Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Research 341 (2006) 2290–2292
Carbohydrate RESEARCH

Note

Enzymatic synthesis of aldonic acids

F. Pezzotti and M. Therisod*

Laboratoire de Chimie Bioorganique et Bioinorganique, UMR 8182, ICMMO, Université Paris-Sud, 91405 Orsay, France

Received 6 April 2006; received in revised form 19 May 2006; accepted 25 May 2006
Available online 27 June 2006

Abstract—Several aldonic acids (D-mannonic, D-galactonic, D-xylonic, 2-deoxy-D-arabinohexonic (2-deoxy-D-gluconic)) were prepared on a scale of several grams by a simple oxidation catalyzed by glucose oxidase in pure water.

Keywords: Aldonic acids; Oxidation; Glucose oxidase; Bioconversion; Green chemistry

Polyhydroxy acids and mostly sugar acids have been proved to be useful in a wide range of practical applications, especially as sequestering agents for metal ions (Zn, Fe, Cu, Ca in the presence of borate).[1,2] These metal–aldonate complexes have found industrial, medical and agricultural applications.[3,4] Their structures and physicochemical properties have been extensively studied.[1] Xylonic acid has recently been shown to be a valuable precursor for an industrial synthesis of 1,2,4-butanetriol through microbial fermentation.[5]

The most common methods of preparation of aldonic acids use stoichiometric amounts of bromine, copper or silver hydroxides in totally non-ecological processes, and their chemical synthesis have been reviewed recently.[6,7] Alternatively, some aldonic acids like gluconic acid and their metabolites have been prepared by fermentation.[8] However, the products must then be purified from complex aqueous media. An efficient synthesis of D-xylonic acid from D-xylose (70%) by oxidation using fermentor-controlled cultures of a particular strain of *Pseudomonas fragi* has been reported.[5]

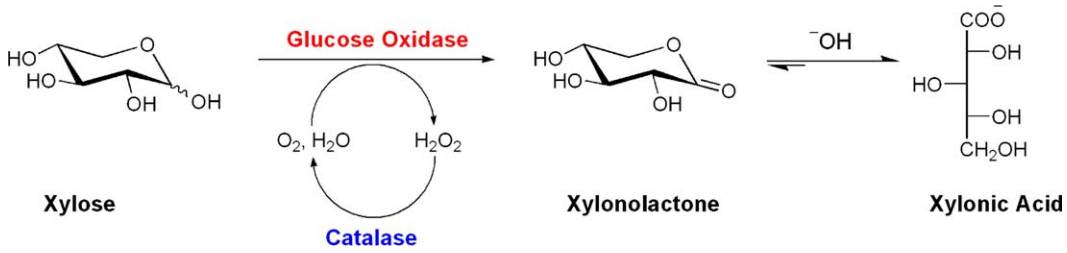
Glucose oxidase (GO; EC 1.1.3.4) has the reputation of being extremely specific for D-glucose to the point of being considered as its single substrate. This characteristic is commonly used in analytical biochemistry for the selective estimation of glucose in complex biological fluids (e.g., blood).[9] Early studies[10,11] indicated that other sugars were oxidized only at a very slow or negligible rate as compared to glucose, ranging from 0.5% for galactose, 1% for mannose, 2% for 2-amino-2-deoxy-D-glucose to 20% for 2-deoxy-D-*arabino*-hexose (2-deoxyglucose). GO therefore appeared to be an unusable enzyme for the preparative bioconversion of sugars, except glucose itself. Chemists using enzymes in organic synthesis more often have to deal with non-natural substrates, on which enzymes have low activity. They overcome this difficulty by extending the reaction time (several days) if the enzyme is stable enough and/or commercially available in notable quantities.

GO from *Aspergillus niger* is a robust enzyme now produced on an industrial scale and marketed in large quantities at reasonable prices from several companies like Novozymes Biotech, Inc., and Amano Enzyme, Inc. (Besides its use in biochemical analysis, it is an important additive in bakery products.) This opens new prospects for the use of this biocatalyst. As an example, we report herein our results relative to the preparative oxidation by GO of 'inert' aldoses, as a continuation of our work previously reported on the enzymatic preparation of 2-amino-2-deoxy-D-gluconic acid from 2-amino-2-deoxy-D-glucose (glucosamine).[12]

Oxidation was performed by simple dissolution of the sugar in water, followed by addition of glucose oxidase and catalase under vigorous agitation [catalase (E.C. 1.11.1.6) was added in order to decompose the hydrogen peroxide formed as a byproduct, which is known to have a deleterious effect on GO; Scheme 1]. The aldonic acid

* Corresponding author. Tel.: +33 16915 6311; fax: +33 16915 7281; e-mail: therisod@icmo.u-psud.fr

0008-6215/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2006.05.023



Scheme 1. Synthesis of aldonic acids (e.g., xylonic acid) catalyzed by a two-enzyme system of glucose oxidase and catalase.

formed during oxidation was continuously neutralized with NaOH, added by means of a pH-stat. As a result, the pH of the medium was maintained constant during the reaction, and the volume of added NaOH was directly proportional to the amount of sugar being oxidized (Fig. 1). The reaction mixture was then filtered through a column of Dowex 1 (AcO^- form), from which the aldonic acid was eluted with 1 M aqueous HCl. NMR (D_2O, Na_2CO_3) indicated that this simple purification gave a pure product, free from the enzymes and from any unreacted substrate. We report a complete assignment of the ^1H and ^{13}C NMR signals of the aldonic acids (salts), which has not been published before.

Not surprisingly, the reaction rate was dependent on temperature (Fig. 2). When conducting the reaction under an atmosphere of pure oxygen, the reaction rate was also enhanced, but incomplete oxidation occurred, probably as a result of an oxidative denaturation of the enzyme.

Oxidations of up to 100% could be obtained within 40 h (e.g., with D-mannose). As long as few grams of products are needed, it is not worth recycling the enzymes, considering their low costs. For large-scale syntheses, however, immobilization of GO and catalase can be considered, for which number of established procedures are available.[13]

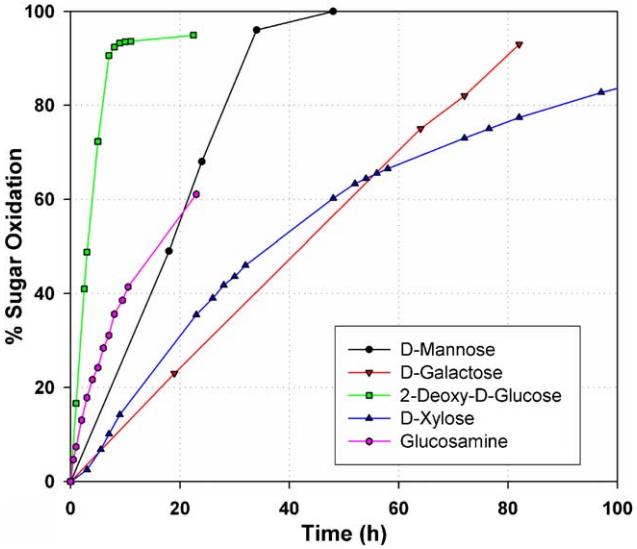


Figure 1. Oxidation of various aldoses into the corresponding aldonic acids catalyzed by glucose oxidase at room temperature (estimated from the volume of NaOH delivered by a pH-stat).

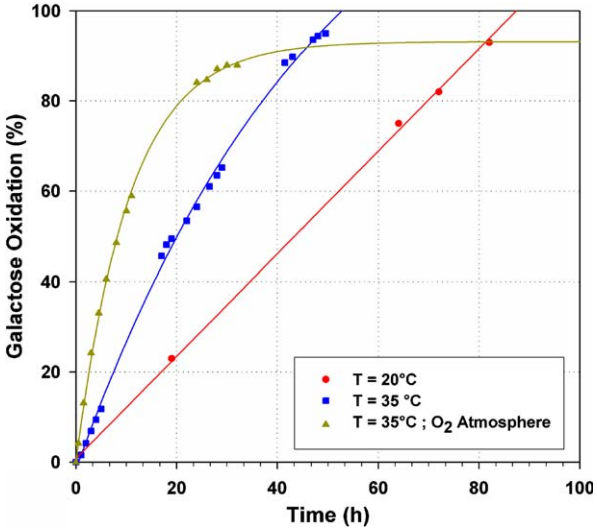


Figure 2. Galactose oxidation dependence on temperature and oxygen atmosphere.

In conclusion, we have devised a very simple procedure for the preparative synthesis of various aldonic acids from the corresponding aldoses. This 'green chemistry' process takes advantage of the availability of cheap, robust industrial enzymes.

1. Experimental

1.1. Enzymes

Glucose oxidase from *A. niger* (Gluzyme®, from Novozymes) had a specific activity of 2 U/mg. Catalase (Catazyme®, from Novozymes) had a specific activity of 25 kU/mL.

1.2. Oxidation procedure

The aldose (11.1 mmol) was dissolved in water to a final concentration of 0.5 M (0.6 mmol and 0.12 M for

2-deoxy-D-glucose) and subjected to oxidation by addition of 200 mg of glucose oxidase (400 U) (10 mg for 2-deoxy-D-glucose) and a large excess of catalase (1 mL, 25 kU). The mixture was vigorously stirred under air, and the pH was kept constant at pH 7.5 by means of a pH-stat adding continuously 1 M NaOH (0.1 M for 2-deoxy-D-glucose). Conversion was directly calculated considering the volume of added NaOH since 1 mol of NaOH neutralizes 1 mol of aldonic acid formed. The reaction mixture was then filtered through a Dowex 1 (AcO^-) column to eliminate the enzymes and any residual substrate. Aldonic acids were then recovered by elution with 1 M aq HCl. After evaporation in vacuo, the identity and purity of each product were confirmed by ^1H and ^{13}C NMR spectroscopy (D_2O–Na_2CO_3). The ^1H and ^{13}C NMR signals were assigned using two-dimensional NMR COSY and HMQC experiments.

1.2.1. 2-Deoxy-D-arabino-hexonic acid (2-deoxy-D-gluconic acid). Yield: 0.1 g, 92%. ^1H NMR (360 MHz, D_2O): δ 2.52 (dd, 1H, *J* 15, *J* 5.8, H2), 2.55 (dd, 1H, *J* 15, *J* 8.3, H2′), 3.52 (dd, 1H, *J* 8.3, *J* 2, H4), 3.76 (dd, 1H, *J* 12, *J* 6.3, H6), 3.8 (m, 1H, H5), 3.9 (dd, 1H, *J* 11.5, *J* 2.9, H6′), 4.3 (ddd, 1H, *J* 8.3, *J* 5.4, *J* 1.8, H3). ^{13}C NMR (90 MHz, D_2O): δ 41.61 C2, 63.07 C6, 67.89 C3, 71.28 C5, 72.87 C4, 180.33 C1. Lit.: ^1H NMR (360 MHz, D_2O): δ 2.49 (m, 2H), 3.51 (m, 1H), 3.69 (m, 1H), 3.79 (m, 1H), 3.87 (m, 1H), 4.29 (m, 1H). ^{13}C NMR (D_2O): δ 38.93, 63.30, 67.16, 71.30, 72.82, 176.36.[14] $[\alpha]_D$ 5.05 (*c* 2.18, H_2O). Lit. $[\alpha]_D$ 4.2 (*c* 2, H_2O).[15]

1.2.2. D-Galactonic acid. Yield: 1.673 g, 77%. ^1H NMR (250 MHz, D_2O): δ 3.56 (dd, 1H, *J* 9.6, *J* 1.5, H4), 3.62 (d, 2H, *J* 6.4, H6-H6′), 3.88 (dd, 1H, *J* 9.5, *J* 1.5, H5), 3.90 (dd, 1H, *J* 9.5, *J* 1.5, H3), 4.2 (d, 1H, *J* 1.5, H2). ^{13}C NMR (62 MHz, D_2O): δ 63.35 C6, 69.82 C4, 70.16 C5, 71.42 C3, 71.57 C2, 179.64 C6. Lit.: ^1H NMR (D_2O): δ 3.70, 3.75, 4.02, 4.03, 4.32. ^{13}C NMR (62 MHz, D_2O): δ 64.79, 71.14, 71.53, 72.84, 72.84, 181.19.[16] $[\alpha]_D$ 1.6 (*c* 10, H_2O). Lit. $[\alpha]_D$ 0.4.[17]

1.2.3. D-Mannonic acid. Yield: 1.5 g, 70%. ^1H NMR (360 MHz, D_2O): δ 3.6 (dd, 1H, *J* 11.5, *J* 2.7, H6), 3.67 (br s, 2H, H5-H4), 3.75 (d, 1H, *J* 11.5, H6′), 3.92 (d, 1H, *J* 5.6, H3), 4.06 (d, 1H, *J* 5.6, H2). ^{13}C NMR (90 MHz, D_2O): δ 62.95 C6, 70.42 C3, 70.49 C5, 70.86 C4, 73.84 C2, 179.25 C1. Lit.: ^{13}C NMR (D_2O): δ 64.0, 70.4, 71.3, 71.8, 72.3, 177.4.[18] $[\alpha]_D$ −8.9 (*c* 10, H_2O). Lit. $[\alpha]_D$ −8.82.[17]

1.2.4. D-Xylonic acid. Yield: 1.98 g, 90%. ^1H NMR (360 MHz, D_2O): δ 3.50 (dd, 1H, *J* 11.8, *J* 5.4, H5), 3.62 (dd, 1H, *J* 11.7, *J* 3.9, H5′), 3.71 (m, 1H, H4), 3.76 (dd, 1H, *J* 5.9, *J* 2.5, H3), 3.99 (d, 1H, *J* 2.55, H2). ^{13}C NMR (90 MHz, D_2O): δ 62.72 C5, 72.54 C3, 73.09 C2, 73.19 C4, 179.19 C1. Lit.: ^{13}C NMR (D_2O): δ 63.9, 73.9, 74.1, 74.3, 180.1.[19] $[\alpha]_D$ 7.05 (*c* 10, H_2O). Lit. $[\alpha]_D$ 7.4.[20]

Acknowledgements

We are grateful to Novozymes Biotech., Inc., for the kind supply of glucose oxidase. This work was supported by a studentship to F.P. from Mexican Government CONACyT (National Council for Science and Technology).

References

1. Gajda, T.; Gyurcsik, B.; Jakusch, T.; Burger, K.; Henry, B.; Delpuech, J.-J. *Inorg. Chem. Acta* **1998**, *275–276*, 130–140.
2. van Duin, M.; Peters, J. A.; Kieboom, A. P. G.; van Bekkum, H. *Carbohydr. Res.* **1987**, *165*, 65–78.
3. Hegenauer, J.; Saltman, P.; Ludwig, J. *J. Agric. Food. Chem.* **1979**, *27*, 868–871.
4. Sawyer, D. T. *Chem. Rev.* **1964**, *64*, 633–643.
5. Niu, W.; Molefe, M. N.; Frost, J. W. *J. Am. Chem. Soc.* **2003**, *125*, 12998–12999.
6. Varela, O. *Adv. Carbohydr. Chem. Biochem.* **2003**, *58*, 307–369.
7. Lederkremer, R. M.; Marino, C. *Adv. Carbohydr. Chem. Biochem.* **2003**, *58*, 199–306.
8. Tanimura, R.; Hamada, A.; Ikehara, K.; Iwamoto, R. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 291–298.
9. Wilson, R.; Turner, A. P. F. *Biosens. Bioelectron.* **1992**, *7*, 165–185.
10. Sols, A.; De la Fuente, G. *Biochim. Biophys. Acta* **1957**, *24*, 206–207.
11. Pazur, J. H.; Kleppe, K. *Biochemistry* **1964**, *578*, 578–583.
12. Pezzotti, F.; Therisod, H.; Therisod, M. *Carbohydr. Res.* **2005**, *340*, 139–141.
13. van de Velde, F.; Lourenço, N. D.; Bakker, M.; van Rantwijk, F.; Sheldon, R. A. *Biotechnol. Bioeng.* **2000**, *69*, 286–291.
14. Freimund, S.; Huwig, A.; Giffhorn, F.; Köpper, S. *Chem. Eur. J.* **1998**, *4*, 2442–2455.
15. Horton, D.; Philips, K. D. *Carbohydr. Res.* **1972**, *22*, 151–162.
16. Ramos, M. L.; Caldeira, M. M.; Gil, V. *Carbohydr. Res.* **1997**, *297*, 191–200.
17. Levene, P. A. *J. Biol. Chem.* **1924**, *59*, 123–127.
18. Horton, D.; Walaszek, Z.; Ekiel, I. *Carbohydr. Res.* **1983**, *119*, 263–268.
19. Serianni, A. S.; Nunez, H. A.; Barker, R. *J. Org. Chem.* **1980**, *45*, 3329–3341.
20. Browne, C. A.; Tollens, B. *Ber. Dtsch. Chem. Ges.* **1902**, *35*, 1457–1467.