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Note

Enantiomeric resolution of 1-[2-(3-hydroxyphenyl)-1-phenylethyl]-4-(3-methyl-2-butenyl)piperazine by reversed-phase high-performance liquid chromatography using a chiral mobile phase

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A new analgesic, 1-[2-(3-hydroxyphenyl)-1-phenylethyl]-4-(3-methyl-2-butenyl)piperazine (Fig. 1), has an asymmetric carbon in the molecule and it has been reported[1] that the pharmacological activities of the *R*(−)- and *S*(+)-enantiomers were different. It is thus important to examine the optical purity of the enantiomers.

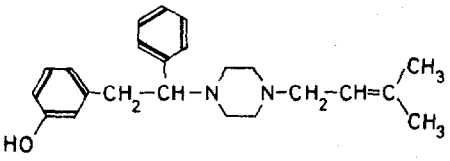

Fig. 1. The structure of 1-[2-(3-hydroxyphenyl)-1-phenylethyl]-4-(3-methyl-2-butenyl) piperazine.

High-performance liquid chromatography (HPLC) is very convenient for the direct resolution of enantiomers and for the examination of optical purity. Recently much work has been reported on the direct resolution of enantiomers by HPLC, using either a chiral stationary phase[2–9] or a chiral mobile phase[10–17]. In the former a particular stationary phase is required, but in the latter a commercial stationary phase can conveniently be used.

This note describes the enantiomeric resolution of the analgesic by reversed-phase HPLC using a chiral mobile phase. β-Cyclodextrin (β-CyD) was added to the mobile phase as a chiral component, which Debowski *et al.*[10] had used for the enantiomeric resolution of mandelic acid.

EXPERIMENTAL

Reagents and materials

Ethanol, sodium acetate, diethylamine and β-CyD of analytical-reagent grade were purchased from Wako (Osaka, Japan).

Enantiomers of 1-[2-(3-hydroxyphenyl)-1-phenylethyl]-4-(3-methyl-2-butenyl) piperazine were synthesized in our laboratories. The *R*(−)-enantiomer dihydro-

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chloride had m.p. 227°C and $[\alpha]_D^{27}$ − 51.5° (c = 1, 0.1 N hydrochloric acid) and the $S(+)$-enantiomer dihydrochloride had m.p. 227°C and $[\alpha]_D^{27}$ + 50.5°C (c = 1, 0.1 N hydrochloric acid).

Apparatus

A Shimazu Model LC-3A liquid chromatograph, equipped with a Model SPD-2A UV detector and a Model SIL-1A injector (Shimazu, Kyoto, Japan) was used. The detection wavelength was fixed at 254 nm and the flow-rate was 0.5 ml/min. The column was a stainless-steel tube (250 × 4.6 mm I.D.), slurry-packed with Develosil ODS-5 (Nomura Chemical, Aichi, Japan).

Mobile phase

Acetate buffer (0.1 M) was prepared by dissolving 136 g of sodium acetate (trihydrate) and 5 ml of diethylamine in 900 ml of water, adjusting the pH to 6.2 with acetic acid and diluting to 1000 ml with water.

β-CyD was dissolved in a mixture of 0.1 M acetate buffer and ethanol.

RESULTS AND DISCUSSION

Selection of organic solvent in mobile phase

Usually acetonitrile, methanol or ethanol has been used as organic solvent in the mobile phase in reversed-phase HPLC, and in such a system the concentration of β-CyD in the mobile phase was very important. When the solubility of β-CyD in the mixture of organic solvent and acetate buffer was examined, it became clear that ethanol was suitable but acetonitrile and methanol were not, because β-CyD was soluble in a mixture of ethanol and the buffer, slightly soluble in a mixture of methanol and the buffer and insoluble in a mixture of acetonitrile and the buffer. As a result, it was decided to use ethanol as the organic solvent in the mobile phase.

Effect of diethylamine in mobile phase

When using a freshly packed column, the peaks showed tailing due to adsorp-

TABLE I

EFFECT OF THE CONCENTRATION OF β-CyD ON THE RESOLUTION (R_S) OF THE ENANTIOMERS

Column, Develosil ODS-5 (250 × 4.6 mm I.D.); flow-rate, 0.5 ml/min.

Mobile phase			*Retention time (min)*		R_S
β-CyD (g)	*0.1 M acetate buffer (ml)*	*Ethanol (ml)*	*S(+)-enantiomer*	*R(−)-enantiomer*	
0.3	63	37	32.2	32.5	0.23
0.5	65	35	35.1	35.6	0.32
0.7	67	33	38.1	39.0	0.62
1.0	69	31	43.4	45.1	0.91
2.0	73	27	36.6	38.6	1.24
4.0	82	18	39.6	43.0	1.81

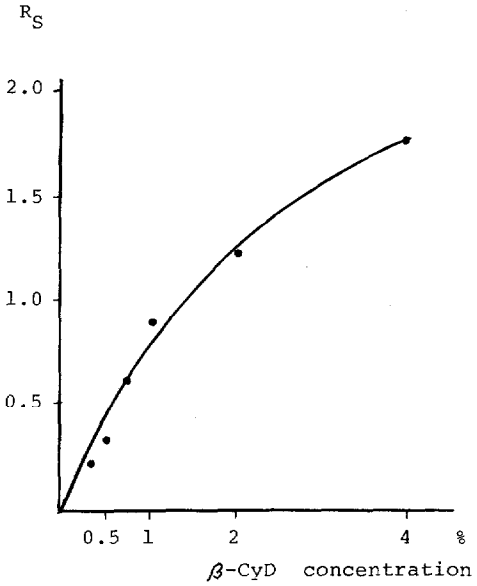

Fig. 2. Relationship between the concentration of β-CyD in the mobile phase and the resolution (R_S) of the enantiomers.

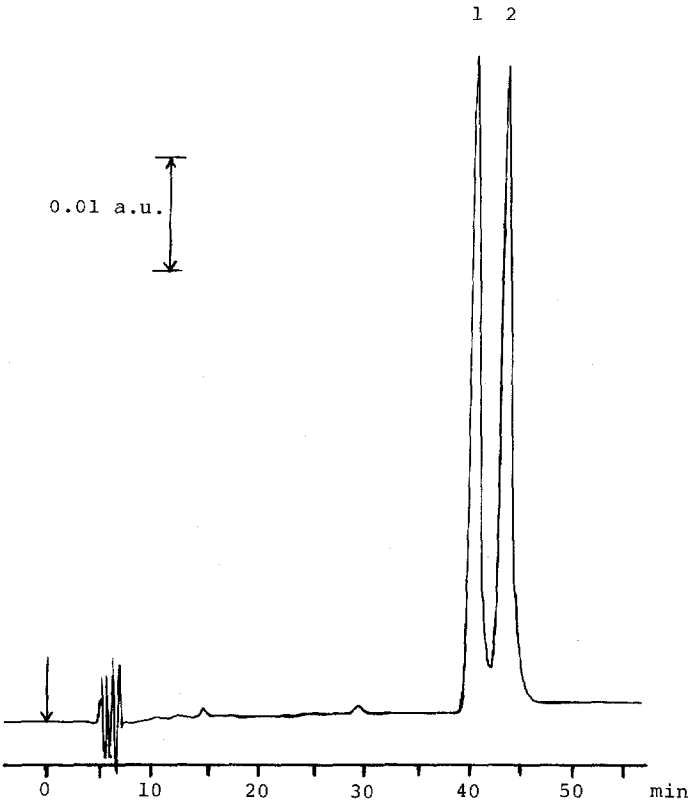

Fig. 3. Chromatogram of the enantiomeric resolution of 1-[2-(3-hydroxyphenyl)-1-phenylethyl]-4-(3-methyl-2-butenyl)piperazine. Column, Develosil ODS-5 (250 × 4.6 mm I.D.); mobile phase, 0.1 *M* acetate buffer–ethanol–β-CyD (82:12:4); flow-rate, 0.5 ml/min. 1 = *S*(+)-Enantiomer (15 μg); 2 = *R*(−)-enantiomer (15 μg).

tion of the stationary phase and the reproducibility of the chromatogram was unsatisfactory. On adding diethylamine to the mobile phase, the peaks became sharp and the reproducibility of the chromatogram was improved. It was therefore decided to add diethylamine to the mobile phase.

Effect of β-CyD concentration

The effect of the concentration of β-CyD in the mobile phase on the resolution of the enantiomers is shown in Table I and Fig. 2; the retention times were varied from 35 to 45 min by adjusting the ratio of ethanol and buffer in the mobile phase.

With increasing concentration of β-CyD the resolution of the enantiomers increased, and when the concentration was more than 4% the separation of the *R*(−)- and *S*(+)-enantiomers was complete. The chromatogram is shown in Fig. 3.

Consequently, the concentration of β-CyD is very important for the resolution of the enantiomers. It is thought that each enantiomer forms completely an inclusion complex on adding a large amount of β-CyD to the mobile phase and the two inclusion complexes have different capacity factors for each other. If the concentration of β-CyD is too low, the formation of the inclusion complex is incomplete and the resolution of the enantiomers is unsatisfactory.

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REFERENCES

1 H. Nakamura, K. Ishii, Y. Yokoyama, S. Motoyoshi, K. Natsuka and M. Shimizu, *J. Pharm. Pharmacol.*, 32 (1980) 635.
2 K. Sugden, C. Hunter and G. Lloyd-Jones, *J. Chromatogr.*, 192 (1980) 228.
3 G. Gübitz, W. Jellenz and W. Santi, *J. Chromatogr.*, 203 (1981) 377.
4 J. Boué, R. Audebert and C. Quivoron, *J. Chromatogr.*, 204 (1981) 185.
5 N. Watanabe, H. Ohzeki and E. Niki, *J. Chromatogr.*, 216 (1981) 406.
6 F. Mikeš and G. Boshart, *J. Chromatogr.*, 149 (1978) 455.
7 H. Yuki, Y. Okamoto and I. Okamoto, *J. Amer. Chem. Soc.*, 102 (1980) 6356.
8 W. H. Pirkle, J. M. Finn, J. L. Schreiner and B. C. Hamper, *J. Amer. Chem. Soc.*, 103 (1981) 3964.
9 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 46 (1981) 2935.
10 J. Debowski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 237 (1982) 303.
11 W. Lindner, J. N. LePage, G. Davies, D. E. Seitz and B. L. Karger, *J. Chromatogr.*, 185 (1979) 323.
12 Y. Tapuhi, N. Miller and B. L. Karger, *J. Chromatogr.*, 205 (1981) 325.
13 S. K. Lam, *J. Chromatogr.*, 234 (1982) 485.
14 E. Gil-Av, A. Tishbee and P. E. Hare, *J. Amer. Chem. Soc.*, 102 (1980) 5115.
15 C. Gilon, R. Leshem and E. Grushka, *Anal. Chem.*, 52 (1980) 1206.
16 C. Gilon, R. Leshem and E. Grushka, *J. Chromatogr.*, 203 (1981) 365.
17 N. Nimura, T. Suzuki, Y. Kasahara and T. Kinoshita, *Anal. Chem.*, 53 (1981) 1380.