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Preliminary communication

Determination of the qualitative host–guest geometrical relationships in cyclomaltohexaose inclusion-complexes on the basis of measurements of intermolecular proton nuclear Overhauser effects

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N.m.r. cross-relaxation data can provide information on molecular structure through the inverse sixth-power dependency of the cross-relaxation rates on internuclear distances[1]. Analysis of the cross-relaxation process between the proton nuclei belonging to two different molecules which are interacting through space, such as in nucleic acids[2] and membrane-bound proteins[3], can also help to determine specific interaction between them. Cyclomalto-oligosaccharides (CDs), which consist of six or more (1→4)-linked α-D-glucopyranosyl residues, have received a great deal of attention as enzyme models because they not only can enclose various guest molecules within their cavities in solution but also can catalyse numerous chemical reactions[4]. Although considerable efforts have been devoted to determine the mechanism of the complexation processes of a CD with guest molecules[5–9], no quantitative description of the interaction of a CD and guest molecules has been given yet.

In order to characterise the inclusion phenomena of a CD, it is necessary to determine the orientation of guest molecules inside the cavity in inclusion complexes. Although such structural information is usually provided from X-ray data, the orientation of guest molecules in solution can be qualitatively deduced from n.m.r. cross-relaxation data. Data on the cross-relaxation between proton nuclei have been obtained through the selective irradiation or inversion of a single resonance in the ^{1}H-n.m.r. spectrum followed by observation of the nuclear Overhauser effect (n.O.e.) on other resonances. With the advent of two-dimensional n.O.e. experiments (NOESY)[10,11], the efficiency of data acquisition and the resolution have been considerably improved. We now report on a NOESY study of cyclomaltohexaose (α-CD) inclusion-complexes with *p*- and *m*-nitrophenol molecules (*p*NP and *m*NP, respectively).

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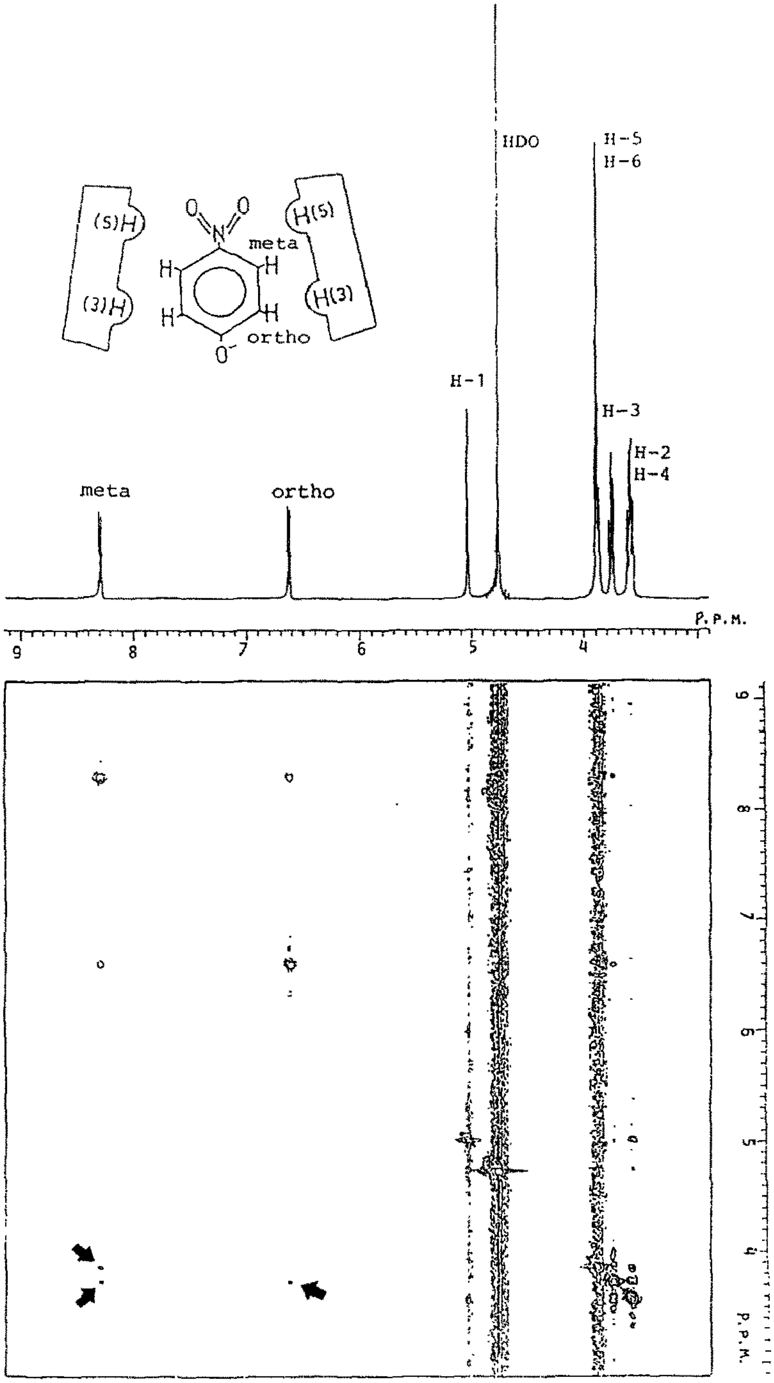

Fig. 1. NOESY spectrum of α-CD–*p*NP in D_2O at pD 10 and 30°. The ^1H spectrum and the signal assignments are shown along the F_2 axis. Cross-peaks connecting H-3 of α-CD to meta and ortho protons of *p*NP and H-5 of α-CD to the meta proton of *p*NP are indicated by arrows. In the inset, the spatial relationships for H-3 and H-5 of α-CD and the meta and ortho protons of *p*NP are illustrated.

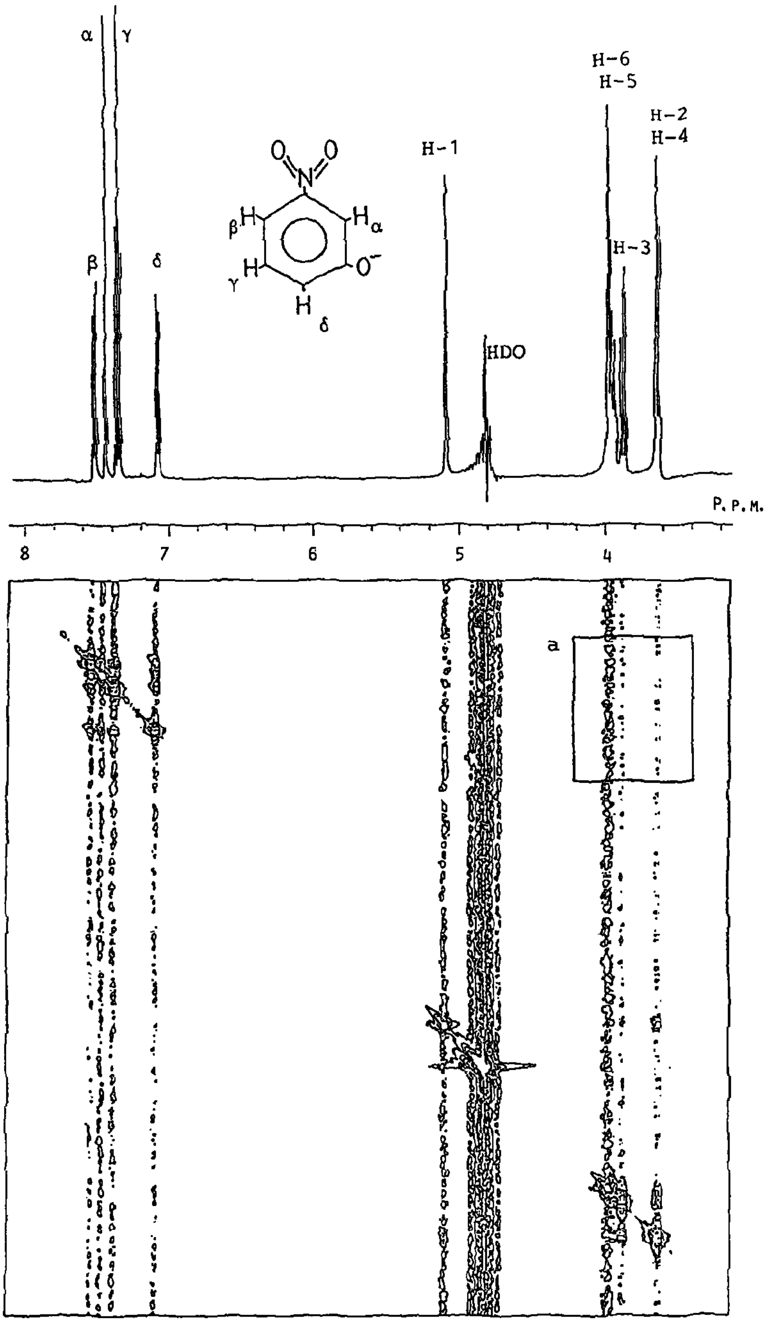

Fig. 2. NOESY spectrum of α-CD–*m*NP in D_2O at pD 10 and 30°. The ^1H spectrum is shown along the F_2 axis. The signal assignments were obtained by using two-dimensional chemical shift-correlated spectroscopy (results not shown).

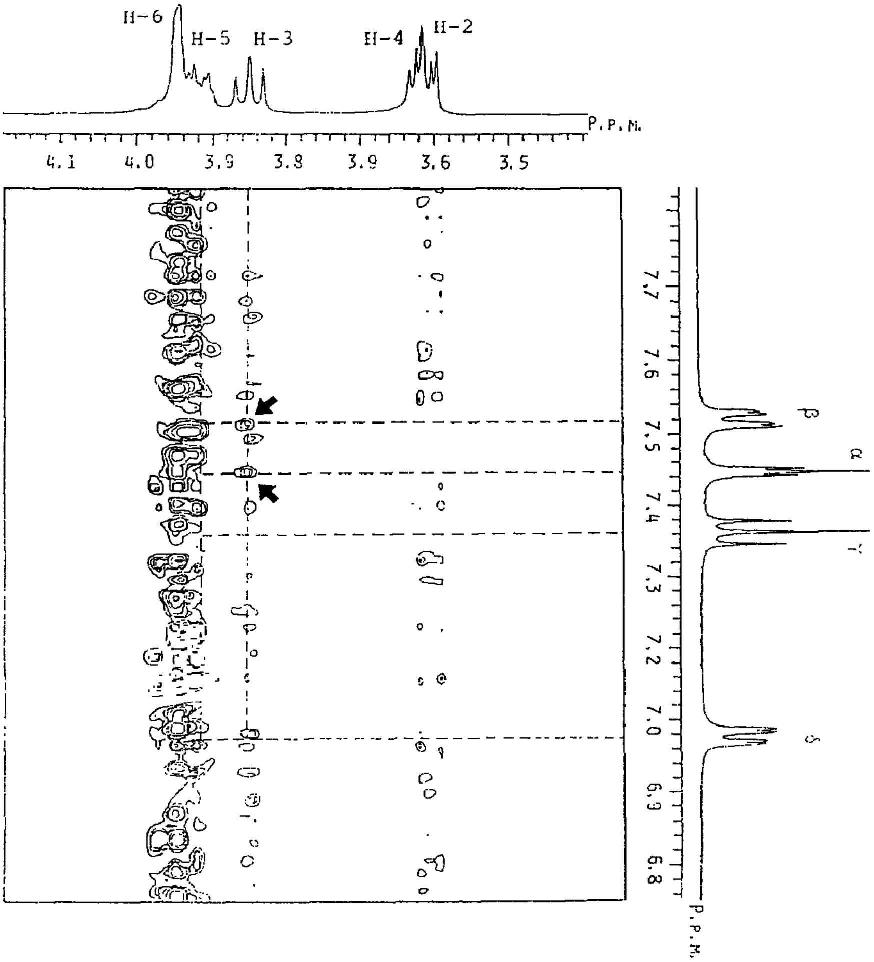

Fig. 3. Expanded spectrum of block a in Fig. 2. Two cross-peaks between α-CD and *m*NP proton resonances are indicated by arrows.

The NOESY and ^{1}H-n.m.r. spectra of α-CD–*p*NP in D_2O at pD 10 and 30° are shown in Fig. 1. In the NOESY spectrum, the cross-peak connecting two proton resonances indicates that those proton nuclei are in close proximity in the ground state of the molecule. Therefore, the cross-peaks in the NOESY spectrum of Fig. 1 connecting the H-3 resonance of α-CD to both meta- and ortho-proton resonances of *p*NP and H-5 to only the meta resonance clearly indicate that *p*NP is preferentially inserted into the cavity of α-CD as shown in the inset of Fig. 1. This result confirms the conclusion drawn from one-dimensional n.O.e. studies[12] and is consistent with the X-ray structure[13] of this complex. In Fig. 2, the NOESY spectrum of the α-CD–*m*NP mixture in D_2O at pD 10 and 30° is given, and the block a is expanded in Fig. 3. Although the intensity of the signal is small, the cross-peaks connecting the H-3 resonance of α-CD to the α and β proton resonances of *m*NP are clear. Since no *m*NP resonance gives a cross-peak with H-5 of α-CD, it can be concluded that *m*NP is incorporated into the cavity of α-CD from the nitro group and that the depth of penetration of *m*NP into the cavity is less than that of *p*NP, possibly due to steric hindrance of the hydroxyl group of *m*NP with the atoms on the wider rim of the α-CD cavity. These results are consistent with the X-ray structure[14], but contrast with the interpretations of earlier n.m.r. studies of aqueous solutions[15], which concluded that *m*NP is incorporated into the cavity of α-CD from the hydroxyl group. Similar NOESY experiments should be applicable to any CD inclusion-complex in solution and should provide the evidence necessary for determining the orientation of guest molecules in CD inclusion-complexes.

EXPERIMENTAL

For the n.m.r. samples, 0.04M and 0.015M solutions of α-CD containing 0.08M *p*NP and 0.09M *m*NP, respectively, were prepared in D_2O and the resulting solutions were adjusted to pD 10 by using NaOD. According to the reported dissociation constants[7] of the α-CD inclusion-complexes with the guest molecules used in the present study, more than 95% of α-CD is considered to be in the complex state under the experimental conditions used. The NOESY spectra were recorded on a JEOL GX-500 F.t.-n.m.r. spectrometer, using the standard pulse sequence with a mixing time of 500 ms. For each t_1 value, 16 and 64 scans for *p*NP and *m*NP systems, respectively, were signal-averaged with a pulse delay of 2 s. 512 Free-induction decays were acquired with 1k data points and a sweep-width of 3125 Hz. The data matrix was apodised with sine bell window functions in both dimensions and the spectrum is shown in the absolute value mode. Chemical shifts are given in parts per million (p.p.m.) downfield from that of Me_4Si.

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