Enantiomer Separation of a Novel Aminothiazolecarboxamide Fungicide Using Polysaccharide-Derived Chiral Stationary Phases

Byoung-Hyoun Kim[1,*] and **Sang Uk Lee**[2]

[1]Analytical Science, [2]Computational Science, Corporate R&D/LG Chemical Research Park, LG Chemical Ltd., 104-1, Moonji-dong, Yusong-gu, Daejeon, 305-380, South Korea

Abstract

The direct enantioseparation of a novel aminothiazolecarboxamide fungicide, ethaboxam, on polysaccharide-derived chiral stationary phases (CSPs) is described. Good resolution is achieved with several polysaccharide-derived CSPs. Chiralcel OD (OD-H) and Chiralpak AS are excellent for direct enantiomer separation of ethaboxam. The elution behavior and the effects of eluent composition on the resolution of ethaboxam are also investigated. Furthermore, the mechanism for chiral recognition using molecular mechanics is discussed.

Introduction

Many fungicides and insecticides contain a mixture of active and inactive isomers. Many of these are now being sold in a resolved form, which contains only the active or a higher ratio of active isomers than the original products. Therefore, the isolation and determination of the active enantiomer is important for improving the efficacy of fungicides and insecticides. Ethaboxam, a novel aminothiazolecarboxamide-derived fungicide, has been found to exhibit strong fungicidal activity (1–3). This fungicide possesses one asymmetric carbon and exists as two stereoisomers. Therefore, the enantiomer separation of these chiral forms and their intermediates is very important for determining and improving the efficacy of this fungicide.

The polysaccharide-derived chiral stationary phases (CSPs) are widely used in high-performance liquid chromatographic (HPLC) separation of enantiomers (4–9). Cellulose and amylose are the most accessible, naturally-occurring, optically-active polymers. These polysaccharides recognize distinct chiral forms, but are not practical for chiral separation. However, derivatization of these polysaccharides produces CSPs that have excellent chiral recognition properties and are able to separate a wide range of racemic compounds. Hydrogen bonding between various racemic compounds and polysaccharide CSPs plays an important role in chiral separation. The mechanism for chiral discrimination on the CSPs has been examined using both spectroscopic (10,11) and computer-aided methods (7,12–14).

The results from a computational study on the chiral discrimination mechanism for polysaccharide-derived CSPs have been reported in the literature (7). Results of those computational calculations were in good agreement with actual chromatographic results that were obtained (7). In the current study, the chiral recognition of ethaboxam enantiomers by polysaccharide-derived CSPs using both chromatographic and computational methods is described.

Experimental

Chemicals and reagents

Novel fungicide, ethaboxam, used was developed and supplied by LG Life Science (Daejeon, South Korea) (Figure 1). All solvent used in this study were HPLC grade and purchased from J.T. Baker (Phillipsburg, NJ).

Chromatographic conditions

Chromatography was carried out on a Waters Alliance 2690 HPLC system and 996 photodiode array detector (Waters, Milford, MA). Signs of optical rotation were determined using a shodex OR-1M optical rotation detector (Showa Denko, Japan). HPLC control and data process were performed by Empower software

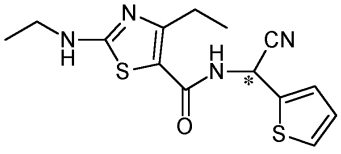

Figure 1. The structure of a novel aminothiazole carboxamide, ethaboxam.

* Author to whom correspondence should be addressed: email bhkime@lgchem.com.

(build 1154, Waters). The chiral columns used were the commercially-available Chiralcel OD, Chiralcel OD-H, Chiralcel OJ, Chiralpak AD, and Chiralpak AS (250- × 4.6-mm i.d., Daicel, Tokyo, Japan). The flow rate was 1.0 mL/min, and detection was set at 240 nm in all experiments. The eluents used were mixtures of *n*-hexane with different alcohols as follows: hexane–*n*-propanol, hexane–isopropanol, and hexane–ethanol, ranging from 70:30 ~ 90:10 (v/v, %). The injection volume of sample dissolved in isopropanol was 10 µL of 1 mg/mL. The dead volume (t_0) of the columns was estimated with 1,3,5-tri-tert-butylbezene as a nonretained compound.

Computational details

The DISCOVER ver. 3.5, module of the CERIUS^2 ver. 4.2 software package (Accelrys, San Diego, CA) was used for investigating the molecular mechanics. Geometry minimizations and energy calculations were performed using the Dreiding force field, which contains a hydrogen-bonding energy term. Minimized monomer units of CSPs were used to construct a helix-shaped polymer with the Polymer Builder module of CERIUS^2. The interaction energy calculations were determined between the 7-mer units of the CSPs and each enantiomer of ethaboxam. The lowest-energy conformations and interaction energies were obtained using molecular mechanics calculations.

Results and Discussion

The chromatographic results for enantioseparation of ethaboxam are summarized in Table I. A consistent elution order of each enantiomer was obtained on both cellulose- and amylose-derived CSPs. Separation factors ranged from 1.09 to 1.93 in hexane containing 20% isopropanol. The enantiomers of ethaboxam were separated very well on Chiralpak AS. However, Chiralcel OD-H was found to be more efficient than others when comparing the separation factor, resolution, and analysis time. Good enantioseparation of ethaboxam was observed on most CSPs (with exception of Chiralpak AD), using a mixture of isopropanol and hexane as an eluent (Figure 2).

It is often difficult to elucidate the mechanism underlying enantiomer discrimination by CSPs. Moreover, the CSPs used in this study were complex polymers. It was recently reported that hydrogen bonding could be a considerable component of the

Table I. Enantioselectivity Data of the Ethaboxam on Polysaccharide Derivied CSPs

		Hexane–*n*propanol (v/v, %)			Hexane–isopropanol (v/v, %)			Hexane–ethanol (v/v, %)		
CSPs		**90:10**	**80:20**	**70:30**	**90:10**	**80:20**	**70:30**	**90:10**	**80:20**	**70:30**
Chiralcel OD	a*	1.29	1.34	1.39	1.33	1.41	1.45	1.34	1.34	1.40
	Rs†	0.95	0.74	0.50	1.13	0.98	0.84	1.13	0.75	0.61
	k'_1‡	2.71	0.94	0.44	4.58	1.72	0.84	2.30	0.76	0.40
	Last eluted§	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)
Chiralcel OD-H	a	1.33	1.38	1.43	1.41	1.48	1.53	1.38	1.40	1.47
	Rs	1.16	0.85	0.61	1.74	1.09	0.95	1.26	0.81	0.63
	k'_1	2.32	0.78	0.40	3.89	1.39	0.72	1.95	0.63	0.34
	Last eluted	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)
Chiralcel OG	a	1.15	1.14	1.13	1.13	1.13	1.11	1.12	1.11	1.09
	Rs	0.83	0.63	0.42	0.81	0.58	0.42	0.76	0.49	0.32
	k'_1	3.7	1.43	0.82	6.79	2.57	1.29	3.30	1.21	0.74
	Last eluted	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)	R(–)
Chiralcel OJ	a	1.76	1.44	1.92	1.24	1.20	–	–	–	–
	Rs	2.46	0.96	1.04	1.07	0.74	–	–	–	–
	k'_1	5.88	2.04	0.96	8.68	3.03	1.79	1.78	1.41	0.88
	Last eluted	R(–)	R(–)	R(–)	R(–)	R(–)	–	–	–	–
Chiralpak AD	a	–	–	–	1.13	1.09	–	1.13	1.09	1.07
	Rs	–	–	–	0.71	0.37	–	0.71	0.44	0.17
	k'_1	5.69	1.61	0.84	4.77	1.61	0.73	5.37	1.61	0.90
	Last eluted	–	–	–	S (+)	S(+)	–	S (+)	S(+)	S(+)
Chiralpak AS	a	1.80	1.74	1.67	2.01	1.93	1.88	1.85	1.79	1.75
	Rs	2.83	1.43	0.94	4.07	2.26	1.51	3.15	1.74	1.04
	k'_1	3.88	1.24	0.64	8.51	3.00	1.45	3.04	1.02	0.57
	Last eluted	S(+)	S(+)	S(+)	S(+)	S(+)	S(+)	S(+)	S(+)	S(+)

* a = separation factor.
† Rs = resolution factor.
‡ k'_1 = capacity factor of first eluted enantiomer.
§ Absolute configuration and sign of optical rotation of more strongly retained enantiomer.

interaction mechanism involved in chiral recognition (15–16). It was previously reported that the extent of steric hindrance, in addition to the strength of hydrogen bonding between the enantiomer and the polysaccharide derived CSPs, was another major factor for chiral recognition (7).

The elution order on cellulose-type polysaccharide-derived CSPs remained constant with the *R*-form of the enantiomer eluting last in all cases. In contrast, the *S*-form of the enantiomer was retained longer on amylose-type polysaccharide derived CSPs. In Figure 3, an interaction mechanism between each enantiomer and each polysaccharide derived CSP is proposed. Stereochemically, the thiophene ring of the enantiomers are on the opposite sides (Figure 3). These geometric configurations (*S*-form/cellulose-type CSPs, *R*-form/amylose-type CSPs) give rise to steric hindrance with the specific CSPs. Figure 4 shows the minimized conformations between enantiomer and polysaccharide CSP. The minimized conformations of the cellulose-type CSP (Chiralcel OD) and amylose-type CSP (Chiralpak AS) are illustrated. The thiophene ring of the *S*-form extends toward the backbone of the cellulose-type CSPs, which results in steric hindrance caused by the thiophene ring of the enantiomer and the alkyl group of the CSPs. The thiophene ring lies on the opposite side of the *R*-form and would be free of this steric hindrance. Thus, the interaction between the *R*-form and the cellulose-type CSP would be sterically favored and have a stronger interaction than the *S*-form. The opposite would be the case for the thiophene ring of the *S*-form and the amylose-type polysaccharide CSPs. The thiophene ring of the *S*-form extends away from the alkyl group of the amylose-type CSP and would be retained longer than the *R*-form. The interaction energies between the enantiomer and the CSPs were ordered and compared with the chromatographic data (Table II). In enantioseparation of ethaboxam, the order of differential interaction energy was Chiralpak AD < Chiralcel OG < Chiralcel OD (-H) < Chiralpak AS. These calculations are in good agreement with the chromatographic data. It appears that the thiophene ring of ethaboxam enantiomers produce steric hindrance with specific CSPs, and this dictates the binding properties of the particular enantiomer.

Good enatioseparation of the ethaboxam was achieved with polysaccharide derived CSPs. The interaction energies between

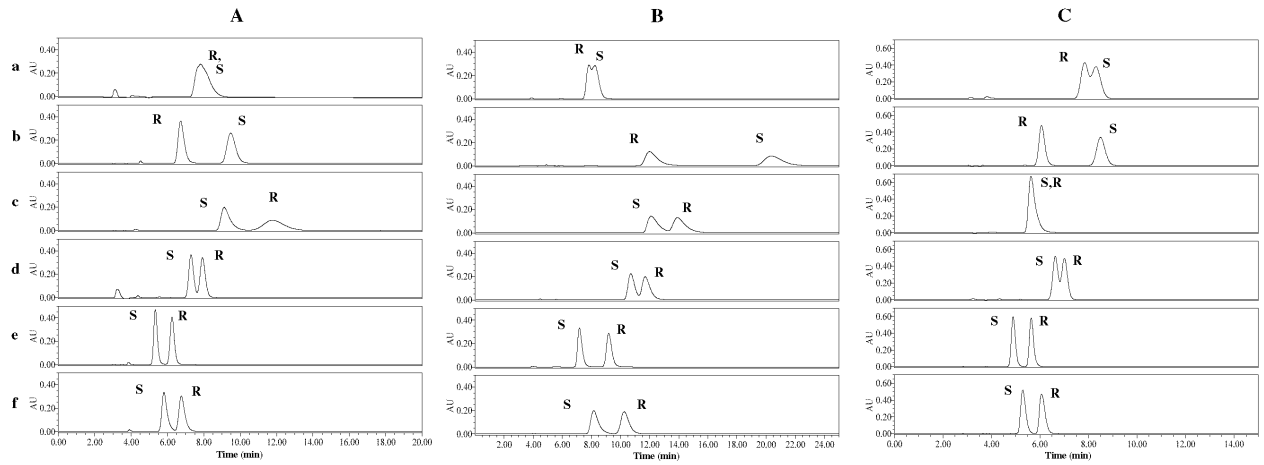

Figure 2. Chromatograms of enantiomer separation of ethaboxam on polysaccharide derived CSPs: 20% *n*-propanol in hexane (A), 20% isopropanol in hexane (B), and 20% ethanol in hexane (C). Column used: Chiralpak AD (a), Chiralpak AS (b), Chiralcel OJ (c), Chiralcel OG (d), Chiralcel OD-H (e), and Chirlacel OD (f).

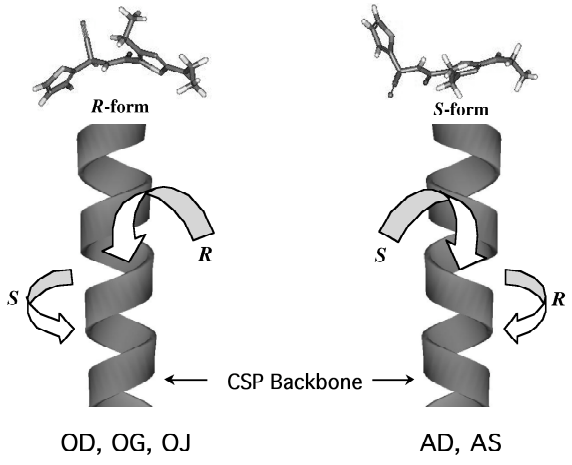

Figure 3. Interaction scheme of the ethaboxam with polysaccharide-derived CSPs.

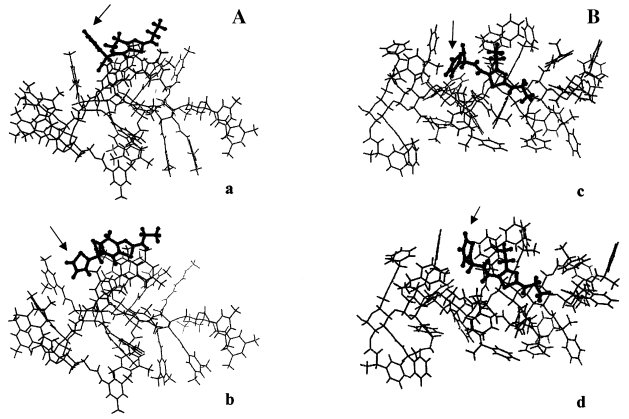

Figure 4. Minimized conformations of the ethaboxam on polysaccharide-derived CSPs: Chiralcel OD (A) and Chiralpak AS (B). The *R*-form of the ethaboxam (a and c), and *S*-form of the ethaboxam (b and d). The arrow indicates the thiophene ring of the ethaboxam.

Table II. The Calculated Interaction Energies (kcal/mol) and Chromatographic Elution Order

CSP		$E_{int.}$*	Last $eluted_{cal.}$†	Last $eluted_{exp.}$‡
Chiralcel OD	*R*	48.53	R(–)	R(–)
	S	44.80		
Chiralcel OG	*R*	52.02	R(–)	R(–)
	S	48.55		
Chiralcel OJ	*R*	34.63	R(–)	R(–)
	S	13.56		
Chiralpak AD	*R*	40.20	S(+)	S(+)
	S	41.27		
Chiralpak AS	*R*	50.25	S(+)	S(+)
	S	58.96		

* $E_{int.}$ = interaction energy (kcal/mol).
† Last $eluted_{cal}$ = more retained enantiomer from computational results.
‡ Last $eluted_{exp}$ = More retained enantiomer from chromatographic results.

enantiomers of ethaboxam and polysaccharide CSPs were calculated using molecular mechanics. The major chiral recognition factor appears to be steric hindrance and hydrogen boding strength between the enantiomer and the CSPs. In this study, it is suggested that steric hindrance was the most important factor for chiral discrimination between enantiomers of ethaboxam and polysaccharide CSPs.

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