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

Vincenzo Cucinotta*, Alessandro Giuffrida, Giuseppe Maccarrone, Marianna Messina, Antonino Puglisi, Alberto Torrisi, Graziella Vecchio

Department of Chemical Sciences, University of Catania, Viale A.Doria 6, 95125 Catania, Italy

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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.
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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

* Corresponding author. Tel.: +39 095 7385094; fax: +39 095 580138.
E-mail address: ecucinotta@unict.it (V. Cucinotta).

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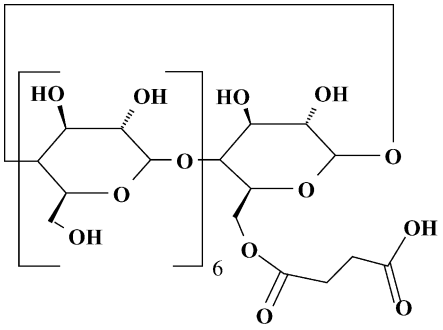


Chart 1.

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.

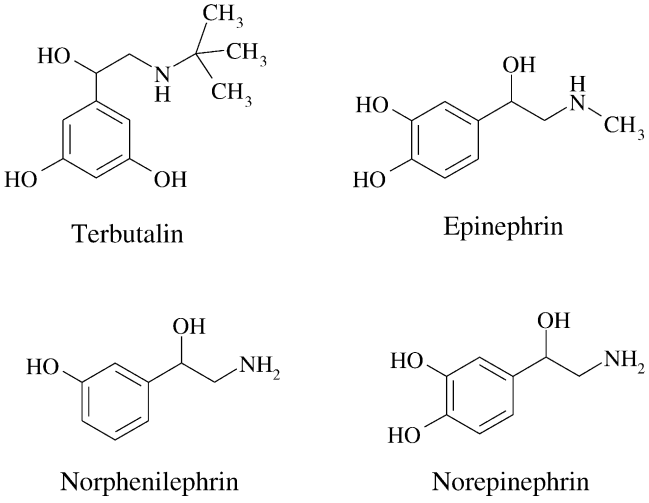


Fig. 1. Molecular formulas of the investigated catecholamines.

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

epinephrine and 0.2 mM for the other analytes) was obtained by dissolving the analyte in the same buffer.

The samples were hydrodynamically injected (0.5 psi, for 5 s). Before each experiment, the capillary was successively flushed (pressure of 2.0106 Pa) with 0.1 M NaOH, 10 mM acetic buffer, and the BGE used in separation.

The enantiomeric resolution (R) and selectivity coefficient (S) were calculated using Eqs. (1) and (2), respectively:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (1)$$

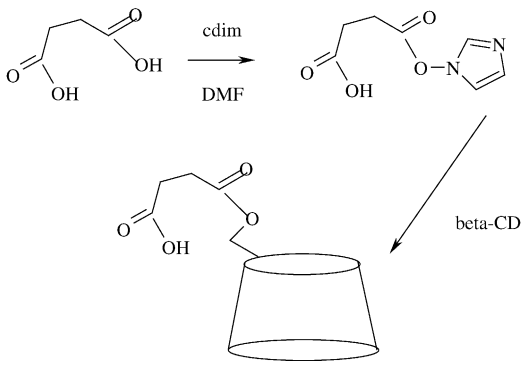
$$S = \frac{2(\mu_2 - \mu_1)}{\mu_1 + \mu_2} \quad (2)$$

where t, w and μ are respectively: migration times, widths at the baseline and the electrophoretic mobilities. Resolution values lower than 1 were reported in the tables, as an estimation of the potential separation capability. However, in our opinion, any value lower than the unity means that the quantitative separation was not obtained, despite how much it is specifically lower than unity is. Furthermore, it is not always obvious how this value can be calculated. In many cases, the usual way to measure the peak width by finding the intersection point between the baseline and the tangent in the peak inflection point can give erroneous results, owing to necessary extrapolation of both the peak and the baseline.

3. Results and discussion

The CDsuc6 was characterized by NMR spectroscopy (COSY, TOCSY, T-ROESY, HSQC, APT) and ESI-MS. The notation system adopted is that in which the glucose rings are named A, B, C, D, E, F, and G considered counter clockwise and viewed from the primary hydroxyl side. A is the functionalized ring.

Various monofunctionalized cyclodextrins have been synthesized starting from 6-*O*-tosyl-CD by nucleophilic substitution reaction [2,25]. In this paper, a 6-derivative, the CDsuc6 was synthesized by a one-pot strategy in a very good yield starting from β-cyclodextrin by the reaction with an imidazole activated ester of the succinic acid (Scheme 1). A



Scheme 1.

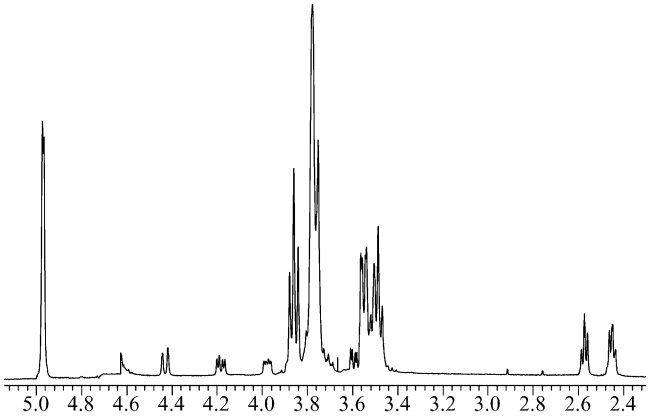


Fig. 2. ^{1}H NMR (D_2O, 500 MHz) spectrum of CDsuc6.

similar strategy has been reported in the literature but in that case the formation of 2- and 3- derivatives is reported [26] probably due to the presence of an organic base in the reaction mixture.

The NMR spectra of the CDsuc6 clearly show the monofunctionalization on the primary site. In the ^{1}H NMR spectra (Fig. 2), we observe the protons due to the functionalized ring in addition to the signals owing to the unfunctionalized glucopyranosinic ring. At 4.43 and 4.18 ppm, there are respectively the diastereotopic H-6As and at 3.97 ppm the H-5A, which are down-field shifted as a consequence of the functionalization with the carboxylic group, as typically found for similar derivatives [27,28]. At higher fields the two triplet signals due to the ethylenic chain are evident. On the COSY (Fig. 3) spectra the assignment of these signals is evident. On the T-ROESY spectra, correlation peaks between the ethylenic chain of the succynate moiety and a proton at

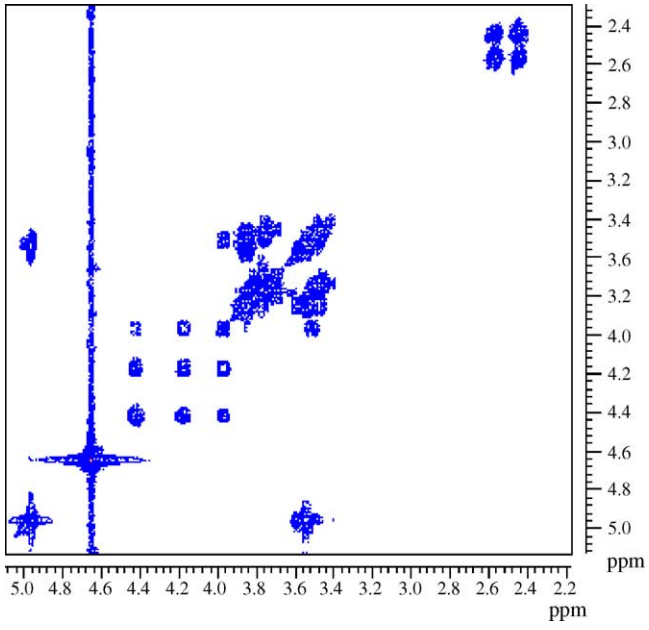


Fig. 3. COSY (D_2O, 500 MHz) spectra of CDsuc6.

Table 1
EKC results concerning experiments carried out at pH 5.6

C_{host} (mM), pH 5.6	Epinephrine			Norepinephrine			Norfenilephrine			Terbutaline		
	t_2	S	R	t_2	S	R	t_2	S	R	t_2	S	R
2.0	6.28	0	0	4.23	0.5	0.15	5.61	0.7	0.42	6.94	1.7	0.61
3.0	6.01	0	0	4.51	0.9	0.50	5.61	0.9	0.59	7.48	2.3	1.00
5.0	6.95	0	0	6.71	0.7	0.48	6.61	1.5	0.95	8.98	2.8	1.16
8.0	10.04	1.5	0.88	9.47	2.2	1.20	9.46	3.1	1.81	15.98	5.8	1.70

t_2: The migration time of the slower enantiomer, S values were multiplied by 100.

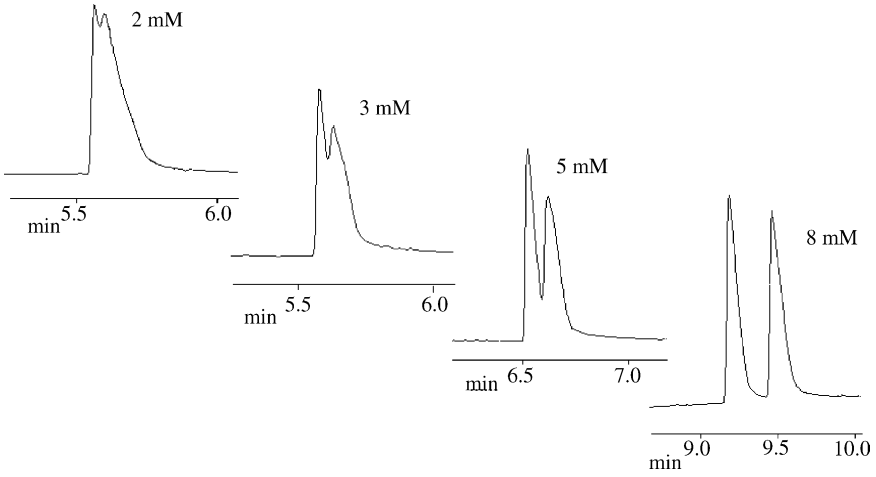


Fig. 4. Electropherograms of norphenilephrine racemate in the presence of CDsuc6 at pH 5.6.

3.76 ppm are found. These can be ascribed to the closeness of the substituting chain to the B or G glucopyranosinic ring. On the ^{13}C NMR a peak due to C-6A carbon is observed at 64 ppm, which shows cross peaks with the H-6As on the HSQC spectra and a peak due to the C-5A is observed at 68.9 ppm, confirming the 6-substitution.

The ability of CDsuc6 as chiral selector by electrokinetic chromatography was tested on four different catecholamine racemates. The results obtained at acidic pH are summarised in Table 1. Examples of electropherograms are reported in Figs. 4 and 5. Four different concentrations of CDsuc6 were used. In all the investigated systems, an improvement in selectivity is observed on increasing the concentration of the selector.

In chiral EKC, the separation is due to the formation of diastereoisomeric complexes that each of the enantiomers forms with the selector. While it cannot be excluded that in some systems separation simply occurs due to differences in their intrinsic electrophoretic mobilities, in most cases separation occurs if the degree formation of the complexes significantly differ from each other. Furthermore, the formation degrees of the complexes must be neither too low, nor too

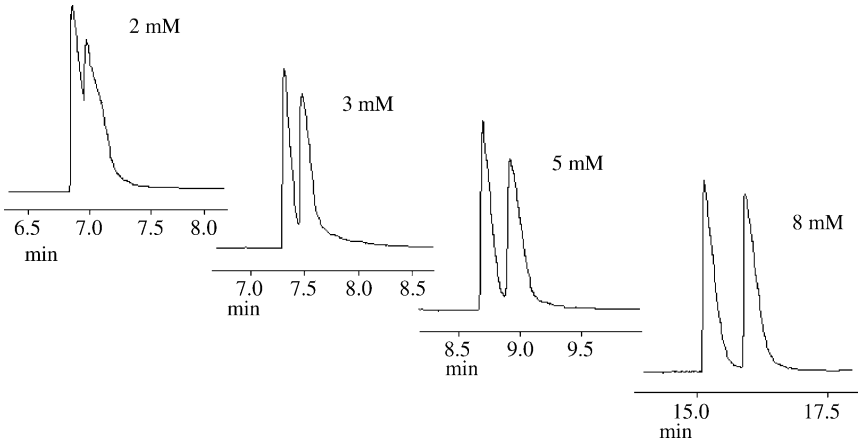


Fig. 5. Electropherograms of terbutaline racemate in the presence of CDsuc6 at pH 5.6.

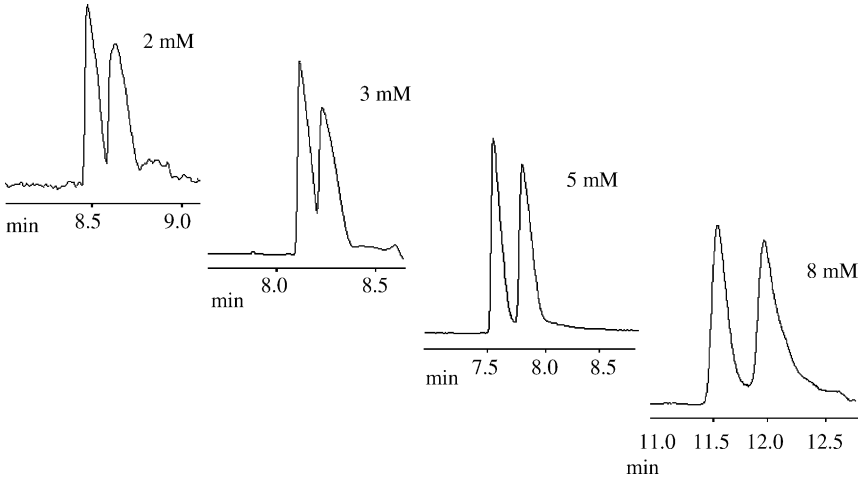


Fig. 6. Electropherograms of terbutaline racemate in the presence of CDsuc6 at pH 9.2.

high, just to assure that the mean migration times that we observe in this kind of experiment will differ significantly. In our case, apparently the complexes are not very stable and, when we increase the selector concentration, the selectivity increases too, and this is valid for all the investigated systems. In the case of epinephrine, the effect is more evident, since separation can only be obtained at the maximum concentration used.

Similar experiments were carried out at alkaline pH too: in this case, however, almost no separation was obtained, with the only exception of terbutaline at the maximum selector concentration, as shown on Table 2, where results concerning the racemates not separated were omitted. We must again take into consideration the formation degree of the involved complexes selector-analyte. It is readily apparent that at this pH, formation degrees are much lower than in acidic pH. The reason for this lowering of stability can likely be ascribed to the fact that at pH 9.2 the catecholamines are present almost exclusively as neutral species. Only terbutaline, probably owing to its methyl groups bonded to the amino nitrogen, gives rise to a complex with the selector, which has a formation constant sufficient to warrant the separation (Fig. 6).

The analysis of the migration times agrees with the hypothesis of low formation degrees of the selector-analyte complexes. While an increase of t(EOF) when increasing the selector concentration is likely, and makes the comparison among data obtained for the same analyte in the presence of different concentrations of selector more difficult, the comparison among the different analytes at the same concentration of selector appears very informative. It is sufficient to consider the comparison between terbutaline and epinephrine, respectively, the best and the worst separated analytes, at the maximum concentration of selector (8 mM) used at acidic pH: it is readily apparent that the very good selectivity observed for terbutaline is accompanied by a migration time much higher than that of the corresponding time for epinephrine. If we take into account that catecholamines, being protonated, at this pH are present as cationic species, and that the selector, on the contrary, as a carboxylic acid, is mainly anionic, any increase in the formation degree of the complexes results in an increase of the observed migration time, the weighted average of the migration times of the free and the complexed analyte.

Analogous comparison among the different analytes at alkaline pH is not so straightforward: at alkaline pH, besides the deprotonation of the amine group, a possible deprotonation of the phenolic groups can occur, differently in each catecholamine, and any detailed explanation of the electrophoretic behaviour should explicity consider these equilibria separately for any analyte.

The results obtained confirm once more how electrophoretic experiments can be fully explained by considering the detailed equilibria occurring in BGE.

In this work, a new chiral selector CDsuc6 was synthesised and characterised. While a similar product is commercially available as a mixture of derivatives at different substitution degrees, in our opinion the availability of a pure product is a significant step forward, since only in this way can we be sure about the reproducibility among different batches: Its behaviour towards catecholamine racemates appears as a promising test and its ability will be further tested towards other analytes.

Table 2
EKC results concerning the separation of terbutaline racemate at pH 9.2

C_{host} (mM), pH 9.2	Terbutaline		
	t_2	S	R
2.0	8.62	1.5	0.90
3.0	8.23	1.3	0.79
5.0	7.81	3.1	1.26
8.0	11.95	3.5	1.26

t_2: The migration time of the slower enantiomer, S values were multiplied by 100.

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