Molecular Recognition by Modified Cyclodextrins. Inclusion Complexation of β-Cyclodextrin 6-*O*-Monobenzoate with Acyclic and Cyclic Hydrocarbons

Lin-Hui Tong,[a] Zhi-Jie Hou,[a] Yoshihisa Inoue*,†,[b] and Akira Tai[b]
[a] *Lanzhou Institute of Chemical Physics, Academia Sinica, Lanzhou, China*
[b] *Group PRESTO, JRDC, Department of Material Science, Himeji Institute of Technology, Kamigori, Hyogo 678-12, Japan*

β-Cyclodextrin 6-*O*-monobenzoate has been synthesized and its complex stability constants (K_s) for acyclic and cyclic hydrocarbons of different size, shape and rigidity evaluated in 1:1 methanol–water and/or water by means of differential circular dichroism spectrometry. In the absence of the dipole–dipole and hydrogen-bonding interactions which usually play significant roles in the inclusion complexation of cyclodextrins, the K_s values obtained for the modified cyclodextrin may be more explicitly understood in terms of the size, conformation and rigidity of the guest molecules.

The study of molecular recognition with model systems is currently a significant topic in chemistry and biochemistry. A wide variety of cyclodextrin derivatives have hitherto been designed and synthesized in order to mimic enzymatic processes chemically.[1–7] Molecular recognition by β-cyclodextrins having fairly rigid and well-defined hydrophobic cavities has also been utilized in chromatographic separation of a wide variety of isomeric guest molecules.[8–10] One of the major characteristics of molecular recognition in these natural and artificial systems is the simultaneous operation of several weak forces working between receptor (host) and substrate (guest), which include dipole–dipole, electrostatic, van der Waals, and hydrogen-bonding interactions. As a consequence of the combined effect of these interactions, some modified cyclodextrins have been shown to be very successful in mimicking biological target systems and in separating several types of guest molecules.[1–10] On the other hand, the specification of major contributor(s) to the guest specificity and the analysis of the recognition mechanism are not always possible in such sophisticated systems.

In this paper, we report our study on the synthesis and inclusion complexation behaviour of a modified β-cyclodextrin carrying one chromophoric benzoate moiety, which is used as a probe for differential circular dichroism (CD) spectrometry. Acyclic and cyclic hydrocarbon guest molecules are employed in order to minimize the possible participation of electrostatic and hydrogen-bonding interactions. Under such circumstances, we can discuss the guest selectivity of β-cyclodextrin monobenzoate and clarify the discrimination mechanism in detail from the viewpoints of molecular shape, hydrophobicity, and conformational rigidity of the guest molecules.

Experimental

General.—Melting points were measured with a YANACO MP-21 apparatus and are uncorrected. Elemental analyses were performed on a Carlo-Erba 1106 instrument. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, in [$^2H_6$]dimethyl sulfoxide (DMSO-d_6) on a JEOL GX-400 spectrometer. IR and UV spectra were obtained on JASCO IR-810 and Ubest-50 spectrometer, respectively. Optical rotations were measured with a Perkin-Elmer 243B polarimeter with a thermostated 10 cm conventional or micro cell. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter equipped with an NEC PC-9801 personal computer for control and data analysis.

Materials.—Guest hydrocarbons employed were mostly commercially available and were used as received, except for the highly strained (*E*)-isomers of cyclooctene and 1-methylcyclooctene. The strained (*E*)-isomers were prepared from the corresponding (*Z*)-isomers by photosensitized geometrical isomerization followed by purification *via* a silver nitrate complex.[11,12] Distilled water and methanol were used as solvents. β-Cyclodextrin purchased from Kanto was dried *in vacuo* for 3 h at 50–70 °C. Commercially available benzoyl chloride was used without further purification. Pyridine was distilled from potassium hydroxide prior to use.

Synthesis of β-*Cyclodextrin* 6-O-*Monobenzoate* (**1**).—To an ice-cooled solution of β-cyclodextrin (25 g, 22 mmol) in dry pyridine (750 cm^3) was added dropwise a solution of benzoyl chloride (2.4 g, 17 mmol) in dry pyridine (70 cm^3) over 20 min with magnetic stirring. The solution was allowed to warm up and stirred for 5 h at room temperature. To the solution was added 2.5 cm^3 of water, and the resultant mixture was evaporated *in vacuo* at 40–50 °C, leaving a white solid. Using a soxhlet apparatus, the solid obtained was first extracted with acetone for 40 h and then repeatedly with methanol. The combined methanol extracts were concentrated by distillation until a trace of white precipitate appeared. After filtration of the precipitate while hot, the methanol solution was allowed to cool to room temperature, yielding the crude product as white crystals. The crude product was recrystallized twice from methanol and then twice from water to give a pure sample (2.0 g): m.p. 289–290 °C; $[\alpha]_D^{25}$ +110.3 (*c* 0.176, MeOH), +111.4 (*c* 0.088, 1:1 MeOH–H_2O); *m/z* [FAB(KI)] 1277 (M^+ + 39); ν(KBr)/cm^{-1} 3375, 2930, 1720, 1640, 1420, 1370, 1335, 1280, 1160, 1080, 1060, 1030, 940, 860, 755, 710, 580; δ_H 7.99 (t, 2 H), 7.66 (d, 1 H), 7.53 (t, 2 H), 5.65–5.85 (m, 14 H), 4.8–4.9 (m, 7 H), 4.54 (d, 1 H), 4.35–4.55 (m, 6 H), 4.0 (br, 1 H), 3.2–3.8 (m, 40 H); δ_C 165.70 (C=O), 133.46 (*ipso*), 129.60 (*p*), 129.56, 128.82 (*o*, *m*), 102.69, 102.07, 102.01, 101.65 (C-1), 82.48, 81.67, 81.60, 81.57, 81.34, 81.29 (C-4), 73.28, 73.13, 73.09, 73.03, 72.94 (C-3), 72.49, 72.28, 72.13, 72.07 (C-2, C-5), 69.06 (C-5′), 64.26 (C-6′), 59.98, 59.72, 59.68 (C-6); (Found: C, 45.3; H, 6.0. Calc. for $C_{49}H_{74}O_{36}\cdot 3H_2O$: C, 45.51; H, 6.24%).

Examinations of the pure sample by both TLC [silica gel; MeCN–MeOH–H_2O (2:2:1) eluent] and HPLC [ODS; EtOH–H_2O (20:80) eluent] showed a single peak.

† JRDC Research Fellow 1992–94.

Table 1 UV and CD spectra of β-cyclodextrin 6-*O*-monobenzoate **1** in methanol, 1:1 methanol–water, and water

Solvent	[**1**]/mmol dm^{-3}	UV λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})	CD λ_{max}/nm (Δε/dm^3 mol^{-1} cm^{-1})
MeOH	0.17	229 (16 300), 273 (1 010), 282 (800)	234 (−0.98), 273 (−0.05), 283 (−0.06)
MeOH–H_2O (1:1)	0.08	231 (11 900, 273 (900), 282 (720)	236 (−1.11), 274 (−0.10), 284 (−0.08)
H_2O[a]	0.043	232 (9 130), 273 (890), 282 (720)	240 (−1.0), 274 (−0.2), 282 (−0.1)
	1.20	232 (9 800), 274 (890), 282 (700)	236 (+1.15), 275 (−0.22), 282 (−0.23)

[a] The CD spectrum was highly concentration dependent in water, only two extreme cases being listed; see Fig. 3 and text for details.

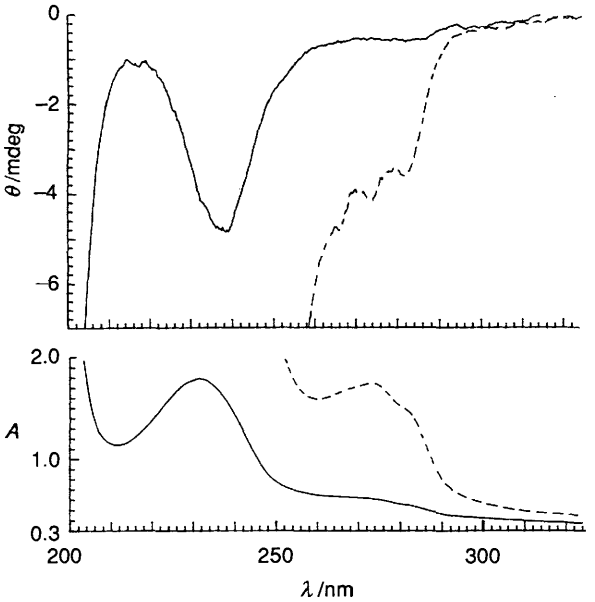

Fig. 1 Absorption and circular dichroism spectra of β-cyclodextrin 6-*O*-monobenzoate **1** (0.789 mmol dm^{-3}) in 1:1 methanol–water in 0.1 (solid lines) and 1 cm cell (dashed lines)

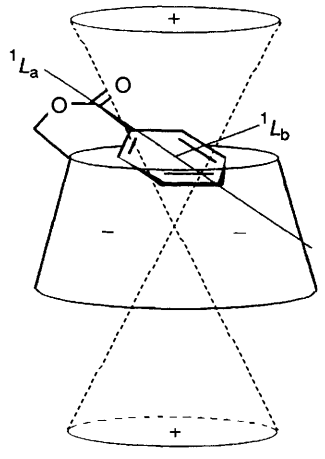

Fig. 2 Kajtar's sector rule (ref. 13) applied to transition moments of ^1L_a and ^1L_b bands of the benzoate moiety in β-cyclodextrin 6-*O*-monobenzoate 1

Measurements.—In the differential CD spectrometry to determine complex stability constants, the CD spectra were measured in 1:1 methanol–water and/or in water at 23–25 °C with a series of solutions containing cyclodextrin monobenzoate (0.087 mmol dm^{-3}) and varying concentrations of guest (0.1–6 mmol dm^{-3}). The differential CD spectrum was obtained by subtracting the original CD spectrum in the absence of guest from that in the presence of a guest on computer memories. Simultaneous examination of each sample solution by UV spectrometry did not show any significant change upon addition of the guests.

Results and Discussion

UV and CD Spectra.—The electronic spectra of cyclodextrin monobenzoate (**1**) in methanol, methanol–water (1:1), and water resemble one another, showing ^1L_a and ^1L_b band maxima at almost the same wavelengths around 230 and 273 nm, respectively, as listed in Table 1. These absorption bands are identical in wavelength to those of methyl benzoate in the same solvents, and no further information about the detailed structure of **1** could be obtained from the UV spectrum. On the other hand, examination of the CD spectra, shown in Fig. 1, enables us to elucidate the conformation of the benzoate moiety in **1**, and further discloses the self-assembly phenomenon in water.

The CD spectra of dilute solutions of **1** (0.04–0.08 mmol dm^{-3}) showed a major negative Cotton effect peak, corresponding to the ^1L_a band at 234, 236 and 240 nm in methanol, methanol–water (1:1) and water, respectively. Also observed was a weak negative Cotton effect maximum around 270–280 nm (^1L_b), which was detectable only at fairly high concentrations of **1**. The negative Cotton effect for the ^1L_a band is of special interest, since inclusion of methyl benzoate by β-cyclodextrin led to a positive Cotton effect for the ^1L_a band and a negative effect for ^1L_b in water and in 1:1 methanol–water. According to the sector rule proposed by Kajtar,[13] these negative Cotton effects observed for the ^1L_a and ^1L_b bands indicate that, in these polar solvents, the benzoate moiety penetrates sideways into the hydrophobic cavity of cyclodextrin. As illustrated schematically in Fig. 2, the transition moment of the ^1L_a band lies in the negative region, inducing a negative Cotton effect. Thus the phenyl group, bonded to the 6-oxygen atom by a short ester linkage, is only shallowly included in the cyclodextrin cavity.

Interestingly, the CD spectrum of **1** in water was highly concentration dependent. As can be seen from Fig. 3, the negative Cotton effect (236 nm) observed at the lowest concentration (0.043 mmol cm^{-3}) gradually decreases to almost zero Δε with increasing concentration up to 0.26 mmol dm^{-3}, and is then inverted to the positive by further increasing the concentration up to 1.2 mmol dm^{-3}. This sequential change was totally reversible, as gradual dilutions of the saturated aqueous solution of **1** reproduced the above-mentioned change. On the basis of the positive Cotton effect observed for the ^1L_a band at high concentrations, this apparently strange behaviour may be reasonably interpreted in terms of the self-assembly phenomenon forming a dimeric complex, in which one or both of the phenyl groups is embedded deeply in the cavity, *i.e.* the positive region, of another host molecule, as illustrated in Figs. 4(*a*) and 4(*b*).

Complexation Equilibrium.—The phenyl group, originally perching on the edge of cyclodextrin cavity, must suffer substantial conformational change upon guest inclusion, probably moving outward to the less-hydrophobic region. This microstructural change could not be detected experimentally from the absorption spectrum, which showed no appreciable changes in the ^1L_a or ^1L_b band even at the highest guest

Table 2 Sensitivity factor (α), complex stability constants (K_S/dm^3 mol^{-1}), and free energy change ($-\Delta G°$/ kJ mol^{-1}) for inclusion complexation of β-cyclodextrin 6-*O*-monobenzoate 1 with acyclic and cyclic hydrocarbon guests **2**–**17** in 1:1 methanol–water or in water at 23–25 °C

Guest	Solvent	α	K_S	$-\Delta G°$
Hexane (**2**)	MeOH–H_2O	5 600	310	14.2
Heptane (**3**)	MeOH–H_2O	4 000	560	15.7
Octane (**4**)	MeOH–H_2O	5 800	1 080	17.3
Cyclohexane (**5**)	MeOH–H_2O	9 000	520	15.5
Methylcyclohexane (**6**)	MeOH–H_2O	9 600	790	16.5
Cycloheptane (**7**)	MeOH–H_2O	9 800	1 150	17.4
Cyclooctane (**8**)	MeOH–H_2O	10 100	2 060	18.9
Cyclohexene (**9**)	MeOH–H_2O	6 200	730	16.3
1-Methylcyclohexene (**10**)	MeOH–H_2O	3 200	1 020	17.2
Cycloheptene (**11**)	MeOH–H_2O	7 300	980	17.1
(*Z*)-Cyclooctene (**12**)	MeOH–H_2O	9 000	1 440	18.0
	H_2O	45 500	2 810	19.7
(*E*)-Cyclooctene (**13**)	MeOH–H_2O	7 400	2 410	19.3
	H_2O	25 900	6 320	21.7
(*Z*)-1-Methylcyclooctene (**14**)	MeOH–H_2O	11 200	500	15.4
(*E*)-1-Methylcyclooctene (**15**)	MeOH–H_2O	8 100	1 790	18.6
(*Z*,*Z*)-1,5-Cyclooctadiene (**16**)	MeOH–H_2O	4 900	940	17.0
(−)-Menthol (**17**)	MeOH–H_2O	4 600	400	14.9

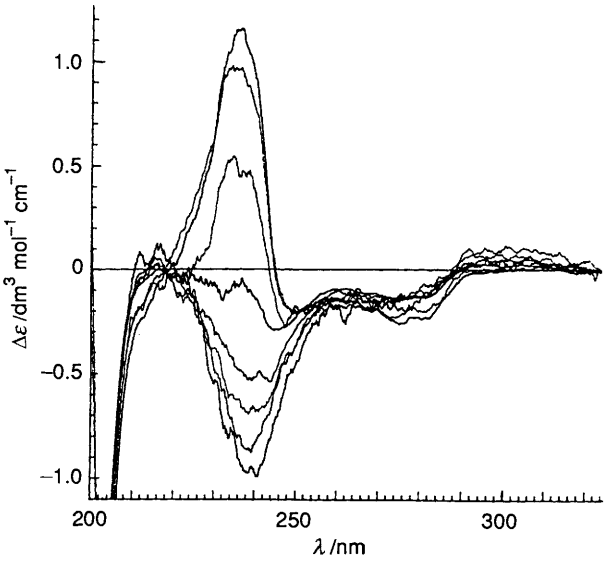

Fig. 3 Concentration dependence of the CD spectrum of β-cyclodextrin 6-*O*-monobenzoate **1** in water; [1] = 1.20, 0.78, 0.56, 0.26, 0.13, 0.086, 0.065 and 0.043 mmol dm^{-3} from top to bottom

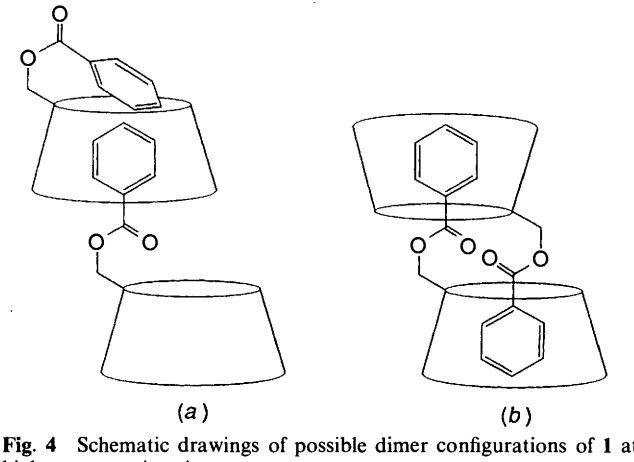

Fig. 4 Schematic drawings of possible dimer configurations of **1** at high concentrations in water

concentration. Hence, the inclusion behaviour of β-cyclodextrin monobenzoate **1** with the hydrocarbon guests was investigated by means of differential CD spectrometry. Gradual addition of a known concentration of guest to a dilute host solution (0.08 mmol dm^{-3}) in 1:1 methanol–water or in water caused a significant change in the ^1L_a band, while the change in the ^1L_b band was minimal at this host concentration. Typical CD and differential CD spectra observed upon addition of cycloheptane are shown in Figs. 5(*a*) and 5(*b*). In methanol solution, only a trivial change was induced by the addition of the guest, although this does not necessarily mean no complex formation occurs, but may rather suggest minimal conformational change of the benzoate moiety upon guest inclusion. These results

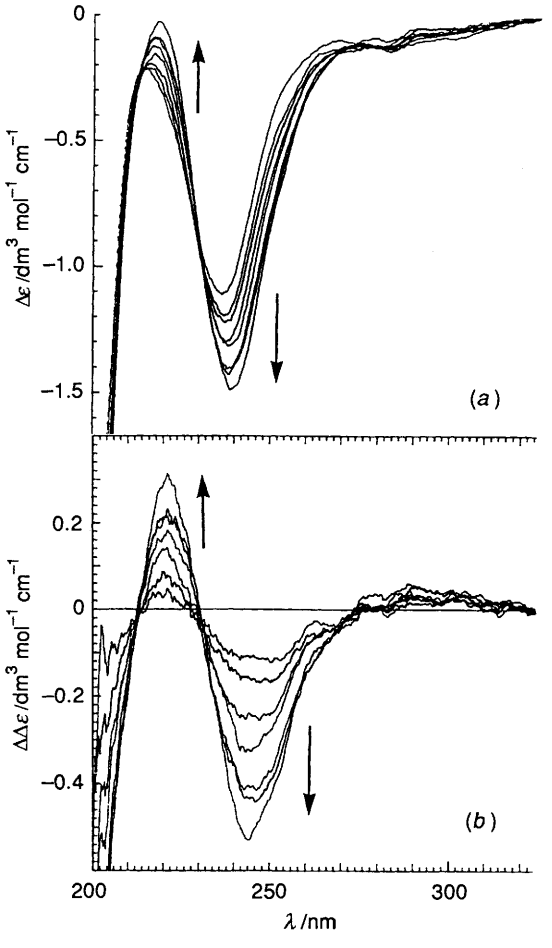

Fig. 5 (*a*) CD and (*b*) differential CD spectra of 1 (0.080 mmol dm^{-3}) upon addition of cycloheptane **7** (0–1.53 mmol dm^{-3}) in 1:1 methanol–water

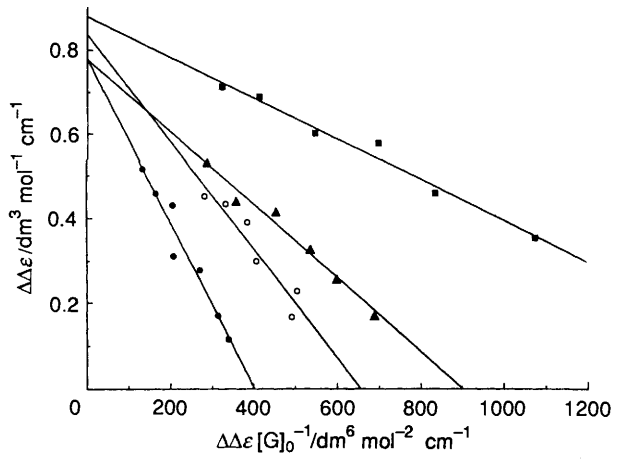

Fig. 6 Plot of $\Delta\Delta\varepsilon$ as a function of $\Delta\Delta\varepsilon/[G]_0$ for the complexation of **1** with cyclohexane **5** (●), methylcyclohexane **6** (○), cycloheptane **7** (▲), and cyclooctane **8** (■) in 1:1 methanol–water

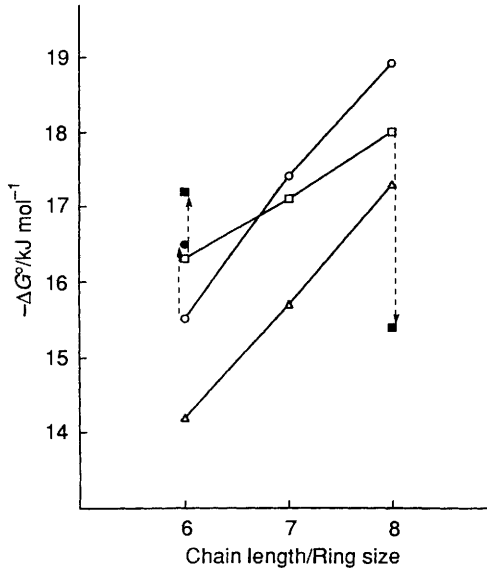

Fig. 7 Free energy change ($-\Delta G^\circ$) on complexation of **1** with a series of alkanes (△), cycloalkanes (○), and cycloalkenes (□) as a function of chain length or ring size; methylated hydrocarbons, indicated by filled symbols, are linked by dashed arrows to the parent hydrocarbons

indicate that the conformation of the benzoate moiety, initially perching on the cavity edge, undergoes considerable changes, inducing CD-spectral changes, upon inclusion of the hydrocarbon guests, in methanol–water and in water.

Assuming 1:1 stoichiometry, the inclusion complexation of a hydrocarbon guest (G) with the cyclodextrin monobenzoate (H) is expressed by eqn. (1).

$$H + G \underset{}{\overset{K_S}{\rightleftharpoons}} H\cdot G \qquad (1)$$

Furthermore, assuming that the change ($\Delta\Delta\varepsilon$) in the CD spectrum upon addition of a guest is proportional to the concentration of inclusion complex produced, *i.e.* $\Delta\Delta\varepsilon = \alpha[H\cdot G]$, the complex stability constant (K_S) is given as a reciprocal slope of eqn. (2), while the proportionality coefficient α may be taken

$$\Delta\Delta\varepsilon = -(1/K_S)(\Delta\Delta\varepsilon/[G]) + \alpha[H]_0 \qquad (2)$$

as a sensitivity factor for the CD change, or a quantitative measure of the conformational change upon complexation. Since the concentration of complex [H·G] is much smaller than the initial guest concentration $[G]_0$ under the conditions employed, *i.e.* $[G] = [G]_0 - [H\cdot G]$ and $[G]_0 \gg [H\cdot G]$, then eqn. (2) reduces to eqn. (3). Plots of observed $\Delta\Delta\varepsilon$ values

$$\Delta\Delta\varepsilon = -(1/K_S)(\Delta\Delta\varepsilon/[G]_0) + \alpha[H]_0 \qquad (3)$$

as a function of $\Delta\Delta\varepsilon/[G]_0$ give good straight lines for all guest molecules examined, confirming the validity of the 1:1 complex stoichiometry assumed above. Typical plots are shown for some cycloalkanes in Fig. 6. The sensitivity factor α and the complex stability constant K_S, calculated from the intercept and slope respectively, are listed in Table 2, along with the free energy change of complex formation ($-\Delta G^\circ$).

Factors Controlling Inclusion Complexation.—Lacking any site-specific dipole–dipole and hydrogen-bonding interactions between host and hydrocarbon guests, the modified cyclodextrin **1** appears to recognize the guest molecule predominantly through its molecular size and shape, maximizing hydrophobic interaction within the host cavity. As a result, the size–fit, or more rigorously shape–fit, relationship between host and guest molecules becomes the major factor governing the complex stability.

As can be seen from Fig. 7, the free energy change ($-\Delta G^\circ$) increases monotonically with increasing chain-length or ring-size in all of the examined cases of C_6–C_8 alkane, cycloalkane, and cycloalkene series; *i.e.* **2** < **3** < **4**; **5** < **7** < **8**; **9** < **11** < **12**. It is noted that the increment per methylene group added is fairly constant within each C_6–C_8 hydrocarbon series and is characteristic of the guest type. The unit increment in ΔG° is much larger for saturated alkanes ($\Delta\Delta G^\circ$ = 1.6 kJ mol^{-1}/CH_2) and cycloalkanes (1.7 kJ mol^{-1}/CH_2) than for unsaturated cycloalkenes (0.9 kJ mol^{-1}/CH_2). As a consequence of the different increments for the saturated and unsaturated cyclic hydrocarbon series, the relative guest selectivity between these two series is inverted between C_6 and C_7, while the preference for cycloalkanes over alkanes is maintained, or even enhanced, over C_6–C_8.

In this context, it seems reasonable to propose that both methylcyclohexane **6** and 1-methylcyclohexene **10** afford more stable complexes with **1** than do the corresponding unsubstituted compounds **5** and **9** ($\Delta\Delta G^\circ$ = 0.9–1.0 kJ mol^{-1}/CH_2) as shown in Table 2 and Fig. 7. By contrast, methyl substitution in (*Z*)- and (*E*)-cyclooctene diminishes K_S substantially, and (−)-menthol, possessing a bulky, as well as hydrophilic, substituent, gives a much reduced K_S value as compared with methylcyclohexane. These reduced stabilities may be attributed to large molecular size, since there must be a best-fit size for the cavity of **1**.

Owing to the combined effect of the reduced flexibility and the distinctly different shape, the geometrical isomers of cyclooctene and 1-methylcyclooctene show substantially different K_S values, yielding fairly good isomer separation; the *E*/*Z* selectivities calculated from the K_S values are 1.7 for **13**/**12** and 3.6 for **15**/**14** in 1:1 methanol–water. The use of water as a more polar solvent not only doubles the complex stability but also enhances the *E*/*Z* selectivity for cyclooctene, probably owing to the strengthened hydrophobic interaction producing more defined complex conformation. Thus, in aqueous solutions, the K_S values for **12** and **13** increase by factors of 2.0 and 2.6, while the *E*/*Z* selectivity rises to 2.2.

By definition, the sensitivity factor α, calculated from the intercept ($\alpha[H]_0$) of the plot of Fig. 6, is a novel quantitative measure of conformational change induced by guest inclusion in the cavity. It is interesting however that the sensitivity factor α shows only a poor positive correlation (correlation coefficient *r* 0.622) with the complex stability, and the most stable complex

does not afford the most drastic CD change and *vice versa*. This is not particularly curious if the benzoyl group in **1** is perching on the edge of cavity and the guest inclusion causes only a small microstructural change, as indicated by the sector rule. For the same reason, the sensitivity factor is much more sensitive to the solvent polarity than the complex stability. Thus, the use of water as solvent dramatically enhances α by a factor of 3.5–5, while K_S is enhanced by a factor of only 2–2.5. It may be interesting to evaluate the sensitivity factor and use it as a quantitative measure of the conformational change induced in other modified cyclodextrins possessing a chromophoric probe at various sites.

Conclusions

The present study demonstrates that a modified cyclodextrin posessing a single benzoate moiety as a probe can recognize minimal differences between acyclic hydrocarbons based on their size, rigidity and shape. The chromophoric probe located on the edge of the cyclodextrin cavity is useful not only in determining complex stability constants, but also in examining microstructural change in different solvents. It is shown that, in addition to the molecular size and shape, the structural flexibility of the guest molecule apparently governs the complexation phenomena to some extent, since the inclusion of guests is inevitably accompanied by the induced fit into the cyclodextrin cavity. Introduction of double bond(s) into cycloalkanes increases the skeletal rigidity of the guest, affording better defined and fitted structures for the unsaturated hydrocarbons. Hence, the complexation of guest molecules with flexible skeletons is more susceptible to changes in molecular size or shape, although the complex stability is governed by the size/shape–fit concept. Experimentally, the C_8 hydrocarbons, especially (*E*)-cyclooctene, have the best-fitted size and shape among the hydrocarbon guests examined.

Acknowledgements

This work was supported in part by grants from the National Natural Science Foundation of China and the Ministry of Science, Culture and Education of Japan, which are gratefully acknowledged.

References

1 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer Verlag, Berlin, 1978.
2 A. P. Croft and R. A. Bartsch, *Tetrahedron*, 1983, **39**, 1417.
3 I. Tabushi, in *Inclusion Compounds*, eds. J. L. Atwood, J. E. D. Davies and D. D. MacNicol, Academic, London, 1984, vol. 3, ch. 13.
4 R. Breslow, in *Inclusion Compounds*, eds. J. L. Atwood, J. E. D. Davies and D. D. MacNicol, Academic, London, 1984, vol. 3, ch. 14.
5 I. Tabushi, *Tetrahedron*, 1984, **40**, 269.
6 J. Szejtli, *Cyclodextrin Technology*, Kluwer-Academic, Dordrecht, 1988.
7 H. Ougar, *Bioorganic Chemistry*, 2nd ed., Springer, New York, 1989.
8 D. Sybilska and E. Smolková-Keulemansová, in *Inclusion Compounds*, eds. J. L. Atwood, J. E. Davies and D. D. MacNicol, Academic, London, 1984, vol. 3, ch. 6.
9 J. Szojtli, *Starch*, 1987, **39**, 357.
10 V. Schurig and H.-P. Nowotny, *Angew. Chem., Int. Ed. Engl.*, 1990, **29**, 939.
11 N. Yamasaki, Y. Inoue, T. Yokoyama and A. Tai, *J. Photochem. Photobiol., A: Photochem.*, 1989, **48**, 465.
12 H. Tsuneishi, Y. Inoue and A. Tai, unpublished results.
13 M. Kajtar, Cs. Harvath-Toro, E. Kuthi and J. Szejtli, *Acta Chim. Acad. Sci. Hung.*, 1982, **110**, 327.

Paper 2/01813F
Received 6*th April* 1992
Accepted 15*th April* 1992