Tandem Mass Spectrometric Characterization of the Conversion of Xylose to Furfural

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Abstract

Thermal decomposition of xylose into furfural under acidic conditions has been studied using tandem mass spectrometry. Two different Brønsted acids, maleic and sulfuric acids, were used to demonstrate that varying the Brønsted acid does not affect the mechanism of the reaction. Two selectively labeled xylose molecules, 1-¹³C and 5-¹³C-xyloses, were examined to determine which carbon atom is converted to the aldehyde carbon in furfural. This can be done by using tandem mass spectrometry since collision-activated dissociation (CAD) of protonated unlabeled furfural results in the loss of CO from the aldehyde moiety. The loss of a neutral molecule with MW of 29 Da (¹³CO) was observed for protonated furfural derived from 1-¹³C-labeled xylose while the loss of a neutral molecule with MW of 28 Da (CO) was observed for protonated furfural derived from 5-¹³C labeled xylose. These results support the hypothesis that the mechanism of formation of furfural under mildly hot acidic conditions involves an intramolecular rearrangement of protonated xylose into the pyranose form rather than into an open-chain form.

Keywords

Xylose • Furfural • Tandem mass spectrometry • Catalytic conversion • Maleic acid • Sulfuric acid • ¹³C labeling

1. Introduction

Plant biomass, mostly plant secondary cell walls, represents an important renewable source of energy as well as valuable organic chemicals [1-3]. For most plants, lignocellulosic biomass consists of cellulose (35-50%), non-cellulosic glycans (25-37%), as well as lignin and other phenolic substances (15-30%) [3]. Cellulose microfibrils give plant cell walls their foundational structure. Glucose derived from cellulose is the major substrate for fermentation of biomass to bioethanol [3]. However, for grasses and certain hardwoods, glucuronoxyran is the principal

glycan that coats cellulose microfibrils and interacts with lignin and other phenolic substances to establish the fundamental architecture of the secondary wall [4]. Hence, the conversion of xylan to more useful molecules is critically important if biomass feedstocks, such as corn and sorghum stover, switchgrass, sugarcane bagasse and poplar, are to be used as a source of energy or as starting materials for valuable organic compounds [6-9].

Furan derivatives, such as furfural and 5-(hydroxymethyl)furfural, have great potential as precursors for different chemicals, including pharmaceuticals, plastics and polymers [6-9].

Furfural, obtained by dehydration of xylose and xylan, is perhaps the most common industrial chemical derived from lignocellulosic biomass [10]. Conversion of xylose under acidic catalyst conditions is the most common method used to generate furfural [11-14]. However, the mechanism of formation of furfural under these conditions is still a subject of debate. Different mechanisms proposed for this reaction [11-13,15,17] are shown in Scheme 1. A quantum mechanical study ruled out mechanisms A and B shown in Scheme 1 and suggested that protonation of C2-OH followed by ring-contraction (mechanism C) is the most likely pathway [17].

Hence, the mechanism for formation of furfural most likely begins with a cyclic form of xylose (cyclic form of xylose, pyranose, is presented based on the mechanism studies by Antal et al. [15] and NMR studies by Drew et al. [16]), which is in agreement with kinetic studies of the conversion of xylose into furfural [15]. According to mechanism C, C-1 of xylose becomes the carbon at the aldehyde moiety in furfural (Scheme 1). However, another mechanism of conversion of xylose to furfural has been proposed [17] (mechanism D, Scheme 1), which was not discussed in the quantum mechanical study mentioned above [18]. Based on this fourth mechanism, C-5 of xylose becomes the carbon at the aldehyde group in furfural.

Further, maleic acid catalyzed dehydration of xylose has been shown to result in a higher selectivity toward furfural production than sulfuric acid catalyzed dehydration [19]. Possible

interactions between maleate and xylose through hydrogen bonds may explain this difference. However, it is unclear at this time whether this interaction would affect the mechanism of dehydration.

We report here on the use of atmospheric pressure chemical ionization (APCI) in combination with tandem mass spectrometry and selectively ^{13}C -labeled xyloses as a way to determine whether C-1 (mechanism C [18]) or C-5 (mechanism D [17]) becomes the carbon of the aldehyde group in furfural upon thermal degradation of xylose under acidic conditions. Further, both maleic and sulfuric acids were used as catalysts to explore whether the type of the acid has an influence on the reaction mechanism.

2. Experimental Section

2.1 Materials

Maleic acid ($\geq 99\%$) and furfural were purchased from Sigma-Aldrich and used as received without further purification. Sulfuric acid (95-98%) was purchased from Mallinckrodt Chemicals and used as received. Selectively isotopically labeled 1- ^{13}C and 5- ^{13}C xylose samples ($\geq 99\%$ enrichment) were purchased from Cambridge Isotope Laboratories, Inc., and used as received.

2.2 General Methods

All reactions were performed in a CEM DISCOVERTM SP Microwave System reactor described previously [19]. The reactor was set to 100 watts and cooled via nitrogen flow to end the reaction. Heat up times from 25 °C up to 200 °C averaged 75 seconds with a two-minute cool down to 60 °C. Reaction temperatures were recorded in real time by an IR fiber optic probe in the reactor.

2.3 Isotopically Labeled Xylose Solutions

Solutions of 50 mmol·L⁻¹ of 1-¹³C and 5-¹³C labeled xyloses (Cambridge Isotope Laboratories, Andover, MA) were prepared in sulfuric acid and maleic acid. The pH of all the solutions was set to 1.3 by adding the corresponding acid until the pH reading of the solution reached the desired value. All these solutions were stored at room temperature until use

2.4 Formation of Furfural

Microwave reaction tubes (10 mL) were loaded with each selectively ¹³C-labeled xylose solution (pH 1.3) and a stir bar. Reactions were performed at 200 °C for 8 minutes. The solutions were filtered through a 0.22 µm cut-off syringe filter (25 mm diameter) and analyzed by HPLC with refractive index for detection using a BioRad HPX-87H column at 65°C with mobile phase of 2mM H₂SO₄ in 95% (w/w) water 5% (w/w) acetonitrile at a flow rate of 0.6 mL/min as described by Kim *et al.* [19]. Consistent with the results previously reported when maleic acid was the catalyst [5,19], 65% of the xylose was consumed by the end of the reaction time with furfural produced at 80% molar yield (based upon sugar consumed). When sulfuric acid was the catalyst, 95% of the xylose was consumed with a molar yield of furfural of 65%.

2.5 Tandem Mass Spectrometry

Tandem mass spectrometry experiments were performed using a Thermo Scientific LTQ linear quadrupole ion trap (LQIT) mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source. All solutions were prepared at 10⁻³–10⁻⁵ mol·L⁻¹ concentrations in HPLC grade H₂O/CH₃OH (50:50, v/v). Typical APCI conditions were: discharge current, 5.0 µA; vaporizer temperature, 400 °C; sheath gas (N₂) flow, 30 (arbitrary units); auxiliary gas flow (N₂), 10 (arbitrary units); sweep gas flow (N₂), 0 (arbitrary units); and capillary temperature, 275 °C.

Voltages for the ion optics were optimized for each analyte by using the tune feature of the LTQ Tune Plus interface.

The experiments were performed using the advanced scan features of the LTQ Tune Plus interface. Collision-activated dissociation (CAD) experiments involved isolation of the analyte ions by using a 2 m/z window (full width) and their kinetic excitation for 30 ms by the application of an appropriate activation voltage (generally 22% of the “normalized collision energy” (NCE), as defined by the LTQ Tune Plus interface). Xcalibur 2.0 software was used for both data acquisition and processing. All mass spectra shown are an average of at least 20 scans.

Exact mass measurements were performed on a Finnigan dual-cell Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer equipped with a 7 Tesla superconducting magnet. Furfural was introduced via an Andonian leak valve into one of the cells of the ICR, followed by ionization via self-chemical ionization to obtain protonated furfural (m/z 97). Protonated furfural was transferred into the other cell and isolated via stored waveform inverse-Fourier transform (SWIFT) excitation pulses, which has been previously described [20-22]. For CAD experiments, the ion of interest was kinetically excited into a larger cyclotron orbit and then allowed to undergo activating collisions with argon admitted through a pulsed valve. The resulting fragment ions were excited and detected.

3. Results and Discussion

(+)APCI mass spectrometry has been used previously for the analysis of formic acid, acetic acid and furfural in preheated wheat straw hydrolysates [23]. Hence, (+)APCI was selected as the ionization method for this study. Authentic furfural was protonated using (+)APCI, isolated and subjected to collision-activated dissociation (CAD) in a tandem mass spectrometry (MS^2) experiment in order to determine its main fragmentation patterns. Loss of a neutral molecule with MW of 28 Da, which corresponds to CO, as confirmed by exact mass analysis, is the main

fragmentation reaction (Figure 1a). This reaction involves elimination of CO from the aldehyde functionality (Scheme 2), based on an extensive earlier study showing that related protonated aldehydes readily lose CO from the aldehyde functionality [24]. Hence, the loss of CO can be used as a diagnostic cleavage to determine which carbon of xylose, C-1 or C-5, becomes the carbon of the aldehyde moiety in furfural after acid degradation. MS analysis of the sulfuric and maleic acid solutions obtained after catalytic conversion of xylose with C-5 labeled as ^{13}C revealed protonated furfural (m/z 98) for both solutions. When the protonated furfural was subjected to CAD, the loss of a neutral molecule with MW of 28 Da was observed for both acid solutions, corresponding to the loss of unlabeled CO (Figure 1c) to give an ion of m/z 70. This result suggests that C-5 of xylose does not become the aldehyde carbon of furfural and instead becomes part of the furan ring.

The acidic solutions obtained after catalytic conversion of xylose with C-1 labeled with ^{13}C were analyzed in the same way as described above. Upon CAD of protonated furfural, a loss of a neutral molecule with MW of 29 Da was observed, which correspond to the loss of ^{13}CO (Figure 1b), to give an ion of m/z 69. This result confirms that C-1 of xylose is the carbon that is converted to the aldehyde moiety of furfural. Hence, mechanism **C** (Scheme 1) is the most likely mechanism for formation of furfural upon acid-catalyzed degradation of xylose. These findings support the earlier quantum mechanical study discussed above [18].

4. Conclusions

The loss of CO from the aldehyde moiety of protonated furfural during CAD allows one to determine which carbon atom in partially ^{13}C -labelled xylose, C-1 or C-5, becomes the carbon atom at the aldehyde moiety in furfural. The CAD results show clearly that C-1 of xylose is converted to the aldehyde carbon during an intramolecular rearrangement of xylose, hence

supporting the mechanism **C** (Scheme 1) as the path for the formation of furfural. Reactions catalysed by maleic and sulfuric acids followed the same mechanism.

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References

- [1] Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM. Ethanol Can Contribute to Energy and Environmental Goals. *Science* 2006;311(5760): 506-8.
- [2] Dodds DR, Gross RA. Chemicals from Biomass. *Science* 2007;318(5854): 1250-1.
- [3] Pauly M, Keegstra K. Cell-wall Carbohydrates and their Modification as a Resource for Biofuels. *Plant J.* 2008;54(4): 559-68.
- [4] Carpita NC, McCann MC. Maize and Sorghum: Genetic Resources for Bioenergy Grasses. *Trends Plant Sci.* 2008;13(8): 415-20.
- [5] Lu Y, Mosier NS. Biomimetic Catalysis for Hemicellulose Hydrolysis in Corn Stover. *Biotechnol. Progr.* 2007;23(1): 116-23.
- [6] Chheda JN, Huber GW, Dumesic JA. Liquid-Phase Catalytic Processing of Biomass-Derived Oxygenated Hydrocarbons to Fuels and Chemicals. *Angew. Chem. Int. Ed.* 2007;46(38): 7164-83.
- [7] Huber GW, Corma A. Synergies between Bio- and Oil Refineries for the Production of Fuels from Biomass. *Angew. Chem. Int. Ed.* 2007;46(38): 7184-7201.
- [8] Román-Leshkov Y, Chheda JN, Dumesic JA. Phase Modifier Promote Efficient Production of Hydroxymethylfurfural from Fructose. *Science* 2006;312(5782): 1933-7.
- [9] Zhao H, Holladay JE, Brown H, Zhang ZC. Metal Chlorides in Ionic Liquid Solvents Convert Sugar to 5-Hydroxymethylfurfural. *Science* 2007;316(5831): 1597-1600.

- 186 [10] Binder JB, Blank JJ, Cefali AV, Raines RT. Synthesis of Furfural from Xylose and Xylan.
187 ChemSusChem 2010;3(11): 1268-72.
- 188 [11] Feather MS. The Conversion of D-Xylose and D-glucuronic Acid to 2-furaldehyde.
189 Tetrahedron Lett. 1970;11(48): 4143-5.
- 190 [12] Feather MS, Harris DW, Nichols SB. Routes of Conversion of D-xylose Hexuronics Acids
191 and L-ascorbic Acids to 2-Furfural. J. Org. Chem. 1972;37(10): 1606-8.
- 192 [13] Harris DW, Feather MS. Evidence for C2 to C1 Intramolecular Hydrogen Transferring
193 during the Acid-Catalized Isomerization of D-Glucose to D-Fructose. Carbohydr. Res.
194 1973;30(2): 359-65.
- 195 [14] Hurd CD, Isenhour LL. Pentose Reactions: I Furfural Formation J. Am. Chem. Soc.
196 1932;54(1): 317-30.
- 197 [15] Antal MJ, Leesomboon T, Mok WS, Richards GN. Mechanism of Formation of 2-
198 Furaldehyde from D-Xylose. Carbohydr. Res. 1991;September 217: 71-85.
- 199 [16] Drew KN, Zajicek J, Bondo G, Bose B, Serianni AS. ¹³C-labeled Aldopentoses: Detection
200 and Quantitation of Cyclic and Acyclic forms by Heteronuclear 1D and 2D NMR
201 Spectroscopy. Carbohydr. Res. 1998;September 307: 199-209.
- 202 [17] Zeitsch KJ. The Chemistry and Technology of Furfural and its Many Byproducts. 1st ed.
203 Netherlands: Elsevier; 2000.
- 204 [18] Nimlos MR, Qian X, Davis M, Himmel ME, Johnson DKJ. Energetics of Xylose
205 Decomposition as Determined using Quantum Mechanics Modeling. Phys. Chem. A
206 2006;110(42): 11824-38.
- 207 [19] Kim ES, Liu S, Abu-Omar MM, Mosier NS. Selective Conversion of Biomass
208 Hemicellulose to Furfural using Maleic Acid with Microwave Heating. Energy Fuels
209 2012;26(2): 1298-1304.
- 210 [20] Amster IJ. Fourier Transform Mass Spectrometry. J. Mass Spectrom. 1996;31(12): 1325-
211 37.
- 212 [21] Chen L, Wang TCL, Ricca TL, Marshall AG. Phase – Modulated Stored Waveform
213 Inverse Fourier Transform Excitation for Trapped Ion Mass Spectrometry. Anal. Chem.
214 1987;59(3): 449-54.

- [22] Marshall AG, Wang TCL, Ricca TL. Tailored Excitation for Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. *J. Am. Chem. Soc* 1985;107(26): 7893-7.
- [23] Davies SM, Linforth RS, Wilkinson SJ, Smart KA, Cook DJ. Rapid Analysis of Formic Acid, Acetic Acid and Furfural in Pretreated Wheat Straw Hydrolysates Ethanol in a Bioethanol Fermentation using Atmospheric Pressure Chemical Ionisation Mass Spectrometry. *Biotechnol Biofuels* 2011; Sept. 4: 28-35.
- [24] Amundson LM, Eismann J, Reece JN, Fu M, Habicht SC, Mossman AB, et al. Identification and Counting of Oxygen Functionalities in Aromatic Analytes Related to Lignin by using Negative Mode Electrospray Ionization and Multiple Collision Activated Dissociation Steps. *Energy Fuels* 2011;25(7): 3212-22.

Figure 1. (a) MS² spectrum obtained after CAD of protonated furfural (m/z 97). The loss of a neutral molecule with MW of 28 Da (CO) yields a cation of m/z 69. (b) MS² spectrum obtained after CAD of protonated, partially ¹³C-labeled furfural (m/z 98) generated upon thermal degradation of 1-¹³C xylose. The loss of a neutral molecule with MW of 29 Da (¹³CO) yields a cation of m/z 69. (c) MS² spectrum obtained after CAD of protonated partially ¹³C-labeled furfural

233 (m/z 98) generated upon thermal degradation of 5- ^{13}C xylose. The loss of a neutral molecule
234 with MW of 28 Da (CO) yields a cation of m/z 70.

235

236

237

a

-28 (CO)

A

B

C

D

239 Scheme 1. Proposed mechanisms for the formation of furfural from xylose. Mechanism A,¹¹⁻¹⁴ B,¹⁵
240 C,¹⁵ and D.¹⁷

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244 Scheme 2. Proposed mechanism of the CO loss from furfural after CAD.