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Preliminary communication

Elucidation of the host–guest geometrical relationship in a branched cyclomaltohexaose inclusion-complex by measurement of the intermolecular nuclear Overhauser effects in the rotating frame

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6-*O*-(α-D-Glucopyranosyl) cyclomaltohexaose (G_1-α-CD; Fig. 1) is a branched cyclomaltohexaose (α-cyclodextrin, α-CD) that consists of six linked (1→4)-α-D-glucopyranose residues forming a macrocycle and one branching D-glucopyranose residue attached to one of the residues of the macrocyle through a (1→6)-α-D-glucopyranosidic linkage. The compound is of interest as it has shown greater water solubility than unsubstituted α-CDs[1,2], and, because α-CDs can accommodate various substances in their cavities by forming inclusion-complexes[3], it was expected that G_1-α-CD will also form inclusion-complexes in a manner similar to α-CD[4]. In order to characterize these CD inclusion-complexes, it was necessary to determine the molecular structure of the conjugates by ^{1}H-n.m.r. spectroscopy, which has been successfully used to obtain such structural information in solution. Measurement[5] of the host–guest intermolecular ^{1}H homonuclear Overhauser effect allows the qualitative determination of the orientation of the guest molecule inside of the CD cavity[6,7]. For example, the orientation of the *p*-nitrophenol (*p*NP) molecule inside the α-CD cavity has been successfully determined on the basis of a two-dimensional n.O.e. (NOESY) spectrum[7]. While an experiment was attempted on the G_1-α-CD–*p*NP complex with experimental parameters similar or identical to those used for the measurement of n.O.e.s of an α-CD system, no cross-peak due to an intermolecular n.O.e. was observed. Thus it was determined that new experimental parameters must be sought for the NOESY experiment on the G_1-α-CD system.

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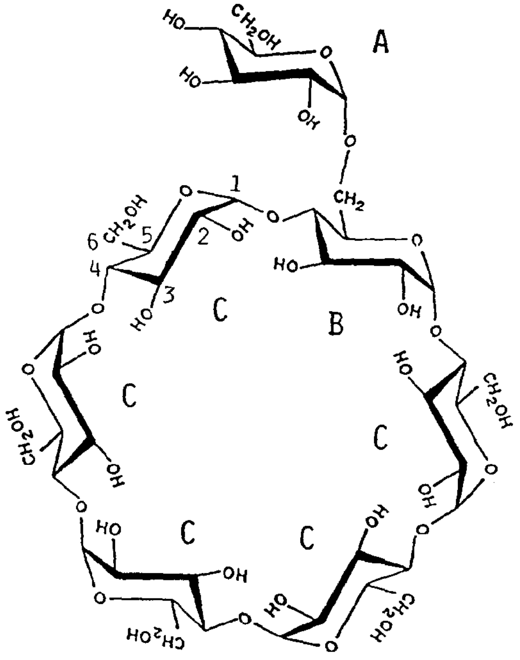

Fig. 1. The structure of G_1-α-CD. The D-glucopyranosyl residues are divided into three groups, A-C, based on the ^{1}H-n.m.r. spectrum.

Measurements of n.O.e.s under spin-locked conditions, an operation termed ROESY[8-11], are particularly useful for molecules with a motional correlation time, τ_c, near the condition $\omega\tau_c = 1$, where ω is the angular Larmor frequency. Although the conventional n.O.e. is very small in such cases, an n.O.e. measured in a rotating frame is always positive and quite large. Under conditions used for ROESY experiments, magnetization transfer between scalar coupled spins can also occur[12], leading to *J* cross-peaks; however, n.O.e. and *J* cross-peaks in 2D ROESY spectra can be differentiated by their relative sign with respect to the diagonal peaks[10]. Thus n.O.e. cross-peaks have an opposite phase independent of τ_c, while cross-peaks due to *J* scalar coupling and chemical exchange are in-phase[9,11], making it possible to distinguish n.O.e. cross-peaks in a 2D spectrum even when complicated *J* cross-peaks are observed, as in the case of COSY experiments on oligosaccharides[13]. We now report the results of a rotating-frame n.O.e. study of a G_1-α-CD inclusion complex with a *p*NP molecule.

Figure 2 shows the ROESY and the 1D proton spectrum of G_1-α-CD- and α-CD–*p*NP mixtures in D_2O at pD 7 and 40°. Conditions for effective suppression of coherence transfer from homonuclear Hartmann–Hahn cross polarization was chosen[10], and assignments of their proton resonances are described elsewhere[7,15].

In Fig. 2b the contour plot displays only resonances that have opposite phase to the diagonal peaks, *i.e.*, resonances due to transverse n.O.e. effects. A set of

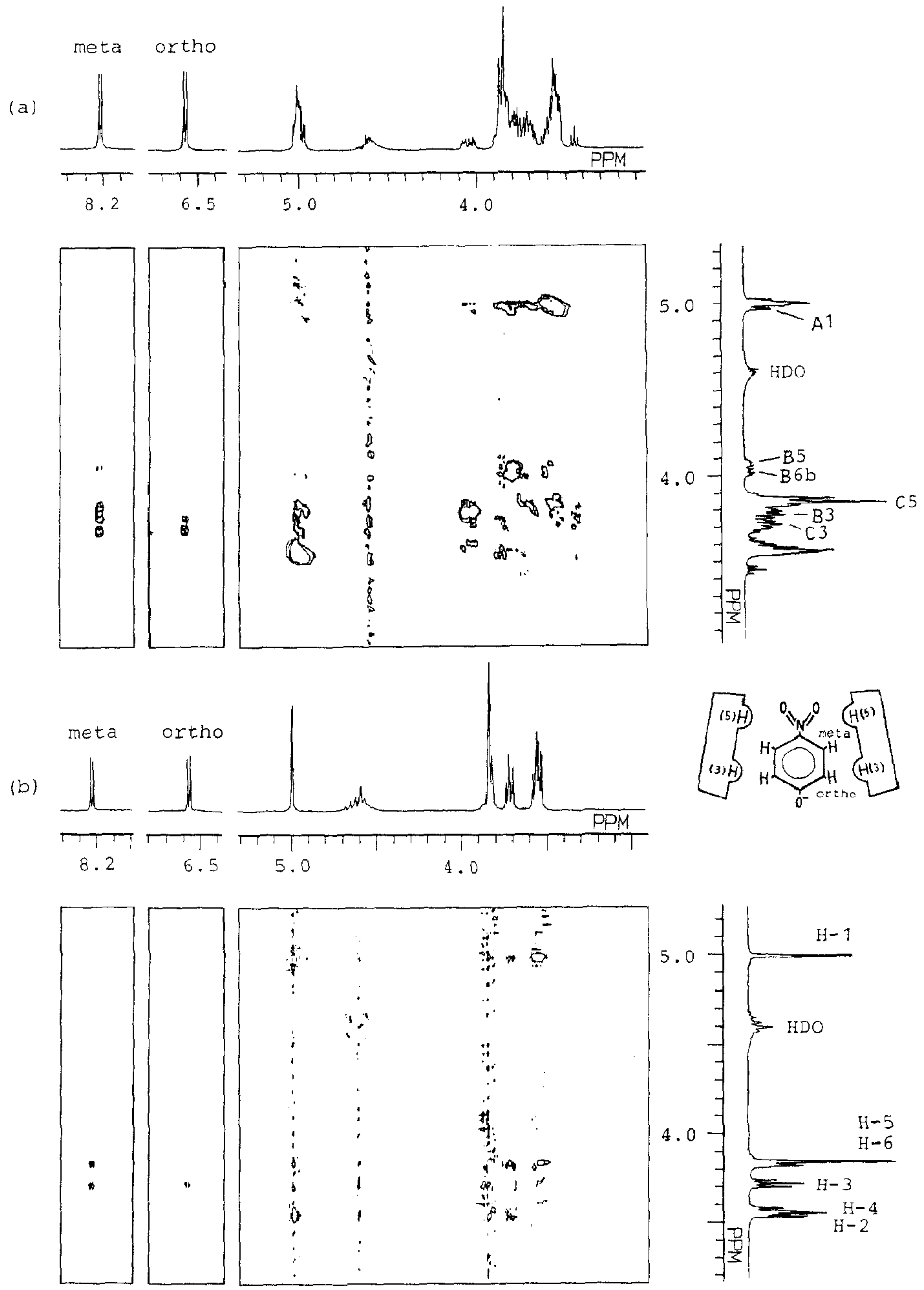

Fig. 2. 500-MHz ROESY spectra with negative contour levels of (a), G_1-α-CD–*p*NP at pD 7 and (b), α-CD–*p*NP at pD 10 in D_2O, at 40° with a spin-lock time of 250 ms. The contour plots show the resonances that have a sign opposite to the diagonal peaks. 1D spectra and the signal assignments are also shown, and proton resonances of G_1-α-CD are expressed as A1, B1, etc., corresponding respectively to the H-1 resonance of the A unit and B unit, etc. In the inset, the spatial relationships for H-3 and H-5 of α-CD and the *meta* and *ortho* protons of *p*NP are illustrated.

cross-peaks that connect the H-3 resonance of α-CD both *meta*- and *ortho*-proton resonances of *p*NP and connect H-5 to only the *meta* resonance, is observed and essentially identical with the NOESY spectrum previously reported[7]. These cross-peaks due to n.O.e. are subject to no contribution from *J* cross-peaks, since they arise between two protons belonging to two different molecules. Therefore, they are pure n.O.e. peaks and lead to the same conclusion about the host–guest geometry.

On the other hand, in the G_1-α-CD–*p*NP complex, the CD macrocylic ring is made up of at least two types of D-glucopyranose residues: B and C units (see Fig. 1), which exhibit different resonances from each other and consequently make the 1D spectrum complicated. Cross-peaks due to host–guest intermolecular n.O.e.s are observed in Fig. 2a. These include the cross-peaks connecting the H-3 resonances of both the B and C units to both *meta* and *ortho* resonances of *p*NP and those of the H-5 resonances of both units that connect to only the *meta* resonance of *p*NP. Therefore, the host–guest geometry in the G_1-α-CD–*p*NP complex appears to be similar to that observed in the α-CD–*p*NP complex[7]. Consequently, *p*NP is preferentially inserted into the cavity of G_1-α-CD with the nitro-end first and the *meta*-protons located close to both the H-3 and H-5 of the B and C units so that they exhibit n.O.e. peaks with both protons in each unit.

Similar ROESY experiments should be applicable to other CD inclusion-complexes in solution and should provide experimental data necessary for determining the orientation or insertion depth of a guest molecule into the CD cavity.

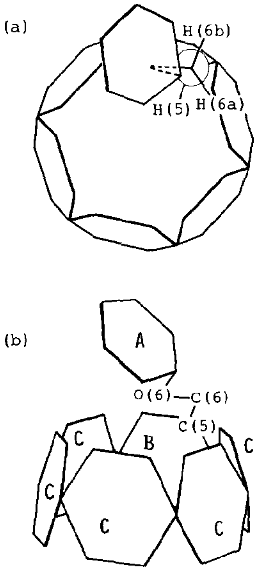

Fig. 3. The molecular structure of G_1-α-CD expected from n.O.e. data: (a), top view; (b), oblique side view. A hexagon represents one D-glucopyranosyl residue. The C-5–C-6 linkage of the B unit is drawn as the gt rotamer.

Information concerning the conformation of the G_1-α-CD molecule can be also obtained from the ROESY spectrum. By scrutinizing Fig. 2a, one can determine that some intramolecular n.O.e. cross-peaks are also identified in the region of the CD resonances. The intramolecular n.O.e. cross-peaks (A1,B6a), (B5,B6a), (B6a,B6b), (B4,B6b), and the somewhat weak cross-peak (A1,B6b), taken collectively, suggest a preferential conformation about the C-5–C-6 linkage of the B unit for which the gt rotamer (Fig. 3), where the branch residue A is located above the B unit, is a satisfactory fit. This conformation is consistent with the X-ray structure[14] established on a crystal of G_1-α-CD$\cdot 8H_2O$, which has the gt conformation of the C-5–C-6 linkage of the B unit with the plane comprised of H-1, H-3 and H-5 of the A unit situated nearly parallel with that comprised of the C-1 carbons of the C units. In other words, the branch is not situated over the entrance of the cavity.

The ROESY experiment is a powerful tool for investigating, not only the conformation of molecules in solution undergoing a motion with various time scales, but also for specific interaction between two different molecules.

EXPERIMENTAL

For the n.m.r. sample, solutions, 0.04M in G_1-α-CD and 0.04M in α-CD, both containing 0.08M *p*NP were prepared in D_2O at pD 7 and pD 10, respectively. According to the reported dissociation constants of the G_1-α-CD- and α-CD–*p*NP complexes[15], >90% of the G_1-α-CD and 98% of the α-CD are considered to be in the complexed state under the experimental conditions used. 2D ROESY spectra were recorded on a JEOL GX-500 spectrometer at 500 MHz and a sweep width of 3500 Hz. The carrier frequency was positioned at 5.58 ppm, and a 4-kHz spin-lock field was used during the mixing period of 250 ms in order to minimize magnetization transfer through scalar couplings[10]. Eight scans were performed for every t_1 value, and two 256 × 2k data sets were acquired. Chemical shifts are given in parts per million (p.p.m.) downfield from that of Me_4Si.

REFERENCES

1 S. Kobayashi, N. Shibuya, B. M. Young, and D. French, *Carbohydr. Res.*, 126 (1984) 215–224.
2 S. Kobayashi, K. Kainuma, and S. Suzuki, *Agric. Biol. Chem.*, 51 (1977) 691–698.
3 W. Saenger, *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344–362.
4 Y. Yamamoto, Y. Kanda, Y. Inoue, R. Chûjô, and S. Kobayashi, *Chem. Lett.*, (1988) 495–498.
5 J. H. Noggle and R. E. Shirmer, *The Nuclear Overhauser Effect*, Academic Press, New York, 1971.
6 R. Bergeron and R. Rowan, III, *Bioorg. Chem.*, 5 (1976) 425–436.
7 Y. Yamamoto, M. Onda, M. Kitagawa, Y. Inoue, and R. Chûjô, *Carbohydr. Res.*, 167 (1987) c11–c16.
8 A. A. Bothner-By, R. L. Stephens, J. Lee, C. D. Warren, and R. W. Jeanloz, *J. Am. Chem. Soc.*, 106 (1984) 811–813.
9 D. G. Davis and A. Bax, *J. Magn. Reson.*, 64 (1985) 533–535.
10 A. Bax and D. G. Davis, *J. Magn. Reson.*, 63 (1985) 207–213.

11 H. Kessler, C. Griesinger, R. Kerssebaum, K. Wagner, and R. R. Ernst, *J. Am. Chem. Soc.*, 109 (1987) 607–609.
12 L. Braunschweiler and R. R. Ernst, *J. Magn. Reson.*, 53 (1983) 521–528.
13 Y. Yamamoto, Y. Inoue, R. Chûjô, and S. Kabayashi, *Carbohydr. Res.*, 166 (1987) 156–161.
14 T. Fujiwara, N. Tanaka, K. Hamada, and S. Kobayashi, *Chem. Lett.*, (1989) 1131–1134.
15 Y. Kanda, Y. Yamamoto, Y. Inoue, R. Chûjô, and S. Kobayashi, *Bull. Chem. Soc. Jpn.*, 62 (1989) 2002–2008.