obtain maximum resolution using racemic N-acetylamino acid methyl esters. Such resolution was possible using the CSP derived from N-formyl-L-valine (CSP 1) with the aid of the maximized chromatographic efficiency of this CSP. Effectiveness of valine or an amino acid with side chain branching at the α position to construct CSP has been confirmed by Akanya and co-workers (14), who found the highest chiral recognition obtainable by CSP 1 and congener comprised of L-isoleucine.

As for the steric bulkiness of the alkyl moiety in each functional group of the solute enantiomers studied in connection with CSP 1, an increase in that of O-alkyl substituent enhanced chiral recognition but in a different part little affected the extent of chiral recognition (13). The separation factors varied from 1.10 for the methyl ester of N-acetylleucine to 1.38 for the tert-butyl ester. In Figure 2, the logarithm of the capacity factors (k') of their enantiomers is plotted against that of the

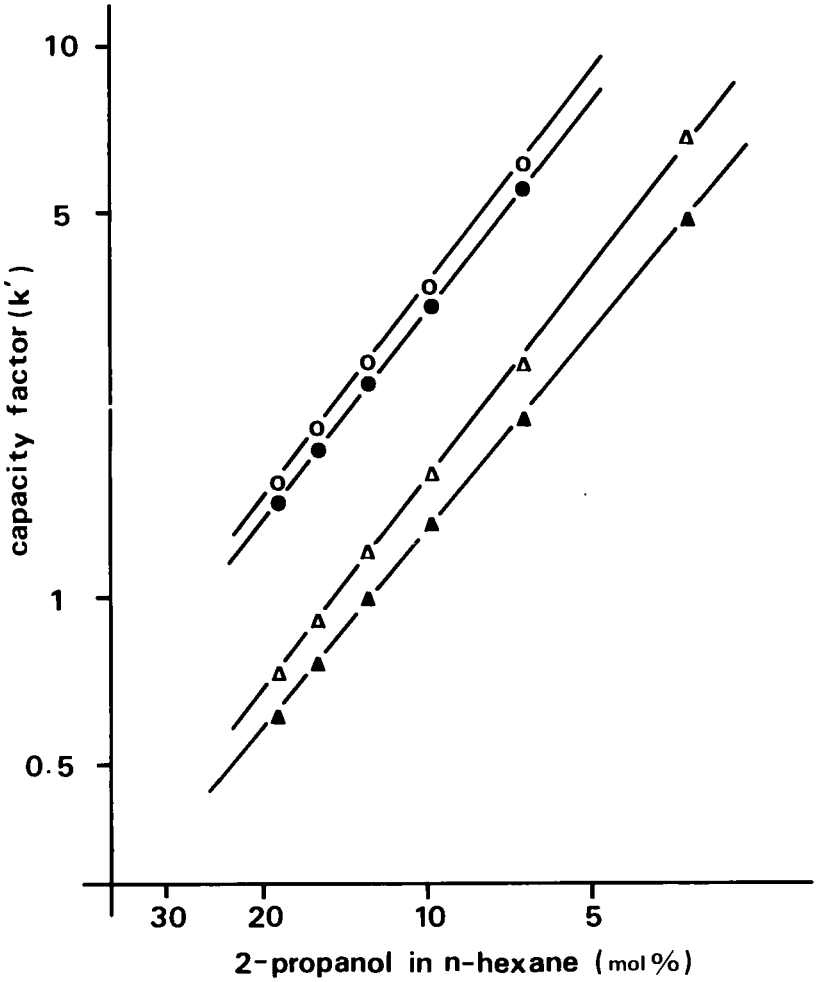


Figure 2 Logarithm of the capacity ratio plotted against that of the molar fraction of 2-propanol in n-hexane: O, N-acetyl-L-leucine methyl ester and ●, its D enantiomer; Δ, N-acetyl-L-leucine tert-butyl ester and ▲, its D enantiomer.

volume fraction for binary solvent systems. Straight linear correlations were obtained for both pairs of enantiomers. This linear relationship indicated the chromatography of the present system to be consistent with the theory of adsorption and desorption (15-17). This is logically due to the hydrogen bonding interactions between the solutes and CSP. The separation factors (α) for the tert-butyl ester are apparently greater than those for the methyl ester throughout the illustrated area.

Thus, nearly all the members of the amino acid family was resolved as N-acetyl O-tert-butyl ester derivatives to give the most effective recognition on CSP 1 (13), as evident from Table I. For all solutes resolved, the L enantiomer was retained more selectively than the corresponding counterpart on the CSP, showing the hydrogen-bond associations in the chiral graft of the L configuration to be more stable for the L enantiomer.

The perturbed derivatives having additional polar functions in their side chains, particularly amide units such as lysine and glutamine derivatives, provided smaller separation factors than unperturbed neutral amino acid derivatives. Substitution of 2-propanol as a stronger solvent for elution to aprotic solvents such as chloroform, dichloromethane, and diethyl ether facilitated chiral recognition because of the possibly less competitive association with solute-CSP interactions. Peak tailing was, however, minimized by a protic solvent systems such as 2-propanol-n-hexane mixtures which afford base-line separations.

Recognition Mechanism in the Enantioselectivity of CSP

The chiral diamide moiety immobilized onto the silica gel surface through a propylene unit may either lie flat on the surface or stand upright. It is likely that these two postures are in equilibrium instead of one in preference to the other. One possible interaction between CSP and a solute enantiomer is hydrogen-bond association of the chiral graft in the upright-standing state. Its two amide units constitute the so-called C_5 and C_7 conformational sites making up bidentate NH--O=C hydrogen bonds

Table I Optical resolution of the enantiomers of N-acetylamino acid tert-butyl esters on the (N-formyl-L-valylamino)propyl silica gel (CSP 1)[a]

entry	amino acid	strong solvent in n-hexane (%(v/v))	k' D	k' L	α[b]
1	Leu	Et_2O (80)	3.12	4.33	1.39
2	Val	Et_2O (80)	3.41	4.69	1.38
3	Nle	Et_2O (80)	3.13	4.28	1.37
4	Nva	Et_2O (80)	3.63	4.89	1.35
5	Abu	Et_2O (80)	4.12	5.37	1.30
6	Ala	Et_2O (80)	4.83	5.95	1.23
7	Ile	Et_2O (80)	3.16	4.32	1.37
8	O-t-BuSer	Et_2O (80)	2.13	2.82	1.32
9	O-AcTyr	Et_2O (80)	7.67	9.33	1.22
10	O-t-BuAsp	Et_2O (80)	2.57	3.10	1.21
11	O-t-BuGlu	Et_2O (80)	3.57	4.33	1.21
12	S-BzlCys	CH_2Cl_2 (30)	1.77	2.31	1.31
13	N-t-BuTrp	CH_2Cl_2 (30)	1.88	2.58	1.37
14	α-PheGly	$CHCl_3$ (30)	2.28	3.02	1.32
15	Phe	$CHCl_3$ (30)	1.96	2.71	1.38
16	N-AcLys	2-PrOH (12)	6.83	7.17	1.05
17	Gln	2-PrOH (8)	17.63[c]		>1.00
18	Pro	2-PrOH (4)	2.77		1.00

[a]The chromatographic conditions are as follows: column, two columns of 20 X 0.4 (i.d.) cm each, linked in series; flow-rate, 1 ml/min; column temperature, 20°C for entries 1-15, and 40°C for entries 16-18; detection, UV at 254 nm for entries 12-15 with all others at 230 nm. [b]α = k' of L enantiomer/k' of D enantiomer. [c]Shoulder was definitely detected.

and are capable of forming distinct associated structures with the solute in which only the C_5 conformational site is offered: "C_5-C_5" and "C_7-C_5" chelate-like associates. A combination of small N-acetyl and bulky O-tert-butyl groups of this solute provides the most effective resolution. Such a derivatization pattern may possibly contribute to making the solute orientate toward the C_5 site of the chiral graft; i.e., it may give rise to the alternating "head-to-tail" association, as shown in Figure 3, more so than the "head-to-head" association in which the large O-tert-butyl moiety of the solute is positioned in front of the propylene

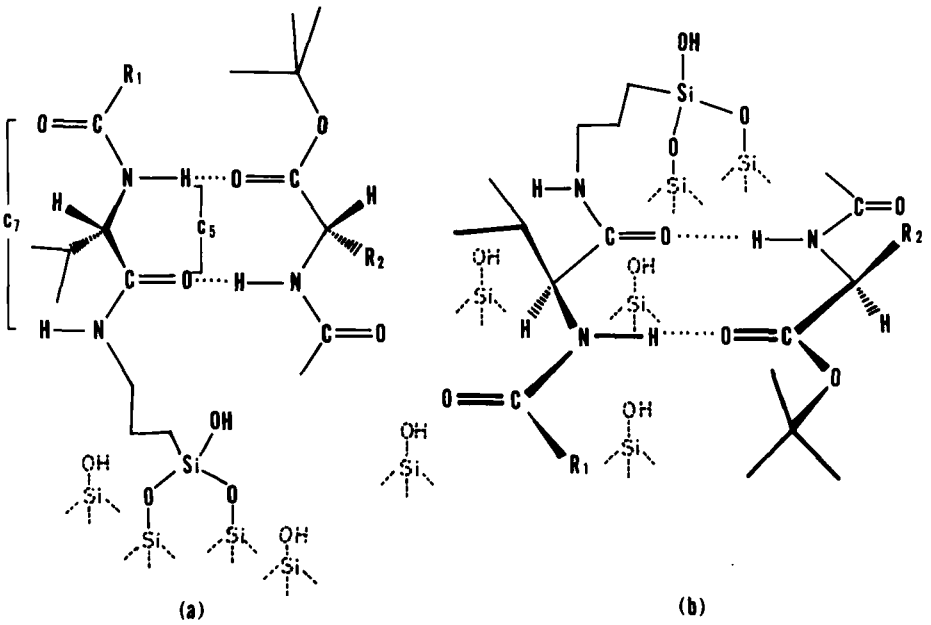


Figure 3 Alternating "head-to-tail" association model proposed to account for the maximal chiral recognition obtained by amino acid derivatives possessing a combination of small N-acetyl and bulky O-tert-butyl groups. The associated complex of the L solute enantiomer which are selectively retained is presented, and it may stand either upright, as depicted in the Figure (a), or lie flat on the surface of the gel, giving rise to the adsorbed state (b). These two postures may possibly be in equilibrium.

spacer adjacent to the silica gel. It seems reasonable to expect that the observed recognition is enhanced through a stricter regulation of the orientation of the solute toward the chiral graft.

Careful attention should be given to the chiral recognition on CSP: the stability difference in the diastereomeric associated complexes may not necessarily be only contributor in the stationary phase process owing to the presence of the silica gel surface responsible for the adsorption of molecules. That is, the surface holding the chiral graft is involved not only in the overall

retention process but may exert a steric effect responsible for enantioselectivity. Assuming the aforementioned hydrogen-bond association to occur in the adsorbed state of the graft with its less hindered face toward the surface of the gel, the associated complex of the L enantiomer can make possible a more stable flat lying posture since its side chain is oriented toward the mobile phase bulk similar to that of the chiral graft. In contrast, the complexed D enantiomer must however orient its large substituent toward the surface of the gel provided it has the same hydrogen-bonded state as that in the L enantiomer. A possibly lesser degree of its adsorption may give rise to the first elution of the D enantiomer. It should be kept in mind that a direct allocation of enantioselectivity based on difference in stability between diastereomeric associated complexes onto a stationary surface is not always possible although the above selectivity contributes in part to the overall stationary phase process. The difference in these stabilities may be elicited more explicitly in the observed enantioselectivity when remaining adsorption sites on the silica gel surface are covered up.

Successful resolution by the chiral diamide-bonded stationary phase in LSC implies that a chiral resolving agent serving as a hydrogen bonding stationary liquid in GLC is also capable of serving as such in LSC. Pains-taking efforts to introduce various fundamental structures of the resolving agent developed in GLC into the LSC system have been made by Ôi and co-workers, although they failed to determine the origin of resolution of enantiomers (18, 19). The α-naphthylethylamide-bonded stationary phase, a CSP prepared by them (18) and modified more effectively by Pirkle and co-workers (20), showed quite well, as expected, alternating relationships between a stationary liquid of GLC and a chiral graft of LSC. The long chain-acylated α-naphthylethylamine is capable of resolving volatile N-acylated amine and amino acid ester derivatives as the GLC stationary liquid (21) and is then incorporated into LSC to bring about the resolution of the corresponding solute enantiomers.

Table II Optical resolution of enantiomeric pairs of N-benzyloxycarbonyl dipeptide methyl esters on CSP 1[a]

entry	dipeptide	strong solvent in n-hexane (%(v/v))	k'	α
1	L-Leu-L-Leu	$CHCl_3$ (30)	2.72	1.12
	D-Leu-D-Leu		3.05	
2	D-Leu-L-Leu	2-PrOH (2)[b]	4.18	1.12
	L-Leu-D-Leu		4.68	
3	L-Val-L-Val	$CHCl_3$ (30)	3.04	1.13
	D-Val-D-Val		3.44	
4	D-Val-L-Val	2-PrOH (2)[b]	4.90	1.07
	L-Val-D-Val		5.23	
5	L-Phe-L-Phe	$CHCl_3$ (30)	3.30	1.11
	D-Phe-D-Phe		3.65	
6	D-Phe-L-Phe	2-PrOH (3)[b]	5.36	1.07
	L-Phe-D-Phe		5.74	
7	L-Ala-L-Ala	$CHCl_3$ (40)	4.40	1.00
	D-Ala-D-Ala		4.40	
8	D-Ala-L-Ala	$CHCl_3$ (40)[c]	4.27	0.91
	L-Ala-D-Ala		3.88	
9	L-Leu-Gly	2-PrOH (1)	5.12	1.00[d]
	D-Leu-Gly		5.12	
10	Gly-L-Leu	2-PrOH (3)	3.75	1.00[d]
	Gly-D-Leu		3.75	

[a]The detection was made by UV at 254 nm. Columns and other operating details are as described in Table I legend. [b]Separation factors between the D-L and L-D enantiomers obtained with chloroform were rather small than those with 2-propanol. [c]Separation was not obtained when using 2-propanol, and the separation observed with chloroform had an inverse elution order. [d]The dipeptide derivatives containing glycine component were not resolved at all.

CSP 1 was also capable of resolving enantiomeric pairs of protected dipeptides (10-12) and tripeptides such as N-benzyloxycarbonyl(Z) O-methyl ester derivatives, as shown in Table II. N-Z dipeptide methyl esters resolved had the same N-terminal amino acids as C-terminal amino acids. The peptide derivatives having L-configuration in C-terminal amino acids eluted more quickly than the corresponding counterparts of the D configuration, except the alanine derivatives. Figure 4 shows the optical resolution of

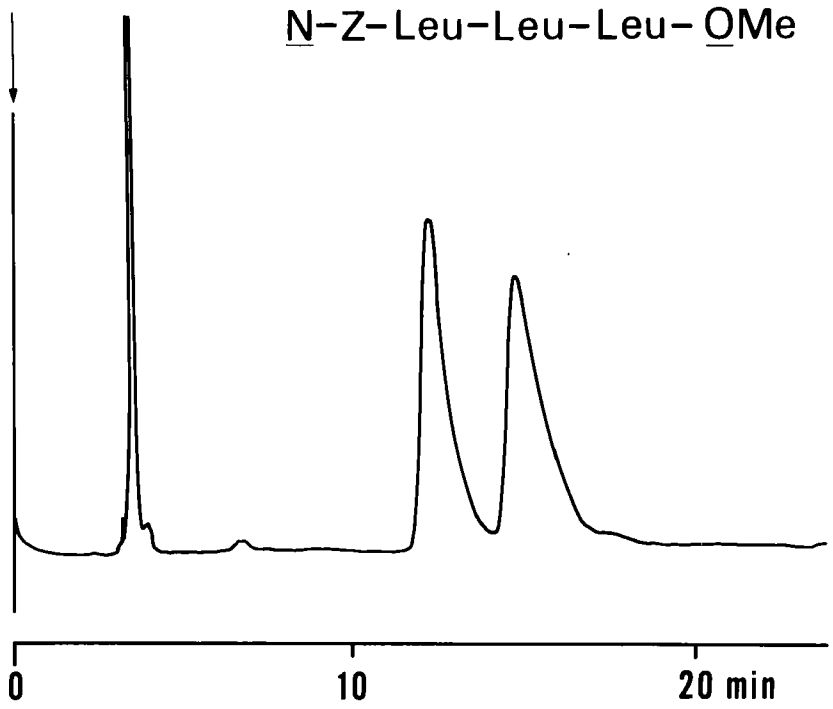


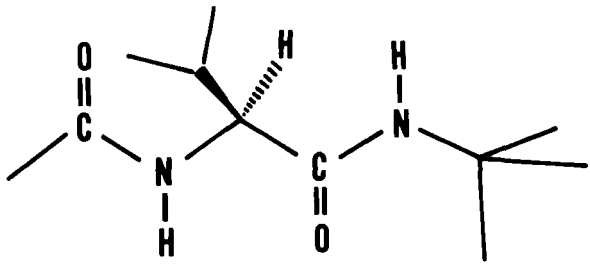
Figure 4 Optical resolution of the enantiomeric pair of N-benzyloxycarbonyl(Z)-leucylleucylleucine methyl ester on CSP 1. The protected peptides of all L configurations eluted faster than the counterparts of the D configurations. The chromatographic conditions are as follows: column, two columns of 24 X 0.4 (i.d.) cm packed with CSP 1 each, linked in series; mobile phase solvent, 3%(v/v) 2-propanol in n-hexane; flow rate, 1 ml/min; column temperature, 40 °C; detection, UV at 254 nm.

the enantiomeric pair of the N-Z-leucylleucylleucine methyl esters on CSP; their separation factor was 1.11 (k'_{L-L-L} 3.17, k'_{D-D-D} 3.51) when using 3%(v/v) 2-propanol in n-hexane as the mobile phase solvent. The solvent effect facilitating chiral recognition was also confirmed in the case of the resolution of homo-configurational peptides. For example, the separation factor for the tripeptide enantiomers increased to 1.29 (k'_{L-L-L} 2.67, k'_{D-D-D} 3.44) on using 40%(v/v) chloroform in n-hexane.

Optical Resolution by CMPA N-Acetyl-L-valine tert-Butylamide

Resolution by CMPAs is of interest not only as a characteristic technique in LSC using a mobile phase solvent but because it facilitates the search for the most effective CSP. A chiral graft in CSP and CMPA are likely to have a reciprocal relationship; i.e., the fundamental structure of a chiral graft constitutes that of a CMPA, and vice versa. In fact, the characteristics of the chiral diamide-bonded phase or two planar amide units connected to an asymmetric carbon were reproduced on a CMPA N-acetyl-L-valine tert-butylamide (1), capable of resolving amino acid enantiomers as their N-acetyl O-tert-butyl ester derivatives on a silica gel column (22, 23). This CMPA has the same large-small derivatization pattern as the solute to be resolved for increasing the degree of the aforementioned "head-to-tail" association mode.

The mobile phase co-solvents used were chloroform-n-hexane mixtures incapable of eluting substantially solute enantiomers within a reasonable time. The addition of about 10 mM of CMPA 1 to the co-solvent facilitated the passage of the enantiomers through the column and provided effective chiral recognition superior to that obtained on CSP 1. The observed order of enantiomer emergence was the same as that obtained by CSP 1. The D enantiomer eluted faster than the L enantiomer. Thus, CMPA 1 has the characteristics of hydrogen-bond solvent as a stronger solvent component responsible for the elution of a solute in LSC and may



CMPA 1

be regarded as the "chiral hydrogen-bond solvent" serving also as a resolving agent.

In the present chromatography, the degree of chiral recognition depended markedly on the concentration of CMPA 1 and that of chloroform in the mobile phase solvent. An increase in the additive concentration afforded greater chiral recognition while causing the solute enantiomers to elute more rapidly. A chloroform concentration low enough to dissolve a particular amount of CMPA 1 in the mobile phase solvent caused higher separation factors of the enantiomers. Chiral recognition may thus possibly be enhanced under a less competitive association of chloroform with either the solute or CMPA.

Outline of the Chiral Recognition Mechanism by CMPA

Enantiomers must partition differently between a mobile and stationary phase in which CMPA associates with each of them. Since CMPA is a hydrogen-bond solvent, it is thus firmly attached to the silica gel surface and forms a hydrogen-bonded phase similar to that of a general polar solvent or hydrogen-bond solvent as the stronger solvent component (24, 25). In fact, CMPA 1 was strongly enriched on the surface of the gel (26). If the adsorbed layer of CMPA 1 is not displaced by the solute and interacts with the solute enantiomers to form diastereomeric associated complexes, it can act as a de facto chiral stationary phase. The actual resolution observed by the separation factor may thus possibly be given by superimposing two distinct processes: recognition induced by stability difference in diastereomeric hydrogen-bond association in the mobile phase bulk and that by the overall stationary phase process in which steric effects are exerted by the surface of the gel to which CMPA 1 is attached; the similar situation was also considered to apply in the case of CSP 1. Chiral recognition occuring in the mobile phase bulk, if any, may not surpass the enantioselectivity obtained on the stationary phase as evidenced by the elution order of the enantiomers observed. The above separation mechanism is also supported by

the fact that a chiral stationary phase comprised of glutaryl-L-valine tert-butylamide monocarboxylic acid containing the CMPA 1 structure ionically bound to an aminopropyl silica showed the same elution order of solute enantiomers as that obtained by CMPA 1. This is reasonable since the immobilization of CMPA 1 on the silica gel surface should make it possible to elicit the enantioselectivity exerted by the stationary phase process for the chiral mobile phase system (26).

Highly Sensitive Resolution of Amino Acid Enantiomers with CMPA 1

The scope of the CMPA method was extended to nearly all members of the amino acid family through effective and conveniently accessible derivatization (27). The amino acid derivatives which improved resolution and detection had the structure of N-(4-nitrobenzoyl) O-isopropyl ester. The 4-nitrobenzoyl group facilitated the chiral recognition process and provided a strongly absorbing chromophore to render highly sensitive UV detection easier. Such solutes were easily prepared from the corresponding amino acids using a vial procedure without any detectable racemization. Chiral recognition of the above solute enantiomers showed chromatographic behavior similar to that obtained in the resolution of N-acetyl O-tert-butyl ester derivatives with CMPA 1; that is, the separation factor was greater when the additive concentration was higher and that of chloroform in the mobile phase solvent was lower. Lower column temperatures resulted in greater separation factors and faster elution of the enantiomers. Thus, rapid resolution of the solute could be performed at 1 °C as shown in Table III. A combination of CMPA 1 and the 4-nitrobenzoylated amino acid derivatives permitted highly sensitive resolutions of complex mixtures of amino acid enantiomers on an efficient silica gel column. On a micro-bore column 1 m in length, a mixture of nine racemic solutes was completely resolved, as the results in Figure 5 indicate.

By our method, the design of a novel chiral resolving agent form various naturally occuring chiral products containing proton-

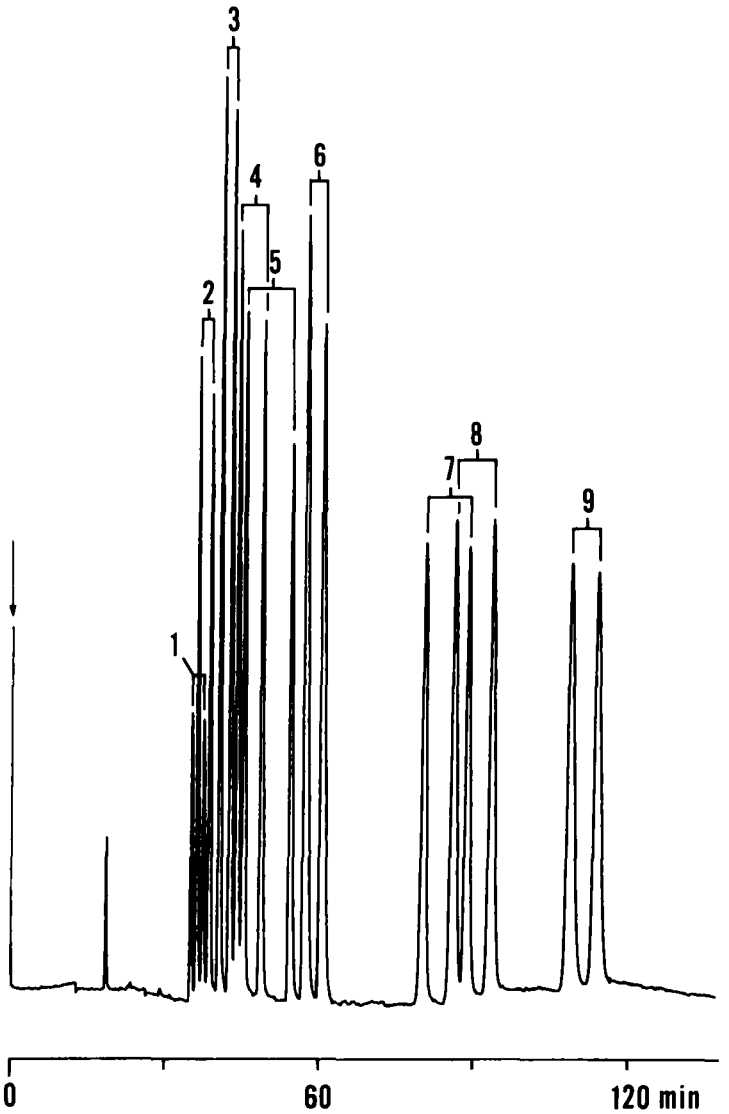


Figure 5 Optical resolution of a mixture containing nine enantiomeric pairs of amino acids as their N-(4-nitrobenzoyl) O-isopropyl ester derivatives. The chromatographic conditions are as follows: column, two columns of 50 X 0.1 (i.d.) cm each, linked in series; flow rate, 30 µl/min; column temperature, 39 °C; mobile phase solvent, 45%(v/v) chloroform in n-hexane containing 14.02 mM of CMPA 1. The packing and detection are described in Table III. Peak identifications are as follows: 1, allo-Ile; 2, Ile; 3, Val; 4, Phe; 5, Leu; 6, Thr; 7, Ala; 8, Tyr; 9, Ser.

Table III Optical resolution of the enantiomers of N-(4-nitrobenzoyl)amino acid isopropyl esters with N-acetyl-L-valine tert-butylamide (CMPA 1) on a silica gel column[a]

entry	amino acid[b]	k' D	k' L	α
1	Leu	1.62	2.61	1.61
2	Ile	0.93	1.19	1.28
3	Val	1.16	1.40	1.21
4	Ala	3.45	4.45	1.29
5	Phe	1.24	1.77	1.43
6	Tyr	3.13	3.93	1.26
7	Ser	4.51	4.94	1.10
8	Thr	1.95	2.25	1.15
9	Asp	1.47	1.61	1.10
10	Glu	2.18	2.65	1.22
11	Met	2.69	3.51	1.30
12	Trp	14.17	16.21	1.14
13	Pro	3.15		1.00

[a]The chromatographic conditions are as follows: column, 25 X 0.1 (i.d.) cm; packing, Spherosil XOA-600 (5 μm); flow rate, 60 μl/min; column temperature, 9 °C; mobile phase solvent, 40%(v/v) chloroform in n-hexane containing 14.02 mM of CMPA 1; detection, UV at 265 nm.
[b]Hydroxyl groups of serine, threonine, and tyrosine were protected as 4-nitrobenzoyl esters, and the carboxyls of aspartic and glutamic acid were protected fully as isopropyl esters.

releasing or proton-accepting groups should become possible. We selected the alternative chiral skeleton tartaric acid as a candidate for CMPA.

Optical Resolution by CMPAs Derived from Tartaric Acid

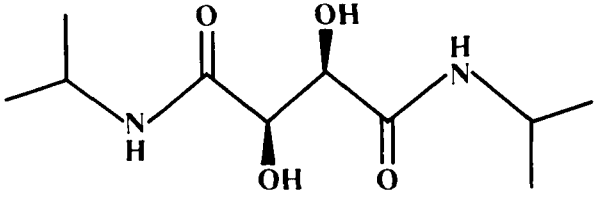
The first instance of chiral recognition of enantiomers through hydrogen-bond associations with tartaric acid derivatives was reported by Bowman and co-workers (28). They applied solvent extraction to enantiomeric resoluiton. The partial resolution of enantiomers such as camphoric acid and 1,2-diol was achieved by partitioning them between (R,R)-dialkyl tartrate (diisopropyl or diisoamyl ester) and aqueous phase. The ratios of the distribu-

tion coefficient of enantiomeric pairs, which correspond to separation factors, were evaluated and found to range from 1.02 to 1.04. A similar approach to chiral recognition has recently been employed by Prelog and his co-workers (29-31) who achieved considerable magnitude of enantioselectivity for the salts of β-amino alcohol with hexafluorophosphoric acid in their distribution process between 1,2-dichloroethane containing (R,R)-di-5-nonyl tartrate and an aqueous solution of sodium hexafluorophosphate. This well designed phase system was incorporated into liquid-liquid partition chromatography or droplet counter current chromatography and semi-preparative scale resolutions of amino alcohol were demonstrated. The diastereomeric associations of dialkyl tartrate and solute enantiomers forming three hydrogen bondings were proposed to account for the resulting resolution. It should be mentioned that the enantioselective process in the liquid-liquid partition system can be discussed only on the basis of molecular association in the organic phase, since the high polarity of the medium and low concentration of the resolving agents in the aqueous phase render hydrogen bond association of solute enantiomers and resolving agents in this phase negligible.

Our initial study dealing with CMPA derived from tartaric acid was also conducted using dialkyl tartrate. This type of derivative was found to function as CMPA in LSC (32). Thus, enantiomeric pairs of 1,2-diol and α-hydroxycarboxylic acid derivatives were resolved with a separation factor ranging from 1.02 to 1.10 using a mixture of chloroform and n-hexane containing dimethyl or diisopropyl tartrate as the chiral eluent. The enantioselections observed here were also considered on the basis of the bidentate interaction between CMPA and the solute by hydrogen bond. Although the ability of dialkyl tartrate to function as CMPA was modest with regard to the scope and degree of chiral recognition, a small modification of the functionality of dialkyl tartrate led to the development of a novel type of CMPA capable of a greater scope of application.

In order that certain chiral molecules function as the resolving agent, they must associate with enantiomeric pairs to trans-

mit a chiral environment to them. That is, the chiral compound with wide adaptability to association with different kinds of enantiomers and the ability to effectively transmit its chiral environment to enantiomers can serve well as a resolving agent. A flexible interaction site is required for the resolving agent to adapt to the association with different kinds of enantiomers. A rigid interaction site is advantageous for the effective transmission of the chiral environment of a resolving agent to enantiomers to be resolved. (R,R)-N,N'-diisopropyltartramide (CMPA 2) embodies a combination of these different type of interaction sites. Hydrogen bonds through hydroxyl groups have a wide scope of direction owing to their free rotation about C-O single bond. Hydrogen bonds through amide units are strong and their direction relatively restricted due to their planalities. The scope of chiral recognition using CMPA derived from tartaric acid was dramatically extended using this derivative as a resolving agent. The addition of CMPA 2 to a nonaqueous mobile phase of chloroform and n-hexane in silica gel chromatography makes possible the chiral recognition of the many categories of enantiomers listed in Figure 6 (33, 34). Figures 7-10 show some typical examples. Among the enantiomers listed in Figure 6, α- or β-hydroxycarboxylic acid and N-acyl α-amino acid were resolved as either amide or ester derivatives. The effect of the bulkiness of the N- and O-alkyl substituents of these derivatives on the degree of enantioselection is summarized as follows. An increase in the bulkiness of the N- and O-alkyl substituents of N-alkyl-β-hydroxycarboxamide and N-acyl amino acid amide or ester enhanced the separation factors. α-Hydroxycarboxamide was resolved well with smaller N-alkyl substituents. It was also recognized that β-



CMPA 2

(R,R)-DIPTA

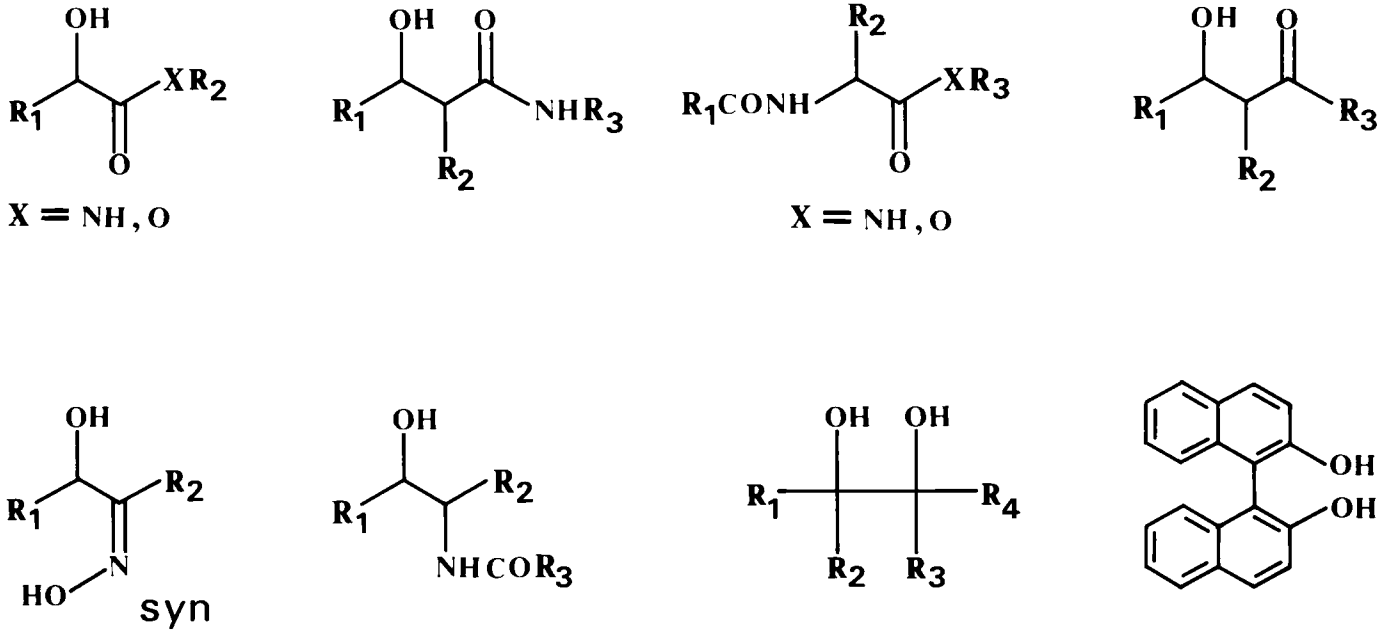


Figure 6 Members of categories of compounds whose enantiomers are resolvable by using CMPA 2.

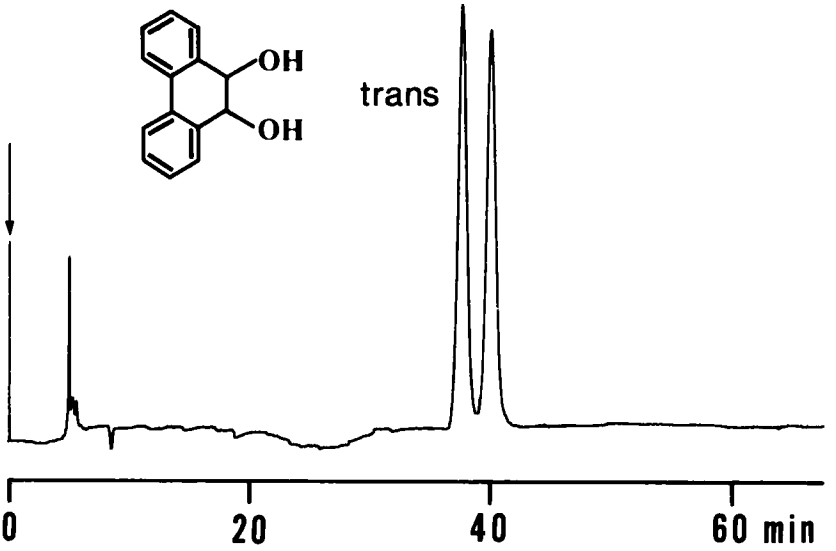


Figure 7 Optical resolution of trans-9,10-dihydroxy-9,10-dihydrophenanthrene. The chromatographic conditions are follows: column, 50 x 0.1 (i.d.) cm stainless steel tube packed with Nucleosil 100-5 (5 μm); mobile phase solvent, chloroform-n-hexane (30 : 7 (v:v)) containing 83.38 mM of CMPA 2; flow rate, 60 μl/min; column temperature, 30 °C; detection, UV at 254 nm. The capcity factor for the first eluted enantiomer and the separation factor are 6.52 and 1.07.

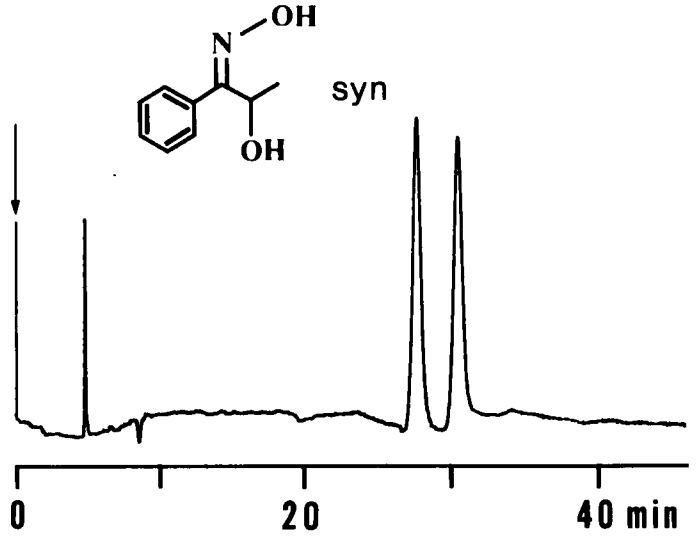


Figure 8 Optical resolution of syn-2-hydroxy-1-phenyl-1-propanone oxime. The chromatographic conditions are the same as those described in the legend of Figure 7. The capacity factor for the first eluted enantiomer and the separation factor are 4.60 and 1.13.

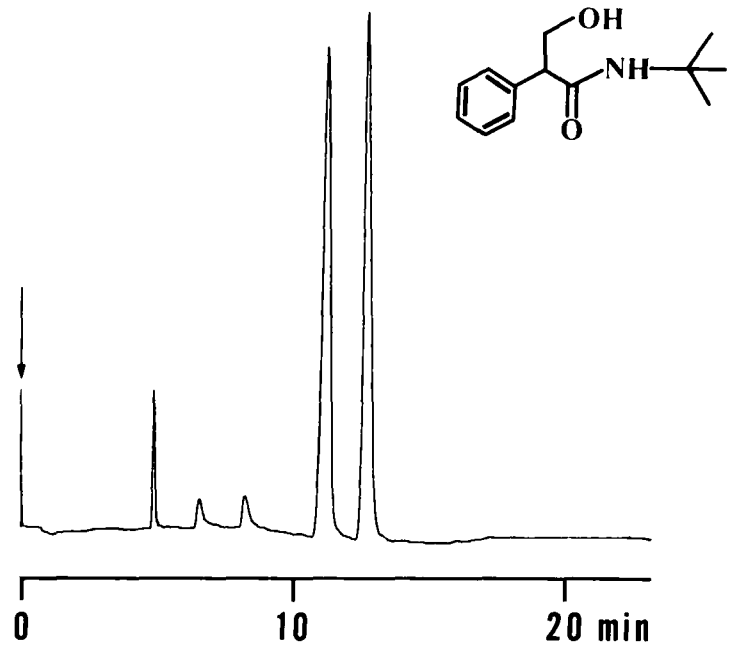


Figure 9 Optical resolution of N-tert-butyl-3-hydroxy-2-phenyl-propionamide. The chromatographic conditions are the same as those described in the legend of Figure 7. The capacity factor for the first eluted enantiomer and the separation factor are 1.30 and 1.23.

hydoxycarboxylic acid derivatives with asymmetric centers at α-positions gave greater separation factors than their isomer with asymmetric centers at β-positions. In the systematic resolution of a series of acyclic 1,2-diols, 1,2-diols, which afford a higher degree of chiral recognition, were found to correspond to those preferring to adopt to the gauche conformation with regard to two hydroxyl groups as shown in Figure 11. Thus 1,2-diols may associate with CMPA by hydrogen bond through their two hydroxyl groups to achieve their enantioselections. The molecular association through two hydroxyl groups was also applicable to the resolution of atropisomers. Thus, bi-β-naphthol was resolved successfully in this manner. Of the two geometical isomes of α-

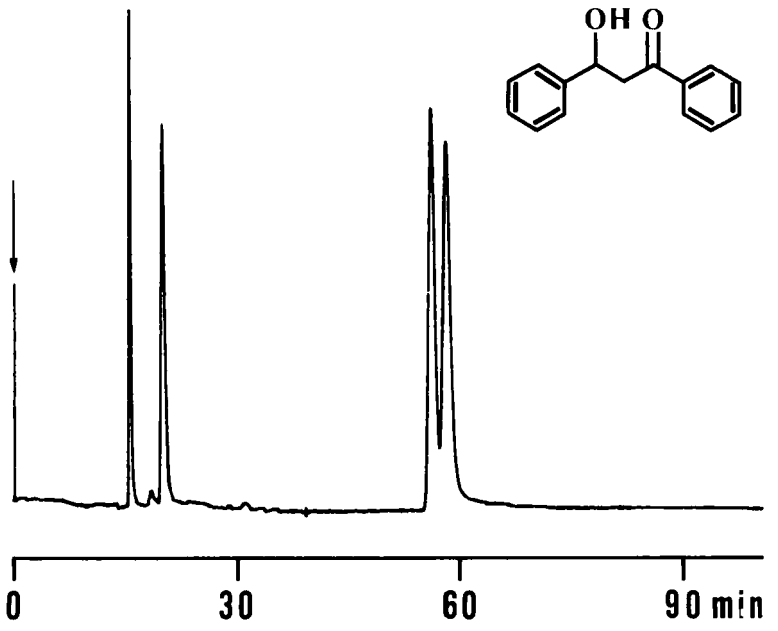


Figure 10 Optical resolution of 3-hydroxy-3-phenylpropiophenone. The chromatographic conditions are as follows: mobile phase solvent, chloroform-n-hexane (3 : 8 (v:v)) containing 3.19 mM of CMPA 2; flow rate, 20 µl/min; column temperature, 20 °C; The other conditions are the same as those described in the legend of Figure 7. The capacity factor for the first eluted enantiomer and the separation factor are 2.61 and 1.05.

hydroxy ketoximes, only the syn-isomers possessing two hydroxy groups at the same side of the C=N double bond could be resolved.

In the above resolutions using tartaric acid derivatives, the CMPA concentration and polarity of the medium had the same effect on the degree of chiral recognition observed in resolution using CMPA 2 derived from amino acid. That is, a higher degree of the chiral recognition was obtained at the higher concentration of CMPA in the co-solvent with lower polarity. The discussion presented in the previous section also seems applicable to the retention mechanism responsible for the observed enantioselection.

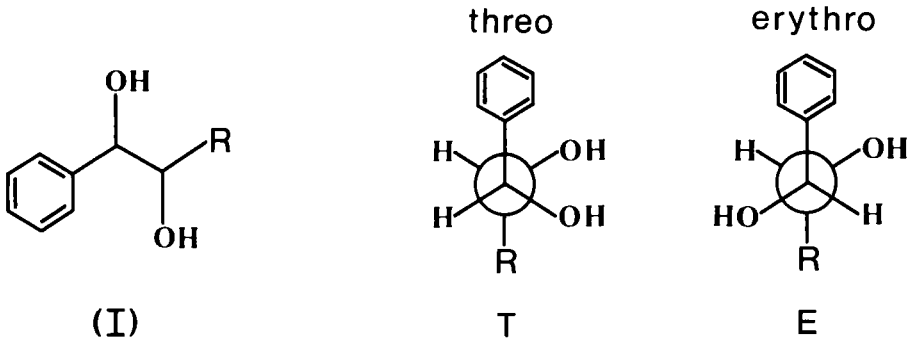
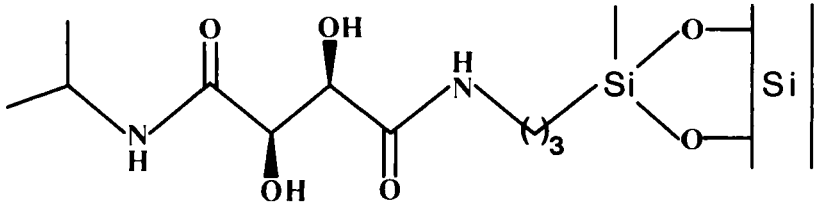


Figure 11 In the threo family of 1.2-diol (I), an increase in the bulkiness of R substituent resulted in greater separation factors. But in the erythro family, the substituent bulkiness had the reverse effect on the separation factors. Variation in the separation factors due to increased bulkiness of R substituent is related to the preferential conformation of these derivatives. Increased bulkiness of R substituent caused the threo diols to adopt the conformation T, whereas, the erythro diols adopt the conformation E (For the sake of convenience, only one of enantiomers is illustrated).

The successful resolution using CMPA 2 showed molecular association based on hydrogen bonds to have wide application to the resolution of enantiomers. This finding has provided a key for the design of a novel type of CSP just as the enantiomeric resolution on CSP 1 provided a basis for the development of CMPA 1. That is, a chiral molecule analogous to CMPA 2 should be applicable to the chiral moiety of CSP. Consequently, we designed CSP 2 derived from tartramide. β-Hydroxycarboxylic acids were re-



CSP2

solved well on this CSP as tert-butylamide derivatives (35). The further application of this phase is now being investigated.

REFERENCES

(1) Blaschke, G., Angew. Chem. Int. Ed. Engl., 19, 13 (1980).
(2) Pirkle, W. H., Finn, J. in "Asymmetric Synthesis", Vol. 1, p. 87, Ed. by Morrison, J. D., Academic Press, New York, 1983.
(3) Schurig, V., Angew. Chem. Int. Ed. Engl., 23, 747 (1984).
(4) Dalgliesh, C. E., J. Chem. Soc., 3940 (1952).
(5) Pirkle, W. H., House, D. W., J. Org. Chem., 44, 1957 (1979).
(6) Pirkle, W. H., Hyun, M. H., Bank, B., J. Chromatogr., 316, 585 (1984).
(7) Feibush, B., J. Chem. Soc. Chem. Comm., 544 (1971).
(8) Charles, R., Beitler, U., Feibush, B., Gil-Av, E., J. Chromatogr., 112, 121 (1975).
(9) Mikeš, F., Boshart, G., Gil-Av, E., J. Chromatogr., 122, 205 (1976).
(10) Hara, S., Dobashi, A., J. High Resolun. Chromatogr., & CC, 2, 531 (1979).
(11) Hara, S., Dobashi, A., J. Liq. Chromatogr., 2, 883 (1979).
(12) Hara, S., Dobashi, A., J. Chromatogr., 186, 543 (1979).
(13) Dobashi, A., Oka, K., Hara. S., J. Am. Chem. Soc., 102, 7122 (1980).
(14) Akanya, J. N., Hitchen, S. M., Taylor, D. R., Chromatographia, 16, 224 (1982).
(15) Hara, S., Miyamoto, S., Anal. Chem., 53, 1365 (1981).
(16) Hara, S., Kunihiro, K., Yamaguchi, H., Soczewiński, E., J. Chromatogr., 239, 687 (1982).
(17) Hara, S., Ohkuma, T., J. Chromatogr., 316, 389 (1984).
(18) Ôi, N., Nagase, T., Doi, J., J. Chromatogr., 257, 111 (1983).
(19) Ôi, N., Nagase, M., Sawada, Y., J. Chromatagr., 292, 427 (1984).
(20) Pirkle, W. H., Hyun, M. H., J. Org. Chem., 49, 3043 (1984).
(21) Weinstein, S., Feibush, B., Gil-Av, E., J. Chromatogr., 126, 97 (1976).
(22) Dobashi, A., Hara, S., Tetrahedron Lett., 24, 1509 (1983).
(23) Dobashi, A., Hara, S., J. Chromatogr., 267, 11(1983).
(24) Scott, R. P. W., Kucera, P., J. Chromatogr., 149, 93 (1978).
(25) Scott, R. P. W., Faraday Symp. Chem. Soc., 15, 49 (1981).
(26) Dobashi, A., Hara, S., J. Chromatogr., in press.
(27) Dobashi, A., Hara, S., Anal. Chem., 55, 1805 (1983).
(28) Bowman, N. S., McCloud, G., Schweitzer, G. K., J. Am. Chem. Soc., 90, 3848 (1968).
(29) Prelog, V., Stojanac, Ž., Kovačević, K., Helv. Chim. Acta, 65, 377 (1982).
(30) Domon, B., Hostettmann, K., Kovačević, K., Prelog, V., J. Chromatogr., 250, 149 (1982).
(31) Prelog, V., Mutak, S., Kovačević, K., Helv. Chim. Acta, 66, 2279 (1983).
(32) Dobashi, Y., Hara, S., unpublished results.

(33) Dobashi, Y., Dobashi, A., Hara, S., Tetrahedron Lett., 25, 329 (1984).
(34) Dobashi, Y., Hara, S., J. Am. Chem. Soc., 107, 3406 (1985).
(35) Dobashi, Y., Hara, S., Tetrahedron Lett., in press.