250-W high-pressure lamp) for 6 h (disappearence of the red color) under nitrogen. Analytical GLC analysis were performed on a HP 5790 A chromatograph with a 25 m OV101 cross-linked fused capillary column (internal diameter 0.2 mm) equipped with a HP 5970 mass selective detector: $Ph_2CN_2CPh_2$ mass spectrum (relative intensity), *m/e* 360 (26.7), 283 (100), 180 (68.5), 166 (21.4), 165 (97.5), 77 (75.1); $Ph_2CN_2C(Ph)SiMe_3$ mass spectrum, *m/e* 356 (1), 180 (16.8), 176 (51.6), 77 (30.5), 73 (100).

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Registry No. 1a, 94930-06-0; **1b**, 94930-07-1; **1c**, 94930-08-2; **1d**, 94930-09-3; **1e**, 94930-10-6; **2a**, 94930-11-7; **2b**, 94930-12-8; **2c**, 94930-13-9; **2d**, 94930-14-0; **3a**, 94943-96-1; **3b**, 94930-15-1; **3c**, 94930-16-2; **3d**, 94930-17-3; **3e**, 94930-18-4; $Ph_2CN_2Ph_2$, 3746-21-2; $Ph_2CN_2C(Ph)SiMe_3$, 94930-05-9; $Ph(Me_3Si)C{=}O$, 5908-41-8; Ph_2CN_2, 883-40-9; benzoyltriphenylsilane, 1171-49-9; (4-chlorobenzoyl)triphenylsilane, 1172-90-3; (4-methoxybenzoyl)triphenylsilane, 1174-56-7; 2-thenoxyltrimethylsilane, 88372-95-6; tetraphenylbiphosphine, 1101-41-3; diphenylphosphine oxide, 4559-70-0; diethyl phosphite, 762-04-9; triethyl phosphite, 122-52-1; tetraethyl pyrophosphite, 21646-99-1; di-*tert*-butyl peroxide, 110-05-4.

Geometry of Cyclohexaamylose Inclusion Complexes with Some Substituted Benzenes in Aqueous Solution Based on Carbon-13 NMR Chemical Shifts

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Abstract: The orientation and position of the guest molecules, benzoic acid, *p*-hydroxybenzoic acid, and *p*-nitrophenol in the cyclohexaamylose (α-CD) inclusion complex have been determined by comparing the α-CD complexation-induced ^{13}C shifts of guests with quantum chemical predictions. The nonpolar environmental effects produced by the α-CD cavity on the ^{13}C shifts of included guest molecules have been formulated by the so-called solvent effect theory. The geometries of the complexes predicted theoretically were consistent with those in aqueous solution determined by ^1H NMR and those in the solid by the X-ray method.

Cycloamyloses, also well-known as cyclodextrins (which will be denoted by CD, hereafter), are cyclic oligosaccharides composed of at least six (1 → 4)-linked α-D-glucosyl residues.[1] Each CD molecules has a toroidal, hollow, truncated cone with primary and secondary hydroxyl groups crowning the narrower and wider rims, respectively. The interior of each CD cavity contains two rings of C–H groups and a ring of glucose oxygen atoms. Hence, the interior is relatively hydrophobic, whereas the exterior is relatively hydrophilic. The most important property of CD is its ability to admit a variety of guest molecules into its hydrophobic cavity, without any covalent bonds being formed. Consequently, CDs have interested many investigators of diverse fields in both science and industry.[1]

High-resolution NMR spectroscopy is one of the most useful methods in the analysis of the structure and molecular dynamics of CD inclusion complexes both in aqueous solution[2] and in the solid state.[3] Earlier ^{13}C NMR studies of α-CD (CD with six glucosyl residues) inclusion complexes with benzoic acid (BA), *p*-nitrophenol (PNP), and *p*-nitrophenolate in aqueous solution have shown that the included lead (head: Figure 1A) carbons are largely shielded (high-field shift) compared to the deshielding (low-field shift) of corresponding para (tail: Figure 1A) carbons.[2c,d] The directions of the carboxyl group of BA and the nitro group of PNP into the α-CD cavity have been clearly demonstrated by ^1H nuclear Overhauser enhancement experiments.[2c,d] Similar distinctive patterns of ^{13}C displacements have been also observed for *p*-hydroxybenzoic acid (PHBA), and it has been concluded that the carboxyl group of PHBA is directed into the α-CD cavity.[2h] A variety of substituted benzenes are known to show quite similar ^{13}C high-field (head) and low-field shifts (tail), irrespective of the kinds of substituents if their sizes are matched.[2h,4] If the origin of these ^{13}C displacements is accounted for, these characteristic ^{13}C displacements induced by complexation with α-CD may be useful in inferring the extent of the host–guest complexation and the orientation of the guest in the α-CD cavity.

In general, there are several kinds of nonbonded interactions, which may influence the ^{13}C chemical shifts of a given carbon.[5,6] Among them, the electrical environmental effects are expected to be the major contributor to the α-CD complexation-induced ^{13}C displacements of head and tail carbons of the guest molecules, as these shifts are induced by transference of the guest molecule from the free state, surrounded by polar water molecules, to the relatively nonpolar α-CD cavity. This expectation is supported by the observations of the different trends of ^{13}C displacements in the high-resolution solid-state ^{13}C NMR spectra of corresponding α-CD inclusion complexes.[3e] In the solid state, the α-CD complexation-induced shifts are caused by transference of the guest molecule from the free state, surrounded by the same molecules, to the CD cavity.

(1) For reviews: (a) Bergeron R. J. *J. Chem. Educ.* **1977**, *54*, 204. (b) Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer-Verlag: New York, 1978. (c) Saenger, W. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344. (d) Hinze, W. L. *Sep. Purif. Methods*, **1981**, *10*, 159. (e) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.

(2) For examples: (a) Demarco, P. V.; Thakkar, A. L. *J. Chem. Soc., Chem. Commun.* **1970**, 2. (b) Bergeron, R. J.; Rowan, R. *Bioorg. Chem.* **1976**, *5*, 425. (c) Bergeron, R. J.; Channing, M. A.; Gibeily, G. J.; Pillor, D. M. *J. Am. Chem. Soc.* **1977**, *99*, 5146. (d) Bergeron, R. J.; Channing, M. A.; McGovern, K. A. *J. Am. Chem. Soc.* **1978**, *100*, 2878. (e) Wood, D. J.; Hruska, F. E.; Saenger, W. *J. Am. Chem. Soc.* **1977**, *99*, 1735. (f) Gelb, R. I.; Schwartz, L. M.; Murray, C. T.; Laufer, D. A. *J. Am. Chem. Soc.* **1978**, *100*, 3553. (g) Bergeron, R. J.; Channing, M. A. *J. Am. Chem. Soc.* **1979**, *101*, 2511. (h) Gelb, R. I.; Schwartz, L. M.; Cardelino, B.; Fuhrman, H. S.; Johnson, R. F.; Laufer, D. A. *J. Am. Chem. Soc.* **1981**, *103*, 1750. (i) Inoue, Y.; Miyata, Y. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 809. (j) Inoue, Y.; Okuda, T.; Miyata, Y. *J. Am. Chem. Soc.* **1981**, *103*, 7393.

(3) (a) Saito, H.; Tabeta, R. *Chem. Lett.* **1981**, 713. (b) Saito, H.; Izumi, G.; Mamizuka, T.; Suzuki, S.; Tabeta, R. *J. Chem. Soc., Chem. Commun.* **1982**, 1386. (c) Inoue, Y.; Okuda, T.; Chûjô, R. *Carbohydr. Res.* **1983**, *116*, C5. (d) Okazaki, M.; McDowell, C. A. *Chem. Phys. Lett.* **1983**, *102*, 20. (e) Inoue, Y.; Okuda, T.; Kuan, F.-H.; Chûjô, R. *Carbohydr. Res.* **1984**, *129*, 9.

(4) Inoue, Y.; Okuda, T.; Miyata, Y.; Chûjô, R. *Carbohydr. Res.* **1984**, *125*, 65.

(5) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972.

(6) Ando, I.; Webb, G. A. *Org. Magn. Reson.* **1981**, *15*, 111.

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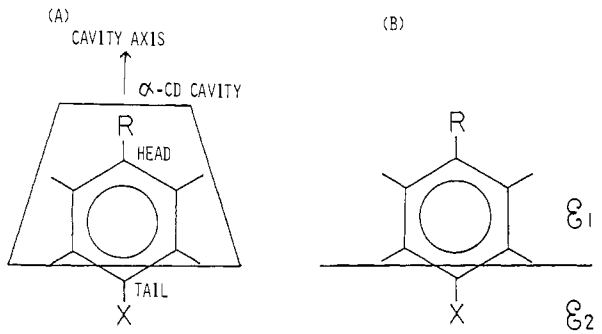

Figure 1. (A) Geometry of α-CD inclusion complexes with benzene derivatives. (B) Double-layer model. The benzene derivative is situated on the borderline dividing the surroundings into two layers of different dielectric constants, ϵ_1 and ϵ_2.

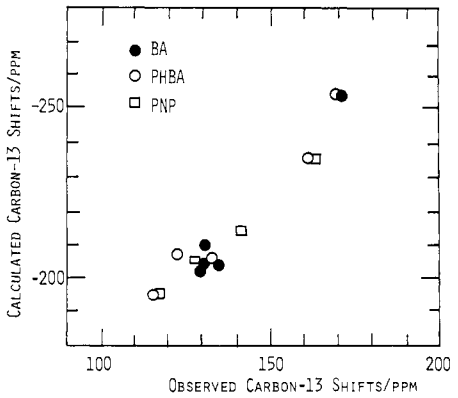

Figure 2. Correlation between calculated (reference: isolated ^{13}C nucleus in vacuum) and observed ^{13}C shifts (reference: external tetramethylsilane, ref 2h) of BA, PHBA, and PNP.

In the case of NMR shielding, such environmental effects could be treated as solvent effects.[5-7] The present paper reports the determination of the structure of α-CD inclusion coplexes with some substituted benzenes in aqueous solution by comparing the α-CD complexation-induced shifts of the guest's carbons with predictions calculated by the application of a quantum chemical model of solvent effects on ^{13}C shielding. BA, PHBA, and PNP were chosen as the guests, since α-CD complexation-induced ^{13}C shifts in aqueous solution were available.[2h] In solution, these compounds form 1:1 inclusion complexes with α-CD. The molecular structures in the crystalline state and in solution of the complexes between α-CD and PNP and PHBA have been characterized by X-ray diffraction[8] and ^1H NMR,[4] respectively.

Selection of a Suitable Molecular Orbital Method for Calculation of ^{13}C Chemical Shifts

For the calculation of ^{13}C chemical shifts, there are several quantum chemical methods with varying levels of approximation.[9] At first we examined the applicability of three well-known methods, i.e., (i) Karplus and Pople's (average excitation energy E) method[10] using CNDO/2 parameters,[11] (ii) sum-over-state method[12] using INDO parameters,[11] and (iii) finite perturbation method[13] using INDO parameters,[11] for the calculation of ^{13}C chemical shifts of BA, PHBA, and PNP. In the calculations, the geometries of these compounds were determined from the standard parameters[10] and the relative orientations of the substituents were taken to be the same as those in the solid-state α-CD inclusion-

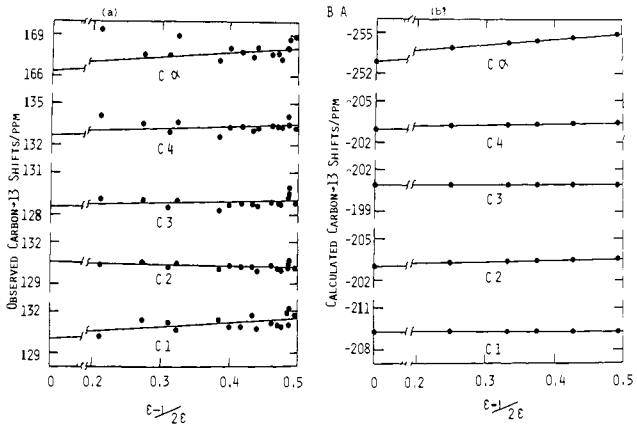

Figure 3. Dependence of (a) observed and (b) calculated ^{13}C shifts of BA on dielectric constants of solvents.

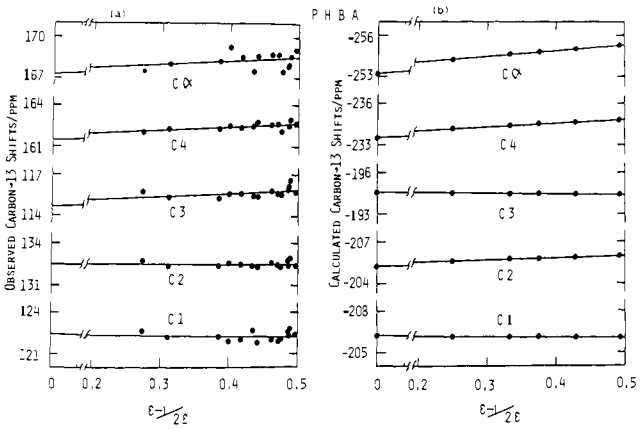

Figure 4. Dependence of (a) observed and (b) calculated ^{13}C shifts of PHBA on dielectric constants of solvents.

complexes determined by the X-ray method.[8] Among the three methods investigated, the first (E = 10 eV[10]) was found to be the best, giving good linearity between the calculated and observed ^{13}C shifts,[2h] as shown in Figure 2. We could not find any explicit correlation between the observed ^{13}C shifts and the shifts calculated by the second or third methods. Therefore, we used the first method for calculations of ^{13}C shifts.

Applicability of the Model of Solvent Effect on ^{13}C Chemical Shifts

As the model of solvent effect on ^{13}C chemical shifts, we used one developed by Ando et al.[6,9] based on a Klopman's "solvaton" theory.[14] This model has been successfully applied to interpret the dielectric solvent effect on ^{13}C chemical shifts of organic compounds, including conjugated polyene compounds.[15] According to this model, the interaction of solute with solvent molecules is incorporated into semiempirical MO calculations by an assumption of a virtual particle called a solvaton, representing the oriented solvent distribution around each atom in the solute molecule. The solvent–solute interactions are approximated by the Born equation.[16]

To examine the applicability and the limits of the CNDO/2-solvaton combined method, the ^{13}C chemical shifts were calculated for BA, PHBA, and PNP as a function of the dielectric constant (ϵ), and the results were compared with those observed in several solvents of a wide range of ϵ values. In the calculations, effective van der Waals radii ($\times 10^{-10}$ m) for hydrogen, carbon, nitrogen, and oxygen were assumed to be 1.20, 1.59, 1.50, and 1.40, respectively. In Figures 3–5, the calculated and observed ^{13}C shifts

(7) Reichardt, C. "Solvent Effects in Organic Chemistry"; Verlag Chemie: New York, 1979; Chapter 6.
(8) Harata, K. *Bull. Chem. Soc. Jpn.* **1977**, *50*, 1416.
(9) Ando, I.; Webb, G. A. "Theory of NMR Parameters"; Academic Press: New York, 1983; Chapter 3.
(10) Karplus, M.; Pople, J. A. *J. Chem. Phys.* **1963**, *38*, 2803.
(11) Pople, J. A.; Beveridge, D. L. "Approximate Molecular Orbital Theory"; McGraw-Hill: New York, 1970.
(12) Pople, J. A. *J. Chem. Phys.* **1962**, *37*, 53.
(13) Ditchfield, R.; Miller, D. P.; Pople, J. A. *J. Chem. Phys.* **1970**, *53*, 613.
(14) Klopman, G. *Chem. Phys. Lett.* **1967**, *1*, 200.
(15) Sakurai, M.; Ando, I.; Inoue, Y.; Chûjô, R. *Photochem. Photobiol.* **1981**, *34*, 367.
(16) Born, M. *Z. Phys.* **1920**, *1*, 45.

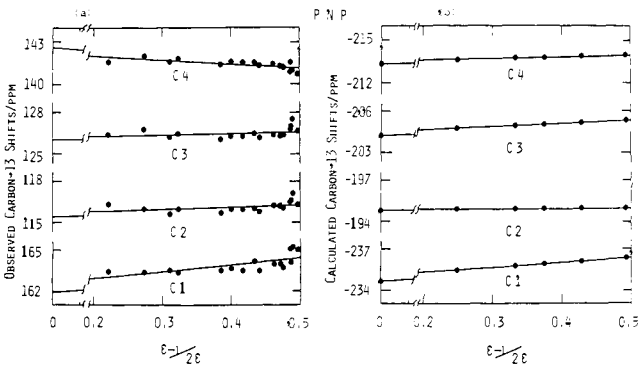

Figure 5. Dependence of (a) observed and (b) calculated ^{13}C shifts of PNP on dielectric constants of solvents.

Table I. Solvents and Their Dielectric Constants

solvents	dielectric const[a]
ethyl oxalate	1.80
dioxane	2.209
dimethoxymethane	2.645
ethyl carbonate	2.820
ethyl ether	4.335
butyl acetate	5.01
ethyl acetate	6.02
tetrahydrofuran	7.58
methylformate	8.5
4-methyl-2-pentanone	13.11
3-pentanone	17.00
acetone	20.70
dimethylformamide	36.71
γ-butyrolactone	39
dimethyl sulfoxide	46.82
methylformamide	182.4

[a] Riddick, J. A.; Bunger, W. B. "Techniques of Chemistry", 3rd ed., Wiley-Interscience: New York, 1970; Vol. II.

are plotted against the Born factor $(\epsilon - 1)/2\epsilon$. The solvents used for this experiment are listed in Table I together with their ϵ values. The linear dependence on $(\epsilon - 1)/2\epsilon$ is found for both observed and calculated ^{13}C shifts. The observed shifts for carboxyl carbons of BA and PHBA show relatively large scattering about the least-square fitted lines. These may be due to different degrees of dissociation of the carboxyl groups in different solvents. The observed shifts of PNP C4 were oppposite to the calculated ones, although the magnitude of shifts was insignificant. It was confirmed that the gross trends of the solvent-dependent ^{13}C shifts of BA, PHBA, and PNP are reproducible by the MO calculation, with some trivial exceptions.

As described above, the interior of the CD cavity is relatively hydrophobic. It is well-known that by forming inclusion complexes in aqueous solution with α-CD, some compounds such as *p-tert*-butylphenol[17] show UV spectral changes almost identical with that observed when the compounds are dissolved in dioxane instead of water. These results lead to a possibility that the ^{13}C shift displacement of the guest induced by complexation with α-CD may be interpretable as shift changes when the guest is transferred from water (ϵ = 80) to the hydrophobic environment of dioxane (ϵ = 2). Therefore, the chemical shift difference, $\Delta\delta = \delta_2 - \delta_{80}$, was calculated, where δ_2 and δ_{80} were chemical shifts in media of ϵ values 2 and 80, respectively. The results are shown in the first column of Table II as the "continuum model", since the CD cavity and the aqueous media were assumed in the calculations to be continua of ϵ = 2 and 80, respectively. Table II also contains the observed ^{13}C shift displacements induced by complexation with α-CD.[2h]

Among the reported α-CD-induced shifts of carbons which show the characteristic ^{13}C complexation shifts, those for BA carboxyl and C1, PHBA carboxyl, and PNP C4 are reproduced qualitatively by the continuum solvent effect model, while those for corresponding para carbons (BA C4, PHBA C4, and PNP C1) are not. The main reason for the failure of the continuum solvent effect model in explaining the α-CD complexation-induced shifts for the latter carbons may be due to the disregard of the actual structure of the inclusion complexes. The ^1H NMR study of α-CD inclusion complexes with para-substituted phenols in aqueous solution has led to their geometries, where a part of the included guest molecules is exposed to the aqueous medium.[4] Therefore, in the following section, the ^{13}C shifts were calculated by the solvaton theory based on a double-layer model. The trends of the α-CD complexation-induced shifts for the protonated carbons, i.e., C2 and C3, of each guest compound are also not always reproducible by the continuum solvent effect model. Since the hydrogen atoms bonded to these carbons can contact with α-CDs H-3 and H-5 located inside the cavity in the complexed state,[8] the steric shielding effect[5] must also be considered for the explanation of the ^{13}C shifts of these carbons.

(17) VanEtten, R. L.; Sebastian, J. F.; Clowes, G. A.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3242.

Table II. ^{13}C Chemical Shift Displacements[a]

compound	carbon	calculated: continuum model[b]	calculated: double-layer model[c]	obsd[d]
BA	Cα	−1.00	−1.01	−1.70
	C1	−0.02	−0.14	−1.18
	C2	−0.27	−0.18	1.59
	C3	−0.03	−0.43	−0.76
	C4	−0.24	0.37	0.93
PHBA	Cα	−1.01	−1.02	−1.65
	C1	0.11	−0.07	−0.78
	C2	−0.36	−0.21	1.70
	C3	0.07	−0.57	−0.85
	C4	−0.64	0.45	0.72
PNP	C4	−0.31	−0.73	−1.21
	C3	−0.54	−0.26	0.87
	C2	−0.07	−1.35	0.23
	C1	−0.85	1.15	1.88

[a] Given in ppm. Negative values indicate upfield shifts. [b] Displacements induced by transference of molecule from medium of ϵ = 80 to that of ϵ = 2. [c] Displacements induced by transference of molecule from medium of ϵ = 80 to double layer of ϵ = 80,2. [d] α-CD complexation-induced shifts cited from ref 2h.

Geometry of α-CD Inclusion Complex Based on ^{13}C Shifts

In the original solvaton model, the surroundings are assumed to be the homogeneous continuum dielectrics. In order to take into account the heterogeneous nature of the surroundings around the guest molecule in the α-CD inclusion complex, a more realistic solvaton model was constructed. It was assumed that the α-CD cavity has the environmental (solvent) effect of the dielectric constant ϵ_1 on the included part of the guest molecule, while the other part of the guest is exposed to the aqueous phase of dielectric constant ϵ_2, as shown in Figure 1B. We will call this the "double-layer model". Here, the interaction between the solvaton induced around the atom A in the medium of ϵ_1 and the atom B in the medium of ϵ_2, where both atoms A and B belong to the same molecule, was approximated by the Born equation by using the ϵ_2 value as the dielectric constant. It was further assumed that the twofold axis of the guest molecule is perpendicular to the borderline between two environments, namely the twofold axis of the guest coincides with the axis of α-CD cavity (Figure 1). Therefore, only two orientations are possible for the guest molecule on complexation; i.e., the substituent R or X is the lead (Figure 1). This assumption is reasonable according to experimental results.[2,4]

The ^{13}C chemical shift displacements, which were defined as the shift changes when the guest is transferred from the water (ϵ_2 = 80) to the double-layer environments (ϵ_1 = 2, ϵ_2 = 80), were

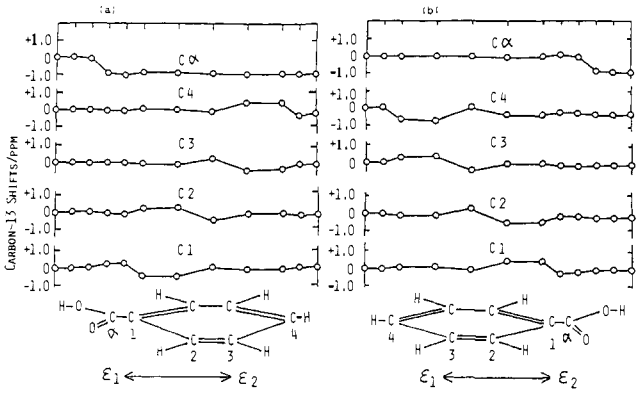

Figure 6. Dependence of ^{13}C shift displacements of BA on the position of the borderline dividing the surroundings around the molecule into two layers with dielectric constants, ϵ_1 and ϵ_2. For each carbon atom, a series of borderlines was chosen. The position of each borderline can be represented on the molecular formula (shown at the bottom) by an extended line which was drawn by joining vertically the points. The ϵ_1 layer is on the left-hand side of the borderline while the ϵ_2 layer is on the right. The directions of the molecule in (a) and (b) are opposite. See also Figure 1B and the text.

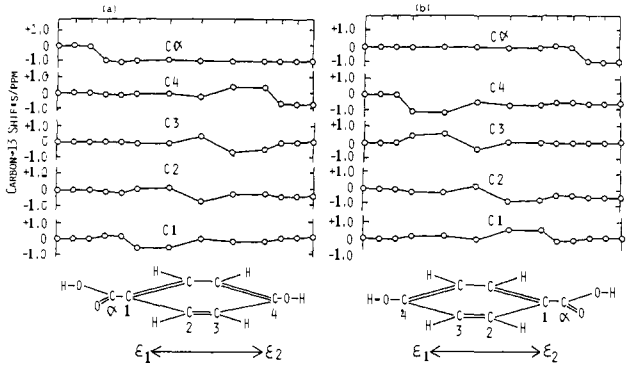

Figure 7. Dependence of ^{13}C shift displacements of PHBA on the position of the borderline. See also the caption of Figure 6.

calculated as the function of the position of the borderline dividing the two layers. In the calculations, the accurate position of the borderline is unimportant, but what is important is which atom is included in the layer of ϵ_1 and which remains in another layer of ϵ_2. A series of calculations was made by shifting the borderline and also by reversing the orientation of the guest molecule relative to the α-CD cavity. The results were reproduced in Figures 6–8, where the positive and the negative signs for ^{13}C displacements indicate low-field and upfield shifts, respectively. The plots on the left-hand side (Figures 6a, 7a, and 8a) correspond to the results for the inclusion mode in which the direction of the guest molecule to the α-CD cavity is the same as that suggested from various experiments,[2c,d,h,4,8] and the right-hand side plots (Figures 6b, 7b, and 8b) to those for the inverse inclusion. We also calculated ^{13}C displacements for cases where the ϵ_2 value of the double-layer model was assumed to be 4 and 7; the same trends were again obtained, although the absolute magnitudes of the displacements were reduced.

By comparing the calculated and observed ^{13}C displacements, we tried to determine the geometry of the inclusion complexes, i.e., the relative orientation and position of the host α-CD and the guest molecule. Here, the ^{13}C displacements of only the carbons on the twofold axis are taken into account, because those of the other carbons may contain the contributions from the steric shielding effects as discussed above, which were not taken into account in our calculations. For three host–guest systems, the observed characteristic ^{13}C displacements, i.e., the high-field shifts of the head carbons and the low-field shifts of the corresponding para carbons, are reproduced only when the borderline in each system is on the shaded region as shown in Figure 9, where the orientation of the guest molecule is the same as that suggested

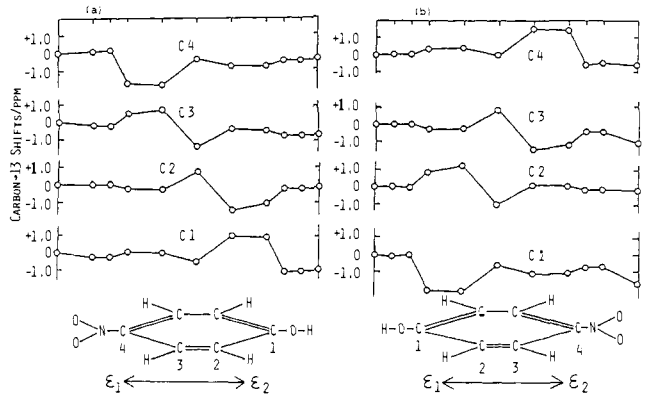

Figure 8. Dependence of ^{13}C shift displacements of PNP on the position of the borderline. See also the caption of Figure 6.

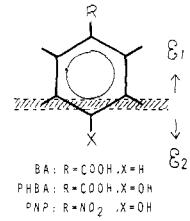

Figure 9. Position of the borderline (shaded region) dividing the surroundings into two layers with dielectric constants, ϵ_1 and ϵ_2, where the characteristic ^{13}C shift displacements are reproduced by the caculations.

experimentally. The ^{13}C displacements calculated for this geometry are listed in the middle column of Table II as the double-layer model. The trends of the characteristic ^{13}C displacements of the carbons on the twofold axis are satisfactorily reproduced by the calculations, although the agreements of the magnitudes of ^{13}C displacements are not always good. If we assume that the borderline corresponds to the α-CDs wider rim consisting of secondary hydroxyl groups, the position of the phenyl ring agrees well with that found in solution determined by ^1H NMR[4] and that in the solid state by X-ray diffraction[8] for PNP and PHBA as shown in Figure 1A.

Discussion

By comparing the calculated ^{13}C shifts based on the quantum chemical solvent effect model with the observed shifts we could determine the geometries of the α-CD inclusion complexes with BA, PHBA, and PNP, which are consistent with those determined experimentally. This agreement assures the working hypothesis that the electrical environmental effects are expected to be the main contributor in the α-CD complexation-induced ^{13}C displacements of the head and tail carbons of the guests. We assumed that the borderline which divides the environment into two layers corresponds not to the α-CDs narrower rim consisting of primary hydroxyl groups but to the wider rim of secondary hydroxyl groups. The theoretical approach which is reported here can not determine from which side of the α-CD cavity the guest molecule is included. The direction of the inclusion, namely, from the wider or narrower side of the α-CD cavity, may be determinable by simple considerations, for example, by building space-filling models, since the major factor determining the inclusion side is considered to be steric acceptibility. It is generally accepted that many benzene derivatives without bulky substituents form 1:1 inclusion complexes with α-CD with the benzene ring inserted into the cavity from the secondary hydroxyl side. From this point of view, our approach is essentially empirical but is useful for the determination of the orientation of the including guest molecules relative to the α-CD cavity.

Here we present theoretical support for the empirical rules that the included head carbons are largely shielded compared with the deshielding para carbons. In consequence, we can conclude that such rules could be used generally for the determination of the orientation of guest mono-substituted and para-disubstituted

benzene derivatives in α-CD complexes.

It is clear from Figures 6–8 that for a given carbon, a large change is induced in the ^{13}C displacement only when the borderline crosses over it. This result is important, since it suggests that the dielectric environmental effects are restricted on ^{13}C shifts of carbon atoms situated within a quite narrow region. In other words, ^{13}C shifts may serve as a sensitive probe of electrical environment change around a given carbon atom in heterogeneous systems such as those investigated here. The method presented here may be applicable to investigations of a variety of complex systems, for examples, the determination of the relative orientation of constituents in a enzyme–substrate complex and the estimation of dielectric nature around a substrate included in a cleft or cavity of the enzyme.

Experimental Section

BA, PHBA, PNP, and all solvents used were commercially available reagent-grade materials. Prior to use, almost all solvents were dried and distilled.

^{13}C NMR spectra of BA, PHBA, and PNP in a series of solvents were recorded at 30 °C on a JEOL JNM PS-100 NMR spectrometer equipped with a PFT-100 Fourier transform system at 25.1 MHz, with instrumental setting conditions of 20° pulse tip angle, 6250-Hz spectral width, 4-s pulse repetition time, 4096 data points, and more than 5000 accumulations of interferograms, using a 8-mm sample tube. The concentration of NMR samples was 0.4 M.

Molecular orbital calculations were carried out on a HITAC-M280 computer at the Information Processing Center of Tokyo Institute of Technology. The calculations of CNDO/2 containing the effects of solvaton and ^{13}C shifts were made by using the program originally written by Prof. Ando and modified by Hoshi to include the effect of the double-layer environment.

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Registry No. α-CD, benzoic acid 1:1 inclusion complex, 15162-62-6; α-CD, *p*-hydroxybenzoic acid 1:1 inclusion complex, 15155-16-5; α-CD, *p*-nitrophenol 1:1 inclusion complex, 61955-25-7.

Conformational Dependencies of Vicinal $^{13}C(O)$–N–C_α–^{13}C and $^{13}C(O)$–N–C_α–^{1}H Coupling Constants in Compounds Which Model the Peptide Backbone

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Abstract: To determine the angular dependencies of vicinal $^{13}C(O)$–N–C_α–^{13}C and $^{13}C(O)$–N–C_α–^{1}H coupling constants on the dihedral angle about the N–C_α bond, a series of ^{13}C-labeled lactams and amides were synthesized and their NMR parameters were measured. The compounds were chosen to serve as model compounds for peptides while providing structural rigidity and covering the entire range of dihedral angles. Both the vicinal ^{13}C–^{13}C and ^{13}C–^{1}H coupling constants are larger in magnitude than the calculated INDO-FPT molecular orbital results over the whole range of dihedral angles. However, the $^{13}C(O)$–N–C–^{1}H coupling constants are only about one-half of those previously obtained in systems which are less appropriate model compounds for peptide systems. Geminal $^{13}C(O)$–N–^{13}C coupling constants are quite sensitive to cis/trans orientations of the amide bond and also offer a potential parameter for structural studies in peptides.

Nuclear spin–spin coupling constants have been used extensively[1–5] for studies of peptide conformations in solution. Alternatives to the ^{1}H–N–C_α–^{1}H coupling constants, which can help in providing a unique specification of ϕ angles in the peptide backbone **1**,[6–11] include vicinal $^{13}C(O)$–N–C_α–^{13}C and $^{13}C(O)$–N–C_α–^{1}H coupling constants. Recent studies from these laboratories have emphasized the conformational and substituent dependencies of geminal,[12,13] vicinal,[14–16] and long-range[17] ^{13}C–^{13}C and ^{13}C–^{1}H coupling constants in cases in which there are only carbon atoms in the coupling path. These studies clearly indicate the complexity of intercarbon coupling constants and emphasize the necessity for experimental measurements in appropriate model systems. The absence of systematic studies of vicinal $^{13}C(O)$–N–C_α–^{13}C coupling would have made it inappropriate, heretofore, to use such data for conformational conclusions in peptide systems.

Several experimental and theoretical studies have treated the angular dependence of vicinal $^{13}C(O)$–N–C_α–^{1}H coupling constants. However, the plot of these, which is based on uridine and

(1) Bystrov, V. F. *Prog. NMR Spectrosc.* **1976**, *10*, 41.
(2) Deslauriers, R.; Smith, I. C. P. In "Topics in Carbon-13 NMR Spectroscopy"; Levy, G., Ed.; Wiley: New York, 1975; Vol. 2.
(3) Thomas, W. A. In "Annual Reports on NMR Spectroscopy"; Mooney, E. F., Ed.; Academic Press: New York, 1976; Vol. 6B.
(4) Urry, D. W.; Onishi, M. In "Spectroscopic Approaches to Biomolecular Conformation"; Urry, D. W., Ed., American Medical Association: Chicago, 1970; pp 260, 270.
(5) Hruby, V. J. In "Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins"; Weinstein, B., Ed., Marcel Dekker: New York, 1974; Vol. 3.
(6) Barfield, M.; Karplus, M. *J. Am. Chem. Soc.* **1969**, *91*, 1.
(7) Bystrov, V. F.; Portnova, S. L.; Tsetlin, V. I.; Ivanov, V. T.; Ovchinnikov, Yu. A. *Tetrahedron* **1969**, *25*, 493.
(8) Ramachandran, G. N.; Chandrasekaren, R.; Kopple, K. D. *Biopolymers* **1971**, *10*, 2113.
(9) Barfield, M.; Gearheart, H. L. *J. Am. Chem. Soc.* **1973**, *95*, 641.
(10) Bystrov, V. F.; Ivanov, V. T.; Portnova, S. L.; Balashova, T. A.; Ovchinnikov, Yu. A. *Tetrahedron* **1973**, *29*, 873.
(11) De Marco, A.; Llinás, M.; Wüthrich, K. *Biopolymers* **1978**, *17*, 637.
(12) Barfield, M.; Walter, S. R. *J. Am. Chem. Soc.* **1983**, *105*, 4191.
(13) Barfield, M.; Della, E. W.; Pigou, P. E. *J. Am. Chem. Soc.* **1984**, *106*, 5051.
(14) Barfield, M. *J. Am. Chem. Soc.* **1980**, *102*, 1.
(15) Barfield, M.; Marshall, J. L.; Canada, E. D., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7.
(16) Barfield, M.; Canada, Jr., E. D.; McDaniel, C. R., Jr.; Marshall, J. L.; Walter, S. R. *J. Am. Chem. Soc.* **1983**, *105*, 3411.
(17) Walter, S. R.; Marshall, J. L.; McDaniel, C. R., Jr.; Canada, E. D., Jr.; Barfield, M. *J. Am. Chem. Soc.* **1983**, *105*, 4185.

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