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HIGH-RESOLUTION, C.P.–M.A.S., ^{13}C-N.M.R. SPECTRA OF SOLID AMYLODEXTRINS AND AMYLOSE POLYMORPHS

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ABSTRACT

High-resolution, solid-state, ^{13}C-n.m.r. spectra of a series of amylodextrins, either linear or cyclic, together with those of the most common polymorphs of linear amylose, have been obtained by cross-polarization–magic-angle-spinning (c.p.–m.a.s.) techniques. The establishment of a correlation between the orientations of the primary hydroxymethyl groups and the ^{13}C chemical shifts displayed by the C-6 atoms shows that a conformational mapping can be attempted through high-resolution, solid-state n.m.r. spectroscopy. A qualitative correlation between the dispersion of the orientations at the glycosidic linkages of the crystals and the dispersion of the chemical shifts is also proposed.

INTRODUCTION

Amylose, the linear fraction of starch, is deposited in the crystalline form within the native granule. It is an α-(1→4)-linked polymer of D-glucose, and has the well established structure depicted in formula **1**. Depending on the origin, the amylose of native starch has two main polymorphs: the A polymorph in cereal starches, and the B polymorph in tuber starches. By precipitation and further drying of the native amyloses, a so-called V structure is obtained. Double-helical chains have been proposed for the A and B forms[1–3], whereas it is currently believed that single-helical amylose chains occur in type V amylose[4,5]. The action of cyclodextrin glucanotransferase (EC 2.4.1.19) on starch yields a series of cyclic maltodextrins containing 6–12 D-glucosyl units per ring. The main fractions contain cyclomaltohexaose, cyclomaltoheptaose, and cyclomalto-octaose (α, β, and γ-

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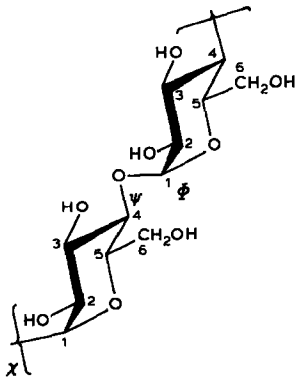

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cyclodextrins). They all form inclusion compounds with small molecules that fit into their 5–8-Å cavity[6], as established from crystal-structure elucidations[7–9]. X-Ray diffraction data on linear maltodextrins[10–16] and conformational analysis[17] revealed a range of orientations about the glycosidic bonds in these compounds.

The purpose of the present work was, first, to examine, systematically, the solid-state, c.p.–m.a.s., ^{13}C-n.m.r. spectra of a series of amylose oligomers, both linear and cyclic, together with those of the most common polymorphs of linear amylose and, ultimately, "branched amylose" such as amylopectin. Highly reliable, structural data are available for most of the amylosic oligomers investigated. Therefore, the chemical-shift data obtained for these compounds by c.p.–m.a.s., solid-state, ^{13}C-n.m.r. spectroscopy might provide correlations and information that could be helpful in the structural understanding of unknown or less-ordered systems.

EXPERIMENTAL

General. — Samples of α-D-glucose, methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, β-maltose monohydrate, and cyclomalto-hexaose, -heptaose, and -octaose were obtained commercially. β-Maltose octaacetate was prepared by conventional acetolysis of amylose, and recrystallized from ethanol. It was a gift from Dr. H. Driguez, CERMAV, France. The high purity of these samples and their anomeric configurations were confirmed by high-resolution, solution n.m.r. spectroscopy.

Crystalline amyloses A, B, and V were prepared as follows[18].

Single crystals of amylose A. — Amylose having a degree of polymerization (d.p.) of 15 was obtained by mild hydrolysis of potato starch[19]. A suspension of the sample in water (<1%, w/v) was heated in an autoclave for 30 min at 130°, allowed to cool, and filtered. The product was recrystallized by precipitation in 7:13 (v/v) water–ethanol at 50°.

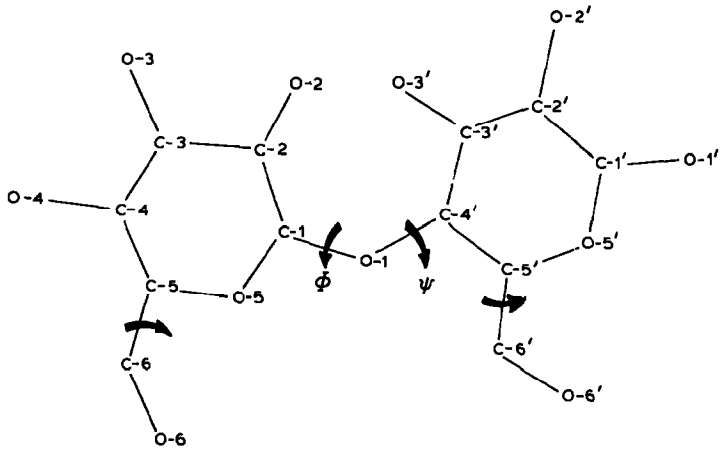

Fig. 1. Schematic representation of the dimeric fragment of amylose, namely, α-maltose, along with the atom numbering and the torsion angles of interest.

Single crystals of amylose B. — Amylose B having a d.p. of 35 was obtained by hydrolysis of wrinkled-pea starch with 2.2M HCl for 35 d at 35°. Batches of a suspension of this amylose in water were heated at 100°, which caused almost total dissolution; these were then filtered into thick-walled, glass ampoules, which were sealed and kept for 15 min at 160°, in order to erase any memory of previous crystal forms. The product crystallized from solution at room temperature.

Single crystals of amylose V. — The procedure was similar to that used for preparation of single crystals of amylose A. The product was recrystallized by precipitation in 1:9 (v/v) water–ethanol at 50°.

Spectra. — Solid-state, ^{13}C-n.m.r. spectra at 25.18 MHz were recorded with a Bruker CXP-100 spectrometer. Proton 90° pulse-widths were 5 μs, and cross-polarization times were 2 ms. Matching conditions were checked with an adamantane standard. The magic angle was set by monitoring the ^{79}Br-n.m.r. spectrum[20] of KBr incorporated in the rotor. Sample containers (Andrew Beams type) were made of deuterated poly(methyl methacrylate) and were spun at 4.4 kHz. Characteristically, 200–10,000 acquisitions were obtained per spectrum, with recycle times of 4 s. All peaks in the spectra were referenced to the peak of linear polyethylene (33.6 p.p.m.). A small amount of polyethylene was added to each sample[21].

The dimeric fragment of amylose, namely, maltose, is shown in Fig. 1, along with the numbering of the atoms and the torsion angles of interest. The numbering* of the atoms proceeds from the nonreducing end (unprimed group) to the reducing end (primed residue). Extension of this numbering to a maltotriose molecule implies the use of double-primed atoms for the reducing residue of the trisaccharide.

Editor's note. It is customary to assign unprimed numbers to the atoms of the glycose residue, and primed numbers to those of the glycosyl group.

The sign of the torsion angles is defined in agreement with the rules recommended by the IUPAC–IUB Comission on Biochemical Nomenclature[22].

The torsion angles of interest are defined as follows.

$\phi = \theta$[O-5–C-1–O-1–C-4′]
$\psi = \theta$[C-1–O-1–C-4′–C-5′]

The orientation in terms of the primary hydroxyl group at C-6 is referred to as either *gauche-trans* or *gauche-gauche*[23]. In this terminology, the torsion angle θ[O-5–C-5–C-6–O-6] is stated first, and then the torsion angle θ[C-4–C-5–C-6–O-6].

RESULTS

C.p.–m.a.s., ^{13}C-n.m.r. spectra of D-*glucopyranoses and linear amylodextrins.*

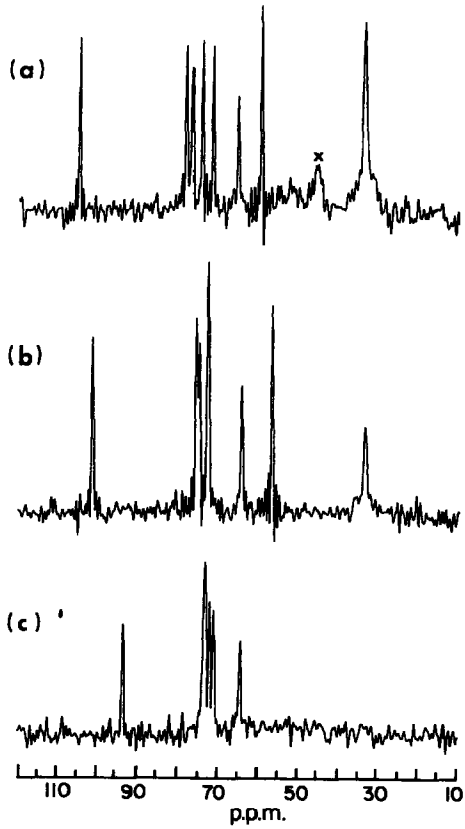

Fig. 2. 25.18-MHz, C.p.–m.a.s., ^{13}C-n.m.r. spectra of (a) methyl β-D-glucopyranoside, (b) methyl α-D-glucopyranoside, and (c) α-D-glucopyranose. Resonances at 40–50 p.p.m. are due to the rotor [deuterated poly(methyl methacrylate)] and are marked ×. [Polyethylene (added to some samples) was assigned a chemical shift at 33.6 p.p.m. relative to liquid Me_4Si. Before all Fourier transformations, a −5-Hz line-broadening was applied.]

TABLE I

^{13}C-CHEMICAL SHIFTS[a] OF SOLID α-D-GLUCOPYRANOSE AND METHYL D-GLUCOPYRANOSIDES AT 25.18 MHz

Compound	*C-1*	*C-2, C-3, C-4, C-5*	*C-6*	*C-Me*
Me β-D-Glc*p*	104.5	78.0–76.5–74.1–71.4	65.0	59.4
Me α-D-Glc*p*	101.5	75.9–75.1–72.8	64.2	56.9
α-D-Glc*p*	93.5	73.5–72.4–71.2	64.4	

[a]Chemical shifts, referenced to liquid Me_4Si, were measured from polyethylene at 33.6 p.p.m.

^{13}C-CHEMICAL-SHIFT ASSIGNMENTS[25] FOR α-D-Glc*p* AND α-D-Glc*p*·H_2O

Compound	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
α-D-Glc*p*	92.8	70.5	73.0	72.6	71.6	63.7
α-D-Glc*p*·H_2O	92.9	70.9	73.0	69.8	71.7	60.8

— The c.p.–m.a.s., ^{13}C-n.m.r. spectra of α-D-glucopyranose, methyl α-D-glucopyranoside, and methyl β-D-glucopyranoside are shown in Fig. 2. The chemical shifts are listed in Table I. The resonances in the spectrum of α-D-glucopyranose are identified with reference to reports by Pfeffer *et al.*[24,25]. The resonance of the anomeric carbon atom (C-1) in the α configuration occurs at 93.5 p.p.m., whereas the C-6 atom (bearing the primary hydroxyl group) gives a peak at 64.4 p.p.m. For the two methyl D-glucopyranosides, the resonance of the methyl carbon atoms occurs at 59.4 (α configuration) and 56.9 p.p.m. (β configuration), whereas the resonance of the C-6 atom is at 65.0 and 64.2 p.p.m. for the α and β configurations, respectively. These two spectra are intended to show that, as observed in high-resolution, n.m.r. spectroscopy of carbohydrates in solution, the resonance of the β C-1 atom (104.5 p.p.m.) is shifted downfield with respect to the resonance of the α C-1 carbon (101.5 p.p.m.).

The c.p.–m.a.s., ^{13}C-n.m.r. spectra of β-maltose monohydrate and β-maltose octaacetate are shown in Fig. 3. The appropriate chemical shifts are summarized in Table II. Ten (almost perfectly resolved) resonances are found in the spectrum of crystalline β-maltose monohydrate. The resonance of C-1 at the α-D-(1→4) linkage occurs at 97.8 p.p.m., whereas the C-1′ atom of the reducing residue gives a peak at 105.2 p.p.m.; this assignment was made by analogy with the c.p.–m.a.s., ^{13}C-n.m.r. spectra mentioned in the preceding paragraph. The resonances at 66.4 and 60.9 p.p.m. can be attributed to the C-6 and C-6′ atoms of the primary hydroxymethyl groups. The rest of the spectrum can be assigned only tentatively, with the presumption that C-4′ resonates at 80.9 p.p.m.

As for β-maltose octaacetate, all of the methyl carbon nuclei resonate at 21.4 p.p.m., whereas two distinct peaks (171.9 and 171.0 p.p.m.) correspond to the carbonyl carbon nuclei of the acetate groups. The resonance at 97.0 p.p.m. is attributed to the β-anomeric C-1′ nucleus at the reducing end. The upfield shift of

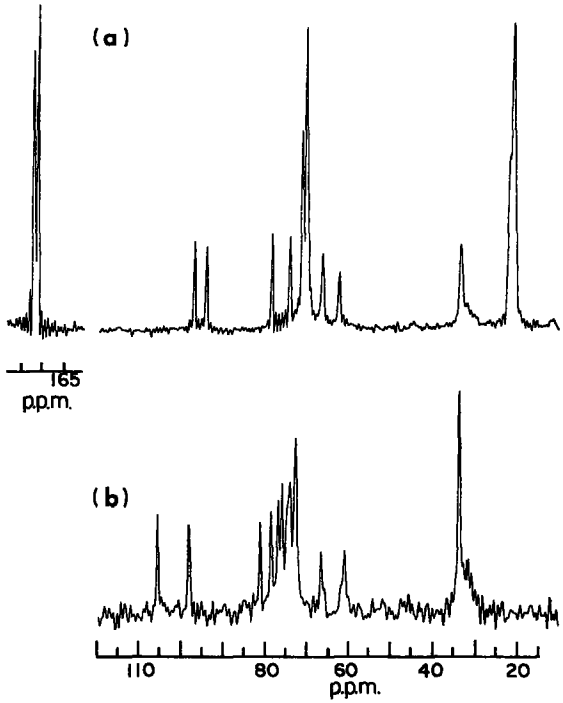

Fig. 3. 25.18-MHz, C.p.–m.a.s., ^{13}C-n.m.r. spectra of (a) β-maltose octaacetate, and (b) β-maltose monohydrate. [Polyethylene (added to the samples) was assigned a chemical shift of 33.6 p.p.m. relative to liquid Me_4Si. Before all Fourier transformations, a −5-Hz line-broadening was applied.]

8.2 p.p.m. found for this atom in β-maltose octaacetate as compared to β-maltose is explained by the influence of the acetate group. Similar behavior is found in solution. Consequently, the resonance at 94.1 p.p.m. can be attributed to the C-1 nucleus at the glycosidic linkage. The two well-resolved peaks at 78.6 and 74.3 p.p.m. undoubtedly correspond to C-6 and C-6′ of the primary acetate groups. The remaining six carbon nuclei [C-1, C-3, C-5, C-2′, C-3′ and C-5′] yield two resolved peaks, at 71.4 and 70.4 p.p.m.

C.p.–m.a.s., ^{13}C-n.m.r. spectra of cyclodextrins. — Cyclomalto-hexaose,

TABLE II

^{13}C-CHEMICAL SHIFTS[a] OF SOLID β-MALTOSE OCTAACETATE AND β-MALTOSE HYDRATE AT 25.18 MHz

Compound	*C-1*	*C-1′*	*C-4*	*C-4′*	*C-2, C-2′, C-3, C-3′, C-5, C-5′*	*C-6*	*C-6′*
β-Maltose octaacetate	94.1	97.0	78.6	74.3	71.4–70.4	66.6	62.7
β-Maltose · H_2O	97.8	105.2	80.9	78.4	76.7–75.7–73.7–72.6	66.4	60.9

[a]Chemical shifts, referenced to liquid Me_4Si, were measured from polyethylene at 33.6 p.p.m.

-heptaose, and -octaose give highly crystalline samples, which, in turn, yield well resolved, c.p.–m.a.s., ^{13}C-n.m.r. spectra (see Fig. 4). As described in the preceding paragraph, four distinct regions of resonances occur consistently in each spectrum. These correspond to the resonances of the C-1 (99–106 p.p.m.), C-4 (77–87 p.p.m.), and C-6 nuclei (60–65 p.p.m.); the resonances of the remaining carbon nuclei (71–76 p.p.m.) are not well resolved, and are centered at ~73 p.p.m. The appropriate chemical shifts observed for cyclomalto-hexaose, -heptaose, and -octaose are summarized in Table III.

In contrast to what is usually observed in the high-resolution, n.m.r. spectra of cyclomalto-hexaose, -heptaose, and -octaose in solution, significant splittings associated with resonances of chemically identical carbon atoms are revealed in the c.p.–m.a.s., ^{13}C-n.m.r. spectra. Six partially resolved peaks are found for the C-1 resonances of cyclomaltohexaose. Four peaks, two of which are of high intensity, are observed for the C-1 resonances of cyclomaltoheptaose, whereas five peaks can be seen for the resonances corresponding to the C-1 nucleus in cyclomalto-octaose. This spectral complexity is also exemplified by the significant splittings (up to 5)

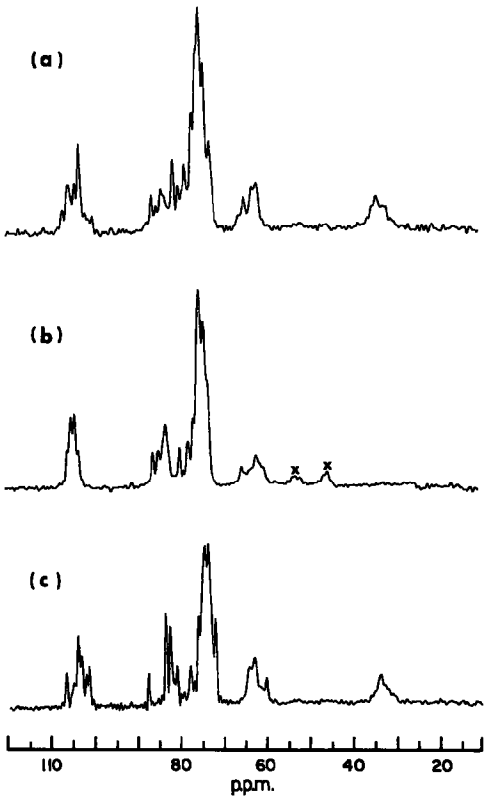

Fig. 4. 25.18-MHz, Cp.–m.a.s., ^{13}C-n.m.r. spectra of (a) cyclomaltohexaose hydrate, (b) cyclomalto-heptaose hydrate, and (c) cyclomalto-octaose hydrate. [All chemical shifts, referenced to liquid Me_4Si, were measured from polyethylene at 33.6 p.p.m. Resonances due to the rotor are marked ×.]

TABLE III

^{13}C-CHEMICAL SHIFTS[a] OF SOLID CYCLOMALTODEXTRINS AT 25.18 MHz

Compound	*C-1*	*C-2, C-3*	*C-4, C-5*	*C-6*
Cyclomaltohexaose	105.7, 104.4 103.0, 102.1	85.4, 84.2 83.1, 80.3 79.4	77.6–75.9–74.1 73.2–72.0	64.0, 62.1 61.1
Cyclomaltoheptaose	104.9, 104.1 103.4, 102.4	85.3, 84.4 83.4, 82.6 79.3	77.2	65.0, 61.5
Cyclomalto-octaose	106.1, 103.4 102.6, 101.7 100.9	87.3, 83.2 82.3, 80.7	77.4–75.7–74.3 73.4–71.8	63.9, 62.7 61.2, 60.0

[a]Chemical shifts, referenced to liquid Me_4Si, were measured from polyethylene at 33.6 p.p.m.

observed for the ^{13}C signal of C-4. The resonances of the C-6 nuclei of the primary hydroxymethyl groups are consistently split into at least two peaks.

C.p.–m.a.s., ^{13}C-n.m.r. spectra of amyloses. — The c.p.–m.a.s., ^{13}C-n.m.r. spectra of amylose V, amylose A (d.p. 15), amylose B (d.p. 35), pure (long chains) amylose, and amylopectin are shown in Fig. 5. The ^{13}C signals of the C-1 and C-6 nuclei are well resolved from the rest of the spectrum. As observed for the linear and cyclic oligomers, the C-2, C-3, and C-5 signals overlap. Because of the increased line-widths, compared with those of related, small, model compounds, the ^{13}C signal of C-4 also overlaps the C-2, C-3, and C-5 signals.

Some consistent features are clear. For amyloses V, B, and A, a line width of ~6 p.p.m. is found for resonances of C-1 nuclei, with some indications of two maxima, at ~100 and 101 p.p.m. The ^{13}C resonance of C-6 consistently occurs at ~62.5 p.p.m. The C-2, C-3, C-4, and C-5 signals are slightly better resolved for amylose A (d.p. 15) and amylose B (d.p. 35) than for the other cases. There is also evidence for a peak at ~71.5 p.p.m. for amylose A. The resolution may be attributed, in part, to the relatively high crystallinity of the two samples. The general

TABLE IV

^{13}C-CHEMICAL SHIFTS[a] OF SOLID AMYLOSES AT 25.18 MHz

Compound	*C-1*	*C-2,. C-3, C-4, C-5*	*C-6*
Amylose A	102.1–100.5	76–72.8–71.7	62.7
Amylose B	100.3	76–73.2–71.6	62.9
Amylose V	101.3	76.7–72.8–71.2	62.9
Amylose	102.1	73.2	62.5
Amylopectin	101.9–100.3	73.4	63.1

[a]Chemical shifts, referenced to liquid Me_4Si, were measured from polyethylene at 33.6 p.p.m.

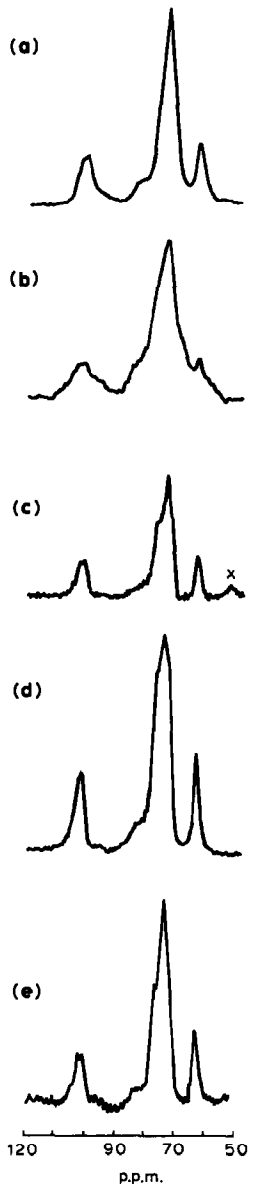

Fig. 5. 25.18-MHz, C.p.–m.a.s., ^{13}C-n.m.r. spectra of (a) amylopectin, (b) pure amylose, (c) amylose V, (d) amylose B (d.p. 35), and (e) amylose A (d.p. 15). [In (c), the resonance near 50 p.p.m. is from the deuterated poly(methyl methacrylate) rotor, and is marked ×.]

features of these spectra are similar to those reported by Saito and Takeda[26]. However, an upfield shift of ~3 p.p.m. for the low-molecular-weight amyloses, as compared with that of the high-molecular-weight amylose, was not seen. Also, the spectrum obtained by Saito and Takeda for amylose V has a peak at ~80 p.p.m.;

such a resolved resonance is not apparent in Fig. 5. The resolution obtained for the c.p.–m.a.s., ^{13}C-n.m.r. spectrum of amylopectin is surprisingly better than that of that of the sample of pure, high-d.p. amylose. The appropriate chemical shifts are summarized in Table IV.

DISCUSSION

Previous investigations on the conformational behavior of amylodextrins have shown that some geometrical alterations could occur within the α-D-glucosyl residues[27]. Nonetheless, the torsion angles ϕ and ψ about the (1→4)-glycosidic junction, as well as the orientations of the primary hydroxymethyl groups, are the most important sites of conformational variations. Tables V and VI demonstrate a tentative correlation between some of the conformational parameters in the crystal structures and the corresponding chemical shifts in c.p.–m.a.s., ^{13}C-n.m.r. spectroscopy.

The correlation between the orientation of the primary hydroxymethyl groups and the ^{13}C-chemical shift of C-6 is striking. Although the existence of such a correlation had been suspected[28], our results establish that the resonance of C-6 in a *gauche-gauche* orientation occurs at ~61.5 p.p.m., whereas the resonance of

TABLE V

^{13}C-CHEMICAL SHIFTS OF THE PRIMARY CARBON NUCLEUS (C-6) *vs.* THE ORIENTATION ABOUT THE EXOCYCLIC, C-5–C-6 BOND AS FOUND IN THE CORRESPONDING CRYSTAL STRUCTURE

Compound	δ	*Orientation*
α-D-Glucopyranose	64.4	*gauche-trans*
Me α-D-glucopyranoside	64.2	*gauche-trans*
Me β-D-glucopyranoside	65.0	*gauche-trans*
β-Maltose · H_2O	60.9	*gauche-gauche*
	66.4	*gauche-trans*
β-Maltose octaacetate	62.7	*gauche-gauche*
	66.4	*gauche-trans*
Cyclomaltohexaose	60.0	*gauche-gauche*
		gauche-gauche
	61.2	*gauche-gauche*
		gauche-gauche
	62.7	*gauche-trans*
		gauche-trans
	63.9	
Cyclomaltoheptaose	61.5	*gauche-gauche*
		gauche-gauche (d)
		gauche-gauche
		gauche-gauche
		gauche-trans
		gauche-trans
		gauche-trans (d)
	65.0	*gauche-trans*

TABLE VI

^{13}C-CHEMICAL SHIFTS OF THE C-1 NUCLEUS *vs.* THE ORIENTATION ABOUT THE GLYCOSIDIC BOND (TORSION ANGLES ϕ AND ψ)

Compound	$\delta_{C\text{-}1}$	ϕ *(degrees)*	ψ *(degrees)*
β-Maltose	97.8	121.7	−107.7
β-Maltose octaacetate	94.0	84.0	−154.8
Cyclomaltohexaose	100.9	112.8	−103.2
	101.7	104.8	−110.5
	102.6	107.5	−115.4
	103.4	88.2	−123.7
		90.4	−69.3
	106.1	100.7	−118.5
Cyclomaltoheptaose	102.4	110.6	−106.5
	103.4	115.0	−99.3
	104.1	103.0	−125.7
		120.0	−109.7
	104.9	110.9	−113.9
		107.6	−109.4
		102.2	−121.0
		109.9	−112.2

C-6 in a *gauche-trans* orientation occurs at ~65.0 p.p.m. Such a difference in the chemical shift is somewhat comparable to that observed between the resonances of a C-1 atom in either the α- or the β-glycosidic configuration. As already suggested in an investigation of the orientational features of primary hydroxymethyl groups in the solid state[23], stereoelectronic effects should be included in the calculation of the stable conformers that result from rotation about the exocyclic C-5–C-6 bond in hexopyranoses.

The occurrence of a correlation between the orientations at the glycosidic linkage and the ^{13}C-chemical shifts of C-1 was looked for. In the case of cyclomaltohexaose, a dispersion of ~5 p.p.m. is observed for the ^{13}C-chemical shifts of the glycosidic C-1, somewhat in agreement with the dispersion of the orientations about the glycosidic linkages observed in the crystal structure[7]. With respect to the ^{13}C-chemical shift of C-1 of β-maltose, all of the resonances observed are shifted downfield. The dispersion of the observed chemical shift agrees with the dispersion of the orientations dictated by (ϕ, ψ) in the solid state. Among these, that at ϕ 90.40° and ψ −69.30° lies away from those centered about ϕ 105° and ψ −110°. Despite its intrinsically enhanced flexibility, the cyclomaltoheptaose molecule in the solid state has a fairly constant orientation at the α-D-(1→4) glycosidic linkage[8]. Such a constancy is reflected in the limited range of ^{13}C-chemical shifts observed for the C-1 atoms (102.4–104.9 p.p.m.). A second class of stable conformers is typified by β-maltose octaacetate[13]. On the average, the ϕ angles are 40° away from those of the preceding class. The ^{13}C-chemical shift of C-1 for β-maltose octaacetate, at 94.0 p.p.m., differs significantly from that of β-maltose, at 97.8 p.p.m. It is not yet

clear whether such a difference reflects orientational changes at the glycosidic linkage or the influence of the primary and secondary acetate groups.

Despite the fact that the samples of amylose A, B, and V used showed high crystallinity[18], the spectra are not well resolved. The systematic splitting of ~1–1.5 p.p.m. observed for C-1 nuclei suggests distinct orientations at the glycosidic linkage. No significant differences can be found in these three spectra; none of them have substantial peaks in the 90–80-p.p.m. region, where all of the C-4 resonances were found in the spectra of the cyclodextrins. However, this is not true for the spectra of long-chain amylose and amylopectin, for which there is a shoulder that can be associated with cyclodextrin-like C-4 resonances. Also, for these two spectra, line widths in the C-1-resonance region are increased. For long-chain amylose, such an increase can be attributed to the superimposition of conformers that deviate from the conformations most commonly adopted. The same argument can be invoked for amylopectin, for which the resonances from the α-D-(1→6) branching of lateral chains on the main backbone can also be invoked.

In conclusion, it is considered that, owing to the complexity of the molecular organization at the glycosidic linkage, the correlation between the observed ^{13}C-chemical shifts of C-1 atoms and the orientations at the glycosidic linkage is complex. Indeed, it cannot be expected that the magnitude of the torsion angle alone can be correlated with the differences in the chemical shifts of C-1. The first degree of approximation should at least take into account the magnitude of both the α- and the β-glycosidic torsion-angles. The dispersion of the chemical shifts observed for the C-4′ atoms, also involved in the glycosidic linkage, should be considered as well.

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