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Short communication

Stereoselective separation of β-adrenergic blocking agents containing two chiral centers by countercurrent chromatography

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ABSTRACT

Four β-adrenergic blocking agents, including 1-[(1-methylethyl)amino]-3-phenoxy-2-propanol (1), 1-[(1-methylethyl)amino]-3-(3-methylphenoxy)-2-propanol (2), 1,1′-[1,4-phenylenebis(oxy)] bis[3-[(1-methylethyl)amino]-2-propanol (3) and 1,1′-[(4-methyl-1,2-phenylene)bis(oxy)]bis[3-[(1-methylethyl)amino]-2-propanol (4), were stereoselectively separated by countercurrent chromatography using di-*n*-hexyl L-tartrate and boric acid as chiral selector. The compounds (3) and (4) have four optical isomers since they contained two chiral centers. A two-phase solvent system composed of chloroform-0.05 mol L^{-1} of acetate buffer containing 0.10 mol L^{-1} of boric acid (1:1, v/v) was selected, in which 0.10 mol L^{-1} of di-*n*-hexyl L-tartrate was added in the organic phase as chiral selector. 20–42 mg of each racemate was stereoselectively separated by countercurrent chromatography in a single run with high purity of 96–98%, and the recovery of each separated compound reached around 87–93%. This is the first time report on successful stereoselective separation of optical isomeric compounds containing two chiral centers by countercurrent chromatography. At the same time, a chiral stationary phase was screened for analytical stereoselective separation of compounds (3) and (4) by high performance liquid chromatography.

1. Introduction

β-Adrenergic blocking agents have been widely used for the treatment of hypertension for the past 50 years, and continue to be recommended as a mainstay of therapy in many national guidelines. They have also been used in a variety of cardiovascular conditions commonly complicating hypertension, including angina pectoris, myocardial infarction, acute and chronic heart failure, as well as conditions like essential tremor and migraine [1,2]. Most of the β-adrenergic blocking agents have one chiral carbon and so they have at least two optical isomers. As shown in Fig. 1, compound (1) and (2) are β-adrenergic blocking agents with one chiral center. Compound (2) was also known under the name of (±)-toliprolol. In order to investigate the structure-activity relationship, two additional β-adrenergic blocking agents containing two chiral centers, compound (3) and compound (4), were synthesized in our lab. Big difference in pharmacologic activities might be found between enantiomers and diastereoisomers due to the stereospecific characteristics of chemical structures, which necessitate a method for preparative stereoselective separation.

No literature about preparative stereoselective separation of β-adrenergic blocking agents containing two chiral centers are available, though quite a few of literature could be found which is about analytical enantioseparation of β-blockers with one chiral center [3–9]. In our previous work, countercurrent chromatography and pH-zone-refining countercurrent chromatography were successfully used for enantioseparation of three β-adrenergic blocking agents containing one chiral centers, propranolol, pindolol and alprenolol, based on borate coordination complex when di-*n*-hexyl L-tartrate was selected as chiral ligand [10]. Herein we want to report our recent study on stereoselective separation of β-adrenergic blocking agents with two chiral centers by countercurrent chromatography. Meanwhile, a chiral column was selected for analytical stereoselective separation of the two synthesized β-adrenergic blocking agents containing two chiral centers.

Drugs with more than one chiral center are of high medicinal values. However, the stereoselective separation of such type of racemate by various kinds of chromatographic technique is still a very challenging task. There is a limited number of scientific reports about stereoselective separation of racemate with more than one

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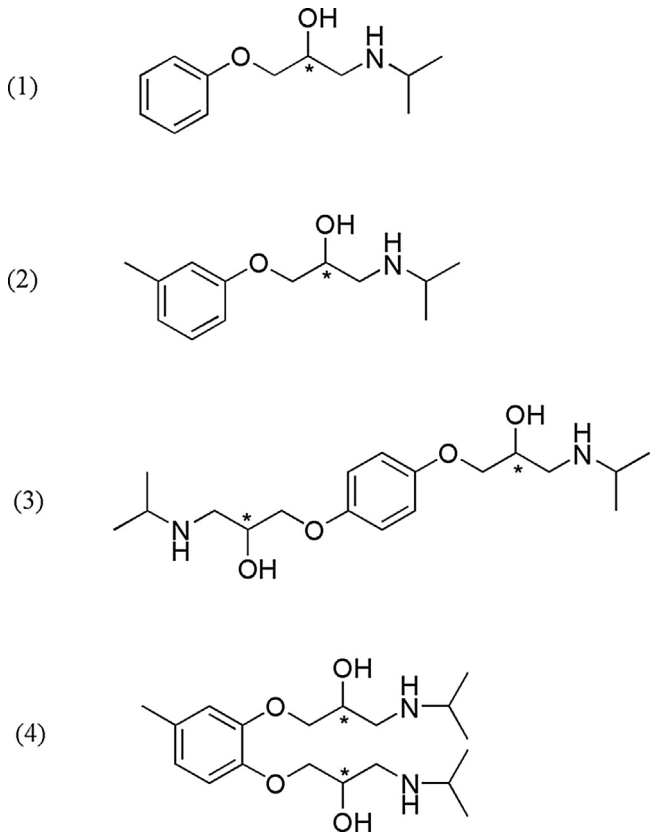

Fig. 1. Chemical structures of four synthesized β-adrenergic blocking agents.
(1) 1-[(1-methylethyl)amino]-3-phenoxy-2-propanol.
(2) 1-[(1-methylethyl)amino]-3-(3-methylphenoxy)-2-propanol.
(3) 1,1′-[1,4-phenylenebis(oxy)]bis[3-[(1-methylethyl)amino]-2-propanol.
(4) 1,1′-[(4-methyl-1,2-phenylene)bis(oxy)]bis[3-[(1-methylethyl)amino]-2-propanol.

chiral center by chromatography and capillary electrophoresis [11–17]. Countercurrent chromatography has been widely used for separation of chemical components from natural products [18]. However, much smaller number of literature concerning enantioseparations and stereoselective separations by countercurrent chromatography is available compared with traditional separation methods because it is difficult to find a suitable biphasic solvent system along with a chiral selector with high enantiorecognition [19]. All the applications of countercurrent chromatography in stereoselective separations in the past decades are about enantioseparation of racemates with one chiral center. Therefore, this is the first time report on successful stereoselective separation of racemates with two chiral centers by countercurrent chromatography.

2. Experimental section

2.1. Apparatus

A model of TBE-200V preparative multilayer coil planet centrifuges (Shanghai Tauto Biotechnique, Shanghai, China) was used in the present work, each equipped with a set of three multilayer coils. The parameters for this apparatus have been described in our previous literature [20]. The separation columns were installed in a vessel that maintains column temperature by a model SDC-6 constant-temperature controller (Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China). The solvents were pumped into the column with a model TBP 5002 constant-flow pump (Shanghai Tauto Biotechnique, Shanghai, China). Continuous monitoring of the effluent was achieved with a model UVD-200 detector (Shanghai Jinda Biotechnology Co., Ltd., Shanghai, China), and SEPU3000 workstation (Hangzhou Puhui Technology, Hangzhou, China) was employed to record the chromatogram.

The high performance liquid chromatography (HPLC) used was a CLASS-VP Ver.6.1 system (Shimadzu, Japan) comprised of a Shimadzu SPD10Avp UV detector, a Shimadzu LC-10ATvp Multisolvent Delivery System, a Shimadzu SCL-10Avp controller, a Shimadzu LC pump, and a CLASS-VP Ver.6.1 workstation.

The pH value was determined with a PB-10 pH meter (Sartorius, Germany).

2.2. Reagents

L-Tartaric acid was purchased from Lanxi Shengda tartaric acid limited company, Zhejiang, China. Glycidyl phenyl ether and potassium hexafluorophosphate were purchased from J&K chemical scientific Co., Ltd, Shanghai, China. Hydroquinone, *m*-cresol, 4-methylcatechol, epichlorohydrin, isopropyl amine, triethylbenzylammonium chloride (TEBA) and boric acid were purchased from Huipu Chemical, Hangzhou, China. All organic solvents used for countercurrent chromatography were of analytical grade. Acetonitrile, methyl *tert*-butyl ether, *n*-hexane and ethanol used for HPLC analysis were of chromatographic grade. *n*-Butyl L-tartrate, *iso*butyl L-tartrate, *n*-hexyl L-tartrate, *n*-octyl L-tartrate and *iso*octyl L-tartrate were prepared according to the literature [21], and their structures were confirmed by ^{1}H NMR.

2.3. Preparation of β-adrenergic blocking agents

Compounds (1)–(4) were prepared according to the literature [22] and their chemical structures were determined by ^{1}H NMR.

Compound **(1)**: A solution of 2 g (13 mmol) of glycidyl phenyl ether and 3 mL (35 mmol) of isopropyl amine was stirred and refluxed at 50 °C for 10 h. Excess amine was evaporated under reduced pressure, and it was further purified by column chromatography (dichloromethane: methanol = 95:5, v/v), yielding 2.15 g (79.13%) of compounds (1).

Compound **(2)**: A solution of 7.56 g (0.19 mol) of sodium hydroxide dissolved in 20 mL of water and 5 mL (0.048 mol) of *m*-cresol was stirred at room temperature for 40 min, and 0.5 g of triethylbenzylammonium chloride (TEBA) was added as phase transfer catalyst in the mixture and 15.04 mL of (0.192 mol) epichlorohydrin was added in 30 min. After stirring at room temperature for 5 h, the aqueous layer was extracted twice with ether, and the combined organic layers were washed, concentrated and purified by silica column chromatography (ethyl acetate: petroleum ether = 1:24, v/v), yielding 2.5 g (31.76%) of intermediate product. A solution of 2.5 g (15 mmol) of intermediate and 3 mL (35 mmol) of isopropyl amine was stirred and refluxed at 50 °C for 14 h. Excess amine was evaporated under reduced pressure, and it was further purified by silica column chromatography (dichloromethane: methanol = 95:5, v/v), yielding 1.1645 g (34.26%) of compounds (2).

Compound **(3)**: A solution of 11.0 g (0.1 mol) of hydroquinone, 37.0 g (0.4 mol) of epichlorohydrin, and 0.4 mL of 10 mol L^{-1} sodium hydroxide was stirred under nitrogen gas under 40 °C for 48 h. After cooling, 42.0 mL of 5 mol L^{-1} sodium hydroxide, saturated with sodium carbonate, was added and the mixture stirred vigorously at room temperature for 20 h. The aqueous layer was extracted twice with chloroform and the combined organic layers were washed, concentrated and purified by silica column chromatography (ethyl acetate: petroleum ether = 1:6, v/v), yielding 0.6 g (2.70%) of intermediate product. A solution of 0.6 g (2.7 mmol) intermediate, 4 mL (50 mmol) of isopropyl amine and 20 mL of absolute ethanol was stirred and refluxed at 50 °C for 8 h. Excess amine was evaporated under reduced pressure, and it was further purified by silica column