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Short communication

Enantioseparation of fourteen O-ethyl O-phenyl N-isopropyl phosphoroamidothioates by high-performance liquid chromatography on a chiral stationary phase

R.Y. Gao*, G.S. Yang, H.Z. Yang, Z.Y. Chen, Q.S. Wang

National Laboratory of Elemento-Organic Chemistry, Institute of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China

Abstract

A series of fourteen O-ethyl O-phenyl N-isopropyl phosphoroamidothioate enantiomers containing a phosphorus atom as a chiral center have been separated by high-performance liquid chromatography on a Pirkle model chiral stationary phase. Chromatography data, the capacity factor (k') and the separation factor (α) of all solutes are presented. The influence of chromatographic conditions was investigated. The probable mechanism for chiral recognition is proposed.

Keywords: Enantiomer separation; Phosphoroamidothioates; Organophosphorus compounds

1. Introduction

Enantiomeric separation methods using high-performance liquid chromatography (HPLC) have been extensively developed. In the last decade, enantiomeric resolution by HPLC using chiral stationary phases (CSPs) have become of interest, and numerous CSPs have been developed [1–4]. A great number of organophosphorus compounds have biological activity and some of these are used as pesticides. The stereoisomers of organic phosphorus compounds that contain a phosphorus atom stereogenic center have obvious differences in biological activity and toxicological effects. Recently, some approaches have been explored for the enantiomeric separation of organophosphorus compounds by HPLC using CSPs [5–7]. In this study, fourteen O-ethyl O-phenyl N-isopropyl phosphoroamidothioates containing a phosphorus atom as a chiral center have been separated. The enantiomers were separated on a Pirkle-type CSP. The influence of the composition of the mobile phase on retention and stereoselectivity was investigated. The probable mechanism for chiral recognition is proposed.

2. Experimental

2.1. Materials

A series of fourteen O-ethyl O-phenyl N-isopropyl phosphoroamidothioates were synthesized in our laboratory. The spectra and elemental analyses were used as a check of the purity. O-Ethyl, O-aryl and N-isopropyl phosphoroamidothioates have herbicidal activity. They act on growing points of susceptible plants and cause severe enlargement of the affected tissues. The general structure of the compounds is shown in Fig. 1.

The structures of the substitiuents are *p*-Cl; 4,6-Cl;

*Corresponding author.

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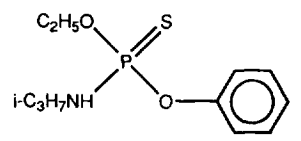

Fig. 1. Structure of the compounds used.

3,4,6-Cl; 2,4,6-Cl; *p*-Br; *p*-I; *p*-OCH_3; *p*-$C(CH_3)_3$; 2-NO_2,4-Cl; 2,6-NO_2,4-Cl; 2,4-CH_3,6-NO_2; 2-NO_2,4-$C(CH_3)_3$; 4-CF_3,6-NO_2 and 3-CH_3,4-Cl. These compounds were dissolved in isopropanol and then were diluted with the mobile phase. A solution with an approximate eluent concentration of 0.1 mg/ml was used for injection. Ethanol, isopropanol, methylene chloride, tetrahydrofuran and hexane were used as the eluent, all of which were redistilled and filtered through a 0.45-μm filter and degassed in vacuo before use.

2.2. Apparatus

The chromatographic system consisted of a Varian model 2010 HPLC pump (Varian, Northeast Florham Park, NJ, USA), a Rheodyne model 7125 injector with a 10-μl loop, a Varian model 2050 UV detector and a HP model 3394 integrator (Hewlett-Packard, Palo Alto, CA, USA).

2.3. Chromatography

The chiral column (CSP) was Sumichiral OA-4700 (25 cm×4.6 mm I.D., particle size 10 μm; Sumika Chemical Analysis Service, Osaka, Japan). The mobile phase was composed of various mixtures of hexane, isopropanol, tetrahydrofuran (THF), ethanol and methylene chloride. (A) isopropanol–hexane (0.05:99.95, v/v); (B) methylene chloride–hexane (0.60:99.40, v/v); (C) isopropanol–methylene chloride–hexane (0.08:3.00:96.92, v/v/v); (D) ethanol–methylene chloride–hexane (0.03:6.00:93.97, v/v/v); (E) THF–methylene chloride-hexane (0.03:6.00:93.97, v/v/v).

A column temperature of 25°C and a flow-rate of 1 ml/min were maintained throughout the study, 10 μl samples were injected and UV detection was at 254 nm.

3. Results and discussion

Enantiomeric separation of fourteen phosphoroamidothioates was tested on an OA-4700 chiral column using various mobile phase compositions. The separation data, capacity factor k_2' and α values are listed in Table 1. The results show that all fourteen phosphorus enantiomers can be separated in all experimental mobile phases. The best combination of k_2' and α values (i.e. small k_2' and large α) for each enantiomer is indicated in Table 1.

Chromatograms of some of the enantiomers are shown in Figs. 2 and 3. However, these results evidently prove that all fourteen of the enantiomers of phosphoramidothioates can be separated under optimum conditions.

The chiral recognition mechanism was investigated. The results suggest that the probable mechanism for chiral recognition is as depicted in Fig. 4.

Obviously enantioselectivity can be strongly dependent on the characteristics (nature of position, count and size of the substituents) of a given compound.

This model involves the formation of a $\pi-\pi$ complex between the weak π-electron donor group of the CSP (OA-4700) and the weak π-electron acceptor aryl substituent of the organic phosphorus compounds and three hydrogen bonding of the CSP and the O-ethyl, O-phenyl and N-isopropyl phosphoroamidothioates. Therefore, the hydrogen bonding is more important. The compounds that lost the hydrogen bonding donor NH group (ex. iso-C_3H_7NH change to iso-C_3H_7NR) had very short retention times and could not be separated.

When the donor group of CSP is changed to an acceptor group (DNB), compounds could not be separated (it has been done in previous work carried out by us). From a mechanistic point of view, the first eluted enantiomer is called (*R*) and the second (*S*).

4. Conclusion

Enantioseparation of a series of O-ethyl O-phenyl N-isopropyl phosphoroamidothioates containing a phosphorus atom as the chiral center has been investigated by HPLC on an OA-4700 chiral station-

Table 1
Values of k_2' and α for fourteen O-ethyl O-phenyl N-isopropyl phosohoroamidothioates obtained with various mobile phase compositions

Number	R	Mobile phase									
		A		B		C		D		E	
		k_2'	α	k_2'	α	k_2'	α	k_2'	α	k_2'	α
1	*p*-Cl	1.421	1.094	1.501	1.109	1.363	1.114	1.332	1.109	1.146[a]	1.129[a]
2	4,6-2Cl	1.727	1.200	2.221	1.190	1.583[a]	1.213[a]	1.477	1.190	1.685	1.213
3	3,4,6-3Cl	1.782	1.302	2.025	1.287	1.587[a]	1.310[a]	1.391	1.278	1.771	1.284
4	2,4,6-3Cl	0.843	1.138	1.246	1.136	0.815[a]	1.143[a]	0.885	1.142	1.021	1.126
5	*p*-Br	2.474[a]	1.114[a]	2.897	1.102	2.257	1.110	1.936	1.104	2.496	1.097
6	*p*-I	2.779[a]	1.097[a]	3.063	1.084	2.479	1.095	2.113	1.095	2.387	1.077
7	*p*-OCH_3	6.018	1.025	6.328[a]	1.066[a]	3.892	1.064	3.054	1.039	3.925	1.054
8	*p*-$C(CH_3)_3$	1.885	1.112	2.097	1.110	1.555	1.118	1.576	1.124	1.661[a]	1.125[a]
9	2-NO_2,4-Cl	3.199	1.068	3.883	1.062	3.049	1.084	2.351[a]	1.092[a]	3.187	1.074
10	2,6-2NO_2,4-Cl	3.031	1.065	4.269	1.067	3.085	1.082	2.826[a]	1.094[a]	2.976	1.085
11	2,4-2CH_3,6NO_2[b]	2.703	1.073	3.521	1.095	2.805	1.098	2.579	1.099	3.427[a]	1.101[a]
12	2-NO_2,4-$C(CH_3)_3$	2.613	1.094	3.264	1.084	2.538[a]	1.099[a]	2.126	1.107	2.624	1.085
13	4-CF_3,6-NO_2	2.869	1.161	3.474	1.174	2.605[a]	1.174[a]	2.373	1.172	2.624	1.165
14	3-CH_3,4-Cl	2.274	1.137	2.331	1.118	2.207[a]	1.137[a]	1.587	1.119	1.728	1.121

Column temperature, 16°C. Flow rate, 1.0 ml/min.
[a] Best separation conditions.
[b] Here C_2H_5O is changed to CH_3O.

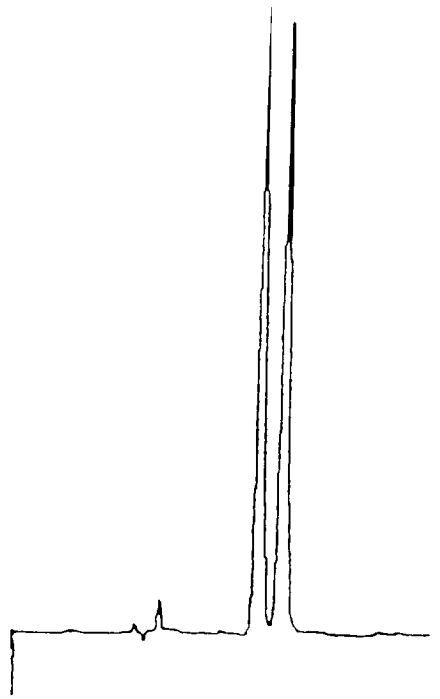

Fig. 2. Chromatogram of compound **2** under optimal conditions.

Fig. 3. Chromatogram of compound **4** under optimal conditions.

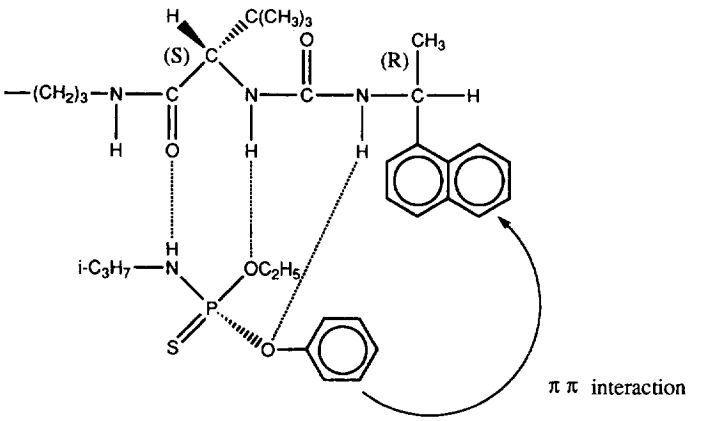

Fig. 4. Proposed mechanism for chiral recognition.

ary phase. A chiral recognition mechanism was proposed. Both hydrogen bonding and weak $\pi-\pi$ interactions are needed. This method should lead to the more rapid development of studies on the biological activity of enantiomers.

References

[1] S.G. Allemark, Chromatographic Enantioseparation. Methods and Applications, Wiley, New York, 1988.
[2] V.A. Davankow, Chromatographia, 27 (1989) 475.
[3] W.H. Pirkle and T.C. Pochapsky, Chem. Rev., 89 (1989) 347.
[4] C.J. Welch, J. Chromatogr. A, 666 (1994) 3.
[5] Yoshio Okamoto, J. Chem. Soc. Jpn., 57 (1984) 168.
[6] Charie E. Kientz., J. High Resolut. Chromatogr., 14 (1991) 461.
[7] R.Y. Gao, G.S. Yang, H.X. Shen, R.Y. Chen, Q. Dai and Q.S. Wang, J. Liq. Chromatogr. Rel. Technol., 19 (1996) 171.