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Separation of brompheniramine enantiomers by capillary electrophoresis and study of chiral recognition mechanisms of cyclodextrins using NMR-spectroscopy, UV spectrometry, electrospray ionization mass spectrometry and X-ray crystallography

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Abstract

Opposite migration order was observed for the enantiomers of brompheniramine {*N*-[3-(4-bromphenyl)-3-(2-pyridyl)propyl]-*N*,*N*-dimethylamine} (BrPh) in capillary electrophoresis (CE) when native β-cyclodextrin (β-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) were used as chiral selectors. NMR spectrometry was applied in order to obtain information about the stoichiometry, binding constants and structure of the selector–selectand complexes in solution. The data were further confirmed by UV spectrometry and electrospray ionization mass spectrometry. The structure of the complexes in the solid state was determined using X-ray crystallography performed on the co-crystals precipitated from the 1:1 aqueous solution of selector and selectand. This multiple approach allowed an elucidation of the most likely structural reason for a different affinity (binding strength) of BrPh enantiomers towards β-CD and TM-β-CD. However, the question about a force responsible for the opposite affinity pattern of BrPh enantiomers towards these CDs could not be answered definitely.

Keywords: Enantiomer separation; Chiral selectors; Pharmaceutical analysis; Brompheniramine; Cyclodextrins

1. Introduction

Cyclodextrins (CDs) belong to the group of the most widely used chiral selectors in CE. Analysis of the recent literature indicated that almost 85% of all chiral CE separations are performed using CDs as chiral selectors [1]. Despite the intensive application of CDs for enantioseparations, not only in CE but also in other instrumental separation techniques [2,3], the chiral recognition mechanisms of these macrocyclic compounds are still not clearly understood [4].

From a mechanistic point of view of special interest are the cases when a chemical modification of a CD causes the reversal of the affinity pattern of

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enantiomers to the host. High separation efficiency and the possibility of using a large excess of a chiral selector in CE allows CDs to be easily screened on their chiral recognition ability and pattern. As previous CE studies indicated, the opposite affinity of enantiomers towards native β-CD and its permethylated analog, heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), is a common rather than exclusive case [5–10]. For instance, the affinity of the enantiomers of the chiral Ca^{2+} channel blocking drug verapamil [6], the chiral antihistaminic drugs dimethindene [7] and chlorpheniramine [8], as well as methadone used as opiate substituent [9] and chiral aromatase inhibitor aminoglutethimide [10] is opposite to native β-CD and TM-β-CD.

In this work the opposite affinity pattern of the enantiomers of the chiral antihistaminic drug brompheniramine {*N*-[3-(4-bromphenyl)-3-(2-pyridyl)propyl]-*N*,*N*-dimethylamine} (BrPh) towards native β-CD and TM-β-CD was studied. The analyte–selector interactions were investigated using NMR spectroscopy, UV spectrometry, electrospray ionization mass spectrometry (ESI-MS) and X-ray crystallography in order to understand the nature of the forces which may be responsible for the significantly different binding strengths and the opposite affinity pattern of BrPh enantiomers towards these two CDs.

2. Experimental

2.1. Chemicals

Racemic BrPh and its (+)-enantiomer as maleate salts were obtained from Sigma (Deisenhofen, Germany). α-, β- and γ-CD, carboxymethyl-β-CD (CM-β-CD) with a degree of substitution (D.S.) 3.5, hydroxypropyl-α-CD (D.S. 4.2) were a gift from Wacker Chemie (Munich, Germany). Heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD) and heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) were from Fluka (Buchs, Switzerland). Deuterium oxide and analytical grade KH_2PO_4, Na_2HPO_4, triethanolamine and H_3PO_4 were purchased from Merck (Darmstadt, Germany).

2.2. Apparatus

2.2.1. CE

CE separations were performed using a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector. The sample was injected by pressure, 0.5 psi for 3 s (1 p.s.i. = 6894.76 Pa). A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 31.2 cm (21 cm effective length) 50 μm I.D., was used. Other experimental conditions are given in the legends to the figures.

2.2.2. NMR spectroscopy

^1H and ^{13}C-NMR spectral analyses were performed using a Varian Gemini 200 NMR-spectrometer at 200 MHz (^1H) and 50 MHz (^{13}C). ^2H_2O was used as a solvent, and a solution of tetramethylsilane (TMS) in tetrachloromethane served as external standard. The stoichiometry of BrPh–CD complexes was determined by the continuous variation method [5–8,10–12] based on the ^{13}C-NMR chemical shifts. The same signals and Scott's method [5–8,10,13] were used for the calculation of the binding constants.

One dimensional (1D) ROESY experiments were performed using 600 MHz Unity Plus equipment from Varian according to the pulse program described in Ref. [14].

2.2.3. UV spectrometry

UV absorbance of the aqueous solutions of (+)-BrPh and β-CD with a constant total molar concentration of 20 M^{-3} and a different ratio of the components was measured using a Shimadzu UV-160 UV–visible spectrometer with 1 cm optical path length.

2.2.4. Electrospray ionization mass spectrometry

ESI-MS spectra of β-CD and TM-β-CD and their complexes with (±)-BrPh were obtained using an ion trap mass spectrometer (LCQ, Finnigan, Branford, CT, USA) equipped with an electrospray interface. The solution of 0.6 mg/ml (±)-BrPh or an equimolar amount of CDs in double distilled water, or alternatively, their 1:1 mixture, were introduced

into the ion source of the mass spectrometer with a flow-rate 5 μl/min using a syringe pump. The ionization voltage was 6 kV for the sample of (±)-BrPh and CD and 4 kV for their mixture. The temperature of the inlet capillary was 200°C. The detection was performed in the positive ion mode.

2.2.5. X-Ray crystallography

Monocrystals of (+)-BrPh maleate were obtained by dissolving it in methanol and slowly evaporating the solution at room temperature. Co-crystals of (+)-BrPh maleate and β-CD were obtained by preparing the equimolar saturated solution at room temperature and storing the solution in a refrigerator at +4°C for several days. This technique did not allow to obtain the co-crystals between (+)-BrPh maleate and TM-β-CD. The crystals were precipitated from this solution. However, as subsequent X-ray crystallographic studies indicated, these were crystals of TM-β-CD without included (+)-BrPh molecules. Therefore, in this case an equimolar amount of TM-β-CD was added to the emulsion of (+)-BrPh as a free base in water (5 mg/ml) and stored in a refrigerator at +4°C for several days. The crystals were isolated by filtration.

Lattice parameters and reflection intensities of (+)-BrPh maleate were measured on an Enraf-Nonius four-circle CAD4 diffractometer (Nonius, Solingen, Germany) with graphite-monochromated Mo-K_α radiation. Unit-cell dimensions and standard deviations were obtained by a least-squares fit to 25 reflections. Reflections were processed using profile analysis and corrected for Lorentz, polarization and absorption effects, the latter was performed by the Ψ-scan method. The heavy atoms were located by Patterson synthesis using the program SHELXS 86 [15] and extended by the difference Fourier method using the SHELXL 93 [16] program. Full-matrix least-squares refinement on F^2 was employed. Hydrogen atoms were placed at geometrically calculated sites and refined with isotropic displacement parameters. All non-hydrogen atoms were refined with anisotropic thermal parameters.

X-Ray diffraction data of the (+)-BrPh maleate-β-CD complex were collected with a Nonius KappaCCD diffractometer (Nonius, Delft, The Netherlands). The structure was solved with data from the isomorphous structure of a β-CD inclusion complex [17]. After several cycles of structure factor calculation followed by Fourier synthesis, the electron density map showed the (+)-BrPh maleate molecule sandwiched between two β-CD molecules. Refinement was carried out using a combination of difference Fourier synthesis and full-matrix and block-diagonal least-squares calculations. During refinement of the anisotropic temperature factors of all non-hydrogen atoms, the guest molecule revealed an occupancy factor of 0.50. In the final cycles of structure factor calculation, hydrogen atoms were calculated at fixed positions and refined with isotropic displacement parameters.

X-Ray diffraction data of the (+)-BrPh–TM-β-CD complex were collected on a STOE imaging plate diffraction system (STOE, Darmstadt, Germany) with graphite-monochromated Mo-K_α radiation. The crystal structure was determined by the replacement method using sets of coordinates of an isomorphous complex [18] and refined by the full-matrix least-squares method with difference Fourier synthesis. All non-hydrogen atoms were refined with anisotropic thermal parameters and the hydrogen atoms were included at fixed, calculated positions. The occupancy factor (0.42) of the guest molecule was estimated from the electron-density map, but was not refined.

Crystal and structure data: (+)-BrPh maleate: triclinic, space group *P1*, $a = 6.070(1)$, $b = 9.826(2)$, $c = 17.803(4)$, $\alpha = 104.37(3)°$, $\beta = 97.12(3)°$, $\gamma = 96.01(3)°$, $V = 1010.5(3)$ Å^3, $Z = 2$, $D_{calcd} = 1.431$ g cm^{-1}, *GooF* $S = 0.98$, final $R_1 = 0.043$ and $wR_2 = 0.085$ for 2291 reflections with $F_o \geq 4\sigma(F_o)$.

(+)-BrPh maleate–β-CD complex: triclinic, space group *P1*, $a = 15.200(3)$, $b = 15.210(3)$, $c = 15.660(3)$, $\alpha = 103.87(3)°$, $\beta = 103.60(3)°$, $\gamma = 103.60(3)°$, $V = 3250.2(1)$ Å^3, $Z = 1$, $D_{calcd} = 1.342$ g cm^{-1}, *GooF* $S = 1.28$, final $R_1 = 0.144$ and $wR_2 = 0.351$ for 10 838 reflections with $F_o \geq 4\sigma(F_o)$.

(+)-BrPh–TM-β-CD complex: orthorhombic, space group $P2_12_12_1$, $a = 14.571(3)$, $b = 21.562(4)$, $c = 27.333(5)$, $V = 8587(3)$ Å^3, $Z = 4$, $D_{calcd} = 1.209$ g cm^{-1}, *GooF* $S = 0.95$, final $R_1 = 0.151$ and $wR_2 = 0.358$ for 2418 reflections with $F_o \geq 4\sigma(F_o)$.

3. Results and discussion

3.1. Separation of the enantiomers

The separations of a (+)/(−)=2/1 mixture of the BrPh enantiomers in the presence of native β-CD and TM-β-CD are shown in Fig. 1. Under the experimental conditions of this CE experiment BrPh (pK_1=3.9–4.9; pK_2=9.2–10.1) [19] is present as a cation charged species. Different concentrations of the CDs were used because the affinity of the BrPh enantiomers towards TM-β-CD is much lower than towards β-CD. The migration order of the enantiomers was opposite in the presence of these two CDs. (+)-BrPh was the more tightly bound enantiomer in the case of native β-CD, whereas (−)-BrPh exhibited higher affinity towards TM-β-CD.

The enantioseparation of BrPh in CE has been studied by several groups before [20–23]. However, the enantiomer migration order has not been addressed in these studies. In HPLC studies with native β-CD and TM-β-CD as chiral additives of the mobile phase [24], the opposite elution order of the BrPh enantiomers was observed. Despite this fact it was suggested that both CDs exhibit the same chiral recognition pattern towards BrPh. The opposite elution order was explained in the following way: β-CD resolves the enantiomers in the mobile phase (it will most likely accelerate the analyte). In contrast, TM-β-CD preadsorbs on the silica and then resolves the enantiomers as an ''in-situ'' stationary phase (it will decelerate the analyte). This would, in principle, result in opposite elution order of the enantiomers.

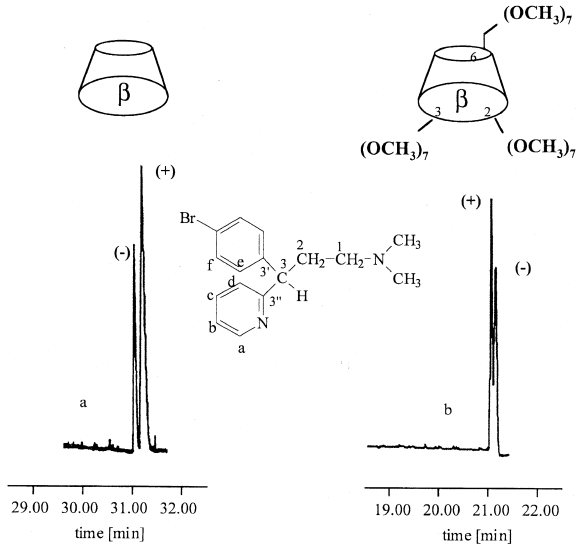

Fig. 1. Electropherogram of the mixture of BrPh enantiomers [(+)/(−)=2/1] in the presence of (a) 18 mg/ml β-CD and (b) 80 mg/ml TM-β-CD. Buffer: 100 m*M* phosphoric acid adjusted to pH 3.0 with triethanolamine. Applied voltage 20 kV. Other conditions as described in the Experimental section.

In contrast to the above mentioned HPLC study, the effect of a stationary phase is absent in CE. Thus, CE studies unambiguously show that the enantiomers of BrPh exhibit the opposite affinity pattern towards β-CD and TM-β-CD. This is perhaps the most likely reason for the opposite elution order of the enantiomers in the aforementioned HPLC study [24].

Several other CDs were also studied (Table 1). It was impossible to observe enantioseparations with native α- and γ-CDs, as well as with DM-β-CD. Interestingly, the enantiomer migration order with HP-α-CD was the same as with TM-β-CD.

3.2. Stoichiometry of BrPh–CD complexes

The stoichiometry of the BrPh complexes with β-CD and TM-β-CD was studied using four different techniques: the continuous variation method based on the chemical shifts in ^{13}C-NMR and on the absorbance in UV-spectra, ^{1}H-NMR analysis of the crystals obtained by precipitation from the solution, and ESI-MS spectra of equimolar solutions of BrPh and CDs.

Table 1
Enantioseparation of (±)-BrPh in the presence of different CDs[a]

CD	Concentration (mg/ml)	Migration time (min)		Migration order
		t_1	t_2	
β-CD	18	30.636	31.438	−/+
DM-β-CD	18	28.807	28.807	
TM-β-CD	80	21.225	21.532	+/−
	120	24.849	25.013	+/−
CM-β-CD	0.4	10.628	11.179	−/+
	0.5	11.094	11.470	−/+
α-CD	25	29.788	29.788	
	50	36.355	36.355	
HP-α-CD	30	27.304	28.304	+/−
γ-CD	25	20.105	20.015	

[a] Experimental conditions as in Fig. 1.

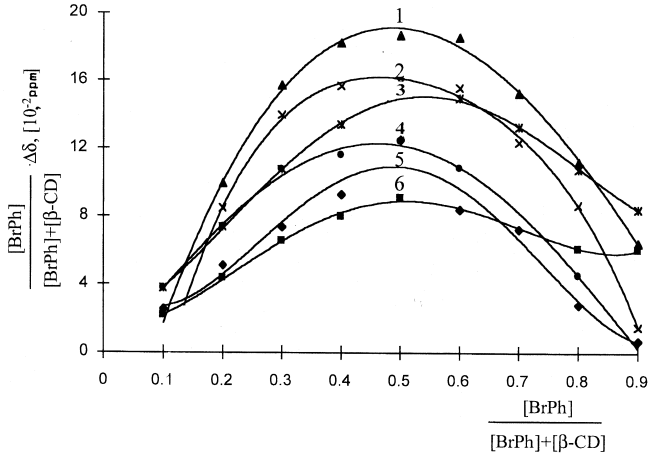

Fig. 2. Continuous variation plots for (±)-BrPh–β-CD complex based on the complexation induced chemical shift (CICS) in ^{13}C-NMR spectra. 1,2-CICS for the carbon atom of CH group in (+)- and (−)-BrPh, respectively; 3,4-CICS for the carbon atom in position **a** in (+)- and (−)-BrPh, respectively; 5,6-CICS for the carbon atom in position **e** in (+)- and (−)-BrPh, respectively.

The results obtained for the complex of BrPh with β-CD were contradictory. As shown in Fig. 2 it was possible to find several sets of well-split ^{13}C-NMR signals indicating that the stoichiometry of the complex is 1:1. The continuous variation plots constructed according to the ^{13}C resonance signals of CH group and carbon atoms in positions **a** of the pyridin moiety and **e** in the *p*-Br-phenyl moiety of BrPh molecule are shown in Fig. 2. On the other hand, there were sets of other signals, for example, of carbon atoms in CH_2 and $N(CH_3)_2$ groups, which were not useful for constructing Job's plot. The latter means that there is not only one uniform complex present in the solution of (±)-BrPh and β-CD. The data obtained using UV spectrometry supported a formation of multiple complexes (data not shown here).

ESI-MS has the potential to be a useful technique for studying individual complexes having different stoichiometry in their mixture. ESI-MS spectra of (±)-BrPh/β-CD solution support a 1:1 stoichiometry but do not give any indication about the existence of a complex of any other stoichiometry (Fig. 3). Thus, based on these results one can conclude that multiple complexation is possible in the solution of (±)-BrPh and β-CD. However, these complexes in solution apparently have a different structure, but most likely all of them favor a 1:1 stoichiometry.

For a more detailed study the monocrystals of (+)-BrPh (maleate salt) β-CD complex were obtained by their co-precipitation from the aqueous solution. The crystals were carefully washed with bidistilled water, methanol and acetone. Then the crystals were dissolved in deuterium oxide and ^{1}H-NMR spectra were taken. The integrals of the selected (+)-BrPh and β-CD protons indicated a complex stoichiometry with two β-CD molecules on each molecule of (+)-BrPh maleate (Fig. 4). Several crystals were analyzed and in most of the cases the above mentioned 1:2 stoichiometry was confirmed.

The stoichiometry of the BrPh complex with TM-β-CD was relatively clear from data from all four techniques. The continuous variation plot shown in Fig. 5 as well as ^{1}H-NMR spectra of monocrystals precipitated from a stoichiometric solution of (+)-BrPh and TM-β-CD (Fig. 6) support this conclusion.

3.3. Binding constants in BrPh–CD complexes

In order to examine the affinity pattern of BrPh enantiomers observed in CE towards β-CD and TM-β-CD more detailed NMR studies were performed. The values of enantioselective complexation induced chemical shifts (CICS) in ^{13}C NMR spectra of the complexes of (±)-BrPh with both β-CD and TM-β-CD were treated according to Scott's technique [5–8,13]. Linear dependencies observed in both cases (data not shown here) indicate that the stoichiometry

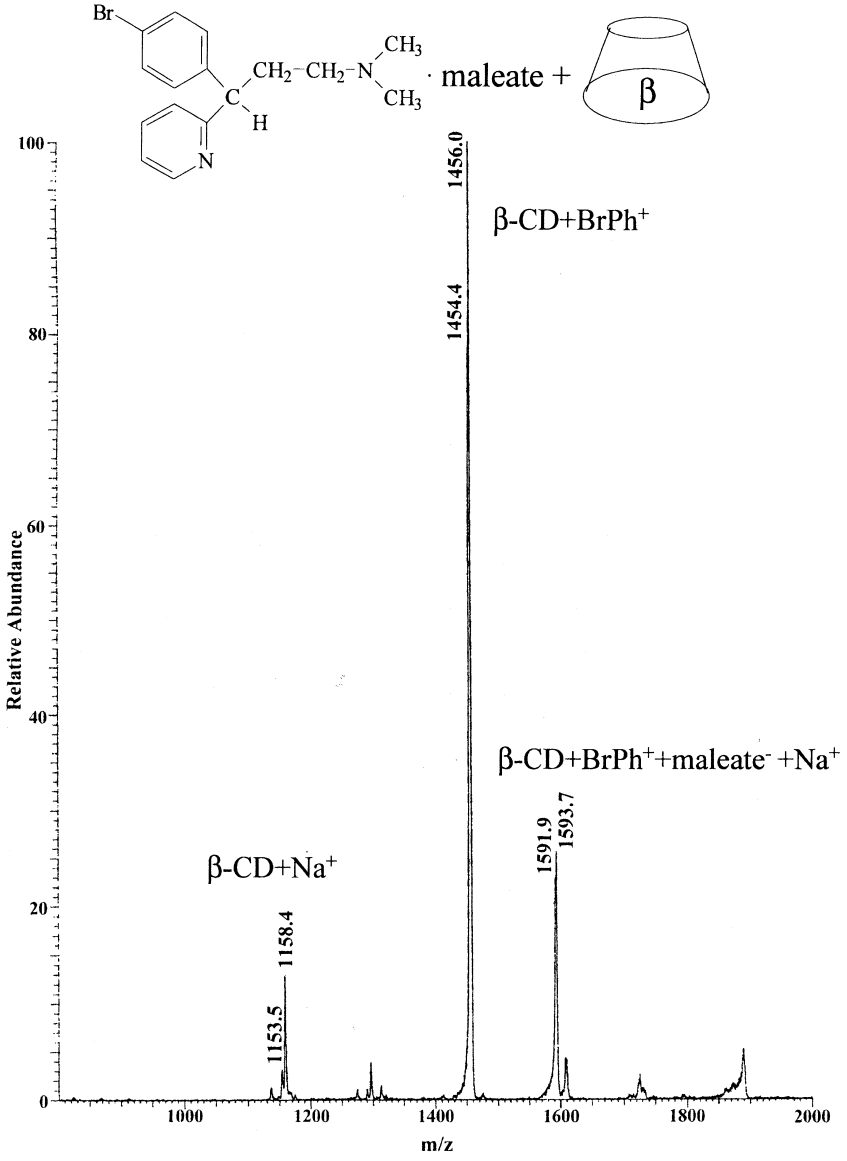

Fig. 3. ESI-MS spectrum of (±)-BrPh–β-CD complex.

of the complexes in solution favors 1:1. The binding characteristics derived from the Scott's plots are summarized in Table 2. As shown in this table the opposite affinity pattern of (±)-BrPh enantiomers towards β-CD and TM-β-CD is confirmed by the binding data obtained from independent NMR experiments. However, it must be noted that the binding constants in NMR spectroscopy were determined assuming 1:1 stoichiometry of the complexes, which is not absolutely unambiguous, in particular in the case of BrPh–β-CD complex, as mentioned above.

3.4. Structure of (+)-BrPh–CD complexes

The structure of the (+)-BrPh–CD complexes in solution and the solid state was studied using 1D ROESY NMR experiments and X-ray crystal-

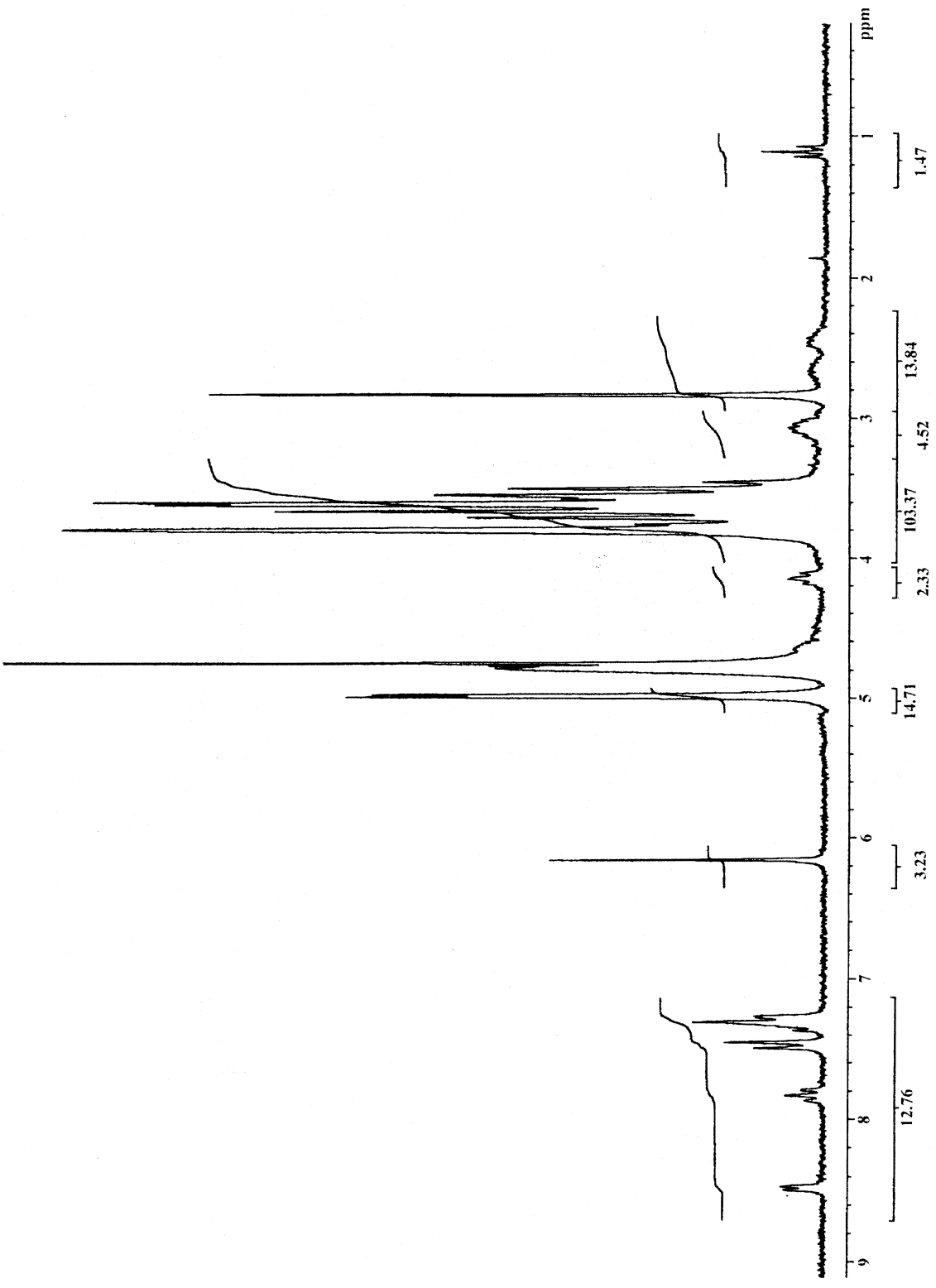

Fig. 4. ^{1}H-NMR spectrum of the (+)-BrPh–β-CD crystals precipitated from the equimolar aqueous solution of the components.

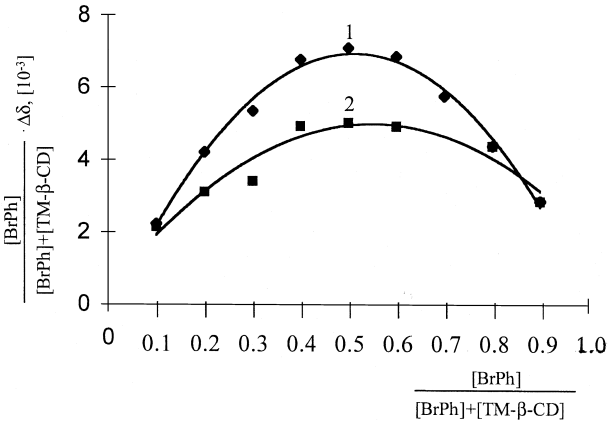

Fig. 5. Continuous variation plots for (±)-BrPh–TM-β-CD complex based on the complexation induced chemical shift in ^{13}C-NMR spectra. 1,2- CICS for the carbon atom of CH_2 group in (+)- and (−)-BrPh, respectively.

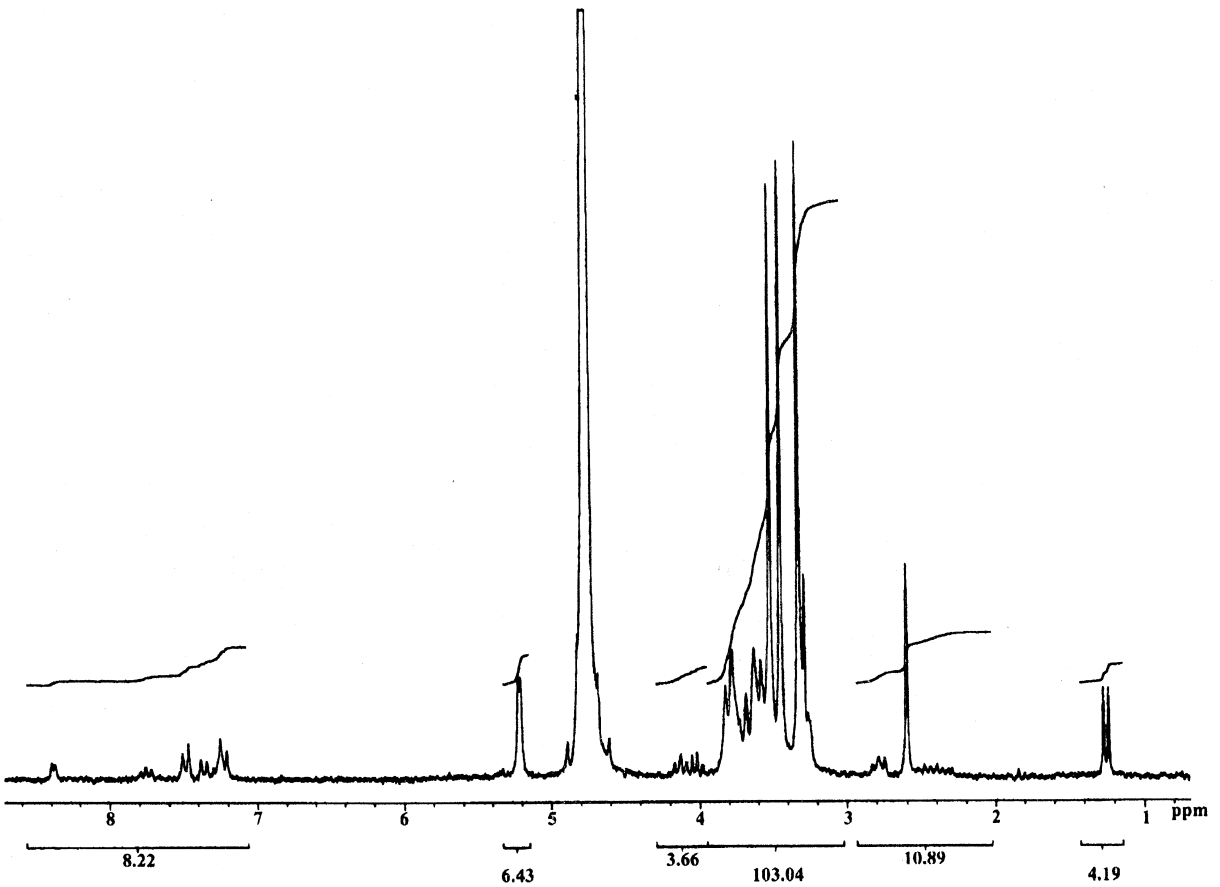

Fig. 6. ^{1}H-NMR spectrum of the (+)-BrPh–TM-β-CD crystals precipitated from the equimolar aqueous solution of the components.

Table 2
Complexation-induced chemical shift (CICS) differences at saturation ($\Delta\delta_s$) and the apparent binding constants (K_a) of (±)-BrPh with β-CD and TM-β-CD

Cyclodextrin	Chemical shift at the saturation, $\Delta\delta_s$ (Hz)		Apparent binding constants K_a (M^{-1})		α[a]
	(+)-BrPh	(−)-BrPh	(+)-BrPh	(−)-BrPh	
β-CD	55.25	47.62	822.7	798.5	1.03
TM-β-CD	13.81	8.71	99.5	207.1	2.08

[a] α was defined as the ratio of higher apparent binding constant to lower one.

lography, respectively. Before studying the structure of the complexes, the structure of (+)-BrPh as a maleate salt was determined using X-ray crystallography. Two different conformations of the BrPh were observed with a distribution 1:1 (Fig. 7). Although the structures in the solid state and in solution will not a priori be the same, it is possible that this structural ''duality'' of BrPh is, at least in part, responsible for the non-uniform complex formation between BrPh and β-CD.

1D ROESY experiments (Fig. 8a) performed on the (+)-BrPh–β-CD complex indicated that the *p*-bromphenyl moiety of BrPh molecule is involved in the complex formation. This group enters the cavity of β-CD from the secondary side and deeply penetrates it. The indications for this conclusion are the following: (a) no significant NOE response was observed on the pyridine protons by irradiating β-CD protons inside a cavity (H-3, H-5); (b) by irradiating H-5 protons (not absolutely selectively) in the β-CD the higher NOE response was observed on H_f protons of BrPh molecule compared to H_e protons. This means that H_f protons are more closely located to H-5 protons of β-CD; (c) in contrast to this, by irradiating the H-3 protons in the β-CD molecule, a higher NOE response was observed on H_e than on H_f protons of (+)-BrPh. These data allow to deduce a structure for the complex of (+)-BrPh and β-CD which is shown in Fig. 9a.

Surpassingly, a weak but positive NOE response was observed also on the protons of the maleate counterion when irradiating protons located inside the cavity of β-CD (Fig. 8a). This indicates an involvement of this ion in inclusion complex formation. However, based on simple geometric considerations, both of the above mentioned groups can not be included inside the cavity of the same β-CD molecule. X-Ray crystallographic studies described below provide a plausible explanation for this observation.

The 1D ROESY experiment on (+)-BrPh–TM-β-CD complex could be performed in a more selective way (Fig. 8b). This experiment showed the complex was formed again by insertion of the *p*-bromphenyl moiety of (+)-BrPh into the cavity of TM-β-CD. However, the analyte does not enter the TM-β-CD cavity as deeply as the cavity of β-CD (Fig. 9b). The indication for this is that by irradiating the H-3 protons in TM-β-CD the NOE response on H_f protons was much stronger than on the H_e protons of (+)-BrPh. In addition, by irradiating the H-5 protons of TM-β-CD a rather weak NOE response was observed only on the H_f protons and no effect was observed at all on the H_e protons. These structural data support a lower affinity of the enantiomers of BrPh towards TM-β-CD compared to β-CD. However, the opposite affinity of the BrPh enantiomers towards these two CDs can not be explained based on these structural data.

In order to obtain information on the structure of the complex(es) between (+)-BrPh and CDs in the solid state the monocrystals precipitated from the selector–selectand solutions were studied using X-ray crystallography.

The solid-state structure of the (+)-BrPh–β-CD complex supports the structural data obtained in the 1D ROESY NMR experiments for a deep inclusion of the *p*-bromphenyl moiety of (+)-BrPh into the β-CD cavity. In addition, the NOE response observed in the ID ROESY experiment for the maleate protons by irradiating the β-CD protons inside the cavity can be explained based on the X-ray crystallographic data in the solid state. As shown in Fig. 10a, a (+)-BrPh molecule is sandwiched between two β-CD molecules. The cavity of one of these β-CDs

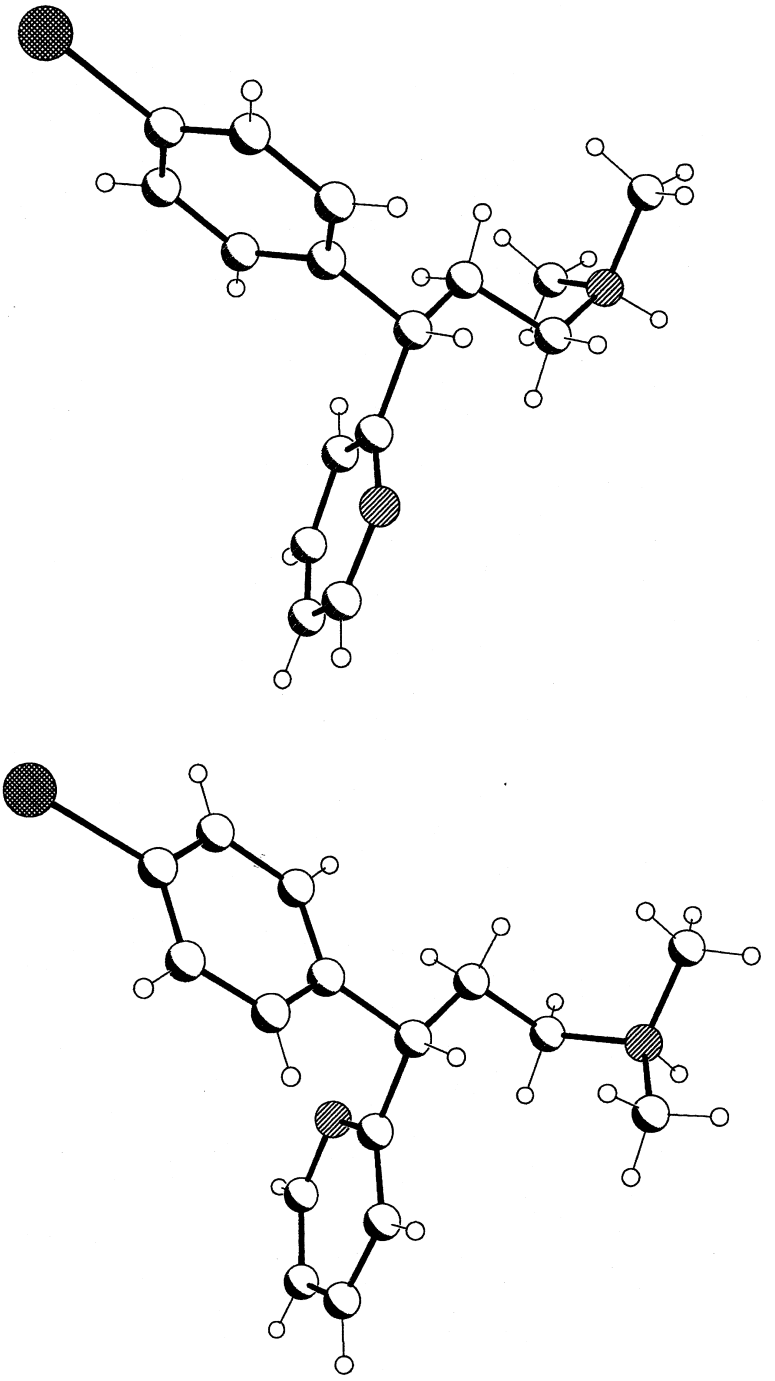

Fig. 7. The structure of two rotamers of (+)-BrPh derived from X-ray crystallographic data.

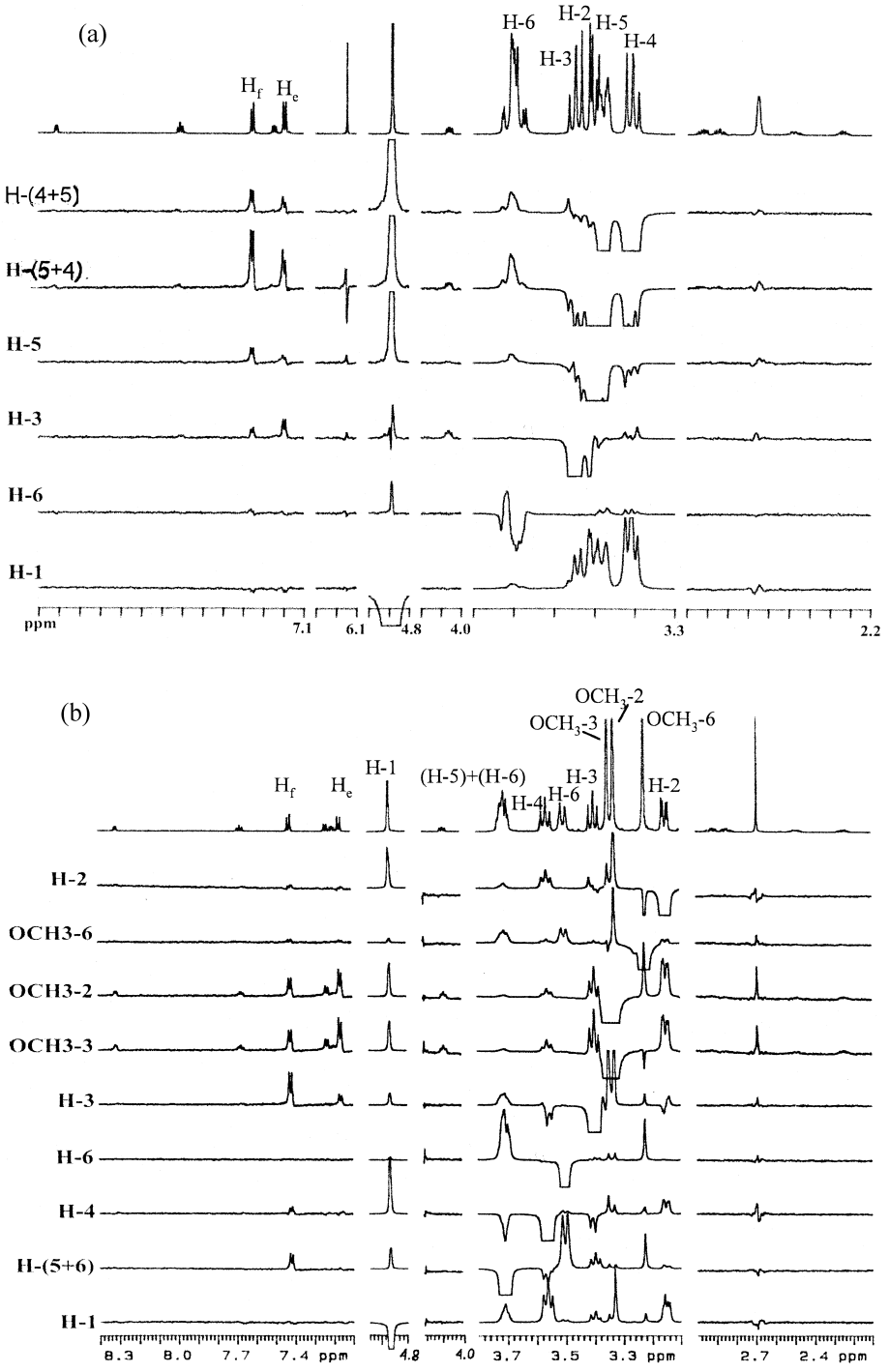

Fig. 8. 1D ROESY spectra of (a) (+)-BrPh–β-CD and (b) (+)-BrPh–TM-β-CD complexes.

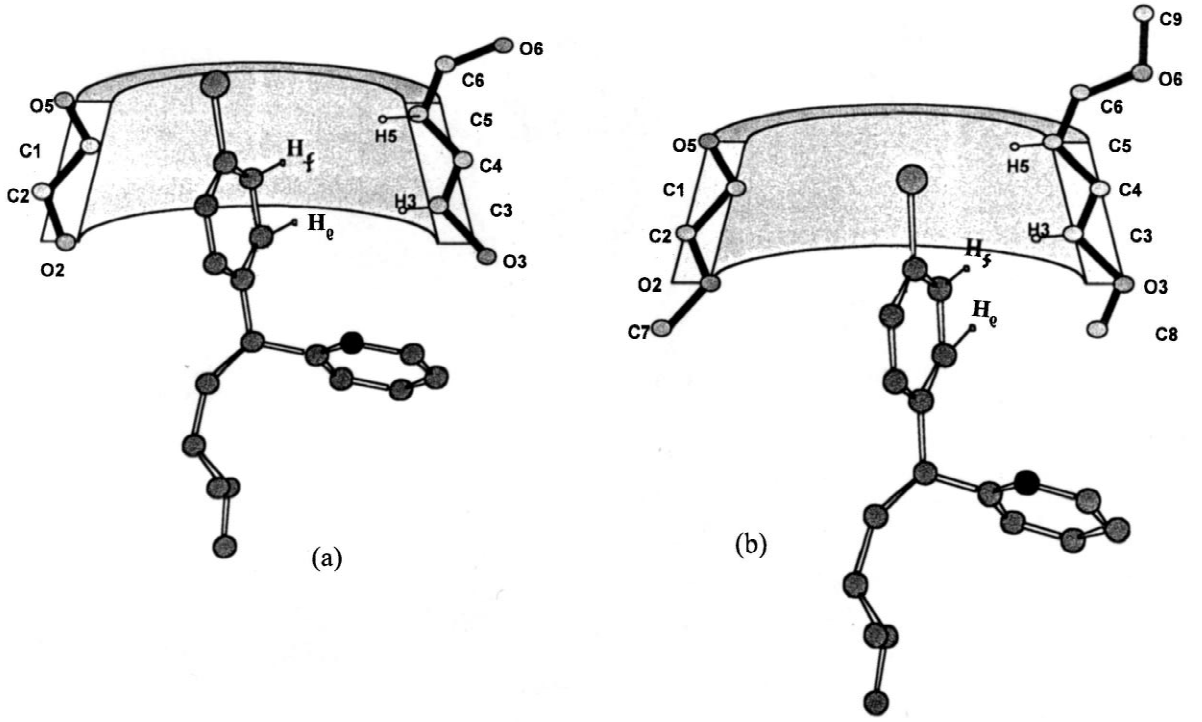

Fig. 9. Structure of (a) (+)-BrPh–β-CD and (b) (+)-BrPh–TM-β-CD complexes derived from 1D ROESY data presented in Fig. 8.

is occupied by a (+)-BrPh molecule, but the maleate counter-ion is included in the cavity of another β-CD molecule. Thus, the stoichiometry of this solid state complex must be 1:2 [(+)-BrPh:β-CD], which contradicts somewhat the 1:1 stoichiometry observed in solution using the continuous variation method and ESI-MS but agrees well with the ^{1}H-NMR data obtained on the crystallized complex (Fig. 4). However, possible differences between the structure in solution and in solid state may have to be considered.

In contrast to correlations observed between 1D ROESY and X-ray crystallographic data of the (+)-BrPh–β-CD complex, the solid-state structure of the (+)-BrPh–TM-β-CD complex was very different compared to that derived based on the 1D ROESY experiment in solution. In particular, the *n-N,N′*-propyldimethylamino moiety of (+)-BrPh appeared to be included in the cavity of TM-β-CD, entering it on the secondary side. A possible reason for this marked structural difference in the liquid and solid phases is perhaps the fact that the maleate salt of (+)-BrPh was studied in the 1D ROESY experiment, whereas the crystals used for X-ray experiments in this particular case (but not in the case of β-CD) were obtained from the aqueous solution (mixture) of free (+)-BrPh (base) and TM-β-CD. This was done because it was impossible to co-crystallize TM-β-CD and (+)-BrPh as the maleate salt.

Thus, this multiple approach allows an explanation of some underlying mechanisms of selector–selectand interaction in chiral CE. In particular, the most likely structural reason for lower affinity of BrPh enantiomers towards TM-β-CD compared to native β-CD could be elucidated. However, further studies are required for a better understanding of the fine effects involved in intermolecular guest–CD interactions.

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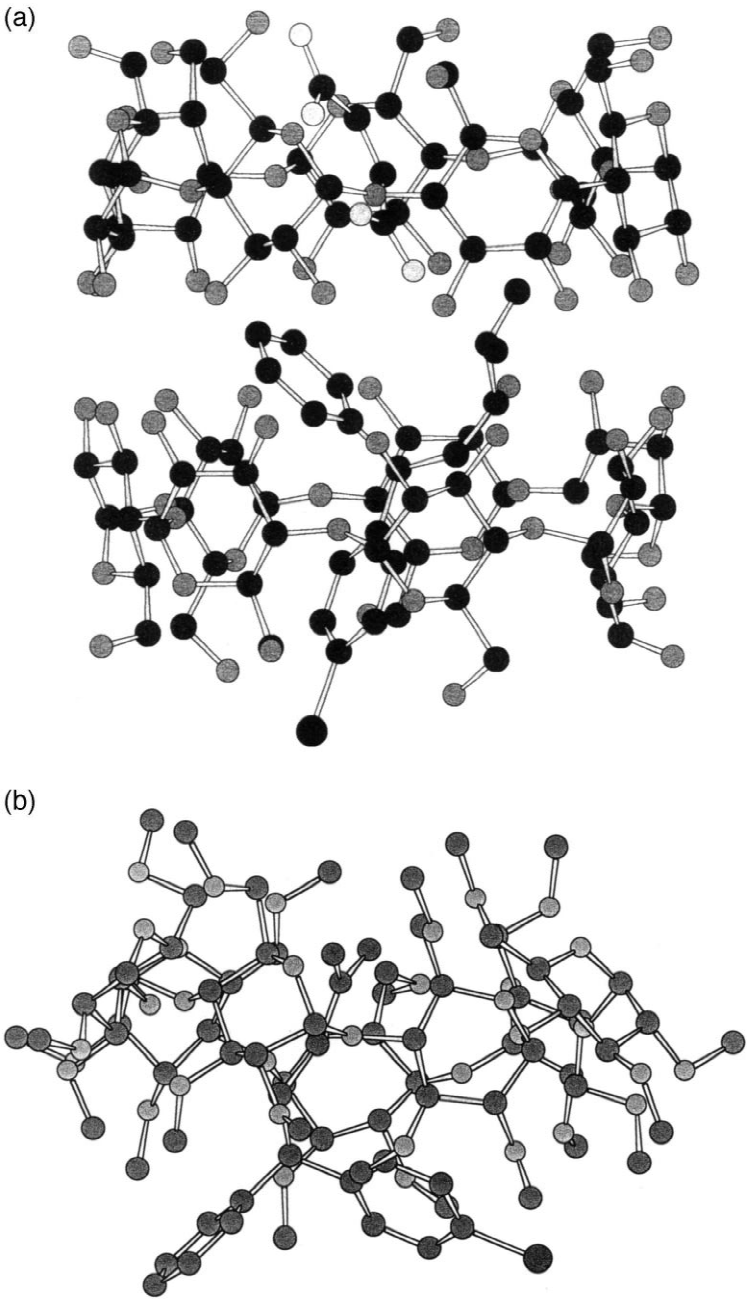

Fig. 10. Structure of (a) (+)-BrPh maleate–β-CD and (b) (+)-BrPh–TM-β-CD complexes in the solid state.

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References

[1] G. Blaschke, B. Chankvetadze, J. Chromatogr. A 875 (2000) 3.
[2] W.A. König, Gas Chromatographic Enantiomer Separation with Modified Cyclodextrins, Wiley, Chichester, 1989.
[3] A.M. Stalcup, in: G. Subramanian (Ed.), A Practical Approach to Chiral Separations by Liquid Chromatography, VCH, Weinheim, 1994, pp. 95–113, Chapter 5.
[4] K.B. Lipkowitz, R. Coner, M.A. Peterson, A. Morreale, J. Shackelford, J. Org. Chem. 63 (1998) 732.
[5] B. Chankvetadze, Capillary Electrophoresis in Chiral Analysis, Wiley, Chichester, 1997.
[6] B. Chankvetadze, N. Burjanadze, G. Pintore, D. Strickmann, D. Bergenthal, G. Blaschke, Chirality 11 (1999) 635.
[7] B. Chankvetadze, G. Schulte, D. Bergenthal, G. Blaschke, J. Chromatogr. A 798 (1998) 315.
[8] B. Chankvetadze, G. Pintore, N. Burjanadze, D. Bergenthal, D. Strickmann, R. Cerri, G. Blaschke, Electrophoresis 19 (1998) 2001.
[9] M. Frost, Ph.D. Thesis, Universität Münster, 1997.
[10] B. Chankvetadze, M. Fillet, N. Burjanadze, D. Bergenthal, C. Bergander, H. Luftmann, J. Crommen, G. Blacshke, Enantiomer, in press.
[11] P. Job, Ann. Chim. (Paris) 9 (1928) 113.
[12] S.K. Branch, U. Holzgrabe, T.M. Jefferiss, H. Malwitz, M.W. Matchett, J. Pharm. Biomed. Anal. 12 (1994) 1507.
[13] R.L. Scott, Rec. Trav. Chim. Pays-Bas 75 (1956) 787.
[14] H. Kessler, U. Anders, G. Gemmecker, S. Steuernagel, J. Magn. Reson. 85 (1989) 1.
[15] G.M. Sheldrick, SHELXS 86, Program for the Solution of Crystal Structures, University of Göttingen, 1987.
[16] G.M. Sheldrick, SHELXL 93, Program for the Refinement of Crystal Structures, University of Göttingen, 1987.
[17] A. Rontoyianni, I.M. Mavridis, J. Incl. Phenom. 18 (1994) 211.
[18] K. Harata, K. Uekama, M. Otagiri, F. Hirayama, Bull. Chem. Soc. Jpn. 56 (1983) 1732.
[19] F. von Bruchhausen, S. Ebel, A.W. Frahm, E. Hachenthal, in: Hager's Handbuch der Pharmazeutisches Praxis, Vol. 7, Springer Verlag, 1993, p. 533.
[20] S. Palmarsdottir, L.-E. Edholm, J. Chromatogr. A 666 (1994) 337.
[21] A.M. Stalcup, K.-H. Gahm, Anal. Chem. 68 (1996) 1360.
[22] A. D'Hulst, N. Verbeke, J. Chromatogr. A 735 (1996) 283.
[23] A.M. Stalcup, N.M. Agyei, Anal. Chem. 66 (1994) 3054.
[24] D.G. Durham, Chirality 8 (1996) 58.