Investigation of retention and chiral recognition mechanism using quantitative structure-enantioselectivity retention relationship in high performance liquid chromatography

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The enantiomers of a series of fourteen *O*-ethyl *O*-(substituted) phenyl *N*-isopropyl-phosphoroamidothioates have been separated by high performance liquid chromatography (HPLC) on the Pirkle-type chiral stationary phase. Seven molecular descriptors were calculated and four significant descriptors were chosen to correlate against the experimental lnk' values in order to form the quantitative structure-enantioselectivity retention relationships (QSERRs). Through the QSERRs, the retention and enantioselectivity mechanism were examined.

Keywords High performance liquid chromatography, quantitative structure-enantioselectivity retention relationship, phosphorus enantiomers, chiral separation

Introduction

The efficient separation of a variety of enantiomers from racemic mixtures has been achieved through utilization of chemically bonded CSPs in high-performance liquid chromatography (HPLC).[1-3] Development of efficient chiral-HPLC methods for enantiomeric separation is extremely important for a multitude of applications in chiral synthesis, catalysis, pharmacology, and biochemistry. However, the majority of the chiral separations achieved were obtained based on the trial and error knowledge of the analyst, and often just by chance. Some researchers developed predictive empirical rules based upon molecular structure.[4-6] The enantiomers of a series of fourteen *O*-ethyl *O*-(substituted) phenyl *N*-isopropyl-phosphoroamidothioates have been previously separated by us.[7,8] But we had not investigated the retention and chiral recognition mechanism. In this paper, we focused our attention on the research of the retention and enantioselectivity mechanism. Here a strategy for prediction of retention for each of the enantiomers was developed through the construction of the QSERRs. These relationships require reliable input data to ensure the validity of the resultant equations. Two types of data are needed for QSERRs studies: (1) relative retention data for a series of the solutes; (2) molecular descriptors reflecting the structural features of solutes. The HPLC capacity factors and solute descriptors are related by using the multiple regression analysis. The physical meaning of the individual descriptors can interpret the driven powers contributing to the retention and enantioselectivity.

Experimental

Enantiomers

A series of fourteen organic phosphorus enantiomers were provided by our laboratory of organic synthesis. The structures of the compounds are shown in Table 1.

Each compound was dissolved in acetone and then diluted with the eluent solvent. Solutions with approximate concentration of 0.1 mg/mL in the eluent solvent were used for injection.

Chromatography

The HPLC system was composed of a Model 2010

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liquid chromatography (Varian, Northeast Florham Park, NJ, USA) with a Model 2050 UV detector and HP-3392 integrator (Hewlett packard, Palo Alto, CA, USA).

The chiral column was Sumichiral OA-4700 (250 × 4.6 mm I.D., particle size 10 μm; Sumika Chemical Analysis Service, Osaka, Japan). All solvents were redistilled and filtered through a 0.45 μm filter and degassed *in vacuo* before use. The mobile phase composition was composed of: 4% ethyl ether, 10% 1, 2-dichloroethane and 86% hexane. The flow rate was maintained at 1.0 mL/min. Injection volume was 20 μL. UV detection was at 254 nm.

Computational chemistry

Molecular structures and conformational searches were performed with Sybyl 6.22 (Silicon Graphics). Molecular geometries were optimized using the program MOPAC6.00 (keywords: mmok). The submolecular polarity parameter (P_{SM}), which was first proposed by Wainer *et al.*,[9] was calculated by MOPAC 6.00. LUMO energy (LUMO), molecular width (W) and the angles formed by the atoms O(3)-P(4)-O(5) (β) were also calculated by MOPAC 6.00 on Silicon Graphics station. The other descriptors were gotten from the studies of Chen *et al.*[10] Multiple regression analysis was performed by STATISTICA on PC.

Results and discussion

Retention data of the experiment and the prediction according to the QSERRs were presented in Table 1. Seven solutes descriptors for 14 racemic compounds in this study were presented in Table 2. Four descriptors were selected using the stepwise multiple regression method. The QSERRs were obtained by multiple regression analysis. The results of multiple regression analysis were presented in Table 3. Correlation coffecients were 99.8% and 99.4%, respectively. In order to examine the reliability of the QSERRs, the predicted and experimental values were correlated and the results were presented in Table 1, which indicated that the QSERRs had strong predictive abilities.

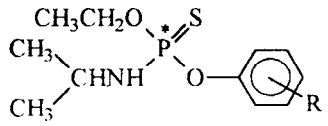
View of the descriptors in the QSERRs models, the hydrophobicity descriptor ($\log P$) describes the lipophilicity with steric and geometric factors. The steric substituent effects (STE) reflect the existence of a steric restriction for retention. The LUMO descriptor indicates the presence of charge transfer interactions between the solutes and the CSP. It has been proven that the LUMO descriptor can be used as a reflection of hydrogen bond donor ability in liquid chromatography.[11] The submolecular parameter (P_{SM}) can be considered as a measure of the submolecular, local dipole, concerned with the stereogenic region of the molecule.

From the results of the multiple regression analysis, we found that the greatest contribution to retention is the term of P_{SM}, which indicates that the strongest interaction between the solutes and the CSP is the local dipole-dipole interactions. Next, the strong hydrogen bondings are formed between the solutes and the CSP according to the greater contribution of the term of LUMO. The lipophilic interactions also have significant effects on retention. The steric substituent effects have the smallest influence on retention.

Comparing the retention of the first and the second eluting peaks on the same column, the coefficient difference of the same descriptors indicates the contribution of enantioselectivity. The most significant difference was observed for f_4, which may be expected to allow for the actual configuration of the more retained isomers with close contact of interaction moieties. The next significant difference is for f_1, which indicates the enantioselectivity is also influenced to the large extent by lipophilic and steric interactions. The negative sign of the f_3 term indicates that the retention increases with the increasing hydrogen bond donor ability. Yet, the small difference of the f_3 term for enantiomers means that the hydrogen bonds formed between the solutes and the CSP contribute little to the enantioselectivity.

The results of the experiment can be simply explained according to the results of the QSERRs. For example, the compounds **10** to **13** can not be separated and the compound **14** just has little separation in such chromatographic condition. The computational chemistry shows that the compounds **10** to **14** all have smaller value of $\log P$ compared with the well separated compounds. The QSERRs indicate that the enantioselectivity is greatly influenced by lipophilic and steric interactions. The smaller the value of $\log P$ is, the poorer the resolution will be. The compound **2** cannot be separated, it may be due to the large value of STE.

Table 1 Structure of the compounds and experimental and predicted retention for the enantiomers



Compound	Substituent R	$\ln k_1'^a$		$\ln k_2'^b$	
		Exp.	Pred.	Exp.	Pred.
1	2-Cl	-0.2574	-0.2846	-0.1732	-0.2171
2	2,6-Cl_2	-0.5108	-0.4982	-0.5108	-0.4142
3	2,4-Cl_2	-0.4700	-0.5130	-0.2944	-0.3774
4	2,4,6-Cl_3	-0.9519	-0.9234	-0.8301	-0.7127
5	2-CH_3	-0.3990	-0.3555	-0.2692	-0.2899
6	3-CH_3	-0.3397	-0.3547	-0.2472	-0.2547
7	4-CH_3	-0.2863	-0.3509	-0.2169	-0.2502
8	4-*t*-Bu	-0.6162	-0.5316	-0.4878	-0.4024
9	2,5-$(CH_3)_2$	-0.5763	-0.6411	-0.4035	-0.5272
10	2-NO_2, 5-CH_3	0.3068	0.3415	0.3068	0.3820
11	2-NO_2, 4-CH_3	0.2654	0.3046	0.2654	0.3449
12	2,6-(NO_2), 4-CH_3	0.9203	0.8895	0.9203	0.8602
13	2,5-$(NO_2)_2$	1.2556	1.2316	1.2556	1.2393
14	2,4-$(NO_2)_2$	0.997	1.0233	1.0375	1.0421
Predicted values for $\ln k'_{1(\text{Pred.})} = -0.0002 + 0.9957 \ln k'_{1(\text{Exp.})}$ $r = 0.9978$					
Predicted values for $\ln k'_{2(\text{Pred.})} = 0.00029 + 0.9886 \ln k'_{2(\text{Exp.})}$ $r = 0.9943$					

[a] Natural logarithm of the capacity factor for the first eluting enantiomer.

[b] Natural logarithm of the capacity factor for the second eluting enantiomer.

Table 2 Solutes descriptor values

Compound	log*P*[a]	STE[b]	P[c]	LUMO[d]	β[e]	P_{SM}[f]	DIP[g]
1	0.590	-0.970	0.300	-1.0743	95.8405	3.8372	7.557
2	1.180	-1.940	2.600	-1.1181	95.8405	3.8323	7.098
3	1.290	-0.970	0.400	-1.2031	95.0093	3.8396	8.016
4	1.880	-1.940	2.500	-1.2409	95.3560	3.7569	7.388
5	0.680	-1.240	-0.100	-1.0168	94.3709	3.8434	7.740
6	0.510	0.000	-0.100	-0.9991	94.0878	3.8460	7.736
7	0.520	0.000	-0.100	-1.0122	93.4325	3.8444	8.108
8	0.820	0.000	0.150	-0.9864	94.1935	3.8440	8.082
9	1.190	-1.240	-0.200	-0.9985	94.5134	3.8413	8.069
10	0.280	-1.010	0.850	-1.5259	97.0287	3.8462	11.752
11	0.290	-1.010	1.100	-1.5133	97.1879	3.8383	11.628
12	0.060	-2.020	4.100	-2.1071	95.2864	3.7664	11.814
13	-0.120	-1.010	0.900	-2.2114	92.3912	3.8516	7.527
14	0.011	-1.010	0.150	-2.7065	93.4620	3.8480	6.055

[a] Hydrophobicity parameter; [b] Substituent steric effect; [c] The chemical shift of phosphorus parameters; [d] Energy of the lowest unoccupied molecular orbital; [e] Angle between O(3)-P(4)-O(5); [f] Submolecular polarity parameter; [g] Total dipole.

Table 3 Summary of the equations obtained from multiple regression analysis

$N=14$	$R=99.8$, $p=0$, $F=519.88$, $s=0.053$			$R=99.4$, $p=0$, $F=195.08$, $s=0.082$		
	First eluting peak			Second eluting peak		
	B	t	p	B	t	p
f	-10.5065	-3.7853	4.3×10^{-3}	-11.0281	-2.6138	2.8×10^{-2}
f_1	-0.5148	-9.9969	4.0×10^{-6}	-0.4192	-5.3560	4.5×10^{-4}
f_2	-0.0611	-1.7793	1.0×10^{-1}	-0.0205	-0.3928	7.0×10^{-1}
f_3	-0.9788	-15.2191	0	-0.9855	-10.0807	3.0×10^{-6}
f_4	2.4536	3.4508	7.2×10^{-3}	2.6008	2.4064	3.9×10^{-2}

General equation: $\ln k' = f + f_1(\log P) + f_2(\mathrm{STE}) + f_3(\mathrm{LUMO}) + f_4(\mathrm{PSM})$; R, correlation coefficient; p, significant level; F, Fischer test; s, standard error of the estimate; B, the coefficient of the descriptors; t, t-test.

Conclusion

A series of 14 chiral phosphorus compounds are separated on the Pirkle-type CSP. The retention and enantioselectivity mechanism were examined through the QSERRs. The successfully predictive ability of the QSERRs shows that the method of QSERRs provides a good and reliable way for the research of the retention and enantioselectivity mechanism.

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