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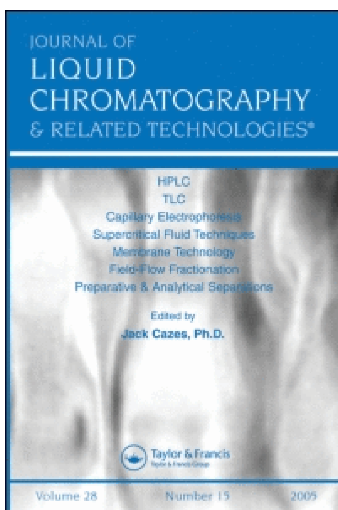

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Liquid Chromatographic Resolution of Enantiomeric Dipeptides on the Chiral Stationary Phase Derived from (S)-1-(6,7-Dimethyl-1-naphthyl)isobutylamine

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JOURNAL OF LIQUID CHROMATOGRAPHY, 11(6), 1249-1259 (1988)

LIQUID CHROMATOGRAPHIC RESOLUTION OF ENANTIOMERIC DIPEPTIDES ON THE CHIRAL STATIONARY PHASE DERIVED FROM (S)-1-(6,7-DIMETHYL-1-NAPHTHYL)-ISOBUTYLAMINE

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ABSTRACT

Liquid chromatographic resolution of fifteen enantiomeric dipeptide methyl esters as their N-3,5-dinitrobenzoyl derivatives was investigated on the chiral stationary phase (CSP) derived from (S)-1-(6,7-dimethyl-1-naphthyl)isobutylamine. The four stereoisomers present in each dipeptide derivative were observed to be separated quite well with the (R,R) isomer being eluted first. The separation factors for two enantiomeric pairs such as (R,R)/(S,S) and (R,S)/(S,R) and the elution orders are explained by two competing "opposite-sense" chiral recognition mechanisms.

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INTRODUCTION

CSP 1 derived from (S)-1-(6,7-dimethyl-1-naphthyl)isobutylamine has been successfully used for the resolution of racemic amino acids, their analogues, aminoalcohols, amines, alcohols, diols and carboxylic acids as their 3,5-dinitrobenzoyl, 3,5-dinitrophenylcarbamate or 3,5-dinitroanilide derivatives.[1-5] Most of the racemates thus far reported to be resolvable upon CSP 1 have only one chiral center and the chromatographic resolution on CSP 1 of racemates having two chiral centers has not yet been extensively studied.

Chiral compounds with two chiral centers exist as four stereoisomers and, upon chromatography on a CSP, such a mixture may show four peaks corresponding to two enantiomeric pairs such (R,R)/(S,S) and (R,S)/(S,R). As an extension of our prior work, we herein report the separation of enantiomeric dipeptides having two configurationally well established chiral centers.

Several small peptides have been nkown to show significant biological and sweetening activities.[6-7] All of these biologically active small peptides are optically active. Therefore, a technique for the easy determination of enantiomeric and diastereomeric purity of small peptides will be useful in the field of peptide chemistry. The resolution of dipeptides on CSP 1 is quite an accurate method for the determination of enantiomeric and/or diastereomeric purity of dipeptides which may either be biologically active themselves or may be precursors in the synthesis of active small peptides.

Previously, several studies concerning the resolution of enantiomeric dipeptides using GC[8] and LC[9-10] have been reported. However, these separations are not yet routine. Very recently, we reported the separation of enantiomeric dipeptide methyl esters as their N-3,5-dinitrobenzoyl derivatives on three CSPs derived from two different (R)-α-arylalkylamines or from (S)-N-(2-naphthyl) valine, and observed that the four stereoisomers of each dipeptide derivative usually are cleanly separated.[11] In this study, we extend the work described above to the resolution

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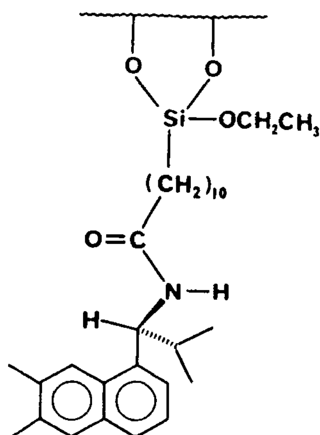
of enantiomeric dipeptide derivatives on CSP 1. The elution order of the four stereoisomers and the degree of chiral recognition for the two enantiomeric pairs will be discussed from the viewpoint of two competing "opposite-sense" chiral recognition mechanisms, earlier postulated by us in mechanistically related work.[1, 12] In the interest of brevity, the details of these mechanisms will not be repeated in this paper.



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MATERIALS AND METHODS

Chromatography was performed by the use of Waters Model 510 Pump, Waters Model U6K Universal Liquid Chromatograph Injector, Waters Model 441 Absorbance Detector and Waters Model 740 Data Module Recorder. The (S)-CSP 1 used in this study has been prepared by the method descrived previously for (R)-CSP 1.[1] This CSP was packed into a 4.6 x 250 mm stainless steel column at the GINSCO Laboratory, Korea, using the conventional slurry method.

All chromatograms were obtained using 2-propanol-hexane (10:90) as the mobile phase with a flow rate of 2 ml/min.

The UV detector moniterd at 254 nm. Preparation of N-3,5-dinitrobenzoyl derivatives of dipeptide methyl esters used in this study has been reported previously.[11]

RESULTS AND DISCUSSION

For convenience, all dipeptide derivatives used in this study have been synthesized by coupling N-(3,5-dinitrobenzoyl) amino acids with amino acid methyl esters using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as a coupling agent. Note that the N-terminal amino group of dipeptides may be derivatized by treating their methyl esters (or other C-terminal derivatives) with 3,5-dinitrobenzoyl chloride in the presence of triethylamine. In every case, the yield for the (R,S)/(S,R) enantiomeric pair was higher than that of the (R,R)/(S,S) pair. The unequal formation of two enantiomeric pairs comes from kinetic fractionation during the coupling reaction as reported previously.[11]

The chromatographic resolution behavior of fifteen dipeptide derivatives on CSP **1** is summarized in Table 1. The elution orders for the four stereoisomers were determined by chromatographing samples which were prepared from different combinations of racemic and optically pure amino acids and comparing the chromatograms of the thus obtained dipeptides. For example, Figure 1 shows how the elution order of the four stereoisomers of N-3,5-DNB-val-leu-OCH_3 is determined. Chromatogram 1a, obtained from the sample which is prepared from N-3,5-dinitrobenzoyl-(R,S)-valine and (R,S)-leucine methyl ester, shows four cleanly separated peaks. Chromatogram 1b, obtained from the sample which is prepared from N-3,5-dinitro-benzoyl-(S)-valine and (R,S)-leucine methyl ester shows two peaks which correspond to the (S,R) and (S,S)-diastereomers. Chromatogram 1c, obtained from the sample prepared from N-3,5-dinitrobenzoyl-(R,S)-valine and (R)-leucine methyl ester, shows two peaks corresponding to the (R,R) and (S,R)-diastereomers. By comparison of these three chromatograms, we can conclude that

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TABLE 1
The Resolution of N-3,5-Dinitrobenzoyl Derivatives of Enantiomeric Dipeptide Methyl Esters on CSP **1**[a]

Entry	N-DNB-a.a.-a.a.-OCH_3[b]	Elution Order[c]	(R,R)/(S,S) k_1^d	(R,R)/(S,S) α^e	(R,S)/(S,R) k_1^f	(R,S)/(S,R) α^e	(R,R)/(R,S) α^e
1	leu-ala	A	6.28	1.88	7.50	1.36	1.21
2	leu-val	E	4.36	1.86	5.72	1.32	1.40
3	leu-leu	A	3.50	2.27	4.57	1.33	1.43
4	leu-pheala	B	5.00	1.55	7.97	1.04	1.77
5	val-ala	A	4.91	2.39	5.94	1.70	1.26
6	val-val	A	3.33	2.37	4.41	1.44	1.47
7	val-leu	A	2.83	2.58	3.75	1.32	1.50
8	val-pheala	F	3.58	2.00	6.16	1.07	2.00
9	ala-val	C	6.79	1.85	8.37	1.77	1.27
10	ala-leu	A	5.21	2.06	6.41	1.47	1.29
11	ala-pheala	C	9.13	1.43	11.46	1.19	1.28
12	phegly-ala	B	11.25	1.40	16.17	1.04	1.49
13	phegly-val	F	6.99	1.69	11.13	1.13	1.69
14	phegly-leu	G	5.71	2.02	13.26	1.00	1.82
15	phegly-pheala	D	11.16	1.26	15.61	-1.12[g]	1.44

[a]All chromatograms were obtained by using 10 % 2-propanol in n-hexane as a mobile phase with a flow rate of 2 ml/min and 254 nm UV detector. [b]N-3,5-Dinitrobenzoyl-amino acid-amino acid-OCH_3. [c]The elution orders are as follows A:(R,R),(R,S),(S,R), (S,S) B:(R,R), (S,S), (R,S), (S,R) C:((R,R), (R,S), (S,S), (S,R) D:(R,R), (S,S), (S,R), (R,S)
E:(R,R), (R,S), (S,R)/(S,S) F:(R,R), (S,S)/(R,S), (S,R) G:(R,R), (R,S)/(S,R), (S,S)

[c]Capacity factor for the first eluted enantiomer of the (R,R)/(S,S) pair. For definition, see reference 13. [e]Separation factor. See reference 13 for definition. [f]Capacity factor for the first eluted enantiomer of (R,S)/(S,R) pair. [g]Negative value indicates that the elution order for the (R,S)/(S,R) pair departs from the usual elution order.

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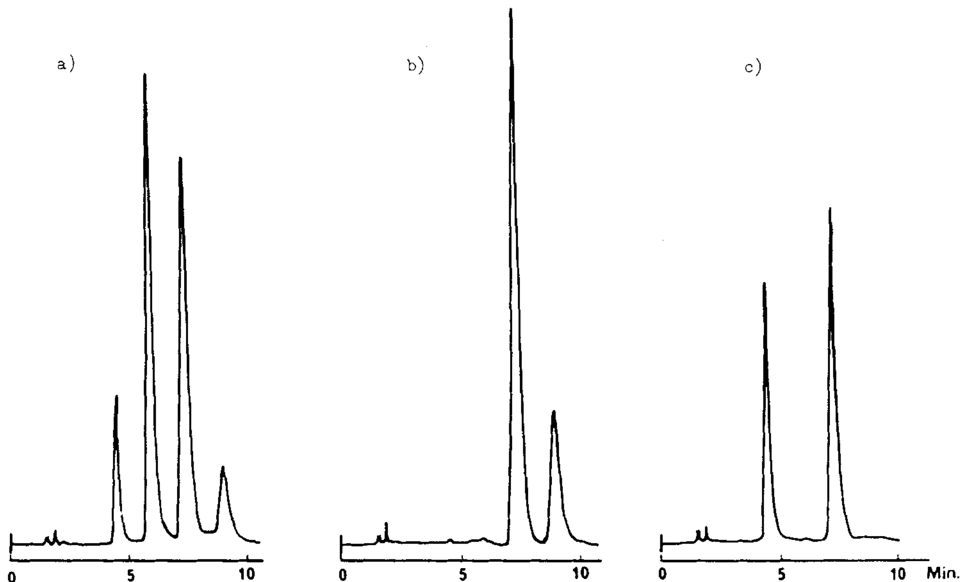

FIGURE 1. The resolution of N-DNB-val-leu-OCH_3 on (S)-CSP **1** using 10 % 2-propanol in n-hexane as a mobile phase with a flow rate of 2 ml/min. Detection was at 254 nm. a) Chromatogram of the sample prepared from N-DNB-(R,S)-valine and (R,S)-leu-OCH_3; b) Chromatogram of the sample prepared from N-DNB-(S)-valine and (R,S)-leu-OCH_3; c) Chromatogram of the sample prepared from N-DNB-(R,S)-valine and (R)-leu-OCH_3

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the elution order of the four stereoisomers is (R,R), (R,S), (S,R), (S,S), where the initially indicated configuration corresponds to that of N-(3,5-dinitrobenzoyl)amino acids. In the cases where the above method was ambiguous, we prepared and chromatographed single stereoisomers prepared from optically pure amino acids, then compared these chromatograms with that of the mixture of the four stereoisomers. By this method, the elution orders shown in Table 1 were established rigorously.

The overall elution order is not regular and may be (R,R), (R,S), (S,R), (S,S) or (R,R), (R,S), (S,S), (S,R) or (R,R), (S,S), (R,S), (S,R). In some cases, two diastereomers may have the

same retention time and the chromatogram shows only three peaks. This problem can be overcome by using a column containing racemic CSP **1** in series with the (S)-CSP **1** column so as to afford greater peak dispersion.[14] Note, however, that the (R,R) isomer elutes first in every case. For the (R,R)/(S,S) enantiomeric pair the (R,R) isomer always elutes first and for the (R,S)/(S,R) enantiomeric pair, the(R,S) isomer elutes first with but a single exception(entry #15 of Table 1).

These results demonstrate that the elution orders for each of the two enantiomeric pairs is determined by the stereochemistry of the N-(3,5-dinitrobenzoyl)amino acid portion of the dipeptide, consistent with the prior observation of the elution order of N-(3,5-dinitrobenzoyl)amino acid methyl esters on (R)-CSP **1**.[1] This elution order has been explained by two competing "opposite-sense" chiral recognition mechanisms termed the "dipole-stacking process" and the "hytrogen-bonding process". When N-3,5-dinitrobenzoyl derivatives of dipeptide methyl esters are resolved on CSP **1**, the amino acid methyl ester portion of the dipeptides may be considered as a simple C-terminal substituent of an N-(3,5-dinitrobenzoyl)amino acid, elution order being determined by the stereochemistry of the latter. Note that the magnitude of the separation factor for the (R,R)/(S,S) enantiomeric pair is always larger than that of the corresponding (R,S)/(S,R) enantiomeric pair, suggestive of an additivity effect. In principle, either amino acid unit can interact with the CSP even though the bulk of the chiral recognition comes from interaction with the N-3,5-dinitro-benzoylated unit. Were the second unit to interact by dipole stacking or hydrogen bonding processes much as the first unit, the contribution of this second effect to the weighted time-average chiral recognition would be of the same sense as that of the first unit and the effects would be additive. For the (R,S)/(S,R) enantiomers, the second effect would partially cancel the first. Hence, the greater separation factors for the (R,R)/(S,S) set can be rationalized. Postulation

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of the "second-effect" draws some support from the observation that the enantiomers of N-acetyl amino acid esters show modest separation on CSP **1**. It is not yet known whether the two amino acids units interact with the CSP in an "either-or" situation or simultaneously. Simultaneous interaction presumably would involve two neighboring strands of bonded phase.

Bearing in mind the two competing chiral recognition mechanisms[1], the reader is reminded that the (R,R) and (R,S)-isomers are retained pricipally by the dipole-stacking process, while the (S,S) and (S,R)-isomers are principally retained by the hydrogen-bonding process. For the isomers principally retained by the dipole-stacking process, the relative elution order may be explained by considering the conformations shown in Figure 2, these being expected to occur as a consequence of intramolecular hydrogen bonding. The two alkyl groups, R_1 and R_2, of the (R,R)-isomer protrude from opposite faces of the semi-rigid backbone of the dipeptide derivative, while the two alkyl groups of the (R,S)-isomer protrude from the same face. Since the dipole-stacking interaction between (S)-CSP **1** and the (R,R)-isomer is a face-to-face approach, R_2 is directed toward the CSP and the adsorbate is consequentially less stable than that of the (R,S)-isomer where both alkyl groups are directed away from the CSP. Consequently, the (R,R)-isomer elutes before the (R,S)-isomer. This explanation is also consistent with the observation that the separation factor noted for the (R,R)/(R,S) pair increases as R_2 becomes larger (Table 1). Indeed, there is some uniformity in the magnitude of the separation factor afforded by a given R_2. This is perhaps a bit surprising, since the nature of R_1 must also play a role here.

Application of the same logic to similar conformations of the (S,S) and (S,R)-diastereomers might lead one to suppose that the (S,R)-isomer would elute before the (S,S)-isomer. Recall however, that these isomers are retained principally by the hydrogen bonding process and that it, unlike the

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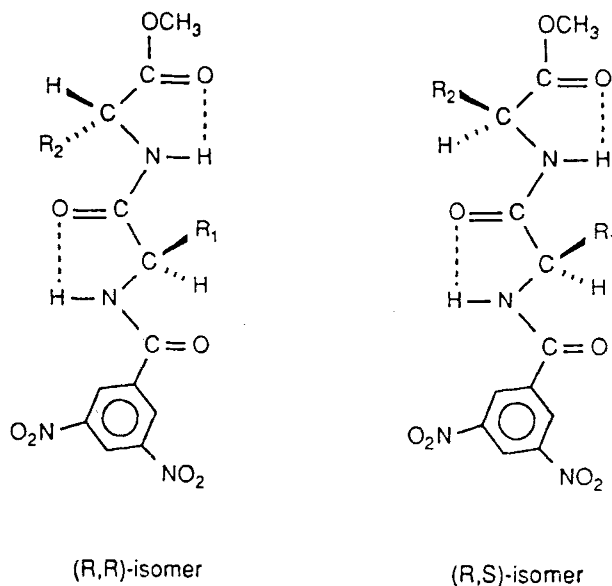

FIGURE 2. The proposed conformations of the (R,R) and (R,S)-isomers

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dipole stacking process, is not an intercalative process so far as the second amino acid unit is concerded. Hence, it is unclear as to which portion of the CSP, if any, the R_2 substituent would interact with during the hydrogen bonding process. In only about half the present instances does the (S,R)-isomer actually elute before the (S,S)-isomer. Hence, the relative order of elution of these isomers is determined by yet unexplained factors.

No clear trends between the size of the R_1 or R_2 substituents and selectivity between enantiomeric dipeptides are noted in Table 1 with the exception that when R_1 is phenyl (entries 12-15), the selectivities noted for enantiomers are less than usual. This general behavior has been noted for other types of analytes and may stem from the phenyl group either altering conformations or serving either as a π-basic site or a basic hydrogen bonding

site. This latter effect has been invoked in a somewhat different CSP system.[15] One does note especially large capacity factors when R_1 is phenyl, suggestive of added bonding interactions.

Arguments have been advanced to account for why the (R,R)-isomer elutes before the (S,S)-isomer and before the (R,S)-isomer. The remainder of the elution order is determined by the extent to which the (R,R)/(S,S) pair of peaks is interleaved with the (R,S)/(S,R) peaks. As mentioned, the extent of interleaving can be altered[14] by using a column of racemic type **1** stationary phase in series with the chiral type **1** column.

In conclusion, CSP **1** has been shown capable of separating the stereoisomers of a number of dipeptide derivatives and shows promise as a means for effecting rapid, accurate, and convenient determinations of enantiomeric purity of dipeptides.

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