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Enantiomeric separation of optically active pyridazinone derivatives by chiral HPLC

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Abstract

Several 4,5-disubstituted 3(2H)-pyridazinone derivatives containing 2-hydroxymethylpyrrolidino moiety as a chiral building block were synthetized. Separation of enantiomers was carried out by chiral HPLC on Chiralcel OJ and OF columns. Mobile phases consisted of hexane, ethanol and 2-propanol. Chiralcel OJ column was capable of separating most of the enantiomeric pairs. For one type of compound, Chiralcel OF column was used for separation.

Keywords: Pyridazinone derivatives; Chiralcel columns; Chiral HPLC

1. Introduction

Over the past few years, a number of 4,5-disubstituted 3(2H)-pyridazinones including *b*-fused [1,5]thiazepines and [1,4]oxazines, have been synthetized and studied in our laboratory [1–5]. In the course of our study on antiarrhythmic and antianginal pyridazine derivatives, some structurally relating pyridazinones possessing a prolinol moiety were also been considered. 2-hydroxymethylpyrrolidino moiety as a chiral building block was introduced by nucleophilic substitution reaction of 4,5-dichloro-2-methyl-3(2H)-pyridazinone with *R*- or *S*-prolinol. Subsequent treatment with thionyl chloride or direct ring closure afforded the chloromethyl derivatives or the oxazines, respectively. For testing the biological activities of the enantiomers, it was necessary to develop an enantiomer separation method, which enables determination of optical purity. For this separation, a chiral HPLC method was elaborated. The chiral centre of this type of compound is situated in the prolinol moiety. The effect of pyridazinone group and its substituents on the retention properties of the derivatives was investigated.

Several methods are given in the literature for the separation of enantiomers of compounds containing N atoms in their heterocyclic rings. For this purpose cellulose ester and cellulose carbamate chiral stationary phases were used [6–9]. In this laboratory, enantiomers of aza-bicyclo derivatives [10] and benzodiazepines [11] were separated on columns containing these types of chiral

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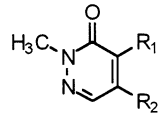
phases. Considering these results, Chiralcel OJ and Chiralcel OF columns were used in the development of a chiral HPLC method suitable to separate the enantiomer forms of pyridazinone derivatives.

2. Experimental

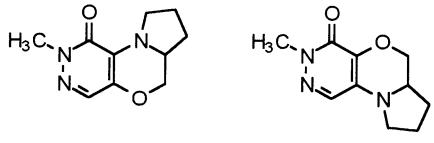
Compounds tested are characterized by the following formula (Scheme 1)



	R_1	R_2
I	$-SO_2-CH_2-C_6H_5$	$-N(CH_2Cl)-$
II	$-SO_2-CH_2-C_6H_5$	$-N(CH_2OH)-$
III	$-N(CH_2OH)-$	$-Cl$
IV	$-Cl$	$-N(CH_2OH)-$

Scheme 1.

In addition, enantiomers of tricyclic compounds formed by ring closure from **III** and **IV** (compounds **V** and **VI**, respectively) were investigated (Scheme 2).



V **VI**

Scheme 2.

Table 1
Chromatographic data of pyridazinone enantiomers[a]

Compound	t_r (min)	k	N	R_s	
	+				
I		>70			
	−				
	+				
II		>70			
	−				
	+	17.09	3.620	5057	
III					3.77
	−	21.13	4.710	5514	
	+	23.16	5.260	4516	
IV					1.56
	−	25.48	5.887	4509	
	+	14.98	3.048	5417	
V					5.59
	−	20.45	4.527	5447	
	+	34.29	8.269	3683	
VI					2.15
	−	39.56	9.691	3971	

[a] Column, Chiralcel OJ; Mobile phase, A. Temperature, 24°C. Flow rate, 0.8 ml min^{-1}.

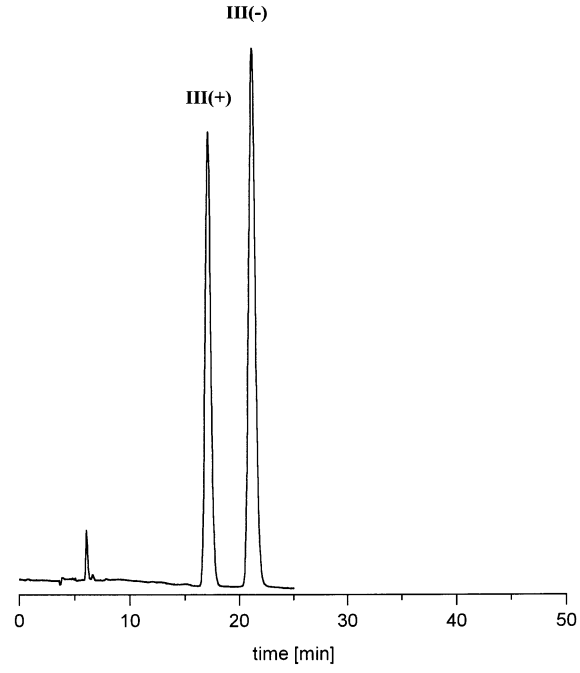


Fig. 1. Separation of $S(+)$ and $R(-)$ enantiomers of compound **III**. (Column, Chiralcel OJ; Mobile phase, A. Flow rate, 0.8 ml min^{-1}. Detection wavelength, 240 nm. Temperature, 24°C.)

Syntheses of (*S*)-enantiomers of **I**, **II** [1] and **III**–**VI** [5] have already been reported. The corresponding (*R*)-enantiomers were prepared in the same synthetic pathway, starting from *R*-prolinol.

3. Chromatographic conditions

Equipment: LKB HPLC pump, UV detector at 240 nm, Rheodyne 7125 injector with 20 μl loop
Data processing: IBM PC/AT, Nelson 5.0 software
Columns: Chiralcel OF, 0.46 × 25 cm (10 μm) Phenomenex; Chiralcel OJ, 0.46 × 25 cm (10 μm) Baker
Mobile phases: A, hexane:abs. ethanol = 9:1 (v/v); B, hexane:abs. ethanol = 65:35 (v/v); C, hexane:2-propanol = 1:1 (v/v). Solvents were of HPLC grade, supplied by Carlo Erba.

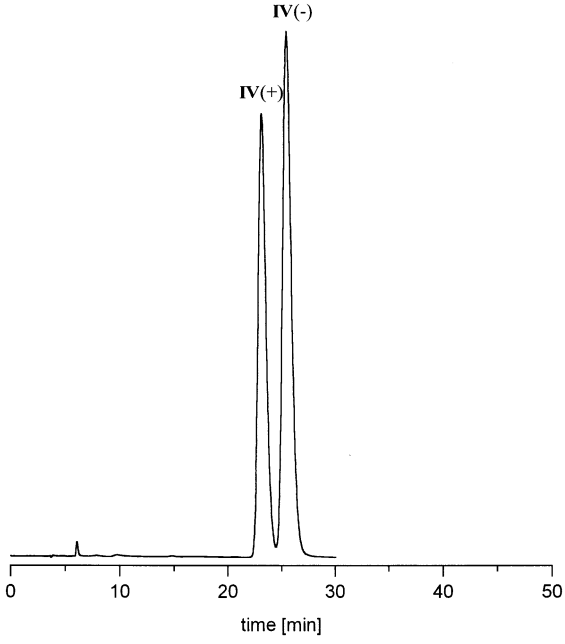


Fig. 2. Separation of *S*(+) and *R* (−) enantiomers of compound **IV**. (Column, Chiralcel OJ; Mobile phase, A. Flow rate, 0.8 ml min^{-1}. Detection wavelength, 240 nm. Temperature: 24°C.)

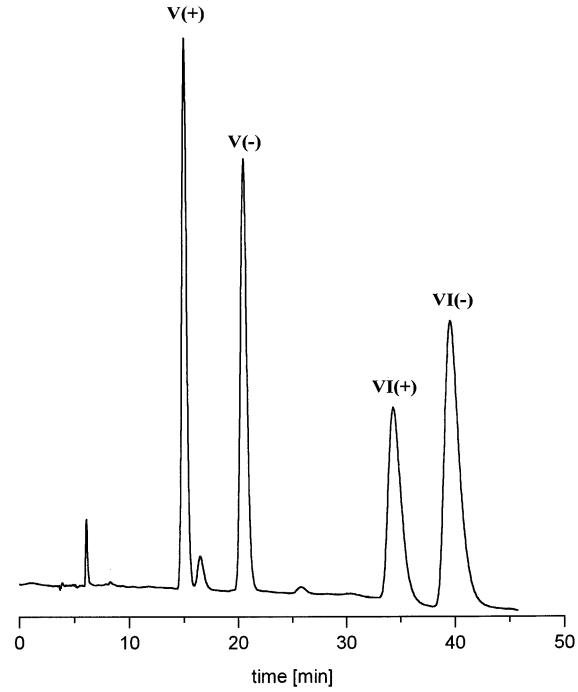


Fig. 3. Separation of *S*(+) and *R*(−) enantiomers of compounds **V** and **VI**. (Column, Chiralcel OJ;. Mobile phase, A. Flow rate, 0.8 ml min^{-1}. Detection wavelength, 240 nm; Temperature, 24°C.)

Table 2
Chromatographic data of enantiomers of compounds **I** and **II**[a]

Compound		t_r (min)	k	N	R_s
I	+	22.90	5.188	2572	1.64
	−	26.17	6.072	2590	
II	+	14.33	2.870	No separation	
	−	14.33	2.870		

[a] Column, Chiralcel OJ; Mobile phase, B. Temperature, 24°C. Flow rate, 0.8 ml min^{-1}.

Flow rate: 0.8 and 1.0 ml min^{-1}, respectively.
Temperature: ambient (24°C) on Chiralcel OJ column, 40°C on Chiralcel OF column.
Sample concentration: ~100 μg ml^{-1} in A mobile phase. Solutions of *S*(+) and *R*(−) enantiomers of the compounds were mixed in proportion of about 1:1 and injected into the chromatograph.

Table 3
Chromatographic data of the enantiomeric forms of compound **II**[a]

Compound		t_r (min)	k	N	R_s
II	+	22.82	5.167	1241	2.26
	–	29.98	7.102	1158	

[a] Column, Chiralcel OF; Mobile phase, C. Temperature, 40°C. Flow rate, 1 ml min^{-1}.

4. Results and discussion

Table 1 shows chromatographic data of the compounds chromatographed on Chiralcel OJ column. The standard deviation of retention times ranged by $\pm 2.5\%$ in the replicate injections.

Resolution (R_s) refers to the corresponding enantiomers. As can be seen from Table 1, mobile phase A is suitable for separation of enantiomer forms of compounds **III**–**VI** in the given experimental conditions. The data show the strong effect of the substituents on retention behaviour of the molecules. Compounds **I** and **II** cannot be studied using this mobile phase because of their strong mutual interaction with stationary phase. Considering the retention of the isomer compounds **III** and **IV** compound **III** shows lower retention when compared to compound **IV** (Figs. 1 and 2).In compound **III**, the prolinol moiety lies nearer to the keto group of pyridazinone than in compound **IV**, so we can suppose the possibility of an intermolecular H bond decreasing polarity of compound **III**.

At tricyclic compounds **V** and **VI**, compound **V** showed the lower retention values (Fig. 3).

In the case of each compounds tested, the $S(+)$ form eluted first.

To separate enantiomers of **I** and **II**, the more polar mobile phase B was used on Chiralcel OJ column. Characteristic data are shown in Table 2.

Data in Table 2 show that enantiomers of compounds **II** co-elute on the Chiralcel OJ column. As a possible explanation of lower retention of compound **II** related to **I**, we may suppose an intermolecular H bond between the sulfo and the OH group of prolinol in **II**. A cellulose carbamate type column (Chiralcel OF) with a strong H donor and acceptor property seemed to be suitable for separating the enantiomers of this compound. Using a suitable mobile phase (C), separation was achieved on Chiralcel OF column. To decrease high viscosity of the mobile phase, the column was thermostated to 40°C. Table 3 shows the retention data of **II** enantiomers.

5. Conclusion

Chiralcel OJ columns were suitable for the separation of enantiomer pairs of compounds **I**, **III**, **IV** and **V**. Enantiomers of **II** could be separated on the Chiralcel OF column. Substituents of the pyridazinone group strongly affect elution order and separation.

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