High-Resolution Nuclear Magnetic Resonance Study of 6-*O*-α-D-Glucopyranosyl-α-cyclodextrin–*p*-Nitrophenol Inclusion Complex in Aqueous Solution

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6-*O*-α-D-Glucopyranosyl-α-cyclodextrin (G1-α-CD)–*p*-nitrophenol (pNP) inclusion complex has been characterized by high resolution ^{1}H and ^{13}C NMR. From ^{1}H NMR measurements it is concluded that G1-α-CD forms a 1:1 inclusion complex with pNP at both pD 7 and 10, and the dissociation constants for their complexation are quite similar to those for α-CD–pNP complexation, suggesting that G1-α-CD and α-CD have a similar ability of complexation with pNP. The geometry of G1-α-CD–pNP complex was found to be similar to that of α-CD–pNP complex. Analysis on the NMR spectral parameters of G1-α-CD have revealed that the asymmetric nature of G1-α-CD molecule is enhanced by complexation with pNP.

6-*O*-α-D-Glucopyranosyl-α-cyclodextrin (G1-α-CD), is a branched α-cyclodextrin (α-CD), produced by limited action of *Bacillus macerans* cyclodextrin glucanotransferase on waxy corn starch.[1,2] It can be isolated and purified from the other sugar by-products using procedures of Kobayashi et al.[1,2] As shown in Fig. 1, G1-α-CD has one branching glucopyranosyl residue attached to one of glucopyranosyl residues constituting α-CD through (1→6)-α-glucosidic linkage and due to this branch G1-α-CD is more water-soluble and more resistant to enzymic degradation than the parent α-CD.[3] It is well-documented that CDs trap various substances as guests in their cavities to form inclusion complexes in solution and because of this property CD has received a considerable attention from a variety of field.[4–6] Although it is expected that G1-α-CD forms inclusion complexes with guest molecules in solution in a manner similar to α-CD, neither the interaction between G1-α-CD and guest molecule nor the structure of its inclusion complexes has been characterized in detail.

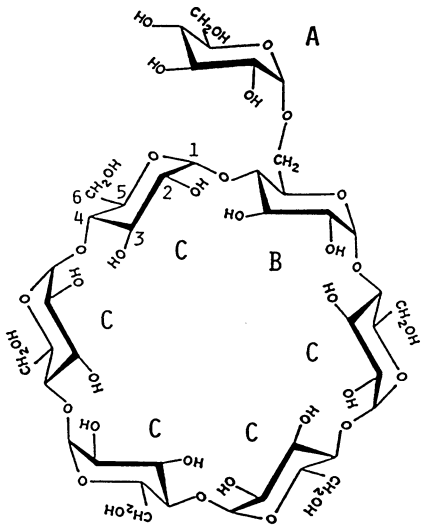

Fig. 1. The structure of G1-α-CD. The glucopyranose residues are divided into three different groups, **A**—**C**, on the basis of ^{1}H NMR spectrum.

NMR spectroscopy is one of the most useful techniques for studying CD inclusion complexes in solution because of its sensitivity.[7,8] The interaction of guest molecule with CD is clearly reflected in changes on various NMR spectral parameters.[9,10] Although ^{1}H NMR spectra of CD derivatives exhibit severely overlapped resonances in the narrow chemical shift range of about δ 3 to 4 from Me_4Si, combined use of various modern two-dimensional NMR spectroscopic techniques can be quite effectively utilized to obtain unambiguous resonance assignments which is essential for detailed structural analysis on CD complexes by NMR.

We report herein the results of 500 MHz ^{1}H and 125 MHz ^{13}C NMR study of the interaction between G1-α-CD and *p*-nitrophenol (pNP) in aqueous solution which not only reveal that pNP is indeed inserted into the cavity of G1-α-CD in a manner similar to α-CD, but also indicate that a considerable conformational change is induced on both the macrocyclic ring structure and constituents of G1-α-CD upon complexation with pNP.

Results and Discussion

Interpretation of the ^{1}H Spectra. The 500 MHz ^{1}H NMR spectra of G1-α-CD and the mixture of G1-α-CD and pNP in D_2O at pD 7.0 and 30 °C are illustrated in traces A and B of Fig. 2, respectively. G1-α-CD exhibits severely overlapped ^{1}H spectrum in the chemical shift region δ 3.4 to 4.0 and two-dimensional NMR techniques were utilized to assign all the resonances in the spectra of G1-α-CD.[11] In trace A there are three sets of independent spin net-work systems arising from three different types of glucopyranose residues, which can be assigned to **A**—**C** units illustrated in Fig. 1, respectively. Due to the presence of the C_6 symmetry axis on α-CD molecule in solution, only single set of NMR signal pattern corresponding to a gluco-

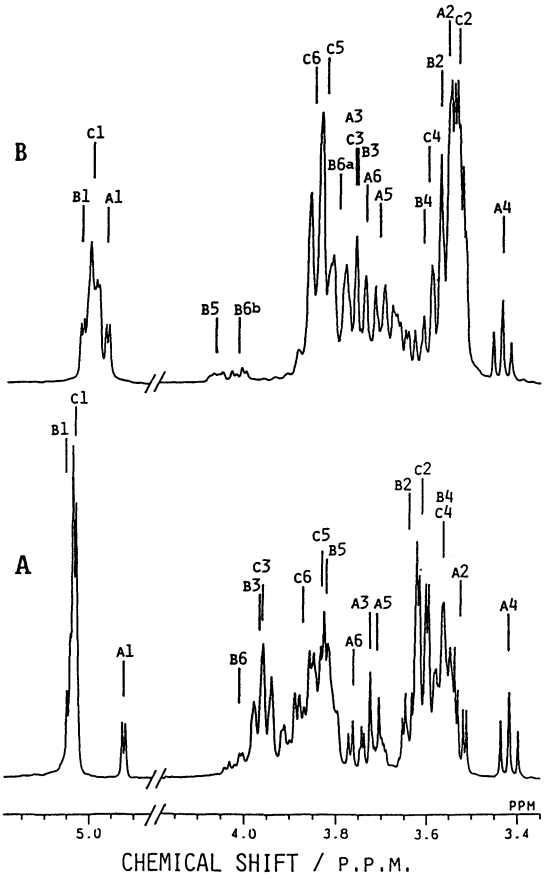

Fig. 2. 500 MHz ^{1}H NMR spectra of (A) 0.04 M G_1-α-CD, (B) 0.04 M G_1-α-CD mixed with 0.08 M pNP, in D_2O at pD 7 and 30 °C. The signals around δ 5 arise from the anomeric protons of the host.

pyranose constituent is observed.[9] Such symmetric property of α-CD is conserved even in the complexed state with pNP owing to the fast averaging of the spatial orientation of pNP with respect to α-CD, although the chemical shifts of α-CD resonances are influenced by the addition of pNP.

On the other hand, in G_1-α-CD the symmetry-breaking constituent, **A** unit, perturbs the C_6 symmetric property of the macrocyclic ring consists of **B** and **C** units, resulting in shifting the resonances of **B** unit relative to those of **C** units, but only single set of NMR signal pattern corresponding to a glucopyranose constituent is observed from **C** units. The observed ^{1}H shifts of the signals of **C** units agree very closely with those of the corresponding resonances of α-CD, suggesting that the substitution of **A** unit perturbs the electronic structure only of **B** unit and all **C** units exist in α-CD-like state.

The complexation of α-CD with pNP in solution leads to a perturbation of NMR spectra of α-CD[9,12] and the ^{1}H resonances of G_1-α-CD are also similarly and even more dramatically perturbed by addition of two-molar excess of pNP as shown in Fig. 2B. The combined use of various 2D NMR techniques (DQF COSY, C-H COSY,[11] HOHAHA,[13] ROESY[14]) makes possible to assign those resonances and their assignment are given with the spectrum in Fig. 2B. Although the resonances arising from **C** units degenerate in only single set of NMR signal pattern, that degeneracy is partially removed by the addition of pNP and the proton resonances, especially H(1) resonance, of **C** unit are split into several peaks.[10]

The resonances of the H(5), H(6a), and H(6b) protons of **B** unit overlap with other proton resonances in Fig. 2A and the individual observation of the spectral patterns of these three proton resonances is difficult. But the H(5) and one of the H(6) resonances are clearly resolved around δ 4.1 in Fig. 2B and the spectral analysis by simulation of these resonances has been carried out to obtain their coupling constants (see below).

Inclusion Complexation between G_1-α-CD and pNP. The induced chemical shift changes of the H(1) and H(3) proton resonances of **C** unit due to the pNP addition are plotted against the molar ratio of pNP to G_1-α-CD in Fig. 3, together with those of α-CD for comparison. Since the H(3) and H(5) protons are located inside the cavity, their resonances are most likely to be influenced by the guest trapped in the cavity. A large upfield shift of H(3) resonance due to the ring-current effect of pNP provides one of the most direct evidence to indicate that pNP is included into the cavity of α-CD,[12] whereas H(1) resonance is slight-

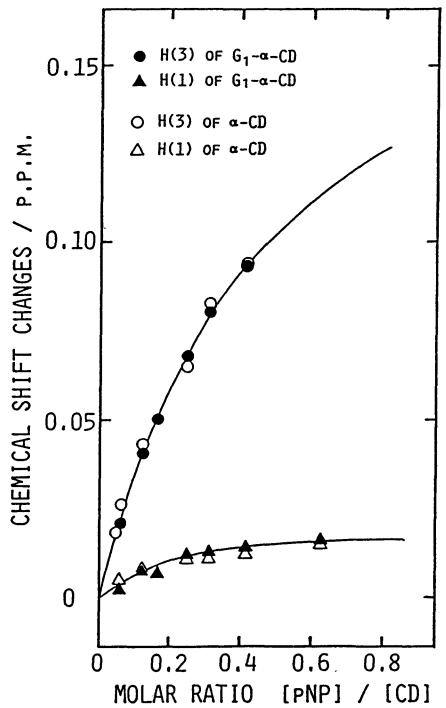

Fig. 3. Effects of *p*-nitrophenol (pNP) on the ^{1}H NMR spectra of G_1-α-CD and α-CD at pD 7 and 30 °C. The pNP-induced changes in chemical shifts of H(1) and H(3) protons of both CDs are plotted relative to the molar ratio [pNP]/[CD]. (▲, ●) G_1-α-CD system; (△, ○) α-CD system.

ly affected and their chemical shift changes are correlated with the conformational changes of the (1→4)-α-linkage and the glucopyranose ring.[10] When pNP was added to G_1-α-CD, H(3) resonances of **C** units as well as **B** unit shift toward upfield considerably due to the ring current effect of aromatic moiety of pNP and the patterns of the chemical shift changes are similar in both G_1-α-CD and α-CD systems, confirming that pNP is inserted into the cavity of G_1-α-CD as in the case of α-CD. The ^{1}H NMR resonances of pNP in free and complexed states can not be observed separately due to a fast exchange process of pNP between the two states and therefore, only single set of pNP resonances, as time-averaged signals, is observed with G_1-α-CD as in the case of α-CD (result not shown).

The chemical shift changes of ortho and meta proton resonances of pNP in aqueous solution of pD 7 are plotted against the molar ratio of G_1-α-CD to pNP and α-CD to pNP in Fig. 4. Downfield shifts are induced for both resonances by G_1-α-CD as well as by α-CD and the meta-proton resonance shifts more significantly in both host-guest systems. The curve indicates a predominant 1 : 1 complexation and the dissociation constant (K_d) of the inclusion complex can be calculated using the chemical shift changes of the meta-proton resonance induced by the host by applying a modified Benesi-Hildebrand equation.[15] Similar procedures were carried out on G_1-α-CD and α-CD-pNP complexes at 30 °C and pD 7 or 10, and the same trend in proton chemical shift changes was also observed in alkaline solution. From these shifts, the

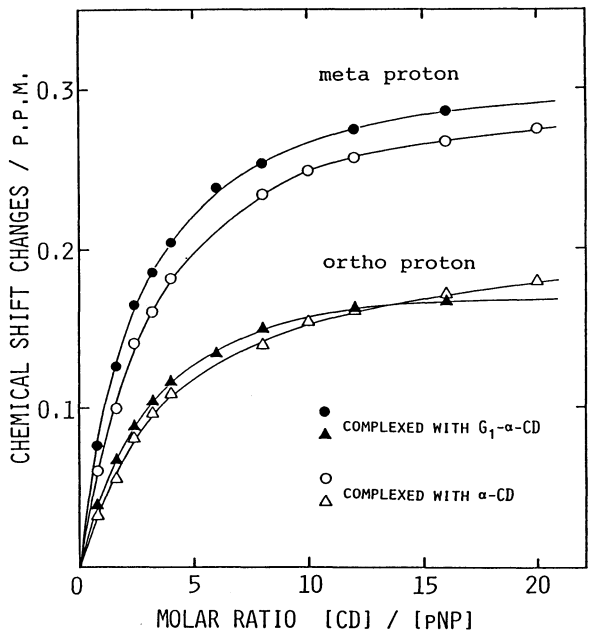

Fig. 4. Effects of G_1-α-CD and α-CD on the ^{1}H NMR spectra of *p*-nitrophenol (pNP) at pD 7 and 30 °C. The CD-induced shifts are plotted as a function of the molar ratio [CD]/[pNP], and [pNP] is constant (2.5 mM).

Table 1. Observed Changes in the Chemical Shift of the ^{1}H Resonances of G_1-α-CD and α-CD Induced by Complexation with pNP

Unit	Chemical shift[a] δ/ppm					
	H(1)	H(2)	H(3)	H(4)	H(5)	H(6)
G_1-α-CD						
A	0.04	0.02	0.03	0.02	0.02	−0.01[b]
B	−0.02	−0.05	−0.22[b]	0.04	0.24[b]	0.00, 0.19[b]
C	−0.03[b]	−0.08[b]	−0.20	0.00	−0.01	−0.03[b]
α-CD	−0.04	−0.07	−0.25	0.00	0.01	−0.04,−0.01

a) Obtained for the solution ([pNP]/[CD]=0.08 M/0.04 M) at pD 7; positive values indicate the downfield shifts. b) Rough estimates due to low resolution of resonances or peak splittings.

K_d values for complexation were estimated to be 4.6×10^{-3}M†† (G_1-α-CD-pNP) and 6.0×10^{-3}M (α-CD-pNP) at pD 7, and 5.1×10^{-4}M (G_1-α-CD-pNP) and 6.1×10^{-4}M (α-CD-pNP) at pD 10. Considering the pK_a value for pNP, the stability of the CD-pNP inclusion complex seems to depend on the ionization state of pNP, and pNP^- ion appears to be more stable than neutral pNP inside the cavity of CD.[16] The K_d values for G_1-α-CD-pNP complex at pD 7 and 10 are markedly close to those of α-CD-pNP complex at corresponding pD values, suggesting that G_1-α-CD and α-CD have a similar ability for complexation with pNP and pNP^-.

Host-Guest Geometry in G_1-α-CD-pNP Inclusion Complex. The orientation of pNP molecule insertion with respect to the cavity of CD can be deduced from the chemical shift changes induced on the resonances of pNP.[17] ^{1}H NMR NOE studies clearly indicated that pNP was preferentially inserted into the α-CD cavity from the 2,3-hydroxyl side with the nitro group first and the respective large and small downfield shifts of the meta- and ortho-proton resonances, respectively, are indicative of inclusion with this orientation.[18,19] The same trend of the chemical shift changes of pNP proton resonances was observed in both G_1-α-CD- and α-CD-pNP complexes, clearly suggesting similar insertion of pNP into the G_1-α-CD cavity as has been suggested for the α-CD-pNP complex. The observed chemical shift differences for the proton resonances of G_1-α-CD between free and complexed states (traces A and B in Fig. 2, respectively) are listed in Table 1, together with the corresponding data of α-CD-pNP complex. The resonances arising from **C** unit of G_1-α-CD are influenced by the addition of pNP like the corresponding resonances of α-CD, and it is consistent with the identical orientation of the pNP insertion in both complexes.

The insertion depth of the guest aromatic molecule into the cavity is determined by using the Johnson-Bovey theory from the chemical shift changes induced by the ring-current effect on the resonances of the H(3)

†† 1 M=1 mol dm^{-3}.

and H(5) protons. The Johnson-Bovey curves for the α-CD-pNP inclusion complex have been reported.[16] Insertion depth is defined as the distance between the center of the benzene ring of pNP and the plane comprised of six H(3) protons and the positive sign representative of the center of the benzene ring lying on the H(5) proton side with respect to the plane of H (3) protons. The depth was found to be 0.8±0.1 Å for α-CD-pNP complex. Assuming the close similarlity of the structural nature of the cavity between both hosts in complexed state, in the case of G_1-α-CD-pNP complex at pD 7 the induced shift changes of the H(3) and H(5) protons of **C** unit, δ −0.22 and −0.01 respectively, suggest that the depth is 0.7±0.2 Å. From these values, the depth of the pNP insertion into the G_1-α-CD cavity is almost the same with that into the α-CD cavity.

Conformation of G_1-α-CD in Complexed State with pNP. The degeneracy of proton resonances of **C** units is removed by complexation with pNP. As already discussed, since the H(1) protons are oriented outside of the cavity, their chemical shift changes may not be explained based on the aromatic ring current effect of pNP. The dispersion of their chemical shift values would be interpreted in terms of small differences in the conformation around the glucosidic bond among the (1→4)-α-linkages of G_1-α-CD in the complexed state with pNP because the magnetic anisotropy effect arising from the lone-pair electrons of the glucosidic oxygen is known to account for the chemical shift of the anomeric proton resonance in glucose derivatives.[20] Conformational changes are presumed to be induced to the different extent among the (1→4)-α-linkages of the macrocyclic ring, and more apparent indications of these conformational changes can be observed on ^{13}C NMR spectra (see below). In addition, the analysis of the vicinal coupling constant, J_{12}, revealed that the structural changes of the glucopyranose C_1 chair conformation of **B** and **C** units appear to be induced by the complexation to the different extent.[10]

It is interesting to investigate where the branch residue is located relative to the macrocyclic ring. The useful information on this location can be obtained from the analysis on the population of the rotamers around C(5)-C(6) bond of **B** unit. Although the spectral parameters for the H(5) and H(6) protons of **B** unit were not easily obtained in the spectrum of G_1-α-CD because of severe overlapping of resonances, two of those proton resonances are resolved in the spectrum of the complexed state with pNP. ^1H vicinal coupling constants, i.e. $^3J_{56a}$ and $^3J_{56b}$, in the spin-network of the glucopyranose residue provide the fractional rotamer populations about the C(5)-C(6) bond[21] and were accurately determined using the LAOCN spin simulation program[22] for **B** unit in the complexed state G_1-α-CD. The obtained spectral parameters are listed in Table 2. The three staggered rotamers, gg, gt, and tg (Fig.5) are considered around the C(5)-C(6) bond of **B** unilt.[23] The

Table 2. ^1H NMR Data for **B** Unit Included in G_1-α-CD Complexed with pNP

Chemical shift δ/ppm	Coupling constant $^3J_{HH}$/Hz	Rotamer population[b]/%
H(5) 4.06		P_{gg} 66 (63)
	J_{56a} 1.8	
H(6a) 3.82[a]		P_{gt} 32 (1)
	J_{56b} 4.6	
H(6b) 4.01[a]		P_{tg} 2 (35)

a) Assignment of H(6a) and H(6b) are interchangeable. The other assignment gives different rotamer populations which are designated in parentheses. b) Calculated with the following equations.[21] $1.7P_{gg}+1.9P_{gt}+11.1P_{tg}=J_{56a}$, $1.5P_{gg}+11.1P_{gt}+2.1P_{tg}=J_{56b}$, $P_{gg}+P_{gt}+P_{tg}=1$.

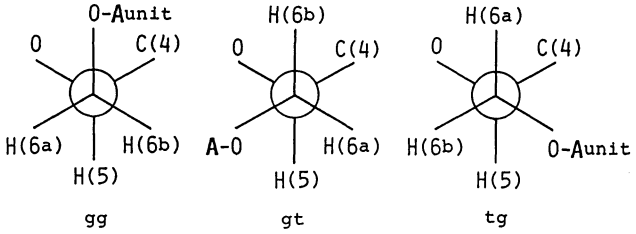

Fig. 5. Stable conformations gg, gt, and tg around C(5)-CH_2O-**A** unit group in **B** unit of G_1-α-CD.

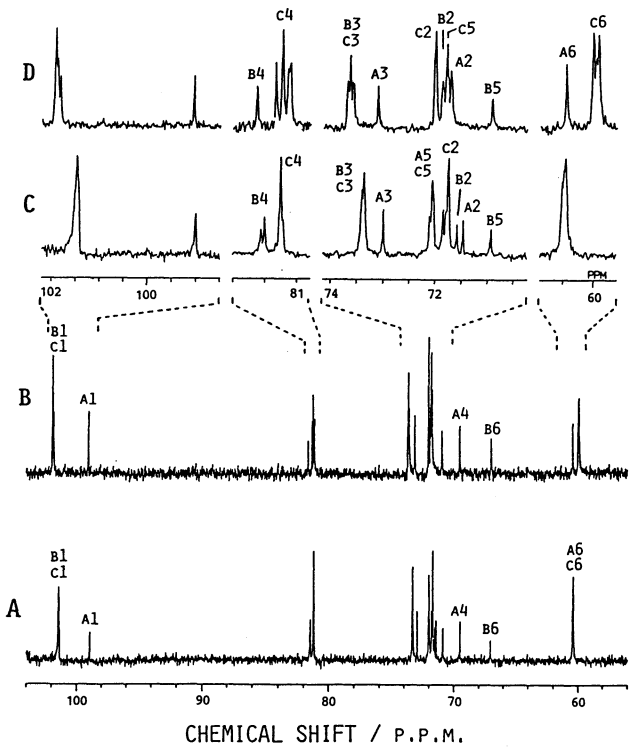

Fig. 6. 125 MHz ^{13}C NMR spectra of (A), (C) 0.04 M G_1-α-CD; (B), (D) 0.04 M G_1-α-CD mixed with 0.08 M pNP, in D_2O at pD 7 and 30 °C.

observed J_{56a} and J_{56b} are expressed with the rotamer populations p_{gg}, p_{gt}, and p_{tg} of the three rotamers by employing the equations in Ref. 21. The assignments of H(6a) and H(6b) are interchangeable, so two alternatives are obtained. In D-glucopyranose units, tg rotamer was very disfavored due to the 1,3-syn repulsion between OR(4) and OR(6).[21] Therefore the data listed in Table 2 represents the most probable rotamer populations for **B** unit. The caluculation indicated predominance of gg rotamer for the **B** unit (p_{gg}=66, p_{gt}=32, and p_{tg}=2), suggesting that the branch residue, **A** unit, is oriented away from the cavity of G_1-α-CD.

Interpretation of the ^{13}C Spectra. The 125 MHz ^{13}C NMR spectra of G_1-α-CD and its complex with two molar excess of pNP in D_2O at pD 7.0 and 30 °C are illustrated in traces A and B of Fig. 6, respectively. From the assignment of the ^1H resonances, the ^{13}C resonances can be assigned using ^1H-^{13}C COSY spectra.[10] The ^{13}C COSY contour plot of G_1-α-CD complexed with pNP, together with ^1H and ^{13}C NMR spectra, are shown in Fig. 7.

In agreement with the results of ^1H NMR, only single set of NMR signal pattern arising from **C** units is observed on ^{13}C NMR spectrum of G_1-α-CD (Fig. 6C), and the observed ^{13}C shifts of the resonances of **C** units are close to those of the corresponding resonances of α-CD (with the differences less than ±0.09 ppm).

Although C_6 symmetric property of α-CD is conserved in the complexed state as described above with the ^1H data, in the case of G_1-α-CD complexed with pNP most of ^{13}C resonances of **C** units are split into several peaks in addition to the shifts of their peaks (Fig. 6D). This indicates that the degeneracy of ^{13}C resonances from **C** units is removed by complexation with pNP, similar to their ^1H resonances, and the splitting of the C(1) and C(4) resonances of **C** units would be interpreted in terms of small differences in the electronic structure of their glucopyranose ring and the conformation of the glucosidic bond among the (1→4)-α-linkages of G_1-α-CD in the complexed state with pNP. It is partly because ^{13}C NMR chemical shifts of the anomeric and aglycone carbon atoms can be correlated with one of the glycoside tortion angles,[23] and partly because conformational distor-

Table 3. Observed Changes in the Chemical Shift of the ^{13}C Resonances of G_1-α-CD and α-CD Induced by Complexation with pNP

Unit	Chemical shift[a] δ/ppm					
	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)
G_1-α-CD						
A	0.06	0.25	0.12	−0.06	n.d.[b]	0.00
B	0.45	0.29	0.28	0.13	−0.02	−0.13
C	0.45	0.26	0.28	−0.01	−0.26	−0.53
	0.41		0.32	−0.14		−0.50
	0.34		0.23	0.09		−0.44
α-CD	0.49	0.27	0.28	−0.07	−0.27	−0.54

a) Obtained for the solution ([pNP]/[CD]=0.08M/0.04 M) at pD 7: positive values indicate the downfield shift. b) Not detected.

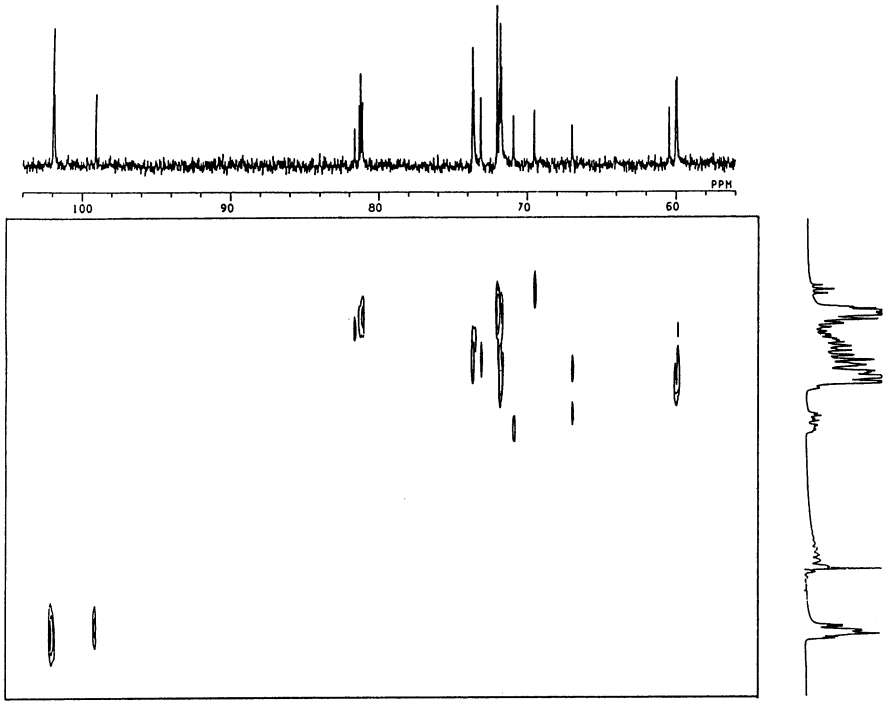

Fig. 7. ^1H-^{13}C COSY spectrum of G_1-α-CD complexed with pNP in D_2O at pD 7 and 30 °C. The ^1H and ^{13}C NMR spectra are attached along F_1 and F_2 axes, respectively.

tion of glucopyranose ring of **C** unit may occur. Due to the lack of C_6 symmetry on G_1-α-CD molecular structure, such conformational changes are not necessarily induced to the same extent among the (1→4)-α-glucosidic linkages of the macrocyclic ring. It is not possible at present to characterize the extent of conformational change of the individual (1→4)-α-linkages. The measurements of long-range C-H J resolved NMR spectra would provide the value of heteronuclear spin-spin coupling constants, $^3J_{COCH}$, which can be interpreted quantitatively in terms of dihedral angles, ϕ, ψ, of the individual(1→4)-α-linkages of G_1-α-CD.[24]

The observed ^{13}C chemical shift differences obtained from the spectra in Fig. 6 are listed in Table 3 with the related data for α-CD. The values for the resonances of **C** unit in G_1-α-CD complex are similar to those of the corresponding resonances of α-CD complex (with differences less than ±0.15 ppm), suggesting that the overall electronic structures are similar between both hosts in complexed state, but the conformational distortions might be induced to the different extent in each (1→4)-α-linkage and each **C** unit as was indicated by the analysis based on the ^1H coupling constant J_{12}. The smaller perturbation induced on the ^{13}C resonances of **A** unit than those of **C** units by the pNP addition indicates that no significant interaction between the branch residue, **A** unit, and pNP inserted into the cavity. This result consistents with those obtained from the analysis on the fractional rotamer populations which lead the conclusion that **A** unit is oriented away from the cavity of G_1-α-CD in complexed state. Although the C(1)-C(3) carbon resonances of **B** unit exhibit almost the same pattern of chemical-shift changes with those of the corresponding resonances of α-CD (with differences less than 0.04 ppm) , those of the resonances of the C(4), C(5), and C(6) carbons are different from those of α-CD (with differences 0.20 to 0.41 ppm). The latter carbons are located nearer the branch residue and the glucopyranose ring conformation of **B** unit is considered to be influenced by the movement of **A** unit. It might be assumed that the difference in the chemical shift changes between **B** unit and others was correlated with some changes of the orientation of **A** unit relative to the macrocyclic ring of G_1-α-CD, and further characterization of this difference and the conformational change is needed.

Experimental

Apparatus and Procedures. The ^1H spectra were recorded at 30 °C on either a JEOL GX-500 (500MHz) or a GX-270 (270 MHz) spectrometers, operating in the quadrature mode. The ^{13}C spectra (125 MHz) and 2D NMR specra were recorded at 30 °C on a GX-500 spectrometer. ^1H and ^{13}C chemical shifts δ are given in parts per million (ppm) downfield from that of Me_4Si with an accuracy of about 0.005 and 0.01 ppm, respectively.

The ^1H-^{13}C COSY spectrum was recorded using the pulse sequence [PD-90° (^1H)-t_1/2-180°(^{13}C)-t_1/2-D_1-90°(^1H, ^{13}C)-D_2-t_2 (with ^1H decoupling)].[25] The delay times D_1 and D_2 were set to 3.4 and 1.7 ms, respectively. A total of 128 transients was accumulated per t_1 value with a pulse delay of 1 s. The initial data matrix was 2K(^{13}C-10000 Hz)×128(^1H-3000 Hz) in the ω_2 and ω_1 dimensions, respectively, and was expanded to the final matrix size 2K×512 by zero filling. The data matrix was apodised with sine bell function and absolute value mode spectrum is presented. DQF-COSY spectra were recorded using the standard procedures.[11]

Spectrum simulations were run on an NEC PC-9801VM personal computer using a LAOCN spin-simulation program.[22]

Materials. G_1-α-CD was prepared using the procedures of Kobayashi et al.[1,2] α-CD was a generous gift from Nihon Shokuhin Kako Co., Ltd. pNP was commercial materials and was purified by high-vacuum sublimation. D_2O with isotopic purity of 99.7% was purchased from Merck Co.

^1H NMR Determination of Dissociation Constants for the Inclusion Complexes. The 270 MHz ^1H NMR spectra were recorded on a GX-270 spectrometer at 30 °C. The change in chemical shift of the pNP aromatic proton resonances was measured as a function of host to guest molar ratio. The pNP and its ionized state, p-nitrophenolate (pNP^-), were prepared in D_2O solution at pD 7.0 and 10.0, respectively. The concentrations of pNP and pNP^- were held constant at 2.5 mM each, and the concentrations of α-CD and G_1-α-CD were varied between 2—50 mM. The data were treated according to a modified Benesi-Hildebrand equation,[15] and linear least-squares analysis were performed on all data.

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