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Note

Chiral stationary phase for the facile resolution of β-adrenoceptor blocking agents

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The relationship between molecular chirality and the pharmaceutical activity of various drugs is of great interest [1]. The separation of a racemic mixture of β-adrenoceptor blocking agents (β-blockers), in particular, has attracted considerable attention [2]. For instance, propranol, a typical β-blocker, has been widely used in the treatment of a variety of cardiovascular disorders and its (*S*)-(−)-isomer is 100 times more potent than its antipode, the (*R*)-(−)-isomer [3]. In addition to the different pharmacological activities, recent studies have shown that the absorption, metabolism and disposition kinetics of drugs also depend greatly on molecular chirality [4]. Therefore, the development of a facile procedure capable of separating and quantifying the enantiomers of of β-blockers is important.

The chromatographic separation of a racemic mixture has been recognized as one of the most effective methods for the above purpose [5]. In addition to the conventional indirect separation method using diastereomeric derivatives, there are two different direct separation approaches, a chiral stationary phase (CSP) method and a chiral ion-pair method. Several kinds of CSPs for the direct chromatographic separation of enantiomeric β-blockers, such as α_1-acid glycoprotein [6], cyclodextrins [7] and cellulose derivatives [8] have been developed recently. An ion-pair chromatographic method for the separation of a racemic mixture of various drugs [9] was developed by Petterson and co-workers, who found (+)-10-camphorsulphonic acid [10] and N-benzyloxycarbonylglycyl-L-proline [11] to be particularly effective as chiral counter ions for a number of β-blockers. These results prompted us to develop a new CSP for the separation of a racemic mixture of β-blockers by immobilizing glycyl-L-proline (GPr) on modified silica gel.

In this paper, the synthesis of a novel chiral stationary phase and the results of the separation of a series of β-blockers are presented.

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EXPERIMENTAL

Materials

All chemicals were analytical-reagent grade, except where indicated otherwise, and were used without further purification.

Develosil 100-5 (5 μm) silica gel, purchased from Nomura Chemical (Aichi, Japan) was used as a support. 3-Glycidoxypropyltrimethoxysilane was obtained from Shin-Etsu (Tokyo, Japan), glycyl-L-proline from the Peptide Institute (Osaka, Japan) or Sigma (St. Louis, MO, U.S.A.), Propranolol, pindolol and atenolol from Sigma, carteolol from Kanto (Tokyo, Japan) and metoprolol from Sterochim (Milan, Italy).

Preparation of the CSP–GPr chiral stationary phase

To a suspension of Develosil 100-5 (20.0 g), dried for 4 h at 120°C, in dry toluene (100 ml) was added 3-glycidoxypropyltrimethoxysilane (12.0 ml) and the resulting mixture was heated under reflux for 20 h with the removal from the mixture of the methanol formed. After cooling, the modified silica gel was filtered with a glass filter (G-4), washed with dry toluene (2 × 50 ml) and dried under vacuum to give 23.6 g of the product. A portion of this modified silica gel (3.70 g) was suspended in dry methanol (20 ml) and then the monosodium salt of glycyl-L-proline (0.512 g) was added and the mixture was allowed to stand for 7 days with occasional stirring under nitrogen at room temperature. The resulting chiral stationary phase, CSP–GPr was collected by filtration and washed exhaustively with methanol. Elemental analysis gave C 7.38, H 1.43, N 0.57; required for silanized silica gel, C 7.28, H 1.47, N $<0.1\%$.

Chromatography

A stainless-steel column (150 mm × 0.46 mm, I.D.) was packed with the modified silica gel using conventional slurry packing techniques. Chromatography was carried out at room temperature at a flow-rate of 1.0 ml/min using a mixture of methanol and dichloromethane containing an amine (1.0 m*M*) as the mobile phase. A Shimadzu (Kyoto, Japan) Model 6A, high-performance liquid chromatograph equipped with a Rheodyne Model 7120 injector with a 20-ml loop and a Shimadzu SPD-6A UV detector measuring at 280 nm was used.

RESULTS AND DISCUSSION

The chiral stationary phase, CSP–GPr was prepared from glycyl-L-proline and Develosil 100-5 (5 μm), pretreated with 3-glycidoxypropyltrimethoxysilane as a coupler, as shown in Fig. 1.

The separation of a racemic mixture of β-blockers on the prepared CSP was first conducted under the conditions used by Pettersson and co-workers with a solution of triethylamine in dichloromethane (1.0 m*M*) as the mobile phase [10]. Pindolol (separation factor, $\alpha = 1.15$), propranolol ($\alpha = 1.15$) and carteolol ($\alpha = 1.18$) were well separated into enantiomers. However, the retention time of these peaks varied with time so that the reproducibility of an analysis was poor. Addition of methanol to the mobile phase solved this problem. Amines added to the mobile phase compete with the β-blocker (β-amino alcohol) for ion pair formation with the carboxyl groups

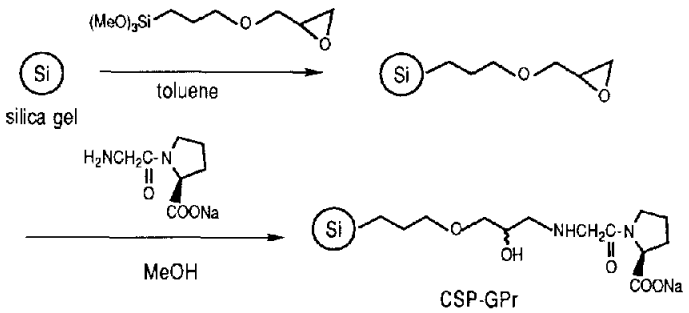


Fig. 1. Synthesis of the chiral stationary phase. Me = methyl.

of CSP–GPr, hence the retention time and separation factor will be affected by the amine structure and basicity. The effects of alcohols and amines added to the mobile phase were further investigated to elucidate the characteristics of CSP–GPr.

Organic solvent in a mobile phase

The results of the separation of propranolol with various mobile phases are given in Table I. When 2-propanol was added instead of methanol, the peaks exhibited tailing, even though the selectivity remained the same as with methanol. Chloroform separated the enantiomers of pindolol better than dichloromethan (Table II).

TABLE I

EFFECT OF ALCOHOLS IN THE MOBILE PHASE ON THE RESOLUTION OF PROPRANOLOL

Chiral stationary phase: CSP–GPr. Mobile phase: alcohol–dichloromethane (3:97) containing 1.0 m*M* triethylamine. Flow-rate: 1.0 ml/min. Temperature: 25°C. UV detection at 280 nm.

Alcohol	δ^a	$k_1{}^b$	$k_2{}^b$	α^c	$R_s{}^d$
Methanol	14.4	1.01	1.11	1.10	0.63
Ethanol	12.7	15.3	17.4	1.13	1.52
2-Propanol	11.5	3.30	3.66	1.11	0.60

[a] δ = Hildebrand's solubility parameter.

[b] Capacity factor of the first eluted enantiomer: k_1 = (retention time − dead time)/dead time.

[c] Separation factor $\alpha = k_2 / k_1$.

[d] Resolution factor R_s = 2 (distance between the two peaks) / (sum of the band widths of the two peaks).

TABLE II

EFFECT OF ORGANIC SOLVENTS IN THE MOBILE PHASE ON THE RESOLUTION OF PINDOLOL

Conditions as in Table I. Mobile phase: methanol–halogenated solvent (3:97) containing 1.0 m*M* triethylamine.

Solvent	δ	k_1	k_2	α	R_s
Dichloromethane	9.7	4.00	4.29	1.07	0.88
Chloroform	9.3	3.80	4.26	1.12	1.17

TABLE III

EFFECT OF VARIOUS AMINES ON THE RESOLUTION OF PINDOLOL

Mobile phase: methanol–dichloromethane (3:97) containing 1.0 m*M* amine. Conditions as in Table I.

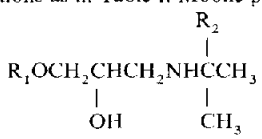
Amine	pK_a[a]	k_1	α	R_s	
Triethylamine	11.01	1.01	1.11	1.10	0.63
Diethylamine	11.09	4.68	5.22	1.12	1.05
Diisopropylamine	11.13	6.56	6.99	1.07	0.59
Butylamine	10.78	4.58	4.92	1.08	0.93
Pyrrolidine	11.27	2.33	2.49	1.07	0.71
Piperidine	11.12	5.74	6.07	1.12	1.63
Morpholine	8.33	26.21	27.90	1.06	0.91
2-Aminoethanol	9.52	1.91	2.10	1.10	0.99
2-(Ethylamino)ethanol	–	5.45	5.84	1.07	1.04

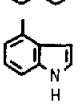
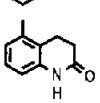
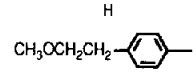
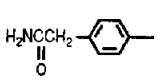
[a] These data were taken from ref. 12.

TABLE IV

RESOLUTION OF VARIOUS β-BLOCKERS ON CSP–GPr

Conditions as in Table I. Mobile phase: methanol–chloroform (2:98) containing 1.0 m*M* diethylamine.



Solute	R_1	R_2	k_1	k_2	α	R_s
Propranolol		H	1.77	2.06	1.16	1.09
Pindolol		H	8.24	9.55	1.16	1.02
Compound I[a]		H	11.7	16.1	1.37	2.12
Compound II[a]		H	2.30	2.77	1.21	1.13
Carteolol		CH_3	4.04	4.82	1.19	1.21
Metoprolol		H	1.18	1.31	1.12	0.41
Atenolol		H	11.2	12.3	1.10	0.58

[a] These compounds are β-blocker analogues.

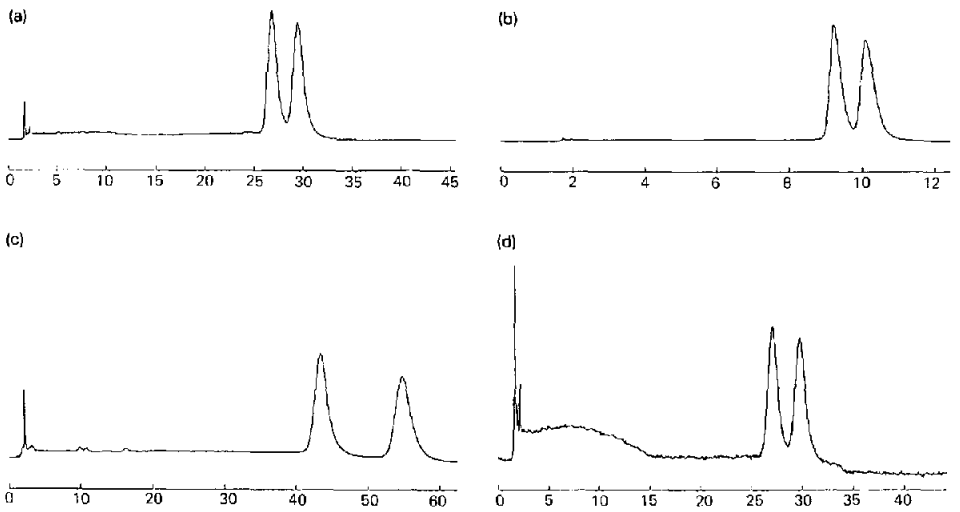


Fig. 2. Chromatograms of (a) pindolol, (b) propranolol, (c) compound I and (d) carteolol using CSP–GPr as a chiral stationary phase. Mobile phase: methanol–dichloromethane (3:97) containing 1.0 m*M* diethylamine. Flow-rate: 1.0 ml/min. Temperature: 25°C. UV detection at 280 nm. Time axis in min.

Influence of amine additives

The separation of a racemic mixture of propranolol was carried out using mobile phase containing nine types of amines, and the results are summarized in Table III. The retention time is greatly affected by the concentration of amines. As the amine concentration in the mobile phase increases, the β-blocker is eluted in a shorter time. Addition of sterically bulky diisopropylamine reduced the separation factor. The relationship between the basicity and the separation factor is not clear at this stage, but triethylamine, diethylamine and piperidine gave optimum separation factors among the nine amines investigated.

A series of β-blockers were separated on CSP–GPr using methanol–dichloromethane (3:97) containing diethylamine (1.0 m*M*) and the results are summarized in Table IV. Typical chromatograms are shown in Fig. 2.

In conclusion, an effective and readily prepared chiral stationary phase has been developed that has potential for application to other adrenergic drugs and other groups of pharmaceuticals with similar chemical structures.

Further studies are in progress to improve the resolution by changing the immobilized peptide structure and the results will be presented elsewhere.

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REFERENCES

1 I. W. Wainer and D. E. Drayer (Editors), *Drug Stereochemistry: Analytical Methods and Pharmacology*, Marcel Dekker, New York, 1988.
2 T. Walle and U. Walle, in V. Marko (Editor), *Determination of β-Blockers in Biological Matrices*, Elsevier, Amsterdam, 1988, Ch. 7.
3 A. M. Barret and V. A. Cullum, *Br. J. Pharmacol.*, 34 (1968) 43.
4 T. Walle, J. Webb, E. Bagwell, U. Walle, H. Daniell and T. Gattney, *Biochem. Pharmacol.*, 37 (1988) 115.
5 S. G. Allenmark, *Chromatographic Enantioseparation, Methods and Applications*, Wiley, New York, 1988.
6 G. Schill, I. W. Wainer and S. A. Barkan, *J. Liq. Chromatogr.*, 9 (1986) 641.
7 D. W. Armstrong, T. W. Ward, R. D. Armstrong and T. E. Beesley, *Science (Washington, D.C.)*, 232 (1986) 1132.
8 Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama and M. Masuda, *Chem. Lett.*, (1986) 1237.
9 C. Pettersson and G. Schill, in M. Zief and L. J. Crane (Editors), *Chromatographic Chiral Separations*, Marcel Dekker, New York, 1988, p. 283.
10 C. Pettersson and G. Schill, *J. Chromatogr.*, 204 (1981) 179.
11 C. Pettersson and M. Josefsson, *Chromatographia*, 21 (1986) 321.
12 D. D. Perrin, *Dissociation Constants of Organic Bases*, Plenum Press, New York, 1965.