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^{13}C-CP/MAS NMR studies of the cyclomalto-oligosaccharide (cyclodextrin) hydrates

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ABSTRACT

The ^{13}C-CP/MAS NMR spectra of cyclomaltohexaose (α-cyclodextrin) hexahydrate, cyclomaltoheptaose (β-cyclodextrin) "undecahydrate", cyclomalto-octaose (γ-cyclodextrin) "octadecahydrate", and of the same materials at lower levels of hydration are compared with solution NMR data, structures obtained from single crystal diffraction studies, and with previous reports of the ^{13}C-CP/MAS NMR spectra. The chemical shifts of the C-1 and C-4 resonances can be correlated with the conformation about the (1 → 4) linkage. The chemical shifts of the C-6 resonances are also sensitive to hydrogen-bonding interactions, as shown by the spectral changes on loss of water from the structures. The results suggest that, for resonances of carbon atoms close to a centre of significant conformational change, chemical shifts may be predicted on the basis of conformation alone, but for the resonances of more distant atoms, changes in chemical shift due to conformational change may be masked by the effects of alterations in the local environment.

INTRODUCTION

The (1 → 4)-linked α-D-glucopyranose residues in cyclomaltohexaose (α-cyclodextrin, αCD), cyclomaltoheptaose (β-cyclodextrin, βCD), and cyclomalto-octaose (γ-cyclodextrin, γCD) produce a left-handed chiral spiral as in amylose[1,2]. The glucose residues always adopt a chair conformation. Moreover, the endocyclic torsion angles are confined essentially to *gauche–gauche (gg)* or *gauche–trans (gt)* values, indicating that the glucose residue is fairly "rigid". Each structure defines a slightly 'V'-shaped toroidal cavity[3,4], with a diameter of 5–8 Å, with secondary hydroxyl groups on the more open side, and primary hydroxyl groups on the other, as shown in **1** for βCD. The primary hydroxyl groups may rotate about the

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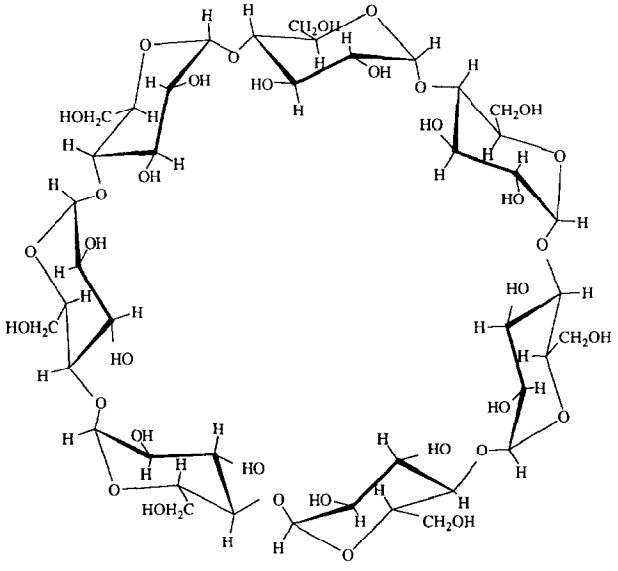
C-5–C-6 bond so as to block the cavity partially. The interior of the torus, which consists of a ring of C–H groups that sandwich a ring of glycosidic oxygens, is relatively apolar.

CDs form inclusion complexes with a wide range of smaller molecules which fit into their cavities[3–5]. In the absence of other guest molecules, the CDs, unless deliberately dehydrated, generally contain water molecules in their cavities, and crystal structures are known for several hydrate forms[6–15]. All the CD structures have an extensively hydrogen-bonded network of water molecules between adjacent CD molecules. Information relating to the structure of complexes in solution has been obtained from ^{1}H and ^{13}C NMR studies[16–23]. In the solid state, preliminary ^{13}C-CP/MAS NMR data for the cyclodextrin hydrates linked chemical shifts tentatively to conformation[24–27]. Recently, more-detailed studies[28–30] of (1 → 4)-α-D-glucans, including the CDs, have shown links between the ^{13}C-CP/MAS NMR-derived chemical shifts and certain glycosidic torsion angles as determined from single-crystal X-ray diffraction studies. Changes in the ^{13}C-CP/MAS NMR spectrum of the CD occur on complexation of a guest molecule and may reflect conformational effects, as well as local magnetic environmental effects caused by that guest[31–36].

We have studied the ^{13}C-CP/MAS NMR spectra of the CD hydrates and now report on the merit of a conformational approach to the explanation of the



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chemical shifts of ^{13}C NMR resonances of CD carbon atoms, and also on the effects of changes in the level of hydration.

EXPERIMENTAL

Cyclodextrin hydrates.—α-, β-, and γ-CD (Sigma) were recrystallised from water, using standard procedures[2]. αCD was recrystallised twice from distilled water. The solution obtained at 60° was filtered through a fine sintered-glass filter and then cooled to 5°. The resulting crystals of αCD hexahydrate often took over a week to appear, despite seeding. An aq 2% solution of βCD was stored at room temperature for 3 days in order to allow aggregation of insoluble impurities, then passed through a fine sintered-glass filter, concentrated to a tenth of its original volume, and allowed to crystallise. This procedure was repeated to give βCD "undecahydrate". γCD was recrystallised from water twice, as for αCD, to give crystals of γCD "octadecahydrate".

The purity of the above CDs was verified by ^1H NMR spectroscopy (500 MHz) of solutions in Me_2SO-d_6. The spectra were assigned by comparison with published data[16,17,20] for solutions in D_2O, in conjunction with selective decoupling experiments. Notable in these spectra were the extremely sharp hydroxyl resonances, and, unlike previous studies[37,38], these showed distinct scalar couplings to other carbohydrate protons. Since all the coupling constants are of different magnitude, unambiguous assignment of each of the two primary hydroxyl resonances was made. The spectrum for αCD is shown in Fig. 1.

The crystalline CD hydrates were characterised by X-ray powder diffraction, recorded on a Phillips PW 1710 powder diffractometer, controlled by a Map 80 microcomputer. Cu-K_α radiation at 40 kV was used and reflections were referenced internally and checked against a silicon standard. The low-angle peaks, referenced to single crystal data[6,11,13], indicated that αCD · $6H_2O$ (form I), βCD · "$11H_2O$", and γCD · "$18H_2O$" were obtained in pure crystalline form. Due to the

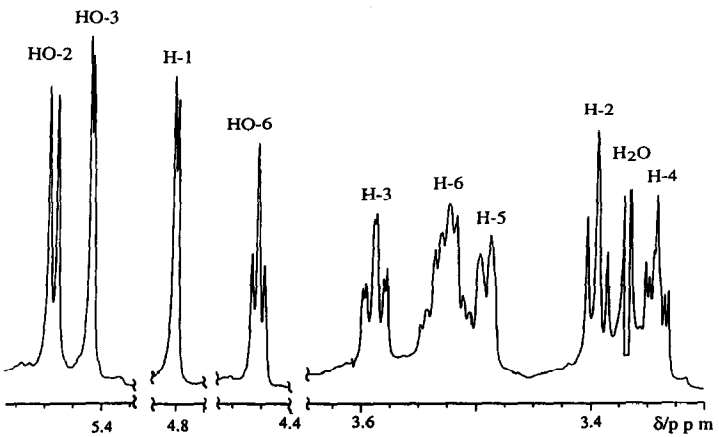

Fig. 1. ^1H NMR spectrum (500 MHz) of a solution of αCD in Me_2SO-d_6.

disorder of the water molecules in these materials, the actual water content of the crystals is a subject of some debate (see below). The crystal hydrates are referred to by the generally accepted notation of Saenger[12], namely, the hexahydrate, "undecahydrate", and "octadecahydrate" for α-, β-, and γ-CD, respectively.

Thermogravimetric analyses (TGA) were carried out using a Stanton Redcroft STA 785 thermal analyser which provides simultaneous TGA, DTG, and DTA curves, on a 10-mg sample in a static, air atmosphere. All samples lost mass gradually between room temperature and $\sim 120°$, which is interpreted as loss of all the water in the structure, and thereafter remained at constant mass up to the mp (dec) of the CD ($\sim 300°$). Calculations from the initial mass loss confirmed the overall hydration numbers as 6.2 ± 0.5 (α), 11.3 ± 0.5 (β), and 17.5 ± 0.5 (γ). Each CD hydrate was stored under the mother liquor until needed, in order to avoid loss of water from the structure. The fully dehydrated materials were obtained by heating the hydrated material at $100°/10^{-2}$ mbar for 3 h. Such materials were handled under dry nitrogen in order to prevent rehydration.

Solid-state NMR spectroscopy.—A Bruker CXP200 pulse NMR spectrometer with an Oxford Instruments 4.7 Tesla wide-bore (98 mm) superconducting solenoid Magnet (200.13 MHz for ^{1}H NMR) and equipped with an Aspect 2000 data system, was used. ^{13}C-CP/MAS NMR spectra were recorded at 50.32 MHz using a multinuclear, proton-enhanced, double-bearing MAS probe (Bruker Z32-DR-MAS-7DB) and a high-power proton decoupler. Chemical shifts are reported relative to $\delta(Me_4Si) = 0$, and were referenced to adamantane as a secondary standard. A single-contact spin-lock CP sequence[39], with alternate cycle spin–temperature inversion, flip back of ^{1}H magnetisation[40], and a proton rf field of 1.7 mT ($\omega_1 = 72$ kHz), resulting in a 90° pulse length of 3.5 μs, was used. Typically, 250–300 mg of each compound was packed into a 7-mm zirconia rotor with a Kel-F top. The optimum contact time for the CD resonances was 0.75 ms, and 800–1200 transients with a recycle delay of 3.5–5 s were acquired for each spectrum. Fully hydrated materials were isolated immediately before packing into the NMR rotors, in order to minimise any unnecessary loss of water from the structure. The anhydrous samples were packed into the rotors under dry nitrogen, and the rotors were sealed with teflon tape and stored under dry nitrogen.

RESULTS

Cyclodextrin hydrates.—The ^{13}C-CP/MAS NMR spectra of αCD hexahydrate, βCD "undecahydrate", and γCD "octadecahydrate" are shown in Fig. 2. The resolution is excellent; individual peaks have half-height line widths ($\Delta\nu_{1/2}$) of < 30 Hz, which illustrates the high crystallinity of the materials. The ^{13}C NMR resonances of the CDs can be assigned directly by comparison with the solution data[41,42]. In solution, there is only one resonance for each set of chemically equivalent carbons because of rapid conformational averaging of the CD structure,

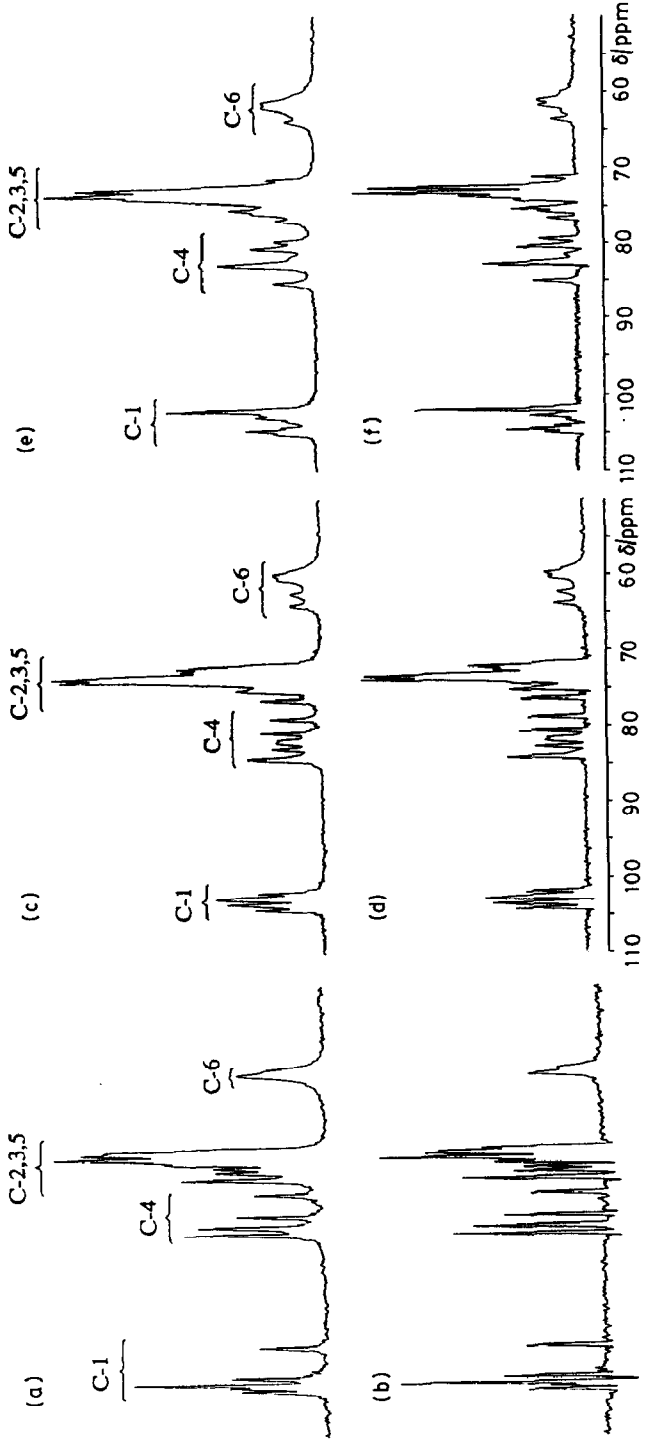

Fig. 2. ^{13}C-CP/MAS NMR spectra: for αCD hexahydrate without (a) and with (b) resolution enhancement, for βCD "undecahydrate" without (c) and with (d) resolution enhancement, for γCD "octadecahydrate" without (e) and with (f) resolution enhancement.

TABLE I

^{13}CP/MAS NMR data (δ/ppm) for the CD hydrates [a]

		Gidley and Bociek[30]	Veregin et al.[28]	Aqueous solution[30,41,42]
α-CD hexahydrate				
C-1	103.7	103.8	103.6	
	103.25	103.2	103.1	
	102.85 (*2*)	102.8 (*2*)	102.7 (*2*)	
	102.0	102.0	101.9	
	98.05	98.1	97.9	
Mean	102.1 (102.95)	102.1 (102.9)	102.0 (102.8)	102.55
C-4	82.9 (*2*)	83.1 (*2*)	82.7 (*2*)	
	81.9 (*2*)	82.0	81.8 (*2*)	
		81.9		
	80.55	80.6	80.4	
	77.7	77.7	77.6	
Mean	81.3 (82.05)	81.4 (82.1)	81.15 (81.9)	82.35
C-6	61.7 (*4*)	No	61.6 (*4*)	
	61.1 (*2*)	assignments	61.0 (*2*)	
Mean	61.5	61.6	61.4	61.6
C-2,3,5	71.2–75.7	71–76	–	
β-CD "undecahydrate"				
C-1	104.3	104.5	104.1	
	103.8	103.8		
	103.5	103.7	103.4 (*2*)	
	103.1	103.1	102.8 (*3*)	
	102.8	102.8	(but clear	
	102.5	102.7	shoulder to peak)	
	101.9	101.9	101.7	
Mean	103.1	103.2	103.0	103.0
C-4	84.4	84.8	84.3	
	84.0	83.7	83.8	
	82.9	82.6	82.6	
	82.1	81.9 (*2*)	81.8 (*2*)	
	81.7	81.3	81.0	
	80.7			
	78.9	78.8	78.8	
Mean	82.1	82.2	82.0	82.25
C-6	64.1		63.8	
	62.5		62.7	
	61.7	59–64	61.0	
	60.55		60.4	
	59.95			
	59.4		59.3	
Ratios are uncertain due to the complex superposition of resonances				
Mean	61.1	61.1	61.0	61.5[5]

γ-CD "octadecahydrate"

	Solid state (this work)								Aqueous solution[41]
C-1	105.1	104.7	104.2	103.0	102.6	110.1 (*2*)	101.5	Mean: 103.2	102.4
C-4	85.3	82.95 (*2*)	81.8	80.65 (*2*)	79.65	76.8		Mean: 81.35	81.2
C-6	63.8	62.9	62.0 (*2*)	61.4 (*2*)	60.7	60.2		Mean: 61.8	61.0

[a] Mean shifts in parentheses ignore the most upfield-shifted resonance. Italic figures indicate intensities.

but, in the solid state, several peaks may be observed for each type of carbon. The C-2,3,5 resonances overlap and cannot be assigned individually in the solid-state spectra.

In the ^{13}C-CP/MAS NMR spectrum of αCD hexahydrate, each carbon resonance shows multiple peaks; for C-1 and C-4 these peaks are spread over the relatively large chemical shift range of 5–6 ppm. Multiple resonances observed in solid-state NMR spectra, when only a single resonance is observed in solution, have been explained[43] as due to there being more than one molecule in the asymmetric unit of the crystal structure, or the imposition of a crystallographic site symmetry which is lower than the overall molecular symmetry, or the "freezing-out" in the solid state of conformational averaging processes. αCD hexahydrate is known from single-crystal X-ray diffraction analysis[6] to have an asymmetric structure with one glucose residue rotated inwards to allow the formation of an O-6 $\cdots$ H_2O hydrogen bond. The chemical shifts of the C-1,4 resonances are thought to be particularly sensitive to the conformation of the CD molecule about the (1 → 4) linkage[28–30,44,45], and therefore are probably dominated by the *intra*molecular torsion angles adopted by the various glucose residues in the crystalline structure. Thus, in principle, a separate peak for each carbon atom in the CD molecule might be expected, although overlap of resonances may reduce the number of resolved peaks. Similarly, the chemical shifts of the C-6 resonances are particularly sensitive to the lattice structure because of the rotation about the C-5–C-6 bond which enables different hydrogen-bonding interactions[44].

The ^{13}C-CP/MAS NMR spectrum obtained for αCD · $6H_2O$ is of a form similar to those reported[28,30] (see Table I). For each of the C-1 and C-4 resonances, the fine structure was suggested to correlate with the different torsion angles about the (1 → 4) linkages. Notably, the most upfield peaks for each of the C-1 (97.8 ppm) and C-4 (77.5 ppm) resonances may be ascribed reasonably to the C-1 and C-4′ involved in that glycosidic linkage which is most strained by the rotation of a single glucose residue into the torus. There is no general method for the assignment of individual peaks to a specific glucose ring because of the inability to explain the shielding mechanism responsible for conformationally dependent shifts. However, Gidley and Bociek[30] have correlated the chemical shifts of the C-1 resonances with the modulus of the torsion angle $|\psi|$, which describes rotation about the O-1–C-4′ bond (see Fig. 3 for definitions of the torsion angles), and with the sum of the moduli of the torsion angles, $|\psi| + |\phi|$, which represents the non-coplanarity of H-1, C-1, O-1, C-4′, and H-4′. Veregin et al.[28] showed that the chemical shifts of the C-1 resonances correlated with ψ, but not with ϕ, and that those of the C-4 resonances correlated with ϕ within each CD, but that the correlation is somewhat different for each CD. Given the scatter in the correlation of the C-1 and C-4 resonances with torsion-angle parameters, and the dispersion in the chemical shifts of the resonances of most of the carbons, it is clear that other factors, in particular *inter*molecular effects, also contribute to the chemical-shift differences. Care must be taken when interpreting small differ-

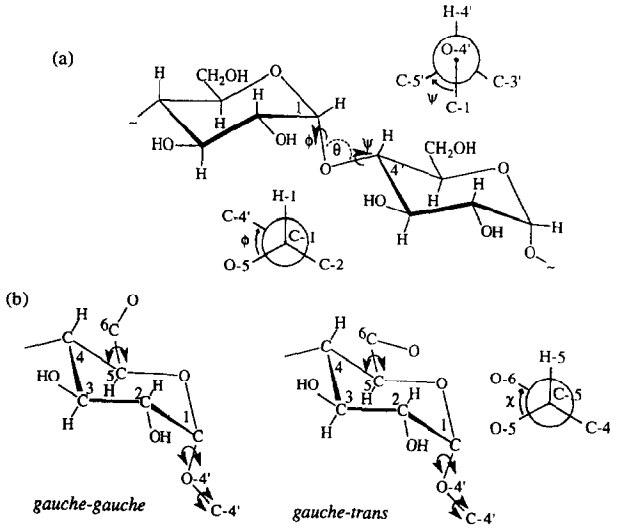

Fig. 3. Conformational parameters of the CDs, showing (a) the torsion angles ϕ and ψ and the corresponding Newman projections along C-1–O-1 and C-4′–O-1, (b) the two possible conformations about the C-5–C-6 bond.

ences in chemical shifts in terms of conformational changes, because this usually assumes implicitly the dominance of a particular shielding mechanism.

Veregin et al.[28] suggested that the chemical shifts of the C-6 resonances are correlated with the torsion angle χ which describes the orientation of the primary hydroxyl group. In all crystal structures, χ is found to be constrained to two orientations, namely *gg* (O-6 *gauche* to O-5 and C-4) and *gt* (O-6 *gauche* to O-5 and *trans* to C-4), with only small angular variations within each orientation[4]. Crystallographically, 2 glucose residues in αCD have χ as *gt* and 4 as *gg*. On the basis of conformationally dependent ^{13}C chemical shifts, two peaks for the C-6 resonance in the ratio 2:1 would be expected, with the more intense peak, accounting for the *gg* conformation, occurring at higher field. In fact, the observed C-6 resonances for $\alpha CD \cdot 6H_2O$ have chemical shifts intermediate of those expected (see Table I). Peaks for the C-6 resonance were observed at 61.5 and 60.9 ppm accounting for 4 and 2 carbons, respectively. Veregin et al.[28] observed a similar structure, although, on resolution enhancement, Gidley and Bociek[30] observed 4 resonances. However, the spectrum reported by Gidley and Bociek[30] is identical to that observed here on very slight water loss from $\alpha CD \cdot 6H_2O$ (see below), suggesting that the "doubling" of the C-6 resonances is due to two slightly different hydrate forms or to disordering of the water molecules in the structure. Veregin et al.[28] interpreted their C-6 resonance as a single peak caused by dynamic averaging of each glucose residue between the *gg* and *gt* conformations, whilst acknowledging that the crystal structure shows no sign of such a disorder. It is concluded that, in addition to conformational features, other factors, including hydrogen bonding, must also influence the chemical shift of the C-6 resonances,

and that the resonance at 61.5 ppm may be assigned reasonably to the 4 *gg* residues and that at 59.9 ppm to the 2 *gt* residues, despite the chemical shifts being reversed from those expected on a purely conformational analysis.

The spectra of the βCD "undecahydrate" and γCD "octadecahydrate" are broadly similar to that of αCD (Fig. 2), but they lack the strongly upfield-shifted resonance in the C-1 and C-4 regions previously assigned to the highly strained linkage peculiar to $\alpha CD \cdot 6H_2O$. Single-crystal X-ray analysis shows that the βCD[11] and γCD[13] hydrates have more-open, circular conformations than the somewhat collapsed structure of $\alpha CD \cdot 6H_2O$. However, the structures do not have complete cylindrical symmetry, as reflected by the chemical-shift dispersion observed for the ^{13}C resonances. For βCD and γCD, the averaged chemical shifts for the C-1,4,6 resonances in the solid-state spectra are similar to those for solutions of the CDs in D_2O. This finding suggests that the solution conformation, as detected by NMR, is a time average of solid-state-like conformations. The averaged chemical shifts for αCD in the solid state are upfield of the corresponding solution values, but are closer if the most-upfield peaks due to the anomalously high energy linkage are ignored, implying that the partially collapsed solid-state structure has little part to play in the conformational equilibrium in solution. The ^{13}C-CP/MAS NMR spectrum of $\beta CD \cdot 11H_2O$ differs slightly in profile from that reported by Gidley and Bociek[30] in the C-4 region and from that reported by Veregin et al.[28] in the C-4 and C-6 regions. However, the chemical-shift correlations are generally the same. Our spectrum for the C-6 region is similar in form to that reported by Gidley and Bociek[30], and both are somewhat different from the spectrum reported by Veregin et al.[28]. As for $\alpha CD \cdot 6H_2O$, the fine structure in this region of the spectrum for β-$CD \cdot 11H_2O$ is hydration-dependent (see below), suggesting that hydrogen-bonding interactions are as important as conformational effects in determining the chemical shifts for the C-6 resonances, thus complicating any conformational analysis. No simple interpretation of the spectrum is available from the crystallographic observations that 2 glucose residues have χ *gt*, 4 residues have χ *gg*, and 1 residue is two-fold disordered approximately equally between the *gt* and *gg* conformations. The spectrum for $\gamma CD \cdot 18H_2O$ (not reported in refs. 28 and 30) shows fair correlation of the chemical shifts of the C-1 and C-4 resonances with appropriate torsional parameters. Again, the C-6 region of the spectrum does not lend itself to simple conformation-dependent chemical-shift analysis based on the information from the crystal structure.

Loss of water from crystalline cyclodextrin hydrates.—On separation of the hydrate from its mother liquor, water is generally lost from the crystals. Indeed, there is some confusion as to the exact hydration numbers of the "fully hydrated" materials of β- and γ-CD. The samples studied here were characterised by X-ray powder diffraction and thermogravimetric analysis and are referred to as βCD "undecahydrate" and γCD "octadecahydrate". In fact, it has been suggested by Zabel et al.[12], from a neutron diffraction study at 120 K, that the crystal form of the βCD hydrate, previously considered from the room temperature study to be

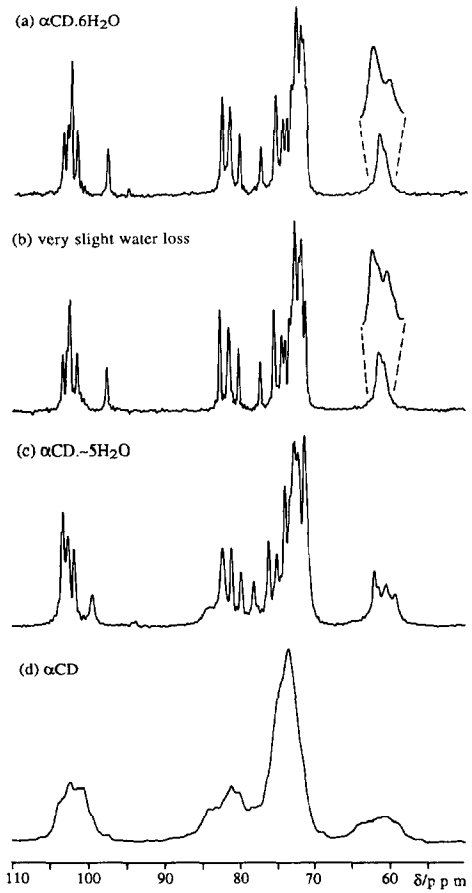

Fig. 4. The ^{13}C-CP/MAS NMR spectra for αCD at various hydrate levels.

the undecahydrate[11], actually contains 11.6 water molecules per CD molecule. The water molecules are more easily located in the ordered structure at 120 K than in the disordered structure at 296 K. A single-crystal X-ray study[9,10] suggested the crystalline form to be the dodecahydrate. Of these values, that of 11.6 from the neutron structure at 120 K is the most precise and also corresponds well to the value of 11.5 that we determined by TGA (see Experimental). X-ray powder diffraction cannot distinguish the small differences in lattice parameters and small changes in the intensities of certain reflections that should be expected from the single-crystal parameters of the various studies. It is assumed that all previous studies were on the same bulk material, which was homogeneous and monophasic. It is also not certain exactly how many water molecules per CD molecule are resident in γCD hydrate: Maclennan and Stezowski[13] suggest 17, Zabel et al.[12] 18, Ding et al.[15] 15.7, Harata[14] 13.3, and the TGA results suggest 17.5. The reason for the greater certainty over the water content of α-CD $\cdot$ $6H_2O$ is that the water molecules are ordered in this structure, whereas the majority are disordered in the

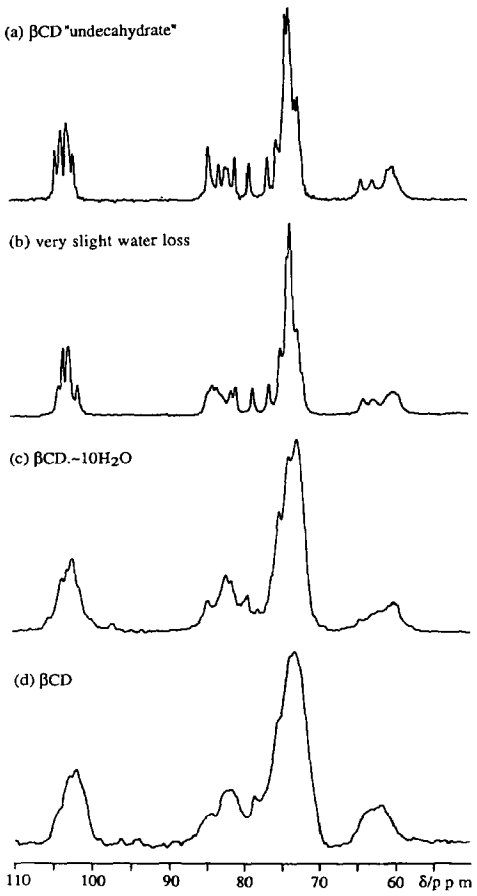

Fig. 5. The ^{13}C-CP/MAS NMR spectra of βCD at various hydrate levels.

other CDs, such that some of the water molecule sites are occupied only partially, and therefore less easily located in the diffraction studies.

The loss of water from the CD hydrates is, however, slow enough to allow the spectrum of the fully hydrated material to be obtained. As water is lost, the hydrogen-bonded structure in the solid changes, and so the conformation of the CD torus, which depends upon the various hydrogen-bonding interactions, will also change. Therefore, it might be hoped that the spectra of the water-depleted samples should allow analysis of the changes in conformation relative to those of the fully hydrated materials. The spectra of the CDs at various hydration levels between fully hydrated and the level which is most stable for each CD at room temperature and humidity, and of the fully dehydrated material are shown for α-, β-, and γ-CDs in Figs. 4–6, respectively. As the first water is removed, the intensities of the resonances change, and new resonances appear. As more water is removed, the resonances broaden, presumably as the conformations of the CD

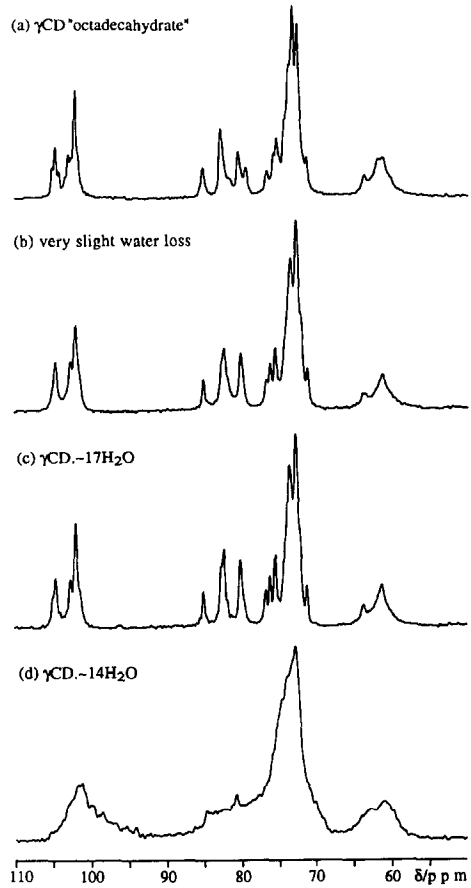

Fig. 6. The ^{13}C-CP/MAS NMR spectra of γCD at various hydrate levels.

rings adapt to changes in the hydrogen-bonding environment, and a dispersion in the torsion angles results.

X-ray powder diffraction patterns are also sensitive to the hydration level of the crystal. Initially, in the materials with very little water loss, the relative intensities of some low-angle reflections change. On increasing dehydration, and corresponding with the grosser changes in the NMR spectra, new reflections appear and previous reflections diminish in intensity. This situation is indicative of a change in phase or the appearance of new phases. However, the NMR spectra do not appear as superpositions of previous and new resonances, and, in addition, the resonances become progressively broader on increasing dehydration. Thus, it may be concluded that dehydration does not lead to any new distinct phases, but probably to an increasing heterogeneity in the sample, such that the available water molecules are not distributed statistically within the crystal lattice. The form of the DTG curves of the TGA analyses suggests the possibility of two separate processes, which might correspond to loss of *intra*- and *inter*-cavity water molecules. It may

be speculated that *intra*cavity water is lost initially and the "emptying" of the CD cavity may cause conformational rearrangement of the CD. This effect is seen most clearly in the spectra for the αCD · ~ $5H_2O$, in which the highest-field C-1 and C-4 resonances, ascribed to the linkage strained by rotation of a glucose ring into the torus in order to hydrogen bond with included water, have shifted significantly to lower field compared with those for αCD · $6H_2O$. As expected, the C-6 region also shows marked changes, due to the effects on the chemical shift of disruption of at least two hydrogen bonds to HO-6 caused by the loss of the *intra*cavity water molecules. For β- and γ-CD, the chemical shift changes on dehydration are not so marked, and may not be interpreted so reliably in conformational terms. For these hydrates, the changes in the macrocyclic conformation are not expected to be as dramatic as those for αCD. Eventually, on removal of nearly all the water, the ^{13}C resonances become very broad, reflecting the more amorphous nature of the material. It would be expected that, as the *inter*cavity water molecules are removed, the stacking order of the CD molecules would be severely disrupted, leading to the amorphous state observed. Indeed, the spectra of the dehydrated materials are very similar to those of lyophilised CDs.

Conclusions.—In conclusion, although conformational changes about the (1 → 4) glycosidic linkage are clearly *prima facie* factors in determining the ^{13}C chemical shifts of the C-1 and C-4 carbons of the CD hydrates, a conformational approach is not so successful for C-6 resonances, particularly as their shifts seem to be very sensitive to hydrogen-bonding interactions, as indicated by the spectral changes on loss of water from the crystalline structure. The scatter in the correlation of chemical shifts with torsional parameters, even for C-1 and C-4, indicates that the shifts of such materials are not caused by intramolecular conformations alone, and we do not expect the chemical shifts to allow prediction of conformation, except for carbons very close to the centre of conformational change for which chemical shift changes due to alterations in local environment are likely to be small. More realistically, the spread of ^{13}C shifts for a particular carbon resonance is a good indication of the range of conformations to be found within any particular structure. This conclusion reflects growing realisation that chemical shifts in the solid state are the result of a subtle interplay of many different NMR interactions, and thus contain much structural information, although it is generally more problematical to extract that information than has been the case for conformational analysis for solutions.

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