^{13}C Cross Polarization–Magic Angle Spinning (CP–MAS) N.M.R. Studies of α- and β-Cyclodextrins: Resolution of All Conformationally-important Sites

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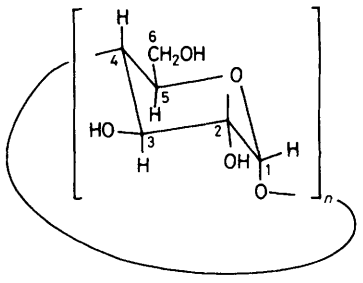
C-1 and C-4 sites in α- and β-cyclodextrin hydrates [six and seven residue cyclic α-(1 → 4) glucans] are resolved into six and seven resonances respectively in ^{13}C CP–MAS n.m.r. spectra, demonstrating that conformational features within these macrocycles are recognised by well-resolved chemical shifts.

In the determination of three-dimensional molecular structures, high resolution solid state ^{13}C n.m.r. spectroscopy provides an important link between the powerful and well-established techniques of crystallography and solution state n.m.r. spectroscopy.[1] The ultimate goal is to correlate crystal structures with ^{13}C chemical shifts in solid and solution states and hence be able to compare directly solid and solution state conformational features.

Two major problems associated with analysing high resolution solid state ^{13}C spectra [routinely obtained using the

combined techniques of cross polarisation and magic angle spinning (CP–MAS)] are firstly that the electronic and spatial factors which affect chemical shifts are difficult to disentangle and secondly that chemical shifts can be influenced by both intramolecular (*i.e.* conformational) and intermolecular (*i.e.* crystallographic) interactions.[2—4] Intermolecular effects are often manifested as multiple resonances reflecting the number of inequivalent sites in the crystallographic unit cell. For direct comparisons between solid and solution state conformations, care must be taken to determine whether solid chemical shifts reflect only intramolecular effects. We now show that ^{13}C CP–MAS n.m.r. spectra of α- and β-cyclodextrin (**1**) hydrates have resonance multiplicities consistent with purely conformational effects. Also chemical shift values can be measured for all C-1 and C-4 sites (*i.e.* those sites most likely to reflect local conformational variations) in both six- (α) and seven- (β) membered cyclodextrin macrocycles. Our observations suggest that cyclodextrins and their complexes may provide useful model systems for understanding the conformational origins of solid state ^{13}C chemical shift effects.



(**1**) n = 6, α
n = 7, β

Figures 1 and 2 show the ^{13}C CP–MAS n.m.r. spectra of α- and β-cyclodextrin hydrates obtained by recrystallisation from water. Resonances in the range δ 97—104 and δ 59—64 are readily assigned to C-1 and C-6 sites respectively. By analogy with chemical shifts in solution,[5] resonances due to C-4 sites should be shifted to low field compared with signals from C-2, -3, and -5 sites. Integration of the spectrum of α-cyclodextrin (Figure 1) shows that resonances in the ranges δ 97—104 and δ 77—83 each have intensities equal to one third of the signal intensity between δ 70 and 76. This strongly suggests that signals in the range δ 77—83 can be assigned to C-4 sites. Similarly, integration of the β-cyclodextrin spectrum (Figure 2) suggests that C-4 sites account for resonances between δ 78 and 85.

Significant splittings of C-1 and C-4 signals in both α- and β-cyclodextrins are apparent from Figures 1 and 2. Thus, following resolution enhancement,[6] seven C-1 resonances are observed for β-cyclodextrin. Similarly six C-4 resonances are

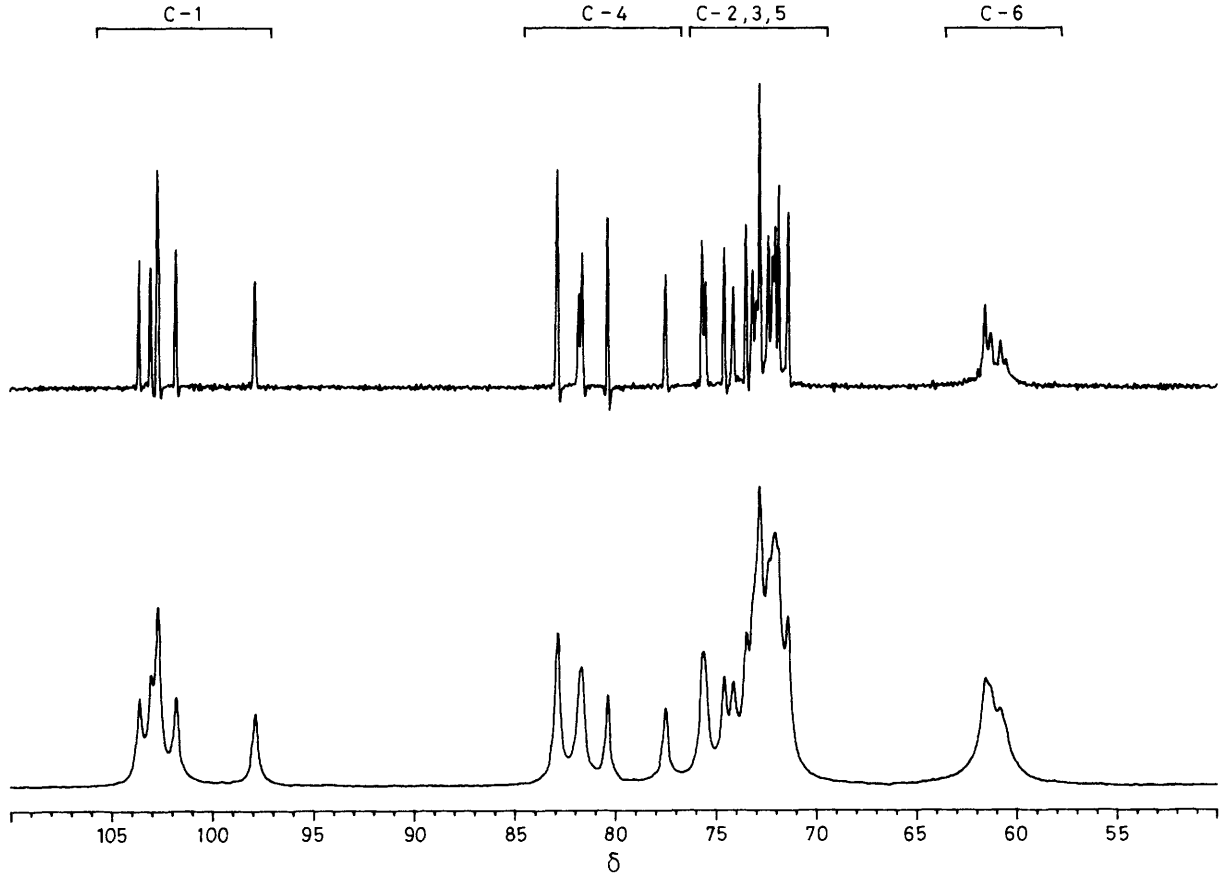


Figure 1. ^{13}C CP–MAS spectrum of α-cyclodextrin hydrate obtained on a Bruker CXP-300 spectrometer operating at 75.46 MHz with a Bruker double bearing MAS probehead. A spinning rate of 4 kHz and spin locking and ^{1}H decoupling fields of ~80 kHz (20 G, 2 × 10^{-3} T) were employed. Spectral parameters were: spectral width 30 kHz, acquisition time 140 ms, single contract time 1 ms, no. of scans 1000, temperature 303 K, recycle delay 4 s, time domain points 8 K, transform size 32 K. Lower trace, 10 Hz line broadening. Upper trace, −15 Hz line broadening, 0.5 Gaussian multiplication factor.

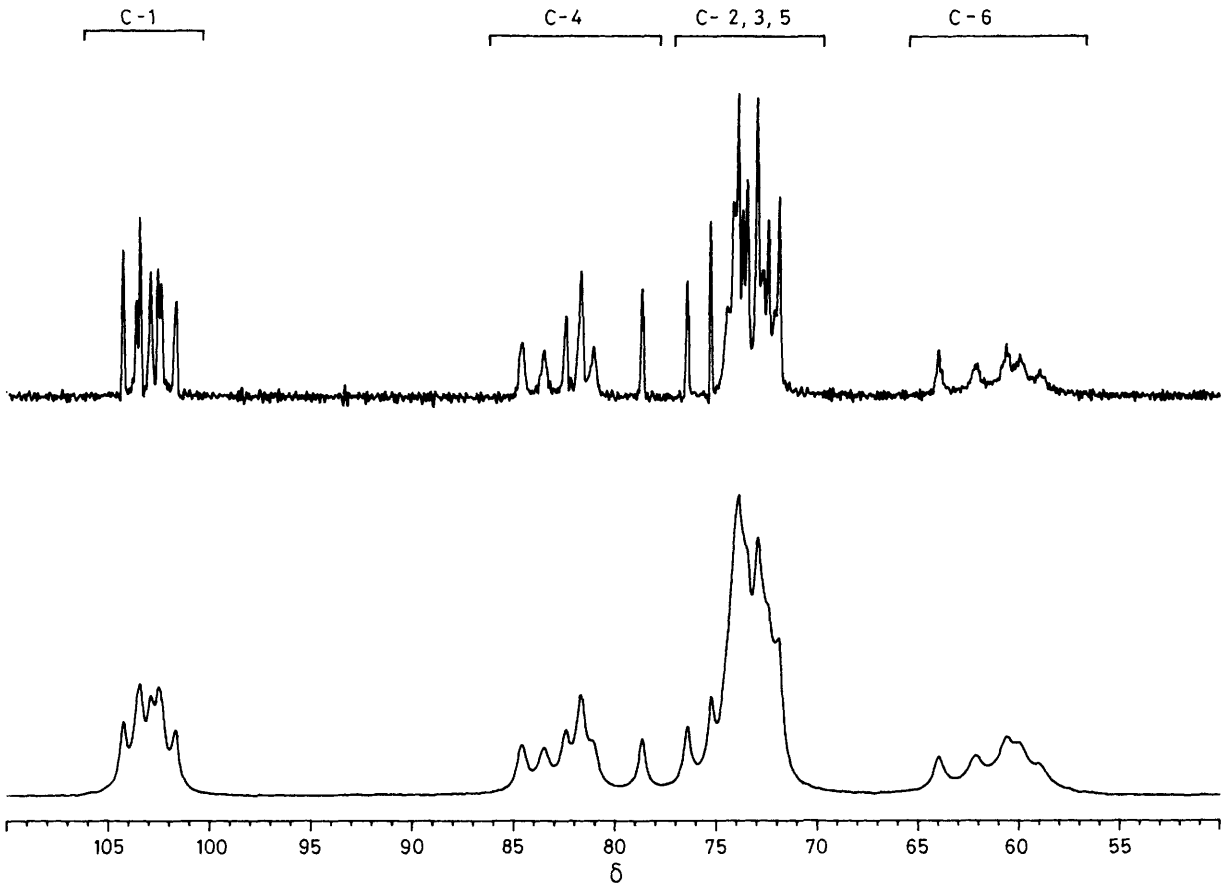


Figure 2. ^{13}C CP–MAS spectrum of β-cyclodextrin hydrate obtained as for Figure 1 except that a line broadening of −20 Hz was used in the upper trace.

clearly resolved, one of which (δ 81.6) has an unresolved shoulder and is of greater intensity (Figure 2) suggesting that it accounts for two sites. For α-cyclodextrin (Figure 1), five C-4 signals are resolved, one of which (at δ 82.8) accounts for one third of the total C-4 signal intensity and is therefore due to two sites. Similarly, five C-1 signals are resolved with the resonance at δ 102.6 accounting for two sites. Discrete chemical shift values can therefore be obtained for all of the conformationally-sensitive C-1 and C-4 sites. The observation of six and seven C-1 and C-4 resonances for the six- (α) and seven- (β) residue cyclodextrin rings respectively provides strong evidence that cyclodextrin solid state ^{13}C chemical shifts are solely determined by intramolecular (conformational) interactions. This result is consistent with *X*-ray crystallographic evidence that individual macrocycles constitute the asymmetric units in both α-[7] and β-[8] cyclodextrin hydrates.

In the α-cyclodextrin hydrate structure determined by *X*-ray diffraction,[7] one of the α-(1 → 4) linkages adopts an unusual high energy conformation. This leads to a partially 'collapsed' structure that is able to expand to a more symmetrical macrocyclic ring on formation of inclusion complexes.[7] It is tempting to assign the resonances at δ 97.8 and 77.4 in Figure 1 to the C-1 and C-4 sites involved in the anomalous linkage as both these resonances are significantly shifted relative to other C-1 and C-4 signals. The β-cyclodextrin hydrate crystal structure,[8] by contrast, shows an open circular conformation which is not dramatically changed upon formation of inclusion complexes.[8] It is interesting that such large chemical shift dispersions (δ 2.6 and 6.0 for C-1 and C-4 sites) are observed for the nearly symmetrical[8] β-cyclodextrin hydrate (Figure 2).

In contrast to the solid state, aqueous solutions of α- and β-cyclodextrins show only one resonance for each carbon site owing to conformational averaging. A comparison of solid and solution conformations can however be made by comparing a weighted average of solid state C-1 and C-4 chemical shifts with solution chemical shifts. For β-cyclodextrin, average solid state chemical shifts for C-1 and C-4 sites (δ 102.86 and 81.89 respectively) are essentially identical with solution chemical shifts[5] (δ 102.81 and 81.95 respectively). This suggests that aqueous solutions of β-cyclodextrin consist of rapidly interconverting conformations which are 'frozen out' in the solid state. Average solid state chemical shifts for α-cyclodextrin C-1 and C-4 sites (δ 101.76 and 81.07 respectively), however, are to higher field than in solution[5] (δ 102.23 and 82.05 respectively) suggesting that solid and solution state conformations may be significantly different. It is interesting to note that the two signals tentatively asigned to sites adjacent to the 'anomalous' linkage[7] in solid α-cyclodextrin are both to higher field than other C-1 and C-4 resonances. Furthermore, average solid state chemical shifts of the five remaining C-1 and C-4 resonances (δ 102.57 and 81.81 respectively) are somewhat closer to the values observed in solution. These observations suggest that the α-cyclodextrin macrocycle which is partially collapsed in the solid state[7] may

expand in aqueous solution to relieve the conformational strain imposed by the single high energy α-(1 → 4) linkage.

The spectral resolution obtained in the present study (Figures 1 and 2) is considerably greater than has been reported in previous ^{13}C CP–MAS n.m.r. studies of cyclodextrins,[9—11] for example resolution of only the highest field C-1 resonance in α-cyclodextrin has previously[9] been achieved. This improvement cannot be ascribed to the ^{13}C operating frequency (75 MHz) and ^{1}H decoupling power (20 G, 2×10^{-3} T) employed as these are comparable to those used previously. It is more likely that greater resolution was achieved through the use of a double bearing probehead and/or by accurately setting the magic angle and ensuring that the ^{1}H decoupling field was set on-resonance.

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