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Study on the chiral recognition of the enantiomers of ephedrine derivatives with neutral and sulfated heptakis(2,3-*O*-diacetyl)-β-cyclodextrins using capillary electrophoresis, UV, nuclear magnetic resonance spectroscopy and mass spectrometry

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Abstract

The enantiomers of methylephedrine, pseudoephedrine and ephedrine showed a different migration behavior in capillary electrophoresis in the presence of β-cyclodextrin (β-CD), *heptakis*(2,3-*O*-diacetyl)-β-cyclodextrin and *heptakis*(2,3-*O*-diacetyl-6-sulfato)-β-cyclodextrin (HDAS). Utilizing UV, MS and NMR spectroscopy, in particular rotating frame Overhauser experiments, an attempt was made to elucidate the chiral recognition mechanism. In the case of the neutral CDs 1:1 complexes were formed with ephedrine and methylephedrine characterized by the inclusion of the phenyl ring in the cavity and the side chain pointing out of the wider rim. In contrast, manifold complexes were formed with HDAS, which on average are characterized by an upside down inclusion of the phenyl ring in the cavity and the side chain pointing out of the narrow rim. This complex geometry is likely be stabilized by an ion–ion interaction between the positively charged nitrogens of the ephedrine derivatives and the negative charges of HDAS. In addition, an attachment of the ligand to the outside of HDAS and other complex stoichiometries are also possible.

Keywords: Enantiomer separation; Nuclear magnetic resonance spectrometry; Job plots; Ephedrines; Cyclodextrins

1. Introduction

β-Cyclodextrins (β-CDs) are widely used as selectors for the resolution of enantiomers of chiral drugs in both separation techniques, e.g. high-performance liquid chromatography (HPLC) (for a review, see Ref. [1]) and capillary electrophoresis (CE) (for a review, see Ref. [2]), and spectroscopic methods, e.g., nuclear magnetic resonance (NMR) spectroscopy [3,4]. In order to enhance the resolution power, a variety of derivatized, often charged cyclodextrins (CDs) were introduced [5]. However, most of these CDs are mixtures characterized by an average degree of substitution in varying locations (for a review, see Ref. [6]). In order to study the mechanism of chiral recognition between the enantiomers of ephedrine derivatives and various β-CDs, we synthesized the

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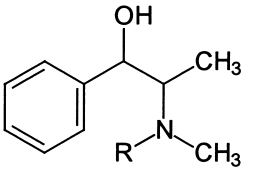
single isomeric *heptakis*(2,3-*O*-diacetyl)-β-CD (Diac) and *heptakis*(6-*O*-acetyl)-β-CD (6-Ac) of high uniformity [7,8]. Using NMR spectroscopy, and in particular the complexation induced chemical shifts (CICSs) of both the ligand and the CD in addition to Job plots and rotating frame Overhauser spectroscopy (ROESY) experiments we could propose a mode of chiral recognition. Generally, it can be stated, that 1:1 complexes were formed with β-CD, Diac and 6-Ac [9]. The aromatic moiety of the ephedrine derivatives is included in the cavity composed of seven glucose units whereas the side chain is located at the wider rim forming hydrogen bondings with the OH and acetyl groups of this rim. Different inter- and intramolecular nuclear Overhauser effects (NOEs) were found for the pairs of enantiomers with β-CD and Diac reflecting the ability of these CDs to discriminate between the enantiomers. Since the $^{1}H–^{1}H$-coupling constants of the ligands did not change upon complexation with both CDs it is likely that a different orientation of the enantiomers rather than a different conformation in the cavity causes the chiral recognition. These results are in accordance with findings reported by other authors [10–12].

Similar inclusion complexes between brompheniramine, chlorpheniramine, dimethindene and verapamil, and neutral and negatively charged β-CDs [13–17] were characterized by Chankvetadze and co-workers by means of NMR spectroscopy, mass spectrometry (MS) and X-ray analysis.

Recently, single isomeric negatively charged CDs were introduced which were found to be powerful tools in chiral CE [18–21]. These CDs are bearing sulfato groups at the narrow rim (6-position) of the cavity. Using *heptakis*(2,3-*O*-diacetyl-6-sulfato)-β-CD (HDAS) in CE the resolution of ephedrines could be impressively enhanced. This raised the question whether this charged CD forms similar complexes as the corresponding diacetylated CD and along with this, whether the mode of recognition is similar. Preliminary studies revealed a heterogenous picture of both the stoichiometry and the complex geometry. Job plots constructed from UV measurements showed plateaus ranging from 0.4 to 0.6 instead of a clear maximum at 0.5 obtained with the neutral CDs. These curved Job plots gave the first evidence for a mixed complex stoichiometry. Preliminary NMR measurements using a 300 MHz spectrometer at a 1:1 ratio of ephedrines and HDAS gave CICSs of both the ligands and the CD which clearly differ from the pattern obtained with Diac [22]: whereas H3 sitting inside the Diac cavity is mostly affected by complexation with the ephedrines, H5 and H6 which are located close to the narrow rim of HDAS are strongly shifted. In addition, these hydrogens showed the highest difference upon complexation with both enantiomers. These results and the different patterns of the CICSs of the ephedrine hydrogens upon complexation with Diac and HDAS further support the hypothesis of a different way of complexation. However, the resolution of a 300 MHz spectrometer is not high enough to derive a defined picture of the complex geometry.

Thus, the purpose of this study was to further characterize the structure of the diastereomeric complexes obtained with the enantiomers of ephedrine and methylephedrine (see Fig. 1) and HDAS in comparison to complexes formed with these ligands and β-CD and Diac. Firstly, concerning CE the migration order of the enantiomers of ephedrine and the diastereomeric pseudoephedrine was determined in the presence of all three CDs. Secondly, it was checked by means of UV spectroscopy whether a change of the complex arrangement takes place over a certain time course. Thirdly, the stoichiometry of the ephedrine and methylephedrine complexes with β-CD, Diac and HDAS was explored by means of the ionspray MS technique. Fourthly, using a 600



	R
(1R,2S), (1S,2R) Ephedrine	H
(1S,2S), (1R,2R) Pseudoephedrine	H
(1R,2S), (1S,2R) N-Methylephedrine	CH_3

Fig. 1. Structural formulae of ephedrine, pseudoephedrine and methylephedrine.

MHz NMR spectrometer, CICSs at varying CD–ligand ratios were measured and ROESY experiments performed.

2. Experimental

2.1. Chemicals

The enantiomers of *N*-methylephedrine, pseudoephedrine and ephedrine were purchased from Fluka (Buchs, Switzerland). Diac-β-CD was synthesized according to Branch et al. [7], β-CD, was a generous gift from the Consortium für Elektrochemische Industrie (Munich, Germany), HDAS-β-CD a gift from Professor G. Vigh, A&M University, College Station, TX, USA. Analytical-grade KH_2PO_4 was obtained from Merck (Darmstadt, Germany). 50 m*M* Phosphate buffer, pH 3 was prepared by mixing appropriate concentrations of H_3PO_4 and KH_2PO_4 solutions. The CDs were dissolved in buffer, the samples subjected to CE were dissolved in deionized water (concentration ≈1 mg/ml). All solutions were filtered through a 0.45-μm syringe (Schleicher & Schüll, Dassel, Germany).

2.2. CE

All experiments were performed on a Beckman P/ACE MDQ system (Beckman Instruments, Fullerton, CA, USA) using a fused-silica capillary with a total length of 60 cm, a detection length of 50 cm, and an internal diameter of 50 μm. Samples were loaded by 5 s of pressure injection and separated at 25°C in the cationic mode, using a constant voltage of 20 kV. The drug solution had a concentration of 50 μg/ml and was detected using a diode array detector at 194 nm. The capillary was conditioned for 20 min with 0.1 *M* NaOH, and 10 min with water. Additionally, the capillary was washed for 2 min with 0.1 *M* NaOH, 1 min with water, and 2 min with the running buffer before each run.

2.3. UV

The UV spectra were recorded on a Hewlett-Packard 8452 A diode array spectrophotometer (Böblingen, Germany). The determination of the stoichiometry of the complexes was done by mixing stock solutions of the enantiomers and the CDs with a molar concentration of 0.1 m*M* in the ratios of 0.2:0.8, 0.33:0.66, 0.4:0.6, 0.5:0.5, 0.6:0.4, 0.66:0.33 and 0.8:0.2. Comparison of these spectra with solutions of the same concentration of the ephedrine derivatives but without CDs gave the required changes in absorbance. All absorbances were determined at the maximum wavelength of 206 nm directly after preparation and time-dependently up to 1 h.

2.4. Ionspray MS

The LC–MS–MS experiments were performed on a Perkin-Elmer Sciex API3000 mass analyzer equipped with a Harvard Syringe Pump, Model 55-1111 (Harvard Apparatus, South Natick, MA, USA), an IonSpray interface (Perkin-Elmer, Toronto, Canada) and LC2 Tune 1.4 software; scan type Q1 MCA (10 scans) was used in positive polarity mode. Adjustments were as follows: nebulizer gas 10, curtain gas 8, ion spray voltage 5000 V, orifice voltage 56 V, temperature 0°C, resolution 1 u.

Stock solutions of 1 mg/ml ephedrines and CDs were prepared in mixtures of water–methanol (1:1) and diluted to 10 μg/ml with the same solvent; 1% HCOOH was added to enhance the ionization. The samples were applied to the LC–MS–MS system using a flow-rate of 10 μl/min. Q1 scans were recorded for each component of the complexes and for each complex; masses from m/z 50 to 2200 were registered.

2.5. NMR spectroscopy

All experiments were performed on a Bruker AMX600 FT NMR spectrometer operating at 600.130 MHz and a sample temperature of 300 K. An appropriate Gaussian function was applied before Fourier transformation to enhance the spectral resolution. All chemical shifts were referenced to the ^2H_2O signal at 4.650 ppm. The (ROESY) experiments were performed using the pulse program ROESYPRTP with an applied mixing time of 225 ms.

The CD derivatives were dried throughout in vacuo over P_2O_5 before use. Solutions having molar ratios of 5.5:0.5, 5:1, 4:2, 3.6:2.4, 3:3, 2.4:3.6, 2:4

and 1:5 (ligand–CD) were prepared in deuterated 100 m*M* phosphate solution (composed of 100 m*M* KH_2PO_4 in deuterated water, equivalent to pH 4.5) in order to measure the induced chemical shifts of the CD and ephedrine signals and to build a Job plot.

3. Results and discussion

3.1. CE studies

In a previous study the experimental conditions for the separation of the enantiomers of ephedrine and pseudoephedrine were already optimized for each CD derivative using a slightly different capillary [22]. All measurements were performed in 50 m*M* phosphate buffer at pH 3.0 at 20 kV. In order to elucidate the migration order the samples were spikes with either isomer. The electropherograms are displayed in Fig. 2.

As observed in previous studies dealing with the resolution of the racemates of various phenethylamines [7–9] the resolution power of β-CD is low. β-CD is not able to baseline separate all enantiomers and diastereomers. However, the separation is good enough to determine the migration order: D-pseudoephedrine, D-ephedrine, L-ephedrine, L-pseudoephedrine. Interestingly, the migration order completely changes when using Diac. L-Ephedrine and L-pseudoephedrine are faster migrating than the corresponding D-enantiomers; in addition, the migration time of all isomers is much shorter than with β-CD. Using HDAS, again the L-enantiomers are migrating faster than the D-enantiomers, but the order of the diastereomers is reversed. As expected [6] the resolution power of the charged CD is very high resulting in a baseline separation of all isomers. Especially, the differences in the migration time between the ephedrine enantiomers is (about 5 min) very high. Moreover, the resolutions obtained in this study are higher than observed with the sulfobutyl ether β-CD by Tait et al. [23].

These CE findings suggest that the mode of complexation must be different for the various CDs.

3.2. UV measurements

In a previous paper [22] we already described Job

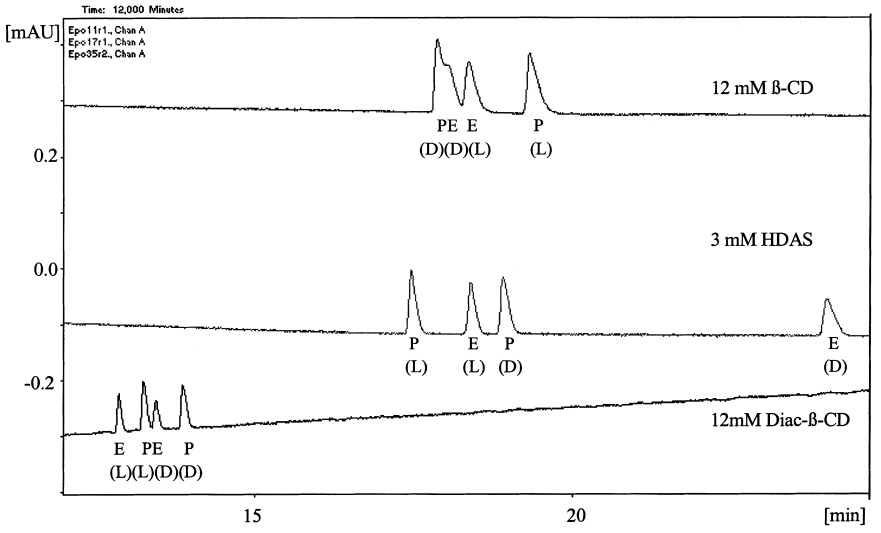


Fig. 2. Electropherograms of the isomeric mixture of ephedrine and pseudoephedrine in presence of 12 m*M* β-CD, 3 m*M* HDAS and 12 m*M* Diac using 50 m*M* phosphate buffer (KH_2PO_4) at pH 3 and 20 kV. Fused-silica capillary, 60 cm (effective length 50 cm)×50 μm I.D.

plots [24] produced for four ephedrine derivatives with all three CDs. Whereas symmetrical plot with a maximum at 0.5 were achieved with β-CD and Diac indicating a 1:1 stoichiometry, the plots obtained from HDAS were rather curved and difficult to reproduce. Recently, Abou-Hamdam et al. [25] followed the complex formation between a dye and α-CD via various intermediates over a time course of 1400 s by means of UV spectrometry. Corresponding time-dependent measurements using β-CD and the ephedrines did not show any change of the UV spectra for 2 h. In addition, the Job plots stay the same for the period, indicating that the defined 1:1 complexes were formed immediately. In contrast, using HDAS the UV spectra showed changes for the observed period but no systematical ones; correspondingly, the appearance of the Job plots changed to more curved non-symmetrical plots (data not shown) which do not give any information about the complex stoichiometry, but may indicate the formation of various complexes.

3.3. Mass spectrometry

In the cases of β-CD and Diac the quasi molecule ions corresponding to 1:1 complexes were found with ephedrine and methylephedrine. In addition, ammonium adducts of these complexes could be detected in the Q1 scan (see Fig. 3). A corresponding ephedrine–ammonium and methylephedrine–ammonium adduct could not be registered. In addition, no

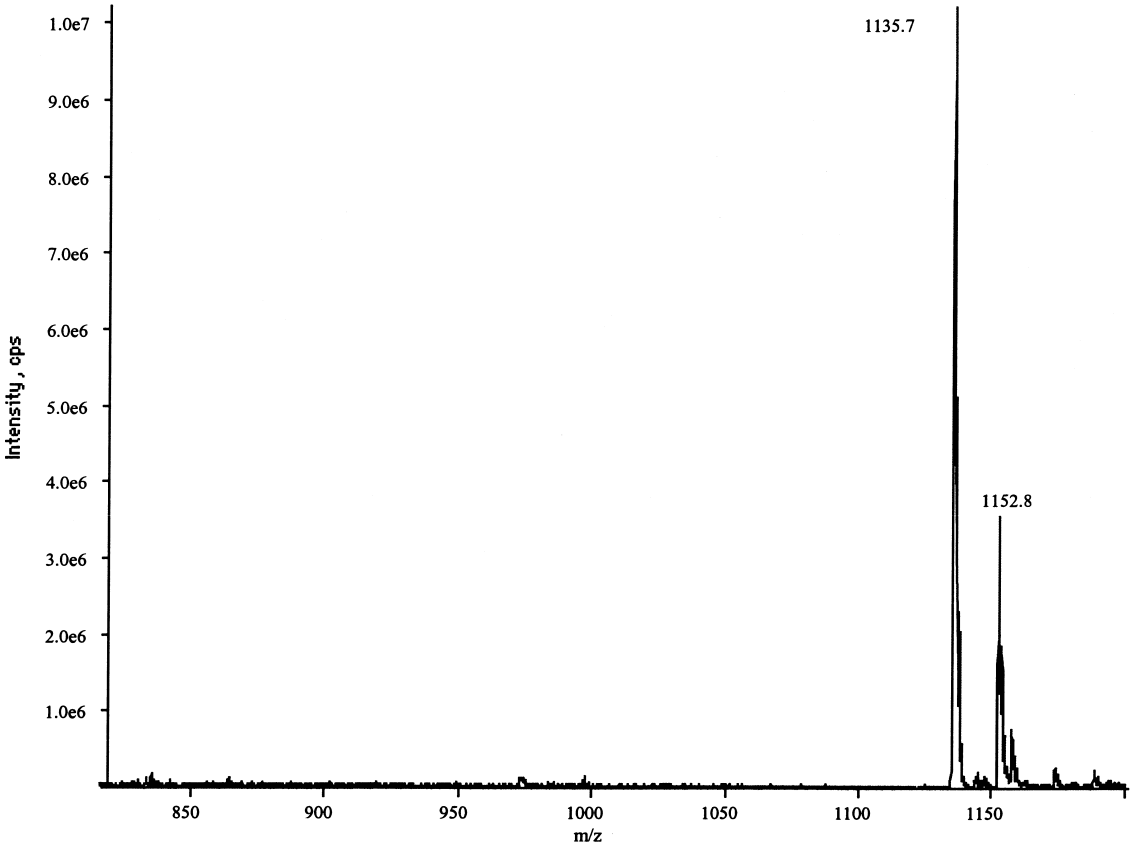


Fig. 3. Electrospray ionization mass spectrum (Q1 scan) of a complex between β-CD and ephedrine.

complex of a stoichiometry different from 1:1 was observed. In the case of HDAS no quasi molecule ions of the complexes could be detected.

3.4. NMR spectroscopy

Even though 600 MHz ^{1}H-NMR spectra of the HDAS complexes were recorded a considerable broadening of all signals is observed which again presumes that a variety of complexes is formed. Correspondingly, no systematical changes can be observed for most of the hydrogens of the ephedrines (see Fig. 4). Interestingly, the Job plots constructed using the CICSs of the methylephedrine hydrogens exhibit a negative broad maximum at a molar fraction of 0.66 (C<u>H</u>N and NC<u>H</u>$_3$; Fig. 4a and b). The CICSs of the aromatic hydrogens are not included in the Job plots because the pattern of the aromatic region strongly changes upon complexation and cannot unambiguously be assigned by correlation spectroscopy (COSY) experiments (Fig. 5). Regarding HDAS, only H5 and H6a/b show a strong downfield shift upon addition of increasing amounts of HDAS (cf. Ref. [22]). Due to this strong shift, the signal of H5 is migrating into the signal of H4 in the

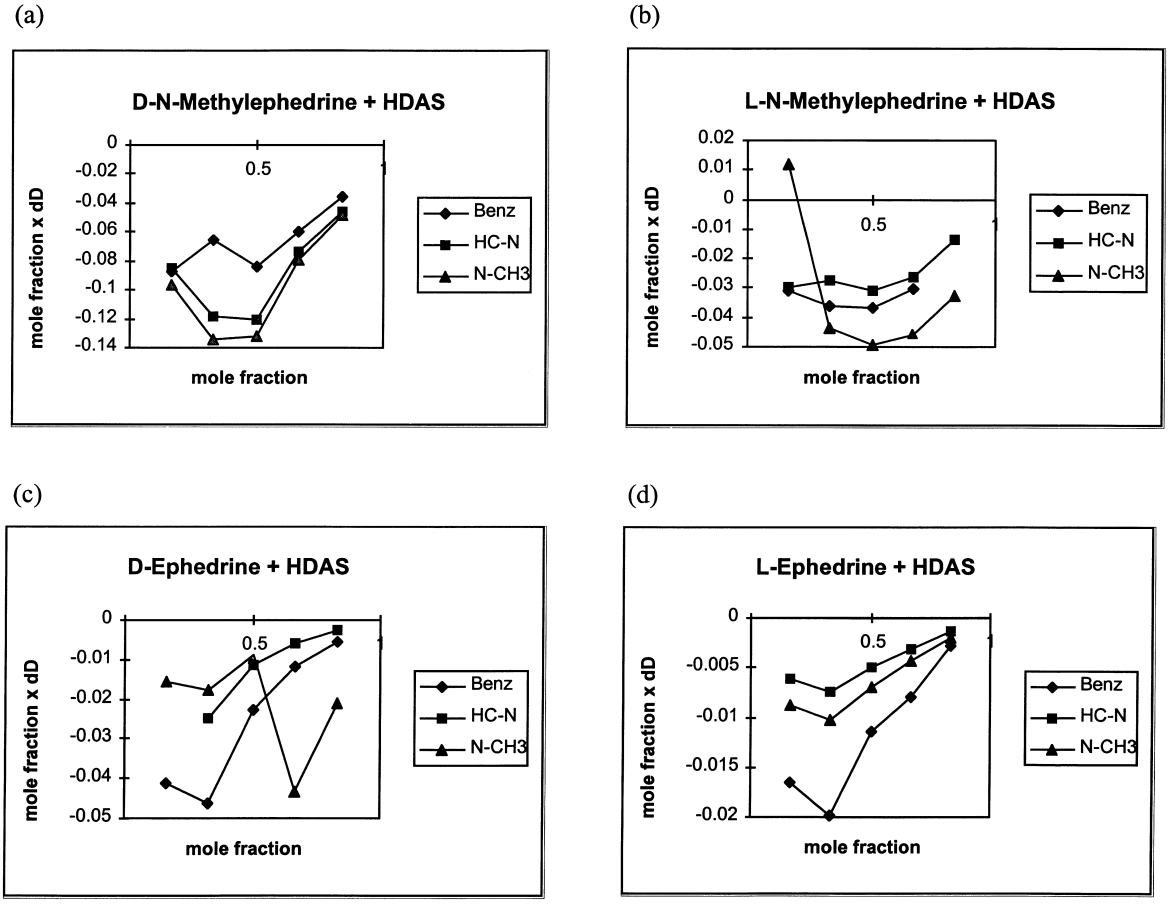


Fig. 4. Job plots of the interaction between HDAS and D-methylephedrine (a), L-methylephedrine (b), D-ephedrine (c) and L-ephedrine (d); *y*-axis=$\Delta\delta$ of the various ephedrine hydrogens, *x*-axis=mole fraction [guest]/([host]+[guest]).

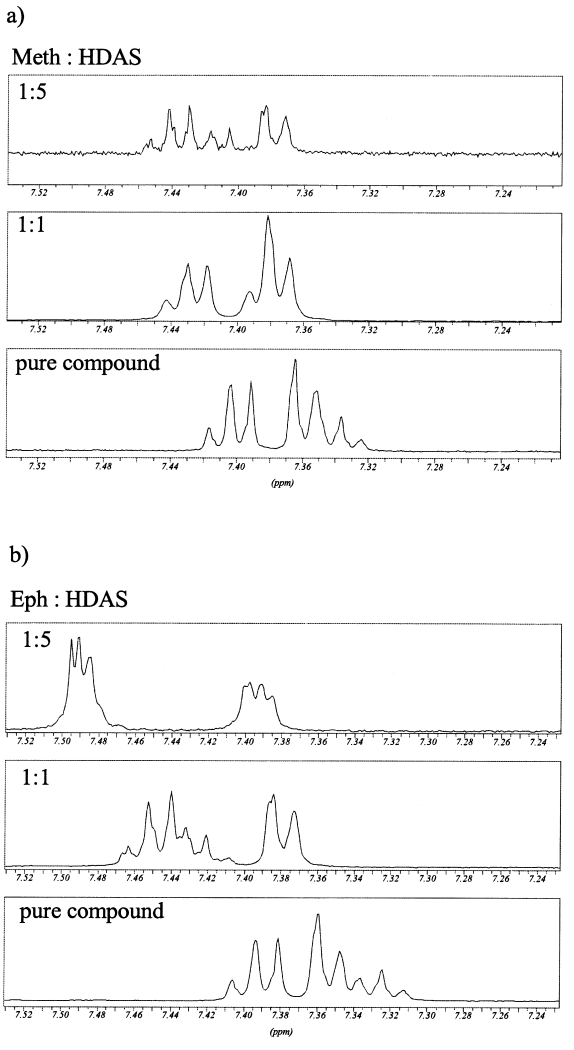


Fig. 5. ^{1}H-NMR spectra: aromatic region of various ratios of L-methylephedrine and HDAS (a) and D-ephedrine and HDAS (b).

case of the D-enantiomers. In addition, the Job plots built with the CICSs of the HDAS hydrogens do not show significant differences between the complexes formed with the enantiomers of ephedrine and methylephedrine.

Taking the CICSs together, it can be stated that H5 and H6a/b of the HDAS and the aromatic hydrogens of the ephedrine derivatives are involved in the complexation.

The ROESY experiments individually performed with the enantiomers of ephedrine and methylephedrine support the picture: whereas a lot of intramolecular NOEs of ephedrine and methylephedrine were observed upon complexation with the neutral Diac [9], only a very few intramolecular NOEs were found with the charged HDAS (see Table 1). Since an average of a variety of conformations can reduce the intensity of intramolecular NOEs the small amount of observed few intramolecular NOEs indicate that the ephedrine derivatives do not take one defined conformation upon complexation.

Even more interesting are the intermolecular NOEs (see Table 2). Again, a clear cut picture was observed upon complexation with Diac. Strong cross peaks between the benzyl moiety of the ephedrines and H3 and H5, both sitting inside the CD cavity, indicate a well defined inclusion complex [9]. With HDAS more or less weak cross peaks between cyclodextrin hydrogens and the ephedrine and *N*-methylephedrine hydrogens seemed to be randomly distributed. Interestingly, both enantiomers of ephedrine showed strong cross peaks between the benzyl moiety and H5, sitting deeply inside the cavity. This clearly indicates that the phenyl ring is located inside the cavity. However, additional cross peaks were found between the benzyl moiety and HCCH3 of the ephedrine and H1, H4, H6 and COCH3 of HDAS which are located outside the cavity. Moreover, most of these cross peaks are only observed with either enantiomer which might explain the chiral recognition. The huge differences in the CICSs of H5 and both H6s between the enantiomers support this hypothesis. From these findings it might be concluded that on average the aromatic ring is sitting inside the cavity and most of the side chain is looking out of the narrow rim of HDAS which might be caused by an ion–ion attraction of the negative charges of HDAS and the positively charged nitrogen of ephedrine (see Fig. 6). The amount of intermolecular NOEs found with methylephedrine is smaller. Again, cross peaks between the phenyl ring and inside hydrogens (H3, H5) of HDAS were observed. In addition, stereoselective NOEs with hydrogens outside the cavity were found resulting in a similar structural interpretation.

Table 1
Intramolecular NOEs observed in D- and L-ephedrine and methylephedrine in the presence of HDAS

			D-Ephedrine		
	Arom.	**H**COH	**H**CCH$_3$	NH-C**H**$_3$	CH-C**H$_3$**
Arom.					
HCOH				+	
HCCH$_3$					
NH-C**H**$_3$					(+)
CH-C**H$_3$**	+				
			L-Ephedrine		
			D-Methylephedrine		
	Arom.	**H**COH	**H**CCH$_3$	N-C**H**$_3$	CH-C**H$_3$**
Arom.			+		
HCOH					
HCCH$_3$				+	+
N-C**H**$_3$		+			+
CH-C**H$_3$**	+	+	+	+	
			L-Methylephedrine		

Table 2
Intermolecular NOEs observed between ephedrine and methylephedrine and HDAS

HDAS-CD	D- and L-Ephedrine				
	Arom.	**H**COH	**H**CCH$_3$	NH-C**H**$_3$	CH-C**H$_3$**
H1′	ll[a]	–	–	d	–
H2′	–	–	–	–	–
H3′	l	–	d	–	–
H4′	d ll	l	–	–	–
H5′	d ll	dd ll	d	–	–
H6a′	–	–	–	–	–
H6b′	d l	–	–	–	–
CH$_3$-CO	–	dd	(d?)	–	(d)
	D- and L-Methylephedrine				
	Arom.	**H**COH	**H**CCH$_3$	NH-C**H**$_3$	CH-C**H$_3$**
H1′	l	–	–	l	–
H2′	–	–	–	–	–
H3′	d l	–	d	d	–
H4′	d l	–	–	–	–
H5′	d l	–	d	–	–
H6a′	l	–	–	–	–
H6b′	l	–	–	–	–
CH$_3$-CO	l	–	–	–	–

[a] ''d'' and ''l'' represent NOEs observed for D- and L-ephedrine, respectively. Two letters indicate that a strong NOE was found, a single letter reflects a weaker NOE. Furthermore, letters given in parentheses indicate that the NOEs are very weak hence are difficult to interpret.

4. Conclusion

Even though we cannot give the geometry of the HDAS–liganded complexes, we can state that a switch of the mode of recognition has taken place when going from neutral CDs, mostly showing inclusion complexes, to negatively charged CDs which seem to form manifold complexes. It is likely, that on average the phenyl ring is sitting inside the cavity and the side chain is pointing outside the narrow rim of HDAS which offers the possibility to form ion–ion interactions between the positively charged nitrogens of the ephedrines and the negatively charged sulfato groups of HDAS. Similar observations were recently reported by Galaverna et al. [26] for phenyllactic acid complexed with a 6-histamine substituted CD. However, complexes formed by an attachment of ephedrine or methylephedrine outside to HDAS as well as 2:3 and 3:2 complexes are also possible. Due to the manifold complexes formed it is impossible to derive a defined picture of the complexation of both enantiomers.

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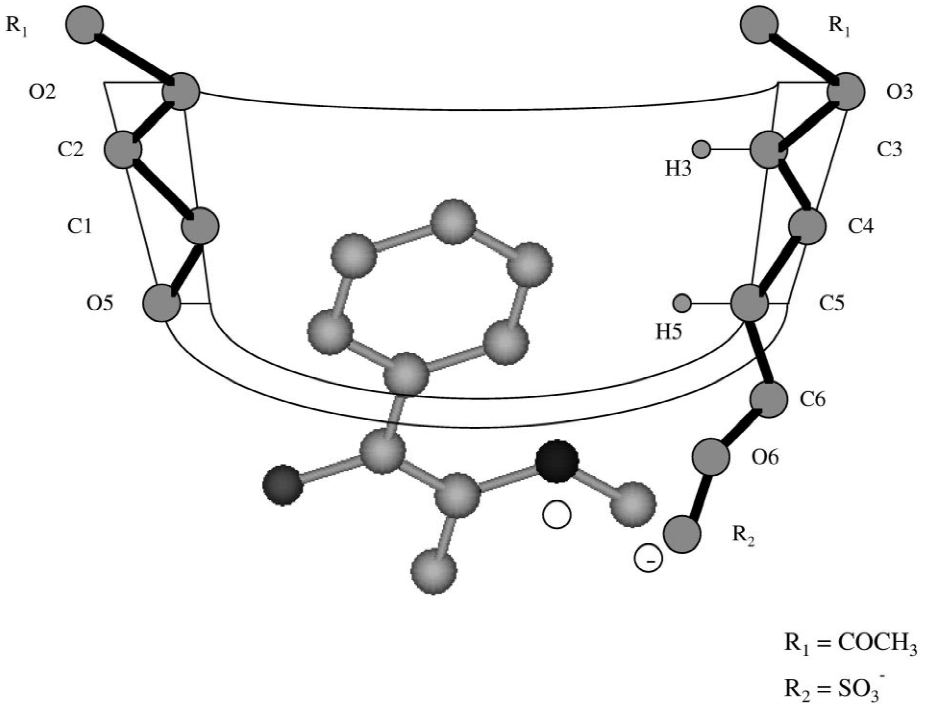

Fig. 6. Proposed structure of the complex between the ephedrine and HDAS derived from the ROESY experiments and the CICSs.

Würzburg, for performing some electrospray ionization MS experiments.

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