C.P.–D.D.–M.A.S. 13C-N.M.R. INVESTIGATIONS OF ANHYDROUS AND HYDRATED CYCLOMALTO-OLIGOSACCHARIDES: THE ROLE OF WATER OF HYDRATION

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ABSTRACT

Hydrated and anhydrous cyclomaltohexaose, cyclomaltoheptaose, and cyclomalto-octaose (cyclodextrins) have been investigated by the c.p.–d.d.–m.a.s. 13C-n.m.r. technique. The chemical shifts of the signals of C-1 and C-6 provide information about conformation and the results agree fairly well with the earlier scattering data on hydrated systems, but some discrepancies have been found for cyclomaltohexaose. The conformation of the macro-rings seems to be determined by the hydration water. The unique role of water in forming crystals of cyclomalto-oligosaccharides is demonstrated.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) are cyclic oligosaccharides containing 6 (cyclomaltohexaose, α-CD), 7 (cyclomaltoheptaose, β-CD), or 8 (cyclomalto-octaose, γ-CD) (1→4)-linked α-D-glucopyranosyl residues. The torus-shaped macro-rings can form inclusion complexes with various guest molecules and are of considerable interest in research and industry[1].

Previous studies of CD inclusion-complexes by 13C-n.m.r. spectroscopy have provided information on the molecular properties[2–15]. Some studies[2–6] refer to solutions where the macro-rings are in rapid exchange between various conformations. The c.p.–d.d.–m.a.s. (cross polarisation–dipolar decoupling–magic angle spinning) technique has enabled solid, crystalline, or amorphous samples to be investigated. Mainly solid complexes with various guest molecules have been investigated[7–15] up to now. However, little information has been gained on the CDs "complexed" only with water molecules. The early work of Saito *et al.*[7,8] and the recent study of Inoue *et al.*[14] described spectra of hydrated CDs with relatively poor resolution.

CD hydrates have been studied by X-ray and neutron scattering[16–28], which

have defined atomic positions and molecular conformations. However, these methods are not suitable for investigating microcrystalline materials and powders. Powder diffraction methods can be applied to CD hydrates[29], but the results refer to the crystal structure only. In order to obtain precise information on conformation, sufficiently large crystals of CD hydrates are needed. the existence of which is strongly determined by their water content.

No such problems exist with n.m.r. spectroscopy, and the chemical shifts of the ^{13}C signals of carbohydrate polymers can give information about molecular conformation[30-32]. Thus, the chemical shifts of the signals for C-1 and C-4, respectively, reflect the ϕ and ψ dihedral angles around the glycosidic linkage, and that for the signal of C-6 is affected by the torsional state around the C-5–C-6 bond.

A study of CD hydrates should give information additional to earlier scattering data[33]. Moreover, a comparative study of anhydrous and hydrated CDs could demonstrate the effect of water on conformation.
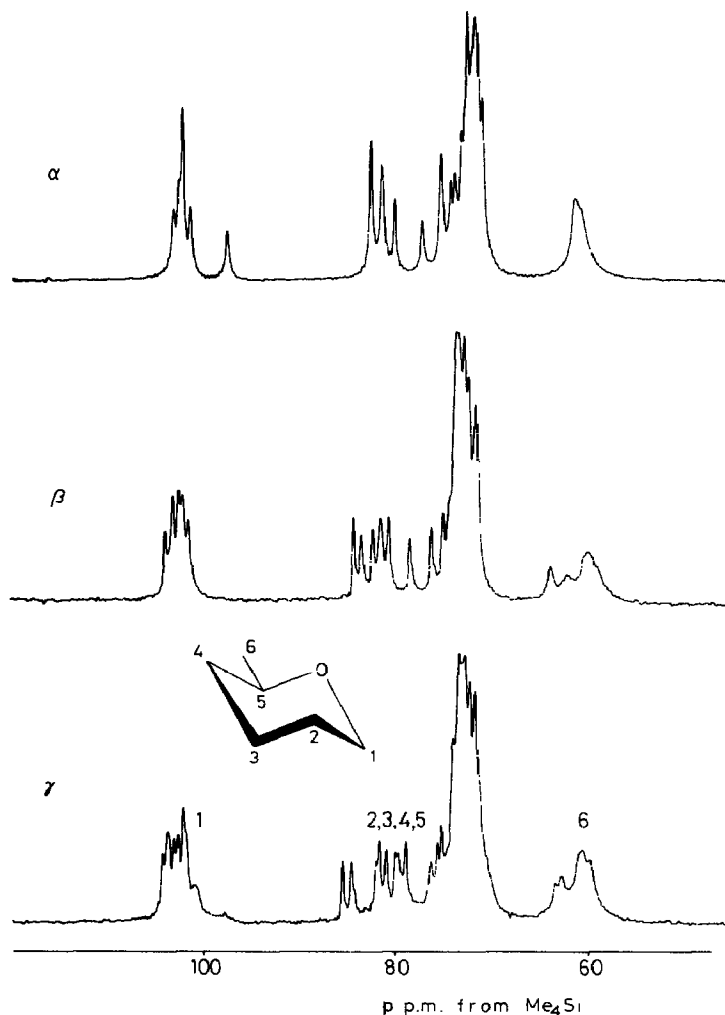

Fig. 1. The c.p.–d.d.–m.a.s. ^{13}C-n.m.r. spectra of α-CD 6.4 hydrate, β-CD 10.0 hydrate, and γ-CD 13.7 hydrate.

EXPERIMENTAL

The samples studied, namely, α-CD 6.4 hydrate, β-CD 10.0 hydrate, and γ-CD 13.7 hydrate were commercial products of Chinoin (Budapest). The high-resolution ^{13}C-n.m.r. spectra (50 MHz) of these materials (Fig. 1) were recorded with a Bruker CXP-200 spectrometer of the Tallinn group. All measurements were performed at room temperature with a spinning rate of 3 kHz. The repetition, contact, and decoupling times were 3 s, 1 ms, and 60 ms, respectively. The anhydrous samples were obtained by vacuum drying at room temperature and their spectra are presented in Fig. 2. The ^{13}C chemical shifts were converted into the Me$_4$Si scale by using the methyl resonance of external hexamethylbenzene at 16.9 p.p.m. The chemical shift data of C-1 and C-6 are presented in Table I.
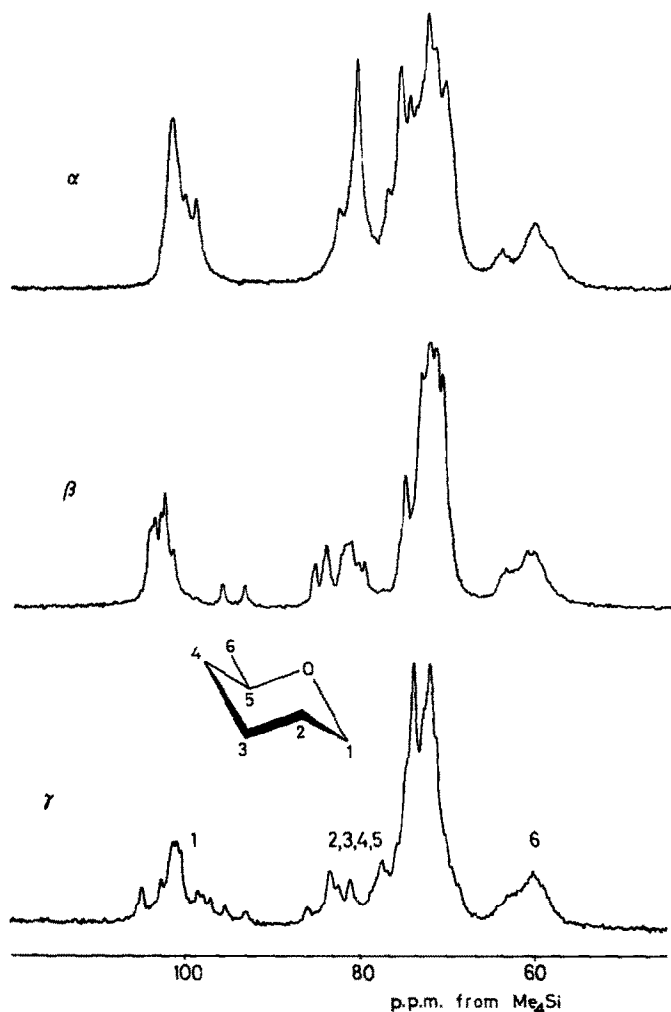

Fig. 2. The c.p.–d.d.–m.a.s. ^{13}C-n.m.r. spectra of anhydrous α-, β-, and γ-CD.

TABLE I

CHEMICAL SHIFT DATA FOR C-1 AND C-6 IN CYCLOMALTO-OLIGOSACCHARIDES

	Hydrated			Anhydrous		
	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
C-1 (p.p.m.)	103.5	104.1	104.6	102.3	104.6	104.9
	103.0	103.4	104.1	100.7	104.3	102.7
	102.6	102.8	103.5	99.5	104.0	101.5
	101.8	102.4	103.1		103.3	101.2
	97.8	101.8	102.5		102.9	100.6
			102.2		101.9	98.8
			101.2		96.3	98.1
					93.8	97.3
						95.6
						93.2
C-6 (p.p.m.)	61.5	64.0	63.6	64.6	64.0	63.3
	61.3	62.3	62.9	61.0	61.5	60.3
	60.8	60.1	61.1	59.1	60.0	
			60.7			
			60.0			

RESULTS AND DISCUSSION

X-Ray and neutron scattering methods have revealed common and dissimilar structural features of α-, β-, and γ-CD hydrates having degrees of hydration similar to those of our samples[26,27]. A strong and extended hydrogen-bonding system involving the hydration water and glucose hydroxyl groups was present in each of the materials investigated. This net-work of hydrogen bonds may determine some important properties of crystalline CD hydrates, e.g., macro-ring conformations.

α-CD includes one or two water molecules in its cavity, depending on the process of crystal growth[16,17,21]. The presence of hydrogen bonds between two HO-6 groups of the macro-ring and the included water molecules has been demonstrated. It has been suggested[16,17] that these hydrogen bonds cause a strain in the macro-ring that affects the conformation of one of the glucopyranosyl residues, in that the ϕ and ψ dihedral angles are 154° and −135° instead of the average values 166° and −169°, respectively[20,21]. The orientation about the C-5–C-6 bond is defined by the torsion angle O-5–C-5–C-6–O-6 and, depending on this angle, the conformations about the C-5–C-6 bond can be gauche–gauche (gg) or −gauche and gauche–trans (gt) or +gauche. In the gg and gt conformations, HO-6 is directed "away" and "toward" the cavity, respectively. Diffraction studies have shown that the two HO-6 groups that are hydrogen-bonded to included water molecules are associated with the gt conformation, whereas the other four have gg orientations.

In contrast to α-CD, the β- and γ-CD hydrates have roughly symmetrical macro-rings and the cavities include five and nine water molecules, respectively[18,22,24,25]; the hydrogen bond to included molecules causes no distortion of the kind observed for α-CD. The hydrogen-bond networks of water molecules in these larger CDs seem to be less rigid than in α-CD[34,35]. The distribution of conformations about the C-5–C-6 bond is four *gg*, two *gt*, and one disordered in β-CD, and four *gg*, three *gt*, and one disordered in γ-CD.

The chemical shifts of the signals for C-1, C-4, and C-6 reflect the conformations of the macro-rings and HO-6. The orientation of HO-6 can be obtained accurately from the chemical shifts by using the scaling reported by Horii *et al.*[31,32]. However, the conformation of the macro-ring can be characterised only qualitatively, because the scaling between the C-1 and C-4 shifts and dihedral angles has been determined only for cellobiose analogues[30,32] (and not for the maltose series) and the cyclic structure of CDs deforms the glycosidic linkages resulting in some up-field shifts[2]. Moreover, only C-1 shifts can be used, because the C-4 lines are close to the range of the C-2,3,5 resonances and cannot be separated clearly.

The scattering and n.m.r. results relating to the conformation of the macro-rings in hydrated systems are in agreement. The C-1 shifts of β- and γ-CDs are in a small range, indicating similar dihedral angles for different glucopyranosyl residues and thus a roughly symmetrical macro-ring. However, a distinct C-1 line is observable in the spectrum of α-CD (Fig. 1).

The information obtained from C-6 shifts agrees well with the scattering data for β- and γ-CD. The reported ^{13}C shift values for *gg* and *gt* conformations (60–62.6 and 62.5–64.5 p.p.m., respectively[31,32]) fit well the observed spectral lines. However, the spectrum of α-CD contains only C-6 shifts that are <61.5 p.p.m., which means that only *gg* ("away" from the macro-ring) HO-6 conformations are present.

This finding contrasts with the scattering results, but it is not due to the C-6 shift scaling since the β- and γ-CD spectra contain both *gg* and *gt* resonances. Inaccuracies in the measurement of chemical shifts can also be excluded because they were accurate to <0.2 p.p.m. The recent report on the field-strength dependence of ^{13}C chemical shifts measured by the c.p.–d.d.–m.a.s. method[36] shows that the different fields used by Horii *et al.*[31,32] and in this work could result in a systematic shift difference of <0.2 p.p.m. On the other hand, the significant scattering results show definite conformations around C-5–C-6 and no disorder. A possible explanation may be the different structure of the samples investigated (specially grown crystals for scattering studies and commercial powders for n.m.r. measurements).

The original and widely accepted explanation of scattering results[16,17,21] is the occurrence of one anomalous glucopyranosyl residue in the α-CD macro-ring. The conformation of this residue is affected by a hydrogen bond between the oxygen of included water and HO-6, which is possible only when the conformation around the C-5–C-6 bond is *gauche–trans*. Our α-CD spectrum shows a distorted macro-

ring and no *gauche–trans* conformations. The question is how can the macro-ring strain exist independently of the included water? Our α-CD hydrate might have a structure in which the macro-ring strain is caused by water molecules outside the CD cavity.

The common feature of the spectra of dry samples is the significant line-broadening which might be due to the originally crystalline samples becoming amorphous. Comparison of the shifts of the C-1 signals for the dry and hydrate samples (Table I) reflects the expected behaviour of the macro-ring of α-CD, but presents surprising effects for β- and γ-CD. A slightly distorted macro-ring was expected in anhydrous α-CD since the water, responsible for the distortion, has been removed. A change in the symmetrical, hydrated macro-ring conformation was not expected during the dehydration of β- and γ-CD.

The C-1 resonances of anhydrous α-CD are in a narrow group, whereas those for the hydrated α-CD spectrum give a distinct peak at 97.8 p.p.m. This narrow group possibly reflects a decrease in the distortion of the macro-ring in α-CD. The opposite effect was observed in the spectra of anhydrous β- and γ-CD, each of which contained a low-shift C-1 peak reflecting marked distortion of the macro-ring. Because of the cross polarisation, the spectral intensities do not reflect rigorously the ratios of different conformations, so we can estimate that the spectrum of β-CD indicates not more than two distorted glucopyranosyl residues in the macro-ring and their shift values are similar to those for hydrated α-CD. Anhydrous γ-CD may have more distorted units.

The collapse of the symmetrical hydrated structures may be due to the strain in the glucopyranosyl residues in hydrated β- and γ-CDs[22,25]. This strain, which has a simple geometric origin, is balanced in the hydrated state by an extended hydrogen-bond system of water molecules. Evaporation of the water restores the regular conformation of the glucopyranosyl residues, causing distortion of the macro-ring.

Added in proof. — Only after submission of this paper did the authors become aware of well-resolved c.p.–d.d.–m.a.s. ^{13}C-n.m.r. spectra of hydrated α- and β-CD[37]. This article also indicated the connection between chemical shifts and the conformation of macro-rings.

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