^{1}H NMR Study of the Inclusion of Aromatic Molecules in α-Cyclodextrin

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Abstract: The proton chemical shifts and coupling constants for cyclohexaamylose (α-CD) in aqueous solution were obtained at 100 and 220 MHz. Comparison of the data with those of methyl α-D-glucopyranoside revealed that the C1 conformation is maintained at the monomer and cyclohexamer level. A slight increase in the gauche-gauche conformer population was noted for α-CD relative to the monomer. Addition of *p*-iodoaniline to an α-CD solution at acid pH resulted in an *upfield* shift of the H_3 and a *downfield* shift of H_5, hydrogens located on the inner surface of the α-CD torus, whereas hydrogens on the outer surface of the torus, H_1, H_2, H_4, were unaffected; the effect on the H_6 hydrogens which are located at the O_6 end of the torus was of intermediate magnitude. These data were interpreted in terms of an inclusion complex between the α-CD and the aromatic. The binding studies were extended to *p*-nitrophenol and its anion and to D,L-phenylalanine. In these instances, though inclusion into the torus was evident from the upfield shift of the H_3 resonances, the H_5 resonances were not affected by complexation.

α-Cyclodextrin (cyclohexaamylose, α-CD)[2] is the smallest member of the family of Schardinger dextrins, cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyranose units. X-ray crystallographic studies[3-5] have established that α-CD assumes in the crystalline state a toroidal topography with a central void of about 5-Å diameter. α-CD is capable of forming adducts[5-7] by including into the cavity a variety of molecular species such as H_2O, benzene derivatives, iodine, etc. Adduct formation in aqueous solution has been studied by NMR,[8] ultraviolet and visible spectroscopy,[9-11] and ESR;[12,13] in addition some fluorescence,[14] ORD, and circular dichroism[15,16] measurements have been made. The x-ray studies demonstrated that in the inclusion complexes α-CD assumes an almost hexagonal structure with a ring of six intramolecular, interglucosidic hydrogen bonds between adjacent O_2 and O_3 hydroxyls. However, in the "empty" α-CD molecule, two water molecules occupy the 5.0 Å wide void which is collapsed to some extent in order to allow a snug fit with the small water molecules, the diminishing of the void being achieved mainly by the rotation of one of the six glucoses.[5] This rotation interrupts the ring of $O_2 \cdots O_3$ hydrogen bonds and causes steric strain at the glucosidic linkages. Thus in the "empty" α-CD·2 H_2O complex, α-CD has increased potential energy which is released upon complex formation.

In view of the conformational changes associated with α-CD complex formation in the solid state, it was of interest to study complexation in aqueous solution using ^{1}H NMR. Because of the magnetic screening environment of aromatic molecules, their inclusion should be particularly susceptible to detection by ^{1}H NMR.[8a] In this work we present: (a) a comparison of the 100- and 220-MHz ^{1}H NMR chemical shift and coupling constant data for α-CD and for the monomeric glycoside (methyl α-D-glucopyranoside) in aqueous solution which is relevant to the discussion of the influence of macrocyclic ring formation on the stereochemistry of the individual glucose units; and (b) a study of the influence of several aromatic molecules (*p*-iodoaniline, *p*-nitrophenol and its anion, and D,L-phenylalanine) on the proton chemical shifts and coupling constant data of α-CD. The solution data are discussed in the light of x-ray,[5,16,17] kinetic,[9] and thermodynamic studies[9] of the inclusion of aromatics into α-CD.

Experimental Section

The following materials[2] were used (source in parentheses): α-CD (Corn Products Development, Englewood Cliffs, N.J.); pIA (Merck Co., Darmstadt, Germany); pNP (Chemical Service Inc., Media, Pa.); D- and D,L-phe (Sigma Chemical Co., St. Louis, Mo.); mglc (Nutritional Biochemicals, Cleveland, Ohio). α-CD was purified by recrystallization once from 1-propanol and twice from water.

For the study of α-CD in the absence of added guest, D_2O solutions (pD adjusted with NaOD and DCl) were lyophilized three times and then 100% D_2O was added to give a 0.1 M solution. No internal ^{1}H NMR reference was added since the possibility of reference binding to the α-CD could not be excluded. An external TSP (in D_2O) sample was used instead.

Spectra of mglc and α-CD (220 MHz) in the *absence* of added guest, Figure 1, were recorded on a Varian HR-220 MHz spectrometer. In aqueous solution only resonances from the nonexchanging hydrogens attached to carbons are detected. The spectral assignment for mglc is that of De Bruyn et al.[19] in a 300-MHz study of this molecule. The assignment of the α-CD spectrum was facilitated by the work of Demarco and Thakar.[8a] The spectra were analyzed with the aid of LAME[20] and computer-simulated spectra were generated as the final test of the assignment and spectral parameters. Our data for mglc are in good agreement with those reported by De Bruyn et al.[19]

For the *binding studies*, D_2O solutions of α-CD (ca. 0.041 M) were prepared with different amounts of aromatic guest. Spectra were obtained on a Varian HD-100 spectrometer, but for selected solutions 220 MHz spectra were obtained as well (Figure 2). Because of the overlap of the bands in the 100-MHz spectra, the chemical shift (δ) and coupling constant (J) data for α-CD could not be obtained by the iterative technique at this frequency. Using the selected 220-MHz spectra as guides, we generated a series of computer-calculated 100-MHz spectra with variable δ values for comparison with the observed 100-MHz spectra. In this manner we obtained δ values sufficiently accurate for our discussion of the guest–host complexation.

The 220-MHz δ and J data for α-CD are presented in Table I. Some of the 100-MHz δ data for the binding study are given in Figures 3 and 4 as graphs of $\Delta\delta_i$ vs. R. R is defined as the guest to α-CD molar ratio of the solution, and $\Delta\delta_i$ is the chemical shift of the ith hydrogen of α-CD relative to the shift of the hydrogen on C_1, a positive value indicating resonance at high field from this reference. A hydrogen on the outer surface of α-CD was chosen as the chemical shift reference, since complexation was expected to occur by inclusion of the aromatic and, therefore, to have minimal influence on the shielding of outer-surface hydrogens. Any changes in $\Delta\delta$ due to added guest for an inner-surface hydrogen may then be related to the nature of the adduct.

Results and Discussion

(a) Conformation of α-CD in Solution. The 220-MHz spectrum of α-CD (Figure 1) is consistent with the postulate that *on the ^{1}H NMR time scale* all six glucose units have identical conformations and the molecule has hexagonal symmetry, in agreement with earlier observations[21] at 60 MHz. Furthermore, the magnitudes of the vicinal coupling constants J_{12}

Table I. 220-MHz Data for α-Cyclohexaamylose (A) and Methyl α-D-Glucopyranoside (B)

	A								B
Guest	None	None	None	None	*p*-Iodo-aniline	*p*-Nitro-phenol	*p*-Nitro-phenolate ion	D,L-Phenyl-alanine	None
R^a	0.00	0.00	0.00	0.00	0.67	1.16	1.72	1.35	—
pD	7.5	7.5	7.5	2.7	2.7	2.6	11.8	2.3	7.5
Temp, °C	10	46	68	30	30	30	30	30	18
$\Delta\delta_2{}^b$	1.43	1.42	1.42	1.42	1.41	1.42	1.45	1.41	1.25
$\Delta\delta_3$	1.08	1.08	1.09	1.08	1.28	1.32	1.29	1.12	1.14
$\Delta\delta_4$	1.47	1.48	1.48	1.49	1.44	1.44	1.43	1.47	1.41
$\Delta\delta_5$	1.22	1.22	1.22	1.22	1.00	1.22	1.17	1.21	1.16
$\Delta\delta_{6a}$	1.16	1.15	1.14	1.15	1.11	1.14	<1.14[d]	1.13	0.94
$\Delta\delta_{6b}$	1.18	1.19	1.20	1.20	1.14	1.17	<1.15	1.18	1.05
$J_{12}{}^c$	3.3	3.5	3.3	3.5	3.4	3.3	3.2	3.5	3.7
J_{23}	9.8	9.8	9.9	10.0	9.8	9.9	9.9	9.9	9.8
J_{34}	8.8	8.8	8.8	8.7	8.9	8.7	8.7	8.9	8.8
J_{45}	9.6	9.4	9.3	9.3	9.8	9.5	9.1	9.7	10.0
J_{56a}	1.8	1.9	2.0	2.3	2.0	1.9	[3.1[e]	2.0	2.3
J_{56b}	3.7	3.8	4.2	3.5	4.3	3.6	[3.1	3.8	5.7
J_{6a6b}	−12.5	−12.4	−12.6	−12.9	−12.6	−13.0	−12.5[f]	−13.1	−12.3

[a] R = moles of guest/moles of α-CD. [b] $\Delta\delta_i = \delta_1 - \delta_i$ where δ_i is the chemical shift of proton i in ppm relative to external TSP. Estimated error is ±0.02 ppm. δ_1 is approximately 5.1 ppm below external TSP (in D_2O). [c] The coupling constants (J) are reported in Hz. Estimated error is ±0.2 Hz. Broadening in the 4 and 6 proton resonances was simulated by including an ~0.5 Hz coupling between these protons. [d] The 0.01 ppm chemical shift difference in the connected values is arbitrary, i.e., $\delta_i \sim \delta_j$. [e] Only the sum of the connected coupling constants is significant. [f] Arbitrary; when $\delta_i \sim \delta_j$ then the spectrum is independent of J_{ij}.

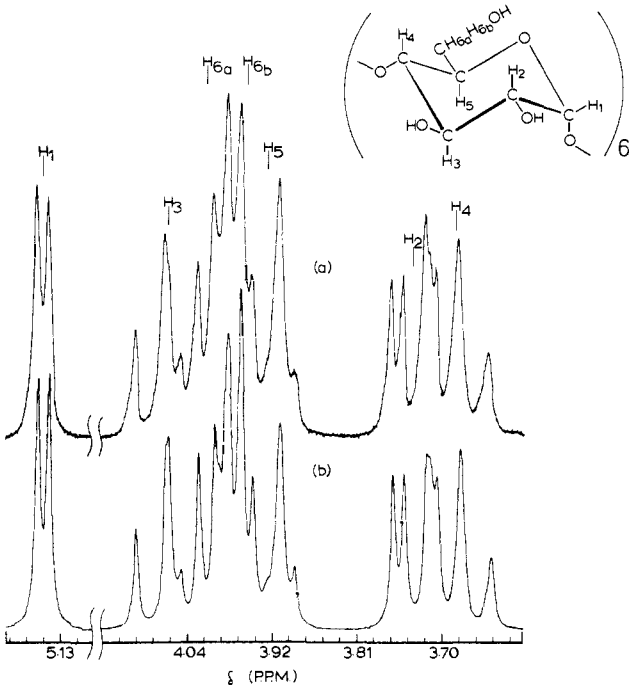

Figure 1. (a) Spectrum (220 MHz) of 0.1 M α-CD in D_2O (68 °C, pD = 7.5); sweep width 250 Hz. (b) Computer-simulated spectrum.

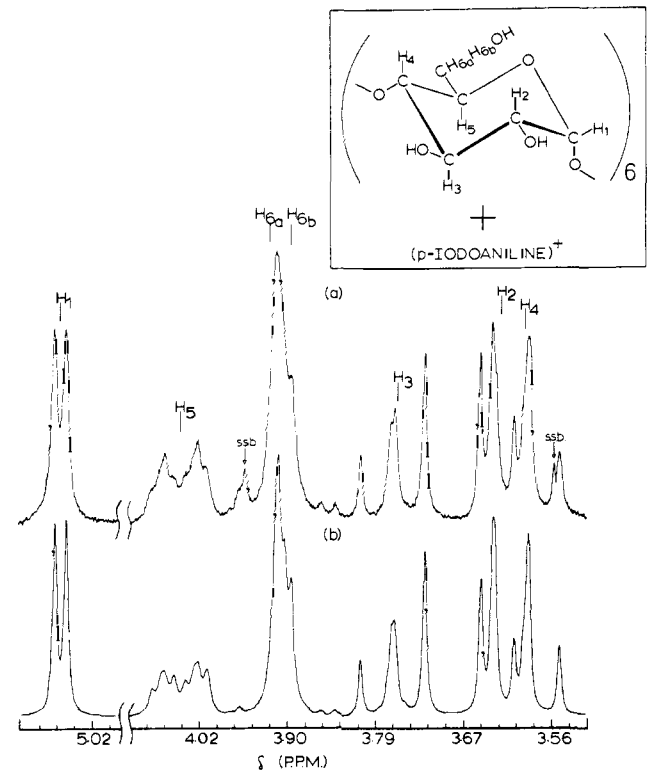

Figure 2. (a) Spectrum (220 MHz) of a 0.042 M α-CD and 0.028 M pIA$^+$ solution (30 °C, pD = 2.7), ssb, spinning side band; sweep width 250 Hz. (b) Computer-simulated spectrum.

through to J_{45} are consistent with the C1 chair form for the glucose units of α-CD as well as for the monomer mglc, again in agreement with early work based on partial analysis of 60-MHz spectra.[21,8c] The striking similarity in corresponding J values for mglc and α-CD demonstrates that incorporation of the monomer unit into the cyclohexaamylose does not lead to significant distortions of the C1 chair form. Furthermore, the pucker of the rings in α-CD is not affected by temperature in the 10–68 °C range, by pD changes in the 2.7–7.5 range, nor by complexation with any of the aromatic guests. X-ray crystallographic studies[5] have demonstrated as well that the glucose units in α-CD behave as relatively rigid building blocks, with the main conformational freedom being rotations about the glucosidic C_1–O_4 and C_4–O_4 bonds and about the C_5–C_6 bond.

In general the conformation about the C_5–C_6 bond of a pyranose can be discussed in terms of the relative contributions from the gauche-gauche (gg), gauche-trans (gt), and trans-gauche (tg) conformers to the gg ⇄ gt ⇄ tg conformer blend (Figure 5). A recent study of the ^{13}C relaxation times of α-CD

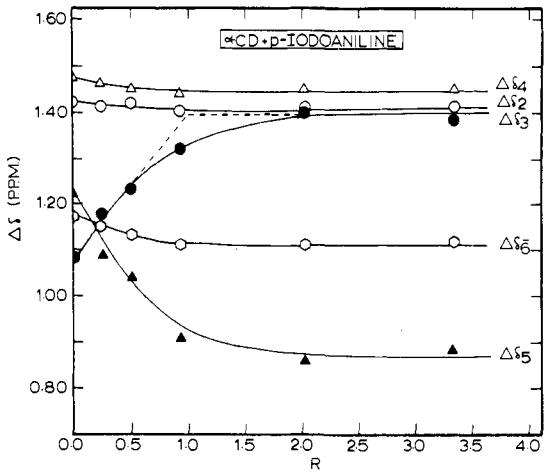

Figure 3. Solid lines: graph of the proton chemical shifts for α-CD in aqueous solutions containing different amounts of plA$^+$. $\Delta\delta_i$ is the chemical shift of the ith proton of α-CD relative to the shift of H_1. R is the plA$^+$:α-CD molar ratio. α-CD is 0.041 M. Dashed lines: variation of $\Delta\delta_3$ with R calculated for 1:1 adduct with an infinite association constant. $\Delta\delta_{\bar{6}} = (\Delta\delta_{6a} + \Delta\delta_{6b})/2$.

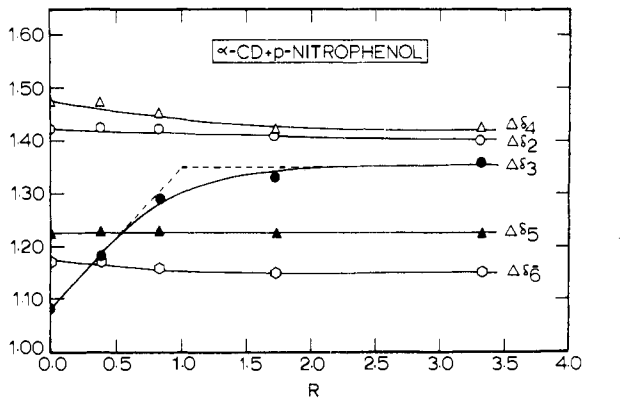

Figure 4. Solid lines: graph of proton chemical shifts for α-CD in aqueous solutions containing different amounts of pNP. $\Delta\delta_i$ is the chemical shift of the ith proton relative to the shift of H_1. R is the pNP:α-CD molar ratio. α-CD is 0.041 M. Dashed lines: variation of $\Delta\delta_3$ with R calculated for 1:1 adduct with an infinite association constant. $\Delta\delta_{\bar{6}} = (\Delta\delta_{6a} + \Delta\delta_{6b})/2$.

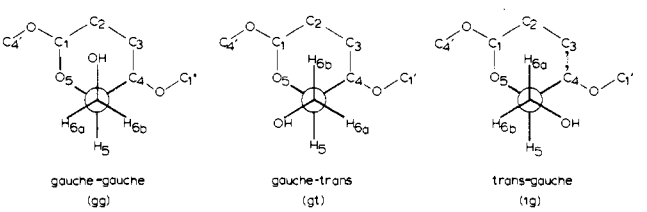

Figure 5. Classically staggered conformations about the C_5-C_6 bond: gauche-gauche (gg), gauche-trans (gt), and trans-gauche (tg).

in aqueous solution led Behr and Lehn[8b] to conclude that rotation about the C_5-C_6 bond was occurring but no conformer populations could be extracted from these data. In principle, the relative contributions can be estimated from the magnitudes of the pair of vicinal coupling constants involving the C_5 and C_6 hydrogens, i.e., J_{56a} and J_{56b}. For the *monomer* mglc one of the couplings is minimal (2.3 Hz), whereas the other is of intermediate magnitude (5.7 Hz). This must mean that one of the C_6 hydrogens is predominantly (if not exclusively) gauche to H_5 whereas the second C_6 hydrogen assumes to significant extents both a trans and a gauche relationship to H_5. This situation can arise only if the gg and only one of the trans conformers (gt or tg) is significantly populated. The most likely trans conformer to exclude is tg. In this conformer there are steric interactions between the hydroxyl groups on C_4 and C_6 which are analogous to the unfavorable parallel 1,3-interactions between oxygen atoms in acyclic sugar derivatives, the alleviation of which is an important factor determining the conformation of these sugars.[22] In our system the interaction between the C_4 and C_6 hydroxyls can be relieved by rotation into either gg or gt. Thus, a conformational blend with significant contributions from gg and gt is consistent with the observed magnitudes of the pair of vicinal couplings involving the C_5 and C_6 hydrogens of mglc.

Gagnaire et al.[23] have shown that the gg and gt conformers contribute significantly to the conformer blend in acetylated mglc in nonpolar solvents. Lemieux and Martin[24] also exclude the tg conformer in this situation. Both the gg and gt forms, but not tg, have been observed in crystalline pyranosides;[25] crystalline mglc itself is gt.[26]

The magnitudes of the pair of vicinal couplings involving the C_5 and C_6 hydrogens of α-CD suggest that the conformational distribution about the C_5-C_6 bond is similar to that of the monomer. The magnitudes of all J_{56a} entries for α-CD in Table I lie in the range 1.8-2.3 Hz while those for J_{56b} lie in the range 3.7-4.3 Hz. Thus we may again conclude that one of the trans conformers, gt or tg, may be excluded as a significant contributor. For the same reasons given above, it seems most reasonable to exclude the tg conformer and thus a gg $\rightleftarrows$ gt conformer blend seems to be the best description for both the monomer and cyclohexamer. (Furthermore, in molecular models of α-CD we find additional close contacts with O_6 which are not present in the monomer mglc and which should further destabilize the tg conformer relative to gg and gt. These new contacts involve the O_5 atom of the contiguous glucose unit linked to the 4 position.) This proposal, however, can ultimately be proven only if an assignment of the individual C_6 hydrogens is made by selective deuteration as has been done by Gagnaire et al.[23] for several pyranosides. The proposal is, however, consistent with the fact that both gg and gt, but not tg, have been observed in the thus far studied crystal structures of α-CD adducts.[5] Further, it is clear from Table I that the magnitudes of J_{56b} for α-CD are *attenuated* by as much as 2.2 Hz relative to that for the monomer. This is consistent with the view that formation of the macrocyclic ring system has a slight stabilizing influence upon the gg conformer. Keeping in mind reasonable estimates for gauche and trans hydrogen-hydrogen coupling constants (about 2 and 10 Hz, respectively),[27] we conclude from the values of J_{56a} and J_{56b} of the monomer (2.3 Hz, 5.7 Hz) that there exist sizable contributions from both gg and gt to the conformer blend. In the case of α-CD the magnitudes of these couplings (as small as 1.8 and 3.5 Hz for J_{56a} and J_{56b}, respectively) are approaching the magnitudes expected for the gg conformer itself and thus reflect a bias for this conformer over gt. However, inclusion of any of the aromatic guests does not lead to any large change in these couplings, and thus in the gg $\rightleftarrows$ gt conformer blend. Interestingly, in the crystalline state we find that mglc is gt,[26] that four of the six glucoses in water-included α-CD are gg while two are gt, and that in plA-included α-CD all glucose units are gg. Thus, the trends in solution and the crystal are identical in that formation of the macrocyclic system of α-CD leads to a stabilization of the gg rotamer. The trends differ in that replacement of water by an aromatic guest does not affect significantly the gg $\rightleftarrows$ gt blend in aqueous solution, but this replacement in the crystal state leads to an all gg situation. In the crystal state the pair of glucoses which are gt utilize their O_6 atoms as proton donors for hydrogen bonding with the included water; such interactions require the gt conformation. Such hydrogen bonding schemes have not been observed for aromatic α-CD adducts and in these instances all units are gg.[5]

(b) Binding of *p*-Iodoaniline. Demarco and Thakar[8a] have shown that ^{1}H NMR can provide evidence for the inclusion

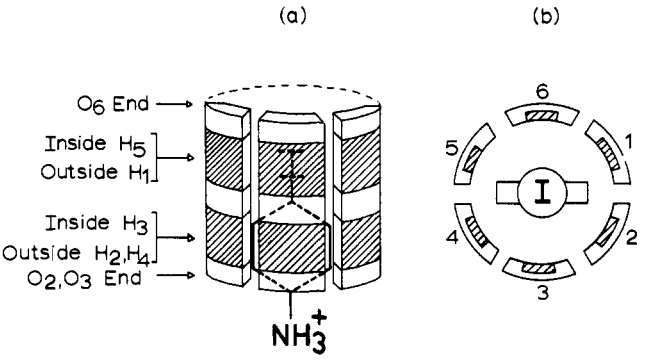

Figure 6. (a) Side view representation of the crystalline pIA:α-CD complex. Shaded areas represent the general location of the α-CD protons. (b) Representation of crystalline pIA:α-CD adduct viewed from the O_6 end. Shaded areas represent the general location of the α-CD protons (H_3 and H_5).

of aromatic substances into cyclodextrins. Their reasoning is based on the expectation that, if inclusion takes place, the screening environment should be sensed by hydrogens on the inner surface (H_3 and H_5) but not by hydrogens on the outer surface (H_1, H_2, H_4). In Figure 3 we have plotted the $\Delta\delta$ values for α-CD as a function of R, the *p*-iodoaniline-to-α-CD molar ratio. (The studies were carried out at pD = 2.0 since difficulties were encountered in dissolving pIA in neutral solution; thus, the predominant species in solution is the *p*-iodoanilinium ion, pIA^+.) $\Delta\delta_2$ and $\Delta\delta_4$ are constant to within 0.02 ppm over the range of R values considered. This means that the relative shifts of the outer-surface hydrogens are unaffected by the addition of pIA^+. (We should also add that the chemical shift of H_1 (used as our reference point for the $\Delta\delta$ values), when measured relative to external TSP, is itself virtually unaffected by inclusion by pIA^+.) However, H_3 and H_5 experience large changes in shielding which are consistent with the inclusion of pIA^+. $\Delta\delta_3$ *increases* with increasing R from an initial (R = 0) value of 1.08 ppm to an upper limiting value of 1.39 ppm while $\Delta\delta_5$ *decreases* with increasing R from 1.22 ppm for R = 0 to a lower limiting value of 0.87 ppm. The H_6 hydrogens which are located on the upper (C_6) surface and directed inwards in the gg conformation are also affected, though to a lesser extent, with $\Delta\delta_6$ *decreasing* from 1.17 ppm to 1.12 ppm. Interestingly the H_5 and H_6 hydrogens which are located in the same region of the torus experience shielding changes in the same direction. Presumably the limiting values of $\Delta\delta_i$ at large R correspond to the chemical shifts of the pIA^+-α-CD adduct whereas the value of $\Delta\delta_i$ for R = 0 are the values for the "empty" water-included adduct.

Further, we conclude from the spectrum in Figure 2 that the α-CD molecule in the pIA^+ adduct possesses an *apparent* hexagonal symmetry. The adduct itself can have at most twofold symmetry. This apparent hexagonal symmetry is consistent with two sets of experimental findings for α-CD inclusion complexes involving para-disubstituted aromatic guests, namely, that the association-dissociation process is rapid[9] (in the microsecond to millisecond range) and that the included guest can undergo appreciable motion within the cavity, presumably by rotation of the plane along its twofold axis.[8b] These dynamic features of the adduct should ensure that, on a time-averaged basis, corresponding hydrogens (i.e., H_3, etc.) on each of the glucose units will receive identical shielding contributions from the included guest.

It is possible to rationalize the observed trends in $\Delta\delta_3$ and $\Delta\delta_5$ if it is assumed that the pIA^+ moiety is oriented in the cavity in a manner similar to that in the crystalline pIA adduct.[5] In the crystal state the orientation of the pIA molecule is such that the glucosidic oxygens bridging glucose units 1 and 2 and units 4 and 5 lie in the plane of the aromatic ring (see Figure 6). The H_3 hydrogens on units 3 and 6 are located on opposite sides of and above the plane, regions where strong ring-current shielding is expected. The H_3 hydrogens on units 1, 2, 4, and 5 are located in regions where a small deshielding is expected. Using the crystalline geometry and the Johnson-Bovey tables[28] for ring-current effects, we have estimated a 2.0-ppm shielding effect for H_3 (units 3 and 6) and a −0.3-ppm effect for H_3 (units 1, 2, 4, and 5). Since the α-CD molecule possesses in solution an apparent hexagonal symmetry, the H_3 hydrogens must be sampling to the same extent the environments provided by the three adducts with twofold symmetry which are equivalent to the adduct described above. Thus the average, calculated shielding expected for each H_3 is 0.47 ppm, i.e., 0.33 (2) + 0.67 (−0.3). This is in fair agreement with the observed change in $\Delta\delta_3$, 0.30 ppm. Though this agreement must be somewhat fortuitous, nevertheless, we feel that the main point is made: the H_3 hydrogens of the pyranose units can locate above the plane of the aromatic ring where shielding by the guest is expected.

In the crystalline pIA adduct the hydrophobic iodine atom is located inside the cavity near to the O_6 side (Figure 6) whereas the polar amino group protrudes 1.5 Å from the O_2, O_3 side. The H_5 hydrogens form a ring encircling the iodine atom at a separation at which they should experience van der Waals deshielding by the iodine atom.[29] Furthermore the H_5 hydrogens lie in a region where a deshielding by the ring current of the pIA^+ moiety is expected. Thus, the downfield shift (0.35 ppm) experienced by the H_5 hydrogens when the adduct is formed in aqueous solution seems reasonable, though it is not easy to estimate the combined effect of the van der Waals and ring-current effects. Furthermore, the small downfield shift of the H_6 hydrogens is also reasonable since they are located at a greater distance from the iodine atom and the benzene ring.

The $\Delta\delta$ data can be used to estimate the binding constant K for the pIA^+-α-CD adduct. If we assume that a 1:1 complex is formed and that the $\Delta\delta_3$ values for the complexed and uncomplexed α-CD are 1.39 and 1.08 ppm, respectively, we may write $\Delta\delta_3 = 1.08N_u + 1.39N_c$ where N_u and N_c are the mole fractions of uncomplexed and complexed α-CD, and $\Delta\delta_3$ is the observed value at a specified value of R. It is a simple matter then to obtain the relationship between $\Delta\delta_3$ and R for a series of K values. In Figure 7 we have reproduced $\Delta\delta_3$ vs. R curves (solid lines) corresponding to K values of 10, 100, 1000, and 10 000 M^{-1}. Included in Figure 7 are the *experimental* $\Delta\delta_3$ vs. R data (solid dots) from Table I. Clearly, for $K > 1000$ M^{-1}, $\Delta\delta$ becomes quite insensitive to changes in K. At best then our data should be used for placing a lower limit to the value of K. Since all experimental points lie to the left of the K vs. R plot calculated for $K \simeq 750$ M^{-1}, we choose this as an estimate of the lower bound. Thus, the negative free energy of formation ΔG^0 for the pIA^+-α-CD adduct is estimated to be at least 3.9 kcal/mol. No estimates by other techniques are available.

(c) Binding of *p*-Nitrophenol, *p*-Nitrophenolate, and D,L-Phenylalanine. The effects of added pNP^2 (pD = 2.1), pNP^- (pD = 11.4), D,L-phe mixture (pD = 1.9), and D-phe (pD = 1.9) on the ^1H NMR spectrum of α-CD in aqueous solution were also examined. In Figure 4 we have plotted the $\Delta\delta$ values for α-CD in the presence of varying amounts of pNP, R again being the guest: α-CD molar ratio. Again $\Delta\delta_2$ and $\Delta\delta_4$ are essentially invariant (to within 0.03 ppm) over the range of R values used. However, the effect of added guest on $\Delta\delta_3$ is substantial, its magnitude increasing by 0.28 ppm from the R = 0 value to the limiting value at large R. In contrast with the observed effect of added pIA^+, we find now that $\Delta\delta_5$ and $\Delta\delta_6$ are invariant to within 0.02 ppm over the range of R values in Figure 4. No $\Delta\delta$ vs. R plots are given here for the pNP^-, D,L-phe, or D-phe guests since their general features are

similar to those of Figure 4. In each instance only $\Delta\delta_3$ is affected by the addition of guest to the solution. The observed increases in $\Delta\delta_3$ from its $R = 0$ value to its upper limiting value are: pNP^-, 0.20 ppm; D,L-phe, 0.10 ppm; D-phe, 0.10 ppm.

Only when actual inclusion of the aromatic guest molecule into the α-CD cavity occurs can the strong shielding environment of the aromatic ring become accessible to the hydrogen atoms which line the cavity. Therefore the strong R dependence of the $\Delta\delta$ value for the H_3 hydrogens should be taken as evidence for the inclusion of pNP, pNP^-, and D,L-phe. (The inclusion of the first two guests has been suggested from thermodynamic and kinetic data.[9]) Furthermore, it is possible that the large spread in the total changes in $\Delta\delta_3$ from 0.31 ppm for the pIA^+ guest to 0.10 ppm for the D-phe guest is reflecting a dependence of the *extent* of insertion of the benzene ring upon the nature of the para substituents. The absence of any influence of these guests on the shielding of the H_5 hydrogens which is comparable to that of pIA^+ (Figure 3) requires explanation. In any reasonable complex involving a para disubstituted aromatic it is likely that the more hydrophobic substituent is inserted into the cavity, i.e., the p-NO_2 and p-H of pNP (pNP^-) and D,L-phe, respectively. Cheney[30] has discussed the evidence that hydrogen atoms subjected to steric compression generally exhibit a chemical shift to low field. Perhaps the deshielding experienced by H_5 upon insertion of pIA^+ and the absence of a similar effect with the other guests is a reflection of close-contact interactions involving the iodine atom and the absence of such interactions with the p-NO_2 (of pNP and pNP^-) or with the p-H (of D-and L-phe). Close contact with the iodine could be a consequence of a larger effective size or could be due to greater penetration of pIA^+ or a combination of these two factors.

If the variations in $\Delta\delta_3$ with R for pNP and pNP^- are treated according to the method described for pIA^+, then the following thermodynamic data for adduct formation may be obtained: pNP guest, $K > 400\ M^{-1}$, $(-\Delta G°) > 3.6$ kcal mol^{-1}; pNP^- guest, $K > 800\ M^{-1}$, $(-\Delta G°) > 4.0$ kcal mol^{-1}. The magnitude of $\Delta G°$ for the association of α-CD with pNP and pNP^- determined from the data given by Cramer et al.[9] is -3.6 kcal mol^{-1} and -4.9 kcal mol^{-1}, respectively, data with which ours are in reasonable agreement. Lewis and Hansen,[31] however, find a smaller value (-2.9 kcal mol^{-1}) for the association with pNP. In the case of D,L-phe no K value calculations were attempted since D,L-phe had a smaller effect upon the H_3 chemical shift and as a result no accurate upper limiting value of $\Delta\delta_3$ could be obtained.

Conclusions

The ^1H NMR data for methyl α-D-glucopyranoside and for α-CD indicate that a similar C1 conformation is maintained by the pyranose rings at the monomer and cyclic hexamer levels, consistent with the view that the individual units of α-CD may be regarded as rigid building blocks.[5] The coupling constant data are consistent with sizable contributions from gg and gt to the C_5-C_6 conformer blend of the monomer and hexamer, though the macrocyclic ring formation leads to an enhanced contribution from gg. Inclusion of an aromatic guest does not lead to significant changes in contributions to the blend. Formation of an aromatic guest-α-CD adduct was revealed by the guest's influence on the magnetic shielding of hydrogens located on the inner surface of the α-CD torus. In the presence of the p-iodoanilinium ion H_3 was shifted upfield by 0.31 ppm while H_5 was shifted downfield by 0.35 ppm. These effects were rationalized on the basis of published crystal structures of the p-iodoaniline adduct. Other aromatic guests, though leading to an increased shielding of H_3, had no significant influence on H_5. This altered behavior could be a reflection of diminished contact, in these instances, between the H_5 hydrogens and the para substituent due to the smaller size of the substituent and to reduced penetration of the guest.

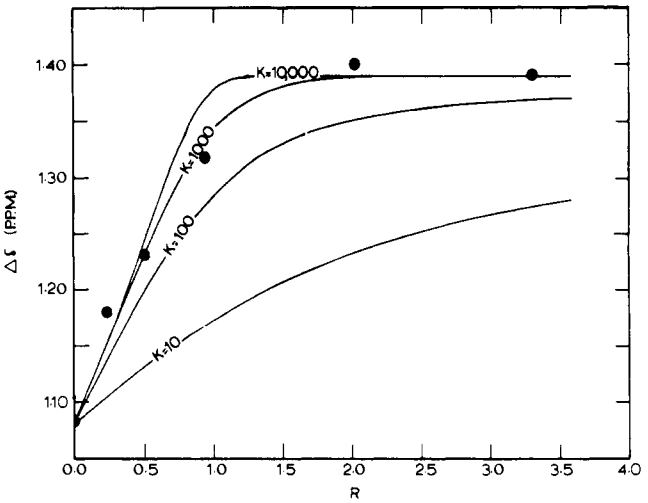

Figure 7. Solid lines: for pIA^+-α-CD solution, variation of $\Delta\delta_3$ with R for several association constants, calculated assuming a 1:1 adduct stoichiometry and using 1.08 and 1.39 ppm for $\Delta\delta_3$ in the uncomplexed and complexed forms, respectively. Solid dots: experimental data for $\Delta\delta_3$ taken from Figure 3.

It is known that adduct formation involving aromatic guests leads in the crystal state to conformational changes in the α-CD molecule, but these changes are due almost entirely to changes in conformation about the C-O bonds linking the glucose units though changes in C_5-C_6 orientations do occur.[5] In solution, conformational change in α-CD due to adduct formation has been revealed by ORD measurements.[16] Our solution study demonstrates that inclusion of the aromatic molecules has significant influence on neither the pucker of the glucose units nor on the C_5-C_6 conformer blend. Unfortunately, our study provides no meaningful information about the conformational situation of the C-O bonds. Viewed together, the ^1H NMR and ORD studies suggest that in solution conformational changes induced by inclusion are restricted to interglucosidic C-O bond rotations.

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References and Notes

(1) (a) Postdoctoral Fellow, (b) The University of Manitoba; (c) Max-Planck-Institut fur Experimentelle Medizin, Abteilung Chemie.
(2) Abbreviations used: α-CD, cyclohexaamylose; mglc, methyl α-D-glucopyranoside; pIA, p-iodoaniline; pIA^+, p-iodoanilinium ion; pNP, p-nitrophenol; pNP^-, p-nitrophenolate ion; D,L-phe, D,L-phenylalanine; TSP, sodium 3-trimethylsilylpropionate-2,2,3,4,-d_4; R, the guest to α-CD molar ratio for D_2O solutions containing both aromatic guest and α-CD.
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(32) After submission of our manuscript, we received a preprint from Drs. R. Bergeron and R. Rowan, III (*Bioorg. Chem.*, in press) describing an NMR study of pNP^- binding to α-CD and to β-CD (cycloheptaamylose). They have, as we have, found that pNP^- insertion into α-CD leads to an enhanced shielding of H_3 while H_5 is affected very little. Insertion of pNP^- into β-CD, however, leads to increased shielding for both H_3 and H_5. Their observations are discussed in terms of the extent of guest penetration. Partial penetration into α-CD was evident also in their nuclear Overhauser studies.

A Pulsed NMR Study of Molecular Motion in Solid 6,12,12-Trimethyl-5,6-dihydro-7*H*,12*H*-dibenzo[*c*,*f*][1,5]silazocine

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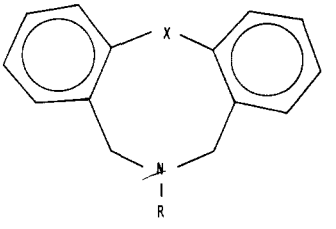
Abstract: 6,12,12-Trimethyl-5,6-dihydro-7*H*,12*H*-dibenzo[*c*,*f*][1,5]silazocine was studied in the solid phase by use of pulsed NMR. Values of T_1, $T_{1\rho}$, T_{1D}, and the second moment were measured over the temperature range 85 to 326 K. The compound exhibits four distinct motions in the solid: methyl reorientation (E_A = 2.34 kcal/mol), ring flexing (E_A = 4.14 kcal/mol), molecular reorientation (E_A = 22 kcal/mol), and an unidentified motion ($E_A \geq$ 6.2 kcal/mol). Experimental second moments agree with values calculated from crystallographic data using reduction factors. The possibility of N inversion in the silazocine is discussed.

The rates of conformational change and the activation parameters for various processes have been studied for a large number of systems, both as neat liquids and in solution, by use of high-resolution NMR line shape analysis.[1] The technique applies to values of conformer lifetime, $\tau \sim (\Delta\omega)^{-1}$, where $\Delta\omega$ is the chemical shift between conformers. Since $\Delta\omega$ for organic molecules is not larger than a few ppm, except in unusual instances, and since the temperature region below the freezing point of the liquid may not be investigated by this technique, there are motional processes that cannot be studied, in particular, low activation energy, rapid motions. The scope of molecular motion studies can be increased dramatically by use of the pulsed NMR technique to measure relaxation times (T_1, $T_{1\rho}$, and T_{1D}) and second moments with solid and glass samples. Studies of this type can probe motional frequencies from $\sim 10^3$ to $\sim 10^8$ Hz. The technique is particularly useful for the study of rapid motions, i.e., processes for which activation barriers are less than 10 kcal/mol.

We wish to report an application of the pulsed NMR technique in the study of the molecular motions in the tricyclic system 6,12,12-trimethyl-5,6-dihydro-7*H*,12*H*-dibenzo[*c*,*f*][1,5]silazocine, IA, which contains a central eight-membered ring with silicon and nitrogen heteroatoms.[2] Two idealized conformations are possible for azocine systems, such as I. The twist-boat (flexible) conformer is exhibited in the solid state by ID[3] and the boat-chair (rigid) conformer was reported for IC.[4] Conformational equilibria of solutions of I (X = CH_2; R = Me, H, $CD_2C_6H_5$, $CHMe_2$, CMe_3) have been studied by means of variable temperature NMR and results rationalized in terms of TB ⇄ TB, BC ⇄ BC, and TB ⇄ BC processes.[5] The twist-boat conformer is exhibited by the silazocine, IB, in the solid state as confirmed by a solid state x-ray



IA X = $SiMe_2$; R = Me
B X = $SiMe_2$; R = t-Bu
C X = CH_2; R = Me
D X = CH_2; R = t-Bu

study.[2] The solution NMR spectra of I (X = $SiMe_2$; R = Me, CMe_3, CH_2Ph, C_6H_{11}) are identical (except for protons attributed to the exocyclic R group) at room temperature and all exhibit a singlet for the benzyl ($-CH_2-$) ring protons and the singlet persists for both IA and IB down to −60 °C. These observations suggest the presence of a single conformer, the flexible twist-boat conformer, in solution; however, we were unable to measure the value of the activation barrier using high resolution NMR. Pulsed NMR studies on a solid sample of IA have enabled the activation barrier for the ring flexing motion to be determined and have also enabled other molecular motions to be identified and characterized. We presently report the results of the study on solid IA.

Experimental Section

Sample. The compound, IA, was synthesized by the reaction of bis(*o*-bromomethylphenyl)dimethylsilane with methylamine in CCl_4.[2] The solid was recrystallized from heptane, and the sample was placed in a closed glass container for study.