^{1}H and ^{13}C NMR and Molecular Dynamics Study of Chiral Recognition of Camphor Enantiomers by α-Cyclodextrin

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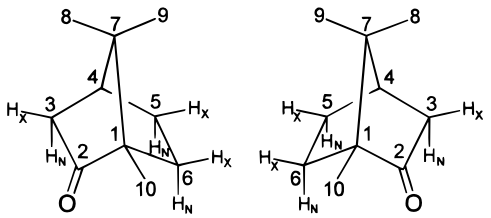
^{1}H and ^{13}C NMR spectra of the complexes of camphor enantiomers with α-cyclodextrin in D_2O manifest splittings due to chiral recognition. The complexes were found to be of 1:2 guest-to-host stoichiometry. Free energies of the complex formation obtained from ^{1}H NMR titration data are equal to -7.95 ± 0.09 kcal mol^{-1} for the complex with (1*S*,4*S*)- and -7.61 ± 0.06 kcal mol^{-1} for that with (1*R*,4*R*)-enantiomer. Thus, the free energy difference between the complexes is equal to 0.34 ± 0.11 kcal mol^{-1}, with the complex involving the (1*S*,4*S*)-camphor more stable. A strong positive cooperativity of the guests binding has been found. In agreement with experimental results, molecular dynamics simulations yielded greater stability of the complex with (1*S*,4*S*)-camphor. However, they reproduced only qualitatively the experimental trend since the corresponding difference in average energies obtained from molecular dynamic simulations carried out in a water solution is equal to 5 kcal/mol with the CVFF force field.

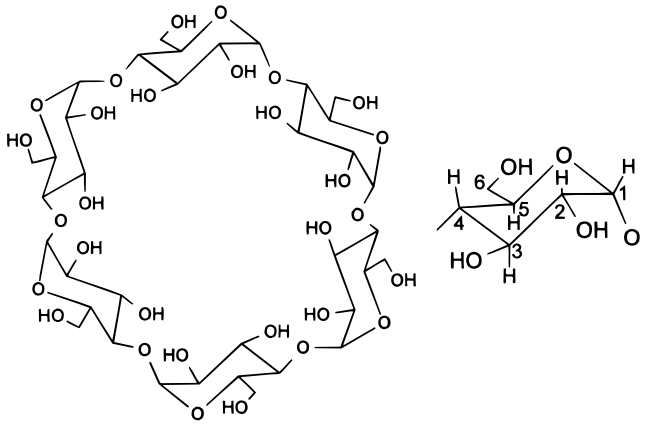
Introduction

Chiral recognition by cyclodextrins, CDs, is of considerable importance in view of, among others, enantiospecificity of action of most drugs.[1] However, despite intense efforts, the mechanism of the recognition is not fully understood [2] and many essential data are missing. In particular, to our knowledge experimental data on the free energy difference between CD complexes involving enantiomeric pairs of guests are scarce.[3] In this paper, NMR and computational results concerning the selective complexation of camphor enantiomers **1** by α-CD **2** in water are reported. They include ^{1}H and ^{13}C spectral manifestations of chiral recognition of **1a** ((+)- or (1*S*,4*S*)-isomer) and **1b** ((−)- or (1*R*,4*R*)-one) by **2**, a 1:2 stoichiometry ratio for the complexes, stability constants, free energies of complex formation, and the difference between free energies of the diastereomeric complexes.

2 was found to discriminate between **1a** and **1b** by both HPLC and gas chromatography[4] with greater stability



1a **1b**



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of the complex involving the former enantiomer. In continuation of our studies of molecular and chiral recognition by CDs,[5] NMR manifestations of chiral

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(3) (a) Liu, Y.; Bin, Zh.; Bu, X.; Li, Y.; Chen, R. *Chin. Sci. Bull.* **1996**, *41*, 117; *Chem. Abstr.* **1997**, *125*, 196298. (b) Liu, Y.; Zhang, Y.; Sun, S.; Li, Y.; Chen, R. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1609. (c) Rekharsky, M. V.; Goldberg, R. N.; Schwartz, F. P.; Tewari, Y. B.; Ross, P. D.; Yamashoji, Y.; Inoue, Y. *J. Am. Chem. Soc.* **1995**, *117*, 8830. (d) Kano, K.; Negi, S.; Kamo, H.; Kitae, T.; Yamaguchi, M.; Okubo, H.; Hirama, M. *Chem. Lett.* **1998**, 151. (e) Arad-Yellin, R.; Green, B. S.; Knossow, M.; Tsoucaris G. In *Inclusion Compounds, Vol. 3;* Atwood, J. L., Davies, J. E., MacNicol D. D., Eds.; Academic Press: London, 1984; p 263. In addition, stability constants calculated from chromatographically determined retention factors for several diastereomeric complexes with enantiomeric guests are available but these data do not contain error estimates that are of importance for the small values of free energy difference between the complexes.

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Table 1. ^{1}H Chemical Shifts of Camphor Enantiomers [1a-(+) and 1b-(−)] in D_2O Free and in the Complexes with α-CD, 2 ($C_{α-CD}$ = 12 mM/L, C_{camph} = 1 mM/L)

assignment	1a/1b	1a⊂2	1b⊂2
H-3N	1.943	2.304	2.185
H-3X	2.458	2.588	2.592
H-4	2.173	2.322	2.282
H-5N	1.378	1.683[a]	1.681[a]
H-5X	1.985	*b*	*b*
H-6N	1.361	1.778 [a]	1.760[a]
H-6X	1.807	1.962	1.962
H-8	0.830	1.141	1.139
H-9	0.982	1.189	1.212
H-10	0.900	1.267	1.246

[a] Assignments may be reversed. [b] Very broad.

recognition of **1a** and **1b** by **2** have been studied together with molecular dynamics simulations of the complexes.

Results and Discussion

NMR Studies. Chemical shifts of free **1** and those of camphor enantiomers complexed with **2** are collected in Table 1. (The camphor signal assignments had to be done *de novo* on the basis of COSY and NOESY spectra since the literature values[6] were measured in different solvents.) Methyl protons exhibit the largest chemical shift changes on complexation considerably higher than the usual ones referring to 1:1 complexes.[7] The magnitude of these changes is enantiomer dependent with $\Delta\delta = \delta_{\mathbf{1a}\subset\mathbf{2}} - \delta_{\mathbf{1b}\subset\mathbf{2}}$ equal to 0.023 ppm and −0.021 ppm for H9 and H10, respectively, while it is within the limits of error for H8.

An NOESY spectrum of the mixture of the complexes exhibits considerable differentiation of the dipolar interactions of H3′ CD protons (pointing inside the cyclodextrin cavity) with those of H9 and H10 methyl groups of the guest (Figure 1). H9 in the complex with **1a** and H10 in that with **1b** show much stronger correlations than the signals involving opposite enantiomers of the guest. This finding points to stronger interactions, thus closer distances, in the corresponding complexes.

The methyl group signals also exhibit enantiomeric differentiation in ^{13}C spectra. A part of the spectrum showing ^{1}H/^{13}C methyl correlations obtained with the gradient-enhanced HSQC technique[8] is presented in Figure 2.

Using the Job method[9] both complexes have been found to be of 1:2 stoichiometry (Figure 3). However, these data do not allow one to discriminate among head-to-head, head-to-tail, and tail-to-tail structures of the dimeric CD capsules.

To obtain a quantitative description of the complex stabilities, ^{1}H NMR titrations of **1a** and **1b** with **2** were performed. The titration isotherms are shown in Figure 4. Table 2 in turn summarizes the data calculated on the basis of the titration experiments. Stability constants of the complexes with enantiomeric guests were determined

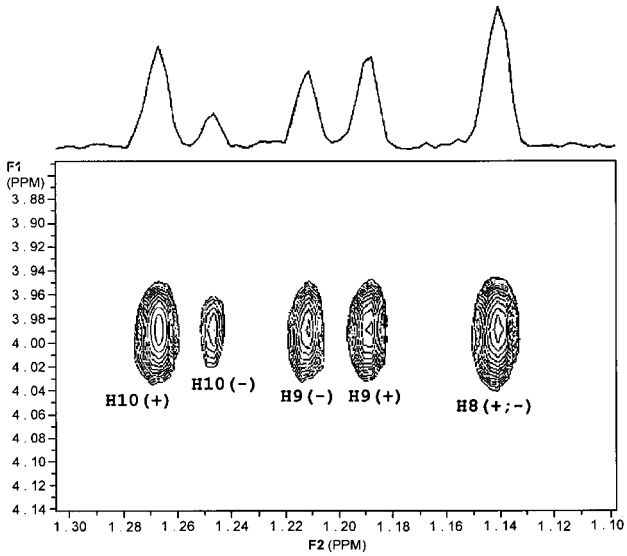

Figure 1. NOESY spectrum of the complexes involving a racemic mixture of camphor enantiomers with α-CD (H3′ CD proton signals irradiated). The concentration of camphor enantiomers is equal to 1 mM/L, that of the host 12 mM/L.

by direct analysis of the titration isotherms using the software developed by Hunter.[10] Computer simulated fitting of theoretical curves to the sets of experimental data obtained from our ^{1}H NMR titrations was performed for eight resolved guests signals. The values of free energy of the complex formation determined by this procedure were equal to -7.95 ± 0.09 and -7.61 ± 0.06 kcal·mol^{-1} for the complexes involving **1a** and **1b**, respectively. A less accurate Benesi−Hildebrand[9b,11] method is frequently used to determine stability constants of CD complexes. Therefore, for the sake of comparison, the Benesi−Hildebrand method modified to take into account the 1:2 stoichiometry was applied for this purpose. It gave values (Table 2) that are slightly different from those obtained from the least-squares fitting with the Hunter program. Free energy differences between the enantiomeric complexes calculated on the basis of both methods were consistent with each other and equal to $0.34 \pm 0.11$and 0.31 ± 0.21 kcal mol^{-1} for simulated fitting by the Hunter program and Benesi−Hildebrand method, respectively. In agreement with the greater stability of the former complex studied by Sybilska and co-workers[4] by HPLC and gas chromatography, the complex with **1a** is more stable than that with the second enantiomer.

A theoretical analysis[10] of the titration isotherms allowed us to estimate stepwise equilibrium constants K_1 and K_2 describing the first and the second stage of the complexation (Table 2). Noteworthy, the values of K_2 are 4 orders of magnitude larger than those of K_1, pointing to a strong *positive cooperativity* of camphor enantiomers binding by α-CD.[12] Instead of the hyperbolic saturation curves that are typical of binding of a wide variety of guests to CDs,[7] the ^{1}H NMR titration data

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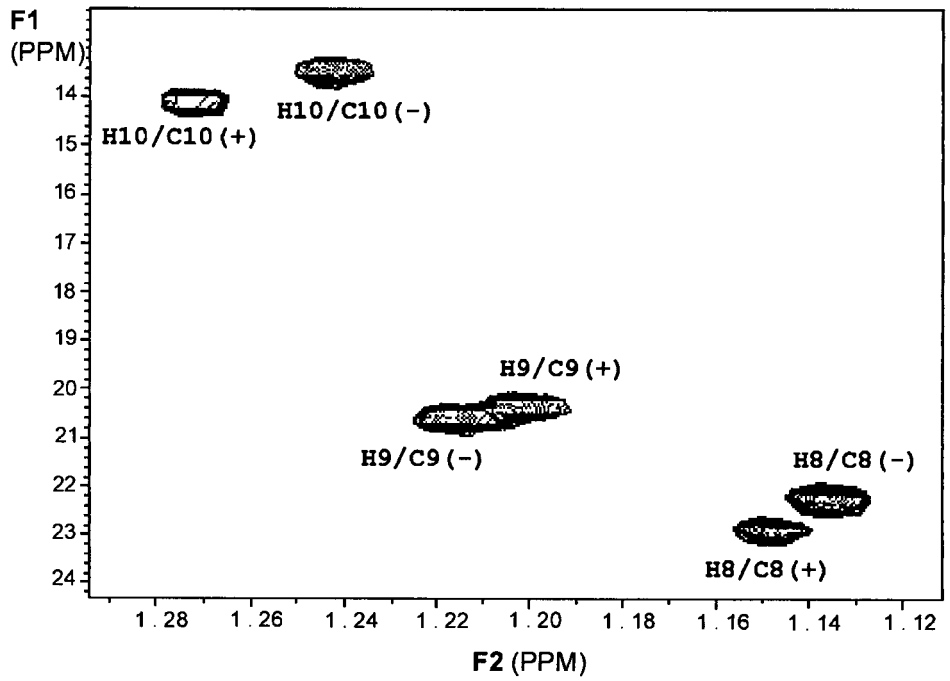

Figure 2. $^{1}H/^{13}C$ methyl correlations (the concentrations used are the same as those in Figure 1).

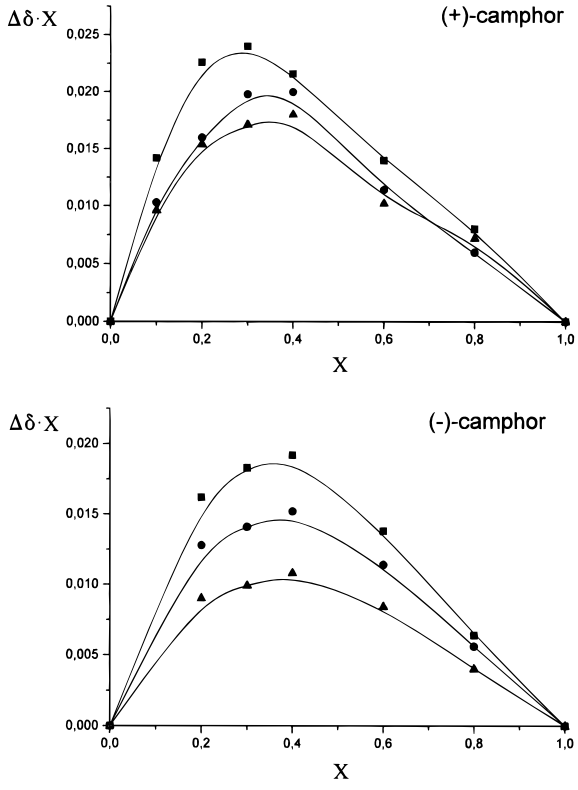

Figure 3. Typical Job plots for the methyl group signals. *X* is a relative amount of α-CD in the mixture.

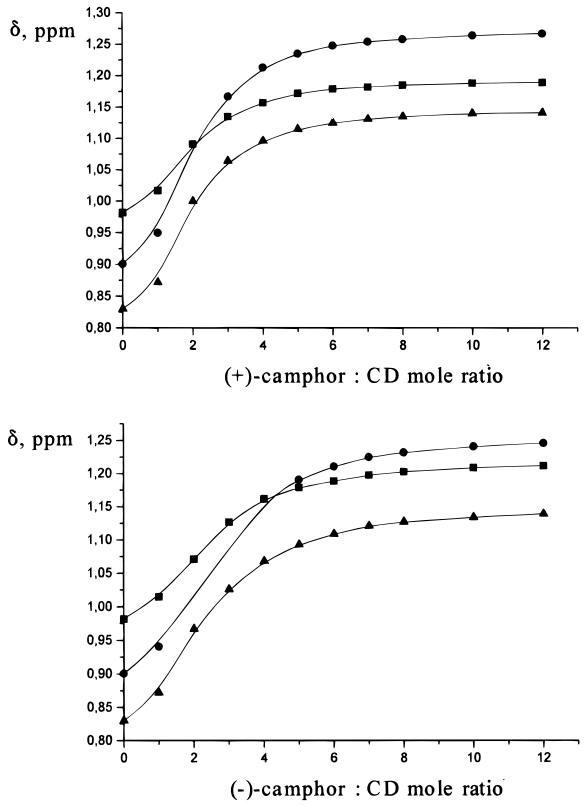

Figure 4. Titration curves for the methyl group signals of the complexes of (+)- and (−)-camphor enantiomers with α-CD.

yielded sigmoidal ones for the shifts of all camphor signals (Figure 4), the hallmarks of positive cooperativity of binding of the enantiomers of **1** by **2**. The Hill coefficients, *h* (positive cooperativity is denoted by $h > 1$),[13] calculated from the corresponding Hill plots are 1.98 ± 0.10 and 1.89 ± 0.05 for (+)- and (−)-camphor, respectively, indicating the allosteric regulation in the formation of CD dimeric capsules.

Dynamic Simulations. The calculations were carried out using the Insight II[14] program package with the

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(14) Insight II (version 97.0) is distributed by BIOSYM Technologies, Inc., 9685 Scranton Road, San Diego, CA 92121-2777.

Table 2. Data on the Complex Stabilities Calculated from the ^{1}H NMR Titrations of 1 by 2[a]

	Hunter program		Benesi−Hildebrand	
	1a⊂2	**1b⊂2**	**1a⊂2**	**1b⊂2**
K_1, M^{-1}	9 ± 1.8	8.6 ± 2.3	*b*	*b*
K_2, M^{-1}	$(7.14 \pm 0.9) \times 10^4$	$(4.53 \pm 1) \times 10^4$	*b*	*b*
K, M^{-2}	$(6.66 \pm 0.1) \times 10^5$	$(3.65 \pm 0.3) \times 10^5$	$(3.30 \pm 0.8) \times 10^5$	$(1.95 \pm 0.5) \times 10^5$
ΔG, kcal mol^{-1}	-7.95 ± 0.09	-7.61 ± 0.06	-7.53 ± 0.17	-7.22 ± 0.16
$\Delta\Delta G$, kcal mol^{-1}	0.34 ± 0.11		0.31 ± 0.21	

[a] The constants describe the following equilibria: K_1, [G] + [H] ⇆ [GH]; K_2, [GH] +[H] ⇆ [GH$_2$]; K, [G] + 2[H] ⇆ [GH$_2$]; $K = K_1K_2$, where [G] and [H] denote equilibrium concentrations of **1** and **2**, respectively. [b] The values could not be computed by this method.

CVFF force field.[15] It should be stressed that we did not use any kind of constraint in any of the computational studies. A visual inspection of the 1:1, camphor:α-CD, energy minimized complexes shows that approximately one-half of the guest's volume could be accommodated by α-CD cavity and the guest seems "to need" the second α-CD "cap" to completely isolate the camphor from the solvent. Molecular dynamics, MD, simulations carried out in a vacuum at 300 K for 1:1 complexes with both enantiomers showed that the complexes decomposed after about 10 ps. Contrary to that, MD runs for all 1:2 camphor:CD dimeric complexes studied both in a vacuum and in water boxes demonstrated extreme stabilities of the molecular ensembles on the nanosecond time scale. An analysis of trajectories of 2 ns MD simulations in a vacuum showed that head-to-head and head-to-tail complexes have almost the same values of Boltzmann average energies, while the tail-to-tail complex is about 20 kcal mol^{-1} less favorable. Contrary to the experimental results, dimeric complexes with **1b** in a vacuum were more stable by ca. 1 kcal mol^{-1} independently on the head-to-head, head-to-tail, or tail-to-tail dimer structure. For the head-to-head complexes of **1a** and **1b** with **2**, MD simulations in a periodic water box during 1.5 ns have been carried out. In agreement with the experimental data, the calculations yielded greater stability of **1a** with **2**, but an unreliably high value of the difference in the Boltzmann average energies between the complexes involving camphor enantiomers $\Delta E = 5$ kcal mol^{-1} has been obtained. This example shows that MD simulations in solvent with relatively long simulation times reproduce only qualitatively the experimental trend.

Conclusions

The diastereomeric complexes of camphor enantiomers **1a** and **1b** with α-CD **2** were characterized by ^{1}H and ^{13}C NMR spectra manifesting differences in signal positions and NOE effects due to chiral recognition. Both complexes differing in energy by ca. 0.3 kcal/mol (determined using Hunter program[10] and Benesi−Hildebrand method[11]) were found to exhibit 1:2 stoichiometry using the Job method. MD simulations in water reproduced only qualitatively the greater stability of the complex involving the **1a** enantiomer.

Experimental Section

Chemicals. α-CD was a gift from Wacker-Chemie, and (+)- and (−)-camphor of 98% optical purity were purchased from Fluka. All chemicals were used without any additional purification.

NMR Spectra. D_2O was used as a solvent for all NMR measurements. All NMR experiments were performed on a Varian Unity Plus 500 MHZ spectrometer ($B_0 = 11.7$ T) at 298 K with 4,4-dimethyl-4-silapentane sodium sulfonate (DSS) used as external standard in both ^{1}H and ^{13}C spectra. NOESY[16] with 500 ms mixing time and DQF-COSY[17] spectra were measured in a phase-sensitive mode[18] with a presaturation of residual HDO signal during relaxation delay (1 s). NOESY spectra with 400 ms mixing time and HSQC[8] spectra in a gradient version[19] were performed for a nonracemic mixture of camphor enantiomers with α-CD enabling the assignment of the signals to the corresponding species.

Complexation Studies. (a) Job Method. Mother solutions of equal concentrations (2 mM in D_2O) of α-CD and both camphor enantiomers were prepared. Samples for ^{1}H NMR measurements were prepared directly in an NMR tube by adding appropriate aliquots of the host and a guest with a microsyringe. For certainty each measurement was repeated twice.

(b) Titrations of Camphor Enantiomers with α-CD. Samples of (+)- and (−)-camphor were dissolved in D_2O (concentrations of 1 mM were used). Each camphor solution was separated into two portions. A portion was used as a guest NMR sample, and the rest was used to dissolve and to dilute the sample of the host, so that the concentrations of camphor enantiomers remained constant during the titrations. Successive aliquots of the host solution were added to a guest sample, and ^{1}H NMR spectra were recorded after each addition. The changes in chemical shift of almost all of the camphor signals as a function of α-CD concentration were then analyzed both with a program written for the Apple Macintosh microcomputer by Prof. C. A. Hunter from Sheffield University (the data are fitted by the program by iterative procedure to the appropriate binding model yielding association constants and the bound chemical shift; a detailed description of the program is given in ref 10) and by the Benesi−Hildebrand method.[9b,11]

Molecular Dynamics. The simulations were carried out on a Silicon Graphics Indigo 2 workstation. The force field used for energy minimizations and molecular dynamics simulations was CVFF[15] as implemented in the Discover module (version 2.9.5) within the Insight II program.[18] Default values were assumed for all parameters. A conjugate gradient procedure was applied in all minimizations. A root-mean-square (rms) gradient of 0.001 kcal mol^{-1} or less was assumed as a condition of energy convergence. All MD simulations were performed at constant volume (*NVT* ensemble) with 1 fs time step. The coordinates were saved every 1 ps. α-CD was built connecting six α-D-glucose units from fragment libraries of the Builder module (Insight II). Its energy was minimized, producing the structure with C_6 symmetry. One of the camphor enantiomers was sketched using 2D Sketcher options within the Builder module with the following transformation to 3D structure and energy minimization. The second camphor enantiomer was

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(19) Kay, L. E.; Keifer P.; Saarinen, T. *J. Am. Chem. Soc.* **1992**, *114*, 10663.

obtained by reflection of the first one. Then, with the aid of Docking module of Insight II two α-CD molecules were placed to form the head-to-head capsule and the camphor enantiomers were accommodated inside the dimer cavities. Next, the energies of the whole systems were minimized. Subsequently, the complexes previously minimized in a vacuum were immersed in cubic cells with dimensions 30 × 30 × 30 $Å^3$ containing 794 water molecules each. The energy of the entire system, consisting of the solvent and solute, was minimized, and the following equilibration steps in the MD simulations were performed using periodic boundary conditions. MD was carried out for the entire system for 5 ps intervals at temperatures 10, 100, 200, 300, 400, and again 300 K with velocity reassignment every 0.5 ps followed by 50 ps simulation at 300 K with no velocity reassignment. After the equilibration, the main simulations were run for 1500 ps. During the simulations the cutoff was 14 Å for all interactions. MD simulations in a vacuum were performed using a similar equilibration procedure without cutoffs. Some computations in a vacuum (see text) were carried out for 2 ns.

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