



Hydroxy protons as structural probes to reveal hydrogen bonding properties of polyols in aqueous solution by NMR spectroscopy

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ABSTRACT

The solution properties of ethylene glycol (ethane-1,2-diol), glycerol (propane-1,2,3-triol), erythritol ((2R,3S)-butane-1,2,3,4-tetraol), D-xylitol ((2R,3R,4S)-pentane-1,2,3,4,5-pentaol), D-mannitol ((2R,3R,4R,5R)-hexane-1,2,3,4,5,6-hexaol), and D-sorbitol ((2S,3R,4R,5R)-hexane-1,2,3,4,5,6-hexaol), constituting a subgroup of polyalcohols/polyols of maximum six carbon atoms have been investigated using ¹H NMR chemical shifts, coupling constants, temperature coefficients, and chemical exchange rates of hydroxy protons in aqueous medium.

Relative within a molecule, minimum two-fold difference in rate of exchange values and higher temperature dependence of chemical shifts of the hydroxy protons on terminal carbon atoms confirm that sustainable hydrogen bonding interactions is accentuated for the hydroxyl groups on secondary carbons. Compared to the primary carbons *i.e.* terminal ones, the hydroxy protons on second and third carbon atoms exhibit much lower rate of exchange and smaller temperature coefficients, indicating that they are further involved in transient hydrogen bonding interactions. Scalar ³J_{OH,CH}-couplings ranging between 3.9 and 7.2 Hz imply that the hydroxyl groups are practically in free rotation regime. Examination of the chemical shift differences with respect to the shift of glycol hydroxy proton reveals that the disparity between terminal and inner hydroxyl groups disclosed by the exchange rates and temperature coefficients is sustained with the exception of 0.003 and 0.053 ppm for O(3)H of mannitol and O(5)H of sorbitol respectively. The experimental findings have been augmented by quantum chemical calculations targeting theoretical NMR chemical shifts, as well as the conformational analysis of the structures.

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1. Introduction

Sugar alcohols *i.e.* polyalcohols or polyols constitute an important class of carbohydrates, and particularly of sweeteners. They are usually derived from monosaccharides by hydrogenation and have been a subject of interest due to the various roles in many biochemical pathways [1–4], as well as in medical applications [5] and food industry [6]. Their molecular recognition is known to be highly dependent on spatial geometry in biological medium [7], yet their conformational structures are poorly known due to predominant temporal dynamics. The presence of hydroxyl groups attached to every carbon atom facilitates strong hydrogen-bond interactions both intra- and inter-molecularly, generating highly water soluble molecules that can penetrate across cell membranes.

Likewise, water promotes complex hydrogen bond networks as being the primary component of physiological media, which makes the conformational studies further ambiguous [8,9]. Water molecules are also known to be an active participant of carbohydrate-protein interactions in molecular recognition [10]. Studies addressing the sweet taste stimulation revealed important factors determined by water molecules such as key polar interactions and perturbed water regions [11], mobility and perturbation of water molecules during protein-ligand complexation [12–14].

Due to the lack of appropriate experimental techniques allowing molecular level structural examinations of polyols in aqueous solutions, the studies on various polyols are either theoretical [15–19] or solid state (X-ray, IR) investigations [20–25]. In contrast to solid state studies resulting in immobile crystal structures, dynamic conformational properties in aqueous solutions can be deduced using NMR spectroscopy [26–31]. Concerning the analyses of molecular structures and dynamics in solution, NMR spectroscopy is currently the most powerful and versatile tool. Similar to other

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biomolecules, the technique has been extensively used for structural determination of carbohydrates [32]. For structural analysis in aqueous solutions by NMR, exchangeable protons are typically omitted due to chemical exchange with deuterated protic solvents e.g. D₂O, even though they are the most valuable messengers regularly exposed to bulk solute-solvent interactions. Therefore, magnetic properties of hydroxy protons are of critical importance to elucidate hydrogen-bonding interactions of polyols in aqueous solution. The observation of hydroxy proton signals is achieved by replacement of D₂O with H₂O, as well as employing techniques in order to decrease the proton chemical exchange [33]. This modification to conventional NMR techniques leads to useful data that facilitates the investigation of molecular properties through hydroxyl groups as probes. In a number of studies, the exchangeable protons have been observed and hydrogen bonding attributes of hydroxy protons were analyzed [26,27,29–31,33–39]. For instance, the hydroxyl groups of Lewis b tetrasaccharide derivative participate in key polar interactions and have distinct NMR features [29]. Suchlike attributes, especially hydrogen bonding interactions of exchangeable protons can be elucidated by interpretation of *i*-chemical shifts (δ), *ii*-spin-spin coupling constants (*J*), *iii*-temperature coefficients ($d\delta/dT$), *iv*-exchange rates (k_{ex}), and *v*-chemical shift differences ($\Delta\delta$). Relatively small temperature dependence, vicinal coupling constants of restricted rotation and slow exchange rates are indications of hydrogen bond interactions [26,29,39,40].

In the current study, specific NMR properties of hydroxyl groups have been attained to explicate the attributes on hydrogen bonding properties of polyols in aqueous solution. The subject molecules are ethylene glycol (ethane-1,2-diol), **1**, glycerol (propane-1,2,3-triol), **2**, erythritol ((2R,3S)-butane-1,2,3,4-tetraol), **3**, D-xylitol ((2R,3r,4S)-pentane-1,2,3,4,5-pentaol), **4**, D-mannitol ((2R,3R,4R,5R)-hexane-1,2,3,4,5,6-hexaol), **5**, and D-sorbitol ((2S,3R,4R,5R)-hexane-1,2,3,4,5,6-hexaol), **6**, which are natural, relatively small and sweet-tasting molecules (for the structures **1–6** see Chart 1). Structural NMR properties of the polyols in aqueous solution are given with the focus on both intra- and inter-molecular interactions through hydroxyl groups both within polyols themselves, and between polyols and water molecules.

2. Experimental

2.1. NMR experiments

All the polyols, **1–6**, and solvents (water and acetone-*d*₆) of the highest possible purity levels were obtained using the readily available commercial sources. The observation of exchangeable hydroxy protons by NMR spectroscopy is accomplished by using H₂O as solvent, and lowering the exchange rate of hydroxy protons with water. The latter was achieved by lowering the experimental temperature to -10°C and sustaining the minimum contamination of the ionic species that increase the chemical exchange. The samples were dissolved in 15% acetone-*d*₆ and 85% H₂O mixture to prevent solutions from freezing.

The reference chemical shift due to residual acetone-*d*₅ signal was set to $\delta = 2.204$ ppm. Sample solutions were prepared to have molarities between 14 and 21 mM. Furthermore, dilute HCl and NaOH solutions with varying concentrations were used to adjust the pH values of sample solutions, since pH values out of the neutral range (usually 6.4 and 6.9) cause faster chemical exchange and, thus, loss of hydroxy proton signals. The resulting pH values were between 6.5 and 6.9 for all samples.

All one dimensional (1D) experiments of ¹H and ¹³C, and 2D homonuclear experiments were performed on a 600 MHz NMR spectrometer that employs 5 mm Penta probe. 2D ¹H-¹³C HSQC experiments were performed on a 300 MHz spectrometer (ID)/PFG

Probe). DPGSE pulse sequence is used for 2D NOESY, ROESY and TOCSY; WATERGATE pulse sequence is used for 1D proton and DQF-COSY experiments.

The processing of 2D NMR data was done by nmrPipe [44] for assignments, peak listing and volume calculations Sparky [45] software was employed. Data processing was done by applying a set of functions, including Fourier transformation (FT), zero filling (ZF), window function (SP), phase correction (PS) and polynomial baseline correction (POLY). For the assignments of hydroxy protons, NOESY, ROESY and TOCSY spectra were iteratively exploited. Unless noted, the chemical shifts are read from 1D ¹H and ¹³C APT spectra. In case of ambiguity, chemical shifts are measured preferably from HSQC spectra.

For determination of temperature coefficients ($d\delta/dT$), 1D proton spectra of each sample were recorded at -10 , -5 , 0 , 5 and 10°C respectively. Chemical shift versus temperature graphs were plotted and temperature coefficients were calculated as the slopes of the fitted line through the data points.

For exchange rates (k_{ex}), NOE volumes of diagonal and cross peaks were measured from data of 2D phase-sensitive NOESY spectra, which were run using six different mixing times of 3–18 ms in 3 ms steps at -10°C . For each compound, data processing of recorded spectra was done with identical parameters. Exchange rate is calculated as the ratio of the initial build-up rates of exchange peaks over the volume of the diagonal peaks at zero mixing time.

2.2. Geometry optimizations

All geometry optimizations are done using Spartan 04 [43] software package. For each polyol, a preliminary set of 100 conformers was obtained by an MMFF conformer search as implemented in Spartan 04 (by Monte Carlo simulation, efficient for polyols [46] as well as oligosaccharides [47]). For each conformer, a single point calculation at B3LYP/6-31G* level of theory was done and the set was sorted according to relative energies. The calculations showed that a hydrogen bond formation lowers the energy of a molecule by maximum about 2.8 kcal/mol at B3LYP/6-31G* level. Considering that the higher the population of a conformer, the more it contributes to the NMR shifts, those with relative energies higher than 7 kcal/mol (ca. two times the energy of moderate hydrogen bonding) were eliminated to lower the computational cost. The remaining number of conformers were approximately half of the preliminary set obtained for each polyol. Geometries and energies were improved by further geometry optimizations; first at B3LYP/6-31G*, consequently at B3LYP/6-311 + G** calculations. An additional geometry optimization was carried out by lowering the gradient tolerance to 1.4×10^{-5} (default value 4.5×10^{-4}) to improve the convergence criterion for NMR shift calculations. Frequency calculations followed to verify no imaginary frequencies for the conformers which were taken to NMR shift calculations.

2.3. NMR chemical shift calculations

Since experimental NMR response is an average of stable conformers, we needed to classify the conformers according to their geometry, i.e. the dihedral angles. Every conformer has a number of dihedral angles, each of which is labelled either with letter "G" for gauche or "T" for trans position (Table 3). The combination of merged labels of all dihedral angles within a polyol leads to labeling of conformers with similar geometry as one single class. Within each class, conformer with the lowest energy is taken for further calculations.

NMR calculations were run on Gaussian 09 [48] package with GIAO method at the B3LYP/6-311 + G** level. TMS was used as the

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