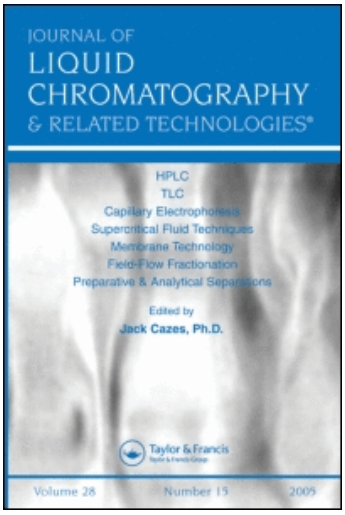

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CHIRAL RECOGNITION BY CHROMATOGRAPHY AND COMPUTATIONAL CHEMISTRY

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SUMMARY

After minimization of the energies of individual compounds including a chiral selector using MM2 calculation of CAChe™ a pair of molecules was brought together to form a complex, and non-bonded energy was minimized. The energy of R- & S-pairs of alanines and phenylalanines was lower than that of the R- & R- and S- & S-pairs of alanines and phenylalanines. The energy of several pairs of R- & S-N-4-nitrobenzoyl amino acids and a chiral selector, N-butylrylvaline-tert-butylamide, also indicated the chiral recognition. The energies of pairs of the selctor and S-amino acids were lower than that of the selector and R-amino acids.

INTRODUCTION

Separation of enantiomers is very important, especially in pharmaceutical compounds. Chiral separation, moreover, has become

a common technique in chromatography. Volatile compounds are separated by gas chromatography, and non-volatile compounds are separated by liquid chromatography, surper clitical fluid chromatography and capillary electrically driven liquid chromatography. The separation mechanism and hence molecular recognition have been studied by chromatography and spectrometry. Molecular interaction is mainly due to hydrogen bonding between a targeted chemical and a chiral selector or a chiral stationary phase. The separation condition is however decided case by case, and the separation of each pair must still be done by try-and-error.

On the other hand, optimization of the molecular structure of molecules becomes possible with a lap-top computer. A molecular interaction of a chiral complex was therefore examined by MM2 calculation of CAChe™. The minimized energy of a complex was measured after trial-and-error fixing of the complex forms on a 3-D colour CRT. When the lowest energy was obtained, a complex form was recognized as being optimized, and the chiral recognition was analyzed.

In this study, N-acetyl-tert-butylamide was selected as a chiral selector among many compounds used in chromatography. A similar chiral selector was applied in gas chromatography [1-4], liquid chromatography [5,6], and super clitical fluid chromatography [7,8].

EXPERIMENTAL

The calculation on a Macintosh IIfx was performed by a CAChe™ program (Sony-Techtronix, Oregon). The optimization was continued until the energy change was less than 0.0001 Kcal/mole. The energies calculated by MM2 for free and derivatized amino acids are summarized in Tables I - III. The minimized structure of three pairs of R- and S-phenylalanines is shown in Fig. 1. The minimized structure of the chiral selector N-butyrylvaline-tert-butylamide is shown with some atomic distances in Fig. 2. The minimnized R- and S-4-nitrobenzoylvalines with the chiral selector are shown in Fig. 3 where molecules with atomic symbols are R-N-butyrylvaline-tert-butylamide.

Table I Calculated Energies of D- & L-Alanines by Molecular Mechanics

Form	Total	H-bonding	Electrostatics	Van der Waals
D	-0.22	-3.79	3.43	1.50
L	-0.22	-3.79	3.42	1.51
DD	-9.84	-13.05	4.18	2.68
LL	-7.14	-11.51	5.09	2.91
DL	-11.37	-13.46	4.24	2.04
DD*	19.79	-9.00	5.73	12.71
LL*	16.72	-10.28	5.66	12.15
DL*	17.52	-10.67	5.03	12.94

unit: Kcal/mole, *: calculated as the locked form

Table II Calculated Energies of D- & L-Phenylalanines by Molecular Mechanics

Form	Total	H-bonding	Electrostatics	Van der Waals
D	-9.94	-5.14	3.11	4.49
L	-9.94	-5.14	3.11	4.49
DD	-31.10	-17.85	3.22	7.46
LL	-30.30	-17.07	3.67	7.26
DL	-28.95	-12.79	2.80	5.54

unit: Kcal/mole

RESULTS AND DISCUSSION

The possibility of the measurement of molecular interactions was first examined in a pair of alkylalcohols. It is well known that the hydrogen bonding of alkylalcohols is affected by the alkyl chain length, and up-to four methylene units can affect the hydrogen bonding. The energy of a pair of alkylalcohols calculated by molecular mechanics indicated that hydrogen bonding contributed to the molecular interaction of less than three carbon alcohols, and the hydrophobic effect was the main energy source for longer alkyl alcohols [9].

Table III Molecular Interaction of Derivatized Amino Acids
AA: Amino acid, TE: Total energy, HB: Hydrogen bonding energy, ES: electrostatic energy, VW: Van der Waals force, All unit: Kcal/mole

AA		iPNBA*1	TE*2	TE	HR	ES	VW
Ala	R	3.83	-19.33	13.39	-10.01	-13.48	11.87
	S	3.83	-18.10	12.33	-9.84	-13.18	11.17
Asp	R	2.46	-18.80	11.51	-9.52	-17.77	10.27
	S	2.46	-17.54	10.80	-9.59	-17.67	8.31
Glu	R	7.75	-16.33	17.06	-9.62	-15.28	13.39
	S	7.75	-14.19	16.00	-9.54	-14.93	12.13
Ile	R	4.71	-15.56	11.44	-9.35	-13.56	11.02
	S	4.71	-15.49	10.42	-9.36	-13.76	10.55
Leu	R	7.50	-15.54	12.67	-9.46	-13.03	8.76
	S	7.50	-13.83	11.77	-9.36	-12.86	7.57
Phe	R	-7.08	-28.97	6.88	-9.96	-13.82	11.54
	S	-7.08	-26.12	5.23	-9.93	-14.19	10.26
Val	R	6.75	-13.55	13.66	-9.33	-13.11	8.46
	S	6.75	-15.66	10.43	-9.46	-14.00	6.43
NBtBA		-3.49					

*1: Total energy of N-4-nitrobenzoyl amino acid isopropylester,
*2: Total energy of free form iPNBA and N-butyrylvaline-tert-butylamide complex.

This result suggested that if a correct model of a chiral complex was given, the optimized form could be given as the difference of energy. Chiral recognition was therefore studied by calculating the energy of pairs of R and S free amino acids.

The energy of R- and S-alanines was the same, and the molecular recognition of R- and S-amino acids became clearer when the calculation was performed on their locked forms as given in Table I. In Table I, RR means that the calculation was performed as a R- and R-pair of alanines, RS means that the calculation was done as a R- and S-pair of alanines.

However the result obtained for R- and S-phenylalanines was interesting as shown in Table II. The total energy of pairs of R- and S-phenylalanines was about -30 Kcal/mole. The individual energy of R- and S-phenylalanines was -11.24 and -11.17 kcal/mole, respec-

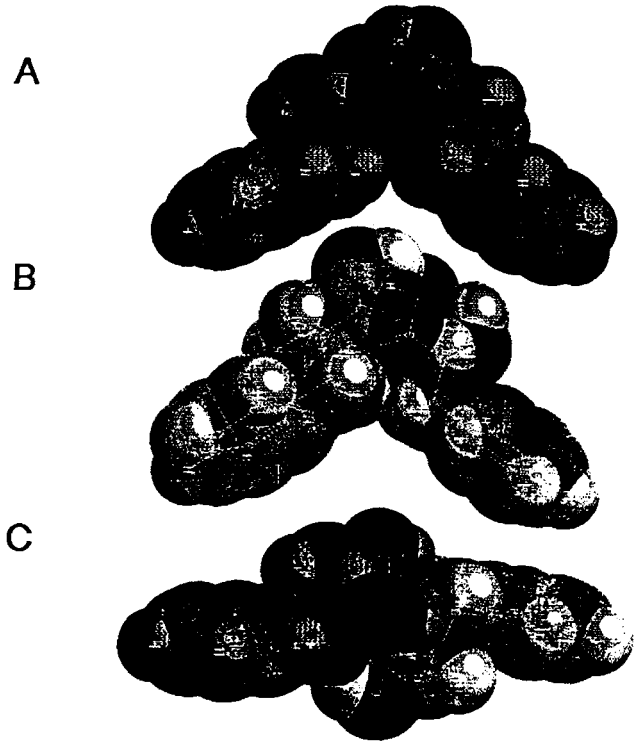

Fig. 1 Chiral recognition of R- and S-phenylalanines.
A: R- and R-pair, B: L- and L-pair, C: R- and L-pair

tively. How stable is the structure of the complex of R- and S-phenylalanines compared to the sum of two phenylalanines?

The Van der Waals energy of a pair of R- and S-phenylalanines was smaller than that of a pair of R- and R-phenylalanines and S- and S-phenylalanines, and especially, the hydrogen bonding and Van der Waals energies were different from those of RR and SS pairs. This R and S recognition model can be explained by three point hydrogen bonding interaction. One interaction site is between the oxygen of the carbonyl group and the hydrogen of the amino group. The other two

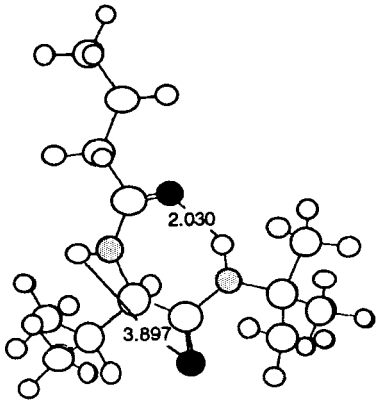

Fig. 2 Minimized structure of N-butyrylvaline-tert-butylamide

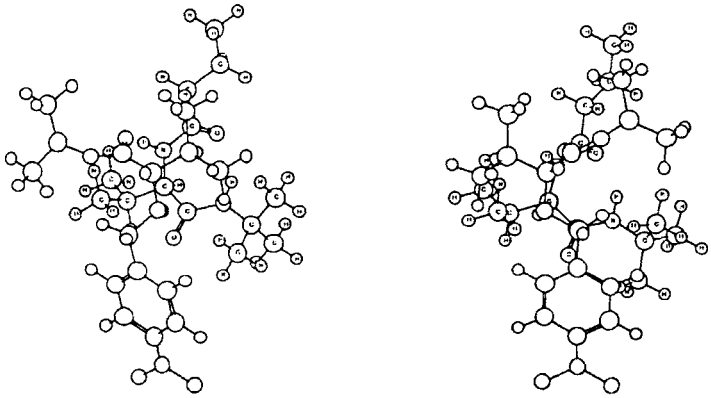

Fig. 3 Minimized structures of R- and S-4-nitrobenzoylvalines and N-butyrylvaline-tert-butylamide.

A: R-4-nitrobenzoylvaline,B: S-4-nitrobenzoylvaline

sites are between the hydroxy groups of these R- and S-phenylalanines. How stable is the structure of the complex of R- and S-phenylalanines compared to other pairs? Other conformations of these R- and S-phenylalanines gave higher energy. This means that the molecular interaction form given in Fig. 1 is recognized as the optimized form.

A chiral separation of amino acids was usually performed on their derivatized forms. The energies and atomic distances were therefore calculated on these forms.

4-Nitrobenzylamino acid isopropylesters were separated on a N-acetyl-tert-butylamide bonded phase in liquid chromatography. The molecular recognition model of this pair was explained as the formation of two hydrogen bonds between secondary amino and carbonyl groups.

The active site of complex formation was proposed as the C5 or C7 ring. The atomic distance of the active hydrogen bonding site of the C5 ring is 3.897 Å and that of the C7 ring is 2.030 Å. The oxygens of carbonyl groups are indicated as dark circles, and the nitrogens of secondary amino groups as grey circles in Fig. 2. Several atomic distance are also given after minimizing the energy. The computationally optimized structure indicated the existence of an intra molecular hydrogen bonding as inferred from NMR and IR [10,11].

The energies of complex forms of pairs of the chiral selector and a derivatized amino acid were calculated by MM2 and the values are given in Table III. The energy was not the sum of the individual energies of the derivatized amino acids and the chiral selector, and the values indicated the level of molecular interaction of their molecular structure. As an example, the sum of total energies of the chiral selector and R- or S-derivatized aspartic acids was -1.03 Kcal/mole, however the tortal energies of a complex of their free form for R and S-aspartic acids were -18.80 and -17.54 Kcal/mole, respectively. The total energy of pairs of the chiral selector (R) and derivatized S-amino acid was smaller than that of their R and R pairs.

Further calculation was performed after partly locking the selector and amino acids, and the complex was formed at the C5 site, in particular, hydrogen bonding sites were locked as shown in Fig. 2. The calculated energies are given in Table III. The hydrogen bonding and electrostatic energies did not indicate the chiral difference, however the total energy and Van der Waals energy demonstrated the possiblity of chiral recognition. This means computational chemistry can help to estimate the enantiomer selectivity. Further a computational synthesis of an enantiomer selective reagent can be performed like a modification of drug which is commonly used in the pharmaceutical industry. The examples of the structure of chiral recognition structure are shown in Fig. 3 where the selector is indicated with atomic symbols.

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