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Visible and ^{13}C nuclear magnetic resonance spectra of azo dyes and their complexes with cyclomalto-oligosaccharides

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Abstract

Inclusion complexes of α-, β-, and γ-cyclomalto-oligosaccharides (CDs), heptakis(2,6-di-*O*-methyl)- and heptakis(2,3,6-tri-*O*-methyl)-βCDs (Me_2–βCD and Me_3–βCD) with methyl orange {sodium 4-[4-(dimethylamino)phenylazo]benzenesulfonate (**3**)}, tropaeolin OO {sodium 4-[[4-(phenylamino)phenyl]azo]benzenesulfonate (**1**)}, metanil yellow {sodium 3-[[4-(phenylamino)phenyl]azo]benzenesulfonate (**2**)}, and orange II {sodium 4-[(2-hydroxy-1-napthalenyl)azo]benzenesulfonate (**4**)} were measured by UV-vis spectrophotometry. Complex formation constants (K) were calculated by a non-linear least-squares method. The order of K in the 1:1 complexes is Me_2–βCD > Me_3–βCD > αCD > βCD. Thus lengthening of the hydrophobic torus by substituting the CD with methyl groups exhibits a greater effect than the sizes of the inner diameter of the cavity. Since the K values correlate with the bulkiness of the hydrophobic parts of the azo dyes, these numbers suggest a direction of inclusion of the azo dye molecules (i.e., hydrophobic groups of the guest molecules associated with hydrophobic groups of the CDs) into the CD cavities. Complex formation induces a hypsochromic shift to the λ_{max} that very closely resembles the shift induced by dioxane, suggesting that the inside of the cavity resembles this solvent. As the order of the shift is **3** > **1**, **2** > **4**, γCD > βCD series > αCD, and the shift scarcely appears in the complexes of **4**, the shift may be mainly derived by the inclusion of an anilino group in **1**, **2**, and **3**. NMR chemical shifts, relaxation times, and rotational correlation times from the ^{13}C NMR studies all support the above conclusions. © 1996 Elsevier Science Ltd.

Keywords: Cyclomalto-oligosaccharides (cyclodextrins, CDs); host–guest complex formation; Azo dyes; host–guest complex formation; UV-vis methods; ^1H NMR spectroscopy; ^{13}C NMR spectroscopy

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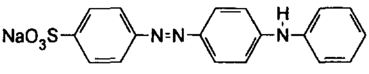
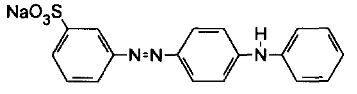
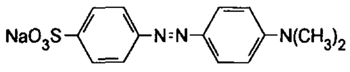
1. Introduction

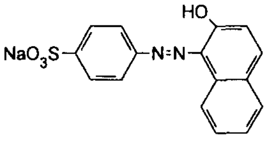
Azobenzene derivatives are included by almost all types of cyclomalto-oligosaccharides (cyclodextrins, CDs), and such compounds are suitable for systematically investigating host–guest interactions. Circular dichroism and ^{1}H, ^{2}H, and ^{13}C NMR spectra have been measured for a large number of azo dye–CD complexes, and much valuable information has been obtained from these studies. However, it is difficult to obtain complex formation constants (K) from visible spectra by ordinary methods, such as that of Benesi and Hildebrand [1], which are applicable only under certain conditions, as the dye itself shows a large absorbance in the visible spectrum, and the hypsochromic shift on the addition of CD is slight.

The aim of this paper is to do the following: *(i)* Use the azo dyes **1**–**4** and measure visible spectra of all inclusion compounds with α, β, and γCDs, 2,6-di-*O*-methyl- and 2,3,6-tri-*O*-methyl-βCDs (Me_2–βCD and Me_3–βCD), and get K values and molar absorption coefficients (ε) by a non-linear least-squares method. *(ii)* Investigate whether or not ^{13}C NMR data (chemical shifts, relaxation times, and correlation times previously obtained [2]) can explain the origin of parameters obtained from the visible spectra.


Tropaeolin OO (**1**)


Metanil Yellow (**2**)


Methyl Orange (**3**)


Orange II (**4**)

2. Materials and methods

Visible spectra.—Materials used were those previously described [3]. In measurements, 2×10^{-5} mol of the guest molecule and between 5.0×10^{-6} and $\sim 2.5 \times 10^{-3}$ mol of the host molecule were dissolved in 0.1 M phosphate buffer at pH 6.0. Spectra were recorded with a Hitachi U-2000 type double-beam spectrometer at 298 K. The spectrometer was connected to a PC-9801 NEC computer. K and ε were calculated by a non-linear least-squares method.

For a 1:1 complex, the calculation was carried out as follows:

$$\text{dye} + \text{CD} \overset{K}{\leftrightarrows} \text{complex} \tag{1}$$

$$K = \frac{[\text{Comp}]}{[\text{Dye}][\text{CD}]} = \frac{[\text{Comp}]}{([\text{Dye}]_0 - [\text{Comp}])([\text{CD}]_0 - [\text{Comp}])} \tag{2}$$

$$[\text{Comp}] = 1/2\Big\{([\text{Dye}]_0 + [\text{CD}]_0 + 1/K) - \big([\text{Dye}]_0^2 + [\text{CD}]_0^2 + 1/K(2[\text{Dye}]_0 + 2[\text{CD}]_0 + 1/K) - 2[\text{Dye}]_0[\text{CD}]_0\big)^{1/2}\Big\} \tag{3}$$

For a 1:2 complex, the calculation was carried out as follows:

$$2\text{dye} + \text{CD} \overset{K}{\rightleftarrows} \text{complex} \tag{4}$$

$$K = \frac{[\text{Comp}]}{[\text{Dye}]^2[\text{CD}]} = \frac{[\text{Comp}]}{([\text{Dye}]_0 - 2[\text{Comp}])^2([\text{CD}]_0 - [\text{Comp}])} \tag{5}$$

$$4[\text{Comp}]^3 - 4([\text{CD}]_0 + [\text{Dye}]_0)[\text{Comp}]^2 + \big(4[\text{Dye}]_0[\text{CD}]_0 + [\text{Dye}]_0^2 + K\big)[\text{Comp}] - [\text{Dye}]_0^2[\text{CD}]_0 = 0 \tag{6}$$

$$E_{\text{calcd}} = \varepsilon_{\text{Dye}}[\text{Dye}] + \varepsilon_{\text{Comp}}[\text{Comp}] \tag{7}$$

$$\text{SumD} = \Sigma(E_{\text{obsd}} - E_{\text{calcd}})^2 \tag{8}$$

where

E_{calcd} = calculated value of absorbance
E_{obsd} = observed value of absorbance
ε_{Dye} = molar absorption coefficient of Dye
$\varepsilon_{\text{Comp}}$ = molar absorption coefficient of the complex
[Dye] = molar concentration of dye
[CD] = molar concentration of CD
[Comp] = molar concentration of complex
$[\text{Dye}]_0$ = initial concentration of dye
$[\text{CD}]_0$ = initial concentration of CD
SumD = the summation of squares of the deviations

When 1:1 and 1:2 complexes are formed, we have eqs (1) and (4), respectively, while the K values are as expressed in eqs (2) and (5), respectively. In the 1:2 complex, the Newton method [4] was used to solve cubic equation (6). Thus, E_{calcd} is expressed as a function of K ([Comp]) and ε [eq (7)]. SumD is minimized by the simplex method [eq (8)].

3. Results and discussion

Visible spectra.—Figs. 1 and 2 show a comparison of the experimental and calculated spectra in the first $\pi \rightarrow \pi^*$ region of the **2**–Me_3–βCD and **2**–γCD complexes, respectively. Isosbestic points indicate the formation of precisely 1:1 and 2:1 complexes, although the change in spectral pattern by complex formation is slight. Continuous variation plots of the above complexes show that the host–guest ratios are 1:1 and 1:2 (Fig. 3). K values are collected in Table 1. Azo dyes whose molecules are longer exhibit larger K values. The order of K in the complexes of **1**, **2**, and **3** is

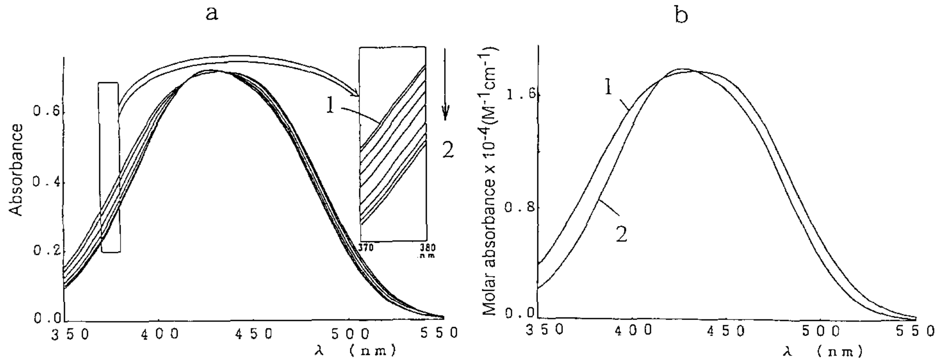


Fig. 1. Comparison of experimental and calculated UV-vis spectra of heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (trimethyl β-cyclodextrin, Me_3–βCD) and metanil yellow (**2**) in the $\pi \rightarrow \pi^*$ region. (a) Experimental spectra of **2** with increasing Me_3–βCD: 1, [**2**] = 2.5×10^{-5} M; 2, [Me_3–βCD] added = $5 \times 10^{-6} \rightarrow 2.5 \times 10^{-2}$ M. (b) Calculated spectrum: 1, **2** only (the experimental spectrum (1) was added for comparison with that of the complex); 2, spectrum calculated from K.

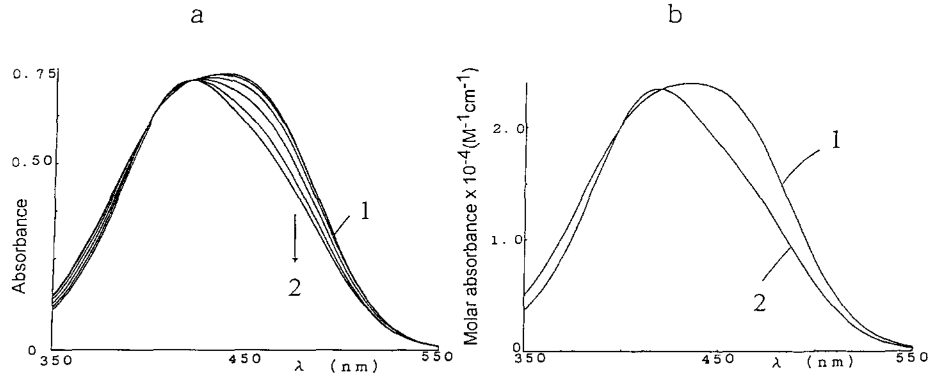


Fig. 2. Comparison of experimental and calculated UV-vis spectra of cyclomalto-octaose (γ-cyclodextrin, γCD) and metanil yellow (**2**) in the $\pi \rightarrow \pi^*$ region. (a) Experimental spectra of **2** with increasing γCD: 1, [**2**] = 2.5×10^{-5} M; 2, [γCD] added = $5 \times 10^{-6} \rightarrow 2.5 \times 10^{-2}$ M. (b) Calculated spectrum: 1, **2** only (the experimental spectrum 1 was added for comparison with that of the complex); 2, spectrum calculated from K.

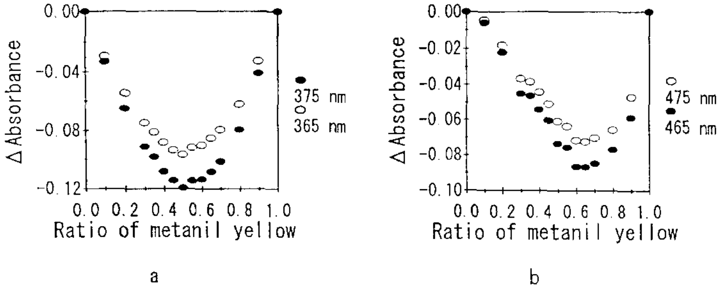


Fig. 3. (a) Continuous variation plots of heptakis(2,3,6-tri-*O*-methyl)cyclomaltoheptaose (trimethyl β-cyclodextrin, Me_3–βCD) and metanil yellow (**2**). [Me_3–βCD]+[**2**]= 10^{-4} M. ○, 365 nm; ●, 375 nm. (b) Continuous variation plots of cyclomalto-octaose (γ-cyclodextrin, γCD) and **2**. [γCD]+[**2**]= 10^{-4} M. ○, 475 nm; ●, 465 nm.

Me_2–βCD > Me_3–βCD > αCD > βCD. Here the guest–host molar ratio and the lengthening of the hydrophobic torus (from ~ 8 to ~ 11 Å) by substituting the CD moiety with methyl groups results in a larger effect than does an increase in the size of the inner diameter of the cavity of the CD. In the αCD complexes, *K* has almost the same value for **1**, **2**, and **3** (data for **4** not included). For the Me_3–βCD complexes, the order of *K* is **1** ~ **2** > **3** > **4**; Me_3–βCD exclusively recognizes an unsubstituted benzene ring. In the complexes of **4**, the γCD complex shows the largest *K* value in the series investigated, while those of the other complexes are either very small or zero. The *K* values correspond to the bulkiness of the hydrophobic sides of the azo dyes and suggest the direction of the inclusion of azo dyes in the CD molecules to favor hydrophobic–hydrophobic interactions. The **4**–γCD complex self-associates and can form a liquid-crystal polymer [5,6]. A large *K* value may suggest such a special characteristic. As shown in Table 2, complex formation induces a hypsochromic shift to the λ_{max} of **1**, **2**, and **3** [7], which is also induced when azo dyes are dissolved in a hydrophobic solvent such as dioxane, indicating that the environment for dye molecules that are included in the CD is more akin to that of being dissolved in dioxane than in water. The **4**–CD complexes scarcely exhibit hypsochromic shifts, and these may be derived mainly from inclusion of the anilino groups in **1**, **2**, and **3**. In each azo dye complex, the shift of λ_{max} is γCD > βCD series > αCD. Exciton theory predicts a hypsochromic shift for the dimer of dyes in γCD complexes [8].

Table 1
Complex formation constants *K* [a] of azo dyes and cyclodextrins (10^4 M^{-1}, M^{-2})

Dye	αCD	βCD	Me_2–βCD	Me_3–βCD	γCD
1	1.1	0.6	8.8	1.6	6800
2	1.33	0.58	10.6	1.88	9380
3	1.09	0.30	3.42	0.41	1920
4	—	0.09	0.54	0.03	21 100

[a] Reproducibility is within 5%.

Table 2
Hypsochromic shifts induced to λ_{max} of azo dyes (nm) by solvent and complex formation

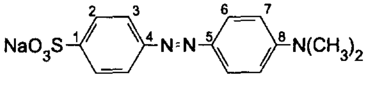
Dye	Dye only in		Complex in H_2O with				
	H_2O	Dioxane [a]	αCD	βCD	Me_2-βCD	Me_3-βCD	γCD
1	444	−27	−2	−9	−8	−10	−18
2	434	−21	0	−7	−6	−6	−16
3	464	−50	−6	−9	−12	−12	−33
4	485	−5	—	+2	0	0	−7
Azobenzene	316						

[a] Dioxane: D_2O = 1:1.

Analysis of host–guest interaction by ^{13}C NMR spectroscopy.—^{13}C NMR spectra have been measured in dioxane [2] and have been used to confirm the type of host–guest interaction that occurs in complex formation. ^{13}C NMR shift data for complexes of **3** are tabulated in Table 3. It is noteworthy that the largest hypsochromic shift observed among the methyl orange (**3**)–CD complexes is that of the **3**–γCD complex.

When the dioxane-induced solvent ^{13}C NMR chemical shift of **3** is compared with the inclusion shift of **3** in the α, β, and γCDs (Table 3), the data for the dioxane-induced shift resembles most closely the inclusion shift in the βCD series, with shifts spread to both lower and higher fields. Chemical shifts observed in the βCD series for the included substrate, which are similar to those observed in dioxane for the strainless hydrophobic state, may be mainly due to the hydrophobic nature of the inside of the CD cavity. In the αCD complex, the shifts in the neighborhood of the N = N group and the *N,N*-dimethylaniline side move to a lower field, perhaps indicating that this part of the molecule is included on the narrow-rim side of the CD and the shift induced by the van der Waals interactions between the **3** and αCD are additive.

Table 3
CD-Induced ^{13}C NMR chemical shifts [a] and the dioxane-induced solvent shifts [b] of methyl orange (**3**) (in ppm)



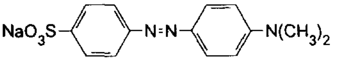
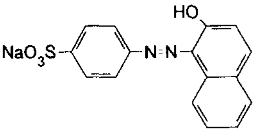
3

Solvent and CDs	Carbon no.								
	1	5	2	Me	4	8	7	3	6
Dioxane	2.4	1.1	0.1	0.0	−0.1	−0.2	−0.2	−0.4	−0.6
αCD	0.9	0.5	−0.2	0.4	0.4	0.2	−0.1	0.4	0.2
βCD	1.1	0.9	0.4	0.1	0.1	−1.0	−0.6	0.0	−0.5
Me_2-βCD	1.6	0.8	0.2	0.0	0.2	−0.2	−0.5	−0.7	−0.3
Me_3-βCD	2.5	1.0	0.3	0.7	−0.1	−1.2	−1.2	−1.0	−0.3
γCD	0.9	−0.3	−0.2	−0.3	−0.8	−1.5	−1.4	−0.1	−0.5

[a] Host molecules (0.05 M) were added to 0.05 M guest molecule in D_2O.
[b] Dioxane:D_2O = 1:1 at 307 K.

As the ^{1}H NMR spectrum shows a broadening of signals for H-3 and H-6, atoms in the neighborhood of the N = N group may be included, but the anilino group may also be partially included and induce a smaller hypsochromic shift to λ_{max} than those observed in the βCD series. γCD-Induced chemical shift clusters at a higher field due to the fact that a ring-current effect is induced by the stacking of molecules of **3** upon each other. Moreover, the positions of dyes included in the CDs and the crowdedness of the inside of the cavity were evaluated by ^{13}C T_1 studies [2]. In Table 4 the mean values of T_1 for the ^{13}C with the directly attached ^{1}H in rings which constitute the dyes, the CH_3 groups, and the CDs, and the T_1 ratio for dye to those in **3** (parenthesized in Table 4) are collected. These data show that in the β series the values of T_1 of the CH_3s in **3** do not decrease substantially; however, γCD gives rise to the largest decrease (amounting to

Table 4
Correlation between λ_{max}, relaxation times [a] (T_1) for azo dyes, their complexes, cyclodextrins and their ratios (333 K)

Compound	λ_{max} (nm)	T_1 (s)				$T_{1(dye)}/T_{1(CD)}$ [c]
		Ring A	Ring B	CH_3	CD [b]	
		 **3**				
3	464	0.75	0.59	6.81		
3+ αCD [d]	−6					
3+ βCD	−9	0.55(0.73) [e]	0.50(0.85)	6.30(0.93)	0.30	0.6
3+ Me_2–βCD	−12	0.54(0.72)	0.44(0.75)	6.69(0.98)	0.28	0.6
3+ Me_3–βCD	−12	0.42(0.56)	0.33(0.56)	5.31(0.78)	0.27	0.8
3+ γCD	−33	0.36(0.48)	0.34(0.58)	3.87(0.57)	0.28	0.8
		Benzene	Naphtalene ring			
		 **4**				
4 [f]	485	0.27	0.26 [g]			
4+ γCD	−7	0.29	0.29		0.28	1.0

[a] ^{13}C T_1 was measured by the inversion-recovery method. T_1 = ±5–10%. 0.1 M host molecules were added to 0.1 M guest molecules [2].

[b] Mean values for the five carbons of cyclodextrin.

[c] Concerning the T_1 of methyl orange (**3**), the calculation was done by using the mean values of T_1 for the carbons of ring B.

[d] αCD complex could not be measured due to precipitation.

[e] Parentheses denote the ratio of T_1 of aromatic rings in complex to those of **3**.

[f] Measured at 307 K.

[g] Mean values for six carbons of the naphthalene ring.

50–60%) in all portions of the molecule, suggesting that the anilino group is included. The T_1 ratio for **3** to that of γCD is $T_{1(3)}/T_{1(\gamma\mathrm{CD})} = 0.8$, which means that two molecules of **3** are packed tightly in the γCD torus. The **4**–γCD complex also exhibits a larger T_1 ratio, indicating that the packing state is tighter in all proportions. However, in this complex, the movement of $\lambda_{\max}$ is negligibly small. The large hypsochromic shift observed in the γCD series is not due to the stacking of the two molecules of azo dyes, but is mainly due to the inclusion of the aniline moiety.

Molecular motions of the complexes of **1** have previously been studied on the basis of ^{13}C T_1^1 studies [2]. The T_1 of a ^{13}C with directly attached ^{1}H is inversely proportional to the effective isotropic correlation time for overall molecular re-orientation (τ_{eff}):

$$1/NT_1 = \hbar^2 \cdot \gamma_C^2 \cdot \gamma_H^2 \cdot r_{\mathrm{CH}}^{-6} \cdot \tau_{\mathrm{eff}} = \mathrm{const} \cdot \tau_{\mathrm{eff}}$$

where N is the number of directly bonded protons, γ_H and γ_C are the magnetogyric ratios of the ^{1}H and ^{13}C nuclei, respectively, and r_{CH} is the length of the C–H bond. τ_{eff} is the time for the whole molecule to rotate one radian. In anisotropic **1**, it is possible to estimate an individual τ value for each aromatic ring that constitutes the azo dye. Fig. 4 shows all the τ values of the aromatic rings in **1** in the included state and the ratios for **1** to the CDs (Fig. 4). The τ values for **1** are smaller than those for the CDs. The ratio for **1** to αCD is 0.5, indicating that **1** rotates up to twice as fast as the αCD molecule.

In Fig. 4, τ_G (shown by ‘) shows more valuable information. τ_G is the correlation time for additional internal motion for an anisotropically overall-tumbling azo dye, such as the phenyl group in azo dyes and the primary alcohol group in the CD, and is given by the equation

$$\frac{1}{NT_1} = \mathrm{const} \cdot \tau_{\mathrm{eff}} \left(A + B \frac{6\tau_G}{6\tau_G + \tau_{\mathrm{eff}}} + C \frac{3\tau_G}{3\tau_G + 2\tau_{\mathrm{eff}}} \right)$$

where $A = 1/4(3\cos^3\theta - 1)^2$, $B = 3\sin^2\theta\cos^2\theta$, and $C = 3/4\sin^4\theta$, where θ is the angle between the C–H vector and the axis of rotation. In the αCD complex, ring C shows a smaller τ_G value compared to that of rings A and B. Compound **1** is included into αCD at the ring A and B side, and ring C may maintain additional internal spinning along the ring B substituent bond. In the βCD complex, the τ values of rings B and C become larger, indicating that the βCD molecule moves to the ring B and C side of the substrate. In the $\mathrm{Me_2}$–βCD and $\mathrm{Me_3}$–βCD complexes, τ values become as a whole larger since **1** rotates more slowly than in the unsubstituted αCD and βCD complex. In the $\mathrm{Me_3}$–βCD complex, the guest and host molecules show essentially the same τ value; thus, the bimolecular complex behaves like a single molecule. Moreover, the τ_G value of ring C is extraordinarily large, indicating that this part is included firmly into the smaller inner cavity of $\mathrm{Me_3}$–βCD. The above fact implies that the larger hypsochromic shift of the $\lambda_{\max}$ of **1** included in the βCD series is due to inclusion of nitrogen or/and the distortion of the anilino group in **1**. The τ_G of the $C\mathrm{H_2OR}$ group in the CD also supports the above results. The τ_G values in both the αCD and βCD complexes (65 and 64×10^{-11} s^{-1}) reflect slower correlation times than those of parent CDs alone (12 and 30×10^{-11} s^{-1}), indicating that ring C of **1** extrudes from or is in contact with the narrow rim of the CDs. On the other hand, the τ_G times in the

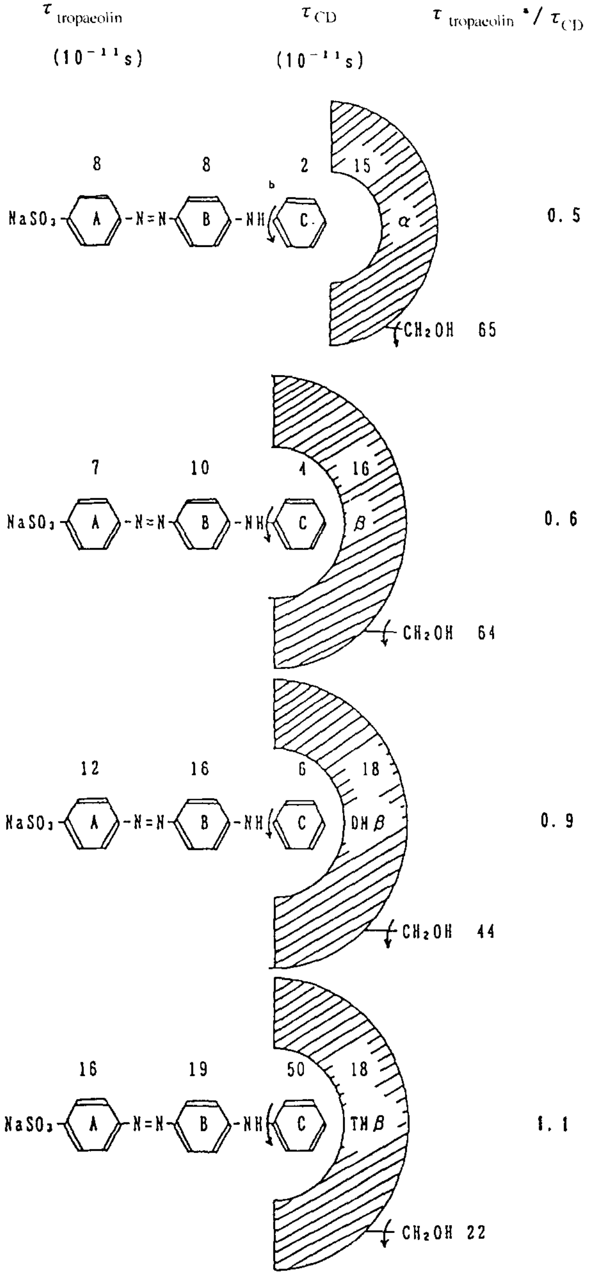


Fig. 4. Rotational correlation times of tropaeolin–CD complexes and motional states of tropaeolin in CDs at 333 K [6]. [a]Concerning the τ of tropaeolin OO (**1**), calculation was done by using the mean value of T_1 for the carbon atoms of ring B. Compound **1** itself could not be measured due to limited solubility in water. [b]Partial internal rotational correlation time.

Me_2–βCD and Me_3–βCD complexes (44 and 22×10^{-11} s^{-1}) are the same or faster than those of the CDs alone (39 and 61×10^{-11} s^{-1}), indicating that ring C is not in contact with the narrow-rim side of the CDs. Thus, all the ^{13}C NMR data point to the fact that the movement of λ_{max} in these compounds depends upon the degree of inclusion of the anilino group in the CD host.

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