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Molecular Recognition of Naphthalenedicarboxylic Acid Regioisomers by Cyclodextrins

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Complexation of cyclodextrins (CD's) with some regioisomers of naphthalenedicarboxylic acid (NDC) was examined in 0.1 mol dm^{-3} $NaHCO_3$ at 25°C by means of ^{1}H NMR spectroscopy, together with the measurement of induced circular dichroism spectra of NDC's with the addition of CD's. The ^{1}H NMR spectra of 2,6- and 2,7-NDC's showed characteristic AB-type patterns, which changed continuously with increasing CD concentration in accord with the Pople theory. The binding constants (K_a) for β-CD complexes with NDC's increased in the order of: 1,4-NDC $\approx$ 1,5-NDC < 1,3-NDC < 1,6-NDC < 2,3-NDC < 2,7-NDC $\leqq$ 2,6-NDC, indicating that β-CD is able to recognize the molecular structures of NDC's. On the other hand, interactions of α- and γ-CD's with NDC's were very weak and nonspecific to NDC's. The molecular structures of β-CD complexes with some NDC's were estimated based on the ROESY spectra of the systems. Column chromatography of NDC's using a β-CD-(2-chloromethyloxirane) polymer column showed that the retention volumes for NDC's increased in the order of: 1,4-NDC < 1,6-NDC < 2,3-NDC < 2,7-NDC < 2,6-NDC, which agreed with the order the K_a values.

Naphthalenedicarboxylic acid (NDC) has ten regioisomers (1,2-, 1,3-, 1,4-, 1,5-, 1,6-, 1,7-, 1,8-, 2,3-, 2,6-, and 2,7-NDC's). Among them, 2,6-NDC is an important raw material for liquid crystals and synthetic fiber. It is generally prepared by the oxidation of 2,6-dimethylnaphthalene (2,6-DMN), which is separated from a petroleum distillate containing a mixture of DMN regioisomers. The present work was undertaken to apply cyclodextrins (CD's) to the separation of 2,6-NDC from a mixture of NDC's obtained by the oxidation of a DMN mixture. The CD's are cyclic oligomers composed of six (α-CD), seven (β-CD), eight (γ-CD), or more α-D-glucopyranose units.[1] They provide relatively hydrophobic interior cavities into which a variety of organic molecules and ions are trapped to form inclusion complexes. Binding constants (K_a) for the inclusion complexes vary widely from one guest to another. Hence, it is anticipated that CD's are available for the separation of NDC's. Unfortunately, the K_a values for the inclusion complexes of CD's with NDC's have never been determined as far as we know, though CD complexation with naphthalene[2] and its derivatives such as naphthalenesulfonates[3] has been extensively studied. In the present work, the K_a values for the inclusion complexes were determined mainly by ^{1}H NMR spectroscopy to evaluate the feasibility of NDC separation by CD's. The molecular structures of CD–NDC complexes were also estimated by recording two-dimensional ROESY (rotating-frame unclear Overhauser enhancement spectroscopy) spectra. Furthermore, we attempted to separate a mixture of NDC's from one another by column chromatography with β-CD-(2-chloromethyloxirane) polymer as packing material.

Experimental

Materials. The α-CD was supplied by Nihon Shokuhin Kako Co., Ltd. The β- and γ-CD's were supplied by Ensuiko Seito Co., Ltd. They were dried overnight in vacuo at 110 °C. DMSO-d_6 (CEA) and D_2O (Isotec) contained 99.8 atm% D. The 1,4-, 2,3-, and 2,6-NDC's were of reagent grade and commercially available. The 1- and 2-naphthalenecarboxylic acids (NCA) were also commercially available. The 1,3-, 1,5-, 1,6-, and 2,7-NDC's were prepared by the oxidation of the corresponding commercially-available DMN's with sodium dichromate in water at 250 °C.[4] The products were identified by ^{1}H NMR spectroscopy and by mass spectrometry after methylation with diazomethane. ^{1}H NMR (400 MHz, DMSO-d_6/TMS): For 1,3-NDC, $\delta = 8.92$ (d, $J = 8.5$ Hz, 1H), 8.81 (s, 1H), 8.60 (d, $J = 1.7$ Hz, 1H) 8.23 (d, $J = 7.8$ Hz, 1H), 7.79 (dt, $J = 1.5$ and 7.08 Hz, 1H), 7.68 (dt, $J = 1.2$ and 8.1 Hz, 1H); for 1,4-NDC, $\delta = 8.78$ (dd, $J = 3.4$ and 6.6 Hz, 2H), 8.10 (s, 2H), 7.70 (dd, $J = 3.4$ and 6.6 Hz, 2H); for 1,5-NDC, $\delta = 9.03$ (d, $J = 8.6$ Hz, 2H), 8.17 (d, $J = 7.1$ Hz, 2H), 7.72 (dd, $J = 7.1$ and 8.6 Hz, 2H); for 1,6-NDC, $\delta = 8.94$ (d, $J = 9.0$ Hz, 1H), 8.67 (d, $J = 1.5$ Hz, 1H), 8.34 (d, $J = 8.1$ Hz, 1H), 8.26 (dd, $J = 1.2$ and 7.3 Hz, 1H), 8.10 (dd, $J = 1.7$ and 9.04 Hz, 1H), 7.68 (t, $J = 7.7$ Hz, 1H); for 2,3-NDC, $\delta = 8.31$ (s, 2H), 8.10 (dd, $J = 3.4$ and 6.4 Hz, 2H), 7.69 (dd, $J = 3.4$ and 6.4 Hz, 2H); for 2,6-NDC, $\delta = 8.68$ (s, 2H), 8.22 (d, $J = 8.8$ Hz, 2H), 8.06 (d, $J = 8.8$ Hz, 2H); for 2,7-NDC, $\delta = 8.75$ (s, 2H), 8.09 (s, 4H). The mass spectra of methylated NDC's gave their molecular ion peak at $m/z = 244$ and base peak at $m/z = 213$. The β-CD-(2-chloromethyloxirane) polymer (β-CDEP) was prepared according to the direction of Wiedenhof et al.[5]

Apparatus. ^{1}H NMR spectra were recorded using a JEOL Model JNM-A400 FT NMR spectrometer (400 MHz) with a sample tube of 5.0 mm diameter at 25.0±0.1°C. Sample solutions were composed of NDC and/or CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$. Methanol (0.4 mmol dm^{-3}) was used as an internal reference ($\delta = 3.343$) for ^{1}H NMR.[6] The phase-sensitive ROESY spectra of CD inclusion complexes with NDC's were acquired with a mixing time of 500 ms, 512 points for t_2, and 256 points for t_1, followed by zero-filling. The induced circular dichroism (ICD) spectra of NDC's in the presence of CD were recorded using a JASCO Model J-720 spectrometer with a quartz cell (1.0 cm) at 25 °C. Column chromatography was carried out with a β-CDEP column (15 mm$\phi \times$190 mm) and 0.1 mol dm^{-3} $NaHCO_3$ as an eluent at the flow rate of 17 mm^3 s^{-1}. To the column, 1 cm^3 of 10 mmol dm^{-3} NDC in 0.1 mol dm^{-3} $NaHCO_3$ was loaded, and each fraction (10 cm^3) was followed by a UV detector at 280 nm. Mass spectra were recorded using a Hitachi Model M-80B mass spectrometer.

Results and Discussion

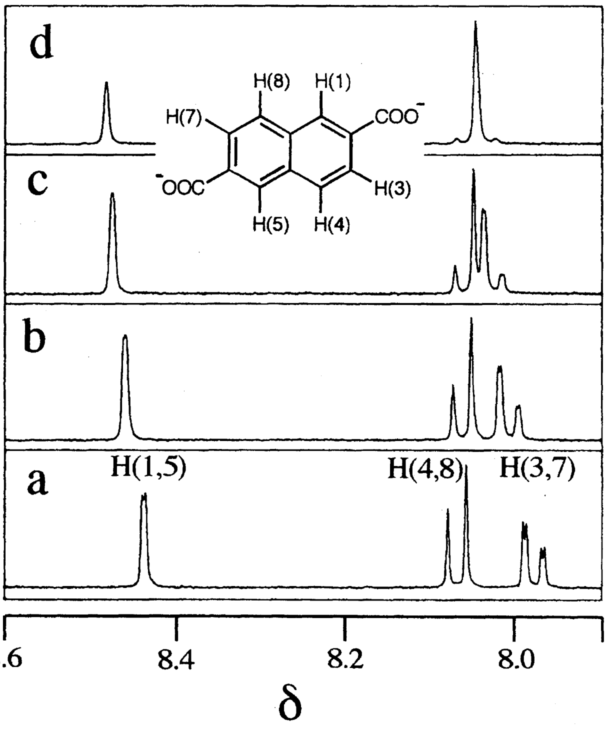


Fig. 1. Effect of the addition of β-CD on the ^{1}H NMR spectra of 3.0 mmol dm^{-3} 2,6-NDC in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.
[β-CD] = 0 (a), 2.0 (b), 4.0 (c), and 6.0 (d) mmol dm^{-3}.

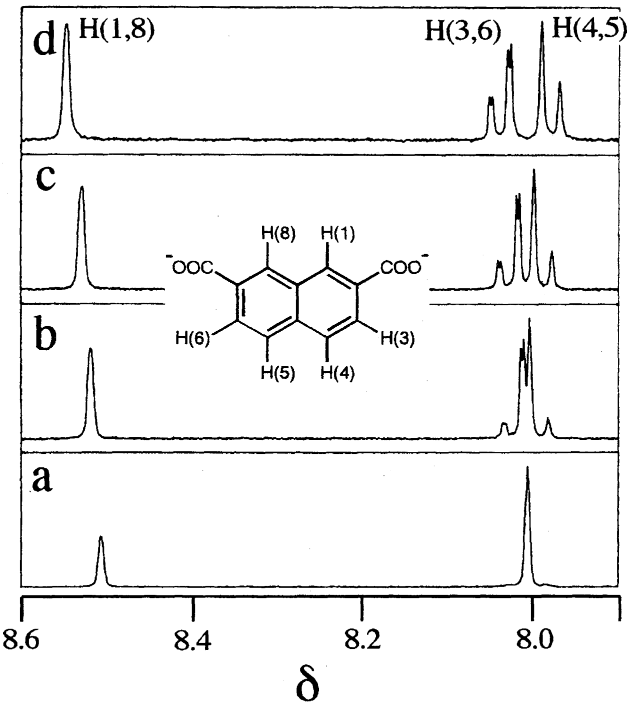


Fig. 2. Effect of the addition of β-CD on the ^{1}H NMR spectra of 3.0 mmol dm^{-3} 2,7-NDC in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.
[β-CD] = 0 (a), 0.5 (b), 1.0 (c), and 2.0 (d) mmol dm^{-3}.

Effect of CD on the ^{1}H NMR Spectra of NDC's. Figures 1 and 2 illustrate changes in ^{1}H NMR spectra of 3.0 mmol dm^{-3} 2,6- and 2,7-NDC's, respectively, in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ with the addition of β-CD. The pD value of 0.1 mol dm^{-3} $NaHCO_3$ in D_2O was recorded to be 8.97, at which the NDC's will completely dissociate into the dicarboxylate ions, since the pK_a values for the NDC's will be similar to or smaller than those of benzene-1,2-dicarboxylic acid (pK_a = 2.66 and 4.74[7]). In the absence of β-CD, 2,6-NDC gave three ^{1}H NMR signals at $\delta = 8.437$ (d, $J = 1.5$ Hz) for H(1, 5), 8.069 (d, $J = 8.5$ Hz) for H(4, 8), and 7.979 (dd, $J = 1.5$ and 8.5 Hz) for H(3, 7). The addition of β-CD caused downfield shift in the signals of H(1, 5) and H(3, 7) and upfield shift in that of H(4, 8). The signals of H(3, 7) and H(4, 8) gradually approached each other and finally coalesced to give a single signal with increasing β-CD concentration. On the other hand, 2,7-NDC gave only two singlet signals in the absence of β-CD at $\delta = 8.505$ for H(1, 8) and 8.007 for degenerate H(3, 6) and H(4, 5). The addition of β-CD caused a splitting of the degenerate signal to give a double-doublet signal due to H(3, 6) and a doublet signal due to H(4, 5). We attempted to explain these changes by the AB system of the Pople notation. According to Pople et al.,[8] the doublets of the AB system approach each other, the inner two peaks increase in intensity, and the outer two peaks decrease, as the ratio ($\Delta\nu/J$) of the chemical shift difference in Hz ($\Delta\nu$) to coupling constant (J) decreases. The ratio (R) of the height (h_1) for an outer peak to that (h_2) for an inner peak is represented by:

$$R = (1 - x)/(1 + x), \quad (1)$$

where $x = J/(\Delta\nu^2 + J^2)^{1/2}$. The observed R values ($R_{obsd} = h_1/h_2$) were plotted against the R values (R_{calcd}) calculated by Eq. 1 (Fig. 3). A good linear relationship was found between R_{obsd} and R_{calcd}, as expressed by:

$$R_{obsd} = 0.089 + 0.890R_{calcd}; \quad n = 56, \quad r = 0.9983. \quad (2)$$

This result indicates that the observed changes are well explained by Pople's theory. The difference between R_{obsd} and R_{calcd} increased with decreasing R values, probably due to a decrease in the signal/noise (S/N) ratio at small h_1 values. In fact, the S/N ratio at the lowest R_{obsd} value for a 2,6-NDC–β-

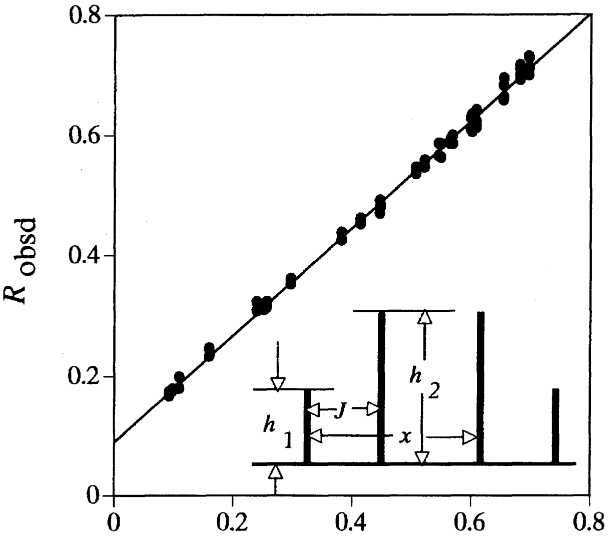


Fig. 3. A plot of R_{obsd} vs. R_{calcd} for the AB-type ^{1}H NMR signals of 2,6- and 2,7-NDC's at various β-CD concentrations.

CD system (Fig. 1d) was ca. 10, though that at the highest R_{obsd} value (Fig. 1a) was larger than 50. Hence, an experimental error of ca. 10% will be inherent in the determination of low R_{obsd} values.

Figure 4 illustrates changes ($\Delta\delta$) in ^{1}H NMR chemical shifts of 2,6-NDC with the addition of β-CD. Based on the assumption that β-CD forms a 1 : 1 complex with 2,6-NDC, the binding constant (K_a) for the complex and the $\Delta\delta$ values ($\Delta\delta_0$) at the infinite concentration of β-CD were determined by a nonlinear least-squares curve-fitting analysis of the change in $\Delta\delta$ with the concentration of β-CD, by means of a microcomputer.[6] The thus-calculated curves (solid lines) were well-fitted to observed data, indicating that the assumption of 1 : 1 complexation was valid. The mean K_a value obtained was 508±27 mol^{-1} dm^3. The $\Delta\delta_0$ values were evaluated to be 0.067 for H(1,5), 0.093 for H(3,7), and −0.023 for H(4,8). Similar measurements and analyses were carried out with regard to β-CD complexes with 2,3- and 2,7-NDC's and α-CD complex with 2,6-NDC. The K_a value for a β-CD–2,7-NDC system was 513±23 mol^{-1} dm^3, which was virtually equal to that of a β-CD–2,6-NDC system. The K_a values for β-CD–2,3-NDC and α-CD–2,6-NDC systems were 309±20 and 12±2 mol^{-1} dm^3, respectively, these were significantly smaller than that of a β-CD–2,6-NDC system.

Effect of NDC's on the ^{1}H NMR Spectra of CD. Figure 5 illustrates changes in a part of ^{1}H NMR spectra of 3.0 mmol dm^{-3} β-CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ with the addition of 2,6-NDC. In the absence of 2,6-NDC, β-CD gave signals at δ=5.068 (d, J=3.7 Hz) for C(1)–H, 3.647 (dd, J=3.7 and 9.8 Hz) for C(2)–H, 3.963 (t, J=9.8 Hz) for C(3)–H, 3.581 (t, J=9.3 Hz) for C(4)–H, 3.863 (m) for C(5)–H, and ca. 3.87 (m) for C(6)–H's. The addition of 2,6-NDC caused upfield shifts in all the signals of β-CD, due to shielding by the ring current of the naphthalene moiety in NDC.[9] Figure 6 shows the plots of $\Delta\delta$ for some protons of β-CD vs. the concentration of 2,6-NDC. The changes in $\Delta\delta$ for the C(5)–H and C(3)–H located at the interior cavity of β-CD were much larger than those for the other protons

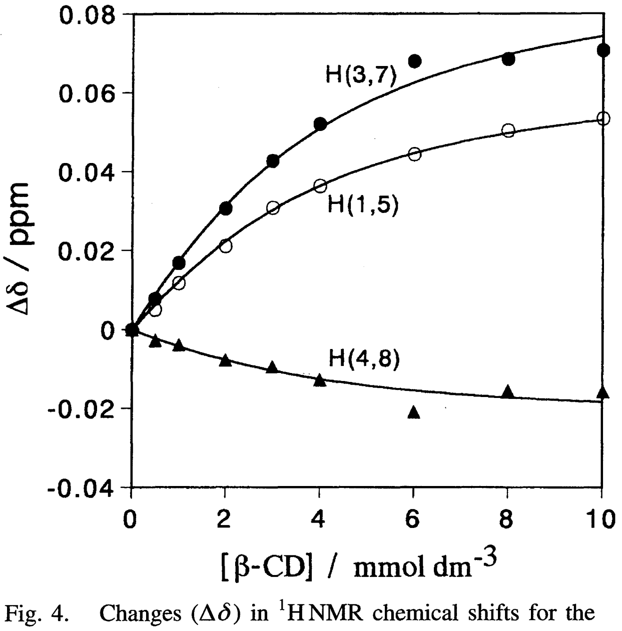


Fig. 4. Changes ($\Delta\delta$) in ^{1}H NMR chemical shifts for the H(1,5) (○), H(3,7) (●), and H(4,8) (▲) signals of 2,6-NDC (3.0 mmol dm^{-3}) upon the addition of β-CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.

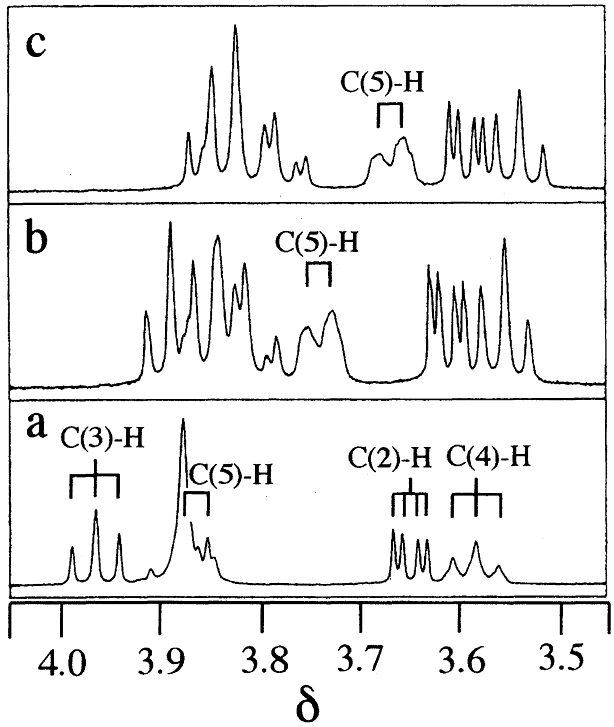


Fig. 5. Effect of the addition of 2,6-NDC on the ^{1}H NMR spectra of 3.0 mmol dm^{-3} β-CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.
[2,6-NDC]=0 (a), 2.0 (b), and 4.0 (c) mmol dm^{-3}.

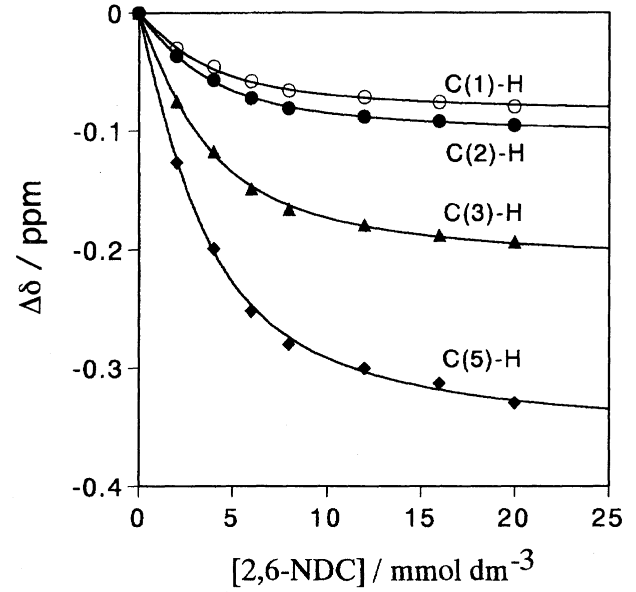


Fig. 6. Changes ($\Delta\delta$) in ^{1}H NMR chemical shifts for the C(1)–H (○), C(2)–H (●), C(3)–H (▲), and C(5)–H (◆) signals of β-CD (3.0 mmol dm^{-3}) upon the addition of β-CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.

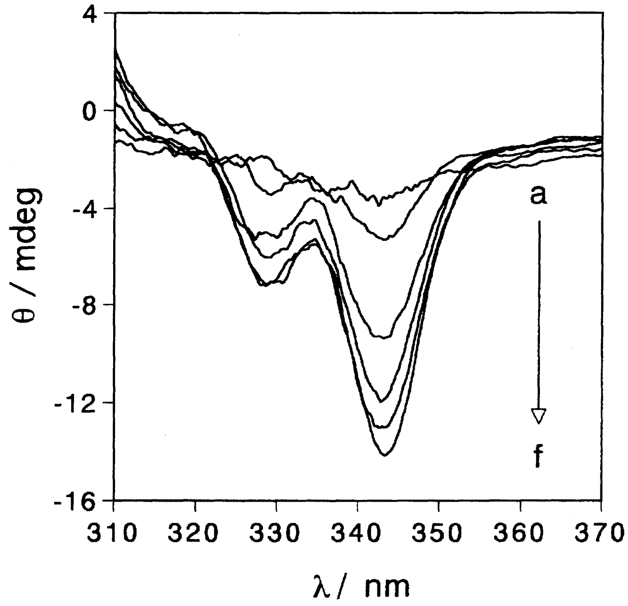


Fig. 7. The ICD spectra of 1.0 mmol dm^{-3} 2,6-NDC with the addition of β-CD in 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C. [β-CD] = 0 (a), 1 (b), 3 (c), 5 (d), 7 (e), and 10 (f) mmol dm^{-3}.

located at the outside of the β-CD cavity, indicating that 2,6-NDC is included within the β-CD cavity. The data were analyzed by a nonlinear least-squares curve-fitting analysis, upon the assumption of 1 : 1 complexation between β-CD and 2,6-NDC. The calculated curves (solid lines) were well-fitted to the observed data. The mean K_a value obtained was 537±26 mol^{-1} dm^3, which agreed well with that (508±27 mol^{-1} dm^3) determined by the above-mentioned changes in $\Delta\delta$ for the protons of 2,6-NDC with the addition of β-CD. The $\Delta\delta_0$ values were evaluated to be −0.087 for C(1)–H, −0.106 for C(2)–H, −0.216 for C(3)–H, −0.087 for C(4)–H, and −0.363 for C(5)–H. Most of the absolute values of $\Delta\delta_0$ for the β-CD protons were significantly larger than those for the NDC protons, suggesting that the β-CD protons are more sensitive to complexation than the 2,6-NDC protons. Hence, the K_a values for CD complexes with the regioisomers of NDC's and NCA's were mainly determined by the analysis of changes in $\Delta\delta$ for CD protons with the addition of NDC's and NCA's. Changes in $\Delta\delta$ for γ-CD protons with increasing concentration of 1,4- and 2,3-NDC's were virtually linear and the K_a values could not be determined by the curve-fitting analysis. The K_a values for them were roughly estimated by using the $\Delta\delta_0$ values for corresponding β-CD complexes.

ICD Spectra of NDC. In order to confirm the validity of the K_a values obtained by NMR measurements, we have tried to determine them by UV spectrophotometry and fluorophotometry. However, the changes in UV and fluorescence spectra of NDC's with the addition of CD were too small to determine the K_a values for CD–NDC complexes. On the other hand, NDC's gave distinct ICD spectra with the addition of CD. Figure 7 shows the ICD spectra of 2,6-NDC (1.0 mmol dm^{-3}) with the addition of β-CD in 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C. Although the S/N ratio of the measurement was relatively low, clear negative ICD peaks appeared at 343 and 300 nm and increased with increasing β-CD concentration. The K_a value for β-CD–2,6-NDC complex was determined by the curve-fitting analysis of a relationship between the observed ellipticity (θ) at 343 nm and β-CD concentration (Fig. 8) to be 511 mol^{-1} dm^3, this agreed well with values obtained by the above-described

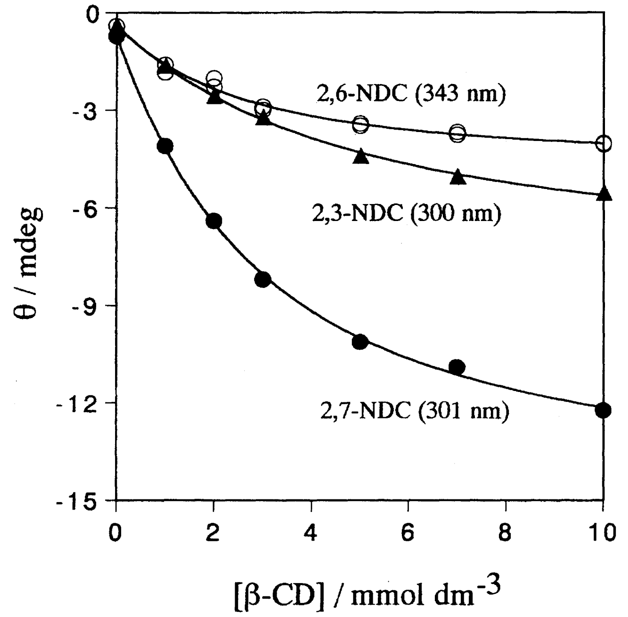


Fig. 8. Changes in the ellipticity (θ) of 1.0 mmol dm^{-3} 2,6-NDC (○) at 343 nm, 2,7-NDC (●) at 301 nm, and 2,3-NDC (▲) at 300 nm upon the addition of β-CD in H_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.

NMR method. Similar experiments were carried out on β-CD complexes with 2,3- and 2,7-NDC's and α- and γ-CD complexes with 2,6-NDC.

Molecular Recognition of NDC's by CD's. Table 1 summarizes the K_a values for CD–NDC complexes determined by the analysis of changes in $\Delta\delta$ for the ^{1}H NMR spectra of CD and NDC and in θ for the ICD spectra of NDC. The K_a values obtained by three different measurements were in good agreement with one another, except that the K_a values by ICD were somewhat smaller than those by NMR in β-CD complexes with 2,3- and 2,7-NDC's. In ICD measurements, the S/N ratio was so small that it is difficult to obtain accurate K_a values. The K_a values for 2,6-NDC complexes with α- and γ-CD's were much smaller than that for β-CD. The interior cavity of α-CD will be too small to accommodate the 2,6-NDC molecule. The γ-CD cavity will be too large to attain close van der Waals contact not only with 2,6-NDC but also with other NDC's so that the obtained K_a values for γ-CD complexes were very similar to one another. On the other hand, the K_a values for β-CD complexes with NDC regioisomers varied widely from 8 mol^{-1} dm^3 for 1,5-NDC to 537 mol^{-1} dm^3 for 2,6-NDC. The β-CD cavity will be suitable for close contact with 2,6-NDC. The K_a value for a β-CD–2,7-NDC complex was similar to or slightly smaller than that for a β-CD–2,6-NDC complex. The K_a value for a β-CD–2,3-NDC complex was also large. These three NDC's bear the carboxylate groups only at the β-positions of the naphthalene moieties. Their

Table 1. The K_a Values for CD–NDC Complexes Determined by the Analysis of Changes in $\Delta\delta$ of CD and NDC Protons in NMR and θ of NDC in ICD[a)]

Host	Guest	K_a/mol^{-1} dm^3		
		NMR(CD)	NMR (NDC)	ICD
α-CD	2,6-NDC	13±2	12±2	*[b)]
β-CD	1,4-NDC	9±2		
	1,5-NDC	8±2		
	1,3-NDC	55±4		
	1,6-NDC	86±2		
	2,3-NDC	298±29	309±20	238
	2,6-NDC	537±26	508±27	511
	2,7-NDC	515±12	513±23	414
	1-NCA	101±14		
	2-NCA	733±82		
γ-CD	1,4-NDC	1[c)]		
	2,3-NDC	3[c)]		
	2,6-NDC	8±1		12
	2,7-NDC	6±1		

a) In aqueous solutions containing 0.1 mol dm^{-3} NaHCO$_3$ at 25 °C. b) The ICD signal was too weak to determine K_a. c) Rough estimation.

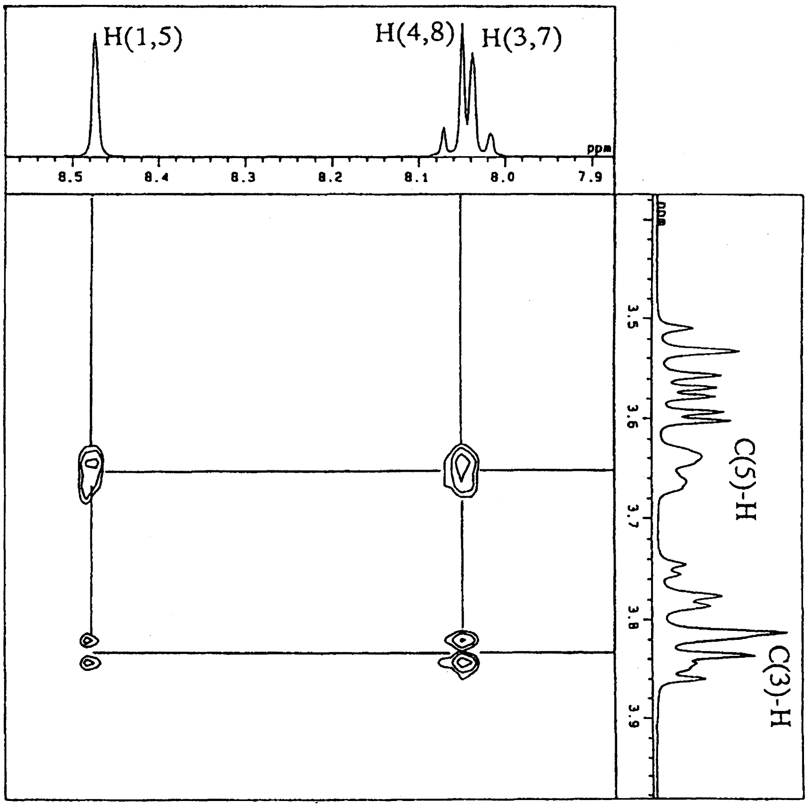


Fig. 9. The ROESY spectrum of a mixture of 5.0 mmol dm^{-3} 2,6-NDC with 5.0 mmol dm^{-3} β-CD in D$_2$O containing 0.1 mol dm^{-3} NaHCO$_3$ at 25 °C.

rod-like molecular structures will be fitted to the β-CD cavity. In contrast, the K_a values for β-CD complexes with 1, 4- and 1,5-NDC's were very small. The carboxylate groups of these NDC's are located only at the α-positions of the naphthalene moieties. Their molecular structures will be too bulky to be included within the β-CD cavity. In fact, the K_a value for a 1-NCA complex with β-CD was much smaller than that for 2-NCA. The K_a values for β-CD complexes with 1,3- and 1,6-NDC's were intermediate. These NDC's contain a carboxylate group at the α-position and another at the β-position of the naphthalene moieties.

The K_a values for a β-CD–2,6-NDC complex increased with increasing concentration of $NaHCO_3$ in solution: 537±26 $mol^{-1}\,dm^3$ at 0.1 $mol\,dm^{-3}$ $NaHCO_3$, 642±5 $mol^{-1}\,dm^3$ at 0.2 $mol\,dm^{-3}$ $NaHCO_3$, and 712±23 $mol^{-1}\,dm^3$ at 0.4 $mol\,dm^{-3}$ $NaHCO_3$. This so-called salting-out effect indicates that hydrophobic interactions between β-CD and 2,6-NDC also contribute to the complex formation.

ROESY Spectra and Molecular Structure of Inclusion Complexes. The molecular structure of β-CD inclusion complexes with NDC's was estimated by the measurement of ROESY spectra. Hirai et al. have estimated the molecular structure of an inclusion complex of β-CD with 2-NCA.[10] Figure 9 shows the ROESY spectrum of a β-CD–2,6-NDC system. Cross-peaks connecting the C(3)- and C(5)–H's of β-CD to the H(1, 5) and H(4, 8) of 2,6-NDC were clearly observed, but those connecting the β-CD protons to the H(3, 7) of 2,6-NDC were not observed. This fact indicates that the C(3)- and C(5)-H's of β-CD lie close to the H(1, 5) and H(4, 8), but far from the H(3, 7), of 2,6-NDC. The ROESY spectrum of a β-CD–2,7-NDC system was very similar to that of a β-CD–2,6-NDC system and indicated that the C(3)- and C(5)–H's of β-CD lie close to the H(1, 8) and H(4, 5), but far from the H(3, 6), of 2,7-NDC. In the ROESY spectrum of a β-CD–2,3-NDC system (Fig. 10), cross-peaks were observed between the C(5)–H of β-CD and the H(5, 8) and H(6, 7) of 2,3-NDC and between the C(3)–H of β-CD and the H(5, 8) and H(1, 4) of 2,3-NDC. In a β-CD–1,6-NDC system (Fig. 11), cross-peaks were observed between the C(5)–H of β-CD and the H(5), H(7), and H(8) of 1,6-NDC and between the C(3)–H of β-CD and the H(4) and H(8) of 1,6-NDC. Based on these facts, we estimated the molecular structures of β-CD inclusion complexes with some NDC's (Fig. 12). In the cases of β-CD complexes with 2,3-, 2,6-, and 2,7-NDC's, the longer axes of NDC's are parallel to the rotational symmetry axis of β-CD. On the other hand, the longer axis of 1,6-NDC is somewhat inclined to the rotational symmetry axis of β-CD, probably due to the steric hindrance of the carboxylate group at the α-position.

Application of the Molecular Recognition Function of β-CD to the Separation of NDC's. Since we determined that β-CD recognizes the molecular structures of NDC regioisomers, we attempted to separate a mixture of NDC's by column chromatography using a β-CDEP column and 0.1 $mol\,dm^{-3}$ $NaHCO_3$ as an eluent (Fig. 13). The fraction number of elution peak maximum, or the retention vol-

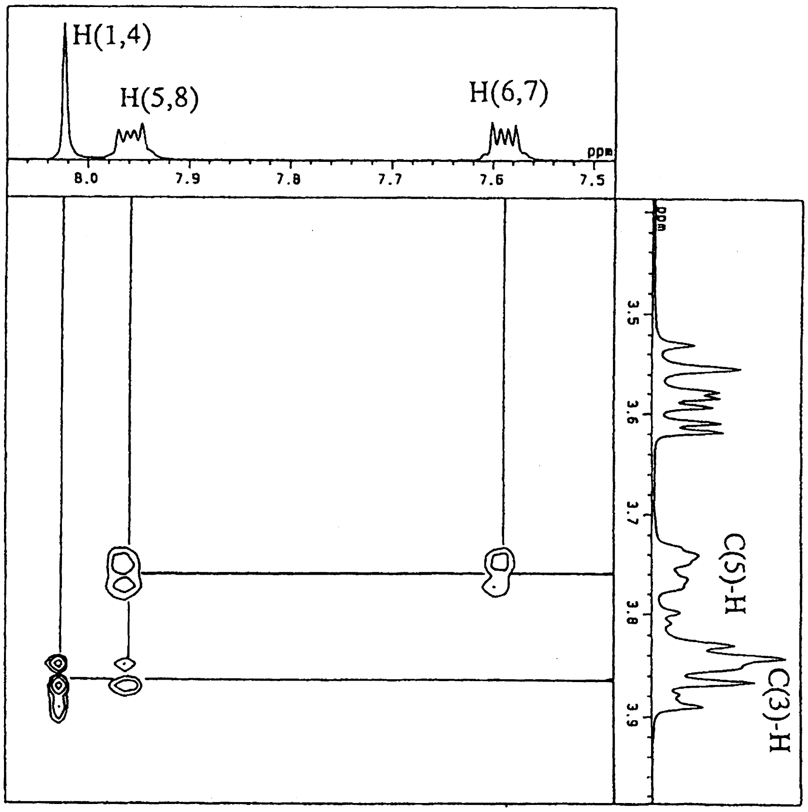


Fig. 10. The ROESY spectrum of a mixture of 5.0 $mmol\,dm^{-3}$ 2,3-NDC with 5.0 $mmol\,dm^{-3}$ β-CD in D_2O containing 0.1 $mol\,dm^{-3}$ $NaHCO_3$ at 25 °C.

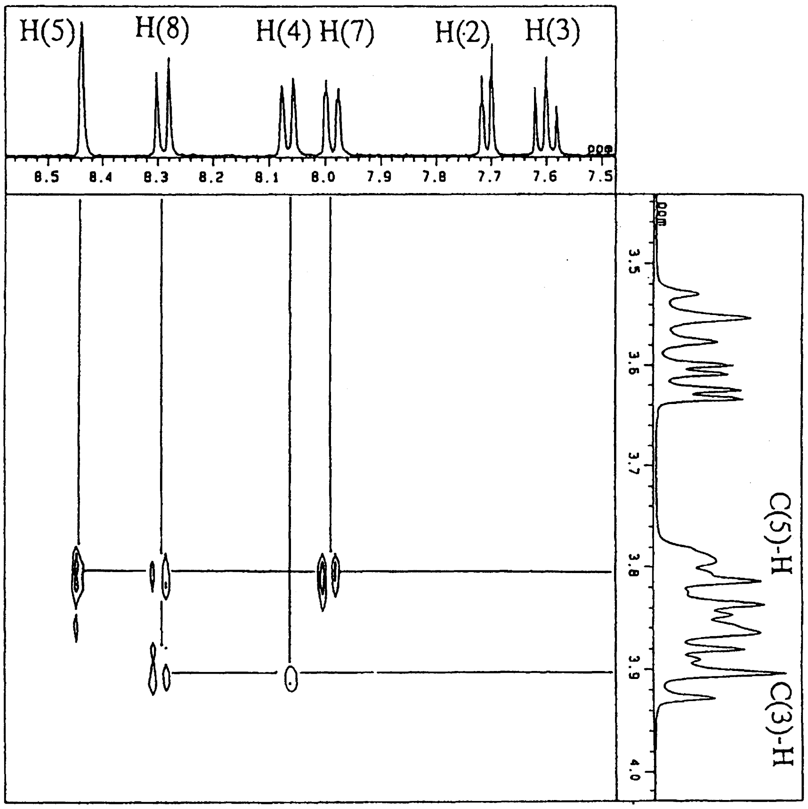
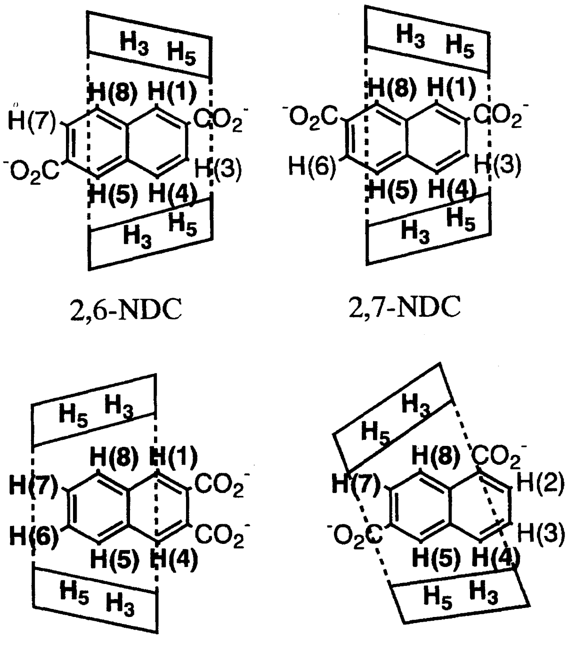


Fig. 11. The ROESY spectrum of a mixture of 5.0 mmol dm^{-3} 1,6-NDC with 5.0 mmol dm^{-3} β-CD in D_2O containing 0.1 mol dm^{-3} $NaHCO_3$ at 25 °C.



2,6-NDC 2,7-NDC

2,3-NDC 1,6-NDC

Fig. 12. Possible molecular structures of β-CD inclusion complexes with some NDC's estimated by the measurements of ROESY spectra.

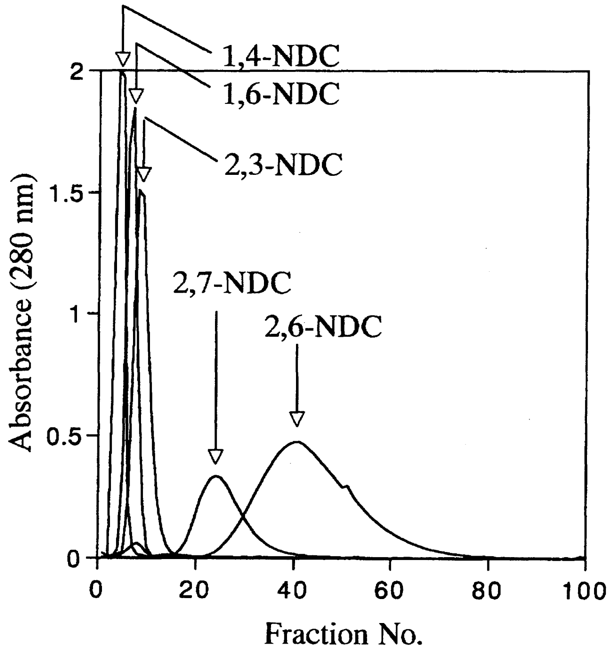


Fig. 13. Column chromatography of some NDC's using a β-CDEP column (15 mmϕ $\times$ 190 mm) and 0.1 mol dm^{-3} $NaHCO_3$ as an eluent (flow rate: 1 cm^3 min^{-1}). To the column, 1 cm^3 of 10 mmol dm^{-3} NDC in 0.1 mol dm^{-3} $NaHCO_3$ was loaded, and each fraction (10 cm^3) was followed by an UV detector at 280 nm.

ume, increased in the order of: 1,4-NDC < 1,6-NDC < 2,3-NDC < 2,7-NDC < 2,6-NDC. The order was in accord with that of the K_a value, indicating that the ability of β-CD to recognize NDC's contributes primarily to the separation of NDC's. Although the K_a value for a 2,6-NDC complex with β-CD was very close to that for a 2,7-NDC, the retention volume for 2,6-NDC was clearly different from that for 2,7-NDC. Complete separation of these NDC's would be attained by using a larger amount of β-CDEP as a column.

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