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Note

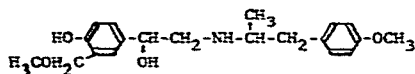
Separation of the diastereoisomers of 1-(4-hydroxy-3-methoxymethylphenyl)-2-N-(p-methoxyphenylisopropyl)aminoethanol by high-performance liquid chromatography

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1-(4-Hydroxy-3-methoxymethylphenyl)-2-N-(p-methoxyphenylisopropyl)ami-noethanol (PAA)[1], a new β-stimulant (I), has two asymmetric carbon atoms and the normal routes of synthesis produce a mixture of two racemic diastereoisomers, racemate A and racemate B. Since the β-stimulant activity of PAA mainly resides in racemate A, a method for the determination of racemate B present in racemate A is required for quality control purposes. Nuclear magnetic resonance spectroscopy (NMR) has been used for the analysis of the diastereoisomeric mixture of PAA, but this method has lacked sensitivity and precision.

PAA (I)

Gas–liquid chromatography and high-performance liquid chromatography (HPLC) are very efficient for the separation of diastereoisomers, and many applications of these techniques to quantitative analysis have been reported[2–6]. Selby and Munden[7], resolved diastereoisomers of labetalol, which is similar to PAA in structure, as the 5-N,N'-dimethylaminonaphthalene-1-sulphonyl (Dns) derivatives. However, this method is complicated and cannot easily be adapted to routine analysis.

Our preliminary experiments revealed that PAA was easily separated by HPLC using the benzyl derivatives and the results are now reported.

EXPERIMENTAL

Material and reagents

PAA was synthesized as described previously[1]. Benzyl chloride was purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan) and all other reagents were commercially available products of analytical grade.

Preparation of benzyl derivatives

To a solution of 0.1 g of PAA in 10 ml of ethanol was added 0.1 g of potassium carbonate and the mixture was heated at 60 °C for 20 min. After successive addition of 0.5 ml of benzyl chloride and 1.0 g of potassium iodide, the mixture was refluxed at 90 °C for 1 h. The solution was cooled, filtered and made up to exactly 10 ml with n-hexane. This solution was subjected to HPLC.

HPLC

The chromatographic system comprised a Model 110A pump (Altex, Berkeley, CA, U.S.A.) and a UVIDEC 100-II multi-wavelength detector (Japan Spectroscopic Co., Hachiōji, Japan). The column (250 × 2.1 mm I.D., stainless steel) was packed with Zorbax SIL (DuPont, Wilmington, DE, U.S.A.). The mobile phase was prepared by mixing n-hexane, methanol and 28% aqueous ammonia in the ratio 500:5:1.5 and drying the mixture on sodium sulphate. The flow-rate was maintained at 1.0 ml/min and the procedure was carried out at ambient temperature. Five microlitres of the sample solution were injected on the chromatographic column and the effluent was monitored at 254 nm.

RESULTS AND DISCUSSION

Diastereoisomers have previously been separated on conventional normal phase partition columns, but those of PAA could not be separated on a silica gel column. In an attempt to enhance the resolution by the introduction of bulky groups on asymmetric centres, derivatization by benzylation, benzoylation and 3,5-dinitrobenzoylation were investigated. Only the benzyl derivative of PAA could be separated on a silica gel column. The separation of benzylated diasteroisomers was examined using Nucleosil 50-5, LiChrospher SI-100 and Zorbax SIL as stationary phase and a mixture of n-hexane, methanol and 28% aqueous ammonia as a mobile phase. The best result was obtained by HPLC using the Zorbax SIL column with the solvent system n-hexane–methanol–28% aqueous ammonia (500:5:1.5).

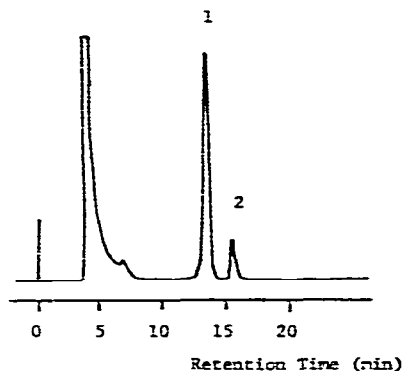Fig. 1 illustrates the chromatogram of the benzyl derivatives of PAA in which

Fig. 1. Separation of diastereoisomers of PPA on a Zorbax SIL column with n-hexane–methanol–28% aqueous ammonia (500:5:1.5) as mobile phase. Peaks 1 and 2 correspond to racemate A and racemate B, respectively.

each peak is baseline resolved (resolution factor, 1.25) and unreacted diastereo-isomers (retention time 8.5 min) are not observed. Components 1 and 2 shown in Fig. 1, which correspond to the benzyl derivatives of racemates A and B respectively, were isolated by HPLC and the structures were confirmed by mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR). MS of components 1 and 2 gave a molecular ion at m/e 507, which suggested the introduction of two benzyl groups. NMR of 1 and 2 showed CH_2 protons of 4-benzyloxyphenyl and N-benzyl-amino groups at δ 5.08 and 3.80 respectively, and the OH proton of CHOH groups at δ 4.80. Consequently, the structure shown in formula II was proposed for components 1 and 2.
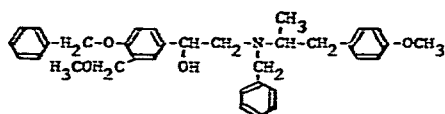
N,O-bis-(benzyl)-PAA (II)

Components 1 and 2 showed the same UV spectra and had identical molar absorptivities. Furthermore, standard calibration curves (plots of peak area *vs.* amount) were also identical for 1 and 2. These results suggest that the ratio of racemate B to racemate A can be calculated from the peak area ratio of component 2 to component 1. The minimum detectable quantity of racemate B in PAA was 0.1% and the standard deviation for the determination of racemate B in PAA was 0.32%.

This method could be simply and accurately used for the quality control of PAA.

ACKNOWLEDGEMENTS

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