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Nuclear Magnetic Resonance and Conformational Studies on Amylose and Model Compounds in Dimethyl Sulfoxide Solution

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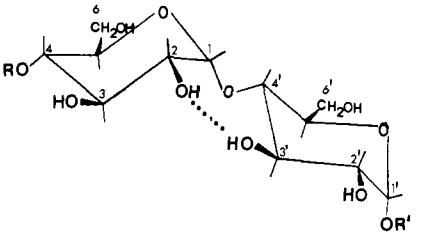
Abstract: The 220-MHz NMR spectra of maltose, cyclohexaamylose, cycloheptaamylose, and amylose were obtained in DMSO-d_6 at several temperatures up to 85 °C. Upfield migration of the signals attributed to the HO(2) and HO(3′) hydroxyl protons was measured and interpreted in terms of an intramolecular hydrogen bond from OH(3′) to OH(2) in all compounds. Analysis of the pertinent coupling constants provides identification of the local environments of donor and acceptor hydroxyl groups by assigning to them a range of $\chi(3')$ torsional (dihedral) angles. Energetically favorable conformations for amylose which satisfy these criteria are defined by steric maps and are discussed. The interpretation of the data in terms of intramolecular hydrogen bonding between contiguous residues leads to the conclusion that the same conformation is perpetuated along the amylose chain and implies substantial right-handed helical character in this solvent.

High-resolution nuclear magnetic resonance (NMR) spectroscopy has been used extensively to determine the conformational characteristics of polypeptides,[1,2] oligopeptides,[3,4] and cyclic peptides.[5-14] Among the pertinent information provided by NMR for such molecules are the presence or absence of intramolecular hydrogen bonded groups deduced from the behavior of the chemical shift with respect to temperature or deuterium exchange and values for the torsional angles on the backbone or side groups deduced from the vicinal coupling constants. Such data have also been used with potential energy calculations to propose a most probable solution conformation for such molecules.[11,15]

In this work, we have sought to determine whether similar NMR techniques will yield information on the solution conformation of some carbohydrates. To avoid complicated spectra arising from compounds containing different sugars and dissimilar linkages, we chose oligo- and polysaccharides which contain only 1 → 4′ linked α-D-glucose as the repeating unit: maltose, cyclohexaamylose, cycloheptaamylose, and amylose.

At present, the short-range conformational characteristics of amylose and related compounds with the repeating sequence I are inferred from the crystalline conformations of maltose[16]



and β-methyl maltopyranoside.[17] Both of these studies reveal an intramolecular hydrogen bond between OH(2) and OH(3′) and the latter study[17] showed specifically that the OH(2) hydroxyl group is the donor in this interaction. (In this paper, O(2), HO(2), and OH(2) denote respectively the oxygen atom, the hydroxyl hydrogen atom, and the hydroxyl group as an entity.) The crystal structure of cyclohexaamylose and its potassium acetate complex[18] showed a similar intramolecular interaction. A secondary structure is also proposed from X-ray

data on amylose coupled with conformational energy calculations.[19,20] By an analysis of X-ray fiber data, Winter and Sarko[21] proposed that OH(2) is the donor in V-anhydrous amylose and in the amylose-DMSO complex.

At present, there exists a paucity of direct results aimed at determining whether such a secondary structure actually persists in solution, at least on a short-range basis. Although the conformational properties of amylose in solution have been extensively investigated, there is still debate concerning the interpretation of much of the data and different experimental methods have led to various models for the conformation of amylose in water or dimethyl sulfoxide (DMSO):[22-28] helical, partially helical (or partially coiled), and random coil. Since the methods of investigation used (hydrodynamic and light scattering) measure dimensions characteristic of the entire macromolecule, it can be argued that both interrupted helical conformations or random coils can have similar overall properties even though short-range order is different.[24] Accordingly, in this case, evidence for an ordered conformation in solution should be founded on a technique which is sensitive to short-range effects.

In the last few years, peptide chemists have used the NMR method as a probe for intramolecular hydrogen bonds. NMR is used to delineate between protons which are solvent shielded compared with those which are exposed to solvent. Three methods are in active use: (a) temperature dependence of the proton chemical shifts; (b) deuterium-proton exchange rates; (c) titration with a weaker hydrogen-bonding solvent. The NMR methods have been applied mostly to cyclic peptides for which it could be shown that particular conformations can be stabilized by intramolecular hydrogen bonds in DMSO solution.[5-14] Such associations are proposed from the fact that certain peptide residues present one or more NH protons which are inaccessible to this hydrogen bond accepting solvent; this has been taken to be due to intramolecular association. A good measure of this inaccessibility is the relative insensitivity of the NH resonance to temperature. The other NH resonances which can form hydrogen bonds more readily with the solvent migrate upfield with increasing temperature and show a substantial temperature coefficient[5-14] of chemical shift. When $d\delta/dT \simeq 0$, the proton in question is said to form an intramolecular hydrogen bond. When $d\delta/dT$ is negative or greater than the reference value observed for the model *N*-methylacetamide, there are problems of interpretation.

Amylose and its model compounds in DMSO have been the subject of a previous study[29] at 60 and 100 MHz where the different resonances were unambiguously assigned and their chemical shifts reported. However, 100-MHz resolution failed to provide accurate coupling constants ($^3J_{HCOH}$) for the hydroxyl groups of the higher molecular weight compounds; in particular the spectrum of amylose yields little or no $^3J_{HCOH}$ data at 100 MHz. Casu and co-workers[29] noted that for amylose and model compounds two hydroxyl protons assigned to HO(2) and HO(3′) were shifted significantly downfield ($\delta >$ 5 ppm). From this fact and the observed correlation between infrared band widths of the OH stretching frequency in DMSO solution and the NMR chemical shift they inferred that there was an intramolecular hydrogen bond between OH(2) and OH(3′) in DMSO solution.

As discussed above, intramolecular hydrogen bonds in peptides have been identified by several NMR experiments. However, a basic difference exists between NMR studies of cyclic peptides and model compounds of amylose in DMSO solution. In the former case NMR reveals mainly associations of the type NH- - -O=C with only one amide proton donating to a carbonyl whereas an intramolecular association between two glucose residues linked $\alpha(1 \rightarrow 4)$ involves protons on both OH(2) and OH(3′). Deuterium exchange rates are also not easily measured for hydroxyl protons in DMSO as they exchange much more rapidly with D_2O than amide protons. The fact that contradictory results sometimes arise from the application of these two methods for peptides[30] stresses the need for an understanding of the hydroxyl–solvent interaction when considering the possibility of intramolecular hydrogen bonds. Therefore, in order to investigate the short-range order and to draw conclusions as to intramolecular hydrogen bonding in amylose and model compounds, the temperature dependence of the HO(2) and HO(3′) signals under conditions of slow chemical exchange (in DMSO-d_6) was examined.

Table I. Selected Chemical Shifts and Coupling Constants for Compounds **1**, **2**, **3**, and **4** at 18 °C[a]

	HO(2)	HO(3′)	H(1)	HO(6)
Maltose (**1**)	5.46 (6.0)	5.47 (2.8)[b]		
Cyclohexaamylose (**2**)	5.55 (7.0)	5.46 (2.5)	4.80 (3.0)	4.53 (6.0)
Cycloheptaamylose (**3**)	5.78 (6.0)	5.70 (2.1)	4.78 (3.0)	4.52 (6.0)
Amylose (**4**)	5.50 (6.0)[c]	5.40 (2.5)[c]	5.10 (~3)[d]	4.58 (~6)[d]

[a] Chemical shifts are expressed in ppm from TMS and coupling constants in Hz are given in parentheses. [b] Because of signal overlap at room temperature, the coupling constants for these two hydroxyl groups were determined at +35 °C. [c] Because of poor resolution at room temperature, the coupling constants were determined at +45 °C. [d] These coupling constants were determined from the spectrum at +60 °C which best resolved the multiplicity of the pertinent signals.

Experimental Section

Commercially available samples of β-D-maltose hydrate (Nutritional Biochemicals Corp.), cyclohexaamylose and cycloheptaamylose (Corn Products Co.), and amylose from the Northern Regional Laboratory of the United States Department of Agriculture ($\overline{DP}$ of 1500) were dissolved in deuterated dimethyl sulfoxide (DMSO-d_6) containing a small quantity of TMS. The concentrations used were 40 mg/ml for amylose and 100 mg/ml for the other three compounds. Small amounts of water in the DMSO-d_6 did not affect the spectra. High-resolution NMR spectra were recorded on a Varian Associates HR-220 NMR spectrometer, operating at 220 MHz, located at the Ontario Research Foundation in Sheridan Park, Ontario. Chemical shifts were determined using the DMSO signal as an intermediate reference. The 100-MHz spectra were recorded on a Jeol JNM-4H-100 spectrometer.

Results and Discussion

The 220-MHz NMR spectra of maltose (**1**), cyclohexaamylose (**2**), cycloheptaamylose (**3**), and amylose (**4**) were recorded at several temperatures up to 85 °C. Figures 1 to 3 show much improved resolution compared with the 100-MHz spectra and the clearly resolved pair of doublets with chemical shifts greater than 5 ppm provide accurate coupling constants ($^3J_{HCOH}$). The assignments of the hydroxyl protons HO(2) and HO(3′) described later is in accord with previous arguments.[29] The spectral behavior of **3** is similar to that of **2** and consequently is not shown.

Analysis of the low-field portion of the spectra provides accurate chemical shifts and coupling constants for the HO(2), HO(3′), and H(1) doublets as well as for the HO(6) triplet. The characteristic parameters are listed in Table I. The amylose spectra (Figure 3) are characterized by a larger line width than those of the model compounds as a consequence of its higher molecular weight and consequently its higher correlation time which broadens the spectral lines. Nevertheless, it is possible to obtain reasonably accurate NMR parameters at several temperatures.

The spectral region between 3.25 and 3.85 ppm for cyclohexaamylose contains the signals of all the ring protons except H(1). The split triplet observed at 3.77 ppm is attributed to

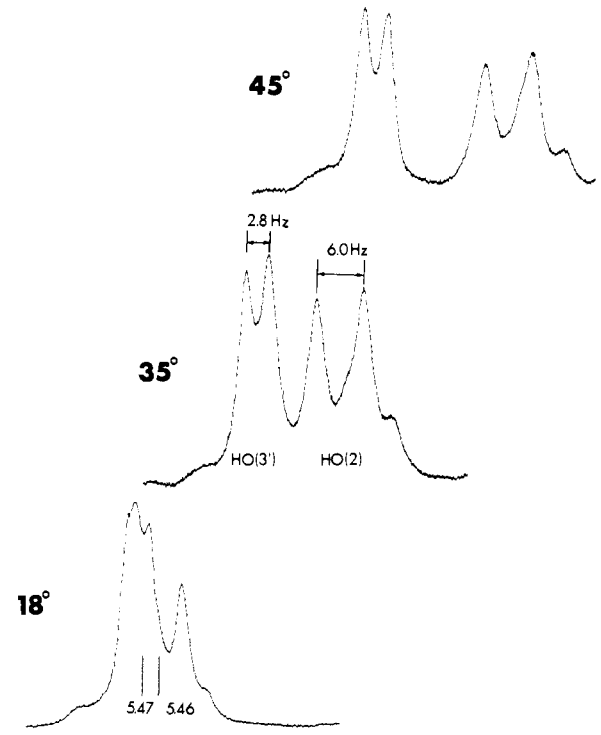


Figure 1. The 220-MHz NMR spectra of the HO(2) and HO(3′) protons in maltose at several temperatures.

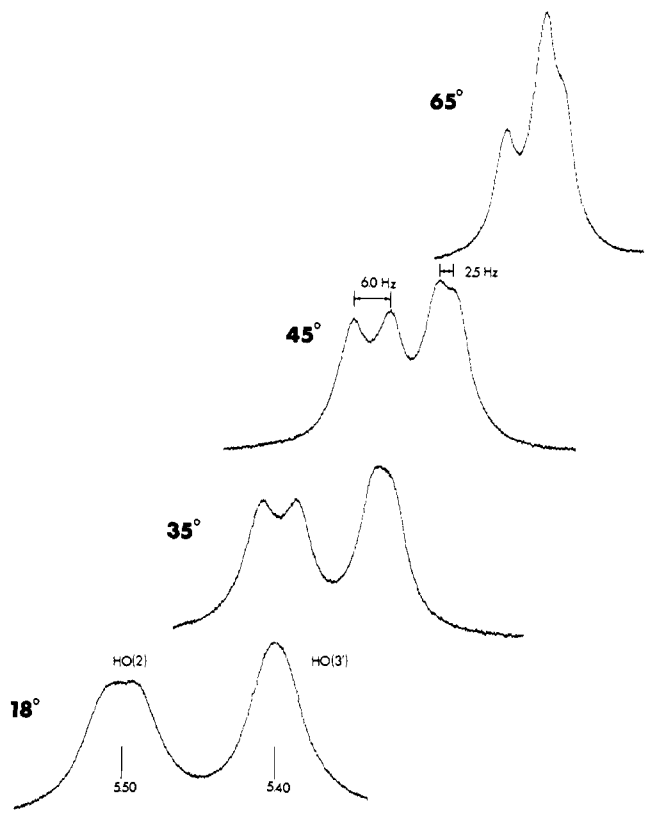


Figure 3. The 220-MHz NMR spectra of the HO(2) and HO(3′) protons in amylose at several temperatures.

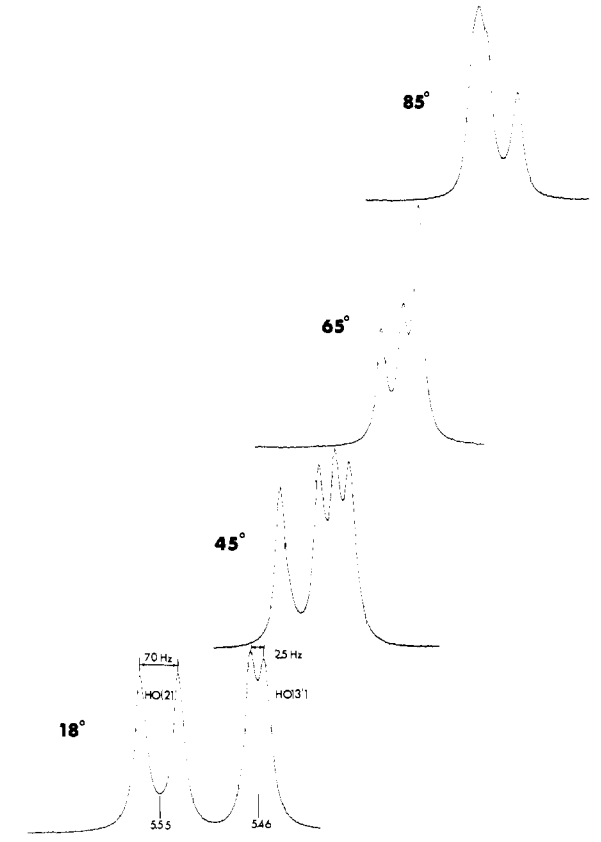


Figure 2. The 220-MHz NMR spectra of the HO(2) and HO(3′) protons in cyclohexaamylose at several temperatures.

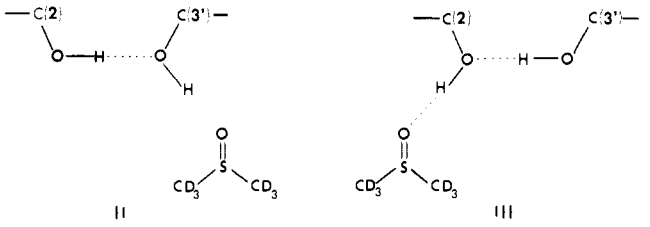
H(3)[31] which is coupled to H(2) and H(4) by 9.0 Hz (axial-axial relationship) and HO(3) by 2.5 Hz. Spin decoupling experiments at 220 MHz confirmed this assignment. Irradiation at 3.77 ppm caused the doublet at 5.46 ppm (J = 2.5 Hz) to collapse to a singlet while irradiation at 3.30 ppm (H(2)) led to the collapse of the H(1) doublet at 4.80 ppm and the HO(2) doublet (J = 7.0 Hz) at 5.55 ppm. Consequently, it appears reasonable to generalize and identify the hydroxyl signals as in Figures 1 to 3 whereby the doublet possessing the larger coupling constant (6-7 Hz) is attributed to HO(2) and the doublet possessing the smaller coupling (2-3 Hz) is attributed to HO(3′) in agreement with a suggestion made in a footnote of ref 29.

Temperature Dependence of Hydroxyl Chemical Shifts. It is well known that when simple alcohols and many polyhydroxyl compounds are dissolved in DMSO, a hydrogen bond is formed between each hydroxyl proton and the solvent as revealed by extensive NMR and infrared studies.[29,32-38] Furthermore, the observed upfield migration of hydroxyl chemical shifts with increased temperature has been ascribed, in part to a decrease in the strength of the solvent-solute association, to shifts in association equilibria as well as to changes in the degree of excitation of the hydrogen bond stretching vibrational mode.[37]

Thus for dilute solutions of alcohols in DMSO-d_6, the equilibrium ROH- - -HOR $\rightleftarrows$ ROH- - -O=S$(CD_3)_2$ is strongly to the right and for β-glucose each hydroxyl group[29,38] forms a hydrogen bond with the solvent whereby the local structural and geometrical factors are believed to be mainly responsible for the differences in chemical shift of the various hydroxyl protons. Our study of the temperature dependence of the OH peaks of β-glucose over the range 25-85 °C at 100 MHz (Figure 4A) revealed an almost constant $d\delta/dT$ value for all hydroxyl signals. This observation suggests that the properties of each -OH- - -O=S$(CD_3)_2$ hydrogen bond change in a similar fashion for OH(1), OH(2), OH(3), OH(4), and OH(6) even though structural factors are significantly different especially for OH(6) which is a primary hydroxyl group.

Similar investigations of the temperature behavior of the OH peaks of maltose (**1**), cyclohexaamylose (**2**), cyclohep-

taamylose (**3**), and amylose (**4**) were carried out at 220 MHz and some of the results are shown in Figures 4B, 4C, and 4D. In all cases, the upfield migration increased in the following order: HO(3′) < HO(2) < HO(6). The contrast between the behavior of glucose with that of **1, 2, 3,** and **4** suggests that for the latter molecules an additional factor contributes to the OH(2) and OH(3′) solvent interaction viz. the favorable geometric arrangement for an OH(2)- - -OH(3′) intramolecular hydrogen bond. This factor may be expected to change both the accessibility and the thermodynamic properties of the interaction of these two hydroxyls with the solvent. The characteristic type of association suggested can be depicted ideally by either structure II and/or structure III.



Identification of the Donor Hydroxyl Group. According to II and III an intramolecular hydrogen bond between OH(2) and OH(3′) could involve either: (i) OH(2) preferentially donating its proton to the oxygen of OH(3′) as in II, (ii) OH(3′) preferentially donating its proton to the oxygen of OH(2) as in III, (iii) an equilibrium involving i and ii.

Careful analysis of the expanded spectra of cyclohexaamylose (Figure 2) from +18 to +85 °C has revealed that both $^3J_{HOCH}$ values of interest are independent of temperature within experimental error (±0.2 Hz). This observation is compatible with the predominance of a particular local conformation for each secondary hydroxyl group. Coupling constants for maltose and amylose could not be determined as accurately over as extended a temperature range, but in the interval studied there is no evidence to suggest a markedly different behavior. Consequently, it appears reasonable to suggest that for all compounds studied, the two $^3J_{HOCH}$ of interest are characteristic of a single predominant local conformation for both OH(2) and OH(3′). This observation rules out possibility iii and is compatible with either i or ii. The identification of the donor hydroxyl group, based on an interpretation of the temperature behavior of the hydroxyl peaks, will permit a choice between i and ii as explained below.

For **1, 2, 3,** and **4,** the HO(3′) doublet with the smaller $^3J_{HOCH}$ (2.5 Hz) is characterized by a smaller upfield migration than that observed for the HO(2) and HO(6) signals. Since the temperature dependence of HO(3′) and HO(6) differs the most (that of HO(2) being intermediate) and since HO(6) is bonded to solvent alone, we conclude that HO(3′) is the donor hydroxyl group in accord with structure III. The particular electrostatic effect experienced by the oxygen atom of OH(2) could well account for the observed difference of OH(2) compared with OH(6).

Thus our interpretation of the experimental NMR results in DMSO solution is most compatible with structure III. It supports the existence of an intramolecular hydrogen bond in which OH(3′) is the donor. This property is apparently similar to the solid state interaction deduced from diffraction studies on methyl maltopyranoside[17] although the direction of the hydrogen bond is opposite.

Estimation of the Torsional Angles χ(2) and χ(3′). Both OH(2) and OH(3′) are equatorial secondary hydroxyls on adjacent $^4C_1$ chairs and yet they are characterized by markedly different coupling constants. The main factor responsible for the variation of $^3J_{HOCH}$ is the dihedral angle (θ) which is related to values of $^3J_{HOCH}$ through the equation:[39]

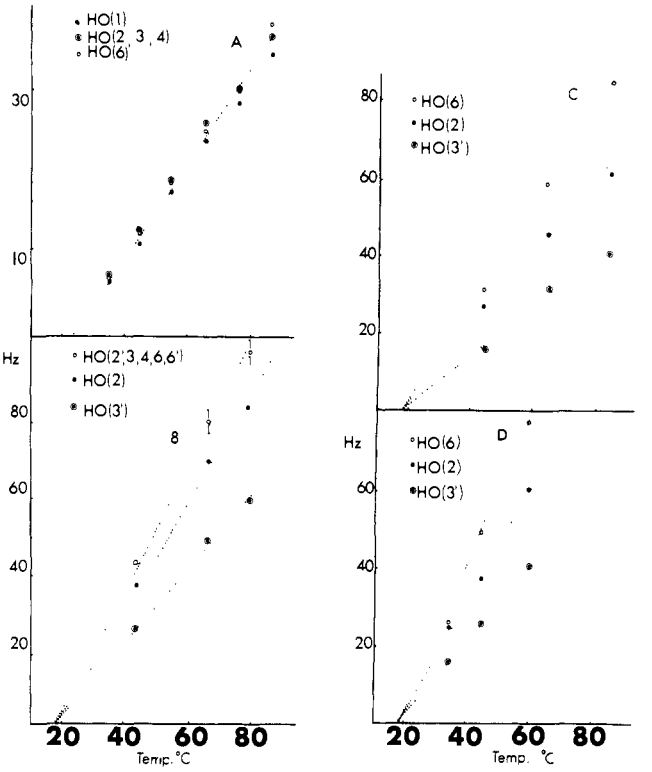


Figure 4. Temperature dependence of the chemical shifts of the hydroxyl protons in glucose (A), maltose (B), cyclohexaamylose (C), and amylose (D). The data for glucose (A) were obtained from 100-MHz spectra, and the ordinate scale is proportional to that for (B), (C), and (D), which are from 220-MHz spectra. The ordinate refers to the magnitude of the upfield shift $\Delta\nu$, measured in Hz, relative to the chemical shift at ambient temperature.

$$^3J_{HOCH} = 10.4\cos^2\theta - 1.5\cos\theta + 0.2 \qquad (1)$$

Equation 1 provides a means of estimating the approximate torsion angle[45] (χ) of the H-O-C-H moiety in the predominant local conformations of both OH(2) and OH(3′). For cyclohexaamylose it is found that χ angles of ±27 and ±138° correspond to $^3J_{HOCH}$ = 7.0 Hz while angles of ±56 and ±114° correspond to $^3J_{HOCH}$ = 2.5 Hz. Furthermore angles obtained from $^3J_{HOCH}$ = 6 Hz, such as for amylose, are ±35 and ±132° while those calculated from $^3J_{HOCH}$ = 2.0 Hz are ±60 and ±110°. Since the uncertainty in each angle obtained from eq 1 might be as much as 10° or greater,[39] the small differences observed from related coupling constants for each compound listed in Table I do not warrant particular attention at this point.

The experimental results show that for the donor hydroxyl OH(3′) in amylose, the following values of χ(3′) are possible: 60, −60, −110, and 110°. A straightforward examination of a molecular model shows that χ(3′) = 60° (V) and χ(3′) = 110° (VII) can be ruled out for OH(3′) donating its proton to O(2), because this hydrogen bond cannot be formed.

Whereas for a free hydroxyl, the value of $^3J_{HCOH}$ is given by the average of the three staggered conformations,[33] the hydrogen-bond geometry required for OH(3′)- - -O(2) suggests that only conformations IV and VI are possible. The value of $^3J_{HCOH}$ is also influenced by the nonbonded interactions with 1,3 substituents;[33,34] in the case of OH(3′) this would mean that the interactions between OH and neighboring atoms in a 1,3-syn relationship might be responsible for the predominance of one conformation as revealed by the temperature independance of $^3J_{HCOH}$. The χ(3′) = −60° conformation involves such a 1,3-syn interaction between the proton and the bridge oxygen. We suggest that the most probable value of χ(3′) is −110°.

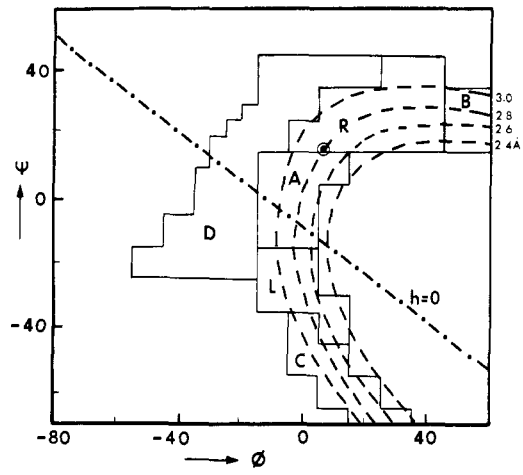
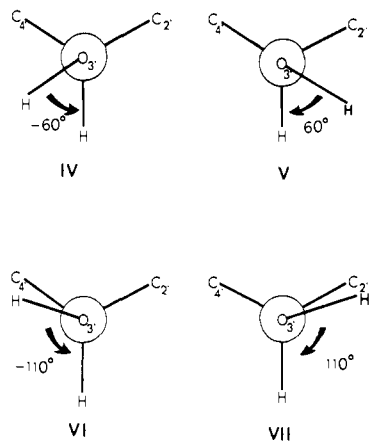


Figure 5. Steric map for maltose as a function of ϕ and ψ. The dashed curves represent iso hydrogen bond O(2)- - -O(3′) distances. The dot-dash line ($h = 0$) shows the conformations of geometrically ideal cycloamyloses. R, L: regions where 2.4 Å ≤ O(2)- - -O(3′) ≤ 3.2 Å. A, B, C: same as R and L, but with one or more close contacts of varying severity between another pair of atoms. D: conformations with no close contact or hydrogen bond. ⊙: crystalline conformation of methyl maltopyranoside.



Of the values ±35 and ±132° deduced for $\chi(2)$, the result $\chi(2) = +35°$ corresponds to the minimum energy position for HO(2) in α-D-glucose.[19] This implies that when the OH(2) group is the acceptor, the position of HO(2) is not perturbed from that in α-D-glucose.

Conformational Calculations. Minimum energy conformations of biopolymers, known to be stabilized by intramolecular hydrogen bonds, can be expressed in terms of steric maps constructed from calculation of nonbonded interactions. Favorable overall conformations may be proposed using experimental NMR data which give estimates of $\chi(3')$.

The atomic coordinates derived from the crystal structure data[17] were used in the calculations. We assume that the glucose ring geometry is fairly rigid and that a slight perturbation is of no appreciable significance. The variable parameters initially are the torsion angles ϕ and ψ around the glycosidic bonds C(1)-O(B) and O(B)-C(4′)[19] (where O(B) is the bridge oxygen atom) which is accessible to a pair of 1 → 4′ linked α-D-glucose units. Favorable conformations are chosen which do not have any steric overlap and in which the oxygen atoms O(2) and O(3′) are at a distance required for hydrogen bonding. This will restrict the range of acceptable ϕ, ψ in further calculations.

A steric map (see ref 41 for a description of the method) for maltose as a function of (ϕ, ψ) is given in Figure 5. Since the positions of hydroxyl hydrogens could be different for various (ϕ, ψ)'s, these atoms were not included in the calculation of

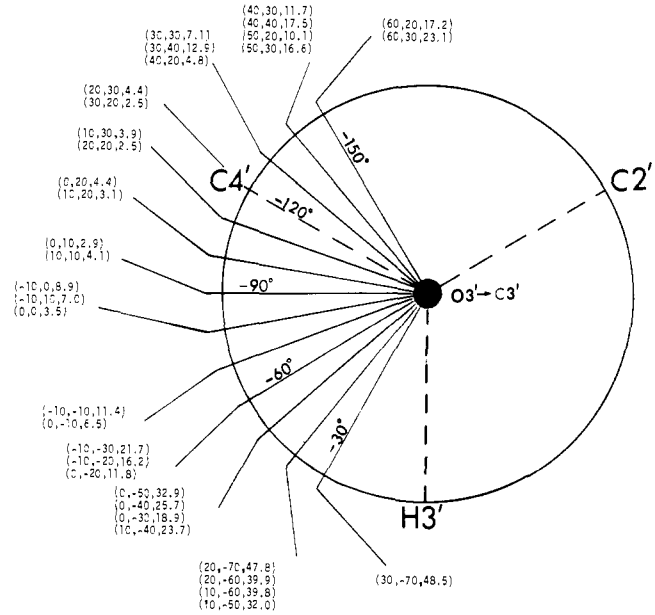


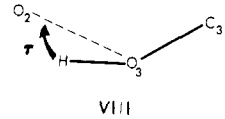
Figure 6. Diagram showing the preferred values of $\chi(3')$ which give the smallest hydrogen-bond angle, τ_{min}, for the various (ϕ, ψ)'s, with 2.4 Å ≤ O(2)- - -O(3′) ≤ 3.2 Å (only $\tau_{min} < 50°$ are shown here). The OH(3′) group was assumed to be the donor in the calculation. The solid lines represent the $\chi(3')$ values. The numbers in brackets denote the ϕ, ψ and τ_{min} values, respectively.

Figure 5. The conformations spanned by the area D are free of any steric overlap. In those included in area R and L, the O(2)- - -O(3′) distance is within the hydrogen bond limit (i.e., 2.4 Å ≤ O(2)- - -O(3′) ≤ 3.2 Å). The areas A and B contain minor close contacts; severe overlaps are found in area C. The dot-dash line represents the set of ϕ, ψ conformations, which, when repeated identically at every glycosidic linkage, would lead to geometrically ideal (i.e., n-fold symmetric, where n is the DP) cycloamyloses with various numbers of glucose residues (h, the advance per monomer, is equal to zero[19]). If regular conformations of amylose are assumed to consist of identically repeated glucose units, then conformations characterized by ϕ and ψ angles above this line constitute right-handed helices and those below are left handed. Since we are not necessarily concerned with regular helices, a random sequence of different ϕ's and ψ's from within the area above the dot-dash line would also yield a right-handed, though irregular, amylose helix. An occasional ϕ, ψ from the area below this line would serve to disrupt this right-handed helix.

It is seen that there are two hydrogen-bond areas identified by R and L, on either side of the $h = 0$ line, indicating that the O(2) and O(3′) atoms are at a reasonable hydrogen-bond distance in both right- and left-handed helices.

The boundaries described in Figure 5 are the result of the selected geometry for the residues. They are somewhat flexible and depend on the positional parameters selected for the atoms in the residues.[42] Within the approximations inherent in such calculations, this figure depicts the mutual steric characteristics of two contiguous residues in maltose and amylose. Having defined the allowed sets of ϕ, ψ, the probable range of the torsion angle $\chi(3')$ was examined within this framework.

Results of these calculations, with OH(3′) as the donor in the OH(3′)- - -O(2) hydrogen bond, are presented in Figure 6. The hydrogen bond angle τ is defined as in structure VIII.



VIII

A conformation defined by (ϕ, ψ) was selected from Figure 5 with the restriction: 2.4 Å $\leq$ O(2)- - -O(3′) $\leq$ 3.2 Å. With this conformation for the disaccharide, the angle $\chi(3')$ was varied from −180 to +180° (i.e., the HO(3′) atom was rotated about the O(3′)-C(3′) bond) at intervals of 10° and for each $\chi(3')$, and the hydrogen bond angle τ (see VIII) was calculated. This enables us to arrive at the value of $\chi(3')$ which gives the smallest value of $\tau(\tau_{min})$ for the chosen (ϕ, ψ). This was repeated for all (ϕ, ψ) combinations in Figure 5 which satisfy the hydrogen bond distance criteria.

Figure 6 shows that for the conformations in area R (in Figure 5), the preferred $\chi(3')$ varies from −100 to −130°, which is in the range deduced from NMR data. For the conformations in area A, the range of $\chi(3') = -90$ to −80° allows good hydrogen-bond angles. This magnitude is somewhat lower than the experimentally deduced value of −110°, but still in reasonable agreement with the experimental $\chi(3')$ from the value of $^3J_{HCOH}$. For the set of ϕ, ψ in area L, the preferred $\chi(3')$ ranges from −70 to −60°, which is significantly different from the value of −110° chosen earlier. Hence, it would seem that for maltose and amylose, the experimental NMR coupling constant data fit the conformations contained within areas R and A in Figure 5; they imply a preferred right-handed helical conformation.

It was suggested earlier that the value of $\chi(3') = -60°$ be eliminated from consideration because of a 1,3-syn interaction. Referring to Figures 5 and 6, we find $\chi(3') \approx -60°$ is preferred for the ϕ, ψ's in area L; however, the hydrogen-bond angles in that area are significantly higher than those for $\chi(3') = -110°$. Thus, on two counts, the value of $\chi(3') = -60°$ may be deemed unfavorable.

The Conformation of Amylose in DMSO Solution. A recent interpretation[21] of x-ray data has suggested that both V-amylose and amylose-DMSO complexes exist as left-handed helices in the solid state with very little difference in the repeat distance and screw symmetry between the individual helical chains for each system. On the other hand, extrapolation of the x-ray data on methyl maltopyranoside[17] provides ϕ and ψ angles which correspond to a right-handed helix as plotted in Figure 5. Data from other studies are in agreement with this.[20,43,44] Similarly, our NMR results, which give information about the local conformation, lead to the conclusion that successive glucose residues in amylose are conformationally like those in areas R and A (above $h = 0$) of Figure 5. When repeated along a chain this leads to right-handed helical segments for amylose.

Proponents for a helical conformation in solution have argued about the specific nature of the helix. Rationalization of hydrodynamic measurements requires an interrupted helical structure whose overall behavior is indistinguishable from that of a random coil. It was at first believed[23] that the helical segments were tight (or stiff) but changes in conformation upon complexation with iodine have led others[24] to infer that the conformation of solvated amylose must be characterized by regions of loose and extended helices alternating with short random-coil segments. The present experimental results allow a range of ϕ and ψ for the helical segments of amylose which are compatible with loose right-handed helices; however, they give no direct information on the length of these segments nor on the overall conformation of the chain.

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