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Chiral discrimination of phenethylamines with β-cyclodextrin and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin by capillary electrophoresis and NMR spectroscopy

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Abstract: The resolution of nine sympathomimetic phenethylamine racemates by β-cyclodextrin and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin has been investigated by capillary electrophoresis and ^{1}H NMR spectroscopy. The NMR and capillary electrophoresis results showed that β-cyclodextrin probably formed stronger complexes with the amines than did *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin but was a poorer chiral discrimination agent in both techniques. The addition of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin to the capillary electrophoresis buffer gave baseline resolution of enantiomer peaks for seven of the compounds studied while β-cyclodextrin resolved only three of the racemates.

Keywords: *Capillary electrophoresis; NMR spectroscopy; derivatized cyclodextrins; modified cyclodextrins;* β-*cyclodextrin;* heptakis*(2,3-di-*O*-acetyl)*β-*cyclodextrin; phenethylamines.*

Introduction

Cyclodextrins are well established as reagents for the chiral discrimination of enantiomers by HPLC, in both the stationary phase [1, 2] and mobile phase [3–5], and by NMR spectroscopy [6, 7]. More recently, they have been used to achieve chiral separations with capillary electrophoresis (CE) [8–11]. Cyclodextrins are particularly useful for the separation of aromatic compounds which form diastereomeric inclusion complexes with the oligosaccharide. It is thought that chiral discrimination is brought about by differential interaction of each enantiomer with the secondary hydroxyl groups (at the 2- and 3-positions of the glucose monomers) which line the wider rim of the cyclodextrin cavity. While both hydrophobic and electrostatic forces are thought to be responsible for complex formation on the basis of theoretical studies [12], the exact nature of the enantiomer discriminating interactions is not well understood. Hydrogen bonding has been proposed from molecular modelling studies of the propranolol-β-cyclodextrin complex [13] and electrostatic forces from an examination of solid-state complex structures [14]. Thus, it is difficult to say precisely which compounds will be resolved and such predictions as are available are based on empirical observations.

As part of an investigation to understand in more detail the structural requirements for chiral discrimination, we have synthesized *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin and compared its ability to resolve enantiomers of a series of sympathomimetic phenethylamines (Table 1) with that of β-cyclodextrin using capillary electrophoresis and NMR spectroscopy. CE was selected in preference to reversed-phase HPLC because resolution can be achieved with smaller quantities of cyclodextrin and because results can be more easily compared with NMR resolution data owing to the absence of organic modifier. In addition, NMR spectroscopy has the potential to give detailed structural information on the inclusion complexes by subsequent NOE measurements. The resolution of some phenethylamines by CE has been reported previously using β-cyclodextrin and *heptakis*(2,6-di-*O*-methyl)β-cyclodextrin in the buffer [15–19]. Data for the

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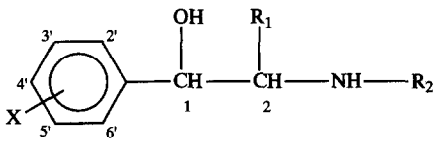
Table 1
Structures of compounds studied



Compound	Name	X	R_1	R_2
1	Ephedrine	H	CH_3	CH_3
2	Oxedrine	4-OH	H	CH_3
3	Oxilofrine (oxyephedrine)	4-OH	CH_3	CH_3
4	Norfenefrine	3-OH	H	H
5	Etilefrine	3-OH	H	CH_2CH_3
6	Orciprenaline	3,5-diOH	H	$CH(CH_3)_2$
7	Noradrenaline (norepinephrine)	3,4-diOH	H	H
8	Isoprenaline	3,4-diOH	H	$CH(CH_3)_2$
9	Salbutamol	3-CH_2OH,4-OH	H	$C(CH_3)_3$

chiral discrimination of a wider range of phenethylamines by β-cyclodextrin and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin are reported here.

Experimental

Synthesis and purification of heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin*

The synthesis was carried out in three stages using β-cyclodextrin as the starting material and required selective protection the primary hydroxyl group, acetylation of the secondary hydroxyls followed by deprotection and purification of the final product. The method was modified from Coleman *et al.* [20].

β-cyclodextrin (a gift from the Consortium für Elektrochemische Industrie) was dried at 0.1 mm Hg and 100°C for 48 h. Pyridine was dried over and distilled from CaH_2; dichloromethane was distilled over $CaCl_2$; boron trifluoride etherate complex was distilled before use. Chlorodimethyl-1,1,2-trimethylpropylsilane (Merck) was used without further purification. TLC was performed on silica gel (F_{254}, Merck) and detected by spraying with H_2SO_4. Silica gel 60 (Merck No. 7734) was used for column chromatography and reversed-phase chromatography was carried out with a LiChroprep RP-18 column (Merck No. 10625). Melting points were determined with a Büchi apparatus (Flawil, Switzerland) and uncorrected values are reported.

Heptakis*(6-O-dimethylsilyl-1,1,2-trimethylpropyl)*β*-cyclodextrin* (**10**). Chlorodimethyl-1,1,2-trimethylpropylsilane (10.0 ml, 51 mM) was added dropwise to a solution of anhydrous β-cyclodextrin (5.0 g, 4.4 mM) in dry pyridine (150 ml) at 0°C. The mixture was stirred at 0°C for 3 h, warmed to room temperature and allowed to stand for 35–40 h. The reaction was monitored by TLC using butanone–1-butanol–water (7:1:1, v/v/v; R_f 0.5). The pyridine was evaporated *in vacuo* and traces of solvent removed by co-evaporation with toluene (30 ml). The residue was crystallized from methanol–dichloromethane to give a product (6.8 g; 79%) with minor impurities. RP-18 chromatography of the product (methanol–dichloromethane, 7:3 v/v) gave pure **10** with analytical data identical to that previously reported [21].

Heptakis*(2,3-di-*O*-acetyl-6-*O*-dimethylsilyl-1,1,2-trimethylpropyl)*β*-cyclodextrin* (**11**). A solution of **10** (3.0 g; 1.41 mM) in acetic anhydride (30 ml) and pyridine (40 ml) was stirred for 4 h at 100°C and then evaporated *in vacuo*. Traces of solvents were removed by co-evaporation with toluene (10 ml). Column chromatography (ethyl acetate–petrol ether; 7:3, v/v) of the residue afforded pure **11** (2.41 g^{-1}; 63%) with analytical data identical to those previously reported [21].

Heptakis*(2,3-di-*O*-acetyl)*β*-cyclodextrin.* A solution of **11** (0.5 g, 0.184 mM) and boron trifluoride etherate complex (0.4 ml, 3.35 mM) in dichloromethane was stirred at room temperature overnight. It was then diluted with dichloromethane (50 ml) and poured into iced water. The organic layer was separated, washed with water (1 × 15 ml), saturated aqueous $NaHCO_3$ (1 × 15 ml) and again with water (1 × 15 ml), then dried over Na_2SO_4 and

evaporated. Column chromatography of the residue with an eluent of ethyl acetate–methanol (85:15, v/v) gave *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin (0.27 g, 87% yield). Analytical data for the product were identical with those already reported [21].

Capillary electrophoresis (CE)

Work was carried out on a BIORAD HPE CE system, using a Biorad coated-capillary of internal diameter 25 μm and a total length of 20 cm. Samples were loaded by electromigration and separated at room temperature using a constant current of 12 μA. Data were recorded at the analyte λ_{max} value with the Biorad 800 HRLC system, version 2.30. Samples of compounds **1–9** were prepared by dissolution in acetonitrile–potassium dihydrogen phosphate (0.1 M, pH 3.0) (10:90, v/v) at about 0.5 mg ml^{-1}. Buffers at pH 3–6 for CE were all prepared from potassium dihydrogen phosphate at 0.1 M using freshly distilled and filtered water, and adjusted with orthophosphoric acid or 1 M sodium hydroxide. The buffer at pH 7.5 was a 50:50 mixture of 0.1 M potassium dihydrogen phosphate and 0.1 M dipotassium hydrogen phosphate, adjusted with 1 M sodium hydroxide. All samples and buffers were filtered through a 0.2 μm cellulose nitrate filter (Whatman, Maidstone, UK) and centrifuged for 5 min before use.

NMR spectroscopy

For the measurement of cyclodextrin-induced shifts, sufficient quantities of compounds **1–9** with and without the appropriate cyclodextrin were dissolved in deuterated 0.1 M phosphate buffer equivalent to pH 4.5, to give 12 mmol concentrations of each. Spectra were obtained on a Jeol EX400 FT NMR spectrometer operating at 399.05 MHz for ^1H. Sixty-four scans with a frequency range of 5000 Hz were collected into 32K data points giving a digital resolution of 0.31 Hz/pt. An appropriate Gaussian function was applied before Fourier transformation to enhance spectral resolution. The temperature was controlled at 30 ± 1°C and the residual protonated water signal was suppressed using homo-gated secondary irradiation (decoupler off during data acquisition). Inverse ^1H-^{13}C correlation spectroscopy with the HMQC pulse sequence [22] on a 12 mmol solution of etilefrine HCl used a final data matrix of 512 × 512 real data points. Frequency widths of 5000 Hz (^1H) and 30120.5 Hz (^{13}C) gave row and column resolutions of 9.77 and 58.8 Hz, respectively. Sixteen scans were accumulated per slice.

The ^1H NMR experiments for the Job plots and measurement of complex formation constants with etilefrine HCl were performed on a Varian XL 300 FT NMR spectrometer operating at 299.956 MHz with a sample temperature of 26°C. Five solutions in deuterated 0.1 M phosphate buffer pH 4.5 having molar ratios (etilefrine HCl:cyclodextrin) of 0.5:1, 0.75:1, 1:1, 1.25:1 and 1.5:1 were prepared for the Job plots. Four similar solutions with molar ratios 1:2.33, 1:3.0, 1:4.0 and 1:5.7 were used to determine the formation constant of the complex between etilefrine HCl and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin.

β-cyclodextrin and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin were dried *in vacuo* over P_4O_{10} before use and all chemical shifts were referenced to the HDO signal at 4.65 ppm.

Results

Capillary electrophoresis

The migration times for all nine compounds using 0.1 M phosphate buffer pH 3.0 are shown in Table 2, which also gives the migration times (t_m) and resolution values (R_s) measured in the presence of β-cyclodextrin or *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin.

The migration times in Table 2 correspond to electrophoretic mobilities ranging from 0.58 × 10^{-4} (t_m = 13.84 min) to 1.72 × 10^{-4} (t_m = 5.39 min) $cm^2\ s^{-1}\ V^{-1}$ which were calculated according to equation 1 [23]:

$$\mu_{app} = \mu_{ep} + \mu_{eo} = lL/Vt_m \qquad (1)$$

where μ_{app}, μ_{ep} and μ_{eo} are the apparent, electrophoretic and endo-osmotic mobilities, respectively ($cm^2\ s^{-1}\ V^{-1}$), l is the length (17.2 cm) of the capillary to the detector, L is its total length (20 cm) and V is the voltage (5700V) across it. In an attempt to measure μ_{eo} under the experimental conditions used, no response was obtained when using acetone as a neutral marker during a time of 90 min over a column distance of 2.8 cm. This indicates that μ_{eo} must be less than 1.8 × 10^{-6} $cm^2\ s^{-1}\ V^{-1}$ which is lower in comparison to a previous report [23] where the effect of μ_{eo} was discounted at 0.04 × 10^{-4} $cm^2\ s^{-1}\ V^{-1}$. Thus the effect of endo-osmotic flow can be ignored so that $\mu_{ep} = \mu_{app}$.

Table 2
CE migration times and resolution values (R_s) for some phenethylamines in 0.1 M phosphate buffer pH 3.0 with and without 12 mmol β-cyclodextrin or *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin

Compound	Buffer alone	Buffer with β-cyclodextrin		Buffer with *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin	
	t_m	t_{m1}, t_{m2}	R_s	t_{m1}, t_{m2}	R_s
1	5.39	9.98	0	6.66, 6.87	1.25
2	8.98	10.91	0	7.55, 7.93	1.62
3	6.09	12.33, 12.71	1.16	6.32, 7.97	1.74
4	8.36	11.95	0	7.43, 7.65	1.31
5	9.49	10.95, 11.11	0.47	8.84, 9.82	4.28
6	7.12	12.61, 12.94	0.89	9.22, 9.48	1.32
7	9.39	11.80	0	7.55	0
8	11.10	13.84	0	9.24	0
9	12.18	12.74	0	9.27, 9.45	0.85

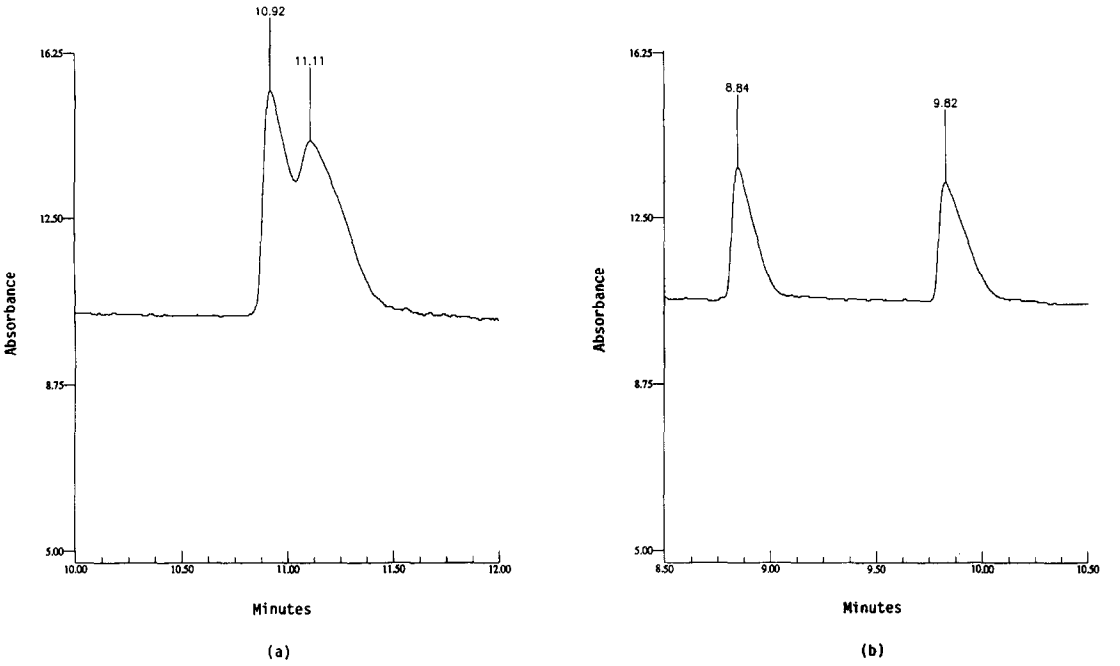


Figure 1
Electropherograms of etilefrine HCl (**5**) in 0.1 M phosphate buffer pH 3.0 in the presence of (a) β-cyclodextrin and (b) *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin.

R_s values were determined using equation 2:

$$R_s = 2\,(R_{t2} - R_{t1})/(w_{b1} + w_{b2}) \qquad (2)$$

where R_{t2}, R_{t1} and w_{b1}, w_{b2} are the migration times and baseline peak-widths, respectively, of the two enantiomers. Typical electropherograms are shown in Fig. 1.

β-cyclodextrin increased the migration times for all compounds relative to the uncomplexed drug whereas *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin caused a decrease in migration except for three compounds (**1**, **3** and **6**). The increase in migration times for these three compounds with the derivatized cyclodextrin was less than that observed with β-cyclodextrin and thus permitted more rapid analysis. β-cyclodextrin resolved only three compounds (**3**, **5** and **6**) and the acetylated cyclodextrin increased the resolution of all racemates compared to the underivatized oligosaccharide except for compounds **7** and **8** which were not resolved by either cyclodextrin. Individual enantiomers of the compounds were not available so the elution order of the R- and S-isomers is not known.

The effect of buffer pH on migration times and resolution was fully investigated for all compounds in the presence of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin. Migration times at

Table 3
Effect of pH on CE migration times for some phenethylamines in phosphate buffer (0.1 M, pH 3.0) in the presence of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin

Compound	pH 3.0	pH 4.5	pH 6.0	pH 7.5
1	6.66, 6.87	7.18, 7.34	8.04, 8.24	12.77, 13.17
2	7.55, 7.93	7.70, 7.97	10.30, 10.66	14.40, 14.81
3	7.97, 8.32	8.12, 8.47	9.13, 9.48	15.94, 16.65
4	7.43, 7.56	7.19, 7.30	9.06, 9.23	15.00, 15.34
5	8.84, 9.82	8.62, 9.26	10.40, 11.10	16.93, 18.42
6	9.22, 9.48	9.73, 10.00	11.20, 11.49	19.12, 19.54
7	7.55	7.71	10.08	18.81
8	9.24	9.53	13.96	20.82
9	9.27, 9.45	9.97, 10.10	13.03, 13.22	16.41

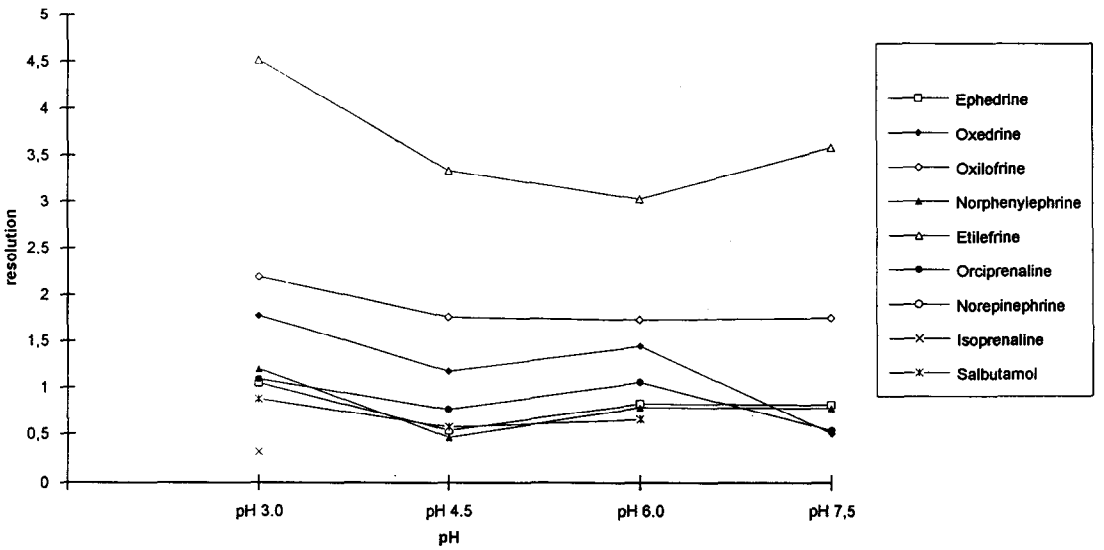


Figure 2
Influence of pH on resolution (R_s) of various racemic phenethylamines by CE in 100 mM phosphate buffer pH 3.0 in the presence of 12 mmol *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin.

pH 3.0, 4.5, 6.0 and 7.5 are given in Table 3 while Fig. 2 shows the effect of pH on resolution. Generally, as the pH was raised migration times increased and resolution decreased, although with etilefrine (**5**) the resolution showed a minimum at pH 6.0.

The effect of adding 5, 10, 15 and 20% v/v acetonitrile to the 0.1 M phosphate buffer at pH 3.0 was investigated for three compounds (**1**, **3** and **6**) to enable comparison with HPLC data (not reported here). Migration decreased to a minimum at 15% v/v and then increased again at 20% v/v. Resolution decreased with increasing concentration of the organic modifier and was accompanied by deterioration of peak shape.

NMR spectroscopy

The ^{1}H NMR spectra of **1–9** were assigned by inspection and, in the case of etilefrine HCl, the two aromatic doublets (H4′ and H6′) were distinguished with the aid of a 2D proton-detected ^{1}H-^{13}C chemical shift correlation spectrum and substituent increment tables. The assignments are shown in Table 4 while Tables 5 and 6 show the change in chemical shift (Δδ) observed on complexation with the two cyclodextrins and the separation between signals arising from the individual enantiomers ($|\delta_R - \delta_S|$). As representative spectra, Fig. 3 shows the NMR spectra of uncomplexed etilefrine and the corresponding signals in the presence of β-cyclodextrin and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin. Separated signals have not yet been attributed to their respective isomers for any of the compounds.

All the compounds show evidence of inclusion by β-cyclodextrin with substantial changes in chemical shift (except **9**) caused by the presence of the oligosaccharide. Δδ was of

Table 4
^{1}H NMR assignments of various phenethylamines

Compound	H2′	H3′	H4′	H5′	H6′	H1	H2	R_2	2-CH_3
1	7.355	—	—	—	—	5.060	3.476	CH_3 2.699	1.062
2	7.245	6.862	—	6.862	7.245	4.904	3.230 3.207	CH_3 2.693	—
3	7.217	6.871	—	6.871	7.217	4.951	3.416	CH_3 2.676	1.078
4	6.861	—	6.826	7.271	6.918	4.885	3.277 3.085	—	—
5	6.847	—	6.812	7.254	6.901	4.906	3.249 3.197	CH_2 3.071 CH_3 1.213	—
6	6.422	—	6.317	—	6.422	4.824	3.204 3.133	CH 3.407 CH_3 1.258 CH_3 1.251	—
7	6.876	—	—	6.868	6.789	4.798	3.206 3.094	—	—
8	6.865	—	—	6.878	6.789	4.811	3.196 3.145	CH 3.407 CH_3 1.260 CH_3 1.244	—
9	7.275	CH_2 4.593	—	6.882	7.205	4.841	3.207 3.128	Bu^t 1.310	—

Table 5
Chemical shift changes ($\Delta\delta$) in ^{1}H NMR spectra of various phenethylamines induced by β-cyclodextrin

Compound	H2′	H3′	H4′	H5′	H6′	H1	H2	R_2	2-CH_3
1			−0.002(*)			0.072 (—)	−0.096 (0.018)	CH_3 0.046 (—)	−0.027 (—)
2	0.000 (—)	−0.024 (—)	—	−0.024 (—)	0.000 (—)	0.008 (—)	~0.037 (*)	CH_3 0.010 (—)	—
3	−0.010 (0.005)	−0.058 (0.005)	—	−0.058 (0.005)	−0.010 (0.005)	0.086 (—)	−0.100 (0.017)	CH_3 0.052 (0.002)	−0.015 (0.003)
4	−0.013 (—)	—	−0.027 (—)	−0.040 (0.003)	0.023 (—)	0.000 (—)	−0.024 (0.013) −0.044 (0.009)	—	—
5	−0.015 (0.005)	—	−0.032 (*)	−0.053 (0.005)	−0.027 (—)	0.005 (—)	*	CH_2* CH_3 0.016 (0.010)	—
6	−0.051 (0.006)	—	−0.045 (0.005)	—	−0.051 (0.006)	−0.003 (—)	−0.071 (—) 0.068 (—)	CH 0.014 (—) CH_3 0.021 (—) CH_3 0.023 (0.008)	—
7	−0.018 (—)	—	—	−0.041 (*)	−0.012 (—)	−0.002 (—)	−0.034 (—) −0.041 (—)	—	—
8	0.001 (—)	—	—	−0.028 (—)	0.019 (—)	0.007 (—)	−0.051 (—) 0.024 (—)	CH 0.007 (—) CH_3 0.012 (—) CH_3 0.011 (—)	—
9	0.000 (—)	CH_2 0.000 (—)	—	−0.007 (—)	0.001 (—)	0.008 (—)	−0.033 (—) −0.005 (—)	Bu^t 0.006 (—)	—

The average change in chemical shift for both enantiomers is given with $|\delta_R-\delta_S|$ in parentheses. A dash in parentheses indicates no signal splitting and an asterisk indicates that full analysis was not possible. The actual chemical shift for each enantiomer can be obtained by adding or subtracting half $|\delta_R-\delta_S|$ to the chemical shift of the uncomplexed drug in Table 4.

Table 6
Chemical shift changes ($\Delta\delta$) in ^{1}H NMR spectra of various phenethylamines induced by *heptakis*(2,3-di-*O*-acetyl) β-cyclodextrin

Compound	H2'	H3'	H4'	H5'	H6'	H1	H2	R_2	2-CH_3
1			0.026(*)			0.063 (0.040)	0.050 (0.036)	CH_3 0.041 (0.026)	0.016 (—)
2	0.015 (0.006)	−0.007 (0.003)	—	−0.007 (0.003)	0.015 (0.006)	0.030 (0.018)	0.001 ( ) −0.003 (—)	CH_3 0.022 (0.007)	—
3	0.039 (0.021)	−0.010 (0.018)	—	−0.010 (0.018)	0.039 (0.021)	0.062 (0.046)	−0.042 (0.037)	CH_3 0.043 (0.031)	0.019 (—)
4	0.009 (—)	—	−0.001 (0.006)	−0.004 (—)	0.023 (0.016)	0.038 (0.030)	0.043 (0.013) −0.008 (0.015)	—	—
5	0.011 (0.006)	—	0.006 (0.006)	−0.019 (0.016)	0.029 (0.018)	0.081 (0.067)	*	CH_2* CH_3 0.034 (0.033)	—
6	−0.005 (—)	—	0.011 (0.008)	—	−0.005 (—)	−0.025 (*)	0.010 (0.004) 0.005 (0.032)	CH 0.039 (0.016) CH_3 0.029 (—) CH_3 0.034 (0.005)	—
7	−0.005 (—)	—	—	0.026 (—)	−0.004 (—)	−0.022 (*)	0.033 (—) −0.018 (—)	—	
8	0.002 (—)	—	—	−0.012 (0.003)	0.006 (0.005)	*	0.003 (—) 0.019 (—)	CH 0.030 (0.016) CH_3 0.025 (0.008) CH_3 0.025 (0.008)	—
9	0.011 (—)	CH_3 0.004 (—)	—	0.002 (—)	0.010 (—)	−0.014 (*)	−0.032 (*)	Bu' 0.036 (0.015)	—

The average change in chemical shift for both enantiomers is given with $|\delta_R-\delta_S|$ in parentheses. A dash in parentheses indicates no signal splitting and an asterisk indicates that full analysis was not possible due to resonance overlap. The actual chemical shift for each enantiomer can be obtained by adding or subtracting half $|\delta_R-\delta_S|$ to the chemical shift of the uncomplexed drug in Table 4.

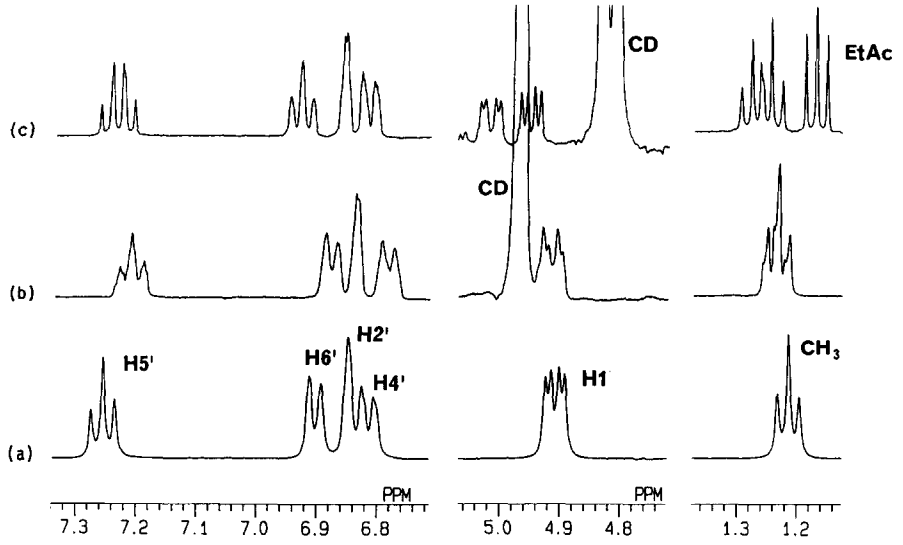


Figure 3
The NMR signals for the aromatic protons, H1 and CH_3 (R_2) group of etilefrine HCl (**5**) in (a) the absence of cyclodextrin, (b) the presence of β-cyclodextrin and (c) the presence of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin. CD indicates cyclodextrin signals and EtAc arises from ethyl acetate impurity.

the same order for all signals, except H1 in those compounds lacking a methyl group at C2, which showed very small changes in chemical shift. In **1** and **3** with the additional side-chain methyl group, H1 had the largest Δδ value in the molecule. A contrasting pattern of Δδ values was observed on the addition of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin. In this case, the largest values of Δδ were observed for H1, although it could not be measured in all cases because the shifted signal was obscured by cyclodextrin peaks. The other signals were shifted to a lesser extent than when β-cyclodextrin was added and the direction of the shift differed in some cases. The aromatic signals showed a different pattern of shifts in most compounds, e.g. in **2** and **3** with a *para*-hydroxy group, the ortho-protons had the smaller shift with β-cyclodextrin but the larger shift with the acetylated cyclodextrin.

Chiral discrimination was much higher with *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin: all compounds had at least one signal that was split when this cyclodextrin was added (although the H1 signal in compound **7** is obscured, it is assumed to be split). The H1 signal had the highest $|\delta_R - \delta_S|$ values in the molecule in the instances where it was not obscured by CD signals and could be measured.

A Job (continuous variation) plot [24, 25] was used to determine the stoichiometry of each CD complex in the case of etilefrine HCl (Fig. 4). Selected data for guest or host are shown but other signals reflect the same pattern with a peak at 0.5 showing that a 1:1

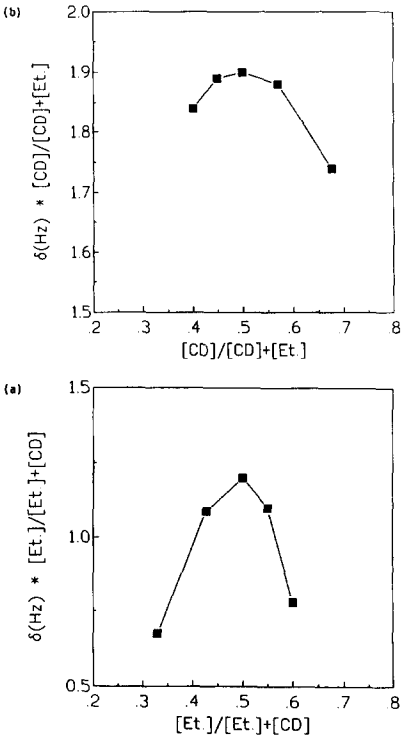


Figure 4
Representative Job plots for complexation between etilefrine HCl (**5**) and (a) β-cyclodextrin (H1 resonance) and (b) *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin (cyclodextrin H3 resonance).

complex is formed with both CDs. It is assumed that the other compounds in the series also form complexes with this stoichiometry due to their structural similarity and the lack of second aromatic rings in the molecules.

Discussion

Capillary electrophoresis

CE has been used in previous studies to resolve enantiomers of phenethylamines. Fanali has described the enantiomeric separation of ephedrine, norephedrine, epinephrine (adrenaline), norepinephrine (noradrenaline) and isoproterenol (isoprenaline) [15], and terbutaline [17] using a coated capillary and a low pH buffer containing *heptakis*-(2,6-di-*O*-methyl)β-cyclodextrin. β-cyclodextrin and *heptakis*(2,3,6-tri-*O*-methyl)β-cyclodextrin were also investigated but, although they increased the migration times of the compounds, they did not cause chiral discrimination except in the case of terbutaline. Ephedrine and related compounds were used for comparison purposes in the chiral separation of some basic drugs by cyclodextrin-modified CE with a fused silica column. Non-alkylated cyclodextrins did not cause discrimination but it was achieved using *heptakis*-(2,6-di-*O*-methyl)β-cyclodextrin [19]. *Heptakis*(2,6-di-*O*-methyl) β-cyclodextrin, added to the buffer, has also been used to determine the enantiomeric ratio of epinephrine in a pharmaceutical formulation by CE with an uncoated fused capillary [18].

The phenethylamines discussed have amine pK_a values in the range 8.6–9.3, and thus under the conditions used here for CE, they are expected to be fully ionized and migrate to the cathode. β-cyclodextrin caused an increase in migration time consistent with complexation reducing the mobility of the drug (Table 2). *Heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin complexes migrated more quickly than β-cyclodextrin complexes with all the phenethylamines studied, which could perhaps be explained by acetylation preventing the formation of complex dimers or by the formation constants being smaller for the derivatized cyclodextrin. Unexpectedly, however, *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin also caused a decrease in migration times relative to the uncomplexed molecule for six of the nine compounds which is difficult to explain. Although migration times were faster with the derivatized cyclodextrin, seven of the nine compounds were resolved compared with only three for β-cyclodextrin.

Overall, increasing pH caused an increase in migration times as would be expected by increasing the proportion of neutral phenethylamine species in the buffer. Addition of up to 15% acetonnitrile to the buffer in the presence of β-cyclodextrin decreased mobility for the three compounds tested (**1**, **3** and **6**) perhaps by adsorption of the organic modifier onto the oligosaccharide. It was not added to the buffer in the final conditions chosen because it lengthened analysis time and gave poorer resolution and peak shape.

On the basis of plots of migration times vs cyclodextrin concentration, Fanali [15] suggested that ephedrine and norephedrine, with unsubstituted phenyl rings, fit into the oligosaccharide cavity better while the catecholamine enantiomers show greater chiral discrimination. There is no evidence in the CE or NMR studies reported here (see later) to support this assertion as ephedrine does not show atypical behaviour with either technique.

NMR spectroscopy

The two cyclodextrins have different overall effects on the NMR spectra of the phenethylamines as shown by chemical shift changes and signal splitting arising from diastereomeric complexation with the enantiomeric guest molecules. Both cyclodextrins demonstrate inclusion of phenethylamines but β-cyclodextrin discriminates poorly between enantiomers and *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin on the whole resolves enantiomers extremely well.

Changes in chemical shift provide evidence of inclusion by both oligosaccharides, although the Δδ values for compound **9** are small or insignificant on complexation with *heptakis*-(2,3-di-*O*-acetyl)β-cyclodextrin. The presence of β-cyclodextrin tends to cause larger shifts in the aromatic region compared to *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin and causes little or no splitting of the signals. On the other hand, *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin splits aromatic signals in several compounds, most notably in **3**, **4** and **5**. It is interesting to note that **3** and **5** have the highest values of R_s in CE.

Mainly upfield shifts (negative Δδ values) were observed in the aromatic region of the

phenethylamine spectra on complexation with either cyclodextrin and are consistent with inclusion of this part of the molecule in the hydrophobic cavity of the macromolecule. Downfield shifts in the H6′ signal of some *N*-(2′,4′-dinitrophenyl)amino acids, together with circular dichroism data, have been interpreted as arising from inclusion complexes where the guest molecule is tilted in the cyclodextrin cavity [7], and are consistent with theoretical calculations on the alignment of guest and host dipole moments [26]. Notable downfield shifts occur for individual aromatic resonances of some phenethylamines and may be indicative of such tilting, for example, the H6′ resonance is shifted downfield on complexation with β-cyclodextrin in compound **4** while other signals move upfield. In the presence of *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin, the H6′ signal for compounds **4**, **5** and **8** shifts downfield to a greater extent than other aromatic signals.

Side-chain interactions with the cyclodextrin (presumably at its surface) are apparently also important for complexation, although dependant on derivatization, and the alkyl Δδ values are larger than for the aromatic signals. The signals of H2 and R_2 are significantly shifted in the presence of either cyclodextrin, and often have large values of $|\delta_R-\delta_S|$. However, the H1 resonance is significantly shifted only by *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin for compounds without a methyl group at C2. *Heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin gives a large splitting of the H1 resonance in those compounds where the signal is not obscured but in the presence of β-cyclodextrin no doubling is observed. These results could be explained by different hydrogen bonding interactions between each cyclodextrin and the guest side-chain. Both hydroxyl and acetyl groups on the cyclodextrin rim could act as hydrogen bond acceptors for the amine NH thus causing chemical shift changes in the guest (although of variable direction) with both cyclodextrins. The downfield shifts of the H1 resonance noted with the derivatized cyclodextrin may be due to hydrogen bonding between the guest hydroxyl group at C1 and the carbonyl oxygen of the macrocyclic acetyl groups. Presumably the structure of the inclusion complex with β-cyclodextrin does not have the correct geometry for a similar interaction with the secondary hydroxyl groups of this oligosaccharide.

In the presence of β-cyclodextrin, compounds **1** and **3** with the 2-Me group also show a large shift of the H1 resonance which could be explained by the side-chain methyl group forcing a conformational rearrangement such that a hydrogen bond can be accommodated. Comparison of the CE data for compounds **2** and **3**, which differ only by the 2-Me group in **3**, shows that the methyl group is required for resolution by β-cyclodextrin. Although **1** also has the 2-Me group, it is not resolved and this may be caused by the lack of aromatic substituents.

Differences in hydrogen bonding patterns could also explain why *heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin is better than the underivatized cyclodextrin at discriminating between phenethylamine enantiomers, as shown by the generally larger values of $|\delta_R-\delta_S|$ obtained with the former oligosaccharide. Further NMR and molecular modelling studies are planned to investigate the structures of the phenethylamine–cyclodextrin complexes.

In general, on the basis of the aromatic Δδ values, the NMR results show that β-cyclodextrin probably forms stronger complexes with the phenethylamines than does *heptakis*-(2,3-di-*O*-acetyl)β-cyclodextrin. Attempts to measure the formation constants of the complexes by NMR spectroscopy have not yet been successful owing to limited solubility of the cyclodextrin. *Heptakis*(2,3-di-*O*-acetyl)β-cyclodextrin is a better chiral discrimination agent and this is reflected in the CE studies.

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References

[1] D.W. Armstrong, W. DeMond, A. Alak, W.L. Hinze, T.E. Riehl and K.H. Bui, *Anal. Chem.* **57**, 234–237 (1985).
[2] S.H. Lee, A. Berthod and D.W. Armstrong, *J. Chromatogr.* **603**, 83–93 (1992).
[3] A.D. Cooper and T.M. Jefferies, *J. Chromatogr.* **637**, 137–143 (1993).
[4] K. Shamada, T. Oe, Y. Hirose and Y. Komine, *J. Chromatogr.* **478**, 339–347 (1989).
[5] D. Sybilska and J. Zukowski, in *Chiral Separation by HPLC: Applications to Pharmaceutical Compounds*, Chapter 7 (A.M. Krstulovic, Ed.), pp. 147–172. Ellis Horwood, Chichester, UK (1989).
[6] A.F. Casy, *TRAC* **12**, No. 4, 185–189 (1993).
[7] S. Li and W.C. Purdy, *Anal. Chem.* **64**, 1405–1412 (1992).

[8] M.J. Sepaniak, R.O. Cole and B.K. Clark, *J. Liq. Chrom.* **15**, 1023–1040 (1992).
[9] I.E. Valko, H.A.H. Billiet, H.A. L. Corstjens and J. Frank, *LC–GC Int.* **6**, 420–427 (1993).
[10] J. Snopek, I. Jelinek and E. Smolkova-Keulemansova, *J. Chromatogr.* **609**, 1–17 (1992).
[11] S.A.C. Wren, *J. Chromatogr.* **636**, 57–62 (1993).
[12] M.J. Sherrod, in *Spectroscopic and Computational Studies of Supramolecular Systems*, (J.E.D. Davies, Ed.), pp. 187–205. Kluwer, The Netherlands (1992).
[13] D.W. Armstrong, T.J. Ward, R.D. Armstrong and T.E. Beesley, *Science* **232**, 1132–1135 (1986).
[14] K.B. Lipkowitz, K.M. Green, J.-A. Yang, G. Pearl and M.A. Peterson, *Chirality* **5**, 51–57 (1993).
[15] S. Fanali, *J. Chromatogr.* **474**, 441–446 (1989).
[16] S. Fanali and P. Bocek, *Electrophoresis* **11**, 757–760 (1990).
[17] S. Fanali, *J. Chromatogr.* **545**, 437–444 (1991).
[18] T.E. Peterson and D. Trowbridge, *J. Chromatogr.* **603**, 298–301 (1992).
[19] M.W. Nielen, *Anal. Chem.* **65**, 885–893 (1993).
[20] A.W. Coleman, P. Zhang, C.-C. Ling, H. Parrot-Lopez and H. Galons, *Carbohydr. Res.* **224**, 307–309 (1992).
[21] K. Takeo, H. Mitoh and K. Uemura, *Carbohydr. Res.* **187**, 203–221 (1989).
[22] A. Bax and S. Subramanian, *J. Magn. Reson.* **67**, 565–569 (1986).
[23] S.A.C. Wren and R.C. Rowe, *J. Chromatogr.* **635**, 113–118 (1993).
[24] P. Job, *Ann. Chim.* **9**, 113–203 (1928).
[25] V.M.S. Gil and N.C. Oliveira, *J. Chem. Educ.* **67**, 473–478 (1990).
[26] M. Kitigawa, H. Hoshi, M. Sakurai, Y. Inoue and R. Chujo, *Carbohydr. Res.* **198**, 181–191 (1990).

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