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Molecular recognition in chromatography aided by computational chemistry

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Molecular interactions such as those involved in hydrogen bonding of alkylalcohols, chiral recognition and hydrophobic interactions in liquid chromatography were analyzed by using energies calculated mainly by MM2. Properties of solutes were calculated by Molecular Mechanics, Molecular Dynamics, Extended Hückel and MOPAC-BlogP of CAChe™. Up to three methylene units affected the hydrogen bonding of alkylalcohols. The position of hydrogen bonding and the atomic distances were important for chiral recognition, and the molecular structure of the adsorbent in hydrophobic interactions demonstrated selectivity if the solutes were saturated hydrocarbons.

INTRODUCTION

Chromatographic separation is based on molecular recognition of a solute, and the components of a mobile and a stationary phase. Solubility properties are therefore fundamental for studying the separation mechanism in liquid chromatography.[1,2] Estimation of molecular interactions on the surface of membranes and proteins is very important for analyzing biomedical reactions and designing drugs.[3–9] The words 'host-guest interaction' and 'affinity' are often used to explain the selectivity, however the details of the interactions have not been well explained except for chiral recognition where hydrogen-binding was experimentally measured for specific compounds.[10]

Computational chemistry opens the way to study molecular interactions, and second-order perturbation theory permits intermolecular interaction energies. These calculations may moreover be assisted by illustrations with 3-D color figures.[11] The free energies of complexes between aminoethanes and (R)-N-diformylbenzoyl-phenylglycine-propyl stationary phase were calculated by MM2,[12] and intermolecular interactions were studied.[13]

Chromatographs are excellent instruments for measuring the relative physico-chemical values of molecules in a short time, and the development of computer software has made it easy to calculate the theoretical properties of molecules. For example, the retention time measured by gas chromatography can be related to the enthalpy and the boiling point of solutes.[14] The retention time measured by reversed-phase liquid chromatography can be related to the enthalpy, relative solubility and dissociation constant. This means the retention time in chromatography can be predicted by a theoretical calculation,[15] and conversely the chromatographic data can improve the precision of the theoretical calculation. In this study, the possible molecular interactions were examined by MM2 calculation in the CAChe™ program.

EXPERIMENTAL

In this study, the molecular properties of solutes were calculated by Molecular Mechanics, Molecular Dynamics, Extended Hückel and MOPAC-BlogP of the CAChe™ program from Sony–Tektronix. The alkyl chain length effect for hydrogen bonding of a pair of alkylalcohols was studied by comparing the MM2 energies of n-alkanes and n-alkylalcohols, and hydrophobic interaction was analyzed from the MM2 energies of a solute and a model adsorbent pair. Chiral recognition was also studied by hydrogen-bonding energy and van der Waals energy calculated by MM2.

RESULTS AND DISCUSSION

The possible molecular interactions were first examined in a pair of alkylalcohols. It is well known that the hydrogen bonding of alkylalcohols depends on alkyl chain length, and up to four methylene units can affect the hydrogen bonding. The total energies of pairs of

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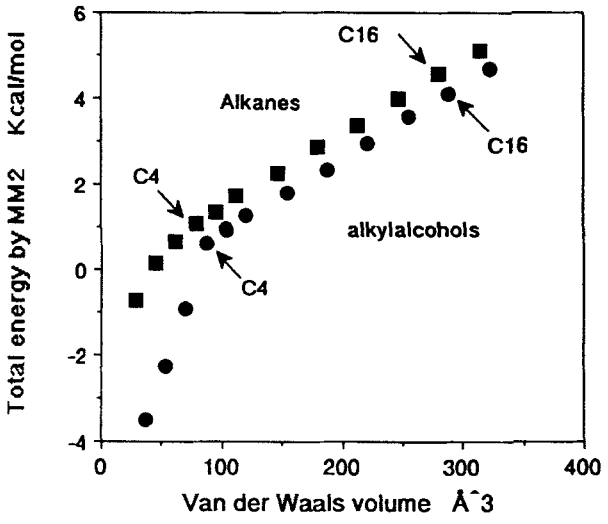

Figure 1 Relation between total energies of pairs of n-alkylalcohols and n-alkanes by MM2 and their van der Waals volumes by MOPAC-BlogP.

Table 1 Molecular interaction energies of alkylalcohols by MM2

Alcohols	*VWV*	*TE1*	*TE2*	*HB*	*ES*	*VW*
Methanol	36.85	0.06	−3.52	−2.91	−1.25	0.36
Ethanol	53.73	0.80	−2.19	−2.90	−1.25	1.51
n-Propanol	70.57	1.47	−0.93	−2.90	−1.27	2.48
n-Butanol	87.41	2.13	0.61	−0.12	−0.06	−0.40
n-Pentanol	104.06	2.78	0.94	−0.10	−0.07	−0.46
n-Hexanol	120.92	3.42	1.22	−0.08	−0.06	−0.55

VWV: van der Waals volume Å 3.
TE1: Total energy of one alcohol.
TE2, HB, ES and VW are total, hydrogen bonding, electrostatic and van der Waals energies of a pair of identical alkylalcohols, respectively.
The unit: Kcal/mol.

identical alkylalcohols were calculated by molecular mechanics (MM2), and the minimized total energies are plotted in Fig 1 against their van der Waals volumes calculated by MOPAC-BlogP. The energies of pairs of alkanes were also used as a reference. Details of calculated values up to n-hexanol are given in Table 1. The total energy of a pair of identical alcohols was rather smaller than twice the energy of one alcohol. This indicates that the values calculated by MM2 can explain the degree of molecular interaction. Methanol, ethanol and n-propanol had similar value, their hydrogen bonding energy being about −2.9 Kcal/mol and their electrostatic energy about −1.3 Kcal/mol. n-Butanol, n-pentanol and n-hexanol also showed similar values, their hydrogen bonding energy being very small (of the order of −0.1 Kcal/mol), and their electrostatic energy about 0.7 Kcal/mol. Their van der Waals energy indicated their complex form. Methanol, ethanol and n-propanol formed pairs through head-to-head hydrogen bonding, like a pair of dancers, while others formed sandwich-like pairs as shown in Fig 2. The total energies of alkylalcohols were corrected to eliminate the molecular size effect by using data from n-alkanes. As seen in Fig 3, their corrected total energy increased dramatically with increasing length of the alkyl chain, then the value levelled off to a constant. Up to three carbons, there was a linear increase in the hydrogen bonding. This means hydrogen bonding contributes to molecular interactions for alcohols up to and including three carbon atoms, and hydrophobic energy is the main energy for alkylalcohols with longer chains. However, the corrected energies did not bear a linear relation to their calculated hydrogen bonding energies as given in Table 1. Further study is necessary to understand such differences. This result can also be

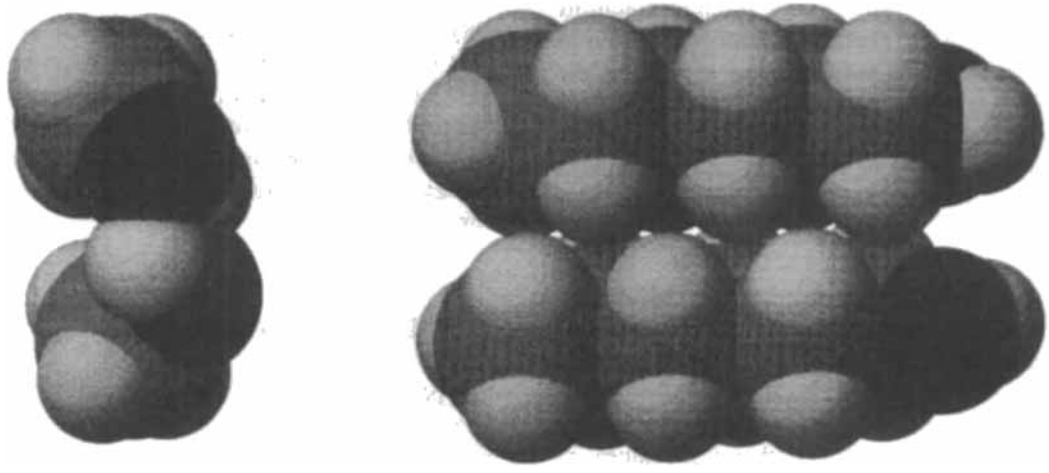

Figure 2 Pair forms of methanol and n-hexanol.
blue: hydrogen, red: oxygen, grey: carbon. (See Color Plate I at the back of this issue.)

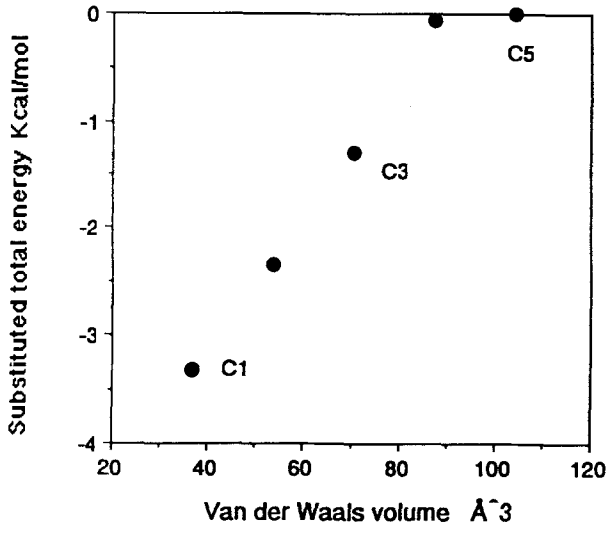

Figure 3 Corrected total energies of paired alkylalcohols.

explained by the fact that the energy change for a chain of five or more carbon atoms was almost parallel to the energy change of alkanes. The above result indicated that if a correct model of chiral recognition was available, the optimized form of the molecule could be declared from the difference of energy of the enantiomers.

Using MM2, a difference emerged between van der Waals energies of amino acid enantiomers. The result however indicated that a much larger energy difference could be obtained if the structure of each enantiomer was locked.[15] A chiral separation model in liquid chromatography was therefore analyzed by MM2 calculation. The energy of a chiral selector, N-butylrylvaline-tert-butylamide, was first minimized by MM2 until the energy change was less than 0.0001 Kcal/mol, and the molecular structure was locked. The total energy was −3.49 Kcal/mol. The structures of nitrobenzoylamino acid isopropyl esters were then optimized until the energy change was less than 0.0001 Kcal/mol. The total energies of these esters ranged from −9.30 for phenylalanine to 7.22 Kcal/mol for leusine. Each amino acid derivative was then forced to make a complex with the locked chiral selector at the C5 and C7 rings by hydrogen bonding on a CRT. The energy of the complex was minimized until the energy change was again less than 0.0001 Kcal/mol. The calculated energies are given in Table 2 and two complex forms of R- and S-phenylalanines are shown in Fig 4. In each case, the total energy of the complex was smaller than the sum of energies of the selector and the amino acid derivative. Although van der Waals energy alone was often smaller when the complex was bonded at the C7 ring, the total energy when it was bonded at the C5 ring was smaller than when it was bonded at the C7 ring. This means that the complex could be formed at the C5 ring, therefore the results for a C5 ring were considered in further detail. When the complex was bonded at the C5, there was no significant energy difference between R and S in terms of total energy, hydrogen bonding energy and electrostatic energy, however the difference of van der Waals energy was large enough to permit chiral recognition. The van der Waals energy of an R,S pair was lower than that of R,R and S,S pairs. This means that R,S pairs form smaller size complexes which facilitate steric recognition.

In reversed phase liquid chromatography, hydrophobicity is one property which can be used to predict the elution order. If the capacity ratio increases the hydrophobicity of the solute increases together with log P. The elution order of compounds with six carbon atoms in reversed-phase liquid chromatography was benzene, cyclohexane and n-hexane. Here, the selectivity of the stationary phase or adsorbent was studied by computer modelling. A saturated hydrocarbon double layer whose structure is shown in Fig 5 was constructed on the computer with 628 atoms (368 carbons and 260 hydrogens), 866 bonds and 1732 connectors, and the molecular weight was 4,676 where one n-pentanol was adsorbed. The minimized total energy by MM2 was 1270.7 Kcal/mol including 276.5 Kcal/mol of van der Waals energy. The properties of three polyaromatic hydrocarbons (benzene (Bz), naphthalene (Na) and anthracene (An)) which were model compounds for studying the effect of aromaticity, three n-alkanes (hexane (C6), decane (C10), tetradecane (C14)) which

Table 2 Molecular interaction energies of chiral complexes by MM2

Amino acid		*TE*	*HB*	*ES*	*VW*
Alanine	R	−16.12	−10.00	−13.55	9.56
	S	−16.74	−9.99	−13.09	8.35
Aspartic acid	R	−23.15	−13.81	−19.37	9.33
	S	−25.03	−13.68	−19.23	7.32
Glutamic acid	R	−15.22	−9.58	−15.33	8.42
	S	−19.77	−9.72	−15.96	6.58
Isoleusine	R	−13.03	−9.32	−13.13	11.02
	S	−16.18	−9.71	−14.00	8.06
Leusine	R	−12.92	−9.03	−13.23	5.48
	S	−17.14	−9.53	−13.35	5.46
Phenylalanine	R	−29.01	−9.80	−14.30	9.60
	S	−30.17	−10.80	−14.15	9.01
Valine	R	−13.26	−9.39	−13.66	6.51
	S	−15.67	−9.72	−14.45	4.69

* Position of complex formation see the detail in text.
TE, HB, ES and VW: Total, hydrogen bonding, electrostatic and van der Waals energies of a chiral complex of amino acid, respectively.
The unit: Kcal/mol.

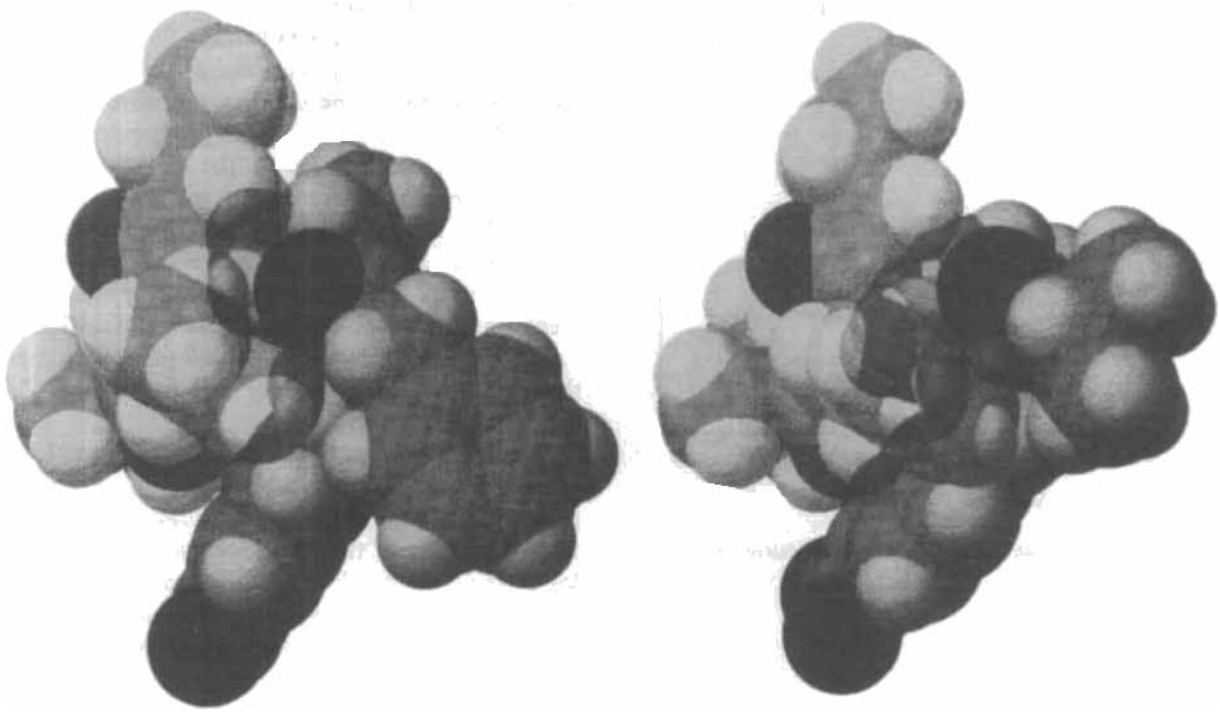

Figure 4 Chiral complexes of derivatized R- and S-phenylalanines with N-butylrylvaline-tert-butylamide.
shadow: (R)-N-butylrylvaline-tert-butylamide, left: R,R complex, right: R,S complex. (See Color Plate II at the back of this issue.)

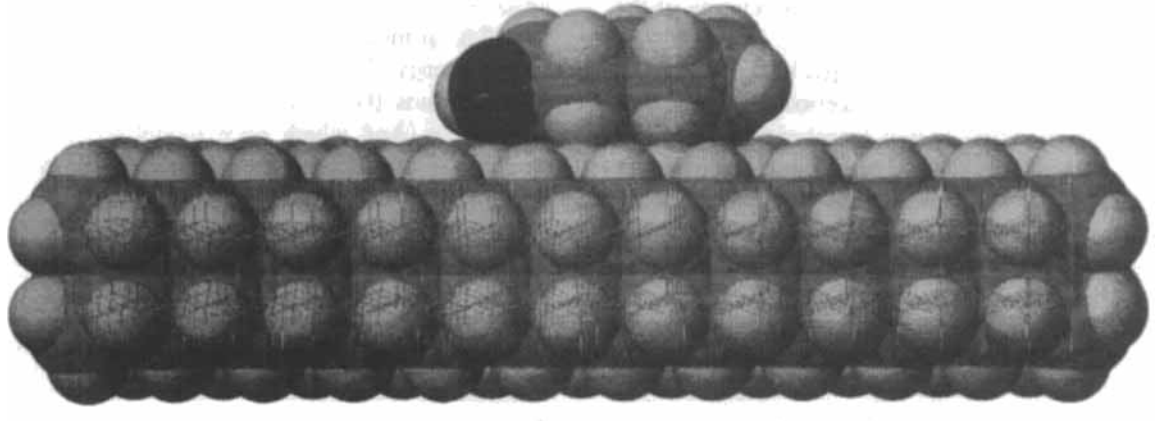

Figure 5 Optimized adsorption form of one n-pentanol on a mode adsorbent. (See Color Plate III at the back of this issue.)

were model compounds for studying the aliphatic effect, three n-alkylalchols (pentanol (C5OH), nonanol (C9OH), tridecanol (C13OH)) which were model compounds for studying the hydrogen bonding effect, and three saturated cyclohydrocarbons (cyclohexane (cyC6), perhydronaphthalene (cyC10), perhydroanthracene (cyC14)) which were model compounds to study steric effect on the adsorbent were calculated by MM2 and MOPAC-BlogP. Appropriate values are given in Table 3.

Although the computer system output a message stating the molecular structure of the model adsorbent was too large so the aromaticity could not be determined, it nevertheless performed the calculation. The calculated values were used as a reference.

These basic properties of the above 12 compounds were first analyzed based on their van der Waals volumes and surface areas. The relation between their van der Waals volumes and enthalpies indicated that these four groups of compounds had individual relationships but could be classified into two groups, one being alkanes and alkylalcohols and the other polyaromatic hydrocarbons and saturated cyclic hydrocarbons. A similar result was obtained in the

Table 3 Hydrophobic molecular interaction and properties of solutes

Solutes	*VWV* Å 3	*SA* Å 2	*log P*	*ΔS* cal/mol	*MM/TE* Kcal/mol	*MM/VW* Kcal/mol	*MD* Kcal/mol	*EH* a.u.	*C/TE* Kcal/mol	*C/VW* Kcal/mol
Ad*	—	—	—	—	—	—	—	—	1270.7	276.5
Bz	83.79	110.08	2.33	3398	−8.08	3.01	0.93	−17.90	1257.2	268.5
Na	127.60	157.29	3.71	4897	−18.79	5.68	−4.30	−28.44	1243.7	264.9
An	171.49	204.66	4.95	6531	−29.49	8.36	−9.61	−38.96	1230.3	261.2
C6	112.79	158.71	3.65	6048	3.47	2.61	19.84	−23.04	1261.9	266.9
C10	180.01	245.56	5.71	9535	6.04	4.47	33.12	−37.48	1257.1	261.4
C14	247.26	333.60	6.95	13030	8.61	6.31	46.69	−51.93	1252.3	256.0
C5OH	104.06	147.60	1.52	5916	2.78	2.05	17.33	−23.56	1262.3	267.3
C9OH	171.44	234.82	3.67	9398	5.35	3.88	30.83	−38.01	1257.5	261.9
C13OH	238.62	322.19	5.02	12896	7.92	5.74	44.08	−52.45	1252.7	256.4
cyC6	101.40	135.47	2.45	4154	6.56	3.63	21.97	−21.65	1269.0	271.8
cyC10	157.27	198.88	3.90	6186	11.44	5.62	34.97	−34.75	1268.3	268.3
cyC14	212.88	259.16	4.87	8579	30.28	11.76	62.84	−47.79	1281.9	268.7

* Ad: Model adsorbent.
VWV: van der Waals volume Å 3.
SA: Surface area Å 2.
ΔS: Enthalpy.
MM/TE: Total energy by MM.
MM/VW: van der Waals energy by MM.
MD: Total energy by MD.
EH: Total energy by EH.
C/TE: Total energy of complex by MM.
C/VW: van der Waals energy of complex by MM.

relation between their enthalpies and surface areas. The total energies of polyaromatic hydrocarbons calculated by molecular mechanics and molecular dynamics differed from the other energies except those found by extended Hückel, and increasing molecular size made the energies negative. Total energies calculated by extended Hückel were roughly related to their van der Waals volumes, and the other energies increased with molecular size. Similar results were also obtained in the relations between surface areas and energies, however more selectivity between compounds of a homologous series was observed in this result than in the relation between their van der Waals volumes and energies. The total energy in the MM2 calculation of each complex was analyzed after correcting for its molecular size and surface area effects. The slope between the van der Waals volume calculated by MOPAC-BlogP and the total energy should indicate the selectivity of the adsorbent. The slopes of n-alkanes and n-alkylalcohols were about the same at −0.11. The slope for polyaromatic hydrocarbons was about one tenth of that for n-alkanes at 0.01. The slope of saturated cyclic compounds differed from other groups, and the value of the slope was −0.17. The van der Waals energy in the MM2 calculation of each complex was also analyzed. The slope between van der Waals volume calculated by MOPAC-BlogP and the van der Waals energy should also indicate the selectivity of the adsorbent. The slope change after the adsorption was −0.11 for n-alkanes, n-alkylalcohols and saturated cyclic compounds, and −0.15 for polyaromatic hydrocarbons. This means that the size effect of solutes was nearly equal for such molecular interactions. The model adsorbent would therefore appear to be selective for saturated cyclic compounds. However, this does not correspond to hydrophobicity, i.e. log P for cyclohexane is 2.446 and log P for n-hexane is 3.654. This result indicates that although the structure of the model adsorbent on the computer demonstrated selectivity, it did not demonstrate simple hydrophobicity. Further basic study of molecular interaction on different adsorbents, and on model adsorbents of biological molecules is therefore desired.

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