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Note

Enantiomeric purity determination of L-proline benzyl ester by chiral column gas chromatography

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The L enantiomer of proline benzyl ester (compound I, Fig. 1) is an intermediate in the synthesis of an angiotensin-converting enzyme (ACE) inhibitor intended for the treatment of hypertension[1,2]. It is necessary to determine the enantiomeric purity of the intermediate to preserve the chiral purity of the final product. The connection between the drug–receptor interaction and drug chiral activity has been documented[3–5].

Gas chromatographic (GC) separation of enantiomers can be achieved by utilizing either chiral or achiral columns. The latter approach requires conversion of the enantiomer to a diastereomer by reacting it with a chiral reagent of specified enantiomeric purity. This procedure has several disadvantages[6–9] which include racemization of the reagent on storage. With the development of thermally stable, commercially available chiral capillary GC columns[10–14], the direct enantiomeric separation is now feasible.

EXPERIMENTAL

Gas chromatography

A Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector and an autosampler was used. The fused-silica chiral capillary column used was Chirasil-Val III, 25 m × 0.32 mm I.D. (Alltech). The inlet pressure of the helium carrier gas was 69 KPa (10 p.s.i.g.) while the flow-rate of the helium make-up gas for the flame ionization detector was 25 ml/min. The oven temperature was held isothermally at 150°C. Injections were made in the split mode at a split flow of 50 ml/min, using a empty split glass insert (Hewlett-Packard). The injector and detector temperatures were maintained at 250°C.

Reagents and chemicals

The L and D enantiomers of proline benzyl ester hydrochloride (L- and D-PBE, respectively) were characterized reference materials obtained from the department of Chemical Process Technology (E. R. Squibb & Sons). Methylene chloride, isopropanol, pyridine and isopropyl isocyanate were obtained from Aldrich. Isopropanol–hydrochloric acid solution (IPA–HCl) was prepared by reacting 20 ml of

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isopropanol with 2.8 ml of acetyl chloride (Alltech). The solution was kept refrigerated for no longer than two weeks.

Sample preparation

To approximately 3 mg of L-PBE, weighed into an autosampler vial, 1.0 ml of IPA–HCl was added. The vial contents, sealed with PTFE-lined cap, were vortexed and then reacted at 100°C for 30 min. The cooled vial was then uncapped and the reagent removed by evaporation at 40°C under a stream of nitrogen. To the residue in the vial, 100 μl of methylene chloride, 100 μl of isopropyl isocyanate and 5 μl of pyridine were added. After sealing and vortexing, the solution was kept at room temperature for 1 h. The vial was then uncapped and the reagent was removed by evaporation at 40°C, under nitrogen. The samples, reconstituted with 0.4 ml of methylene chloride, were placed into the autosampler after recapping the vials. A 1.0-μl aliquot was then injected.

Quantitation

The percentage of D-PBE in the L-PBE sample was calculated by area normalization of the two peaks of the enantiomers, assuming unity relative response for the enantiomer peaks.

RESULTS AND DISCUSSION

Underivatized, the L and D enantiomers eluted at 6.6 min but did not separate. The method developed is based on a two-step derivatization of PBE, transesterifica-

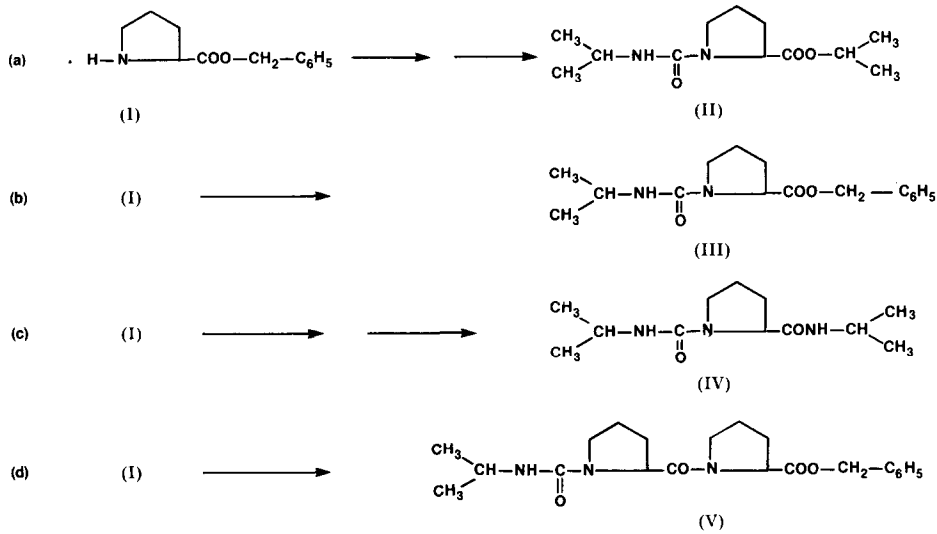

Fig. 1. Reactions of proline benzyl ester (compound I). (a) Compound I reacted with IPA–HCl and subsequently with isopropyl isocyanate to form compound II (refer to sample preparation). (b) Compound I reacted with isopropyl isocyanate at room temperature to form compound III. (c) Compound I reacted with isopropyl amine and subsequently with isopropyl isocyanate to form compound IV. (d) Compound I reacted with isopropyl isocyanate at 100°C to form compound V.

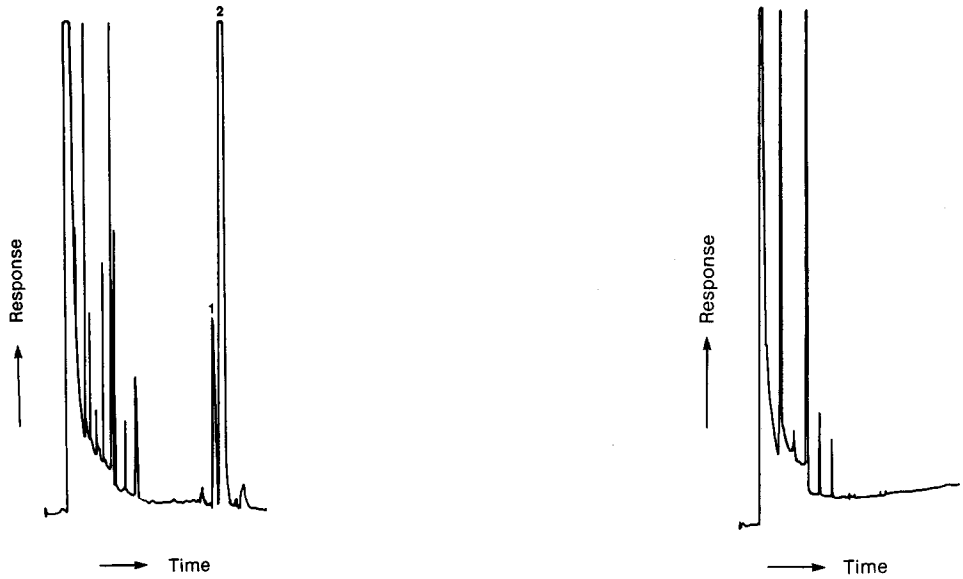

Fig. 2. Chromatogram of L-PBE spiked with 3.0% of D-PBE. Peaks: 1 = D enantiomer of compound II (10.2 min); 2 = L enantiomer of compound II (10.9 min).

Fig. 3. Chromatogram of a reagent blank, corresponding to Fig. 2, with no interfering peaks at the retention times of the enantiomers of compound II.

tion[15] of PBE to the isopropyl ester and subsequent reaction of the amino group to form the ureido derivative[13]. The resulting product (compound II, Figure 1a) was resolved into its enantiomers on a Chirasil-Val III capillary GC column (Figs. 2 and 3).

The accuracy of the method was established by analyzing L-PBE samples spiked with varying amounts of D-PBE. As shown in Table I, added D-PBE was quantitatively recovered. The precision of the method was excellent, with a coefficient of variation ($n = 6$) of 6.0% at the level of 0.50% D enantiomer and 2.0% at the level of 3.0% D enantiomer. The limit of detection was approximately 0.05% of the D enantiomer.

Various derivatization reactions were investigated. Reaction of compound I

TABLE I

RECOVERY OF D-PBE ADDED TO L-PBE

Each replicate represents a new sample preparation.

Added (%)	*Recovered (%)*	*Added (%)*	*Recovered (%)*
0.00	0.00	3.03	2.91
0.50	0.56	3.03	2.83
0.50	0.48	10.10	9.85
1.01	0.99	10.10	9.92
1.01	1.05		

TABLE II

IDENTIFICATION OF REACTION PRODUCTS OF BPE BY POSITIVE CHEMICAL IONIZATION MASS SPECTROMETRY

Compound	$[M+H]^+$	$[M+C_2H_5]^+$
II	243	271
III	291	319
IV	242	270
V	388	416

with isopropyl isocyanate at room temperature, in the presence of pyridine or triethylamine, yielded compound III (Fig. 1b). This derivative required a column temperature of 205°C to elute with the same retention time as compound II at 150°C. The separation between the enantiomers of compound III was only 0.2 min. Formed also as a side product of reaction 1a, described under sample preparation, compound III eluted much later than compound II at 150°C column temperature. Several 13-min chromatographic runs could be performed without encountering interferences from the elution of compound III from prior sample injections. After approximately every ten runs, at 150°C, accumulated compound III was removed from the column by heating it at 210°C for 15 to 20 min.

A third scheme investigated involved the reaction of compound I with 200 μl of isopropylamine at 100°C for 30 min[10] followed by reaction with 100 μl of isopropyl isocyanate at 100°C, for 30 min (Fig. 1c). The same compound (IV) was obtained when proline was reacted with isopropyl isocyanate at 100°C, for 30 min. Compound IV required a 20°C higher column temperature than compound II to elute with the same retention time. The separation between the L and D enantiomers of compound IV was excellent, and significantly better than that of the enantiomers of compound II. This derivatization scheme was not adopted because derivatized samples gave several extraneous peaks, including one at the retention time of the D enantiomer.

Reaction of compound I with isopropyl isocyanate, at 100°C, gave compound V (Fig. 1d), in addition to compound III. Compound V, however, has no practical application in the separation of the enantiomers because of its prolonged retention on the chiral column, operated near the maximum allowable temperature. The identities of compound V and the other reaction products were confirmed by positive chemical ionization mass spectrometry (Table II).

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REFERENCES

1 M. A. Ondetti, B. Rubin and D. W. Cushman, *Science (Washington, D.C.)*, 196 (1977) 441.
2 D. W. Cushman, H. S. Cheung, E. F. Sabo and M. A. Ondetti, *Biochemistry*, 16 (1977) 5484.
3 P. Jenner and B. Testa, *Drug Metab. Rev.*, 2 (1973) 117.
4 E. J. Ariens, *Eur. J. Clin. Pharmacol.*, 26 (1984) 663.

5 A. J. Hutt and J. Caldwell, *Clin. Pharmacokin.*, 9 (1984) 371.
6 W. A. König, *Chromatographia*, 9 (1976) 72.
7 C. Wiecek, B. Halpern, A. M. Sargeson and A. M. Duffield, *Org. Mass Spectrom.*, 14 (1979) 1981.
8 W. A. König, K. Steolting and K. Kruse, *Chromatographia*, 10 (1977) 444.
9 I. L. Payan, R. Cadilla-Perezrios and G. H. Fisher, *Anal. Biochem.*, 149 (1985) 484.
10 H. Frank, G. Nicholson and E. Bayer, *J. Chromatogr.*, 146 (1978) 197.
11 W. A. König, *J. High Resolut. Chromatogr., Chromatogr., Commun.*, 5 (588) 1982.
12 H. Frank, J. Gerhardt, G. J. Nicholson and E. Bayer, *J. Chromatogr.*, 270 (1983) 159.
13 W. A. König, I. Benecke, N. Lucht, E. Schmidt, J. Schulze and S. Sievers, *J. Chromatogr.*, 279 (1983) 555.
14 V. Schurig, *Angew. Chem., Int. Ed. Engl.*, 23 (1984) 747.
15 S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 97 (1974) 19.