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Alizarin Yellow-Modified β-Cyclodextrin as a Guest-Responsive Absorption Change Sensor

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Alizarin yellow-modified β-cyclodextrin (ACD), in which alizarin yellow is linked to β-cyclodextrin via an ethylenediamine spacer, was synthesized as a new absorption change indicator for molecules. Alizarin yellow is a pH indicator that exhibits absorption peaks at 360 and 480 nm in the neutral region and in the alkaline region, respectively, with pK_a = 10.98 for an equilibrium between two forms. ACD has two parts to be deprotonated: one is the phenolic hydroxyl group of alizarin yellow residue and the other is the secondary amine group of the spacer. ACD exhibits pH dependency very different from that of alizarin yellow. We obtained two pK_a values, 4.88 (pK_{a1}) and 8.89 (pK_{a2}), for ACD by pH titration of its absorption intensity. The pK_{a1} and pK_{a2} values were suggested to be the pK_a values of the phenolic hydroxyl group of alizarin yellow residue and the secondary amine group, respectively. Upon addition of guest species, the pK_{a1} and pK_{a2} values shifted to 5.11 and 7.56, respectively, indicating a larger shift in the pK_a for the amine group than for the hydroxyl group. The guest-induced pK_a shift in the alkaline region suggests that deprotonation of the amine group of ACD occurs when the alizarin yellow moiety is excluded from the cyclodextrin cavity associated with guest accommodation and exposed to an alkaline environment. The sensitivities of this host to various guests were examined by absorption changes at 475 nm in pH 8.3 phosphate buffer, and the order of the sensitivities was found to be adamantane derivatives > borneol > bile acids. This order is not parallel with that of the binding constants, suggesting that the structural features of the host−guest complexes are important. All these results demonstrate that ACD can be used as an effective chemosensor for molecules.

During the past decade, much effort has been devoted to developing analysis systems with which chemical and biological compounds can be detected without any chemical modification of analytes. Absorption and fluorescence changes of appropriately designed hosts associated with guest binding are important in this respect.[1−5] Cyclodextrins (CDs), which are cyclic oligosaccharides consisting of six or more members of D-(+)-glucopyranose units and form inclusion complexes with a variety of organic compounds in aqueous solution,[6−8] may be useful for constructing such molecule-sensing systems.[9−15] Recently, we have shown that methyl red-modified cyclodextrin (MRCD) exhibits a guest-responsive color change, and it works as a chemosensor for detecting organic species. Since the color change of MRCD occurs only in the acidic solution, we have attempted to find other CD systems that work in solutions of different pH regions, and previously we prepared *p*-nitrophenol-modified CD as the sensor that exhibits color change in neutral solution.[16−19] Here, we report an alizarin yellow-modified β-CD (ACD) as one example of such CD systems, since ACD changes its absorption in alkaline solution (Figure 1). In ACD, the alizarin yellow moiety is covalently linked to β-CD through an ethylenediamine linkage, and we found that the guest-responsive absorption change arises not from the simple dissociation of the hydroxyl group of alizarin yellow moiety but from the protonation−deprotonation equilibrium shift of the amine group of the ethylenediamine part.

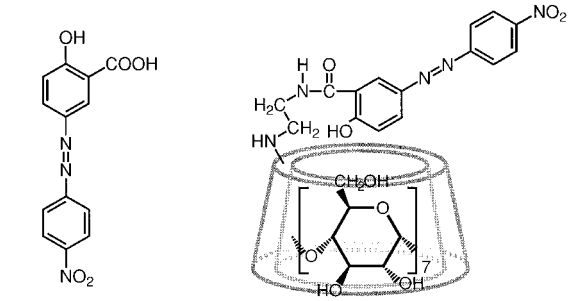

Figure 1. Structures of alizarin yellow and ACD.

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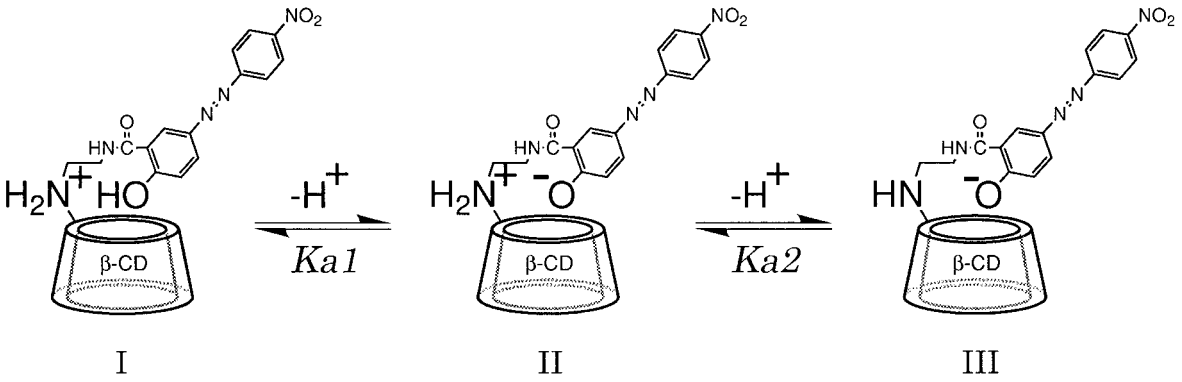

Figure 2. Two-step deprotonation equilibria of ACD.

EXPERIMENTAL SECTION

Materials. β-CD was kindly donated by Nihon Shokuhin Kako Co., Ltd., and was used as received. All the guest compounds were purchased from Tokyo Kasei and were used without further purification.

Synthesis. (i) 6-Deoxy-6-[(2-aminoethyl)amino]-β-CD (CDen). The synthesis of 6-*O*-6-(*p*-tolylsulfonyl)-β-CD (Ts-CD) was previously reported.[20] Ts-CD (1.5 g, 1.16 mmol) was dissolved in *N*,*N*-dimethylformamide (DMF, 8 mL), and this solution was added dropwise to a mixed solution of ethylenediamine (15 mL, 0.224 mol) and DMF (10 mL). The resultant solution was stirred for 2.5 h at 65 °C. The reaction mixture was then poured into acetone (700 mL) to form a precipitate, and the precipitate was collected by filtration. The precipitate was dissolved in water (15 mL), and this solution was poured into acetone (500 mL) to form precipitate again. The precipitate was collected by filtration and dissolved in water (20 mL). After filtration, ion-exchange column chromatography on Sephadex CM-25 with 1N-NH_3 aqueous solution afforded the fractions that contain 6-deoxy-6-[(2-aminoethyl)amino]-β-CD (CDen). The fractions were concentrated on a rotary evaporator and then lyophilized to give the white powder of CDen (890 mg, 0.756 mmol, 65%).

(ii) Alizarin Yellow-Modified β-CD (ACD). A solution of CDen (200 mg, 0.170 mmol), alizarin yellow (60 mg, 2.09 mmol), dicyclohexylcarbodiimide (DCC, 53 mg, 2.57 mmol), and 1-hydroxybenzotriazole (HOBt, 34 mg, 2.52 mmol) in DMF (8 mL) was stirred for 3 h at 0 °C and subsequently for 10 h at room temperature. Insoluble materials were removed by filtration. The solution was poured into acetone (300 mL), and the resulting precipitate was collected by filtration. The precipitate was dissolved in dimethyl sulfoxide (5 mL) and reprecipitated by pouring into acetone (300 mL). This reprecipitation from acetone was repeated until no alizarin yellow was observed by TLC. The product was collected and dried in vacuo at 80 °C to give the red powder of ACD (90 mg, 0.062 mmol, 37%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.90−4.00 (m), 4.50−4.75 (m, 6H, O_6H), 4.80−5.00 (m, 7H, C_1H), 5.60−5.90 (m, 14H, O_2H, O_3H), 6.50−6.60 (s, 1H, aromatic), 7.74−7.82 (d, 1H, aromatic), 7.84−7.92 (d, 2H, aromatic), 8.29−8.36 (d, 2H, aromatic), 8.41−8.45 (d, 1H, aromatic). Anal. Calcd for $C_{57}H_{83}O_{38}N_5 \cdot 9H_2O$: C, 42.62; H, 6.32; N, 4.36. Found: C, 42.90; H, 6.13; N, 3.99.

Measurements. Absorption spectra were recorded on a Shimadzu UV-3100 spectrophotometer. During the spectroscopic measurements, the temperature of the cell was kept at 25 °C, and the ACD concentration was 0.05 mM. The pH of the solution was measured with a pH meter (TOA HM60-S), calibrated at room temperature with pH standard solutions of pH 4.01 ± 0.01, 6.86 ± 0.01, and 9.22 ± 0.01. Hydrochloric acid was used to set the pH in the acid region, and sodium hydroxide solution was used in the alkaline region. Phosphate buffer of pH 8.3 was used for measurements of sensitivity parameters and binding constants.

In this system, there are two equilibria involving three species, **I**, **II**, and **III**, as shown in Figure 2. The acid−base dissociation constants, K_{a1} and K_{a2}, are defined by the following equations:

$$K_{a1} = [\mathbf{II}][H^+]/[\mathbf{I}] \quad (1)$$

$$K_{a2} = [\mathbf{III}][H^+]/[\mathbf{II}] \quad (2)$$

Letting ϵ^+ and ϵ^- be the molar extinction coefficients of totally protonated (**I**) and deprotonated (**III**) forms of ACD, respectively, and letting ϵ^0 be the molar extinction coefficient of ACD when the amino group is protonated and the phenolic hydroxyl group is deprotonated (**II**), the absorption intensity of our sensory system is

$$I_{obs} = \epsilon^+[\mathbf{I}] + \epsilon^0[\mathbf{II}] + \epsilon^-[\mathbf{III}] \quad (3)$$

where I_{obs} is the absorbance at 500 nm under the conditions of path length of 1 cm.

Here, the total ACD concentration, $[ACD]_0$, is the sum of the concentrations of the three species,

$$[ACD]_0 = [\mathbf{I}] + [\mathbf{II}] + [\mathbf{III}] \quad (4)$$

If the absorbances for **I**, **II**, and **III** are abbreviated as I^+, I^0, and I^-, respectively, the absorbances are expressed as

$$\epsilon^+[ACD]_0 = I^+ \quad (5)$$

$$\epsilon^0[ACD]_0 = I^0 \quad (6)$$

$$\epsilon^-[ACD]_0 = I^- \quad (7)$$

From eqs 1−7, I_{obs} is expressed by the following equation:

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$$I_{obs} = \frac{I^- + ([H^+]/K_{a2})I^0 + ([H^+]^2/K_{a1}K_{a2})I^+}{1 + ([H^+]/K_{a2}) + ([H^+]^2/K_{a1}K_{a2})} \quad (8)$$

The pK_a values were estimated by curve-fitting analysis using eq 8.

The binding constants were estimated as follows. Equation 9 describes the complexation equilibrium, where ACD, G, and

$$ACD + G \rightleftarrows ACD{-}G \quad (9)$$

ACD−G represent free host, free guest, and host−guest complex, respectively. This means that ACD forms only a 1:1 complex with a guest. The binding constant of ACD for a guest, *K*, is estimated by fitting the following equation to the data obtained:[19]

$$\frac{\Delta I}{\Delta I_{max}} = (K[G]_0 + 1 + K[ACD]_0 - \sqrt{(K[G]_0 + 1 + K[ACD]_0)^2 - 4K^2[ACD]_0[G]_0})/2K[ACD]_0 \quad (10)$$

where $[G]_0$ represents the initial concentration of guest, ΔI represents the difference of absorption intensity at 475 nm for ACD alone and ACD in the presence of guest, and ΔI_{max} is its value when ACD exists totally as the inclusion complex.

RESULTS AND DISCUSSION

pH Dependence in Absorption Spectra of ACD. Alizarin yellow, which is a pH indicator, is known to change its color from yellow to red when the solution becomes basic. This color change is associated with the dissociation of the phenolic hydroxyl group in the alkaline solution, forming a phenolate form.[21−23] Figure 3 shows the absorption spectra of alizarin yellow at various pH values. The absorption intensity at 360 nm decreases, while that at 500 nm increases with increasing pH of the solution. This observation reflects the fact that the phenol type, which exists in the acid or in neutral solution, shows yellow color with an absorption around 360 nm, while the phenolate form, which exists in the alkaline solution, shows red color with an absorption around 500 nm. All the spectra exhibit isosbestic points (280, 315, and 420 nm), demonstrating that alizarin yellow is in equilibrium between the two forms, and the analysis of the pH dependency of its absorption at 500 nm gave pK_a = 10.98.

Figure 4 shows the absorption spectra of ACD measured at various pH values. The spectral variation associated with the change in the pH value seems essentially the same as that of alizarin yellow. However, the pH values where the spectral variation occurs are quite different, as shown by the absorption around 500 nm in the acidic region. Another observation is that isosbestic points are not clear, suggesting the existence of two equilibria. It is surprising that the absorption around 500 nm of ACD appears even in the acidic region below pH 6.0. Therefore, it is obvious that there exists a somewhat different deprotonation scheme, which cannot be seen for alizarin yellow itself. Considering that the phenol and phenolate forms show absorptions around 360 and 500 nm, respectively, the absorption change of ACD occurring in the acidic region should be caused by the dissociation of the hydroxyl group, and that in the alkaline region must be caused by the deprotonation of the amine group. Figure 2 shows the scheme of this equilibrium for ACD, in which the species **I**, **II**, and **III** are included and K_{a1} and K_{a2} represent dissociation constants. K_{a1} and K_{a2} are the values for the phenolic hydroxyl group of alizarin yellow residue and the secondary amine group located near the CD cavity, respectively. Figure 5 shows the absorption intensities of ACD at 500 nm as a function of pH for ACD alone and in the presence of 1-adamantanol as a guest. From the data of ACD alone, pK_{a1} and pK_{a2} were estimated to be 4.88 and 8.92, respectively, by fitting the theoretical equation to the data obtained. The result indicates that there is a large difference in the pK_a values of the phenolic hydroxyl group between alizarin yellow (10.98) and ACD (4.88). This may be related to their structural differences. Alizarin yellow has a carboxyl group at the ortho position of the hydroxyl group, but the carboxyl group was changed to the amide bond in ACD. This structural difference

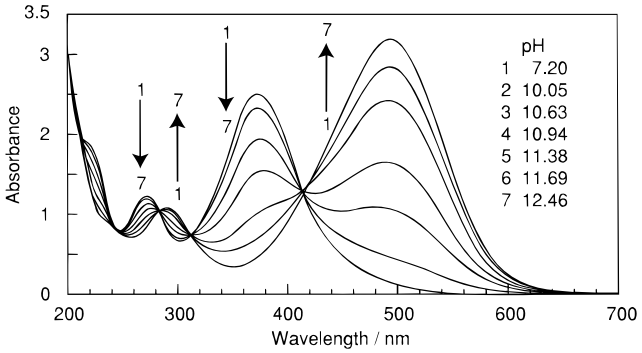

Figure 3. Absorption spectra of alizarin yellow (150 μM) at various pH values.

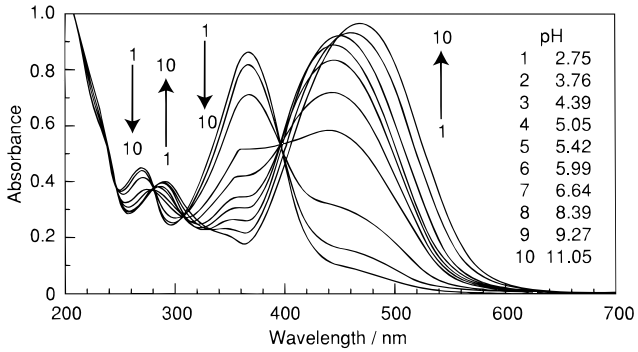

Figure 4. Absorption spectra of ACD (50 μM) at various pH values.

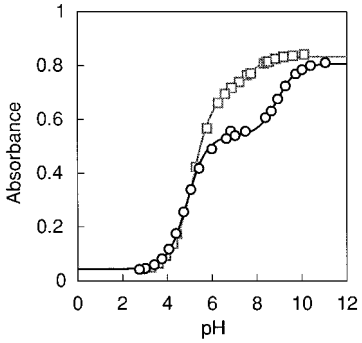

Figure 5. pH dependency of the absorption intensities of ACD alone (○, 50 μM) and in the presence of 1-adamantanol (□, 1 mM) at 500 nm.

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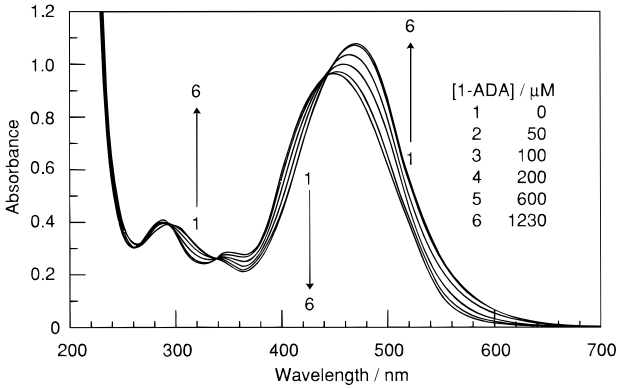

Figure 6. Absorption spectra of ACD (50 μM), alone and in the presence of 1-adamantanol (1-ADA), at pH 8.3.

Table 1. pK_a Values of ACD Alone, ACD in the Presence of 1-Adamantanol (1-ADA), and Alizarin Yellow[a]

	pK_{a1}	pK_{a2}
ACD	4.88	8.92
ACD + 1-ADA	5.13	7.59
alizarin yellow	10.98[b]	

[a] [ACD], 50 μM; [1-ADA], 1 mM; and [alizarin yellow], 150 μM. [b] pK_a 10.69 in ref 24.

Table 2. Sensitivity and Binding Parameters of ACD for Various Guests[a]

guest	$\Delta I/I^0$	K (M^{-1})	ΔI_{max}	$C_{0.01}$ (μM)
(1) chenodeoxycholic acid (CDCA)	0.091	9180	0.102	16.8
(2) ursodeoxycholic acid (UDCA)	0.099	93200	0.088	7.0
(3) cholic acid (CA)	0.021	682	0.074	234.1
(4) deoxycholic acid (DCA)	0.035	1360	0.079	112.8
(5) hyodeoxycholic acid (HDCA)	0.094	66600	0.084	8.0
(6) 1-adamantanol (1-ADA)	0.192	8160	0.214	8.3
(7) 2-adamantanol	0.226	10300	0.242	6.3
(8) 1-adamantanecarboxylic acid	0.184	9240	0.189	8.7
(9) (+)-menthol	0.052	1010	0.138	81.3
(10) (−)-menthol	0.051	918	0.142	86.0
(11) (+)-camphor	0.035	460	0.168	141.3
(12) (−) camphor	0.032	393	0.178	154.1
(13) (+)-borneol	0.136	2530	0.205	22.7
(14) (−)-borneol	0.134	2450	0.204	23.5
(15) cyclohexanol	0.005	266	0.037	1408.0
(16) cyclooctanol	0.029	364	0.179	165.6
(17) nerol	0.029	289	0.199	185.5
(18) geraniol	0.027	178	0.296	198.2

[a] Measured at 25 °C at pH 8.3. ΔI, guest-induced increase of absorption intensity of ACD; I^0, absorption intensity of ACD alone (λ = 475 nm). The concentration of ACD was 50 μM. The concentration of guests was 500 μM. K is binding constant. ΔI_{max} is the ΔI value when ACD exists as a complex. $C_{0.01}$ is the guest concentration where ACD gives 0.01 as the ΔI value.

at the ortho position may be the main reason for the large difference in pK_a between the two compounds. Figure 6 shows the absorption variations of ACD in pH 8.3 phosphate buffer induced by 1-adamantanol, which is well known to fit the cavity of the β-CD. There occur progressive red shifts in the band maximum and increases in the absorption intensity with increasing concentrations of 1-adamantanol. Isosbestic points are seen at 295, 340, and 440 nm. The pH dependency of the absorption at 500 nm in the presence of 1-adamantanol (1 mM) is shown in Figure 5. From the analysis of the pH dependency, pK_{a1} and pK_{a2} were estimated to be 5.13 and 7.59, respectively. The result indicates that pK_a shifts with the guest addition, from 4.88 to 5.13 for pK_{a1} and from 8.92 to 7.59 for pK_{a2} (Table 1). The shift in pK_{a1} means that the dissociation of the phenolic hydroxyl group is depressed, and that in pK_{a2} means that deprotonation of the amine group is facilitated in ACD. This may arise from the environmental change associated with the conformational change caused by the guest inclusion. The effect of the guest on pK_a is larger for pK_{a2} than for pK_{a1}, indicating that the amine group is more influenced than the hydroxyl group by the guest. As is the case for 1-adamantanol addition to the ACD system, we observed remarkable differences in the absorption intensity around 500 nm upon addition of various guests. Therefore, we used ACD as a new absorption change sensor for detecting molecules.

Binding Constants. The binding constants (K) of ACD were determined from the guest-induced absorption variations at 475 nm for 18 guests based on 1:1 complexation stoichiometry. The K values for these guests are summarized in Table 2, together with sensitivity parameters. The selected guests are five steroids, three adamantane derivatives, six cyclic terpenes, two acylic terpenes, and two cyclic alkanols. Their structures are shown in Figure 7 with abbreviations for several guests. Among steroid guests, chenodeoxycholic acid (CDCA), ursodeoxycholic acid (UDCA), deoxycholic acid (DCA), and hyodeoxycholic acid (HDCA) are isomers with one hydroxyl group attached to different carbons or with one hydroxyl group attached to the same carbon but stereochemically inverted. Cholic acid (CA) has one more hydroxyl group than these steroids. The order of K for the steroidal compounds is UDCA > HDCA > CDCA > DCA > CA, and it is remarkable that there exist large differences among the isomeric steroids UDCA, HDCA, CDCA, and DCA, ranging from 93 200 M^{-1} for UDCA to 1360 M^{-1} for DCA. CA has one additional hydroxyl group and gives a smaller K value of 682 M^{-1}, reflecting its less hydrophobic nature. The three adamantane derivatives give the K values of about 10 000 M^{-1}, these large values being consistent with the well-known fact that adamantane derivatives are good guests for β-CD. The order of K values for monoterpenes is borneol > menthol > camphor > nerol > geraniol, ranging from 2530 M^{-1} for (+)-borneol to 178 M^{-1} for geraniol, with a negligible or slight preference of (+)-enantiomer for the former three monoterpenes and cis preference of 1.62 for the latter two open-chain monoterpenes. The K values were also examined for cyclooctanol and cyclohexanol as guests, and the result indicates that cyclooctanol is a better guest than cyclohexanol.

Sensitivity and Selectivity. The sensitivity of this system is shown by $\Delta I/I^0$, where $\Delta I = I - I^0$ and I^0 and I are the absorption intensities at 475 nm of ACD in the absence and presence of a guest, respectively, measured under the conditions of pH 8.3 phosphate buffer.

The data of ACD (0.05 mM) for 18 guests (0.5 mM) are summarized in Table 2. For steroidal guests, the order of $\Delta I/I^0$ for steroidal guests is UDCA (0.099) > HDCA (0.094) > CDCA (0.091) > DCA (0.035) > CA (0.021), parallel to the order of their binding constants. The order for monoterpenes is also parallel to the order of their binding constants. However, detailed examination of the data reveals that the $\Delta I/I^0$ values are not totally

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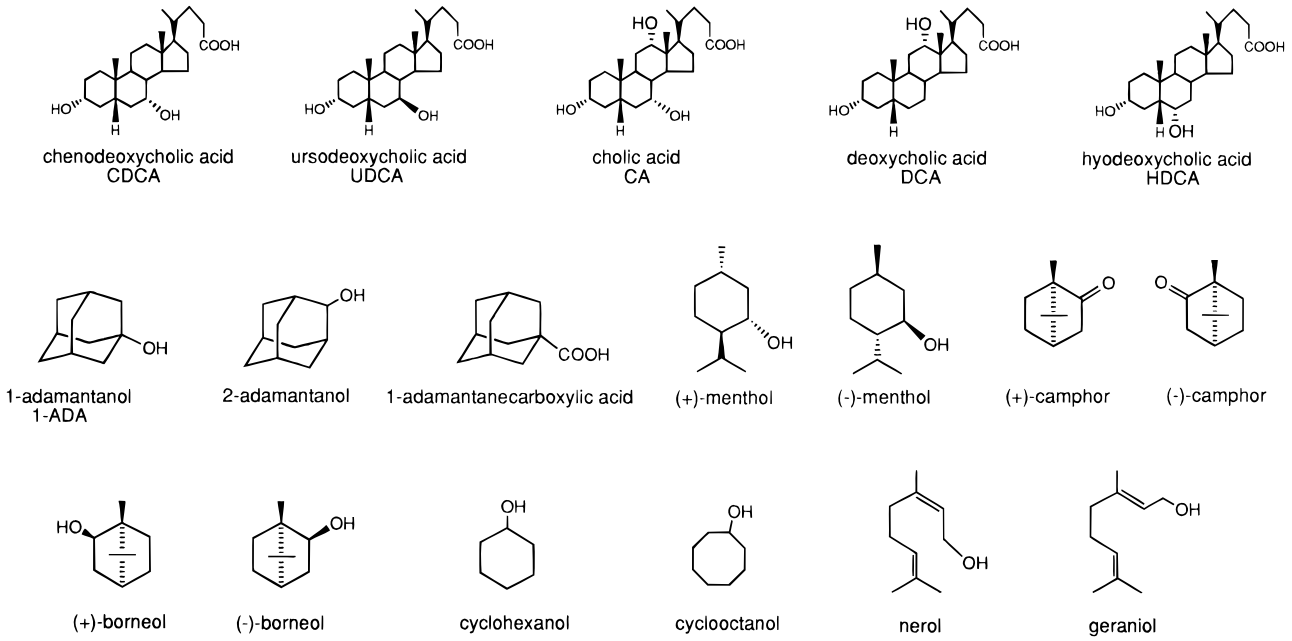

Figure 7. Structures of guests used in this study.

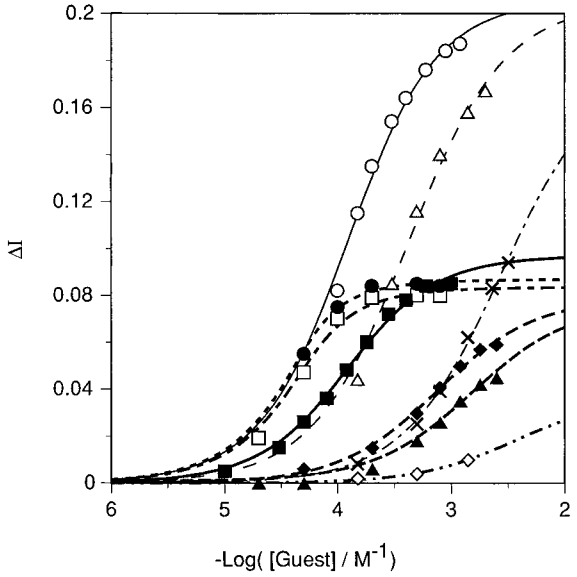

Figure 8. Absorbance of ACD as a function of guest concentration. ■, CDCA; ●, UDCA; ▲, CA; ◆, DCA; □, HDCA; ○, 1-ADA; △, (+)-borneol; ◇, cyclohexanol; and ×, cyclooctanol.

parallel to the binding constants. For example, the $\Delta I/I^0$ value of 2-adamantanol (0.226) is much larger than that of UDCA (0.099), in spite of the fact that the binding constant of UDCA (93 200 M^{-1}) is 9-fold larger than that of 2-adamantanol (103 000 M^{-1}). It is noted that the values give no information on the limit of detection of the guests. Therefore, we have attempted to get whole views on the sensitivity and selectivity employing the curve-fitting data used for determining binding constants. Figure 8 shows the guest-induced absorption changes against the guest concentration for nine guests, including five steroidal guests. This figure indicates that these guests give various saturation values. The values are shown as ΔI_{max} in Table 2, and it is surprising that the guest compounds that give larger ΔI_{max} values are not those that give large binding constants. All steroidal guests give smaller ΔI_{max} values, in contrast to the fact that small guests such as nerol and geraniol give larger values. This result should be related to the structures of the complexes. One possible explanation is that steroidal guests may not be inserted enough into the cavity, resulting in partial exclusion of the alizarin yellow moiety from the cavity. Figure 8 also indicates the concentration ranges for the guests to be detected. The limit of detection may be regarded as the guest concentration to be detected over the noise levels of the instruments used. Here, we use the guest concentration $C_{0.01}$, which is the guest concentration that gives an absorption change of 0.01, as the detection limit, and the values are listed in Table 2. The $C_{0.01}$ values are inversely related to the binding constants, showing UDCA < HDCA < CDCA < DCA < CA for steroids, borneol < menthol < camphor < nerol for monoterpenes, and cyclooctanol < cyclohexanol for alkanols. The values of $C_{0.01}$ for adamantane derivatives are similar to each other, reaching the level of UDCA. Figure 8 indicates that the ability of ACD for detecting a particular compound from a mixture of several substances is limited, but in some cases, such as a mixture of UDCA, cyclooctanol, cyclohexanol, nerol, and geraniol, UDCA would be detected by this host selectively. All these data demonstrate that ACD has remarkable utility as a pH-indicator-based chemosensor. On this basis, many spectroscopically inert compounds may be detected from the absorption changes of the host. Sensitivity and selectivity could be improved by appropriate designing of the dye moiety, and we are now exploring various types of dye-modified CD chemosensors.

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