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The 6-derivative of β-cyclodextrin with succinic acid: a new chiral selector for CD-EKC

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Abstract

6-*O*-Succinil-β-cyclodextrin (CDsuc6) was synthesized with very good yield by one pot synthesis and characterized by NMR spectroscopy and ESI-MS. It was used as a chiral selector in capillary electrophoresis to resolve catecholamine racemates, namely norepinephrine, epinephrine, terbutaline and norphenilephrine.

The CE experiments at pH 5.6 show very promising selector ability by 6-*O*-succinil-β-cyclodextrin for the chiral recognition of all the catecholamines tested, while at pH 9.2, only racemic terbutaline was successfully separated.

Keywords: Cyclodextrin; NMR; Capillary electrophoresis; Chiral separations; Electrokinetic chromatography; Catecholamines

1. Introduction

Over the last few years, a series of derivatives of β-cyclodextrins showing very good properties both as achiral and chiral selectors have been synthesised [1–10]. In particular, in our laboratory the synthesized compounds include amino groups in the substituting moiety [2–10]. Thus, they show affinity for both protons and several metal ions [2–10]. We have exploited the coordinating ability of some of them both in ligand exchange chromatography (LEC) [2–11] and, more recently, in ligand exchange capillary electrophoresis (LECE) [11–14]. Furthermore, also in the absence of metal ions, some have shown very good properties as chiral selectors in electrokinetic chromatography by cyclodextrins (CD-EKC) [15–18]. Nonetheless, if used in alkaline BGE, they are neutral and so the free analyte and its complex have the same charge and thus cannot differ so sharply in their electrophoretic mobility, making the separation less easy: worse, if the analyte is neutral, obviously, no separation at all can occur. If, on the other hand, we use selectors with amino groups at lower pH, they become cationic and well suited for separating both neutral and anionic analytes: in every case, a change in the electrical charge will occur between the free analyte and its complex with the selector. However, in the case of cationic analytes, while even in this case a change of electrical charge occurs, the electrostatic repulsion between selector and analytes, both cationic, will make the stability of complexes low, preventing the possibility of successful separation. In order to extend the applicability of our cyclodextrin selectors to cationic analytes, we have synthesised a new cyclodextrin derivative, which, bearing a carboxylic function in the substituting moiety, can give rise to anionic species in BGE. Further, the anionic species in capillary electrophoresis have an additional advantage: as a consequence of their charge, they have a lesser tendency to interact with the capillary wall and rinsing between runs can be minimised producing a significant saving of time.

Catecholamines are an important class of drugs. They are involved in a variety of regulatory systems in our body, and analytical procedures of their quantitation in tissues and

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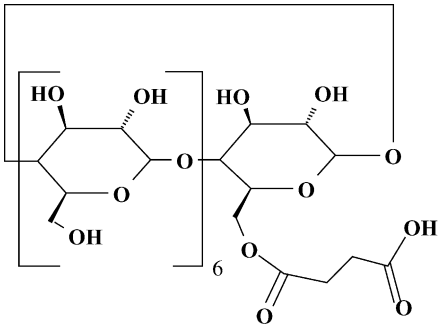

Chart 1.

in body fluids have been developed [19]. Besides the chromatographic methods, more recently, capillary electrophoresis methods have also been developed [20–22]. Since it is well known that biological activity strongly depends on the chirality of the involved substance, it is important to separately quantify the two enantiomers [23,24].

Here, we report the synthesis of 6-*O*-succinil-β-cyclodextrin (CDsuc6), the schematic formula of which is reported in Chart 1, its identity was confirmed by ESI-MS and NMR spectroscopy, and its use is as a chiral selector in capillary electrophoresis towards some catecholamines racemates, namely norepinephrine, epinephrine, terbutaline and norphenilephrine (see Fig. 1).

2. Experimental

2.1. Materials

The racemic mixtures of catacholamines: epinephrine and terbutaline (Fig. 1) were purchased from Sigma and norepinephrine, norphenilephrine (Fig. 1) and β-cyclodextrin were purchased from Fluka. Anhydrous *N*,*N*-dimethylformamide was purchased from Aldrich. β-Cyclodextrin was dried in vacuo ($\sim 10^{-2}$ mmHg) for 24 h at 80 °C by using a P_2O_5 trap. Thin layer chromatography (TLC) was carried out on silica gel plates (Merck 60-F254). CD derivatives were detected on TLC by UV and by the anisaldehyde test. CDsuc6 was synthesised in our laboratory. Double-distilled water was used for solution preparation.

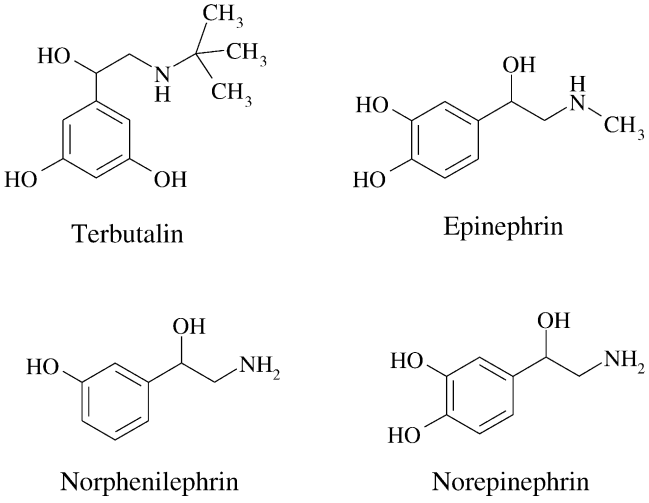

Fig. 1. Molecular formulas of the investigated catecholamines.

2.2. Synthesis of 6-*O*-succinil-β-cyclodextrin (CDsuc6)

A solution of succinic acid (100 mg) and of carbonyldiimidazole (cdim, 138 mg) in anhydrous DMF (1 ml) was stirred for 15′ at r.t. After this time, dried β-cyclodextrin (970 mg) was added and the reaction was carried out under stirring. After 3 h, the solvent was evaporated to dryness in vacuo, and the solid obtained was purified by elution from a column (35 mm × 600 mm) of DEAE-Sephadex C-25 (HCO_3^- form) eluting with water (1 l), then with a 0–0.2 M NH_4HCO_3 linear gradient (total volume 1 l). The appropriate fractions were concentrated to give CDsuc6, R.f. = 0.35 (5/1/3/2 PrOH/AcOEt/H_2O/NH_3), yield: 40%. ESI-MS *m*/*e* 1234 ($M-1$).

^{1}H NMR (D_20, 500 MHz) δ (ppm): 2.44 (t, 2H, CH_2 suc in α to COOH), 2.57 (t, 2H, other CH_2 suc), 3.43–3.56 (m, 13H, H-2, -4), 3.59 (dd, 1H, H-2), 3.07 (m, 25H, H-5, -6-3), 3.97 (m, 1H, H-5A $J_{5A,4A}$ = 9.8 Hz, $J_{5A,6A}$ = 4.5 Hz), 4.18 (dd, 1H, H-6aA, $J_{6A,5A}$ = 4.5 Hz, $J_{6aA,6bA}$ = 11.5 Hz), 4.43 (d, 1H, H-6bA, $J_{6aA,6bB}$ = 11.5 Hz), 4.97 (m, 7H, H-1); ^{13}C NMR (D_20, 125 MHz): 179.2 (COO), 175.1 (COO), 102.1 (C-1), 81.4 (C-4), 73.3 (C-3), 72.3 (C-2), 72.0 (C-5), 69.80 (C-5A), 64.1 (C-6A), 60.5 (C-6), 31.0 (*C*H_2 suc in β to COOH), 30.1 (other *C*H_2 suc).

2.3. NMR

NMR spectra were recorded at 25 °C in D_2O with a Varian Inova 500 spectrometer ^{1}H at 499.88 MHz and ^{13}C at 125.70 MHz. The NMR spectra were measured by using standard pulse programs from the Varian library. In the case of ^{1}H the length of the 90° pulse was c.a. 7 μs. 2-D experiments were acquired using 1 K data points, 256 increments and a relaxation delay of 1.2 s. T-ROESY spectra were obtained using a 300 ms spin-lock time. DSS was used as the external standard.

2.4. CE measurements

CD-EKC measurements were carried out on a Beckman P/ACE MDQ equipped with a diode array detector. An uncoated fused-silica capillary (Beckman; 60 cm total length, 49 cm effective length, 75 mm i.d.) was held at a constant temperature of 25 °C. The system operated at a constant voltage of 25 kV.

BGEs for the chiral separation experiments were prepared by dissolving CDSuc6 (2.0–8.0 mM) in 10.0 mM acetic buffer, (pH 5.6). The sample solution (0.1 mM for