Notes

A Chiral Axis Derivatization Reagent for the Resolution of β -Adrenergic Blockers by Liquid Chromatography with Fluorescence Detection

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The simultaneous determination of β -adrenergic blocker enantiomers in biological fluids is urgently needed for pharmacokinetic studies of the racemate. The commonly used method for the quantitation of enantiomers involves the formation of diastereomeric amides with a chiral derivatization reagent through their amino groups, followed by liquid chromatographic separation.^{1,2} This procedure, however, has disadvantages of less reactivity for β -adrenergic blockers having a bulky N-substituent, such as a *t*-butyl group, owing to a steric hindrance. In previous studies we proposed a novel derivatization method for forming diastereomers through the inherent secondary alcohol attached to the asymmetric center with chiral axis derivatization reagents.^{3,4} This note deals with the preparation of a highly sensitive derivatization reagent, (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide, having binaphthyl as a fluorophore and carbonyl cyanide as a reacting group toward the hydroxyl function (Fig. 1), and its applicability to the resolution of β -adrenergic blocker enantiomers by high performance liquid chromatography (HPLC) with fluorescence detection.

Ia : *aS*

b : *aS*, *aR*

Fig. 1 Structures of 2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanides.

Experimental

HPLC

The apparatus used for this work was a 510 solvent delivery system (Waters Chromatography Div., Millipore Co., Milford, MA, USA) equipped with a 650-10LC fluorescence spectrophotometer (excitation wavelength of 330 nm and emission wavelength of 420 nm; Hitachi Ltd., Tokyo). A Cosmosil 5SL (150 mm×4.6 mm i.d., 5 μ m; Nacalai Tesque Inc., Kyoto) column was used at ambient temperature.

Materials

(*aS*)-2'-Methoxy-1,1'-binaphthalene-2-carboxylic acid (optical purity >99.5%) and its racemate were prepared in these laboratories by a previously reported method.⁵ Optically active bufuralols were kindly donated by Roche Products Ltd. All other chemicals were of analytical-reagent grade.

Synthesis of 2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide

To a solution of (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (250 mg) in anhydrous benzene (10 ml) was added oxalyl chloride (1.5 ml); the solution was then refluxed for 10 h. The reaction mixture was evaporated down *in vacuo*. To a solution of the crystalline product in anhydrous benzene (10 ml) were added trimethylsilyl cyanide (1 ml) and ZnI₂ (1 mg); the mixture was then stirred at room temperature for 5 h. Recrystallization of the crude product from hexane/acetone gave (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide (180 mg) (Ia) as orange yellow needles. m.p. 143–146°C. IR $\nu_{\max}$ (KBr disc): 2225 (C $\equiv$ N), 1689 cm⁻¹ (C=O). Anal. Calcd for C₂₃H₁₅NO₂: C, 81.88; H, 4.48; N, 4.15. Found: C, 81.68; H, 4.42; N, 3.95. MS *m/z* (% relative intensity): 337 (M⁺, 100), 306 (M⁺–OCH₃, 21), 250 (M⁺–OCH₃–COCN, 5). In this manner, the

racemic carbonyl cyanide (Ib) was also prepared from the corresponding carboxylic acid. m.p. 170–172°C.

Preparation of diastereomeric esters from propranolol with racemic 2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide

To a solution of ($\pm$)-propranolol (10 mg) in acetonitrile (2 ml) were added Ib (6 mg) and triethylamine (100 μ l); the mixture was then stirred at room temperature for 5 h. The reaction mixture was concentrated *in vacuo* and the obtained crude product was subjected to column chromatography on silica gel (1 g). Elution with hexane/ethyl acetate (1:1, v/v) and recrystallization of the eluate from hexane/chloroform gave the desired diastereomers (6 mg) as colorless powders. m.p. 215–220°C. MS m/z (% rel. int.): 569 (M^+ , 0.5), 426 ($M^+ - C_{10}H_7O$, 1.2), 328 ($M^+ - C_{16}H_{19}NO$, 100). High resolution MS: 569.2582 ($C_{38}H_{35}NO_4$, required 569.2566), 328.1083 ($C_{22}H_{16}O_3$, required 328.1099).

Results and Discussion

In previous papers we reported the preparation of chiral axis derivatization reagents, (+)- and (–)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanides³, and their use for the separation and determination of propranolol enantiomers in plasma by HPLC with fluorescence detection.⁴ Unfortunately, the sensitivity of the resulting diastereomeric esters is still insufficient for the quantitation of β -adrenergic blockers at low levels in biological fluids. It is well known that the substitution of an electron-donating group, such as $-N(CH_3)_2$ and $-OCH_3$, on the aromatic nucleus could

enhance the fluorescence intensity. Accordingly, our initial effort was directed to the preparation of a chiral axis derivatization reagent having binaphthyl with a methoxyl group as a fluorophore and carbonyl cyanide as a characteristic group reactive toward the alcoholic hydroxyl function. (*aS*)-2'-Methoxy-1,1'-binaphthalene-2-carboxylic acid⁵ was treated with oxalyl chloride to form the corresponding acid chloride which, in turn, was transformed into the desired (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide (Ia) by an exchange reaction with trimethylsilyl cyanide in the presence of ZnI_2 in benzene. The optical purity of the chiral reagent was found to be over 99.5%, as judged by the usual criteria using (*R*)-menthol.⁵ The racemic chiral axis reagent (Ib) was also prepared in a similar fashion.

The applicability of this reagent to the derivatization of β -adrenergic blocker enantiomers was the examined (Fig. 2). Propranolol, one of the typical β -adrenergic blockers possessing an isopropylamino group, was subjected to a condensation reaction with the racemic reagent (Ib) in acetonitrile in the presence of triethylamine; the resulting esters were characterized by microchemical reactions and mass spectral analyses. The derivative was susceptible to hydrolysis with 3% KOH in aqueous methanol, readily providing the intact drug. In the electron impact ionization mass spectrum the derivative exhibited a molecular ion at m/z 569 and a base peak at m/z 328 derivable from methoxybinaphthalenecarboxylic acid which would be formed by a McLafferty rearrangement of the ester moiety. These results lent support to the assumption that a derivatization reaction would occur selectively at the secondary alcohol of the 1-amino-2-hydroxypropoxy

Fig. 2 Derivatization of β -adrenergic blocker enantiomers with (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide.

Fig. 3 Chromatograms of diastereomeric esters derived from β -adrenergic blocker enantiomers with (*aS*)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide. Conditions: column, Cosmosil 5SL (150 mm $\times$ 4.6 mm i.d., 5 μ m); mobile phase, a) 0.1% triethylamine in hexane/ethyl acetate (2:1, v/v), b) and c) 0.1% triethylamine in hexane/ethyl acetate (5:1, v/v); flow rate, 2 ml/min.

group in β -adrenergic blockers.

Separation of the resulting diastereomers was then performed by means of HPLC. It has been sufficiently substantiated that hydrogen bonding between an ester group of the solute and silanol residues of the stationary phase is effective for the efficient separation of the diastereomers. The diastereomeric esters formed from β -adrenergic blockers with the chiral reagent exhibited leading and/or tailing of peaks on the chromatogram owing to the secondary amino residue. Therefore, the use of a normal phase column combined with hexane/ethyl acetate/triethylamine was undertaken.

Condensation of β -adrenergic blockers with the chiral reagent (1a) was effected at 60°C for 20 min in acetonitrile in the presence of 0.1% quinuclidine. The reaction mixture in 90% ethanol was passed through a column (18 mm $\times$ 6 mm i.d.) packed with 100 mg of lipophilic cation exchange gel (carboxymethyl Sephadex LH-20 (CM-LH-20)) in order to remove excess reagents; the desired diastereomeric esters were then eluted with 0.1 M methylamine in 90% ethanol.⁴ The dried eluate was redissolved in ethyl acetate, an aliquot of which was injected into the HPLC system. No racemization of the product and chiral reagent occurred during the derivatization process. As illustrated in Fig. 3, three pairs of diastereomeric esters formed from propranolol, bufuralol and penbutolol were distinctly resolved on a Cosmosil 5SL column with 0.1% triethylamine in hexane/ethyl acetate as mobile phases, respectively. The yielded esters were highly responsive to a

fluorescence detector (excitation wavelength 330 nm; emission wavelength 420 nm), the limit of detection being 100 fmol (at a signal-to-noise ratio of 5).

The newly developed derivatization reagent is of great use for the resolution of β -adrenergic blocker enantiomers by HPLC. Applications of the present method to the determination of these drugs in biological fluids are being conducted; the details will be reported elsewhere.

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