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Solution Structures of 1:1 Complexes of Oxyphenonium Bromide with β- and γ-Cyclodextrins

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Abstract: The solution structures of complexes of oxyphenonium bromide (OB) with β- and γ-cyclodextrins (β- and γ-CDs, respectively) in deuterium oxide have been investigated by 500 MHz proton NMR spectroscopy and molecular mechanics calculations. The chemical shifts induced by complex formation provide the 1:1 binding constants and the chemical shift variations, $\Delta\delta_{OB-CD}$, with complexation for the protons of OB and the CDs. The observed binding constants are very close to those obtained by other methods and are in the following order: β-CD > γ-CD > α-CD. Initial structures of the complexes are constructed on the basis of the ROESY spectra and the $\Delta\delta_{OB-CD}$ values and are optimized by molecular mechanics calculations. The intermolecular distances between the protons of OB and CD calculated for these structures are well-correlated with the observed ROESY intensities. The cyclohexyl group of OB penetrates deeply into a β-CD cavity, and the phenyl group is close to the wide rim of the cavity. The phenyl and cyclohexyl groups of OB are both incorporated into a γ-CD cavity. Furthermore, these structures of the complexes are consistent with the suppression of bitter taste and basic hydrolysis of OB by CDs and the polarity of binding sites of OB.

Keywords: Oxyphenonium bromide; cyclodextrin; NMR; structure

Introduction

Cyclodextrins (CDs) have homogeneous toroidal structures of different molecular sizes. Most typical are cyclohexaamylose (α-CD), cycloheptaamylose (β-CD), and cyclooctaamylose (γ-CD). The toroidal structure has a hydrophilic surface, making it water soluble, whereas the cavity is composed of the glucoside oxygens and methine hydrogens, giving it a hydrophobic character. As a consequence, the CDs can include other hydrophobic molecules of appropriate dimensions and shapes.[1−3] Because CDs are practically nontoxic, they have widespread applications in pharmaceuticals, cosmetics, and foods: solubility enhancement, stabilization of labile drugs, control of volatility and sublimation, physical isolation of incompatible compounds, long-term protection of color, odor, and flavor, and suppression of hemolysis and bitter tastes of drugs.[2,3]

Very recently, we investigated the suppression of the bitter taste of oxyphenonium bromide (OB, Figure 1), an anticholinergic drug having a parasympatholytic activity, by α-, β-, and γ-CD and showed that this suppression can be predicted on the basis of ultraviolet (UV) spectroscopic and electromotive force data. UV data allowed us to estimate the effective dielectric constants of the CD cavities where the phenyl group of OB is located.[4] Electromotive force data indicated the 1:1 stoichiometry of OB with α-, β-, and γ-CD.[5] The basic hydrolysis of OB is suppressed by the CDs.[6] To analyze these applications of CDs at the molecular level, we need

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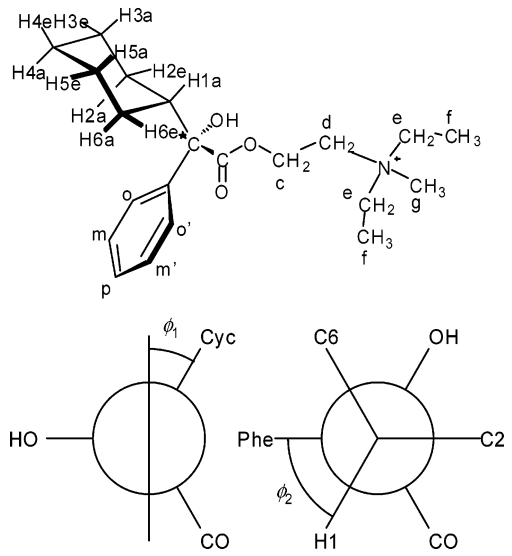

Figure 1. Chemical structures of oxyphenonium bromide and the definition of dihedral angles around the C1−C* and C(phenyl)−C* bond axes of (*S*)-OB in the Newman projection.

the solution structures of OB and its complexes with the CDs. Previously, the solution structures of OB and its complex with α-CD were determined by NMR and calculations of molecular mechanics, molecular dynamics, and molecular surface areas.[7,8]

The solution structures of CD inclusion complexes have been estimated mainly by chemical shifts, vicinal spin−spin coupling constants, and NOE of NMR.[9,10] However, because most of the structures were determined on the basis of qualitative estimations of NOE cross-peak intensities, the precision in atomic coordinates is low. On the other hand, although detailed structures of CD complexes have been supplied by calculations of molecular mechanics and molecular dynamics,[6,11] they have not fully been compared to NMR data.[9,10] The solution structures of biopolymers have been determined by molecular mechanics or dynamics calculations using the structural information (interproton distances, dihedral angles, and others) obtained by NMR.[12,13] This strategy has not been applied to determine the solution structures of CD complexes. Very recently, we have determined rather fine solution structures of α-CD complexes on the basis of quantitative evaluation of NOE cross-peak intensities.[8,14] Because β- and γ-CDs have wider cavities than α-CD, their complexes can have many plausible structures. For this reason, reports on solution structures of inclusion complexes of β- and γ-CDs are limited.[9,10]

In this work, we investigated the solution structures of complexes of OB with β- and γ-CDs by a combination of proton NMR spectroscopy and molecular mechanics calculations. As Figure 1 shows, OB has the phenyl and cyclohexyl groups. These groups can bind to the cavities of the CDs. Because β- and γ-CDs have wider cavities than α-CD, their 1:1 complexes with OB are expected to have many plausible structures. To estimate the solution structures of the complexes of OB with β- and γ-CD, we quantitatively analyzed ROE intensities of the complexes and energy-optimized the solution structures by molecular mechanics calculations. On the basis of these solution structures, bitter taste reduction, chemical stabilization, and microenvironmental changes of OB by CDs, previously reported,[4−6] will be discussed.

Experimental Section

Materials. Commercial samples of 99% deuterium oxide (Aldrich) and the racemic mixture of (*R*)- and (*S*)-2-[(cyclohexylhydroxyphenylacetyl)oxy]-*N*,*N*-diethyl-*N*-methylethanaminium bromide (OB, Sigma) were used as received. This sample of OB gave a single peak on a reversed-phase high-performance liquid chromatogram. Tetramethylammonium chloride (TMA, Nacalai Tesque, Kyoto, Japan) was a specimen of guaranteed grade. Commercial samples of β- and γ-CD (Nacalai Tesque, Kyoto, Japan) were used.

NMR Measurements. All ^{1}H NMR spectra were recorded with a JEOL Lambda 500 MHz spectrometer at 309.7 ± 0.1 K, and the data processing was performed with JEOL standard software. All one-dimensional spectra obtained were deconvoluted with Nuts NMR data processing software (Acorn NMR Inc.). The temperature was chosen for comparison with our experiments of the bitter taste of OB.[4,5] The proton chemical shifts of OB and β-CD were determined for a series of deuterium oxide solutions containing 4 mmol/dm^3 (mM) OB and β-CD in the concentration range of 0.5−9

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mM. The chemical shifts (δ) were referenced to the internal signal of 1 mM TMA at 3.176 ppm.[7] For mixtures of OB and γ-CD, NMR spectra of two series of solutions were recorded: the first series contained 2 mM OB and variable concentrations of γ-CD up to 100 mM, and the second contained 2 mM γ-CD and variable concentrations of OB up to 30 mM.

The 500 MHz ROESY spectroscopy (phase-sensitive rotating frame Overhauser enhancement spectroscopy) for a solution containing 20 mM OB and 10 mM β-CD was performed with the JEOL standard pulse sequences; data consisted of eight transients collected over 2048 complex points. A mixing time of 400 ms, a repetition delay of 1.2 s, and a 90° pulse width of 11.0 μs were used. The ROESY data set was processed by applying an exponential function in both dimensions and zero-filling to 2048 × 2048 real data points prior to the Fourier transformation. Under similar conditions, the ROESY spectrum of 40 mM OB and 40 mM γ-CD was recorded. Small cross-peaks on the ROESY spectra were neglected, because their magnitude was close to that of noise. The volume of a ROESY cross-peak was calculated by summation of spectrum intensities with a certain region around the cross-peak and slightly depended on the region of integration, the peak overlap, and the signal-to-noise ratio.

Molecular Mechanics Calculations. The solution structure of OB determined by NMR was employed.[5] The initial structures of β- and γ-CD were taken from the literature.[15,16] These structures were regarded as being flexible, whereas that of OB was regarded as a rigid body. The starting structures of the complexes of OB with β- and γ-CD were generated on the basis of NMR data with our own software,[8,16] and energy minimization of this structure was carried out by molecular mechanics calculations. These calculations were performed with Molecular Simulation Insight II/Discover (98.0) on a Silicon Graphics Octane workstation. The Discover III CVFF force field was used for energy minimization.[17] The energy minimization was performed using the conjugate gradients method to a derivative of 0.001 kcal/mol with a cutoff distance for van der Waals and electrostatic forces of 1.6 nm.

The complex was placed into a unit cell of 2.5 nm × 2.5 nm × 3.5 nm, where the symmetry axis of CD is parallel to the long axis. The complex was then soaked in 642 water molecules. The periodic boundary condition was applied on this cell. The total potential energy of this system was minimized. Simulated annealing of the system was carried out every 1 fs for 1000 fs at a final temperature of 500 K in the NVT ensemble, after the initial velocities of atoms were given at 10 K. After the molecular dynamics simulation, the total potential energy of this system was again minimized to obtain the final structure of the complex.

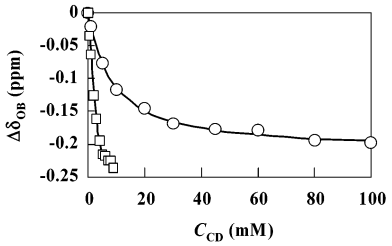

Figure 2. Dependence of the chemical shifts of the OB H1a proton on the CD concentration, where the OB concentration was kept at 4 and 2 mM for β-CD (□) and γ-CD (○), respectively. The solid lines were calculated using the values of K_1 and $\Delta\delta_{OB-CD}$(H1a) given in Tables 1 and 2.

Results

Chemical Shifts and Binding Constants. Assignments of all protons of OB on the 500 MHz NMR spectrum have already been established, and those of β- and γ-CDs have been made in reference to the α-CD spectrum.[7,8] In Figure 2, the chemical shift of the H1a proton of OB is shown as a function of the concentration of β- or γ-CD. This variation is induced by the 1:1 complexation of OB with these CDs, because the absence of other complexes has been established by electromotive force measurements.[5]

When this complexation is rapid on the NMR time scale, the observed chemical shift of an OB proton can be written as

$$\delta = ([OB]\delta_{OB} + [OB{-}CD]\delta_{OB-CD})/C_{OB} \qquad (1)$$

where C_{OB}, [OB], and [OB−CD] denote the total concentration of OB and the concentrations of the free OB molecule and the 1:1 complex, respectively, and δ_{OB} and δ_{OD} stand for the chemical shifts of the free OB molecule and the 1:1 complex, respectively. The observed chemical shift of a CD proton can be written as

$$\delta = ([CD]\delta_{CD} + [OB{-}CD]\delta_{OB-CD})/C_{CD} \qquad (2)$$

where C_{CD}, [CD], and δ_{OB-CD} denote the total concentration of CD, the concentration of the free CD molecule, and the chemical shift of the complexed CD molecule, respectively.

Using a δ_{OB-CD} value and the equilibrium constant, K_1, of this 1:1 complexation, one can calculate a theoretical chemical shift at a given set of C_{OB} and C_{CD} values. Regarding K_1 and δ_{OB-CD} as adjustable parameters, one can best fit this theoretical chemical shift to the observed δ values by a nonlinear least-squares method. The H1a proton signal of OB did not overlap with the other signals of OB and β- and γ-CD, to allow us to determine accurate chemical shifts. Further, because this signal exhibited the largest shift on complex formation, the H1a proton was chosen to determine the K_1 value. Thus, we determined the best fit values of the δ_{OB-CD}(H1a) and K_1 values to the observed $\Delta\delta$ values of H1a (Figure 2). These values are given in Tables 1 ($\Delta\delta_{OB-CD}$

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Table 1. Chemical Shifts of Free OB and Chemical Shift Variations of OB Induced by Complexation with α-, *β*-, and *γ*-CD

		$\Delta\delta_{OB-CD}$		
proton	δ_{free}	α-CD[a]	*β*-CD	*γ*-CD
H1		−0.016	−0.030	−0.027
H2		0.006	0.027	0.036
H3		−0.103	−0.083	−0.086
H4		−0.005	−0.027	−0.003
H5		−0.058	−0.139	−0.166
H6		−0.010	−0.029	−0.046
Ho	7.611	0.049	−0.040	−0.080
Hm	7.469	0.074	0.079	0.053
Hp	7.404	0.026	0.059	0.074
H1a	2.422	0.080	−0.238	−0.210
H2a	1.369	0.141	0.080	−0.080
H2e	1.491	0.128	0.069	0.016
H3a	1.271	0.339	−0.009	−0.038
H3e	1.815	0.209	−0.122	−0.015
H4a	1.120	0.386	−0.026	−0.023
H4e	1.658	0.125	0.026	0.011
H5a	1.245	0.169	−0.011	−0.055
H5e	1.675	0.201	0.041	0.022
H6a	0.991	0.132	−0.059	0.074
H6e	1.339	0.214	0.010	−0.073

[a] The data for OB and α-CD are taken from ref 8.

Table 2. Observed Binding Constants, Dielectric Constants, and Dihedral Angles for the OB−CD Systems at 310 K

	K_1 (M^{-1})					
state	NMR	UV[b]	EMF[c]	D_{eff}[a]	φ_1 (deg)	φ_2 (deg)
α-CD	70[d]	94	58	64.7−61[d]	67[d]	
β-CD	6800 ± 3300[e]	7350	8500	2.9	−41	89
γ-CD	134 ± 8[e]	140	96	30.7	−58	92
OB					−64[f]	102[f]

[a] Effective dielectric constant determined in a 154 mM sodium bromide solution by UV.[4] [b] Determined in a 154 mM sodium bromide solution by UV.[4] [c] Determined in a 154 mM sodium bromide solution by electromotive force measurements.[5] [d] Taken from ref 8. [e] Standard deviation. [f] Taken from ref 7.

$= \delta_{OB-CD} - \delta_{OB}$ or $\Delta\delta_{OB-CD} = \delta_{OB-CD} - \delta_{CD}$) and 2. Furthermore, we determined the best fit δ_{OB-CD} values for all protons of OB and the CDs using the K_1 values given in Table 2. For *γ*-CD, we determined the best fit δ_{OB-CD} values for the *γ*-CD protons from two sets of the observed chemical shift data obtained at a constant *γ*-CD concentration and a constant OB concentration. Because the former δ_{OB-CD} values were more accurate than the latter, the former values are given in Table 1.

The binding constants of OB with *β*- and *γ*-CD have already been determined in 154 mM sodium bromide solutions at 309.7 K by electromotive force measurements[5] and UV spectroscopy.[4] As in Table 2, these binding constants are close to those obtained by the NMR method. This fact indicates that the present K_1 values are reliable.

Table 3. ROE Intensity/$n_{OB}n_{CD}$ Values of Intermolecular Cross-Peaks between the Protons of the Phenyl and Cyclohexyl Groups of OB and the H3, H5, and H6 Protons of *β*- and *γ*-CD in 500 MHz ROESY Spectra

	β-CD			*γ*-CD		
OB	H3	H5	H6	H3	H5	H6
H1a	0.0	0.0	0.0	0.6	1.2	0.9
H2a	2.4	12.7	3.9	0.0	0.7	0.5
H2e	5.8	16.1	6.3	1.7	1.9	0.8
H3a	0.9	7.4	3.4	1.0	2.3	1.4
H3e	1.9	12.7	6.3	2.3	1.8	0.7
H4a	3.3	10.1	4.4	1.3	1.0	0.6
H4e	0.5	5.1	1.8	2.6	2.4	1.3
H5a	3.3	10.1	4.4	1.3	1.0	0.6
H5e	5.8	16.1	6.3	2.6	2.4	1.3
H6a	3.3	10.1	4.4	1.3	1.0	0.9
H6e	9.3	14.5	3.9	3.2	2.3	1.4
Ho	9.5	3.7	0.7	3.5	2.7	1.7
Hm	2.1	0.8	0.2	7.4	2.2	0.0
Hp	0.0	0.0	0.0	2.5	0.3	0.0

All $\Delta\delta_{OB-CD}$ values for OB and α-, *β*-, and *γ*-CD are given in Table 1. It is generally difficult to directly correlate $\Delta\delta_{OB-CD}$ values with the three-dimensional structure of a CD inclusion complex.[9,10] From the relative magnitude of the $\Delta\delta_{OB-CD}$ values for H3, H5, H6, and H1a, however, one can imagine the structures of the complexes of OB with *β*- and *γ*-CD. Although the hydrophobic groups of OB in an α-CD cavity are partially incorporated from the wide rim (the secondary alcoholic side) of the α-CD cavity,[4,8] these groups in the complexes with *β*- and *γ*-CDs, however, are close to the wide rim (the secondary alcoholic side).

ROESY Spectra. To determine the structures of the 1:1 complexes of OB with *β*- and *γ*-CD, we recorded the ROESY spectra. As shown in Figures 3 and 4, intermolecular cross-peaks between OB and *β*- or *γ*-CD are observed in ROESY spectra. For the OB−*β*-CD system (Figure 3), the bound fraction ([OB−CD]/C_{OB}) of OB is 0.652, as calculated from the K_1 value. For the OB−*γ*-CD system (Figure 4), the bound fraction ([OB−CD]/C_{OB}) of OB is 0.493. The volume (ROE intensity) of a cross-peak was determined by integration. The intensities of the cross-peaks among protons H3, H5, and H6 of the CDs and the phenyl and cyclohexyl protons of OB are summarized in Table 3. No significant cross-peak between the other CD protons and the OB protons is observed.

The ROE intensity of the cross-peak is proportional to the number of equivalent protons. In Table 3, ROE/$n_{CD}n_{OB}$ is given, where n_{CD} and n_{OB} denote the numbers of equivalent protons of CD and OB, respectively. When internal rotations are slower than overall tumbling, we can expect eq 3:[12,13]

$$\text{ROE intensity} = k\sum_{i=1}^{n_{CD}}\sum_{j=1}^{n_{OB}} d_{CD_iOB_j}^{-6} \qquad (3)$$

where $d_{CD_iOB_j}$ denotes the distance between a proton (CD_i) of CD and a proton (OB_j) of OB. For simplicity, the effective

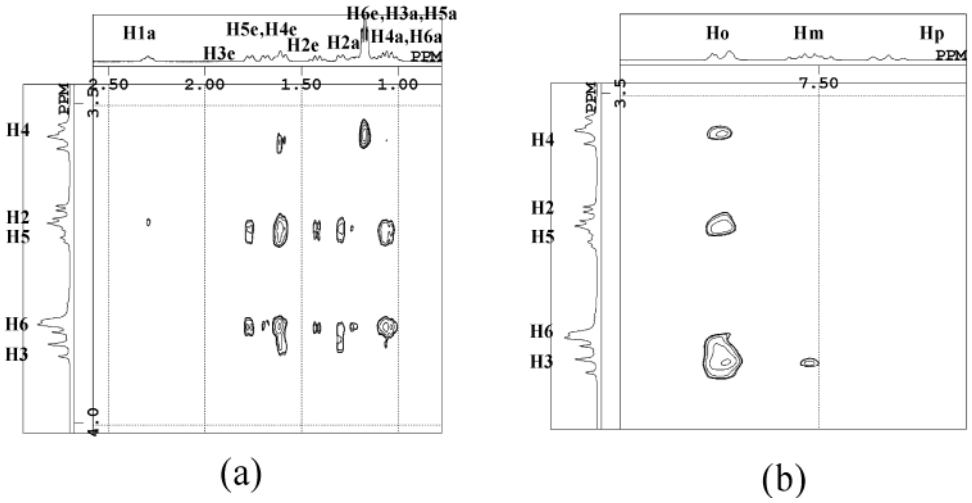

Figure 3. Partial ROESY spectrum of a mixture of OB (C_{OB} = 20 mM) and β-CD (C_{CD} = 10 mM).

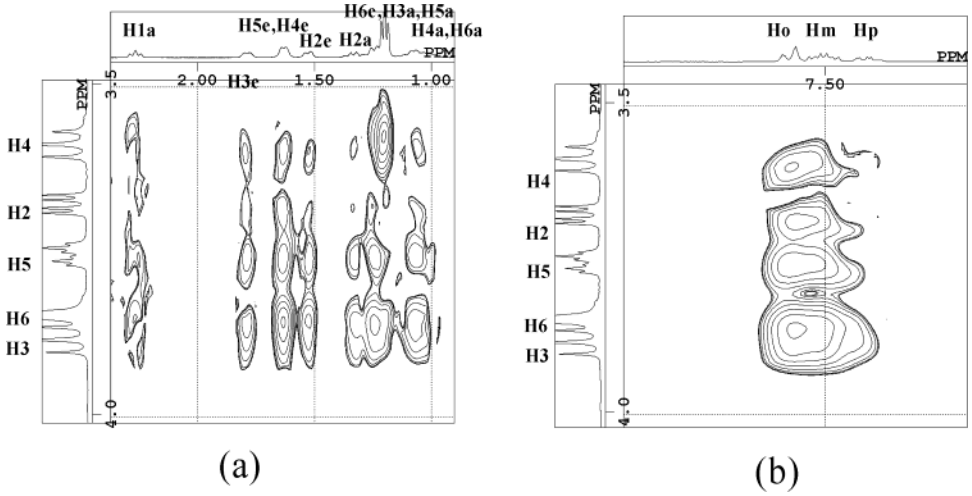

Figure 4. Partial ROESY spectrum of a mixture of OB (C_{OB} = 40 mM) and γ-CD (C_{CD} = 40 mM).

distance, d_{eff}, is defined as

$$(d_{eff})^{-6} = (1/n_{CD}n_{OB})\sum_{i=1}^{n_{CD}}\sum_{j=1}^{n_{OB}} d_{CD_iOB_j}^{-6} \qquad (4)$$

From eqs 3 and 4, we can expect that ROE/$n_{CD}n_{OB}$ increases, as two protons become closer. The ROE intensity was set to 100 for the intramolecular cross-peak between H1a and H2e (d_{eff} = 0.25 nm) of OB. From Table 3, it is suggested that both of the cyclohexyl and phenyl groups are more or less incorporated in the cavities of β- and γ-CDs.

Structures of Complexes. To estimate the structures of the complexes of OB with β- and γ-CD on the basis of the chemical shift and ROESY data, we considered many feasible structures that are different in the direction of penetration of OB and in the number (one or two) of hydrophobic groups that are incorporated. In the complex of OB and β-CD, because H5 exhibits a larger shift with complexation than H3 (Table 1), OB is rather deeply incorporated into the cavity. The protons of the cyclohexyl group exhibit ROE intensities that are larger than those of the phenyl group. A β-CD cavity does not have a space that is sufficiently large to include both of the cyclohexyl and phenyl groups.

Keeping these data in mind, we constructed an appropriate structure of the OB and β-CD complex and optimized it by molecular mechanics calculations. In the energy-optimized structure (Figure 5), the cyclohexyl group penetrates deeply into the cavity and the phenyl group is close to the wide rim of the cavity. This structure gives a good correlation between the ROE intensity and the effective distance (Figure 6). Although many other structures were tested, no correlation better than that shown in Figure 6 was obtained.

In the complex of OB and γ-CD, both of the phenyl and cyclohexyl groups are incorporated, as estimated from the ROESY spectrum (Figure 4 and Table 3). The cross-peaks of the protons of these groups with the H6 protons of γ-CD are smaller than those with the H3 protons of γ-CD (Table 3). The ROESY cross-peak between Hm and H3 is rather large. These findings indicate that both of the phenyl and cyclohexyl groups are inside the wide rim of γ-CD. On the basis of these images, we constructed an initial structure of

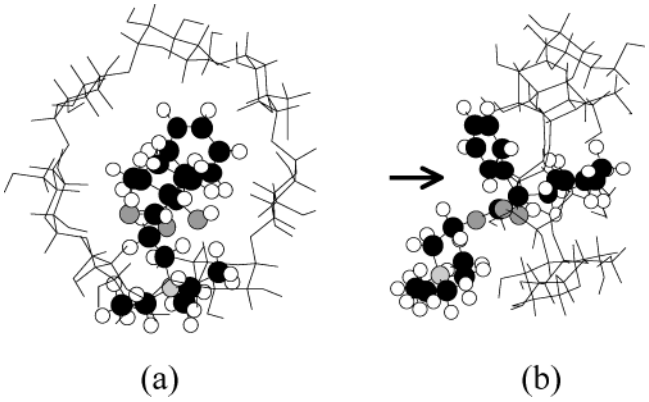

Figure 5. (a) Top and (b) side views of the solution structure of the complex of OB and β-CD. The arrow indicates the direction of insertion of OB into the β-CD cavity.

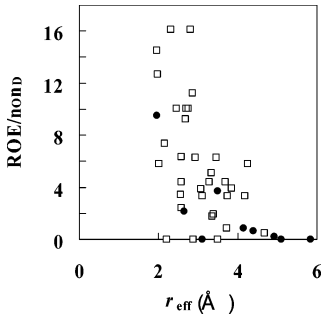

Figure 6. ROE intensities among H3, H5, and H6 and the protons of the phenyl (●) or cyclohexyl (□) groups, plotted against the effective distances for the OB−β-CD complex.

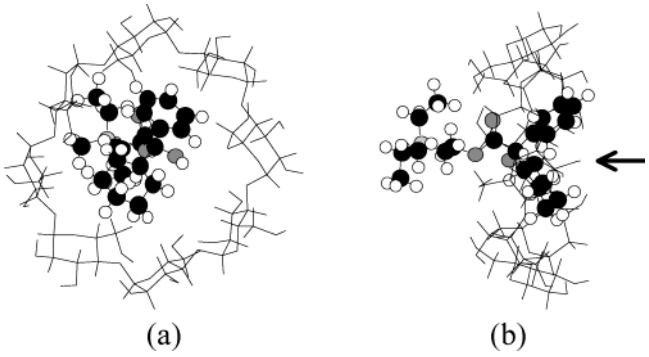

Figure 7. (a) Top and (b) side views of the solution structure of the complex of OB and γ-CD. The arrow indicates the direction of insertion of OB into the γ-CD cavity.

the OB−γ-CD complex and energy-optimized it. The energy-optimized structure is shown in Figure 7. In Figure 8, the ROESY intensities are plotted against the effective distances calculated for this energy-optimized structure.

Conformational Changes of OB with Inclusion of CD. On the basis of NMR measurements and calculations of molecular mechanics, molecular dynamics, and molecular surface areas, we determined the structure of OB in aqueous solution.[7] The chemical shifts of the cyclohexyl protons are very sensitive to the configuration of the phenyl group, namely, to the dihedral angles, φ_1 and φ_2, defined in Figure 1. OB has four pairs of corresponding protons: H2a and

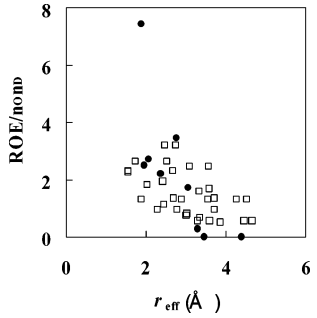

Figure 8. ROE intensities among H3, H5, and H6 and the protons of the phenyl (●) or cyclohexyl (□) groups, plotted against the effective distances for the OB−γ-CD complex.

H6a, H2e and H6e, H3a and H5a, and H3e and H5e. As Table 1 shows, the chemical shifts of the paired protons are different from each other. This magnetic nonequivalence was ascribed to the difference in ring current effects of the phenyl group on the paired protons. The observed chemical shift differences, $\Delta\delta_{OB-CD}$, for four pairs of the protons, were best fitted to those calculated on the basis of the ring current effect by regarding the dihedral angles as adjustable parameters to determine the structure of OB in the free state.[7] This approach was applied to the OB complexes with β- and γ-CD to determine conformational changes of the phenyl and cyclohexyl groups with complex formation. The dihedral angles, φ_1 and φ_2, of OB in the free state and in the complexed states with α-, β-, and γ-CD are given in Table 2. The conformational changes in the phenyl and cyclohexyl groups on complex formation with β- and γ-CDs are smaller than that in the case with α-CD.

Discussion

Chemical Shifts. In this work, we used the racemic mixture of (*R*)- and (*S*)-OB, but we have analyzed the *S*-form alone. Because the sites of binding of OB to β- and γ-CD are the phenyl and cyclohexyl groups, the difference between (*R*)- and (*S*)-OB will be minor.

The chemical shift change, $\Delta\delta_{OB-CD}$, with complexation has been used for the estimation of the solution structures of CD complexes, particularly with aromatic guests.[9,10,15,18] To determine this value accurately, we must pay special attention to chemical shift references. Chemical shifts referred to an external standard changed linearly with the concentration of CD.[19,20] To determine the binding constant, we must correct this change.[19] Internal reference compounds often interact with CD and guest molecules by hydrophobic and

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electrostatic interactions.[20] To avoid these uncertainties, we employed tetramethylammonium chloride as an inert, internal reference.[19,20] Using chemical shift changes, we could determine reliable binding constants (Table 2).

Estimation of Solution Structures of Complexes of OB with *β*- and *γ*-CD. Rough solution structures of CD inclusion complexes have been determined by NMR chemical shifts and ROESY spectra.[9,10] On the other hand, detailed structures have been estimated by molecular mechanics and molecular dynamics calculations, but were not tested quantitatively against experimental data.[11] We quantitatively determined the ROESY intensity and correlated it with the effective distance estimated from the energy-optimized structure. This novel analysis allowed us to determine rather detailed solution structures of OB complexes with β- and γ-CD. Because β- and γ-CDs have large cavities, many structures of their complexes are usually taken into consideration. Then, molecular mechanics calculations related with ROE intensities can predict reliable structures.

Application of traditional NOE difference methods is limited by the unfavorable tumbling rates of compounds with molecular masses of ~1500 Da. ROESY experiments overcome this limitation. However, because CD complexes have loose structures, eq 3 does not hold rigorously.[10] To determine the structures of complexes of OB with β- and γ-CD, we assumed that the ROE intensity decreases with an increase in proton−proton distance.

Binding Constants. In the OB−β-CD (Figure 5) complex, the cyclohexyl group is incorporated into the cavity and the phenyl group is in contact with the rim of the secondary alcohol of β-CD; this may be named the cyclohexyl-in complex. The averages of several literature binding constants for binding of cyclohexanol and phenol to β-CD are 560 and 77 M^{-1}, respectively.[21] Therefore, the cyclohexyl group has a higher affinity for β-CD than the phenyl group. This result is consistent with the preference of the cyclohexyl-in complex over the phenyl-in complex.

OB has a ca. 100-fold larger binding constant for binding to β-CD than to α-CD (Table 2). The most reliable binding constants for binding of cyclohexanol and phenol to α-CD are 56 and 19.8 M^{-1}, respectively, though these values are not available for γ-CD.[22] Comparison of the binding constants suggests that cyclohexanol has an affinity for β-CD 10 times higher than its affinity for α-CD. Furthermore, the phenyl group of OB interacts with the secondary alcohol rim of β-CD. This interaction of the phenyl group will reinforce binding between OB and β-CD. These two effects will be responsible for a much higher affinity for β-CD than for α-CD.

Implications of This Work. Bitter compounds are generally hydrophobic. The bitter taste of OB must originate from two hydrophobic groups, the phenyl and cyclohexyl groups. As already reported, α-, β-, and γ-CDs suppressed the bitter taste of OB in the following order: β-CD > γ-CD > α-CD.[4,5] It was assumed that no complexes of OB with α-, β-, and γ-CD taste bitter, because they are hydrophilic. As Figures 5 and 7 show, the cyclohexyl and phenyl groups are more or less are incorporated in the β- and γ-CD cavities. Therefore, these complexes will not taste bitter. Then, the bitter taste of a mixed solution of OB and CD is determined by the concentration of free OB molecules, regardless of the concentration and kind of CD. This free OB concentration can be estimated by measurements of either electromotive forces or UV absorbance and allows us to predict the suppression of the bitter taste by CDs.[4,5]

From a comparison of the UV maximum wavelength of the 1:1 complexes of OB with α-, β-, and γ-CD, we estimated the effective dielectric constant, D_{eff}, of the microenvironment surrounding the phenyl group (Table 2).[4] These values are between those of water ($D_w = 74.5$) and dioxane ($D = 2.1$). The OB−α-CD complex consists of the cyclohexyl-in complex and the phenyl-in complex.[8] Because the phenyl group in these complexes is exposed fully or mostly to water, the dielectric constant would be slightly smaller than that of water. Because the phenyl group in the OB−β-CD complex is in contact with the wide rim of the cavity (Figure 5), a small dielectric constant may be reasonable (Table 2). The phenyl group in the OB−γ-CD complex is completely incorporated in the γ-CD cavity (Figure 6). However, this cavity has enough room to accommodate several water molecules. Therefore, the phenyl group would sense a mixture of water and dioxane with a dielectric constant of 30.7 (Table 2).

The basic hydrolysis of OB was suppressed by α-, β-, and γ-CD.[6] This will be due to shielding of the ester linkage from hydroxide ions by the CDs, as shown in Figures 5 and 7 for β- and γ-CD. If the ester linkage of OB was located near the secondary hydroxyl groups of β- and γ-CD, the hydrolysis would be accelerated by these CDs.[1]

The determination of the solution structures of OB complexes with β- and γ-CD provides the basis for understanding the mechanisms of suppression of bitter taste and hydrolysis and microenvironmental changes with CD inclusion of OB. This approach will be able to be applied to other systems of a drug and CDs.

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