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A new enzyme catalysis: 3,4-dioxidation of some aryl β-D-glycopyranosides by fungal pyranose dehydrogenase

Petr Sedmera,[a] Petr Halada,[a] Clemens Peterbauer[b] and Jindřich Volc[a,*]

[a]*Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic*
[b]*Department of Food Sciences and Technology, University of Natural Resources and Applied Life Sciences Vienna, Muthgasse 18, A-1190 Vienna, Austria*

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Abstract—Quinone-dependent pyranose dehydrogenase presents a new tool for versatile conversions of numerous carbohydrates to their di- and tricarbonyl derivatives. This enzyme purified from the basidiomycete *Agaricus meleagris* catalysed dioxidation of several aromatic β-D-glucopyranosides and a β-D-xylopyranoside into the corresponding 3,4-didehydro-β-D-aldopyranosides (β-D-aldopyranosid-3,4-diuloses) in high yields, typically >80% for 4-nitrophenyl glycosides. These new compounds were doubly hydrated in aqueous solution. According to in situ NMR investigations, the reaction intermediates were the corresponding 3- and 4-dehydro compounds. The analogous anomeric α-glycosides underwent one-step oxidation only at C-3 to 3-dehydro-α-D-aldopyranosides (α-D-pyranosid-3-uloses).

Redox enzyme catalysis offers great potential for modification of sugars to reactive derivatives (possible intermediates for chemical synthesis) by regiospecifically introducing carbonyl function(s).[1] Syntheses of surfactants and polymer building blocks may serve as an example.[2] A powerful tool in this context is now provided by a novel fungal enzyme, the quinone-dependent pyranose dehydrogenase (PDH, pyranose:acceptor oxidoreductase, EC 1.1.99.29), acting on a vast array of mono- and oligosaccharides. Previously we showed that selective monooxidations (at C-1,[3] C-2,[3,4] C-3[5,6]) or dioxidations (at C-2,3[4,7]) of the sugar molecule can be performed depending on the nature of the sugar, the reaction conditions and the enzyme source (mainly some *Agaricales*). In addition, the primary oxidation can proceed specifically at one position only (C-3 for Me α/β-D-Glc*p*, sucrose, trehalose[5] and D-glucose,[6] C-2 for D-galactose[4]) or in parallel at positions C-2 and C-3 (D-xylose,[7] D-glucose[4]) or C-1 and C-2 (lactose[3]) in various proportions with individual PDHs. In this work, we extended our investigation to the reaction of PDH with aromatic glycosides, some of which proved to be especially good substrates for the unique C-3,4 dioxidative conversions to new glycosides of tricarbonyl sugars. Using in situ NMR methodology,[8] we detected and elucidated the structures of the oxidation products of salicin (2-hydroxymethylphenyl-β-D-glucopyranoside, **1**), arbutin (4-hydroxyphenyl-β-D-glucopyranoside, **2**), 4-nitrophenyl-β-D-glucopyranoside (4NP β-Glc*p*, **3**), 4-nitrophenyl-β-D-xylopyranoside (4NP β-Xyl*p*, **4**) and iridoid glycoside harpagoside (**5**, Fig. 1), respectively. High substrate conversions approaching 95% were achieved within 2–24 h.

Setting the aglycone resonances aside, the ^{1}H NMR spectrum (Table 1) of typical end-products consisted of an AB system (H-1, H-2) and an ABC system

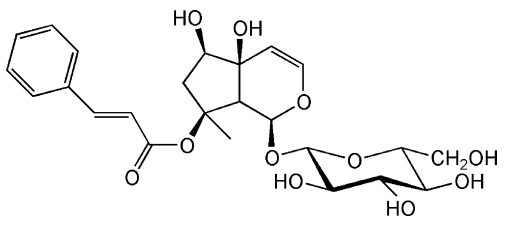

Figure 1. Structure of harpagoside **5**.

Keywords: 3,4-Didehydroglucopyranoside; 3,4-Didehydroxylopyranoside; Aldopyranosid-3,4-diuloses; Aryl glycoside oxidation; Sugar 3,4-dioxidation; Pyranose dehydrogenase; *Agaricus*.
* Corresponding author. Tel.: +42 0241062298; fax: +42 0296442384; e-mail: volc@biomed.cas.cz

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Table 1. NMR data of 3,4-dioxidation products (399.89 [^{1}H] and 100.55 [^{13}C] MHz, D_2O, 30 °C); sugar part only

	1h	**2h**	**3h**	**4h**	**5h**
Proton			δ_H		
1	4.984	4.846	5.119	5.110	4.676
2	3.693	3.583	3.267	3.692	3.386
5	3.712	3.652	3.782	3.687	3.618
6	3.772	3.756	3.787	3.503[a]	3.788
	3.604	3.612	3.603		3.670
H_i,H_j			$J(i,j)$, Hz		
1,2	8.0	8.0	8.0	7.6	8.2
5,6d	2.7	2.2	2.8		2.3
5,6u	7.2	5.1	7.9		7.3
6d,6u	−11.9	−11.6	−12.7	−12.0[b]	−11.9
Carbon			δ_C		
1	100.28	101.00	99.29	99.63	98.5[c]
2	72.66	72.66	75.45	72.44	72.2[c]
3	94.88	94.86	94.81	94.39	94.7[c]
4	94.25	94.26	94.19	92.96	94.1[c]
5	76.02	75.92	76.21	67.53	76.0[c]
6	60.94	59.74	59.63		59.7[c]

For compound identification, see Scheme 1.
[a] H-5u.
[b] J(5d,5u).
[c] HMQC and HMBC readouts.

(comprising H-5 and both H-6's) or two AB systems (with **4**). These properties are consistent with 3,4-dioxidation. Positive-ion ESI mass spectra[9] showed $[M+Na]^+$ ions at masses of 4 mass units lower than those of the parent compounds (*m/z* 305.2, 291.2, 320.3, 290.2 and 513.4 for the final reaction mixtures upon biotransformation of **1**, **2**, **3**, **4** and **5**, respectively). However, no ketone signals were present in the ^{13}C NMR spectra and chemical shifts both of C-3 and C-4 (Table 1) indicated the hydration of both keto groups. HMBC was used to distinguish these quaternary carbons (Fig. 2).

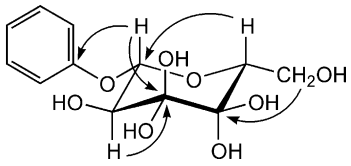


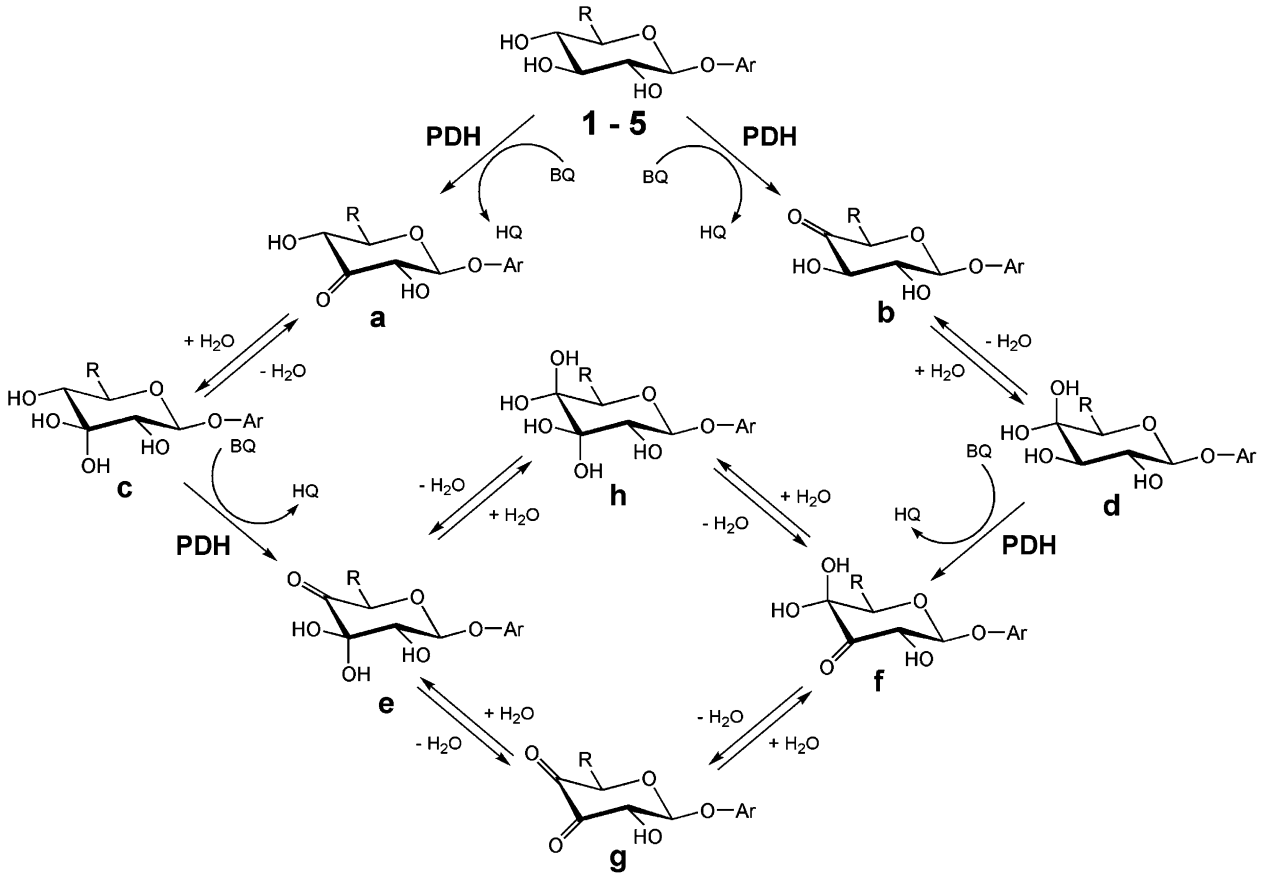
Figure 2. Diagnostic HMBC contacts in the biotransformation product of arbutin **2**.

Careful monitoring of the biotransformations revealed that the reaction intermediates were 3-dehydroglycosides together with their hydrated forms; a hydrate of a 4-dehydroglycoside was found by NMR with **4** (see Table 2 for the spectroscopic characterization). Some low abundance transient intermediates that might be due to **4b**, **4e** or **4f** (Scheme 1) were also observed. The NMR data of the 3-oxidation products (**1a**, **1c**, **2a**, **2c**, **3a**, **3c**) were similar to those reported for methyl β-D-glucopyranoside.[5,10] The structure of compound **4d** is unambiguous: its ^{1}H NMR spectrum consisted of a con-

Table 2. NMR data of reaction intermediates (399.89 [^{1}H] and 100.55 [^{13}C] MHz, D_2O, 30 °C); sugar part only

	1a	**1c**	**2a**	**2c**	**3a**	**3c**	**4c**	**4d**	**5a**	**5c**
Proton						δ_H				
1	5.013	4.964	4.860	4.828	5.172	5.102	5.107	5.041	4.708	4.656
2	4.436	3.492	4.315	3.366	4.435	3.479	3.453	3.528	4.134	3.174
3	—	—	—	—	—	—	—	3.485	—	—
4	4.275	3.997	4.274	3.355	4.290	3.401	3.598	—	4.197	3.304
5	3.449	3.537	3.441	3.462	3.584	3.595	3.752	3.615	3.407	3.431
6	3.796	3.690	3.783	3.679	3.710	3.817	3.467[a]	3.520[a]	3.848	3.739
	3.659	3.530	3.643	3.520	3.540	3.636			3.674	3.550
H_i,H_j						$J(i,j)$, Hz				
1,2	7.9	8.0	8.0	8.0	7.9	8.0	7.2	7.0	8.1	8.2
2,4	1.7	—	1.7	—	1.7	—	—	9.5[b]	1.7	—
4,5	10.3	9.7	10.3	9.8	10.3	9.8	9.8[c]	—	10.4	10.1
5,6d	2.2	4.8	2.2	2.0	2.0	2.1	4.9[d]	—	2.2	2.3
5,6u	4.8	4.5	4.8	5.5	5.5	4.9		—	5.0	5.8
6d,6u	−12.6	−12.6	−12.6	−12.2	−12.1	−12.5	−11.1[e]	−12.4[e]	−12.5	−12.5
Carbon						δ_C				
1	102.02	100.06	102.85	100.79				100.2	100.1	98.4
2	76.60	73.88	76.67	73.98				72.0	72.2	73.5
3	206.20	94.51	n.d.	94.48				75.2	n.d.	n.d.
4	72.16	70.30	72.25	70.42				92.2	76.1	70.6
5	76.25	75.40	76.29	75.43				68.6	76.3	77.4
6	60.72	60.54	60.81	61.07					60.8	61.0

For compound identification, see Scheme 1; n.d., not determined.
[a] H-5u.
[b] J(2,3); carbon chemical shifts reported to one decimal point are HMQC and HMBC readouts.
[c] J(4,5d).
[d] J(4,5u).
[e] J(5d,5u).



Scheme 1. Reaction scheme for double oxidation of aryl glycosides **1**, **2**, **3**, **4** and **5** by pyranose dehydrogenase (PDH). Ar, aryl; **1**, Ar = 2-hydroxymethylphenyl, R = CH_2OH; **2**, Ar = 4-hydroxyphenyl, R = CH_2OH; **3**, Ar = 4-nitrophenyl, R = CH_2OH; **4**, Ar = 4-nitrophenyl, R = H; **5**, Ar = see Figure 1, R = CH_2OH; BQ, benzoquinone, HQ, hydroquinone.

Table 3. Oxidation of glycosides **1**–**5** by PDH

Substrate	Reaction time (h)	Products identified by NMR (%)			
		a	**b**	**c**	**d**
1	24	5	—	8	81
2	2	23	—	60	17
3	2	3	—	4	92
4	24	—	1	17	82
5	20	58	—	8	42

Compounds identified by NMR after substantial (>99%) substrate conversion.

tiguous three-spin system involving H-1 to H-3 and an AB system for the two methylene protons at C-5 (proved by HMQC); C-4 resonates at 92.2 ppm, that is the corresponding carbonyl is hydrated. Contrary to the commonly held belief that double oxidation takes place only after prolonged reaction time, the signals of 3,4-dioxidised products were present from early stages of the reaction. The results of NMR analysis of the reaction mixtures at the time when almost all substrate (>99%) had been consumed are given in Table 3. At this stage, however, both hydration/dehydration reactions and the 3,4-dioxidation (see Scheme 1) were still taking place so that these results are only illustrative of multiple product forms to be analysed for each substrate. Nevertheless, all the data provided confirm that PDH catalyses eventual double oxidation of aryl β-D-glycosides **1**, **2**, **3**, **4**, **5** to the corresponding aryl β-D-3,4-didehydroglycosides (aryl β-D-glycopyranosid-3,4-diuloses) following Scheme 1, preferentially via 3-dehydro intermediates.

The double oxidation is obviously related to the presence of an aromatic moiety in the molecule since both methyl β-D-Glc*p* and methyl β-D-Xyl*p* were transformed into their 3-keto derivatives only, even after long contact with an excess of the enzyme.[5] On the other hand, 4NP derivatives of α-Glc*p*, α-Xyl*p*, α-Gal*p*, β-Gal*p*, α-Ara*p* and β-Ara*p* also yielded 3-oxidised products only (to be described elsewhere), so that both the anomeric and sugar configuration are important. The furanose ring in 4NP α-Xyl*f* and 4NP α-Ara*f* prevented reaction with PDH. The effect of the aromatic ring may be a kind of co-operative binding at the enzyme active site facilitating a better and longer contact. Interestingly, **5** also gave the 3,4-dioxidised product (**5h**, Scheme 1) even though the aromatic part here is the cinnamic acid ester of one of the aglycone hydroxyls.

C-3 oxidation of the glycosyl moiety is known also to be catalysed by other enzymes: bacterial glycoside-3-dehydrogenase (EC 1.1.99.13) from *Agrobacterium tumefaciens* (e.g., sucrose, maltose, isomaltulose, leucrose, lactose),[1] and fungal pyranose oxidase (EC 1.1.3.10)

from *Peniophora gigantea* (Me α/β-D-Glc*p*).[10] Several examples of naturally occurring iridoid 3-dehydroglucosides are also known.[11,12] In addition to PDH, sugar dioxidation at adjacent positions has earlier been reported for C-2 and C-3 of some monosaccharides using pyranose oxidase (D-glucose,[13,14] D-galactose,[15] 1,5-anhydro-D-glucitol and 1,5-anhydro-D-galactitol[10]). Only recently, a unique glycoside of a contiguous tricarbonyl sugar derivative, 2-(3,4-dihydroxyphenyl)ethyl 2,3-didehydro-β-D-glucopyranoside, was isolated from a natural plant source.[16] Here, we report for the first time enzymatic C-3,4 double oxidation of sugars, D-glucose and D-xylose in their glycosidic form. According to our literature search, chemical oxidation of sugars to the corresponding 3,4-didehydroderivatives has not yet been described. On the contrary, both solid state and solution structures of a related diketose, D-*threo*-hexo-3,4-diulose are well understood.[17]

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References and notes

1. Stoppok, E.; Walter, J.; Buchholz, K. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 706–712.
2. Pietsch, M.; Walter, M.; Buchholz, K. *Carbohydr. Res.* **1994**, *254*, 183–194.
3. Volc, J.; Sedmera, P.; Kujawa, M.; Halada, P.; Kubátová, E.; Haltrich, D. *J. Mol. Catal. B: Enzym.* **2004**, *30*, 177–184.
4. Volc, J.; Sedmera, P.; Halada, P.; Přikrylová, V.; Daniel, G. *Carbohydr. Res.* **1998**, *310*, 151–156.
5. Volc, J.; Sedmera, P.; Halada, P.; Daniel, G.; Přikrylová, V.; Haltrich, D. *J. Mol. Catal. B: Enzym.* **2002**, *17*, 91–100.
6. Volc, J.; Kubátová, E.; Daniel, G.; Sedmera, P.; Haltrich, D. *Arch. Microbiol.* **2001**, *176*, 178–186.
7. Volc, J.; Sedmera, P.; Halada, P.; Přikrylová, V.; Haltrich, D. *Carbohydr. Res.* **2000**, *329*, 219–225.
8. The experiments were performed in a 5-mm NMR sample tube (solution volume 0.7 mL) containing substrate (10 mM), 1,4-benzoquinone (30 mM) and 0.4 U PDH in deuterium oxide (no buffering). The enzyme catalyst was PDH purified from mycelial cultures of the fungus *Agaricus meleagris* to apparent homogeneity (53 U mg protein^{-1}, ferricenium assay; 121 U mL^{-1} distilled water; 3 μL per reaction mixture) using a procedure described previously.[6] Monitoring the reaction time course by ^{1}H NMR spectroscopy provided pseudokinetic data. When the concentration of the products was sufficient for their detection 'snapshots' were taken using 2D NMR techniques (COSY, TOCSY, HOM2DJ, HMQC) or their selective one-dimensional variants (1D-TOCSY, 1D-NOE). Upon substantial substrate depletion and reaching a steady state, the complete analysis (including ^{13}C NMR and HMBC) was undertaken to determine the structure and proportions of the products.
9. The spectra were recorded on a LCQ Deca ion trap mass spectrometer equipped with a nanospray ion source. Samples, dissolved in 30% aqueous acetonitrile, were sprayed directly from EconoTip™ emitters. Spray voltage was 1.2 kV and the heated capillary was kept at 150 °C. Only the final reaction mixtures were studied.
10. Freimund, S.; Huwig, A.; Giffhorn, F.; Köpper, S. *Chem. Eur. J.* **1998**, *4*, 2442–2455.
11. Kiuchi, F.; Liu, H. M.; Tsuda, Y. *Chem. Pharm. Bull.* **1990**, *38*, 2326–2328.
12. Gering, B. P., Jr.; Wichtl, M. *Phytochemistry* **1987**, *26*, 3011–3013.
13. Volc, J.; Sedmera, P.; Havlíček, V.; Přikrylová, V.; Daniel, G. *Carbohydr. Res.* **1995**, *278*, 59–70.
14. Sedmera, P.; Volc, J.; Havlíček, V.; Pakhomova, S.; Jegorov, A. *Carbohydr. Res.* **1997**, *297*, 375–378.
15. Volc, J.; Sedmera, P.; Halada, P.; Dwivedi, P.; Costa-Fereira, M. *J. Carbohydr. Chem.* **2003**, *22*, 207–216.
16. Franzyk, H.; Olsen, C. E.; Jensen, S. R. *J. Nat. Prod.* **2004**, *67*, 1052–1054.
17. Angyal, S. J.; Craig, D. C.; Kuszman, A. *J. Carbohydr. Res.* **1989**, *194*, 21–29.