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Mechanistic study of opposite migration order of dimethindene enantiomers in capillary electrophoresis in the presence of native β-cyclodextrin and heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin

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Abstract

The possible mechanisms of the opposite affinity pattern of the enantiomers of dimethindene {(*R*,*S*)-*N*,*N*-dimethyl-3[1(2-pyridyl)ethyl]indene-2-ethylamine} (DIM) towards native β-cyclodextrin (β-CD) and heptakis(2,3,6-tri-*O*-methyl-)-β-CD (TM-β-CD) were studied using capillary electrophoresis (CE), NMR spectrometry, electrospray ionization mass spectrometry (ESI-MS) and X-ray crystallography. NMR spectrometry allowed to estimate the stoichiometry of the complex and to determine the binding constants. As found using ESI-MS, together with more abundant 1:1 complex, a complex with 1:2 stoichiometry may also be present in a rather small amount in a solution of DIM and β-CD. One-dimensional ROESY experiments indicated that the geometry of the complexes of DIM with native β-CD depends on the ratio of the components in the solution. In the 1:1 solution of DIM and β-CD the complex may be formed by inclusion of the indene moiety of DIM into the cavity of β-CD on the primary side and into the cavity of TM-β-CD into the secondary side. The most likely structural reason for lower affinity of the enantiomers of DIM towards the cavity of TM-β-CD compared to native β-CD could be elucidated. The indene moiety does not enter the cavity of TM-β-CD as deeply as the cavity of β-CD. This may be the most likely explanation of significantly higher affinity constants of DIM enantiomers towards the latter CD compared to the former one. The marked difference between the structure of the complexes may also be responsible for the opposite affinity pattern of the DIM enantiomers towards β-CD and TM-β-CD.

Keywords: Enantiomer separation; Chiral selectors; Dimethindene; Cyclodextrins

1. Introduction

Capillary electrophoresis (CE) allows not only to separate effectively enantiomeric mixtures [1,2] but also to observe fine differences in the intermolecular interactions between chiral analyte and a selector. For example, it has been observed in several CE studies that the affinity of some enantiomeric compounds may revert towards a cyclodextrin (CD) host depending on the substituents on the CD rim [2–14]. The understanding of the mechanisms of an affinity reversal of the enantiomers towards CD hosts may help to understand the nature of the forces involved

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in the binding interactions and chiral recognition by CDs. From this viewpoint special interest represent well-characterized native α-, β- and γ-CDs and their single isomer neutral and charged derivatives such as commercially available heptakis(2,6-di-*O*-methyl)-β-CD (DM-β-CD), heptakis(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD), heptakis-6-sulfato-β-CD (6-SU-β-CD) [15], heptakis(2,3-diacetyl-6-sulfato)-β-CD [DACSU-β-CD [16], heptakis(2,3-dimethyl-6-sulfato)-β-CD (DMSU-β-CD) [17], etc.

In this study previously observed opposite migration order of dimethindene (DIM) [(*R*,*S*)-*N*,*N*-dimethyl-3[1(2-pyridyl)ethyl]indene-2-ethylamine] enantiomers towards native β-CD and TM-β-CD [11] is investigated in detail using a combination of CE, NMR spectrometry, electrospray ionization-mass spectrometry (ESI-MS) and X-ray crystallography.

2. Experimental

2.1. Chemicals

Racemic DIM as maleate salt was a gift from Zyma (Munich, Germany). *R*(−) and *S*(+) enantiomers of DIM were obtained in our laboratory using diastereomeric crystallization with optically pure tartaric acid in ethanol as described previously [18]. β-CD was a gift from Wacker Chemie (Munich, Germany) and heptakis-(2,3,6-tri-*O*-methyl)-β-CD (TM-β-CD) was from Fluka (Buchs, Switzerland). Deuterium oxide, analytical-grade 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 p.s.i. for 3 s (1 p.s.i.= 6894.76 Pa). A fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 31.2 cm (21.0 cm effective length)×50 μm I.D. was used. Applied voltage was 20 kV. Other experimental conditions are given in the legend of the Fig. 1.

2.2.2. NMR spectroscopy

^1H and ^{13}C NMR spectral analysis 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 the DIM–TM-β-CD complex was determined by the continuous variation method [2,11–14,19] based on the ^1H NMR chemical shifts. The ^1H NMR resonance signals and Scott's method [2,11–14,20] 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 the pulse program proposed in Ref. [21].

2.2.3. Electrospray ionization-mass spectrometry

ESI-MS spectra of β-CD, TM-β-CD and their complexes with (±)-DIM 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 (±)-DIM or an equimolar amount of CDs in double distilled water as well as, alternatively, their 1:1 mixture were introduced into the ion source of the mass spectrometer with a flow-rate of 5 μl/min using a syringe pump. The ionization voltage was 6 kV for the sample of (±)-DIM and CD and 4 kV for their mixture. The lower ionization voltage was applied in the case of complexes in order to avoid their destruction under hard ionization conditions. The temperature of the inlet capillary was 200°C. The detection was performed in the positive ion mode.

2.2.4. X-ray crystallography

Monocrystals of (+)-DIM maleate were obtained by dissolving a powder of this compound in methanol and slowly evaporating this solution at room temperature.

Lattice parameters and reflection intensities of (+)-DIM maleate were measured on an Enraf-Nonius four-circle CAD4 diffractometer (Nonius, Solingen, Germany) with graphite-monochromated Mo Kα radiation. Unit-cell dimensions and standard deviations were obtained by a least-square fits to 25 independent reflections. A total of 2724 reflections were collected, of which 2486 reflections remained independent (R_{int}=0.108 giving 659 observed reflec-

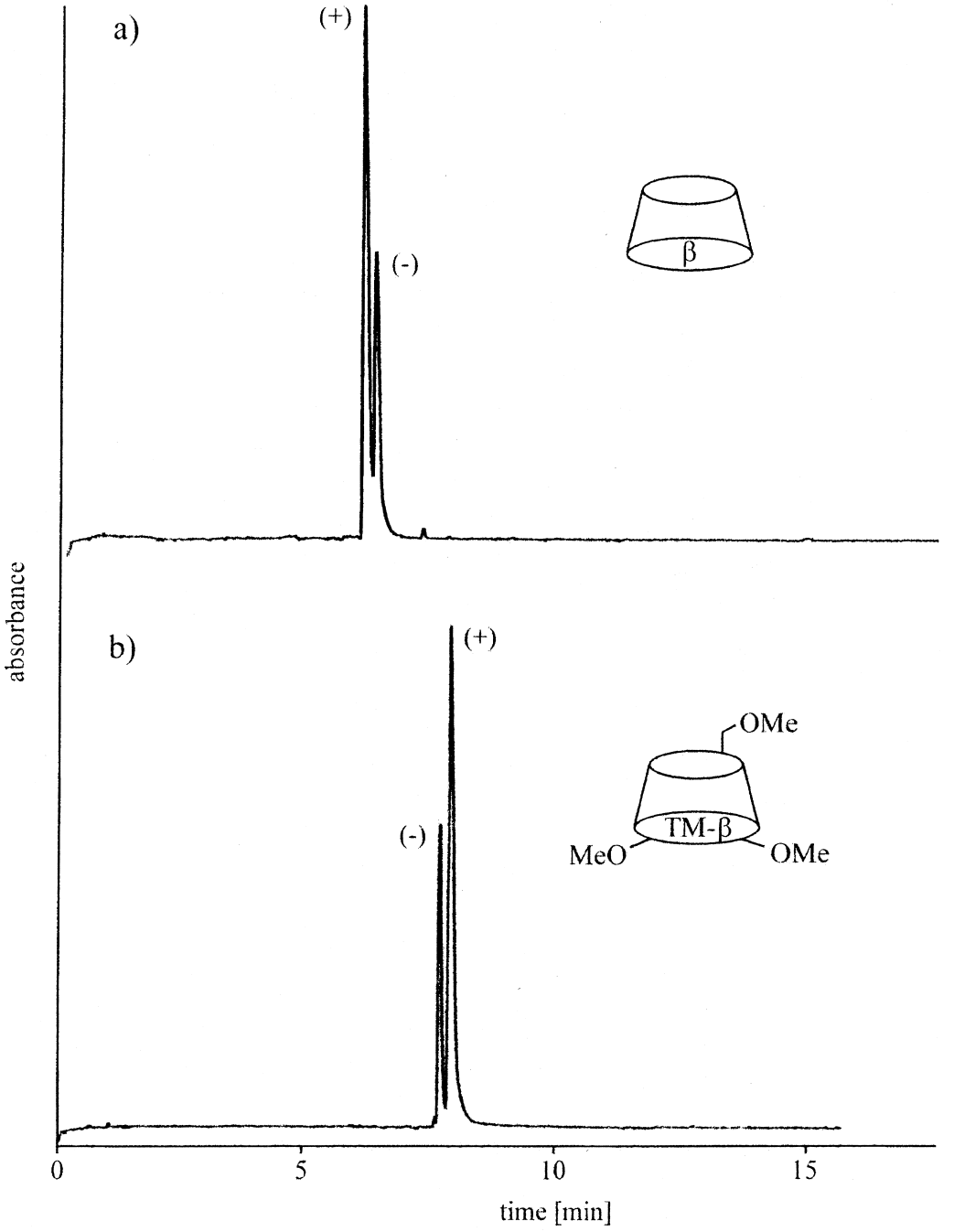

Fig. 1. Electropherograms of the mixture of DIM enantiomers [*R*(−)/*S*(+)=1/3] in the presence of 15 mg/ml β-CD (a) and 150 mg/ml TM-β-CD (b). Buffer: 100 m*M* phosphoric acid adjusted pH 3.0 with triethanolamine. Other conditions as indicated in Section 2.

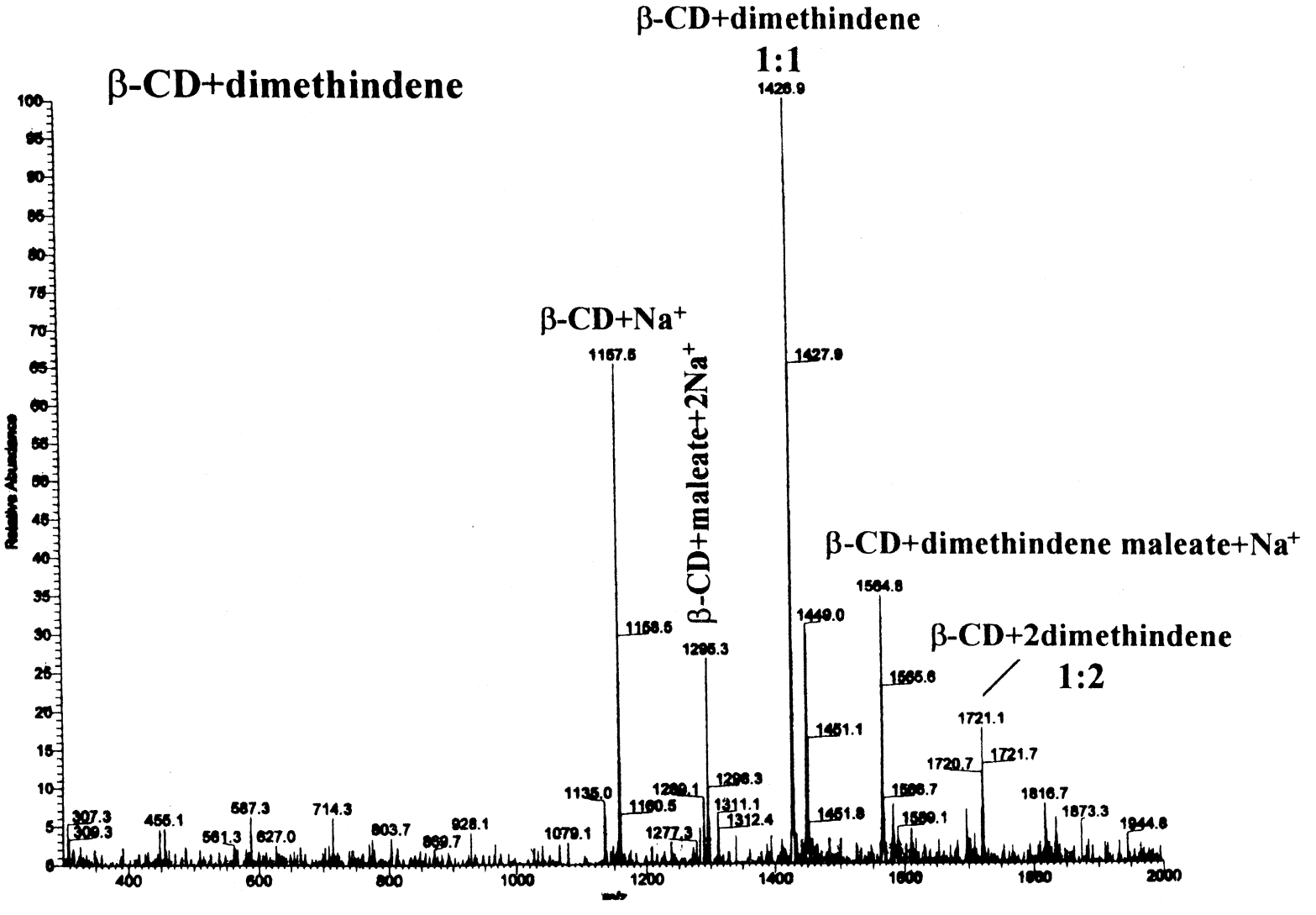

Fig. 2. ESI-MS of DIM–β-CD complex; the conditions are as described in Section 2.

tions with $F_0 4\sigma \geq (F_0)$. The reflections were processed using profile analysis and corrected for Lorentz, polarization and absorption effects, the last was performed by the Φ-scan method. The structure was solved by direct method using the program SHELXS 86 [22] and extended by difference Fourier method using the SHELXL 93 program [23]. 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.

Crystal data: $C_{22}H_{28}N_2O_4$, M_r=408.48, monoclinic, space group $P2_1$, a=5.798(1), b=19.438(4), c=9.953(2) Å, β=99.28(3)°, V=1107.0 Å^3, $F(000)$=436, μ=0.84 cm^{-1}, Z=2, D_C=1.23 g cm^{-1}; crystal dimensions: 0.25×0.20×0.15 mm.

Our multiple experiments for obtaining co-crystals between (+)-DIM and β-CD or TM-β-CD were unsuccessful.

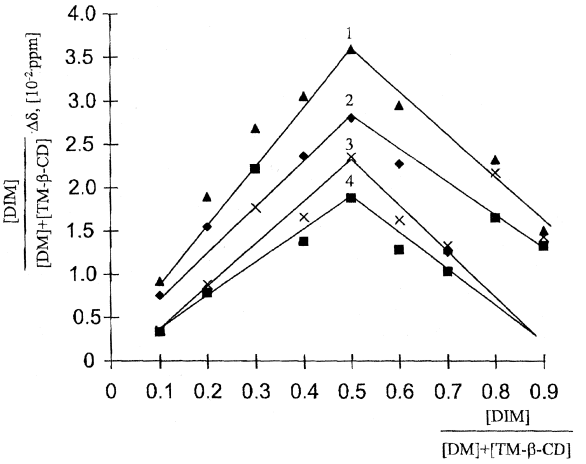

Fig. 3. Job's plots for (±) DIM–TM-β-CD complex constructed based on the complexation-induced chemical shifts (CICS) of the protons in C–CH_3 and N–CH_3 groups in ^{1}H NMR spectra. (1,3) CICS of C–CH_3 protons for (+)- and (−)-DIM, respectively; (2,4) CICS of N-CH_3 protons for (+)- and (−)-DIM, respectively.

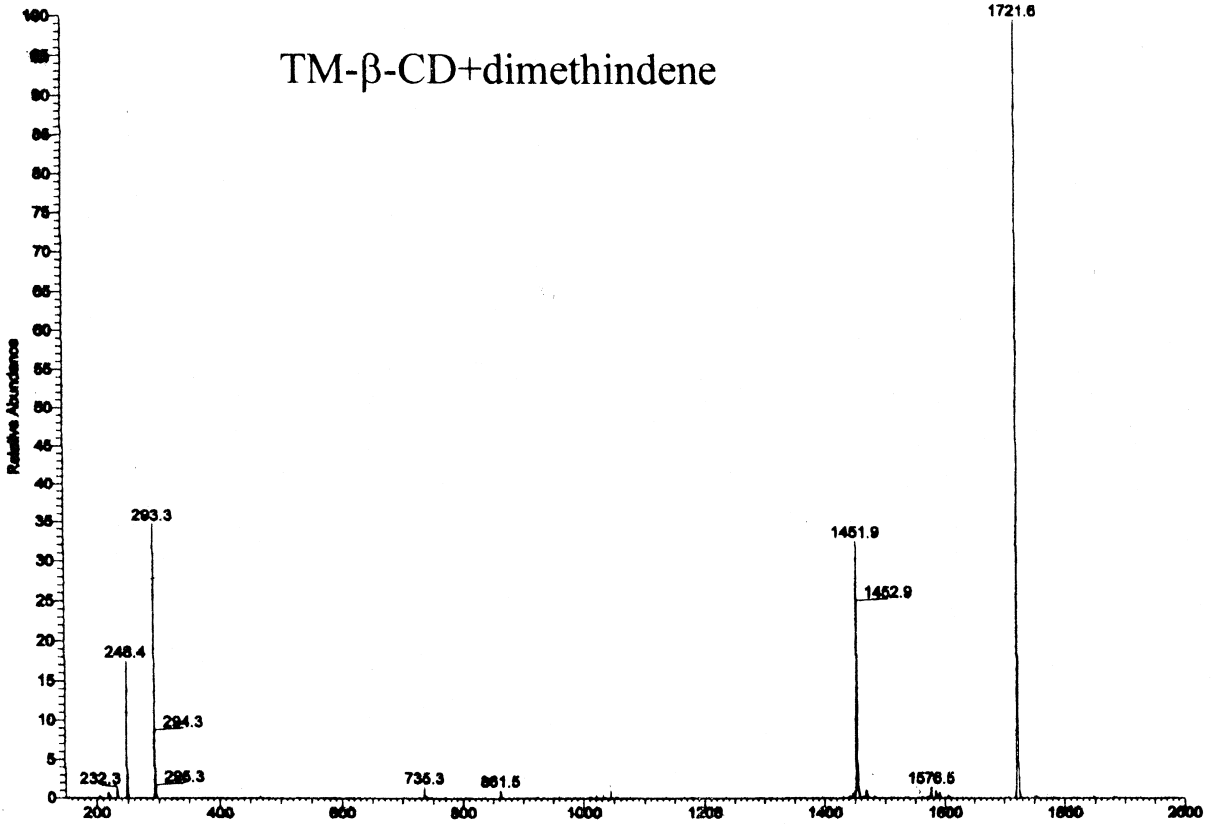

Fig. 4. ESI-MS of DIM–TM-β-CD complex; the conditions are as described in Section 2.

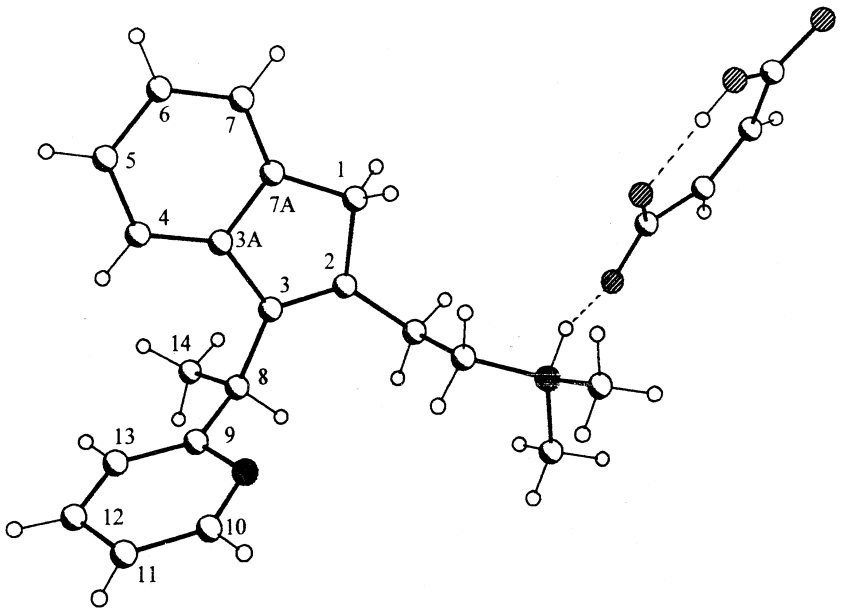

Fig. 5. The crystal structure of DIM maleate.

3. Results and discussion

3.1. Chiral separation of DIM enantiomers

Electropherograms of a nonracemic mixture of the DIM enantiomers in the presence of 15 mg/ml β-CD (a) and 150 mg/ml TM-β-CD (b) are shown in Fig. 1. Under these conditions DIM (pK_1=4.4 and pK_2= 9.0) [24] is present as a positively charged species. As shown in Fig. 1 the affinity of the DIM enantiomers is much lower towards TM-β-CD compared to β-CD. Furthermore, these two CDs exhibit opposite

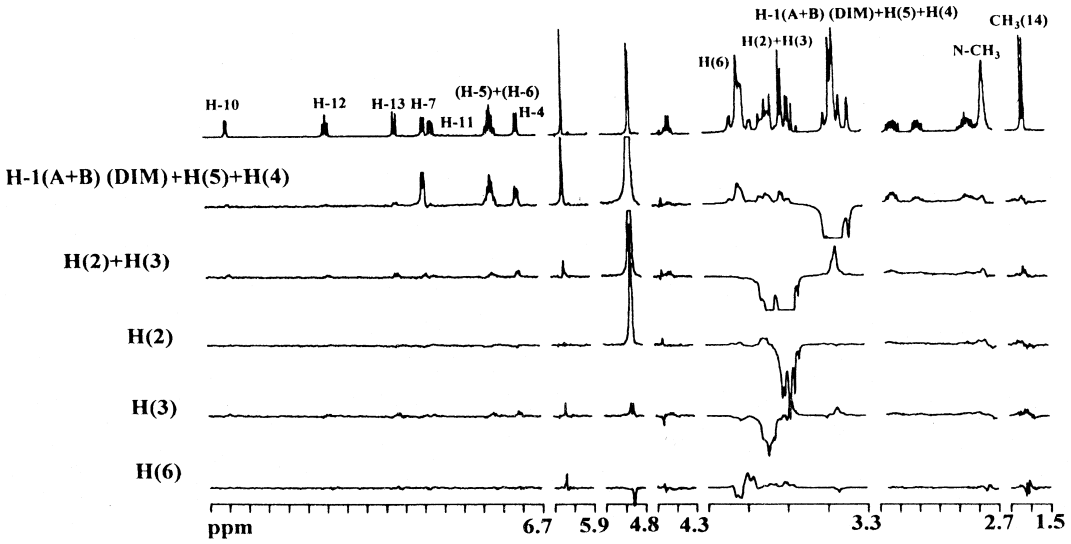

Fig. 6. 1D ROESY spectrum of equimolar solution of (+)-DIM and β-CD; the conditions are as described in Section 2.

chiral recognition patterns in this particular case. Thus, the *R*(−)-enantiomer of DIM is preferentially complexed with β-CD (Fig. 1a), whereas the *S*(+) enantiomer is more tightly complexed with TM-β-CD (Fig. 1b).

3.2. Stoichiometry of DIM complexes with β-CD and TM-β-CD

It was found in the previous NMR study that the complex having 1:1 stoichiometry prevails in the solution of (±)-DIM and β-CD [11]. However, the continuous variation plots do not have a sharp maximum of 1:1 DIM–β-CD ratio. This can be an indication for the presence in the same solution of a minor amount of the complex with other stoichiometry. In order to examine this hypothesis ESI-MS was used as an alternative technique. As shown in the ESI-MS spectrum depicted in Fig. 2, the complex with 1:1 stoichiometry prevails in the solution but a relatively small amount of a complex with 1:2 stoichiometry (β-CD:DIM) may also be present. However, the problem whether a peak with the *m*/*z* ratio 1721.1 corresponding to two molecules of DIM complexed with one molecule of β-CD originates from the complex existing in solution or if it is formed in the ESI-MS interface (false peak) requires further studies.

In contrast to β-CD the Job's curves plotted for DIM–TM-β-CD complex based on complexation-induced chemical shifts (CICS) of the protons in C–C$\underline{H}_3$ and N–C$\underline{H}_3$ groups in ^{1}H NMR spectra provide more definite information on the complex with a 1:1 stoichiometry. There is a clear maximum for the 1:1 DIM–TM-β-CD ratio (Fig. 3). In addition, the ESI-MS spectrum supported the more

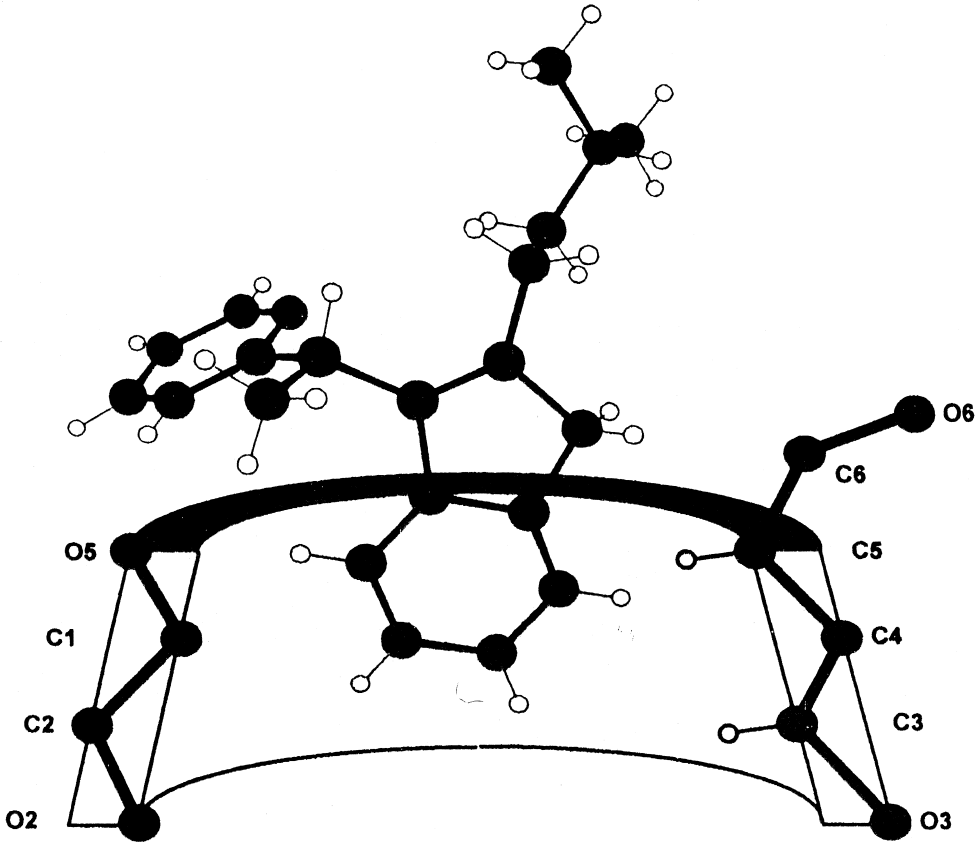

Fig. 7. Structure of DIM–β-CD complex derived from 1D ROESY spectrum shown in Fig. 6.

uniform complex formation (Fig. 4). There was no other peak beside those corresponding to DIM (m/z 293.3), the 1:1 sodium–TM-β-CD adduct (m/z 1451.9) and the 1:1 DIM–TM-β-CD complex (m/z 1721.6).

Thus, the uniform 1:1 complex is most likely formed between DIM and TM-β-CD, whereas in the case of β-CD together with the more abundant 1:1 complex, a complex with 1:2 (β-CD:DIM) stoichiometry may perhaps also be present.

3.3. Binding constants between DIM and TM-β-CD

As shown in Fig. 1 the affinity of the enantiomers of DIM is much lower towards TM-β-CD compared to β-CD. NMR spectrometry was used as an alternative technique in order to obtain more detailed information on the binding constants between the enantiomers of DIM and TM-β-CD.

Scott's plots of DIM–TM-β-CD complex were constructed based on the chemical shifts of C–C$\underline{H}_3$ protons in (−)-DIM and N–C$\underline{H}_3$ protons in (+)-DIM. These plots were linear which is an additional indication that the stoichiometry of the complex is 1:1. The binding constants determined based on the Scott's plots for the complex of *R*(−) DIM–TM-β-CD and *S*(+) DIM–TM-β-CD were 17 and 77 M^{-1}, respectively. These data are much lower than obtained for native β-CD complexes with *S*(+)-DIM and *R*(−)-DIM (457 and 504 M^{-1}, respectively) [11]. This difference in the affinities allows to explain the reason why β-CD in much lower (10 times) concentrations retains the enantiomers of DIM almost as strong as TM-β-CD in the concentration of 150 mg/ml. In addition, the opposite affinity of the enantiomer of DIM towards β-CD and TM-β-CD observed in CE is well supported by the value of the binding constants determined using NMR spectrometry.

3.4. Structure of the complexes between DIM and CDs

Before studying the structure of the DIM complexes with β-CD and TM-β-CD in solution, an attempt

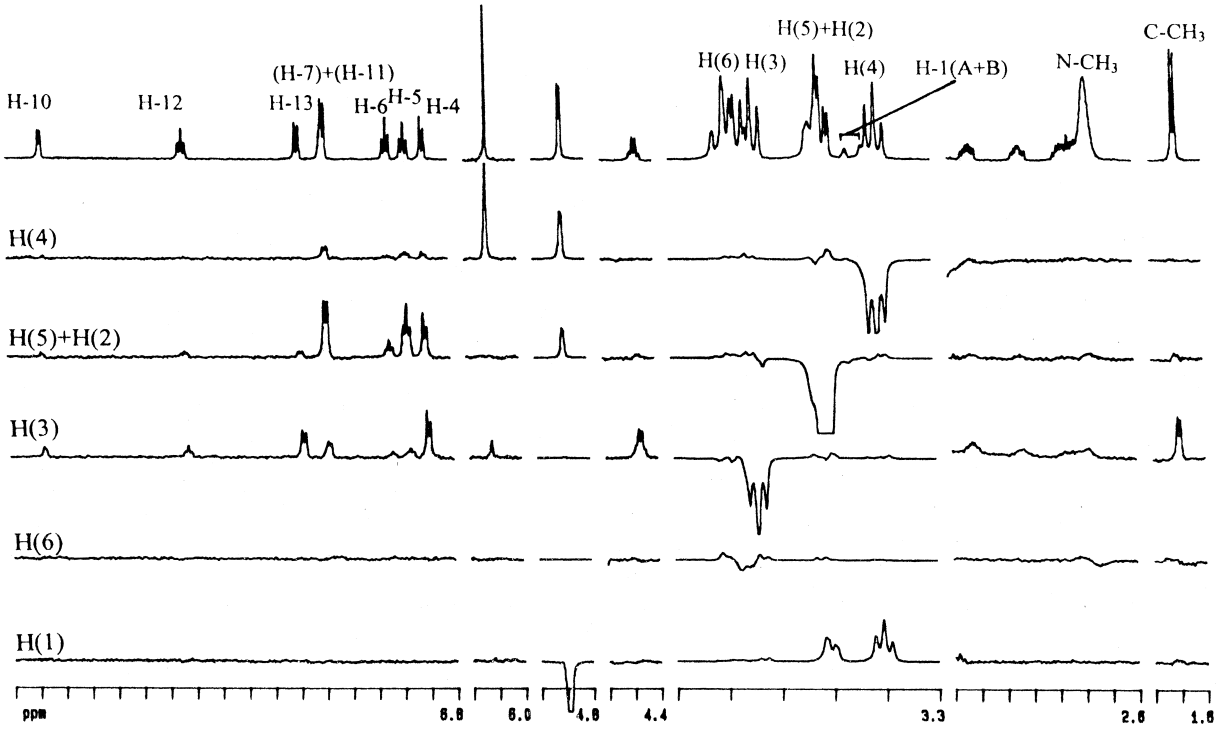

Fig. 8. 1D ROESY spectrum of a solution of (+)-DIM and β-CD with a ratio 1:2.

was made to characterize the guest molecule using X-ray crystallography. This was done in order to have some idea about the geometrical fit of the DIM molecule with the cavity of the CDs. The X-ray structure of DIM maleate is shown in Fig. 5.

The information on the structure of the DIM complexes with β-CD and TM-β-CD in solution was obtained from 1D ROESY NMR measurements. Several experiments were performed in order to get a clear idea on the structure of the intermolecular complexes. Initially, a 1D ROESY experiment was performed on the equimolar solution of (+)-DIM tartrate and β-CD. As shown in Fig. 6, the proton resonance signals in the aromatic area of DIM molecule are quite well resolved. The only overlapping signals are those of protons H-5 and H-6 of the indene moiety. The resonance signals of β-CD protons are quite heavily overlapped. However, based on the C–H heterocosy experiment it was possible to clearly identify the H(6), H(2)+H(3) and H(5)+H(4) protons of β-CD. By irradiation of CD protons in the complex (Fig. 6) the NOE effect was observed basically on the indene protons. The effect was very weak by irradiating H(2)+H(3) protons of β-CD and was significant by irradiating the H(5) protons. This would indicate that the complex is formed by inclusion of the indene moiety into the cavity of β-CD from the primary side [25]. One critical point in this discussion is that the resonance signals of the protons H(4)+H(5) of β-CD overlap to some extent with the (H-1A)+(H-1B) protons of the indene moiety. The intramolecular NOE effect is possible between the H-1A and H-1B and other protons (especially H-7) of the indene moiety. However, the structure depicted on Fig. 7 can be deduced from the 1D ROESY experiment shown in Fig. 6.

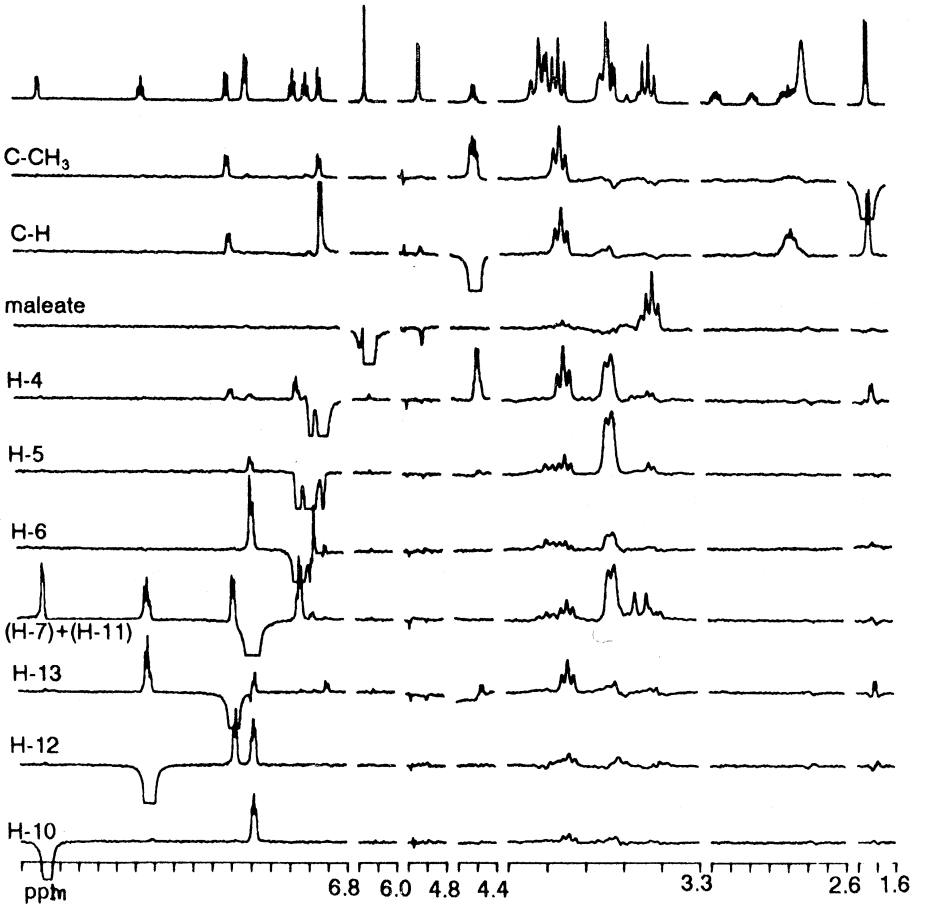

Fig. 9. 'Reverse' 1D ROESY spectrum of a solution of (+)-DIM and β-CD with a ratio 1:2.

The fact that both compounds β-CD and DIM significantly affect the location of the resonance signals of each other allowed to select a DIM–β-CD ratio to avoid the overlapping of the host and guest protons. For this reason a 1D ROESY experiment was also performed on a (+)-DIM–β-CD solution of 1:2 ratio. The ^{1}H NMR spectrum of this mixture (Fig. 8) was quite different to that observed for a 1:1 solution (Fig. 6). At first, in the ^{1}H NMR spectrum of the 1:2 mixture of DIM and β-CD the resonance signals of the indene protons H-5 and H-6 were well resolved. However, the signal of the indene proton H-7 partially overlapped with the signal of H-11 in the pyridine moiety. The most significant difference was that the resonance signals of H-1A and H-1B protons of the indene moiety were located quite far from the resonance signals of the H(3) and H(5) protons of β-CD and partially overlapped with the resonance signals of H(4) β-CD protons, which are located on the outer rim of β-CD. Thus, it was possible to perform 1D ROESY experiments more selectively.

From the 1D ROESY experiment depicted in Fig. 8 it becomes clear that the indene moiety of the DIM molecule enters the cavity of β-CD very deeply from the secondary side. The indication for this is that upon the irradiation of the H(3) protons of the cavity of β-CD the NOE was observed for the H-4 indene proton, for the proton H-13 of the pyridine moiety, as well as for the protons of the methine and methyl groups attached to the center of chirality. A rather small but characteristic NOE was also observed for the protons of the pyridine moiety. When irradiating the H(5) β-CD protons a strong NOE response was observed more selectively for the protons of the indene group. Interestingly, the effect for the H-6 was much less compared to that for the H-5. This most likely means that the H-6 protons protrude so far from the cavity of β-CD on the primary side that the NOE effect becomes very weak. The experiments performed on a 1:2 mixture of DIM and β-CD provided a contradictory structure compared to that derived based on the 1D ROESY experiment performed on 1:1 mixture of DIM and β-CD (Fig. 7). Therefore, a so-called 'reverse' 1D ROESY experiment was performed by irradiating the protons at DIM and observing the response on the protons of β-CD. This experiment (Fig. 9) definitely supported the structure shown in Fig. 10. Thus, almost no NOE response was observed for the CD protons when

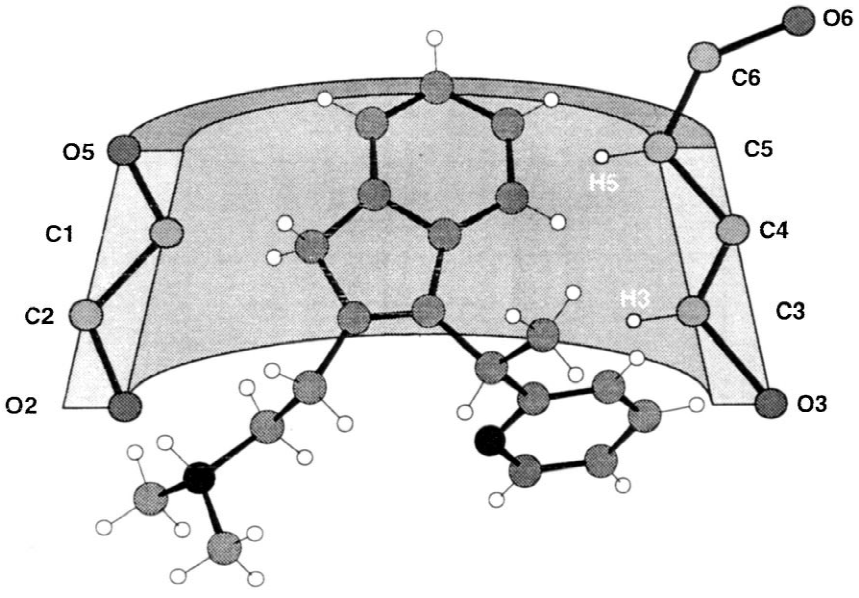

Fig. 10. Structure of DIM–β-CD complex derived from 1D ROESY data of Figs. 8 and 9.

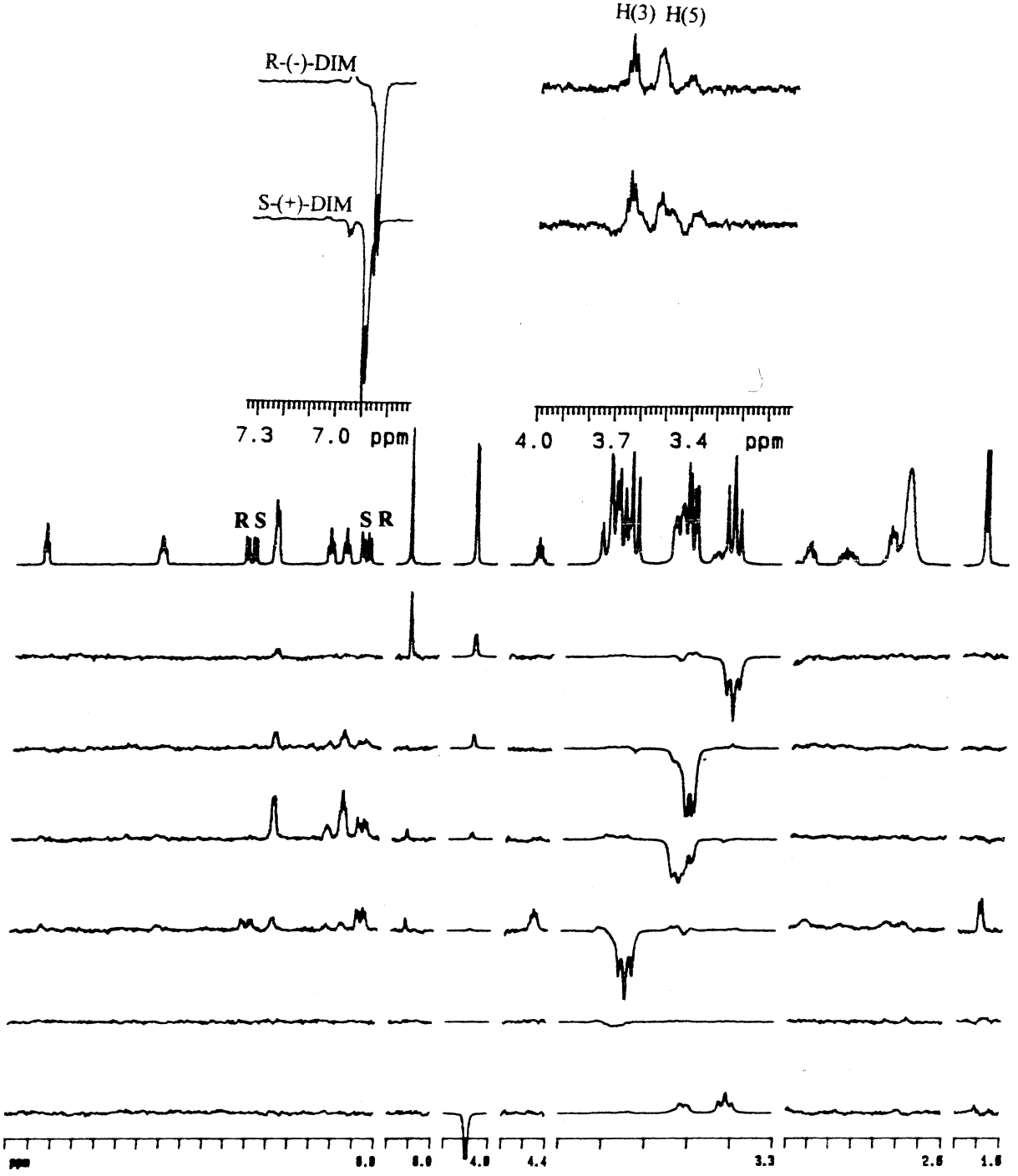

Fig. 11. 1D ROESY spectrum of a solution of (±)-DIM and β-CD with a ratio 1:2.

irradiating the protons H-10 and H-11 in the pyridine group. However, a NOE response on the H(3) protons of β-CD was observed when irradiating the H-13 pyridine protons (Fig. 9). A more pronounced NOE was observed for the H(5) protons of β-CD by irradiating the (H-7)+(H-11) DIM protons. The intramolecular NOE was significant for H-1(A,B) protons after irradiation of H-7 protons. This confirms the aforementioned suspicion on this respect. However, no significant NOE was observed between H1(A,B) and other indene protons, except for H-7. These data agree also with interatomic distances determined based on the X-ray crystal structure of DIM. The distances between H-1(A,B) and H-7 indene protons vary between 2.91 and 2.95 Å, whereas these exceed 4.8 Å for all other pairs. Thus, intramolecular NOE cannot be completely responsible for the effect shown in Fig. 6.

Very weak interactions between the H-6 indene protons and the β-CD protons observed in Fig. 8 have been confirmed in the 'reversed' 1D ROESY experiment (Fig. 9). This experiment also supported a partial inclusion of the proton and the methyl group at the center of chirality into the cavity of β-CD on the secondary side. Thus, based on the data shown in Figs. 8 and 9, the structure of the complex can be proposed as depicted in Fig. 10.

A significant and characteristic intermolecular NOE observed in the (+)-DIM–β-CD solutions indicated a possibility to perform a preliminary study of stereoselective effects for this complex. In order to achieve this goal a 1D ROESY experiment in analogy to the (+)-DIM–β-CD complex was performed on the racemic (±)-DIM–β-CD complex. A complexation-induced chemical shift nonequivalence was observed for the several protons in the aromatic

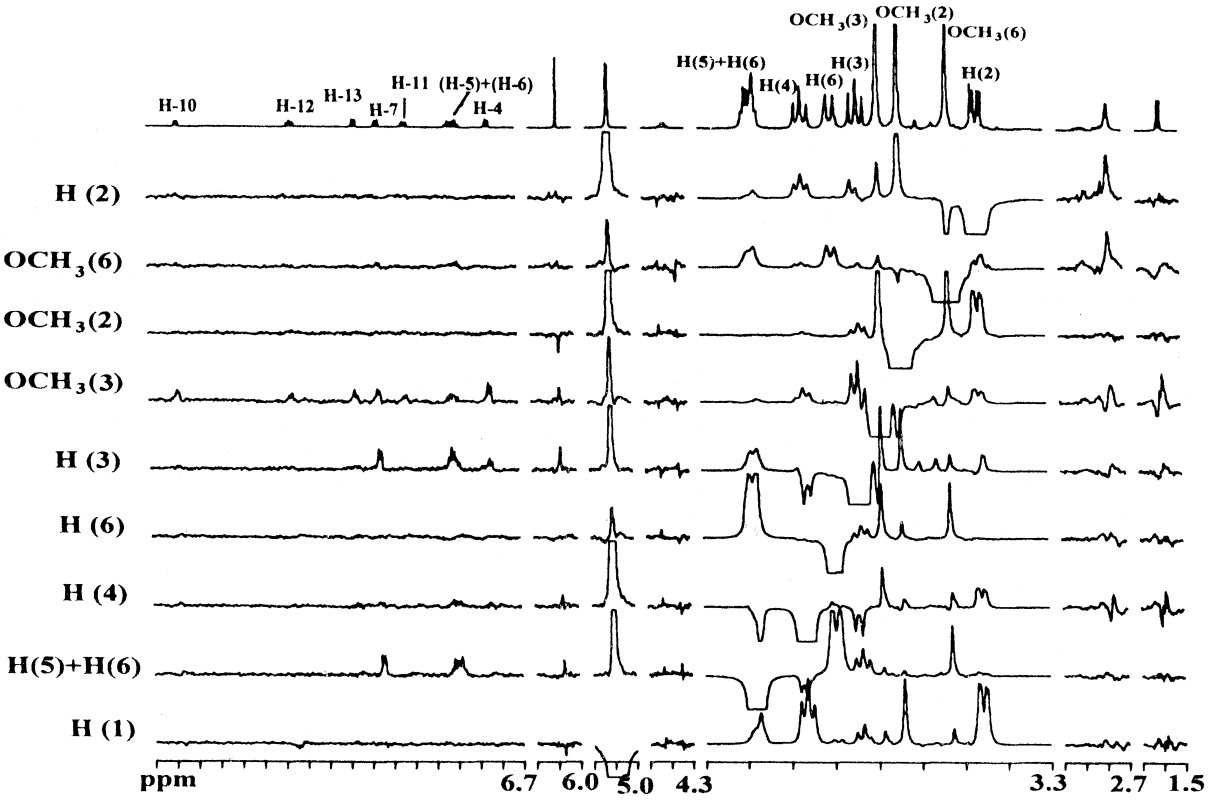

Fig. 12. 1D ROESY spectrum of equimolar solution of (+)-DIM and TM-β-CD.

area. However, the most pronounced effect was observed for the indene protons H-4 and the pyridine proton H-13. The resonance signals of these protons were split into two sets of doublets corresponding to each enantiomer (Fig. 11). It was possible to irradiate a signal of a given H-4 or H-13 proton enantioselectively. A relatively short accumulation time does not allow to draw clear conclusions concerning enantioselective complexation. However, a more strong NOE response for the H(5) β-CD protons when irradiating the H-4 protons of (*R*)-DIM compared to (*S*)-DIM indicates a more deep penetration of (*R*)-DIM into the cavity of β-CD. This could be explanation for the migration of (*R*)-DIM as the second peak in the presence of β-CD as a chiral selector in CE, as well as for the higher affinity of this enantiomer towards β-CD which has been reported before [11].

A 1D ROESY experiment performed on the (+)-DIM–TM-β-CD complex (Fig. 12) indicated that the DIM molecule enters the cavity of TM-β-CD on the secondary side with the indene moiety ahead. Furthermore, H-7 and H-6 protons seem to penetrate the cavity more deeply because the NOE response was pronounced for the H-4 proton of the indene group when the OCH_3 group in position 3 of TM-β-CD was irradiated, only a weak NOE response was observed for the same indene proton when the H(3) protons were irradiated in TM-β-CD, but the effect increased for the (H-5)+(H-6) and the H-7 indene

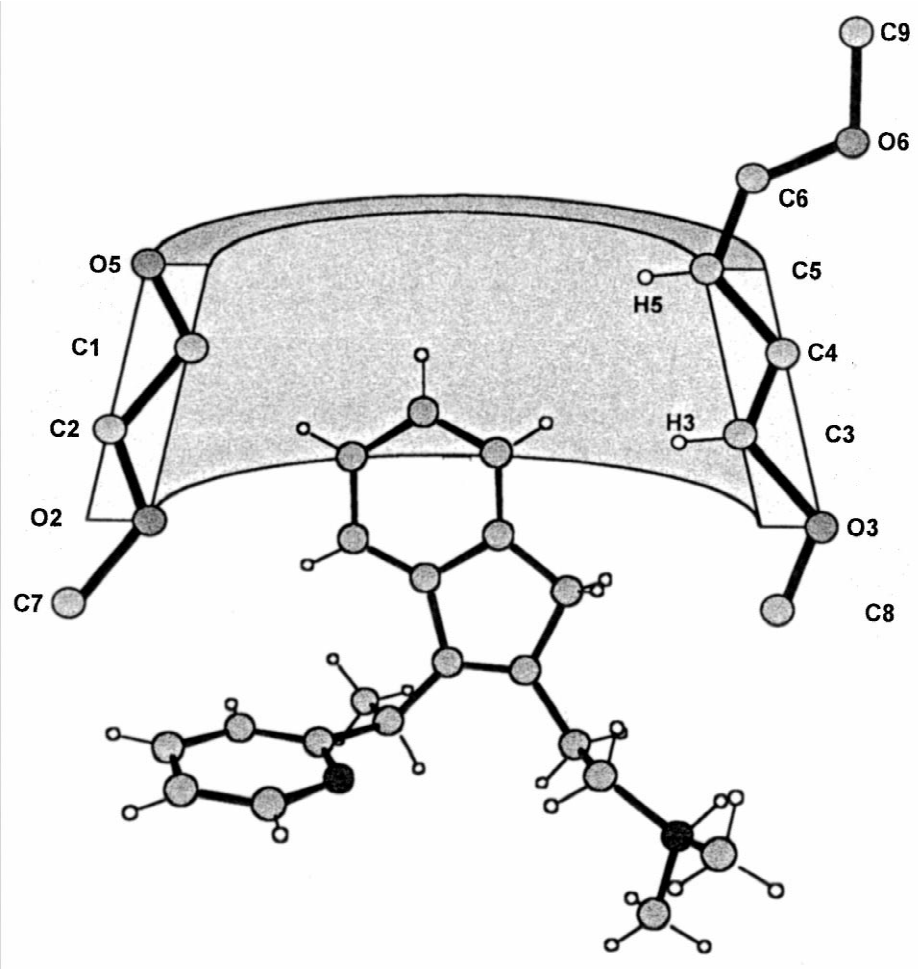

Fig. 13. Structure of DIM–TM-β-CD complex derived from 1D ROESY data of Fig. 12.

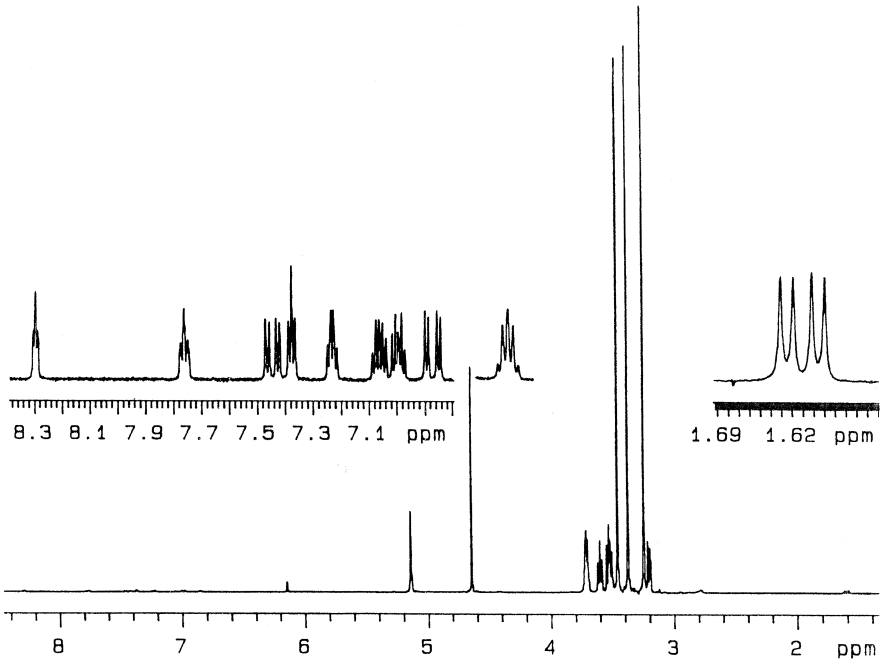

Fig. 14. ^{1}H NMR spectrum of (±)-DIM–TM-β-CD solution (ratio, 1:7) in $^{2}H_2O$ adjusted to apparent pH 3.0.

protons. When irradiating the H(5) protons of TM-β-CD the NOE response was observed only for the (H-5)+(H-6) and the H-7 indene protons. Thus, based on these data the structure depicted in Fig. 13 may be proposed for the (+)-DIM–TM-β-CD complex. In general, the NOE response observed for the (+)-DIM–TM-β-CD complex was significantly weak compared to that for (+)-DIM–β-CD complex. This 1D ROESY experiment clearly indicated that the (+)-DIM molecule does not enter the cavity of TM-β-CD as deeply as the cavity of β-CD. This seems to be the most likely explanation of the lower binding constants of the DIM enantiomers with TM-β-CD compared to β-CD.

An attempt was also made to perform the enantioselective 1D ROESY experiment on the (±)-DIM–TM-β-CD complex. The significant CICS nonequivalence was observed for most protons (Fig. 14) and the resonance signals of the H-4 and H-13 protons as well as the resonance signals of the CH_3 groups on the chiral center split in a clear set of well-resolved doublets for each (*R*)- and (*S*)-DIM enantiomers. However, the NOE effect was too weak for any reliable judgement of stereoselective effects.

Thus, structure differences derived based on the 1D ROESY data allow to a certain extent to explain the reasons for quantitative differences in the binding of DIM with β-CD and TM-β-CD.

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