

1 Tandem Mass Spectrometric Characterization of the 2 Conversion of Xylose to Furfural

3 Nelson R. Vinueza,^{a,e} Eurick S. Kim,^a Vanessa A. Gallardo,^a Nathan S. Mosier,^b Mahdi M. Abu-
4 Omar,^{a,c} Nicholas C. Carpita^d and Hilkka I. Kenttämäa^{*a}

5 [a] Dr. N. R. Vinueza, E. U. Kim, V. A. Gallardo, Prof. M. M. Abu-Omar Prof. H. I. Kenttämäa.

6 Department of Chemistry, Purdue University, West Lafayette, IN 47907 USA

7 Fax(+1)765- 494-0239

8 E-mail: hilkka@purdue.edu

9 [b] Prof. N. S. Mosier.

10 Department of Agricultural and Biological Engineering, Laboratory of Renewable Resources
11 Engineering, Purdue University, West Lafayette, IN 47907 USA

12 [c] Prof. M.M. Abu-Omar.

13 School of Chemical Engineering, Purdue University, West Lafayette, IN 47907 USA

14 [d] Prof. N. C. Carpita.

15 Department of Botany and Plant Pathology and Bindley Biosciences Center, Purdue
16 University, West Lafayette, IN 47907 USA

17 Author Present Address

18 [e] Dr. N. R. Vinueza

19 Department of Textile Engineering, Chemistry and Science, North Carolina State University,
20 Raleigh, NC 27695 USA

21

22

23 **Abstract**

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

36 **Keywords**

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

39 **1. Introduction**

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

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

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

53 Furfural, obtained by dehydration of xylose and xylan, is perhaps the most common industrial
54 chemical derived from lignocellulosic biomass [10]. Conversion of xylose under acidic catalyst
55 conditions is the most common method used to generate furfural [11-14]. However, the
56 mechanism of formation of furfural under these conditions is still a subject of debate. Different
57 mechanisms proposed for this reaction [11-13,15,17] are shown in Scheme 1. A quantum
58 mechanical study ruled out mechanisms A and B shown in Scheme 1 and suggested that
59 protonation of C2-OH followed by ring-contraction (mechanism C) is the most likely pathway
60 [17].

61 Hence, the mechanism for formation of furfural most likely begins with a cyclic form of xylose
62 (cyclic form of xylose, pyranose, is presented based on the mechanism studies by Antal et al.
63 [15] and NMR studies by Drew et al. [16]), which is in agreement with kinetic studies of the
64 conversion of xylose into furfural [15]. According to mechanism C, C-1 of xylose becomes the
65 carbon at the aldehyde moiety in furfural (Scheme 1). However, another mechanism of
66 conversion of xylose to furfural has been proposed [17] (mechanism D, Scheme 1), which was
67 not discussed in the quantum mechanical study mentioned above [18]. Based on this fourth
68 mechanism, C-5 of xylose becomes the carbon at the aldehyde group in furfural.

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

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

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

80 **2. Experimental Section**

81 2.1 Materials

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

86 2.2 General Methods

87 All reactions were performed in a CEM DISCOVERTM SP Microwave System reactor described
88 previously [19]. The reactor was set to 100 watts and cooled via nitrogen flow to end the reaction.
89 Heat up times from 25 °C up to 200 °C averaged 75 seconds with a two-minute cool down to 60
90 °C. Reaction temperatures were recorded in real time by an IR fiber optic probe in the reactor.

91 2.3 Isotopically Labeled Xylose Solutions

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

96

97 .4 Formation of Furfural

98 Microwave reaction tubes (10 mL) were loaded with each selectively ¹³C-labeled xylose solution
99 (pH 1.3) and a stir bar. Reactions were performed at 200 °C for 8 minutes. The solutions were
100 filtered through a 0.22 µm cut-off syringe filter (25 mm diameter) and analyzed by HPLC with
101 refractive index for detection using a BioRad HPX-87H column at 65°C with mobile phase of
102 2mM H₂SO₄ in 95% (w/w) water 5% (w/w) acetonitrile at a flow rate of 0.6 mL/min as described
103 by Kim *et al.* [19]. Consistent with the results previously reported when maleic acid was the
104 catalyst [5,19], 65% of the xylose was consumed by the end of the reaction time with furfural
105 produced at 80% molar yield (based upon sugar consumed). When sulfuric acid was the
106 catalyst, 95% of the xylose was consumed with a molar yield of furfural of 65%.

107 2.5 Tandem Mass Spectrometry

108 Tandem mass spectrometry experiments were performed using a Thermo Scientific LTQ linear
109 quadrupole ion trap (LQIT) mass spectrometer equipped with an atmospheric pressure chemical
110 ionization (APCI) source. All solutions were prepared at 10⁻³ –10⁻⁵ mol·L⁻¹ concentrations in
111 HPLC grade H₂O/CH₃OH (50:50, v/v). Typical APCI conditions were: discharge current, 5.0 µA;
112 vaporizer temperature, 400 °C; sheath gas (N₂) flow, 30 (arbitrary units); auxiliary gas flow (N₂),
113 10 (arbitrary units); sweep gas flow (N₂), 0 (arbitrary units); and capillary temperature, 275 °C.

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

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

122 Exact mass measurements were performed on a Finnigan dual-cell Fourier-transform ion
123 cyclotron resonance (FT-ICR) mass spectrometer equipped with a 7 Tesla superconducting
124 magnet. Furfural was introduced via an Andonian leak valve into one of the cells of the ICR,
125 followed by ionization via self-chemical ionization to obtain protonated furfural (m/z 97).

126 Protonated furfural was transferred into the other cell and isolated via stored waveform inverse-
127 Fourier transform (SWIFT) excitation pulses, which has been previously described [20-22]. For
128 CAD experiments, the ion of interest was kinetically excited into a larger cyclotron orbit and then
129 allowed to undergo activating collisions with argon admitted through a pulsed valve. The
130 resulting fragment ions were excited and detected.

131 **3. Results and Discussion**

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

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

149 The acidic solutions obtained after catalytic conversion of xylose with C-1 labeled with ^{13}C were
150 analyzed in the same way as described above. Upon CAD of protonated furfural, a loss of a
151 neutral molecule with MW of 29 Da was observed, which correspond to the loss of ^{13}CO (Figure
152 1b), to give an ion of m/z 69. This result confirms that C-1 of xylose is the carbon that is
153 converted to the aldehyde moiety of furfural. Hence, mechanism **C** (Scheme 1) is the most likely
154 mechanism for formation of furfural upon acid-catalyzed degradation of xylose. These findings
155 support the earlier quantum mechanical study discussed above [18].

156 **4. Conclusions**

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

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

163 **Acknowledgements**

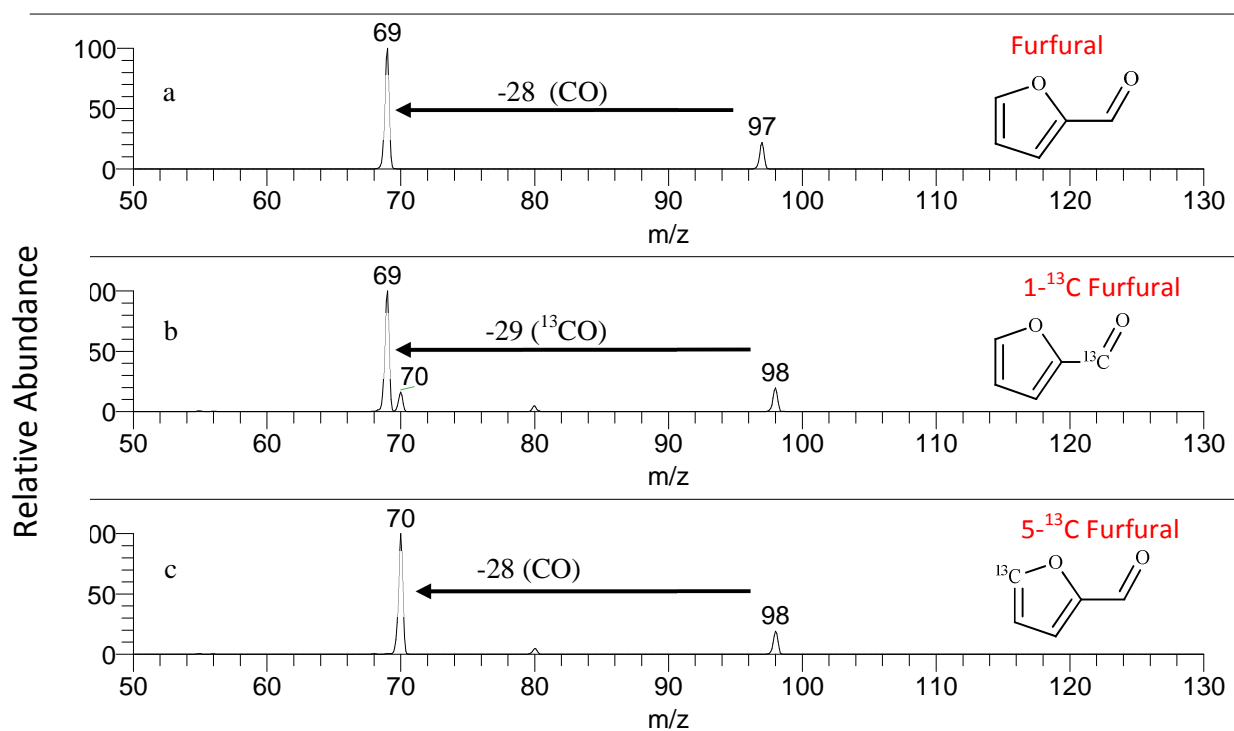
164 This work was supported by the Center for Direct Catalytic Conversion of Biomass to Biofuels
165 (C3Bio), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office
166 of Science, Office of Basic Energy Sciences, Award Number DE-SC0000997.

167 **References**

- 168 [1] Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM. Ethanol Can
169 Contribute to Energy and Environmental Goals. *Science* 2006;311(5760): 506-8.
- 170 [2] Dodds DR, Gross RA. Chemicals from Biomass. *Science* 2007;318(5854): 1250-1.
- 171 [3] Pauly M, Keegstra K. Cell-wall Carbohydrates and their Modification as a Resource for
172 Biofuels. *Plant J.* 2008;54(4): 559-68.
- 173 [4] Carpita NC, McCann MC. Maize and Sorghum: Genetic Resources for Bioenergy
174 Grasses. *Trends Plant Sci.* 2008;13(8): 415-20.
- 175 [5] Lu Y, Mosier NS. Biomimetic Catalysis for Hemicellulose Hydrolysis in Corn Stover.
176 *Biotechnol. Progr.* 2007;23(1): 116-23.
- 177 [6] Chheda JN, Huber GW, Dumesic JA. Liquid-Phase Catalytic Processing of Biomass-
178 Derived Oxygenated Hydrocarbons to Fuels and Chemicals. *Angew. Chem. Int. Ed.*
179 2007;46(38): 7164-83.
- 180 [7] Huber GW, Corma A. Synergies between Bio- and Oil Refineries for the Production of
181 Fuels from Biomass. *Angew. Chem. Int. Ed.* 2007;46(38): 7184-7201.
- 182 [8] Román-Leshkov Y, Chheda JN, Dumesic JA. Phase Modifier Promote Efficient
183 Production of Hydroxymethylfurfural from Fructose. *Science* 2006;312(5782): 1933-7.
- 184 [9] Zhao H, Holladay JE, Brown H, Zhang ZC. Metal Chlorides in Ionic Liquid Solvents
185 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.

- 215 [22] Marshall AG, Wang TCL, Ricca TL. Tailored Excitation for Fourier Transform Ion
 216 Cyclotron Resonance Mass Spectrometry. *J. Am. Chem. Soc* 1985;107(26): 7893-7.
- 217 [23] Davies SM, Linforth RS, Wilkinson SJ, Smart KA, Cook DJ. Rapid Analysis of Formic
 218 Acid, Acetic Acid and Furfural in Pretreated Wheat Straw Hydrolysates Ethanol in a
 219 Bioethanol Fermentation using Atmospheric Pressure Chemical Ionisation Mass
 220 Spectrometry. *Biotechnol Biofuels* 2011; Sept. 4: 28-35.
- 221 [24] Amundson LM, Eismen J, Reece JN, Fu M, Habicht SC, Mossman AB, et al. Identification
 222 and Counting of Oxygen Functionalities in Aromatic Analytes Related to Lignin by using
 223 Negative Mode Electrospray Ionization and Multiple Collision Activated Dissociation
 224 Steps. *Energ Fuels* 2011;25(7): 3212-22.
- 225
- 226



227

228 **Figure 1.** (a) MS² spectrum obtained after CAD of protonated furfural (m/z 97). The loss of a
 229 neutral molecule with MW of 28 Da (CO) yields a cation of m/z 69. (b) MS² spectrum obtained
 230 after CAD of protonated, partially ¹³C-labeled furfural (m/z 98) generated upon thermal
 231 degradation of 1-¹³C xylose. The loss of a neutral molecule with MW of 29 Da (¹³CO) yields a
 232 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

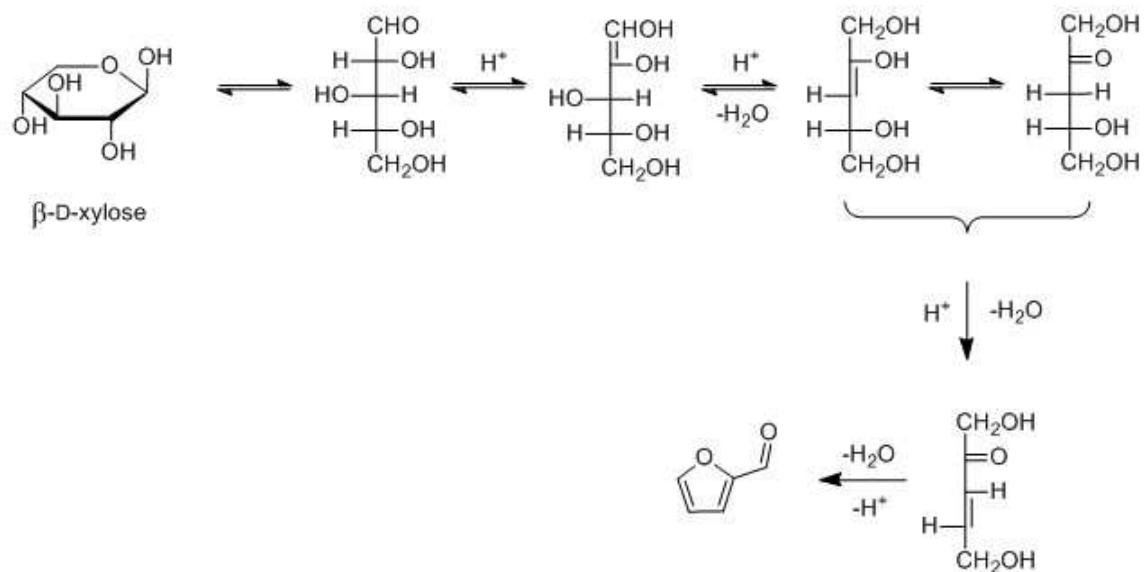
236

237

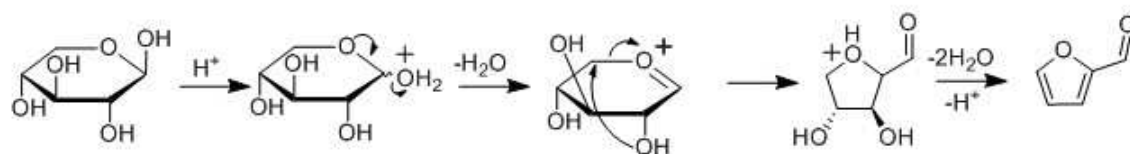
a

-28 (CO)

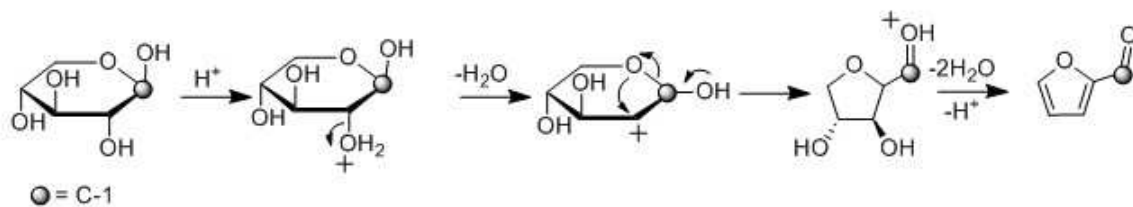
A



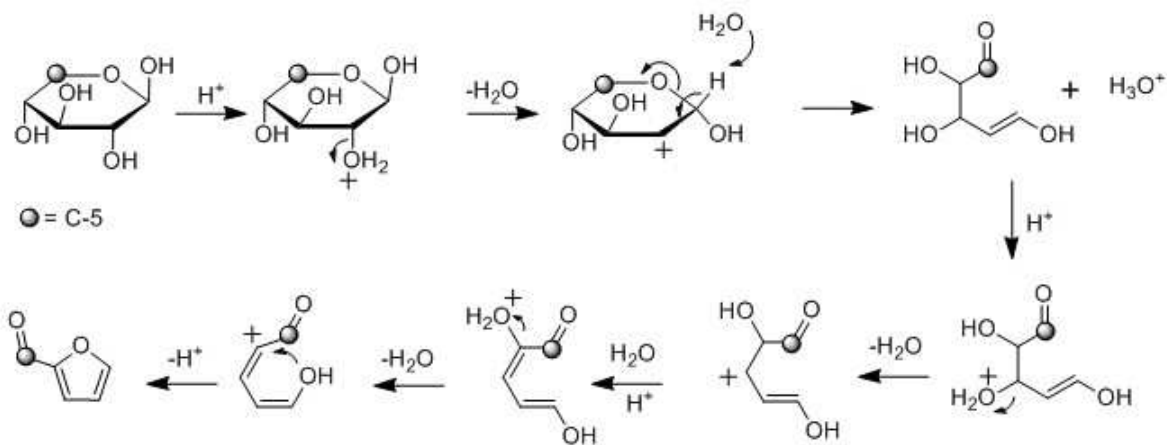
B



C



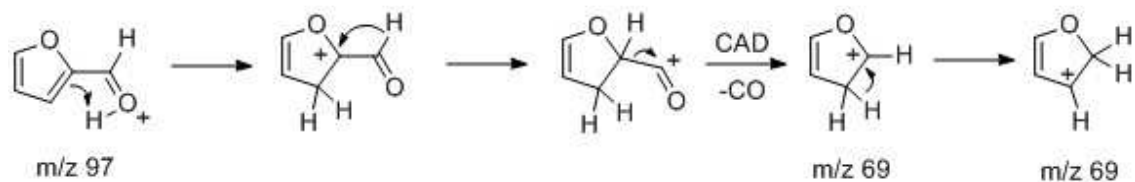
D



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

241

242



243

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