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Short Communication

Direct stereochemical resolution of 3,4-dihydroxyphenylserine using a chiral crown ether stationary phase

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Abstract

The direct stereochemical resolution of the four stereoisomers of 3,4-dihydroxyphenylserine was achieved on a high-performance liquid chromatographic chiral stationary phase based on a chiral crown ether. The effects of pH and temperature were investigated. The role of the crown ether ring in separating the analyte is also described.

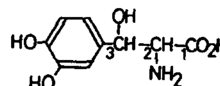
1. Introduction

3,4-Dihydroxyphenylserine (DOPS) is a synthetic amino acid that can exist in four possible stereoisomeric forms: *threo*- and *erythro*-DOPS and their enantiomeric isomers, the L- and D-forms (Table 1). Only L-*threo*-DOPS is postulated to have beneficial effects on the freezing phenomenon or akinaesia in Parkinson's disease [1,2] and orthostatic hypotension in familial amiloid polyneuropathy [3].

The enantiospecific synthesis of L-*threo*-DOPS requires the determination of enantiomeric purity, often when one enantiomer is present in a large excess of the other. For this purpose, a method has to be developed that would allow the chromatographic separation of these optical isomers, preferably without derivatization.

This is the first report on the direct optical resolution of DL-*threo*-DOPS. The enantiomeric ratio of L-*threo*-DOPS can be determined accurately by this method. The system is also able to resolve the diastereomeric pairs of DOPS and the four corresponding isomers.

Table 1
Structures of the four stereoisomers of 3,4-dihydroxyphenylserine (DOPS)



Compound	Absolute configuration
L-*threo*-DOPS	2*S*,3*R*
D-*threo*-DOPS	2*R*,3*S*
L-*erythro*-DOPS	2*S*,3*S*
D-*erythro*-DOPS	2*R*,3*R*

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2. Experimental

2.1. Chemicals

DL-*threo*-DOPS was purchased from Sigma (St. Louis, MO, USA). The four stereoisomers of DOPS was kindly provided by Research Laboratories, Sumitomo Pharmaceuticals (Osaka, Japan). Perchloric acid was obtained from Wako (Osaka, Japan).

2.2. Apparatus

The chromatographic system consisted of a Hitachi L-6000 solvent-delivery module, a Rheodyne Model 7125 injector equipped with a 20-μl loop, a Hitachi L-4000 UV–Vis detector set at 220 nm and a Shimadzu C-R3A integrator. The column temperature was controlled by an Eyela Uni Cool UC-65 circulating water-bath (Tokyo Rikakikai, Tokyo, Japan). The column consisted of a 150 × 4 mm I.D. stainless-steel column packed with a chiral stationary phase composed of a chiral crown ether coated on a polymeric support [Crownpak CR(+) and CR(−); Daicel, Tokyo, Japan].

2.3. Chromatographic conditions

The mobile phase was prepared by addition of perchloric acid to HPLC-grade water until the required pH was obtained. The flow-rate was 0.4 ml/min and the injection volume was 5 μl. To prevent corrosion and decomposition of the stationary phase, the column was washed every night with HPLC-grade water.

2.4. Samples

The chromatographic standards were prepared in distilled water (1 mg/ml) and filtered through a 0.45-μm Millipore filter before injection on to the column.

3. Results and discussion

Fig. 1a shows the enantiomeric separation of DL-*threo*-DOPS using a Crownpak CR (+) column and perchloric acid at pH 1.0 as the mobile phase, a column temperature of 2°C and a flow-rate of 0.4 ml/min. Shinbo *et al.* [4] have reported that this column is very powerful for the direct optical resolution of a variety of natural amino acids. No resolution was obtained using several other columns, such an Enantiopac and Ultron ES-OVM and Cyclobond-I, -II and -III. The resolution factor (R_s) from the data in Fig. 1a was calculated as 2.58. The enantiomer with the longest retention time was assigned as L-*threo*-DOPS by co-chromatography with authentic samples of the respective enantiomers.

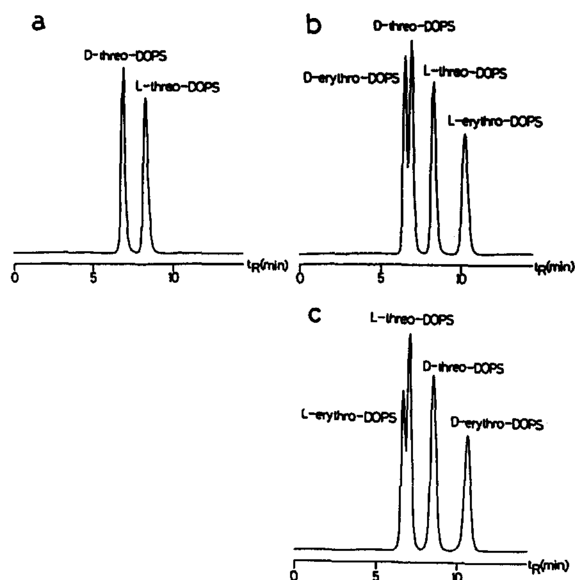

Fig. 1. (a) Chromatographic resolution of DL-*threo*-DOPS. Column, Crownpak CR(+). (b) Chromatogram obtained for the four possible stereoisomers of DOPS. Column, Crownpak CR(+). (c) Separation of the four possible stereoisomers of DOPS. Column, Crownpak CR(−). Other conditions are given in the text.

We carried out recovery tests of the D-*threo*-isomer from L-*threo*-DOPS to establish whether the enantiomeric ratio could be determined accurately. D-*threo*-DOPS was added to L-*threo*-DOPS to give a concentration of the former between 0.05% and 0.5%. The added D-*threo*-DOPS was recovered quantitatively at all concentrations by this procedure (Table 2). Hence the method allows the proportion of D-*threo*-DOPS in a sample to be measured precisely.

The resolution of DL-*threo*-DOPS can be reg-

Table 2
Results of recovery tests of D-*threo*-DOPS from L-*threo*-DOPS

Calculated (%)	Found (%)	Recovery (%)
0.05	0.05	100.0
0.1	0.10	100.0
0.2	0.21	105.0
0.5	0.48	96.0

ulated in two ways, by varying either the column temperature or the pH of the mobile phase. Temperature has a strong influence on the retention and chiral selectivity of this column [4]. The retention and resolution changes in the temperature range 2–40°C are given in Table 3. The stereoselectivity and retention decrease with increasing temperature. At 40°C the chiral resolution effect of the column was removed.

Variation of the pH of the mobile phase between 1.0 and 2.0 influences the resolution of DL-*threo*-DOPS, as demonstrated in Table 4. The resolution increases with decreasing pH, whereas the retention times are hardly affected. This effect is consistent with the proposed chiral recognition mechanism in which inclusion complexes are formed between a protonated primary amino group in the vicinity of the chiral centre of the analyte and the polyether rings of the chiral stationary phase [5]. In a highly acidic media, both the primary amino and the carboxylic acid groups of the solute appear to be completely protonated. Therefore, an inclusion complex with the chiral stationary phase would be readily formed and not be repelled by the oxygen atom of the crown ether ring.

Table 3
Influence of temperature on the enantiomeric resolution of DL-*threo*-DOPS

Temperature (°C)	t_R(min)	R_s
2	7.0, 8.5	2.61
10	6.3, 7.1	1.74
20	5.6, 6.0	1.00
40	4.8	No resolution

Column, Crownpak CR(+); mobile phase, perchloric acid (pH 1.0); R_s = resolution factor, calculated from $R_s = 2(t_2 - t_1)/(W_1 + W_2)$, where t_1 and t_2 are the retention times of the enantiomers and W_1 and W_2 are the widths of the peaks at their bases.

Table 4
Influence of pH on the enantiomeric resolution of DL-*threo*-DOPS

pH	t_R(min)	R_s
1.0	7.0, 8.5	2.58
1.3	7.1, 8.3	2.18
1.5	6.9, 7.9	1.86
2.0	5.9, 6.5	1.20

Mobile phase, perchloric acid; temperature, 2°C; other conditions as in Table 3.

Under the optimum conditions mentioned above, we attempted to separate the four possible stereoisomers of DOPS. A typical chromatogram is shown in Fig. 1b. In these separations, the elution of the D-isomer prior to the L-isomer was observed, as described in the manufacturer's instruction manual. The enantiomeric elution order is reversed on using the Crownpak CR(−) column, under the same conditions (Fig. 1c). In the determination of the optical purity of L-forms in D-forms, we can use these columns effectively by switching from the (+) to the (−)-column.

In conclusion, excellent resolution can be obtained for the stereoisomers of DOPS by using a chiral crown ether as the chiral bonded phase. The optical purity of small amounts of D-*threo*-DOPS can be also determined directly, rapidly and accurately by this HPLC method. This method will be applicable to probing the enantiomeric distribution of the antipode of L-*threo*-DOPS in biological media and to determining the enantiomeric purity of synthetic L-*threo*-DOPS.

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