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# CORRELATION OF $^{13}C$ CHEMICAL SHIFTS WITH TORSIONAL ANGLES FROM HIGH-RESOLUTION, $^{13}C$-C.P.–M.A.S. N.M.R. STUDIES OF CRYSTALLINE CYCLOMALTO-OLIGOSACCHARIDE COMPLEXES, AND THEIR RELATION TO THE STRUCTURES OF THE STARCH POLYMORPHS

RICHARD P VEREGIN, COLIN A FYFE,
*Guelph-Waterloo Centre for Graduate Work in Chemistry, Guelph Campus, University of Guelph, Guelph, Ontario N1G 2W1 (Canada)*

ROBERT H MARCHESSAULT, AND MICHAEL G TAYLOR
*Xerox Research Centre of Canada, Mississauga, Ontario L5K 2L1 (Canada)*

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## ABSTRACT

The chemical shifts and multiplicities of the resonances in high-resolution, $^{13}C$-c.p.–m.a.s. n.m.r. spectra of cyclomalto-oligosaccharide inclusion complexes are characteristic of the crystalline structure of the different complexes. In particular, the $^{13}C$ chemical shifts of C-1 and C-4 correlate with the torsion angles $\phi_2'$ and $\phi_1$, respectively (related to $\psi$ and $\phi$, respectively, in an alternative terminology), which describe the orientation of the D-glucosyl residues about the $\alpha$-D-(1→4) glycosidic linkage. The $^{13}C$ chemical shift of C-6 correlates with the torsion angle $\chi$, which describes the orientation of O-6 about the exocyclic, C-5–C-6 bond. The cyclomalto-oligosaccharide inclusion complexes are good models for the interpretation of the characteristic chemical shifts and multiplicities previously observed in the $^{13}C$-c.p.–m.a.s. n.m.r. spectra of the natural starch polymorphs. From these chemical-shift correlations, values for the torsion angles $\phi_2'$, $\phi_1$, and $\chi$ are predicted for starches that crystallize as "A" and "B" structures. These predicted values are in agreement with the limited data currently available from X-ray fiber diffraction studies.

## INTRODUCTION

The cyclomalto-oligosaccharides ("cyclodextrins") are cyclic oligosaccharides related to starch, consisting[1] of six or more $\alpha$-D-(1→4)-linked D-glucosyl residues (**1**). The two most common cyclomalto-oligosaccharides are cyclomaltohexaose, consisting of six, and cyclomaltoheptaose, consisting of seven residues. The cyclomaltopolyoses are torus-shaped, and can form crystalline inclusion complexes with small molecules that can fit inside the annular cavity (diameter 5.0 Å in cyclomaltohexaose, and 6.2 Å in cyclomaltoheptaose). The crystalline structures of a number

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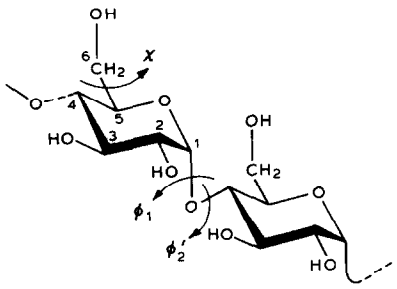

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of these inclusion complexes have been accurately described, using the techniques of single-crystal X-ray diffraction[1]. An important application of these structures is to use the cyclomaltopolyoses as model compounds for the crystalline structure of starch [a polymer of mainly α-D-(1→4)-linked D-glucosyl residues], where single-crystal, X-ray diffraction techniques cannot be used and the crystalline structure is thus poorly known.

The recently developed technique of $^{13}C$ cross-polarization and magic angle spinning (c.p.–m.a.s.), nuclear magnetic resonance (n.m.r.) spectroscopy yields high-resolution n.m.r. spectra in the solid state[2]. The chemical shifts that are obtained from these spectra are the "isotropic" values for the solid state. Thus, these shifts are similar in nature to those obtained in solution, and may be used for structural elucidation in terms both of molecular and crystal structure. Although the c.p.–m a.s. n.m.r spectra of solids yield isotropic shifts as in solution, the solid-state spectra can be more complex, due to inequivalences that may be induced by the solid between nuclei that are "equivalent in solution". The interpretation of these spectra may be complex, but much information on the crystalline structure may, in principle, be obtained, as will be discussed later in more detail. A previous $^{13}C$-c.p.–m.a.s. n.m.r. study of a number of cyclomaltooligosaccharide inclusion complexes has shown apparent multiplicities of the $^{13}C$ resonances for the cyclomaltopolyoses molecule[3]. The suggestion of that study was that these multiplicities may be related to the symmetry and molecular conformation of the cyclomaltopolyoses macrocycle.

The purposes of the present work were to demonstrate, firstly, that $^{13}C$ c.p.–m.a.s n.m.r. spectroscopy is sensitive to the crystalline structure of cyclomaltopolyose inclusion complexes; secondly, that the $^{13}C$-n.m.r. spectra correlate with the molecular conformations of the host molecules as determined by single-crystal X-ray diffraction analysis; and, thirdly, to use these correlations to predict the molecular conformations of starch, the predominantly α-D-(1→4) polymer of D-glucosyl residues.

## EXPERIMENTAL

The cyclomaltohexaose and cyclomaltoheptaose samples were obtained from

Aldrich Chemical Company, and were recrystallized from aqueous solution. The inclusion complexes were obtained as crystals by cooling hot, saturated, aqueous solutions of the cyclomaltopolyoses and the guest, following the procedures previously described for the preparation of samples for single-crystal X-ray diffraction studies[4]. In all cases, the single crystals obtained showed the characteristic habits of the particular inclusion complex. For the complex of cyclomaltohexaose with propanol, a powder diffraction pattern was obtained. The calculated *d*-spacings for this complex were in good agreement with those calculated from the unit cell reported in the literature[4g]

The $^{13}$C-c.p–m.a.s. spectra were recorded at 50.3 MHz with a Bruker CXP-200 spectrometer at room temperature. Spin-locking and decoupling fields of ~15 mT, and spinning rates of ~2.5 kHz were used in all cases. The spectra were referenced to external hexamethyldisiloxane (by substitution) and converted into values with $Me_4Si$ as the reference by adding 2.1 p.p m. to the measured chemical shifts.

## RESULTS AND DISCUSSION

### *Cyclomalto-oligosaccharide complexes*

The $^{13}$C-c.p.–m.a.s. n.m.r. spectra for a series of crystalline cyclomaltopolyose inclusion complexes are shown in Fig. 1. The assignments for the C-1, C-4, and C-6 atoms were made from the corresponding solution data[5]; however, the resonances due to C-2, C-3, and C-5 could not be assigned, due to their extensive overlap. The chemical shifts for C-1, C-4, and C-6 are listed in Table I. Each different inclusion complex gives rise to a characteristic, $^{13}$C-n.m.r. spectrum, the multiplet splittings of the C-1 and C-4 signals being especially characteristic. Thus, the $^{13}$C-c.p.–m.a.s. spectra are, indeed, sensitive to the crystalline structure of the cyclomaltopolyose inclusion complexes.

It is well known that many of the cyclomaltopolyose inclusion complexes lose water and any included solvent on drying[4]. However, the spectra obtained for the complexes studied here remained unchanged after the time needed for recording a spectrum, although samples that were allowed to dry for a few days showed broadened line-shapes, presumably due to loss of crystalline organization.

In the absence of good X-ray diffraction data, the interpretation, in terms of crystalline structure, of the multiplet splittings that arise in $^{13}$C-c.p.–m.a.s. n.m.r. spectra is not straightforward. There are two possible reasons why multiplet peaks may be observed in solid-state n.m.r. spectra, both due to effects of the crystal lattice. The first possibility is that the unit cell might contain a number of crystallographically inequivalent sites, in principle yielding multiplet signals for all resonances (as for 2,4-dinitrotoluene or calcium acetate[6]). This possibility was precluded in the present instance by the results of single-crystal, X-ray diffraction studies, which characterized the structure of these inclusion complexes extremely well[4]. The second possibility is that the packing in the solid state could lessen the symmetry of

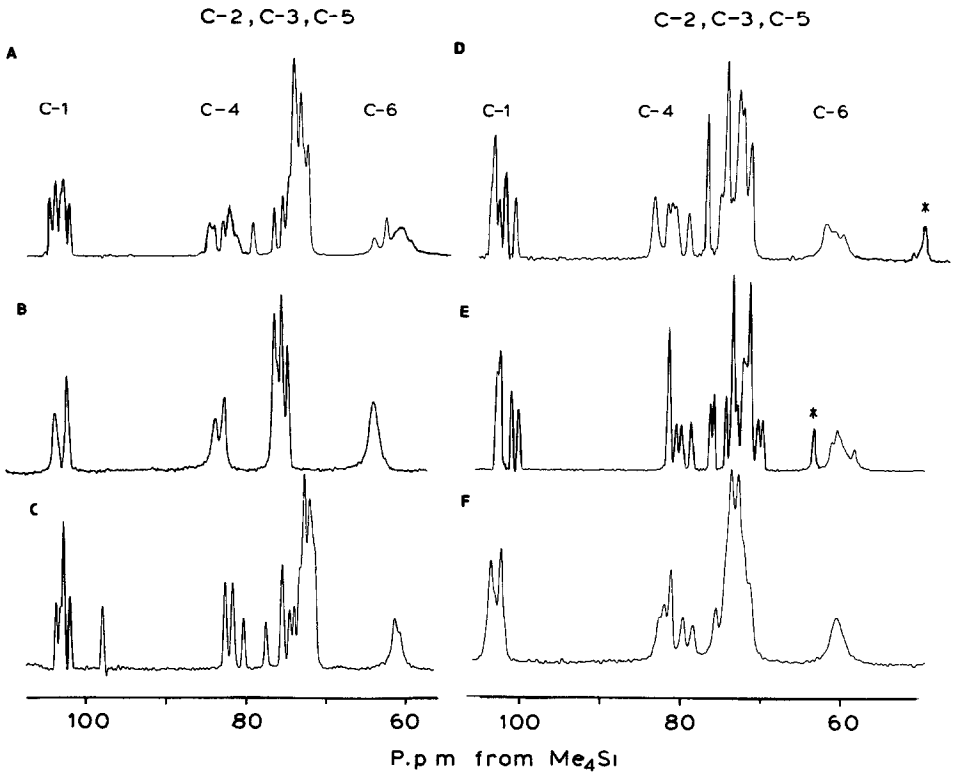



Fig. 1 $^{13}$C-C.p –m a s n.m.r spectra of various cyclomalto-oligosaccharide complexes A, cyclomaltoheptaose dodecahydrate; B, cyclomaltohexaose–potassium acetate; C, cyclomaltohexaose hexahydrate, D, cyclomaltohexaose–methanol; E, cyclomaltohexaose–1-propanol, and F, cyclomaltohexaose–*p*-iodophenol. [All spectra were obtained at 50 3 MHz with a 3-s recycle delay, 1-ms contact time, and, typically, 500 accumulations All spectra are plotted with −20-Hz gaussian resolution-enhancement The symbol "*" indicate peaks due to the guest ]

the molecule, either by its placement in a site whose symmetry is lower than the molecular symmetry of the isolated molecule (as for the heptamethylbenzenium ion[6]), or by inducing a fixed conformation (as for 1,4-dimethoxybenzene[8]). In either case, the result would be more than one signal for some, but not all, resonances. The former possibility can be ruled out here, as the D-glucosyl residue has no symmetry. These effects have been observed for carbohydrates[9] and for such carbohydrate polymers as cellulose[19], although there is still debate on the interpretation of the experimental data. A simple rule would appear to be that the number of signals should be related directly to the number of carbon atoms in the asymmetric unit. The $^{13}$C-n.m.r. spectra of crystalline and methyl $\alpha$- and $\beta$-xylopyranosides follow this rule, with the $\beta$ anomer giving rise to a single resonance for each carbon atom, whereas the spectrum of the $\alpha$ anomer shows two resonances per carbon atom[11]. These results correlate well with the X-ray diffraction data, which show a single molecule in the asymmetric unit of the $\beta$ anomer, and two

TABLE I

$^{13}C$ SOLID-STATE N M R CHEMICAL SHIFTS FOR CYCLOMALTO-OLIGOSACCHARIDE INCLUSION COMPLEXES

| *Complex* | *Observed $^{13}C$ chemical shifts*[a] | | |
|---|---|---|---|
| | *C-1* | *C-4* | *C-6* |
| Cyclomaltoheptaose dodecahydrate | 104.1 | 84 3 | 63 8 |
| | 103 4 | 83 8 | 62 7 |
| | 102 8 | 82.6 | 61 0 |
| | 101 7 | 81 8 | 60 4 |
| | | 81.0 | 59 3 |
| | | 78.8 | |
| Cyclomaltohexaose hexahydrate | 103 6 | 82 7 | 61 6 |
| | 103 1 | 81 8 | 61 0 |
| | 102 7 | 80 4 | |
| | 101.9 | 77 6 | |
| | 97 9 | | |
| Cyclomaltohexaose methanol | 102.9 | 83 0 | 61 8 |
| | 102.2 | 81 4 | 60 7 |
| | 101.5 | 80 9 | 59 6 |
| | 100.2 | 80 4 | |
| | | 78 8 | |
| Cyclomaltohexaose–potassium acetate | 103 1 | 82 9 | 62 9 |
| | 102 0 | 82 0 | |
| Cyclomaltohexaose–1-propanol | 103 3 | 82 1 | 61 7 |
| | 102 9 | 81 1 | 61 1 |
| | 101 5 | 80 5 | 60.7 |
| | 100 6 | 79 2 | 59 9 |
| | | | 58.9 |
| Cyclomaltohexaose–*p*-iodophenol | 103 9 | 82 9 | 61 2 |
| | 103 3 | 82 4 | |
| | 102 6 | 81 6 | |
| | | 80 1 | |
| | | 78 9 | |

[a]Chemical shifts are in p p m. from $Me_4Si$

molecules in the asymmetric unit of the $\alpha$ anomer.

Previous $^{13}C$-n.m.r. studies of the crystalline structure of carbohydrates have shown that the $^{13}C$ resonances of the carbon atoms involved in the glycosidic linkage, and of C-6 (bonded to a primary hydroxyl group) are particularly sensitive to the molecular conformation and lattice structure[2d,10,12,13]. The C-1 and C-4 resonances in cellulose [a polymer of $\beta$-D-(1→4)-D-glucosyl residues] and its oligomers show multiplicities that are sensitive to the crystalline structures of these carbohydrates[12]. Recently, $^{13}C$-c.p.–m.a.s. n.m.r. spectroscopy has been used to characterize the molecular-chain conformations of a series of native and regenerated celluloses and various (1→4)-$\beta$-D-glucans with some success[10b]. The chemical shifts of C-1 and C-4 were respectively found to correlate fairly well with the torsional angles $\phi$ and $\psi$, which describe the orientation of the glycosidic linkage. The chemical shift of C-6 was found to correlate with the torsional angle, $\chi$, O-6–C-6–C-5–C-4,

the angle describing the orientation of the primary hydroxyl group. The problem with these correlations is the heterogeneity of the structures of these different (1→4)-β-D-glucans. In contrast, for the cyclomaltopolyose inclusion complexes, the X-ray structural data are very good indeed, and the structures are closely related. The X-ray diffractions results[1,4] show, to a close approximation, that the structures of the cyclomaltopolyose complexes can be described in terms of the two angles $\phi_1$ and $\phi_2'$ defining the α-D-(1→4)-glycosidic linkage and the angle $\chi$ about the exocyclic, C-5–C-6 bond. All of the D-glucosyl residues have the $^4C_1$ conformation[1b], and the endocyclic bond-angles and bond lengths are, in general, very similar for all of the residues in all of these inclusion complexes. In addition, most of the cyclomaltohexaose complexes in this study crystallize in the space group $P2_12_12_1$, with the same cage-type packing. The X-ray data suggest that the observed multiplet splittings and characteristic shifts in the $^{13}$C-c.p.–m.a.s. spectra reflect the conformational changes described by the angles $\phi_1$, $\phi_2'$, and $\chi$. The X-ray results also clearly demonstrate the structural similarities between the various cyclomaltopolyose complexes, which greatly simplifies the correlation of the $^{13}$C-n.m.r. spectra with the angles determined from the X-ray diffraction studies.

Proceeding on the assumption that the $^{13}$C chemical shifts reflect changes in

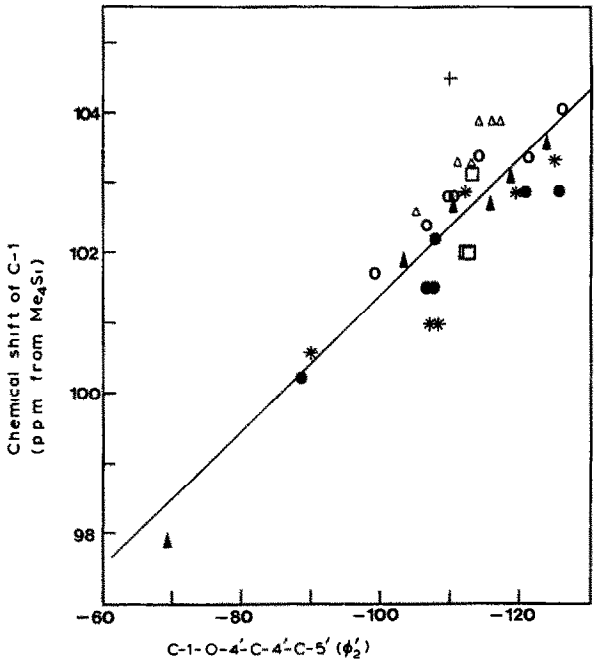



Fig. 2. Correlation of solid-state, $^{13}$C C-1 chemical shifts of various cyclomalto-oligosaccharide complexes with the torsion angle C-1–O-4′–C-4′–C-5′ ($\phi_2'$) determined from single-crystal, X-ray diffraction studies[4]. [Key: ○ = cyclomaltoheptaose dodecahydrate; * = cyclomaltohexaose–1-propanol; ● = cyclomaltohexaose–methanol, ▲ = cyclomaltohexaose hexahydrate; □ = cyclomaltohexaose–potassium acetate; △ = cyclomaltohexaose–*p*-iodophenol; and + = β-maltose[15b,c].]

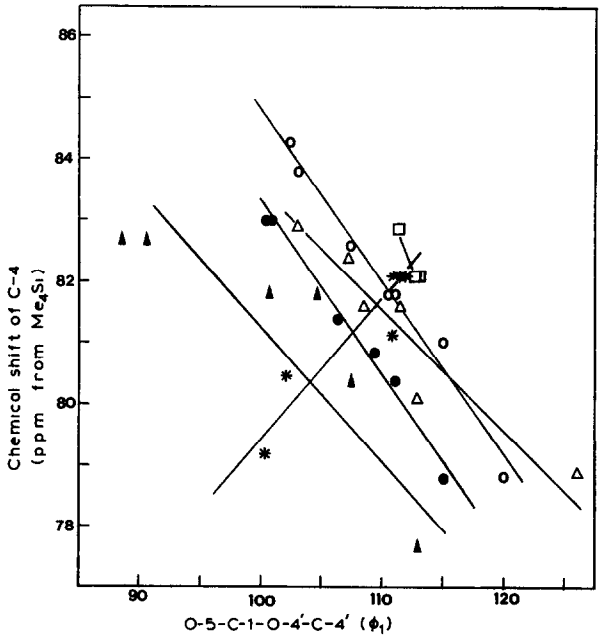
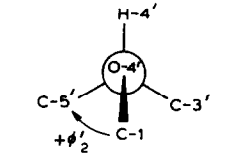



Fig 3 Correlation of solid-state, $^{13}C$ C-4 chemical shifts of various cyclomalto-oligosaccharide complexes with the torsion angle O-5–C-1–O-4′–C-4′ ($\phi_1$) determined from single-crystal, X-ray diffraction studies[4] [Key· ○ = cyclomaltoheptaose dodecahydrate; * = cyclomaltohexaose–1-propanol, ● = cyclomaltohexaose–methanol, ▲ = cyclomaltohexaose hexahydrate, □ = cyclomaltohexaose–potassium acetate, and △ = cyclomaltohexaose–*p*-iodophenol ]

the angles $\phi_1$, $\phi_2'$, and $\chi$, a quantitative correlation between these torsion angles and the $^{13}C$ chemical shifts of C-1, C-4, and C-6 can be developed. The $^{13}C$-c.p.–m.a.s. spectra of the cyclomaltopolyose inclusion complexes can be assumed to be essentially quantitative[14]. Each of the areas of the resonances for C-1, C-4, and C-6 was normalized to 7 carbon atoms for the cyclomaltoheptaose inclusion complex (which consists of 7 residues), and to 6 carbon atoms for the cyclomaltohexaose inclusion complexes (which consist of 6 residues). Thus, in principle, if each residue has a different conformation, seven and six peaks, respectively might be observed for each of the different carbon atoms in the D-glucosyl residue. However, overlap of the resonances may lower the number of peaks resolved. In accordance with their relative intensities of 1:1:1:2:1:1, the C-4 resonances for cyclomaltoheptaose hexahydrate at 84.3, 83.8, 82.6, 81.0, 81.0, and 78.8 p.p.m. were respectively assigned to 1, 1, 1, 2, 1, and 1 carbon atom in the seven residues of the cyclomaltoheptaose molecule (see Fig. 1).

Each of the C-1, C-4, and C-6 chemical shifts was tested for a correlation with each of the torsion angles $\phi_1$, $\phi_2'$, and $\chi$. The general procedure was to plot the $^{13}C$ chemical shifts for a particular carbon atom along the ordinate, and the torsion angles (derived from X-ray studies) for all of the residues in the inclusion complex along the abscissa. For any chemical shift and torsion angle pair, there are only two



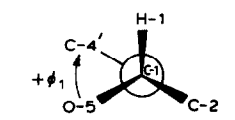

C-1–O-4'–C-4'–C-5'

2

possibilities, corresponding either to a negative or a positive correlation between the two variables. Thus, in total, there are 18 possible correlations that were tested (*i.e.*, 3 shifts × 3 angles × 2 correlations). However, for a particular torsion angle and chemical shift, only one correlation was found to be possible. It was possible to preclude many of the possibilities, as the correlation must not only explain the $^{13}C$ chemical shifts, but also the relative intensities of the peaks within the $^{13}C$ multiplets. That is, the degeneracies shown by the intensities of the peaks in the multiplet must correspond to degeneracies in the torsion angles. This requirement is fulfilled by the correlations discussed later, as is apparent by comparing the spectra in Fig. 1 with the correlations in Figs. 2 and 3. Thus, comparison of the single-crystal, X-ray data with the n.m.r. spectra leads to the correlations between the torsional angles and the $^{13}C$ chemical shifts to be discussed.

The linear correlation between the $^{13}C$ chemical shifts of the C-1 atoms and the torsional angles $\phi'_2$ is shown in Fig. 2. The angle $\phi'_2$ is defined[4d] here as C-1–O-4'–C-4'–C-5'; the angle is zero for C-1–O-4 and for C-4'–C-5' in the *cis*-planar conformation **2**. This correlation is very good ($\rho$ = 0.90 on 21 points), whereas the chemical shifts do not correlate at all with the other torsional angle ($\phi_1$) involved in the glycosidic linkage. Also included in this correlation is 4-*O*-$\alpha$-D-glucopyranosyl-$\beta$-D-glucopyranose ($\beta$-maltose), which consists of a D-glucosyl group and a D-glucose residue joined by an $\alpha$-D-(1→4)-glycosidic linkage. Although many of the resonances in the $^{13}C$ c.p.–m.a.s. spectrum of this disaccharide could not be assigned, the C-1 atom involved in the glycosidic linkage could be assigned, on the basis of solution $^{13}C$-n.m r. studies[15a], to the lowest-field peak, at 104.5 p.p.m. From single-crystal X-ray studies[15b,c], the value of $\phi'_2$ is −109.7°, which fits well in this correlation. Because this peak can be assigned absolutely to a single value of $\phi'_2$, this is a good test of the proposed correlation.

There is also a correlation between the $^{13}C$ chemical shifts of the C-4 atoms in the various cyclomaltopolyose complexes and the torsional angles $\phi_1$, which is shown in Fig. 3 The angle $\phi_1$, is defined[4d] here as O-5–C-1–O-4'–C-4' with the




O-5–C-1–O-4'–C-4'

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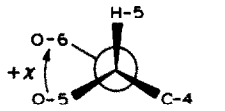


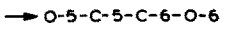


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angle zero for O-5–C-1 and O-4′–C-4′ in a *cis*-planar orientation (see **3**). The data here are more scattered, but it appears that each different cyclomaltopolyose complex gives a slightly different linear correlation. This effect may be due to differences in the orientation about the C-6–O-6 bond; that is, to changes in the C-4–C-5–C-6–O-6 torsional angle, although there is no obvious correlation. Again the chemical shifts do not correlate at all with the other angle ($\phi_2'$) involved in the glycosidic linkage.

The interpretation of the $^{13}C$ resonance of C-6 is a more complex problem. In the cyclomaltopolyoses, the torsional angle $\chi$, C-4–C-5–C-6–O-6, is basically constrained to two different orientations. Within each orientation, angular variations are often quite small. Thus, the angle $\chi$ (shown in **4**) can be fairly accurately characterized by the two allowed orientations, called the *gg* and *gt* orientations. In the *gg* conformer, O-6 is *gauche* to both O-5 and C-4: in the *gt* conformer, O-6 is *gauche* to O-5 and *trans* to C-4, as in **4**. A *tg* conformer, with O-6 *trans* to O-5 and *gauche* to C-4 has been suggested in cellulose, but has not been observed in the cyclomalto-oligosaccharides.

The cyclomaltohexaose–potassium acetate complex has all residues with $\chi$ in the *gt* conformation[4f], and the $^{13}C$ n.m.r. spectrum shows a single peak at 62.9 p.p.m., and thus assigned the *gt* orientation. In the cyclomaltoheptaose dodecahydrate complex, two residues have $\chi$ *gt*, 4 residues have $\chi$ *gg*, and 1 residue is two-fold disordered between the *gt* and *gg* orientations, with 1:1 populations in the two sites[4a]. The observed peak at 62.7 p.p.m. can be assigned to the *gt* orientation, based on the cyclomaltohexaose–potassium acetate spectrum. Presumably, then, the peak centered at 60.4 p.p.m. can be assigned to the 4 residues with a *gg* orientation. The ratio of the $^{13}C$ peak intensities is ~2:1, in agreement with the X-ray data. In β-maltose[15b], one residue has $\chi$ *gg*, the other has $\chi$ *gt*. The two resonances for C-6 in the $^{13}C$-c.p.–m.a.s. spectrum of β-maltose can thus be assigned to the *gg* conformer for the peak at 60.5 p.p.m. and to the *gt* conformer for the peak at 65.9 p.p.m. Thus, for the two possible values of $\chi$, the $^{13}C$ chemical shifts for C-6 are ~60.4 p.p.m. for *gg*, and 62.7–65.9 p.p.m. for *gt*. For comparison, a similar correlation has recently been found in β-(1→4)-linked saccharides and polysaccharides, showing[10b] peaks at 62 p.p.m. for *gg*, 62.7–64.5 p.p.m. for *gt*, and 66 p.p.m. for *tg*. The remaining peak for C-6 in the spectrum of cyclomaltoheptaose dodecahydrate, at 63.8 p.p.m., must then be assigned to the residue with $\chi$ disordered between the *gt* and *gg* orientations. Thus, the disorder observed by X-ray diffraction is not a static disorder due to a distribution of *gg* and *gt* orientations for this residue throughout the crystal, as two signals would then be observed. Rather, there is,

between the two orientations for this residue, an exchange that is fast on the n.m r. time-scale (fast compared to the chemical-shift difference, that is, >80 Hz), which should give rise to a single, $^{13}$C-n.m.r. peak at the average chemical shift, 61.5 p p.m. The peak is actually observed at 63.8 p.p.m. The dynamic nature of this disorder has been confirmed[16] by a neutron-diffraction study at 120 K. There are two possible explanations for this difference of 2.3 p.p.m. between the observed and calculated chemical shifts. Firstly, the changes in the hydrogen bonding of O-6 in this residue compared to the other residues may result in a chemical shift of the attached C-6 carbon. These effects have been previously observed for solid-state $^{13}$C n.m.r. of small organic molecules[17]. In a series of hydroxybenzaldehydes, the chemical shift of the aldehyde carbon atom was found to be inversely proportional to the distance between the hydrogen-bonded oxygen atoms[17b]. The $^{13}$C shifts were extremely sensitive to this distance, showing a shift of ~4 p.p.m. for a change of 0.2 Å. Secondly, the model deduced from X-ray diffraction studies has a number of anomalous bond-lengths and bond angles for this residues. The C-6–O-6 bond-length is 1.26 and 1.33 Å for the *gg* and *gt* conformers, respectively, for this residue (the average of the other 6 residues is 1 41 ±0.01 Å), and the C-5–C-6–O-6 bond-angle is 125.4° for the *gt* conformer of this residue (the average of the other 6 residues is 111 ±2°). These anomalies may be due either to problems in the refinement of the X-ray model, due to the disorder of the O-6 atom, or they may reflect real distortions, also as a result of the disorder. It should be noted that, with the exception of this one residue, the bond lengths and angles observed in the residues of the cyclomaltohexaoses do not vary significantly between the different complexes. The observed chemical-shift difference may then be a result of this distortion, or the inadequacies of the X-ray-determined model. In the latter case, the n.m.r. results could be explained if C-6 is actually in the *tg* orientation in this residue, contrary to the model suggested by the X-ray study. Finally, it should be noted that the resonance assigned to the 4 residues in the *gg* orientation is quite broad, and appears to show some additional fine structure. This may be due to the slightly different $\chi$ angles for each of these four residues ($\chi$ ranges from −60.8 to −70.9), or may be due to changes in the hydrogen bonding of O-6 in these residues.

The cyclomaltohexaose–*p*-iodophenol complex has 5 residues with $\chi$ *gg*, and 1 residue with $\chi$ disordered between *gg* and *gt* with[4i] 1:1 populations. If the disorder is static on the n.m.r. time-scale, there should be two $^{13}$C-n.m r. peaks for C-6, in the ratio of 11:1 for the *gg* and *gt* conformers, respectively; if the disorder is fast, there should be two peaks, in the ratio of 5:1, due to the *gg* conformer and the disordered *gt, gg* conformer, respectively. The observed spectrum favors the former possibility, showing a large peak, at 61.2 p.p.m. assigned to the *gg* conformer (the expected shift is 60.4 p.p.m.); and no obvious peak due to the disordered residue when in the *gt* orientation, presumably a result of its low intensity.

The cyclomaltohexaose–methanol complex has[4b] 4 residues with $\chi$ *gg*, and 2 residues with $\chi$ disordered between *gg* and *gt*, with 1:1 populations. If the disorder is static on the n.m.r. time-scale, there should be two $^{13}$C-n.m.r. peaks for C-6, in

the ratio of 5:1, for the *gg* and *gt* conformers, respectively; if the disorder is fast, there should be two peaks, in the ratio of 2:1 due to the *gg* conformer and the disordered *gt, gg* conformer, respectively. The peak observed is very broad, and appears to consist of at least three peaks centered at 61.8, 60.7, and 59.6 p.p.m. It is not possible to make a good assignment of these peaks. It should be noted that the $\chi$ angles for the different residues in the *gg* conformation for this complex show a very large range from $-56.6$ to $-82.9°$, and this may account for the presence of three peaks, rather than the two peaks expected.

The cyclomaltohexaose–1-propanol complex similarly has 5 residues with $\chi$ *gg*, and 1 residue with $\chi$ disordered between *gg* and *gt*, with 4:1 populations[4g]. In the static case, the ratio of the two $^{13}$C-n.m.r. peaks should be 29:1; in the fast-exchange case, the ratio should be 5:1. The C-6 resonance shows at least three peaks, of intensity 1:4:1 at 61.7, 61.1, and 58.9 p.p.m., respectively. Tentatively, the peak at 61.7 p.p.m. is assigned to the *gt* conformation (a shift of 62.7 to 65.9 p.p.m. has been seen for other complexes with residues in the *gt* conformation), and the 5 carbon atoms, at 61.1 and 58.9 p.p.m., are assigned to the *gg* conformers. Again the wide range of $\chi$ angles in the *gg* conformation, or changes in the hydrogen bonding of O-6 of one residue may account for the large change in shift of one residue to high field.

The cyclomaltohexaose hexahydrate complex[4d] has 4 residues with $\chi$ *gg*, and 2 residues with $\chi$ *gt*, predicting two peaks for C-6 in the $^{13}$C-n.m.r. spectrum, in the ratio of 2:1 at shifts of 60.4 and 62.7–65.9 p.p.m. However, only a single peak is observed at 61.7 p.p.m. If there were fast exchange on the n.m.r. time-scale, the peak would be expected at 61.5–63.1 p.p.m., in fair agreement with the shift observed. However, it is difficult to reconcile this conclusion with the X-ray diffraction results, which do not show this disorder.

It is important to note, also, that the $^{13}$C-c.p.–m.a.s. n.m.r. results apply to a polycrystalline sample, whereas the X-ray results apply to a single crystal only. Thus, comparisons using the two techniques must be applied with caution. For example, cyclomaltohexaose is known to crystallize from water mainly as form (I), although some single crystals have been found[4h] to adopt the form (II) structure, having a different set of $\phi_1$ and $\phi_2'$ angles.

Solution $^{13}$C-n.m.r. studies give some support for the correlations of the angles $\phi_2'$ and $\phi_1$ with the $^{13}$C chemical shifts. The work of Tonelli and others[18] on the "$\gamma$-effect" in hydrocarbons showed that there is an upfield shift of $-5.4$ p.p.m on replacement of a $\gamma$-carbon atom that is *trans* to the carbon atom observed by one that is *gauche*. In addition, a number of $^{13}$C-c.p.–m.a.s. studies of solid polymers have found conformationally dependent chemical shifts that are well explained[19] by the $\gamma$-effect. Examination of the correlations in Figs. 2 and 3 (see also, **2** and **3**) shows that the chemical shift of the carbon atom being observed goes upfield as the angle between the $\gamma$-carbon atoms varies from orientations that approach *trans* to orientations that are closer to *gauche* (the only exception is the $^{13}$C chemical shift of C-4 in the cyclomaltohexaose–1-propanol complex).

*Starch*

Starch consists of two components, amylose and amylopectin, both of which are polymers of mainly α-D-(1→4)-linked D-glucosyl residues. Because these two components are polymers, investigations of the crystalline structure of starches is hampered by the limited information available from X-ray fiber diffraction studies[20,21a,b]. In this regard, the known structures of the cyclopolyose inclusion complexes determined from single-crystal studies have proved invaluable as models for the structure of starches[1].

The correlation of $^{13}$C-c.p.–m.a.s. n.m.r. chemical shifts with the X-ray-determined torsional angles for the cyclomaltopolyose inclusion complexes can be used as a model for the structure of starches. Natural starches occur in two different crystalline forms, the "A" and "B" polymorphs. Recent work in this laboratory[13] has shown that the $^{13}$C-c.p.–m.a.s. spectra of starches are sensitive to the crystalline structure. The characteristic $^{13}$C-c.p.–m.a.s. spectra of hydrated "A" and "B" starches are shown in Fig. 4. The "B" starch gives rise to a characteristic doublet for C-1, whereas "A" starch gives a characteristic triplet. This is in accord with the recently proposed space groups for the "A" and "B" starches[20], as shown schematically in Fig. 5, illustrating the asymmetric unit in each case. Both starches are thought to crystallize as double helixes with parallel strands. The assigned $P2_1$ space-group for "A" starch has the $2_1$ axis perpendicular to the six-residue per turn strands of the double helix, and has a twofold axis in the helix direction. The consequence is that maltotriose (or one-half a strand) must be taken as the asymmetric unit[22]. The assigned $P3_121$ space-group for "B" starch has the $3_1$ axis down the

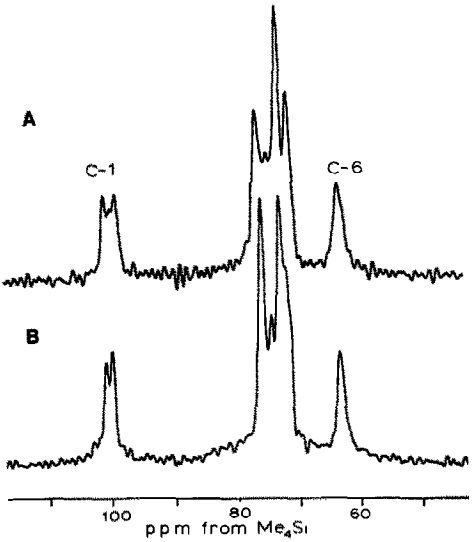


Fig. 4. $^{13}$C-c.p.–m.a.s n.m.r. spectra of hydrated starches: A, Nägeli amylodextrin (an "A" starch); B, a lintner starch (a "B" starch). [The spectra were recorded at 22.6 MHz with a 1-s recycle delay, 1-ms contact time, and 10,000 accumulations. The spectra are plotted with −25-Hz gaussian resolution-enhancement.]

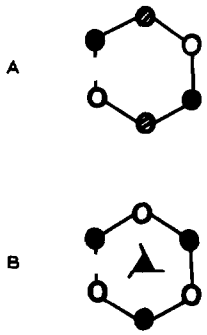


Fig 5 Schematic diagram of the asymmetric unit of· A, "A" starch with space group $P2_1$; the twofold screw axis perpendicular to the helix is not shown, maltotriose is the repeat unit; B, "B" starch, with space group $P3_121$, showing $3_1$ screw axis and maltose repeat unit. [The view shown is down the helix axis, with only one of the two strands of the double helix shown, as the two helices are identical, and are related by a twofold axis in the helix direction ]

strand, specifying two D-glucosyl residues in the asymmetric unit (or one-third of a strand). Thus, in "B" starch, there are two sets of the conformational angles $\phi_1$, $\phi_2'$; whereas, in "A" starch, there are three sets of conformational angles $\phi_1$, $\phi_2'$. These differing conformational angles then give rise to the characteristic multiplets observed in the $^{13}$C-c.p.–m.a.s. spectra.

Based on the relationship between the chemical shift of C-1 and the torsional angle $\phi_2'$ shown in Fig. 2, the torsional angles for the different residues in the asymmetric unit of starch can be calculated. These values, and an average value, for each of the starch polymorphs are shown in Table II. Unfortunately, the most recent X-ray fiber diffraction data for starch were refined[21] in terms of single-residue asymmetric units, yielding only one set of values for the torsional angles $\phi_2'$

TABLE II

PREDICTED TORSIONAL ANGLES $\phi_2'$ IN A AND B STARCHES[a]

| *Starch polymorph* | *C-1 $^{13}$C chemical shift of starches* | *Predicted torsional angles, $\phi_2'$* | |
|---|---|---|---|
| | | *N m r* [b] | *X-r.d* [c] |
| A | 102 3 | −105 7 | — |
| | 101 5 | −97 2 | — |
| | 100 3 | −84 5 | — |
| | average = 101 4 | −96.0 | −84 |
| B | 101 4 | −96.0 | — |
| | 100 4 | −85 0 | — |
| | average = 100 9 | −91 0 | −84 |

[a]From ref 13 Chemical shifts are in p.p m from $Me_4Si$. [b]Predicted on the basis of $^{13}$C chemical shift of C-1. [c]Predicted, on the basis of X-ray refinement of Sarko, from ref. 21

and $\phi_1$. However, the average value of $\phi_2'$ from the n.m.r. results agrees well with that from the X-ray study, as shown in Table II.

The resonance due to C-4 in the spectra of the starches could not be assigned as a result of the overlap of resonances arising from C-2, C-3, C-4, and C-5. However, the C-4 resonance must lie between 77 and 72 p.p.m. Using these values, and the relationship between the chemical shift of C-4 and the torsion angle $\phi_1$ shown in Fig. 3, a range of values for $\phi_1$ in the starch polymorphs can be predicted. Each different complex shows a different correlation in Fig. 3; however, the two complexes that include water only (cyclomaltohexaose hexahydrate, cyclomaltoheptaose dodecahydrate) should be the best models for the starch polymorphs, which are also hydrate structures. Using these two complexes as models, a value of $\phi_1$ in the range from 130 to 190° is predicted for both the "A" and the "B" starches. The values from X-ray diffraction studies are 142° for "A" starch, and 142° for "B" starch, which are within this predicted range. Note that the X-ray-determined value for $\phi_1$ in the "A" and "B" starches is very much larger than that typically found in the cyclomaltopolyose inclusion complexes.

The chemical shift of C-6 in "A" starch is 62.8 p.p.m., and in "B" starch is 62.1 p p.m. In the cyclomaltopolyose inclusion complexes and $\beta$-maltose, the chemical shift of C-6 is 60.4, 62.8–65.5 p.p.m., respectively, if the torsion angle $\chi$ is *gg* and *gt* (the torsion angle $\chi$ is shown in **4**, and the conformation shown is *gt*). Thus, $\chi$ appears to be predominantly *gt* in both the "A" and the "B" starches. However, the resonance for C-6 in "A" starch is definitely broader, and shifted to lower field, compared to the very sharp resonance for "B" starch. There are three possible explanations for this difference Firstly, in "A" starch, there might be a broad distribution of $\chi$ angles that are approximately in the *gt* orientation. Secondly, there may be differences in hydrogen bonding to O-6 in "A" and "B" starches. Thirdly, there may be an exchange occurring between $\chi$ angles that are *gt* and *tg* in the "A" starch, with a rate of exchange similar in magnitude to the chemical-shift difference (~60 Hz). In the latter case, there will be a single, broadened resonance at an average chemical-shift, $\delta_{obs}$, given by $\delta_{obs} = \delta_{gt}p_{gt} + \delta_{tg}p_{tg}$. Here, $\delta_{gt}$, $\delta_{tg}$ are the chemical shifts for C-6 in the two conformations, and $p_{gt}$, $p_{tg}$ are the corresponding populations of the two conformers. If $\delta_{gt}$ is ~62.1 p.p.m (assuming "B" starch has $\chi$ solely *gt*, $\delta_{obs} = \delta_{gt}$), and $\delta_{tg}$ is ~66 p p.m. (assuming $\delta_{tg}$ is the same as in $\beta$-(1→4)-linked saccharides[10b]), then $\delta_{obs}$ = 62.8 p p.m. for "A" starch corresponds very roughly to $p_{tg} = 0.20$ and $p_{tg} = 0.80$. These n.m.r. results are consistent with X-ray fiber diffraction studies of the "A" and "B" starches. The structure proposed for "B" starch has $\chi$ in the *gt* conformation[21b], as the n.m.r. results indicate For "A" starch, two model structures were found to be in equally good agreement with the fiber diffraction pattern, packing analysis, and hydrogen-bonding considerations[21a]. The essential difference between the two models is that $\chi$ is *gt* in one, and *tg* in the other. An equally likely possibility is that the structure of "A" starch is a mixture of the two model structures, containing both the *gt* and the *tg* conformers[21a]. The n.m.r. data for "A" starch fits the latter

possibility best, with the *gt* conformer being considerably the more populous, and with the two conformers being in dynamic equilibrium.

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