Evidence of two-step deprotonation of D-mannitol in aqueous solution

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Received 22 July 2004; received in revised form 24 March 2005; accepted 24 March 2005

Abstract—Deprotonation of D-mannitol was studied in aqueous basic solutions by means of potentiometry and 13C NMR spectroscopy. Two-step dissociation in the pH range from 12 to 13.8 was shown, and successive dissociation constants \( K_{a1} \) and \( K_{a2} \) were determined. In a solution with ionic strength \( I = 1.0 \) M (NaOH + NaNO3) \( pK_{a1} = 13.1 \pm 0.1 \) and \( pK_{a2} = 13.8 \pm 0.2 \). With increasing ionic strength from 0.75 to 3.0 M, both \( pK_{a1} \) and \( pK_{a2} \) values decrease. Deprotonation-induced chemical shifts in pH-variable 13C NMR spectra show that the OH-groups next to internal carbon atoms C-3 and C-4 dissociate to a greater extent compared to OH-groups next to external carbon atoms C-1 and C-6.

Keywords: \( pK_a \); 13C NMR; Potentiometry; Metal ion chelation

D-Mannitol (1) is a widespread hexitol found in a variety of plants, algae and fungi. It is endogenous in humans and is a widely accepted food additive. Although its biological role is not fully understood, evidence suggests that D-mannitol is an important intermediate in the physiology of plants, animals and humans. In aqueous solution D-mannitol adopts a flat zigzag conformation, and sequesters metal ions forming surprisingly stable chelates. D-Mannitol complexes with oxometallates are stable in a wide pH range, however, complexes with simple non-oxo metal ions are rather weak in acidic and neutral media owing to the weak acidic D-mannitol properties. Stable non-oxo metal complexes with D-mannitol exist only in strongly basic solutions where hexitol deprotonation takes place. Acidic properties of D-mannitol were shown, and one-proton per D-mannitol molecule deprotonation is widely accepted. Reported \( pK_{a1} \) values range from 13.1 to 13.7. Two-step deprotonation was also suggested, however, no \( pK_{a2} \) value was reported. Series of studies have shown that D-mannitol chelates metal ions in bidentante, tetradenate and even hexadentate fashion, suggesting that deprotonation of multiple D-mannitol OH-groups is possible in strongly basic solution. Since no step-wise deprotonation constants for D-mannitol were known, D-mannitol deprotonation in strongly basic solutions by means of potentiometry and 13C NMR spectroscopy was investigated in the present study.

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0008-6215/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2005.03.006
Deprotonation of d-mannitol in basic media was studied potentiometrically, and the average number of dissociated OH groups per one d-mannitol molecule \( (N) \) was determined as a function of pH (Fig. 1). The \( N \) values larger than 1 clearly show that at least two-proton dissociation per one molecule takes place at high pH. The average number of dissociated group \( (N) \) for two deprotonating sites with successive dissociation constants \( K_{a1} \) and \( K_{a2} \) depends on solution pH according to Eq. 1. The experimental data points were fitted to Eq. 1 by a generalized reduced gradient procedure, and although high dispersion is observed for individual data points, the entire data set of 20–40 points allowed both \( K_{a1} \) and \( K_{a2} \) value determination with relatively small error. \( pK_a \) values obtained in solutions of various ionic strength are summarized in Table 1. With increasing ionic strength both dissociation constants increase, showing the propensity of d-mannitol to dissociate into ions in solutions with higher ionic strength. This trend is typical for weak acids. The experimental data points were also fitted to the simple one-proton dissociation model \( (N = \frac{10^{-pH} K_{a1} + K_{a1} K_{a2}}{10^{-2pH} + 10^{-pH} K_{a1} + K_{a1} K_{a2}}) \), however, the misfit indicated that at least two-step dissociation has to be considered to describe d-mannitol deprotonation at high pH.

\[
N = \frac{10^{-pH} K_{a1} + 2 \cdot K_{a1} K_{a2}}{10^{-2pH} + 10^{-pH} K_{a1} + K_{a1} K_{a2}}
\]  

(1)

The \(^{13}\text{C} \) NMR spectrum of d-mannitol obtained in aqueous solution is shown in Figure 2. It exhibits three signals at 63.9, 71.46 and 69.86 ppm, which were readily assigned to C-1, C-6, C-2, C-5 and C-3, C-4 carbon atoms, respectively. Observed chemical shifts are similar to the previously published values, with small differences caused by the solvent. Identical chemical shifts were observed for all signals in the pH range from 8 to 12, indicating that the d-mannitol molecule is not affected in neutral and weakly basic media. This observation supports our potentiometric study (Fig. 1). Upon further

<table>
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<tr>
<th>( I ) (M)</th>
<th>( pK_{a1} )</th>
<th>( pK_{a2} )</th>
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<tbody>
<tr>
<td>0.75</td>
<td>13.3 ± 0.2</td>
<td>13.9 ± 0.3</td>
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<tr>
<td>1.0</td>
<td>13.1 ± 0.1</td>
<td>13.8 ± 0.2</td>
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<tr>
<td>2.0</td>
<td>13.0 ± 0.2</td>
<td>13.7 ± 0.2</td>
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<tr>
<td>3.0</td>
<td>13.0 ± 0.1</td>
<td>13.5 ± 0.2</td>
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**Figure 1.** The average number of dissociated OH groups per one d-mannitol molecule \( (N) \) as a function of pH. The ionic strength (NaOH + NaNO\(_3\)) is equal to 0.75 M (a), 1.0 M (b), 2.0 M (c) and 3.0 M (d). Open circles represent experimental data points, and solid lines are best fits to Eq. (1).

**Figure 2.** The \(^{13}\text{C} \) NMR spectrum of aqueous 0.3 M d-mannitol solution (pH 11.5).
increase in solution pH all three signals shift downfield, showing that hydroxyl groups adjacent to carbon atoms are deprotonating (Fig. 3).

The deprotonation-induced chemical shift (DIS = $\delta_{\text{high\,pH}} - \delta_{\text{low\,pH}}$), similarly to coordination induced shift (CIS), allows determination of the most affected sites in a molecule. The pH increase has significantly higher effect on internal atoms C-2,C-5 and C-3,C-4 than on terminal atoms C-1,C-6 (Fig. 3). For instance, the DIS in solution with pH 13.8 compared to pH 11 solution is equal to 0.20, 1.57 and 2.57 ppm for C-1,C-6, C-2,C-5 and C-3,C-4 atoms, respectively. We conclude that dissociation degree of OH-groups decreases in the following order: C-3(OH)–C-4(OH) > C-2(OH)–C-5(OH) > C-1(OH)–C-6(OH). The consistent downfield shift was observed for pH values above 13.8 (data not shown), and all resonances continuously moved downfield without reaching the limiting value $\delta_{\text{high\,pH}}$. This observation suggested the deprotonation of multiple alkoxide groups.

Although there is a large repulsion in the d-mannitol molecule between two neighbouring C-O$^-$ moieties, dianion Man$^{2-}$ also forms in aqueous solution. The fast proton exchange among all OH-groups in the hexitolate anion, and the possible structures of deprotonated d-mannitol species Man$^-$ and Man$^{2-}$ are shown in Figure 4. Based on our pH-variable NMR study the form III is the most likely structure for Man$^-$ anion, whereas forms I and II represent possible, but less likely structures. Respectively, Man$^{2-}$ dianion form IV is expected to be more favourable than eight other possible forms (only two of them, V and VI are shown in Fig. 4). The dissociation impact on the chemical shift of C-1,C-6 and C-2,C-5 pairs can be attributed to the influence of minor forms I and II in monoanion, and forms V and VI in dianion (Fig. 4). Electrostatic shielding and conformational changes in the ionized molecule are also possible reasons of deprotonation-induced chemical shift.

Both potentiometry and pH-variable $^{13}$C NMR spectroscopy supports two-proton per d-mannitol molecule deprotonation in aqueous solution. The well-known formation of metal complexes with multiply deprotonated hexitols is usually referred to metal-promoted deprotonation, where negatively charged alkoxide groups are stabilized by transition metal vacant d-orbitals. We show that d-mannitol dianion forms in aqueous solution, where dissociation is caused solely by strong nucleophilicity of hydroxide ion.

1. Experimental

D-Mannitol (Aldrich) was dried under vacuum for 48 h. Aqueous CO$_2$-free standard solutions of NaOH (1.0 and 10 M) were prepared from 50% NaOH stock. The NaOH concentration was determined by diluted sample titration with strong acid. NaNO$_3$ (5 M) stock solution was prepared from high purity solid salt. Potentiometric titrations were carried out as follows: to the known amount of d-mannitol, NaOH and NaNO$_3$ stock solutions were added, and the pH values of resulted

![Figure 3](image-url)  
**Figure 3.** The $^{13}$C NMR chemical shift as a function of pH for aqueous d-mannitol solution.

![Figure 4](image-url)  
**Figure 4.** Possible structures of d-mannitol monoanion Man$^-$ (I, II and III) and dianion Man$^{2-}$ (IV, V and VI). See the text for details.
solutions were measured. The total molar concentration of NaOH and NaN\textsubscript{3} was kept constant. The difference between initial and final NaN\textsubscript{3} concentrations yielded the amount of NaOH reacted with d-mannitol, and the molar ratio of reacted NaOH to d-mannitol yielded the average number of dissociated OH-groups per one hexitol molecule.

\(^{13}\text{C}(^1\text{H})\) high-resolution NMR spectra were acquired on a Varian Inova 400 spectrometer operating at 100.6 MHz and temperature 22 ± 0.5 °C. Ethanol methylene signals at 58.05 ppm\textsuperscript{45} were used as internal reference. All chemical shifts were converted to TMS (tetramethylsilane) scale, with an estimated precision of ±0.01 ppm. Samples contained 0.3 M of d-mannitol, 0.1 M of ethanol, NaOH with total concentration from 0.01 to 9.2 M and 10 vol % of D\textsubscript{2}O (lock). The pH values were measured directly with Orion 427 pH-meter equipped with Corning glass electrode. The electrode was calibrated in pH region from 10 to 14 using three standard solutions. The sample pH was checked before and after a spectrum was recorded, and the difference between obtained values was 0.02 pH units or less. The spectra were run within 30 min after solution preparation.

Acknowledgements

E.N. thanks the Ministry of Education and Science of Republic of Lithuania for awarding the State Fellowship for Scientists. E.G., E.N. and G.B. are also indebted to Lithuanian State Science and Studies Foundation for financial support (Project C-03047).

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