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Determination of Binding Constants for Cyclodextrin Complexes with Alkanols by the ^{1}H NMR Measurements of Longitudinal Relaxation Time Using Tetramethylammonium Chloride as an Internal Reference

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Abstract. The longitudinal relaxation times (T_1) of ^{1}H NMR signals for a variety of alkanols in D_2O markedly decreased with increasing concentrations (c) of α- and β-cyclodextrins (CD). Tetramethylammonium chloride (TMA) used as an internal reference was available for evaluating an effect of solution viscosity on relaxation rates $R(= 1/T_1)$, since TMA showed no appreciable interaction with CD. Changes in the ratio of R for alkanol protons to R for TMA protons with c were analyzed by the curve-fitting method to give K_a. These K_a values agreed well with those obtained by the analysis of changes in δ, indicating that T_1 measurement is available for the determination of K_a for CD complexes. 2D ROESY spectra provided definite information on the molecular structures of CD complexes with alkanols.

Key words: cyclodextrin, alkanol, inclusion complex, binding constant, NMR spectroscopy, longitudinal relaxation time, ROESY spectrum.

1. Introduction

Cyclodextrins (CDs) provide hydrophobic cavities into which a variety of organic molecules are trapped to form inclusion complexes [1]. The formation of a CD inclusion complex is often studied by means of nuclear magnetic resonance (NMR) spectroscopy [2, 3]. For example, the complexation of CD with a guest molecule generally causes changes in chemical shifts (δ) of ^{1}H and ^{13}C involved in CD or guest. The changes in δ with the concentration of CD or guest are numerically analyzed to afford the binding constant (K_a) of a CD complex. This so-called NMR shift titration [3] is very useful for the determination of K_a. In this connection, we have previously reported [4] that accurate K_a values are obtained by the use

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of tetramethylammonium chloride (TMA) or methanol as an internal reference in NMR measurement. Interactions of CD with TMA and methanol are too weak to affect the δ values of these reference compounds. External referencing for the measurement of δ often leads to erroneous K_a values unless adequately corrected, since the magnetic susceptibility of a D_2O solution changes with the addition of CD, especially when high CD concentrations are used.

Additional NMR parameters available for the study of CD inclusion complexes are the longitudinal and transverse relaxation times (T_1 and T_2, respectively) of nuclei such as ^{1}H [5, 6], ^{2}H [7, 8], ^{13}C [7, 9–15], and ^{81}Br [16]. CD complexation usually prolongs the rotational correlation times of these guest nuclei and reduce their relaxation times. The change in correlation or relaxation time is a good indication for the molecular motion of a guest in the CD cavity. In addition, a relationship between a change in relaxation time and CD concentration is available for the determination of K_a [5, 16]. This method for the determination of K_a is especially useful for guest systems in which CD complexation induces too small changes in δ to be numerically analyzed [5]. However, only a few examples have been reported using this method. Furthermore, an effect of solution viscosity on T_1 or T_2 was neglected or regarded as negligible in these works, despite the fact that solution viscosity generally changes with a change in concentration of host or guest. In the present work, we evaluated an effect of solution viscosity on T_1 by using TMA as a control probe and determined the K_a values for α- and β-CD complexes with various alkanols by analyzing the corrected T_1 values of guest protons. These K_a values were compared with those obtained by δ measurements, as well as those in the literature, to confirm the validity of this method. We selected ^{1}H as a nucleus to be observed. The NMR sensitivity of ^{1}H is so high that its T_1 is easily and accurately measured even at a relatively low concentration of a guest molecule. On the other hand, the natural abundance of ^{13}C is so low that its T_1 has to be measured at such a high concentration that self-association of substrate occurs [11]. Alkanols were selected as guests, since alkanols are relatively soluble in D_2O, various structural isomers and homologs of alkanols are easily available, K_a values for α- and β-CD complexes with various alkanols are available in the literature [17] for comparison, and there is no report dealing with the effect of CD on T_1 of alkanol ^{1}H.

2. Experimental

2.1. MATERIALS

The α- and β-CDs were supplied by Nihon Shokuhin Kako Co., Ltd. and Ensuiko Seito Co., Ltd., respectively. They were dried overnight in vacuo at 110 °C. The D_2O used (Isotec) contained 99.8 atm% D. TMA and alkanols examined were of reagent grade and commercially available.

2.2. APPARATUS

^{1}H NMR spectra were recorded using a JEOL Model JNM-A400 FT NMR spectrometer (400 MHz) with sample tubes of 5.0 mm diameter at 25.0 ± 0.1 °C. Sample solutions were composed of 5–10 mmol dm^{-3} alkanols and 0–120 mmol dm^{-3} α-CD or 0–10 mmol dm^{-3} β-CD in D_2O. TMA (1.0 mmol dm^{-3}) was used as an internal reference (δ = 3.176) for ^{1}H NMR [6]. Immediately before NMR measurements, N_2 gas was bubbled into the sample solutions for 10 minutes to remove dissolved oxygen. The longitudinal relaxation times (T_1) of alkanol protons were measured by the inversion recovery method with a pulse sequence of $\pi - \tau - \pi/2$ (τ: interval between π and $\pi/2$). The phase-sensitive two-dimensional ROESY (rotating-frame nuclear Overhauser enhancement spectroscopy) spectra of CD inclusion complexes with alkanols were acquired with a mixing time of 500 ms, 256 points for t_2, and 128 points for t_1, followed by zero-filling.

3. Results and Discussion

3.1. EFFECT OF α-CD ON THE T_1 OF TMA AND ETHANOL METHYL PROTONS

Figure 1 illustrates changes in the T_1 of TMA and ethanol methyl protons with the addition of α-CD. The T_1 of TMA apparently decreased with increasing α-CD concentration, in spite of negligibly weak interactions of TMA with α-CD [4]. Thus, the observed decrease in T_1 is attributed to an increase in the relative viscosity (η_{rel}) of the solution with increasing α-CD concentration. The relaxation rate R ($= 1/T_1$) generally increases in proportion to an increase in solution viscosity [12]. Figure 2 shows the plot of R for TMA vs. η_{rel}, where η_{rel} was evaluated by an equation relating η_{rel} of an aqueous solution to α-CD concentration (c/mol dm^{-3}): $\eta_{rel} = 1+2.39c+7.8c^2$ [18]. The plot gave a virtually straight line with an intercept approximately equal to zero ($R = -0.005 + 0.105\eta_{\mathrm{rel}}$, n = 6, r = 0.997), indicating that a change in solution viscosity is responsible for the observed decrease in T_1 and increase in R of TMA methyl protons with the addition of α-CD.

Figure 1 also illustrates a change in T_1 of the ethanol methyl protons with the addition of α-CD. The T_1 value remarkably decreased with increasing α-CD concentration. Ethanol is known to form a 1 : 1 inclusion complex with α-CD [4, 17]. Thus, the decrease in T_1 will be due not only to an increase in solution viscosity but also to complexation of ethanol with α-CD. The complexation of ethanol with α-CD is so rapid on the NMR time scale that the observed R (R_{obsd}) is expressed as

$$R_{obsd} = (1-\alpha)R_f + \alpha R_c, \tag{1}$$

where R_f and R_c refer to the relaxation rates of free and complexed ethanol, respectively, and α to the mole fraction of complexed ethanol. R_f will be proportional to solution viscosity, though its proportionality constant will be different from that of R (R_0) for TMA. It is difficult to judge whether R_c is proportional to

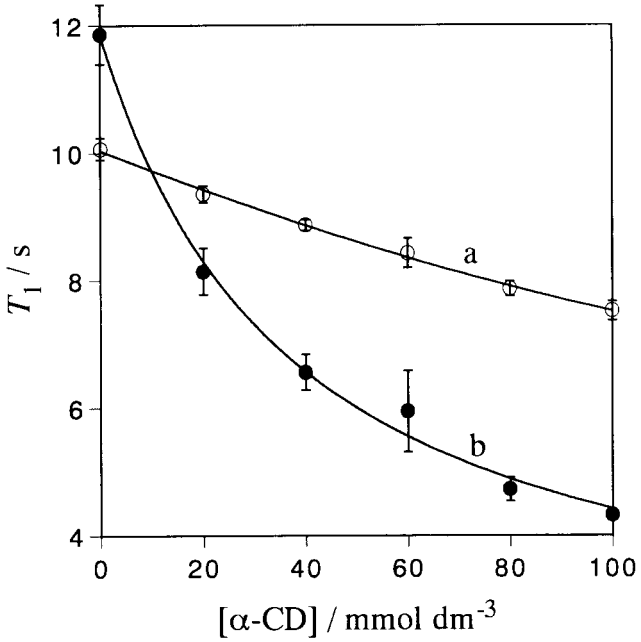

Figure 1. Effect of the addition of α-CD on the T_1 values for 1.0 mmol dm^{-3} TMA (a) and 10.0 mmol dm^{-3} ethanol (b) in D_2O at 25 °C.

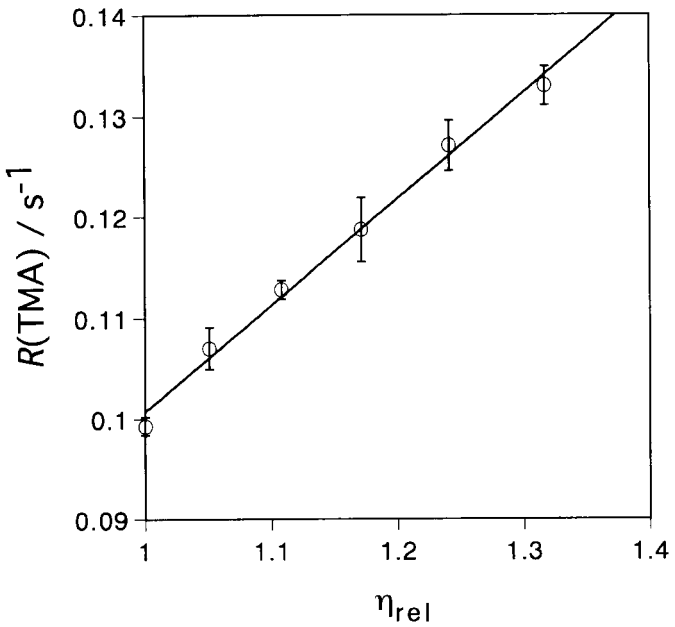

Figure 2. A plot of R for TMA vs. relative viscosity (η_{rel}) of the α-CD solution.

solution viscosity or not, since the molecular motion of included ethanol will be significantly different from that of free ethanol. However, we assumed that R_c is also proportional to solution viscosity, though its proportionality constant will be significantly different from that of R_0 for TMA. The validity of the this assumption will be judged by the validity of the K_a values determined on the basis of this assumption. Thus, we can derive a following equation from Equation (1):

$$r_{\text{obsd}} = (1 - \alpha)r_f + \alpha r_c, \tag{2}$$

where r_{obsd}, r_f, and r_c are the ratios of R_{obsd}, R_f, and R_c to R_0, respectively. If R_0, R_f, and R_c are proportional to solution viscosity, r_f and r_c are constants over the entire concentrations of α-CD, irrespective of a change in solution viscosity. Equation (1) is rewritten as

$$\Delta r = \Delta r_0 \alpha, \tag{3}$$

where $\Delta r = r_{\text{obsd}} - r_f$ and $\Delta r_0 = r_c - r_f$. This equation is similar to that derived for the numerical analysis of changes in chemical shifts (δ) of the guest protons with the addition of CD [4]:

$$\Delta\delta = \Delta\delta_0 \alpha, \tag{4}$$

where $\Delta\delta = \delta_{\text{obsd}} - \delta_f$ and $\Delta\delta_0 = \delta_c - \delta_f$, the meaning of the subscripts being the same as that of r. Thus, a K_a value for a CD complex can be determined by analyzing a relationship between Δr and CD concentration (c) in a similar manner to the analysis of $\Delta\delta$ [4]. Figure 3 illustrates the plot of r_{obsd} vs. c, together with that of $\Delta\delta$ vs. c, obtained for ethanol methyl protons (δ = 1.172 at c = 0 mmol dm^{-3}). Corresponding data for ethanol methylene protons could not be obtained, since the ^{1}H NMR signal overlapped with α-CD proton signals. It is evident that the standard errors of r_{obsd} are fairly larger than those of $\Delta\delta$. The inaccuracy of the T_1 measurement itself, together with the incomplete removal of oxygen from the sample solution, will be responsible for the larger standard errors of r_{obsd}. On the basis of an assumption that ethanol forms a 1 : 1 complex with α-CD, changes in r_{obsd} and $\Delta\delta$ with c were analyzed by the nonlinear least-squares curve-fitting method (solid lines). The K_a value obtained from r_{obsd} was 5.2 mol^{-1} dm^3, which agreed well with that (5.6 mol^{-1} dm^3) obtained from $\Delta\delta$ and those (5.6 [17] and 4.6 [19] mol^{-1} dm^3) previously reported. This fact indicates that the assumption on a relationship between R and solution viscosity is valid and the T_1 measurement of ethanol methyl protons is available for the determination of K_a for an inclusion complex of ethanol with α-CD. This numerical analysis also provided the r_c value for the methyl protons of complexed ethanol to be 4.16, from which the corresponding T_1 value was evaluated to be 2.42 s. This value is ca. four times smaller than that (10.07 s) of free ethanol, suggesting that the internal rotation of ethanol is significantly retarded by complexation. According to Cahill and Bulusu [5], the T_1 value of a guest proton approaches ca. 0.35 s, when the guest is bound to α-CD

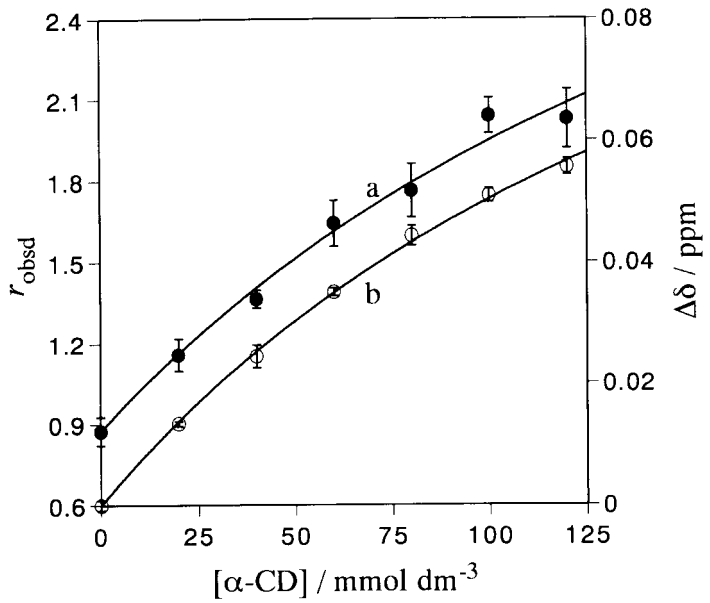

Figure 3. A plot of r_{obsd} (a) and $\Delta\delta$ (b) vs. α-CD concentration.

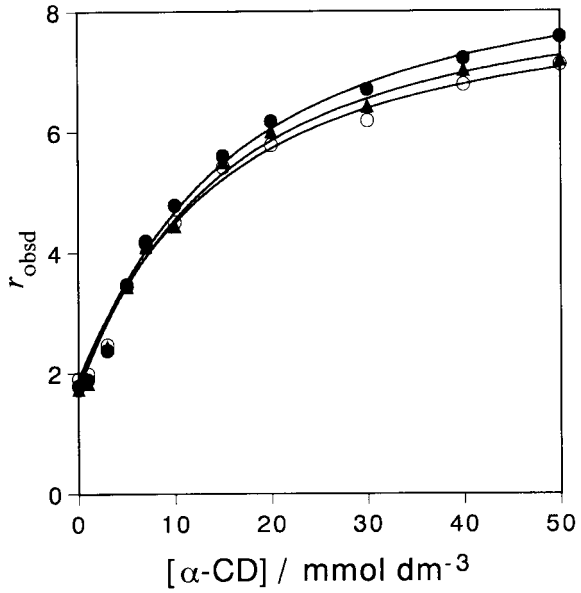

Figure 4. A plot of r_{obsd} for the 2-CH_2 (○), 3-CH_2 (●), and CH_3 (▲) protons of 1-butanol vs. α-CD concentration.

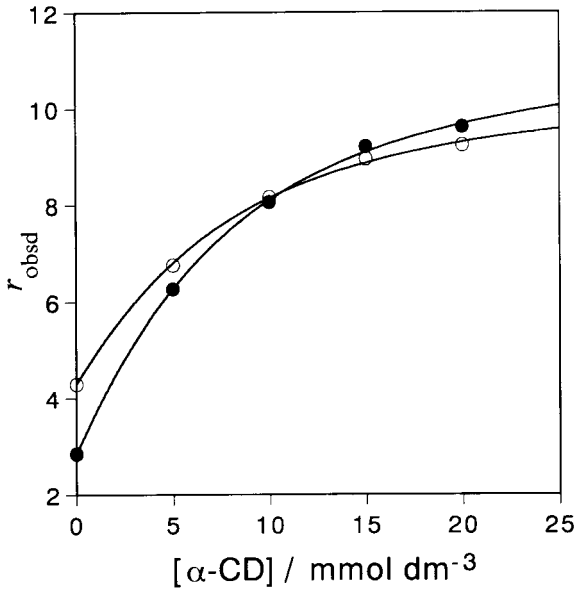

Figure 5. A plot of r_{obsd} for the 1-CH_3 (○) and 5-CH_3 (●) protons of 2-pentanol vs. α-CD concentration.

too tightly to rotate freely within the CD cavity. The observed T_1 value for ethanol indicates that complexed ethanol is weakly coupled with α-CD from the dynamic point of view.

3.2. COMPLEXATION OF VARIOUS ALKANOLS WITH CD

In order to confirm the validity of this approach, the K_a values for complexes of various alkanols with α- and β-CD were determined and compared with those determined by spectrophotometric examination of inhibitory effects of alkanols on CD complexation with a dye [17]. Figure 4 illustrates the plots of r_{obsd} vs. c for the 2- and 3-methylene and methyl protons of 1-butanol. These protons gave ^{1}H NMR signals at δ = 1.515, 1.335, and 0.891, respectively, in the free state. The numerical analysis of the change in r_{obsd} with c gave virtually the same K_a value of 84 $\pm$ 2 mol^{-1} dm^3 and the same T_1 value of 1.16 $\pm$ 0.03 s for complexed 1-butanol. The K_a value fairly agreed with that (96 $\pm$ 2 mol^{-1} dm^3) obtained by the analysis of changes in δ, together with that (89 mol^{-1} dm^3) reported [17]. Thus, we could confirm the validity of this approach by means of three kinds of protons. Figure 5 shows the plots of r_{obsd} vs. c for two kinds of methyl protons (1- and 5-CH_3) of 2-pentanol. These protons gave ^{1}H NMR signals at δ = 1.145 and 0.881, respectively, in the free state. The T_1 value (2.07 s) for 1-CH_3 of uncomplexed 2-pentanol was significantly smaller than that (3.13 s) of 5-CH_3. However, the numerical analysis gave virtually the same T_1 value of 0.90 $\pm$ 0.03 s for complexed 2-pentanol. This fact suggests that the rotational freedoms of these methyl groups are virtually equal

Table I. The K_a values for CD-alkanol complexes determined by the analysis of changes in T_1 and δ of alkanol protons in D_2O at 25 °C

Host	Guest	K_a/mol^{-1} dm^3		
		T_1	δ	Lit.[a]
α-CD	Ethanol	5.2 5.7[b]	5.6	5.6
	1-Propanol	19 ± 1	9 ± 2	23
	2-Propanol	4.2	4.3	4.9
	1-Butanol	84 ± 2 90 ± 5[b]	96 ± 2	89
	2-Butanol	20 ± 3	28 ± 2	26
	2-Methyl-1-propanol	46 ± 7	24 ± 1	28
	2-Methyl-2-propanol	6.7	5.5	4.4
	1-Pentanol	434 ± 68	405 ± 1	324
	2-Pentanol	205 ± 1	206 ± 2	135
	2-Methyl-1-butanol	101 ± 5	118 ± 2	110
	2,2-Dimethyl-1-propanol	17 ± 1	19 ± 1	30
β-CD	1-Butanol	10 ± 2	14 ± 1	17
	2-Methyl-2-propanol	60	66	48
	1-Pentanol	180 ± 10	169 ± 6	63
	2,2-Dimethyl-1-propanol	596	466	575
	1-Hexanol	280 ± 50	220 ± 2	220

[a] Reference [17]: In H_2O solutions at 25 °C.
[b] Determined in the presence of dissolved oxygen.

to each other in the α-CD cavity, though they are significantly different in a bulk solution. The obtained K_a value (205 ± 1 mol^{-1} dm^3) agreed well with that (206 ± 2 mol^{-1} dm^3) obtained from changes in δ, though somewhat different from that (135 mol^{-1} dm^3) reported [17].

Table I summarizes K_a values determined by T_1 and δ measurements for complexes of various alkanols with α- and β-CD. The K_a values determined by T_1 measurement agreed, on the whole, with those determined by δ measurement and those reported, indicating that T_1 measurement is available for the determination of K_a. We also examined the effect of dissolved oxygen on T_1 and K_a for complexes of α-CD with ethanol and 1-butanol. The T_1 values for the protons of these alkanols in the presence of dissolved oxygen were significantly smaller than those in the absence of dissolved oxygen. However, the K_a values obtained agreed well with those in the absence of oxygen (Table I). Degassing is not necessarily required for the determination of K_a by means of T_1 measurement. In conclusion, the determination of K_a by T_1 measurement has the advantage that an effect of CD on T_1 of guest protons is very large. Although the precision of T_1 measurement is less

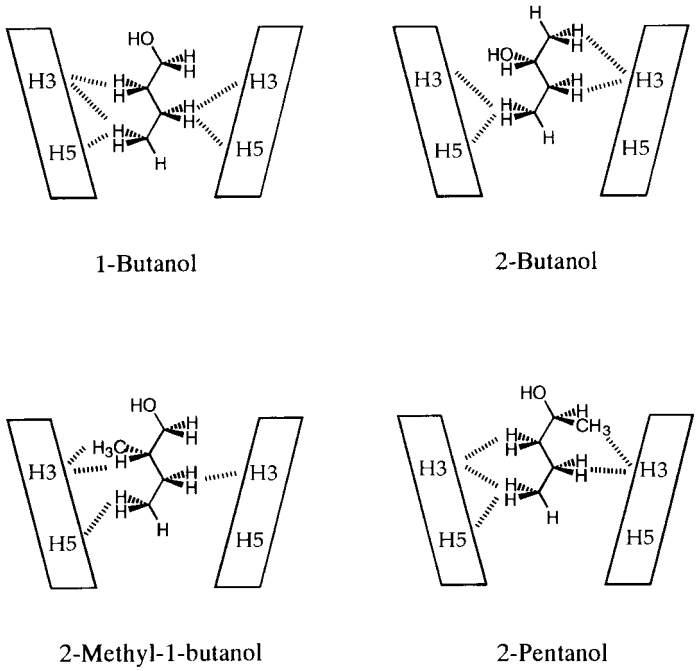

Figure 6. Possible molecular structures of α-CD inclusion complexes with some alkanols estimated by the measurements of ROESY spectra. Cross-peaks were observed between protons connected by dashed lines.

than that of δ measurement, the precision of K_a determined by T_1 measurement is similar to that by δ measurement. In the present study, the effect of alkanol structure on K_a was not discussed, since it was fully discussed in a previous paper [17] which showed that hydrophobic and van der Waals interactions are of primary importance in the complexation of alkanols with CD.

3.3. MOLECULAR STRUCTURES OF CD-ALKANOL COMPLEXES

The molecular structures of CD inclusion complexes with alkanols were estimated by the measurement of ROESY spectra in D_2O solutions. In an α-CD-1-propanol system, cross-peaks were observed between the C(3)- and C(5)-H of α-CD and the 2-CH_2 and CH_3 of 1-propanol. The ^{1}H NMR signal for the 1-CH_2 of 1-propanol overlapped with the signals of α-CD, so that we could not judge whether there were cross-peaks between the 1-CH_2 and α-CD protons or not. The cross-peak connecting the C(5)-H of α-CD to the CH_3 of 1-propanol was significantly higher than that connecting to the 2-CH_2 of 1-propanol, suggesting that 1-propanol is included in the α-CD cavity in such a manner as the CH_3 and 1-CH_2 of 1-propanol being close to the narrower and wider rims of the α-CD cavity, respectively. The ROESY spectrum of an α-CD-1-butanol system gave us more definite information on the

molecular structure of its complex. Very similarly to 1-propanol, we found cross-peaks between the C(3)- and C(5)-H of α-CD and the 3-CH_2 and CH_3 of 1-butanol. On the other hand, the 2-CH_2 of 1-butanol gave only a cross-peak connecting to the C(3)-H but not to the C(5)-H of α-CD, indicating that 1-butanol is included in the α-CD cavity in such a manner as the CH_3 and 1-CH_2 of 1-butanol being close to the narrower and wider rims of the α-CD cavity, respectively (Figure 6). In the case of an α-CD-1-pentanol system, the CH_3 of 1-pentanol gave only a cross-peak connecting to the C(5)-H but not to the C(3)-H of α-CD, indicating that 1-pentanol is more deeply included in the α-CD cavity than those of 1-propanol and 1-butanol. Figure 6 illustrates the estimated molecular structures for inclusion complexes of α-CD with a few alkanols. Cross-peaks were observed between protons connected by dashed lines in the figure. In all the examined alkanols, relatively hydrophobic parts are close to the narrower rim of α-CD, and relatively hydrophilic hydroxy groups are close to the wider rim of α-CD. It is clear that hydrophobic interactions between α-CD and alkanols take part in the orientation of alkanols within the CD cavity.

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