Separation of chiral phosphorus compounds on the substituted β-cyclodextrin stationary phase in normal-phase liquid chromatography

CHEN, Hui(陈慧) LU, Xian-Yu(吕宪禹) GAO, Ru-Yu(高如瑜) ZHOU, Jia(周嘉)
WANG, Qin-Sun*(王琴孙)
National Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China

The separation of enantiomers of a series of eighteen novel nitrogen mustard linked phosphoryl diamide derivatives was investigated on the prepared phenyl carbamate derivative β-cyclodextrin bonded phase in normal-phase HPLC. Some of the enantiomers could be separated in baseline. The chiral recognition mechanism was also suggested for the separation of chiral phosphorus organic compounds.

Keywords Chiral stationary phase, nitrogen mustard linked phosphoryl diamide derivatives, normal-phase LC, β-cyclodextrin

Introduction

The efficient separation of a variety of enantiomers from racemic mixtures has been achieved through utilization of chemically bonded chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC).[1-4] Development of efficient CSP-HPLC methods for enantiomeric separation is extremely important for a multitude of applications in chiral synthesis, catalysis, pharmacology and biochemistry. Owing to the high specificity for the discrimination of enantiomers and their availability in large amounts, cyclodextrins (CDs) are now widely used as chiral selectors for the separation of enantiomers with various chromatographic techniques. Up to now, there are few papers dealing with the separation of chiral phosphorus compounds under the normal phase conditions on substituted CDs. In this paper, a phenyl carbamate derivative β-CD bonded CSP has been synthesized for the separation of organic phosphorus chiral compounds in normal phase condition.

It is believed that the chiral recognition on the derivative CD CSPs can arise from two sources: the base CD and the derivative substituents on CD.[2] According to this property about derivative β-CD bonded CSPs, the phenyl carbamate derivative β-CD bonded CSP was synthesized in order to achieve two aims: first, we tried to add more effective interaction sites to enhance the selectivity; second, we attended to increase hydrophobic effect to form a hydrophobic pocket possessing enantioselectivity. Phosphoryl mustard derivatives (POM) were first separated on this CD-type CSP. Such compounds were reported to have good antitumor activity.[5,6] Some of these chiral phosphorus organic compounds could be separated in baseline. The chiral recognition mechanism was proposed according to the experimental data.

Experimental

Preparation of the phenyl carbamate derivative β-CD *bonded* CSP

β-CD was recrystallized in water for three times. (3-Aminopropyl) triethoxysilane (99%) was purchased from Fluka (Buch, Switzerland). The silylating reagent (3-isocyanatopropyl-triethoxysilane) and the modifying reagent (phenyl isocyanate) were synthesized by our laboratory. The silica gel is totally irregular silica gel with a partial diameter of 5 μm, a mean pore size of 6—8 nm and a specific surface area of 340 m^2/g (Second Reagent

* Received November 22, 1999; accepted January 31, 2000.
Project supported by the Fundation of National Laboratory of Elemento-Organic Chemistry, Nankai University.

Factory, Tianjin, China). All the reaction solvents used were anhydrous and were dried according to conventional methods before use. The method of preparation of the phenyl carbamate derivative β-CD bonded CSP has been published before.[7]

Enantiomers

The compounds of a series of eighteen chiral phosphorus organic enantiomers were provided by our laboratory of organic synthesis.[6] The structures of the compounds were shown in Table 1. Each compound was dissolved in isopropyl alcohol and then diluted to a concentration of 0.1 mg/mL.

Apparatus

The HPLC system was composed of a series IV liquid chromatography with a Model 506 UV detector and ANASTAR chromatography data system (SSI, Scientific Systems Inc., U.S.A.). The phenyl carbamate derivative β-CD bonded CSP was packed into 250 × 4.6 mm I. D. stainless-steel column by conventional high-pressure slurry-packing procedure.

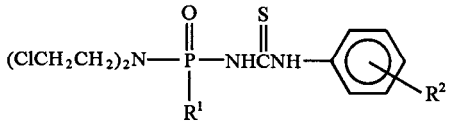
Chromatography

All solvents used were redistilled and filtered through a 0.45 μm filter and degassed *in vacuo* before use. The mobile phase composition was 15 vol% of isopropyl alcohol/*n*-hexane mixture with a constant flow rate of 1.0 mL/min. Injection volume was 20 μL. The column was thermostated at 25℃ and UV detection was used at 230 nm.

Results and discussion

In order to study the retention and enantioselectivity of this series of chiral phosphorus organic compounds, the whole screening was done under the identical chromatographic condition. The experiment results obtained were shown in Table 1.

Table 1 Structures of the compounds and values of the capacity factor k', R_s and α



Compd.	R^1	R^2	k_1'	k_2'	R_s	α
1	-OCH_2CH_2Cl	*p*-Cl	2.509	2.616	0.314	1.043
2	-OCH_2CH_2Cl	*p*-Br	2.489	2.513	0.091	1.010
3	-OC_6H_5	*o*-Cl	1.802	1.845	0.203	1.024
4	-OC_6H_5	*p*-Cl	2.435	2.763	1.562	1.135
5	-OC_6H_5	*p*-Br	1.823	2.178	1.785	1.195
6	-$OC_6H_4Cl(p)$	*o*-Cl	1.724	1.791	0.343	1.039
7	-$OC_6H_4Cl(p)$	*p*-Cl	2.220	2.473	1.519	1.114
8	-$OC_6H_4Cl(p)$	*p*-Br	2.115	2.494	1.770	1.179
9	-$OC_6H_4Cl(p)$	*m*-NO_2	5.407	5.603	1.408	1.103
10	-$OC_6H_4Me(o)$	*o*-Cl	1.440	1.531	0.375	1.063
11	-$OC_6H_4Me(o)$	*p*-Cl	1.707	1.884	1.413	1.104
12	-$OC_6H_4Me(o)$	*p*-Br	1.538	1.794	1.512	1.166
13	-$OC_6H_4Me(o)$	*m*-NO_2	4.758	5.305	1.589	1.115
14	-$OC_6H_4Cl(o)$	*o*-Cl	1.830	2.162	1.663	1.181
15	-$OC_6H_4Cl(o)$	*p*-Me	2.137	2.357	1.516	1.103
16	-NHPr-*i*	*p*-Me	1.758	1.758	0	1.000
17	-NHPr-*i*	*p*-Cl	1.618	1.618	0	1.000
18	-NEt_2	*p*-Cl	1.429	1.429	0	1.000

Retention

From Table 1, retention tends to decrease when the *para*-substituent of R^2 changes to *ortho*-substituent (*e. g.*, compounds **4** and **3**; compounds **7** and **6**; compounds **11** and **10**). This reduction of capacity factor may be due to the steric effect of repulsive interaction resulting from the substituent R^2 of the solute coming into the hydrophobic pocket and attaching to the entrance of β-CD. Such conclusion can also be supported by the retention of the compounds **9** and **13**. When the substituent R^2 is nitro-group (compounds **9** and **13**), the retention becomes much more longer than that of other compounds. Such phenomenon may result from the additional hydrogen-bondings which are formed between the nitro-group of the solute and the hydroxyl located on the entrance of the derivative β-CD.

Selectivity

The enantiomers of **1** and **2** ($R^1 = -OCH_2CH_2Cl$) have a little separation. The group OCH_2CH_2Cl replaced by *O*-aryl group (compounds **3**—**15**) leads to enhance the enantioselectivity. When the group *O*-aryl changes to NR′R″ or NHR, there will be no separation for the compounds **16**—**18**, which may be due to the small difference of the interactive energies between the two diastereoisomers.

For the compounds **3**—**15**, it appears that the enantiomers of *ortho*-substituent R^2 always get less separation compared with those of *para*-substituent R^2. However, the substituent position of R^1 has no significant effect on separation. It implies that R^2 may insert into the hydrophobic pocket which can afford repulsive interactions and R^1 interacts with the CSP through the other external active sites. There is no significant difference whether the substituent group on R^1 is a donor group or an acceptor group. It suggests that the dipole stacking contribute to the enantioselectivity which can enhance the selectivity but is not the indispensable factor for separation because the compounds **1** and **2** can also be separated.[8] Hydrogen-bonding interactions may be involved between N—H or C = O of the CSP and P = O or N—H of the compounds. It seems that the best overall selectivities are obtained when the substituent of R^2 was located on *para*. The experimental separation of compound **5** was given in Fig. 1. The results suggesting the mechanism for chiral recognition were shown in Fig. 2. The model involves two hydrogen-bonding interactions, strong dipole stacking, and inclusion between the substituent R^2 and the hydrophobic pocket.

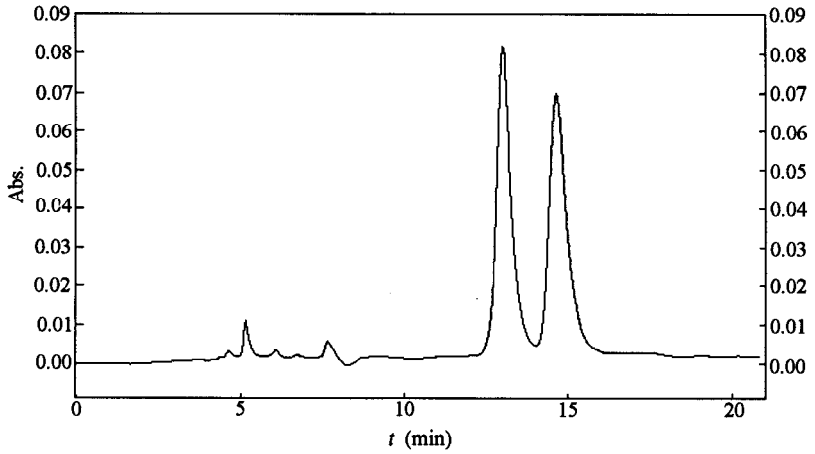


Fig. 1 Experimental separation of compound **5**.

Conclusion

A phenyl carbamate derivative β-CD bonded CSP has been developed for the effective separation of the novel chiral phosphorus compounds which can exhibit significant antitumor activity. The R_s value of some of the compounds exceeds 1.5 suggesting that the enantiomers can be separated in baseline. It may afford a method for the preparative separation of such chiral

phosphorous compounds. The retention and separation mechanism involves the external association and inclusion between the substituent R^2 and the hydrophobic pocket.

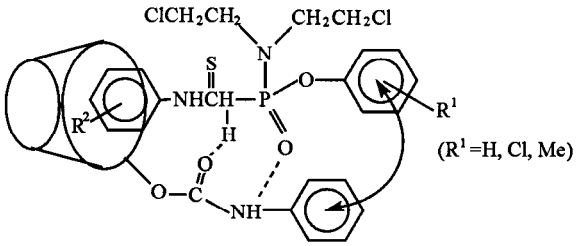


Fig. 2 Proposed mechanism for chiral separation.

References

1. Gao, R.Y.; Huang, J.M.; Yang, G.S.; Yang, H.Z.; Wang, Q.S., *Chin. J. Chem.*, **17**, 92(1999).
2. Gao, R.Y.; Yang, H.Z.; Huang, J.M.; Wang, Q.S., *Chin. J. Chem.*, **16**, 145(1998).
3. Armstrong, D.W., *Anal. Chem.*, **59**, 84A(1987).
4. Pirkle, W.H.; Pochapsky, T.C., *Chem. Rev.*, **89**, 347 (1989).
5. Friedman, M.; Carter, S.K., *Semin. Oncol.*, **5**, 193 (1987).
6. Chen, R.Y.; Wang, H.L.; Zhou, J., *Heteroatom Chem.*, **5/6**, 497(1994).
7. Chen, H.; Lu, X.Y.; Gao, R.Y.; Wang, Q.S., *Chin. J. Chem.*, **17**, 644(1999).
8. Cachau, C.; Thienpont, A.; Soulard, M.-H.; Félix, G., *Chromatographia*, **44**, 411(1997).

(E9911164 JIANG, X.H.; LING, J.)