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Determination of association constants at moderately fast chemical exchange: Complexation of camphor enantiomers by α-cyclodextrin

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Abstract

Association constants in weak molecular complexes can be determined by analysis of chemical shifts variations resulting from changes of guest to host concentration ratio. In the regime of very fast exchange, i.e., when exchange rate is several orders of magnitude larger than the Larmor angular frequency difference of the observed resonance in free and complexed molecule, the apparent position of averaged resonance is a population-weighted mean of resonances of particular forms involved in the equilibrium. The assumption of very fast exchange is often, however, tacitly admitted in literature even in cases where the process of interest is much slower than required. We show that such an unjustified simplification may, under certain circumstances, lead to significant underestimation of association constant and, in consequence, to non-negligible errors in Gibbs free energy under determination. We present a general method, based on iterative numerical NMR line shape analysis, which allows one for the compensation of chemical exchange effects, and delivers both the correct association constants and the exchange rates. The latter are not delivered by the other mentioned method. Practical application of our algorithm is illustrated by the case of camphor-α-cyclodextrin complexes.

Keywords: Chemical exchange; α-Cyclodextrin complexes; NMR titration; Association constants; Chiral recognition of camphor enantiomers

1. Introduction

Association constants K_a in weak molecular complexes in solution can be determined using a number of experimental methods such as microcalorimetry [1], chromatography [2], or NMR spectroscopy [3,4]. The latter method is, probably, most widely used in the quantitative studies of complex formation in which variation of a given NMR parameter *vs*. change of a molar ratio of complex components, sometimes termed NMR titration, is observed and quantitatively analyzed. Chemical shifts are most often applied for this purpose, but variation of nuclear magnetic relaxation rates [5], or self-diffusion coefficients [6], if sufficiently pronounced, can be used as well. Since almost always the exchange between complex components in the free and bound (complexed) state is fast on the NMR timescale, it is widely assumed that the observed NMR parameter value is the mole fraction weighted average of the parameter values in the free and complexed molecule [3]. This assumption includes chemical shift averaging as well and can be expressed by the equation:

$$\delta_{obs} = p_f\delta_f + p_c\delta_c \quad (1)$$

where δ and p are the chemical shifts and the mole fractions, respectively; c and f indices denote complex and free component, respectively. This assumption is valid for the chemical shift averaging provided the exchange is very fast on the chemical shift timescale [7], i.e.,

$$|\omega_f - \omega_c| \ll k \quad (2)$$

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where $\omega_f - \omega_c$ is Larmor angular frequency difference of the observed resonance in free and complexed molecule and k is the exchange rate.

If the complex formation is fast enough to coalesce resonances at ω_f and ω_c, but not sufficiently fast to fully average chemical shifts, the Eq. (1) will not be valid anymore. For $p_f \neq p_c$ an apparent resonance position can be shifted from the mole fraction weighted average towards the position of the resonance corresponding to the more populated species. Such effect is shown in Fig. 1. Since for very small and very large molar ratios of complex components the relations $p_f \gg p_c$ and $p_f \ll p_c$ hold, respectively, such a titration curve systematically results in too low value of association constant as can be deduced from data presented in Fig. 2. In consequence, too low Gibbs free energy values of complex formation are calculated. One order of magnitude change of K_a value results in 5.74 kJ/mole change of ΔG_0 at room temperature.

Due to a relative simplicity and practical applications of camphor enantiomers **A** and **B**, their complexes with α-cyclodextrin, α-CD, **C** (Scheme 1) have been frequently studied by different experimental methods [8–13]. The complexes have been found to be of 1:2 stoichiometry [11], that is the enantiomeric guest is embedded in the capsule formed by two α-CD molecules most probably connected by numerous hydrogen bonds between hydroxyl groups. The complexes are especially interesting objects for NMR study because of relatively large Gibbs free energy of complex formation [10,11] and pronounced Gibbs free energy of activation resulting in the broadening of a number of ^{1}H resonances owing to the exchange of camphor molecules between free and complexed species at room temperature [11,13].

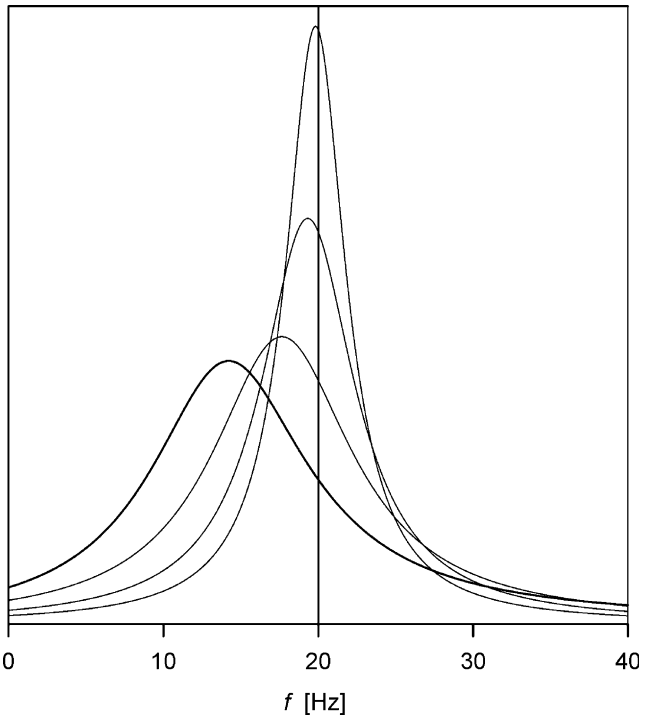


Fig. 1. Theoretical lineshapes of the exchange averaged resonances with the following parameters: $x_f = 0.9$, $f_f = 10$ Hz, $x_c = 0.1$, $f_c = 110$ Hz calculated for the chemical exchange rates (of 50, 100, 200, and 400 s^{-1} from left to right). Mole fraction weighted average of the resonance is equal to 20 Hz and its position is represented by a vertical solid line in the figure. The corresponding frequency deviations from the mole fraction weighted average were equal to: 5.79, 2.35, 0.68, and 0.17 Hz, respectively.

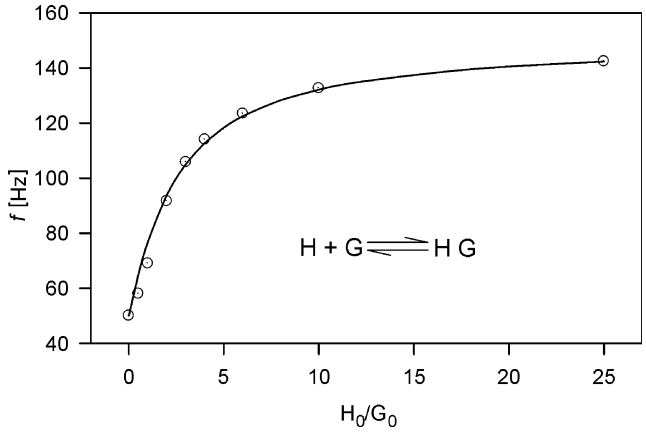


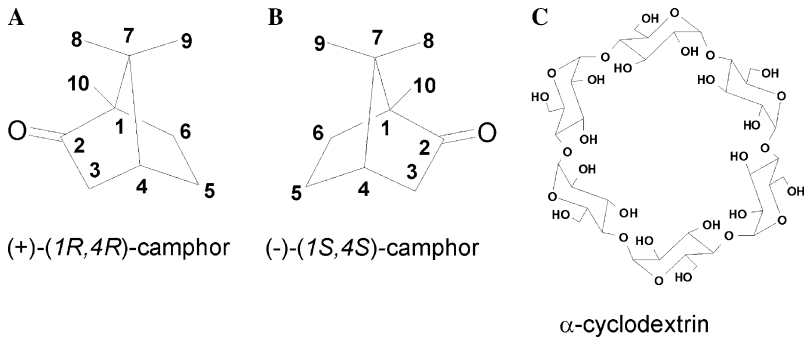
Fig. 2. The resonance frequencies as a function of the initial concentration ratios. Open circles represent resonance frequencies in component G *vs.* initial concentration ratios H_0/G_0 for the complex formation. Calculations were done assuming: $k = 256\ s^{-1}$ $(mM/dm^3)^{-1}$, $G_0 = 1$ mM/dm^3, $K_c = 500\ (M/dm^3)^{-1}$, $f_f - f_c = 100$ Hz. Solid line represents expected resonance positions at very fast chemical exchange limit. The least-square fit to the open circle data results in: $K_c = 418\ (M/dm^3)^{-1}$ and $f_f - f_c = 104$ Hz. K_c deviation owing to not compensated chemical exchange exceeds 16%.

2. Method

Correct K_a values can be obtained if chemical shifts used for their determination will be corrected for a chemical exchange effect, when necessary. Simultaneous fit of theoretical exchange broadened lineshapes calculated assuming constant values of K_a, exchange rate k, and chemical shift in complex δ_c, to all NMR spectra measured in a titration experiment seems to be a general and the most obvious procedure. It should be remembered, however, that the exchange rate k appearing in the theoretical formulation of the chemical exchange is the exchange rate of pseudofirst order. For the association described by a higher order reaction one has to take into account concentration of the complexing component in an appropriate power [14].

The theory of the influence of chemical exchange effect on NMR spectra has been well established for many years and can be found in numerous papers [7,15–18]. Software packages allowing one to simulate exchange influenced NMR spectra or fit them to the experimental ones are also available. In order to keep a close control of all stages of calculations we decided to create own computer program as described below.

The lineshape of each exchanging line can be numerically calculated in the frame of discrete two-site exchange model, where the magnetization $M(\omega)$ is described by the equation similar to Eq. (1) from Ref. [19]:



Scheme 1.

$$M(\omega) = N\mathbf{P}^{\mathrm{T}}[-\mathrm{i}(\mathbf{H} + \omega\mathbf{E}) + \mathbf{R} + \mathbf{X}]^{-1}\mathbf{1} \tag{3}$$

where N is the normalization constant, $\mathbf{P}^{\mathrm{T}} = [p_{\mathrm{f}}, p_{\mathrm{c}}]$ is the transposed column vector of mole fractions of species involved in the exchange under consideration, **H** denotes Hamiltonian operator of the spin system, **E** is a unit matrix, **R** is a relaxation matrix, **X** is a chemical exchange matrix, **1** is a column vector consisting of units, and ω denotes angular frequency. In the case of two exchanging singlets **H** is a diagonal matrix involving frequencies of resonances solely, **R** is also diagonal matrix and involves effective transverse relaxation rates

$$R_2^* = R_2 + \gamma\Delta B_0/2 \tag{4}$$

comprising also effect of external magnetic field inhomogeneity ΔB_0. **X** is composed of the following elements: $x_{11} = k_{\mathrm{fc}}$, $x_{12} = -k_{\mathrm{fc}}$, $x_{21} = -k_{\mathrm{cf}}$, and $x_{22} = k_{\mathrm{cf}}$, where k_{fc} denotes the exchange rate of pseudofirst order from site f to site c. Owing to the steady-state condition $p_{\mathrm{f}}k_{\mathrm{fc}} = p_{\mathrm{c}}k_{\mathrm{cf}}$, matrix **X** can be expressed as a function of only one exchange rate.

Simultaneous least-squares iterative analysis of all measured spectra was performed using an in-house written Fortran routine, based on the Newton–Raphson algorithm. Three local parameters: baseline position, zero-order phase correction, and scaling factor were fitted separately for each spectrum, while chemical shifts of the methyl groups in both forms (free—δ_{f} and complexed–δ_{c}), association constant K_{a}, and exchange rate k_{12} for complex formation were treated as global parameters identical for all analyzed spectra. The routine delivered also variance-covariance matrix whose diagonal elements were used to calculate standard errors of all the fitted parameters at the 99% confidence level.

3. Results and discussion

The approach presented above has been applied to study complex formation of either camphor enantiomer **A** and **B** with α-cyclodextrin **C**. Resonances of three methyl groups were observed for a wide range of α-CD-to-camphor molar ratios. Since the resonances broadened by chemical exchange were partially superposed, the deconvolution was applied in order to obtain accurate, but not corrected for chemical exchange, chemical shifts. Their values were further used for the association constant calculations (computational details are given in Experimental) assuming 1:2 stoichiometry of the complexes of camphor enantiomers with α-CD [11]. Then the K_{a} values were obtained independently by means of the procedure which took into account chemical exchange as described in Section 2. All global parameters obtained for both diastereomeric complexes were given in Table 1. The data obtained in this way were then compared with K_{a} values calculated without allowance for the shift compensation originating from the chemical exchange (Table 1). The results confirmed the claim that association constants obtained without taking into account the exchange were systematically lower (8–14%) due to the lack of chemical shift correction. Several experimental spectra for **A@C** complex and their best theoretical fits calculated using the procedure described in Section 2 were shown in Fig. 3.

It should be pointed out that association constants reported in the literature are mostly solvent uncorrected (further denoted as K_{c}). That is why association constants K_{c} are expressed in units $(\mathrm{M/dm^3})^{-n}$ where n stands for the sum of stoichiometry coefficients. When a complex of 1:2 stoichiometry is formed, $n = 2$. For diluted solutions the following relation between K_{a} and K_{c} holds:

$$K_{\mathrm{a}} = K_{\mathrm{c}}\, M_{\mathrm{s}}^{n} \tag{5}$$

where M_{s} is solvent molarity. In such a case, however, the use of widely applied formula [7]:

$$\Delta G_0 = -RT\ \ln(K) \tag{6}$$

with $K = K_{\mathrm{c}}$ is not justified and therefore the calculated Gibbs free energy values ΔG_0 are too low. Since molarity of ^2H_2O is equal to 55.19 $(\mathrm{M/dm^3})$ at 300.6 K [20], ΔG_0 change for the complexes studied here in ^2H_2O is equal to $-RT\ \ln(M_{\mathrm{s}}^2) = -20.05$ kJ/M. No doubts that to use $K_{\mathrm{a}} = 8.58 \cdot 10^8$ instead of $K_{\mathrm{c}} = 2.81 \cdot 10^5\ (\mathrm{M/dm^3})^{-2}$ makes a big difference.

It seems interesting to compare our present results with those obtained in other studies of the camphor@α-CD complex. First, the problem of stoichiometry should be briefly discussed. Chromatography studies showed that α-CD discriminates between the camphor enantiomers **A** and **B** [8]. Each of the complexes was shown to exhibit equilibrium of free species and those of 1:1 and 1:2

Table 1
Global parameters obtained in the fitting procedure allowing for the compensation of chemical exchange induced shift in diastereomeric complexes of camphor enantiomers **A** and **B** with α-cyclodextrin (**C**) as compared with those obtained without compensation.

Parameter	**A**@**C**[a]	**A**@**C**[b]	**B**@**C**[a]	**B**@**C**[b]
K_a (×10^8)	20.67 (0.14)	17.7 (0.4)	8.58 (0.03)	7.9 (0.2)
k_{12} [s^{-1}(mM/dm^3)$^{-2}$]	59.7 (0.6)	Not available	47.6 (0.4)	Not available
δ_c(H8) [ppm]	1.1240 (0.0003)	1.125 (0.001)	1.1271 (0.0004)	1.132 (0.002)
δ_c(H9) [ppm]	1.1699 (0.0003)	1.174 (0.002)	1.1979 (0.0002)	1.204 (0.002)
δ_c(H10) [ppm]	1.2499 (0.0004)	1.253 (0.001)	1.2355 (0.0003)	1.239 (0.001)

Experimental errors are given in parentheses.
[a] Compensation of chemical exchange as described in Section 2.
[b] No compensation of chemical exchange.

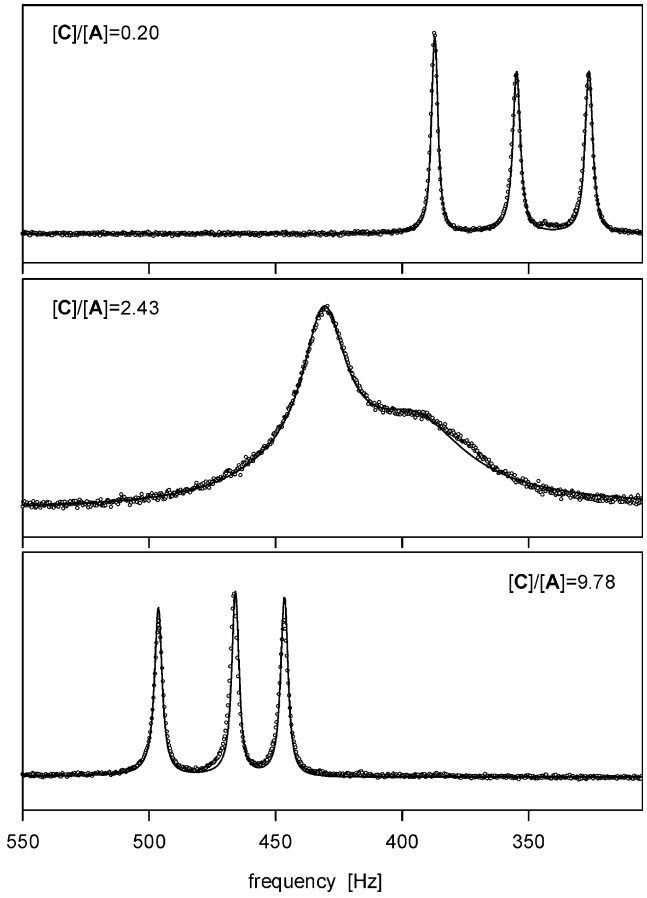


Fig. 3. Part of the experimental spectrum of three methyl groups (open circles) for three molar ratios of α-CD to (+)-camphor-**C**/**A** and the best theoretically calculated corresponding lineshape (line). Molar fractions of complexed **A**-p_c were equal to 0.02, 0.55, and 0.98 for **C**/**A** ratios of 0.20, 2.43, and 9.78, respectively. Frequency axis referenced to zero in the δ scale.

stoichiometries [9]. Although the subsequent measurements yielded the association constants for both complexes [21], the values obtained cannot be compared with those determined using other experimental techniques since the measurements were carried out in mixed solvents. We obtained the association constants of the complexes **A**@**C** and **B**@**C** on the basis of NMR titration [11]. However, the subsequent careful analysis showed that in the case of the former complex the decomposition

Table 2
Gibbs free energy values ΔG_0 (kJ/M) of complex formation of camphor enantiomers with α-CD and their differences $\Delta\Delta G_0$ (kJ/M) describing chiral recognition

Source	Method	T [K]	ΔG_0 (**A**@**C**)	ΔG_0 (**B**@**C**)	$\Delta\Delta G_0$
Ref. [10][a]	ITC	300.6	−54.0	−52.7	1.3
Ref. [11][b,c,d]	NMR	298	−53.1 (0.2)	−51.6 (0.8)	1.5 (0.8)
Present work[d]	NMR	300.6	−53.2 (0.3)	−51.2 (0.3)	2.0 (0.4)
Present work[e]	NMR	300.6	−53.6 (0.2)	−51.4 (0.1)	2.2 (0.2)

Experimental errors are given in parentheses.
[a] Interpolated to 300.6 K in ^2H_2O.
[b] Recalculated using solvent molarity $M_s = 55.22$ M/dm^3.
[c] Measurements at 298 K in ^2H_2O. K values obtained with Hunter program [23].
[d] Without compensation of chemical exchange.
[e] With compensation of chemical exchange.

of the association constant K_c into the components K_1 and K_2 corresponding to 1:1 and 1:2 complex formation was not unequivocal [22]. It took place because $K_1 \ll K_2$ and the concentration of 1:1 complex was practically negligible for all values of α-CD concentrations justifying the use of 1:2 stoichiometry solely in the conditions typical for NMR studies. Therefore, the claim of simultaneous appearance of 1:2 complex and two 1:1 complexes of different geometry concluded from a ROESY spectrum at the **A**:**C** = 3:1 ratio was obviously unfounded [13].

The association constants of the complexes **A**@**C** and **B**@**C** were also measured using ITC calorimetric technique [10]. The values of Gibbs free energy for the complexes of camphor enantiomers with α-CD obtained using NMR and ITC methods are collected in Table 2. Despite the small temperature difference between our two NMR studies, the ΔG_0 values obtained without chemical exchange compensation are consistent. However, taking into account the chemical exchange compensation resulted in larger K_a and, therefore, larger ΔG_0 values. Owing to the different data processing, the latter approach allowed us to reduce significantly corresponding experimental errors, a very important improvement when $\Delta\Delta G_0$ was considered.

4. Conclusions

We have presented a general method of determination of association constants K_a in weak molecular complexes

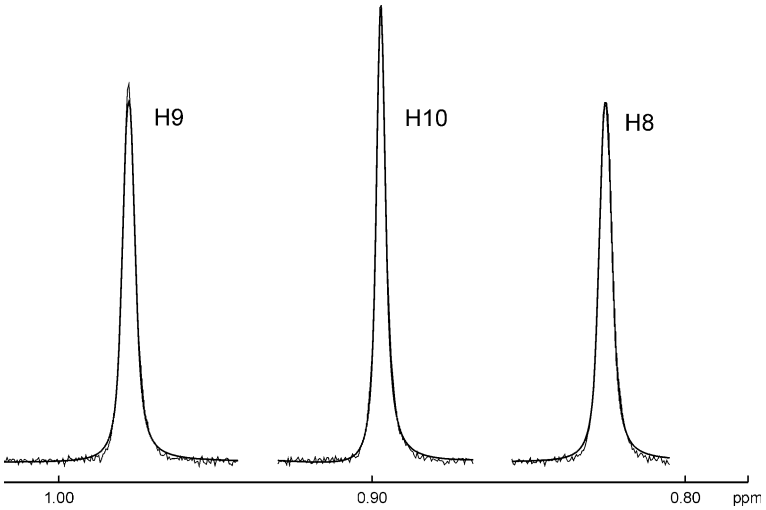


Fig. 4. Experimental and theoretical lineshapes of three methyl resonances in 1 mM solution of camphor in ^2H_2O. Experimental lineshapes and those obtained in the deconvolution procedure are superposed. Determined long range coupling constants were as follows: $^4J(H8,H9) = 0.6$ Hz and $^4J(H10,H6) = 0.4$ Hz.

when observed chemical shifts are not fully averaged during NMR titration experiment. The method relies on the simultaneous fit of theoretical exchange broadened lineshapes calculated assuming constant values of K_a, exchange rate k, and chemical shift in complex δ_c, to all NMR spectra measured in a titration experiment. If the apparent chemical shifts are not compensated for the chemical exchange effect, too low values of association constant and Gibbs free energy of complex formation will be obtained. Then the conclusions drawn from the comparison of data determined for different but similar complexes could be questionable.

5. Experimental

3.1 mg of (+)-camphor (**A**) enantiomer was dissolved in 20 ml of ^2H_2O (99.8 atom percent of deuterium) in order to obtain 1.0 mM solution. Five millilitres of this solution was separated from the rest and 97 mg (20-fold excess) of α-CD (**C**) was added. These basic solutions were mixed afterwards together in order to prepare NMR samples of molar ratios of **C** to **A** varying from 1:0.2 to 1:20. Accurate values of molar ratios were as follows: **C**/**A** {0.20, 0.48, 0.97, 1.56, 1.95, 2.43, 2.93, 3.91, 9.78, 19.57} and for **C**/**B** {0.21, 0.53, 1.07, 1.71, 2.14, 2.65, 3.20, 4.27, 5.34, 10.68, 21.32}. These values were checked by integration of signal of six anomeric protons in α-CD *vs*. those of nine methyl protons in camphor. Standard ^1H spectra of these samples were recorded on a Varian Inova 400 MHz spectrometer at 300.6 K. The temperature was carefully calibrated using reference ethylene glycol sample and its accuracy was better than 0.2 K. The recycle delay was 14 s and acquisition time 6.5 s which was altogether long enough to allow the observed spins to return to the thermal equilibrium ($T_{1,max} = 3.24 \pm 0.02$ s [12]) and provide sufficient digital resolution. The basic solutions used for sample preparation contained also ca. 20 mM of acetone whose ^1H NMR signal was used as the indicator of external magnetic field inhomogeneity ΔB_0 and internal secondary reference ($\delta = 2.22$). Transverse relaxation rates R_2s were taken from elsewhere [12].

Since most of the NMR resonances in camphor ^1H NMR spectrum were strongly scalar-coupled multiplets, only the signals of three methyl groups were further analyzed. At the first glance all of them were singlets, but careful lineshape analysis revealed that they bear unresolved, long-range scalar couplings, whose magnitudes were obtained by the deconvolution procedure. In further spectral analysis an approximation of weakly coupled nuclei was applied, i.e., the signals of H8 and H9 were treated as two mutually coupled sets of four lines of relative intensities 1:3:3:1 separated by long-range coupling of 0.6 Hz and the signal of H10 was treated as three line set 1:2:1 separated by 0.4 Hz. In the latter case, coupling constants to protons H_6^{eq} and H_6^{ax} were found to be equal (Fig. 4). Another approximation was that the chemical exchange of each component of methyl signals was independent. In other words, it was assumed that each of 11 lines constituting three methyl signals in uncomplexed **A** exchanged with respective lines of **A**@**C**. The latter spectrum, of course, also consisted of 11 lines. A similar procedure was repeated for the (−)-camphor enantiomer **B**.

Values of the association constants delivered from the chemical shifts uncorrected for the chemical exchange were computed by grid search in the space of the following parameters: association constant K_a and three chemical shifts of methyl groups δ_c in a complex. Parameter uncertainties were obtained as standard deviations from 200 Monte Carlo simulations.

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