ELSEVIER

Carbohydrate Research 297 (1997) 375–378

CARBOHYDRATE RESEARCH

Note

Structural analysis of crystalline D-*erythro*-hexos-2,3-diulose (2,3-diketo-D-glucose) prepared enzymatically from D-glucose [1]

Petr Sedmera [a,*], Jindřich Volc [a], Vladimír Havlíček [a], Svetlana Pakhomova [b], Alexandr Jegorov [c]

[a] *Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic*

[b] *Department of Solid State Chemistry, Prague Institute of Chemical Technology, 166 28 Prague 6, Czech Republic*

[c] *Galena Co., Research Unit, 370 05 České Budějovice, Czech Republic*

Received 20 June 1996; accepted 5 November 1996

Keywords: D-Glucose; 2,3-Diketo-D-glucose; Bioconversion; NMR; Electrospray; X-Ray diffraction

Pyranose 2-oxidase (P2O, EC 1.1.3.10) of the wood rot basidiomycete *Oudemansiella mucida* oxidizes D-glucose, D-galactose, and D-xylose to the corresponding aldos-2-ulose (2-ketoaldose) [1]. TLC monitoring of D-glucose oxidation using partially purified P2O showed that accumulation of D-*arabino*-hexos-2-ulose (**1**, 2-keto-D-glucose) had a transient character only. Upon reaching the peak (ca. 90% conversion), **1** was oxidatively converted, apparently by the same enzyme [2], to another compound exhibiting high reducing activity. Recently, we have demonstrated the formation of a new tricarbonyl sugar, D-*erythro*-hexos-2,3-diulose (**2**, 2,3-diketo-D-glucose), in the course of this reaction, that was isolated and characterized as its *N*,*N*-diphenylhydrazone upon derivatization of the crude reaction mixture. In this communication we describe ESI-MS, NMR, and X-ray structure determination of **2** obtained in a crystalline underivatized form.

Sodium and potassium adduct ions *m/z* 235 and 249 dominated in the first-order positive-ion electrospray mass spectrum of this compound. The molecular formula $C_6H_{12}O_8$ was deduced from the high resolution measurement of the former ion (calculated 235.0430, measured 235.0432). There was no carbonyl band in the IR spectrum. The ^{1}H NMR spectrum of a fresh $(CD_3)_2SO$ solution of **2** contained a triplet of one primary, a doublet of one secondary, and four singlets of tertiary OH groups. COSY and delay-COSY [3] revealed four spin systems: one -CH(OH) (C-1), two $>$C(OH)$_2$, and one -CH(OH)CH(O-)CH$_2$OH (C-4 to C-6). The ^{13}C NMR spectrum showed that the molecule is built from two -OCO-, one -OCHO-, two OCH, and one CH_2O unit. A large $J_{4,5}$ 9.8 Hz corresponds to an axial–axial coupling in a six-membered ring adopting a chair conformation. Both the NOE observed between H-1 and axial H-4 and the direct coupling $^1J_{C\text{-}1,H\text{-}1}$ 164.2

* Corresponding author. Tel.: 42-2-4752646; Fax: 42-2-4715743; E-mail: sedmera@biomed.cas.cz.

[1] Tables of atomic coordinates, bond lengths, and bond angles have been deposited with the Cambridge Crystallographic Data Centre. These tables may be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

PII S0008-6215(96)00293-5

Table 1

Crystal data

$C_6H_{12}O_8 \cdot H_2O$, mol wt 230.17, orthorhombic; space group $P2_12_12_1$, $Z = 4$

$a = 8.352(5)$, $b = 10.076(2)$, $c = 10.592(7)$ Å

$V = 891.4(8)$ Å^3, $D_c = 1.715$ g cm^{-3}, $F(000) = 488$

Structure determination and refinement data

Crystal dimensions: $0.28 \times 0.28 \times 0.35$ mm

Radiation: Mo-K_α ($\mu = 0.166$ mm^{-1}, $\lambda = 0.71073$ Å)

1090 reflections were measured ($0 < h < 9$, $0 < k < 11$, $0 < l < 12$)

762 considered as unique with $[I > 1.96\sigma(I)]$

Function minimized $\Sigma w(F_o^2 - F_c^2)^2$, $w = 1/[\sigma^2 F_o^2 + (0.0181P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$

190 Parameters were refined, ratio of max. least-squares shift to esd $< 1 \times 10^{-3}$

Final agreement factors: $R = 0.043$, $R_w = 0.066$, $S = 0.947$ for 897 reflections

Largest residual electron density peaks were at -0.23 and 0.24 e Å^{-3}

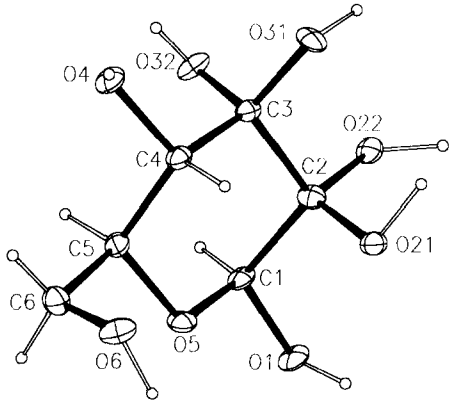


Fig. 1. X-ray structure of the hydrate of compound **2**.

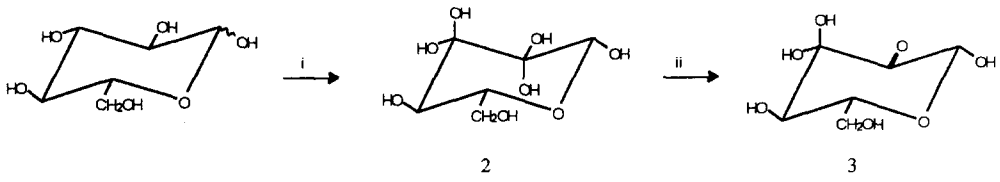
Hz [4,5] indicate an axial orientation of H-1 and thus determine the β-configuration at the anomeric center. Therefore, the isolated compound **2** is β-D-*erythro*-hexos-2,3-diulose-(1,5) (2,3-diketo-β-D-gluco-pyranose), having both carbonyls hydrated.

The X-ray structure determination (Table 1, Fig. 1) confirmed the pyranose form, chair conformation of the six-membered ring, axial orientation of H-1, and equatorial orientation of the CH_2OH group. Both C-2 and C-3 carry two geminal OH groups each. As is common with carbohydrates [6], all hydroxyl hydrogens, all oxygen atoms, and a water molecule are involved in the hydrogen bonding (Table 2) forming

Table 2
The structural parameters of the hydrogen bonds in D-*erythro*-2,3-diulose hydrate

Donor · · · Acceptor	Distance			Angle
	D–H (Å)	D · · · A (Å)	H · · · A (Å)	D–H · · · A (°)
OW-H2OW · · · O32	0.77(6)	3.045(4)	2.43(6)	138(5)
OW-H2OW · · · O22	0.77(6)	2.929(4)	2.24(6)	149(6)
O21-H21 · · · O31	1.09(6)	2.816(4)	2.39(7)	102(4)
O32-H32 · · · O4	0.76(4)	2.791(4)	2.45(4)	109(4)
OW-H1OW · · · O1 [a]	0.90(6)	2.722(4)	1.83(7)	171(6)
O32-H32 · · · OW [a]	0.76(4)	2.846(4)	2.12(4)	162(4)
O1-H1O1 · · · O6 [b]	0.82(4)	2.624(4)	1.83(6)	162(6)
O6-H1O6 · · · O31 [b]	1.01(7)	2.703(4)	1.87(7)	138(6)
O21-H21 · · · OW [c]	1.09(6)	2.774(4)	1.73(6)	161(6)
O22-H22 · · · O5 [d]	0.97(8)	2.996(4)	2.25(8)	132(6)
O31-H31 · · · O4 [e]	0.93(6)	2.835(4)	1.93(6)	165(6)
O4-H1O4 · · · O21 [f]	0.83(5)	3.003(4)	2.24(5)	153(5)

For numbering of atoms see Fig. 1. Symmetry codes: (a) $x + 1/2$, $-y + 3/2$, $-z + 1$; (b) $x - 1/2$, $-y + 3/2$, $-z$; (c) $-x + 1/2$, $-y + 2$, $z - 1/2$; (d) $-x$, $y + 1/2$, $-z + 1/2$; (e) $-x + 1$, $y + 1/2$, $-z + 1/2$; (f) $-x$, $y - 1/2$, $-z + 1/2$.



2 3

Scheme 1. *Reagents and conditions*: i, pyranose 2-oxidase, 18 h, 30 °C; ii, standing in Me_2SO, 24 h, 25 °C.

a three-dimensional network accounting for the high density (1.715 $g\,cm^{-3}$) found for the crystal. The equatorial hydroxyl at C-2 forms a three-center bond towards the oxygen atom of the equatorial hydroxyl at C-3 and to the water oxygen. Similarly, the equatorial hydroxyl at C-3 is intramolecularly bonded to the C-4 oxygen and also to the water oxygen. Finally, one water hydroxyl is also engaged in a three-center bond to the oxygen atoms of the axial hydroxyls at C-2 and C-3.

Though **2** is formally a tricarbonyl sugar, all its carbonyls are masked; two by hydration, one forms a hemiacetal. Several compounds containing two [7–9] or even four [10] vicinal hydrated carbonyls were found in Cambridge Data File but none of them was a sugar. Although **2** is moderately stable in Me_2SO, signals of another compound appeared after 1-day standing. This compound was identified as **3** (Scheme 1) by NMR spectroscopy. Although a much more complex mixture of products is observed in water, **2** and **3** still predominate.

1. Experimental

General.—The melting point was determined with a Kofler apparatus and is not corrected. The infrared spectrum was measured in KBr pellet on a Nicolet FT IR 205 spectrometer. The electrospray mass spectrum was recorded on a Finnigan MAT-90 mass spectrometer. High-resolution measurement was performed with a peak-matching technique against propylene glycol Mw 425 (Aldrich Chemie, Steinheim, Germany). The products of collisionally induced decompositions (He as the collision gas) in the first field-free region of the instrument were analyzed by the daughter-linked scan (B/E = const). NMR spectra in $(CD_3)_2SO$ were measured on a Varian VXR-400 spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C) at 25 °C. Manufacturer's software was used for 2D NMR experiments (COSY, delay-COSY, NOESY, HETCOR).

Organism and growth conditions.—Cultures of *Oudemansiella mucida* (Schrad.:Fr.) Höhn., strain III (CCBAS 428), collection of Institute of Microbiology, Prague were cultivated on a liquid synthetic medium [2] for 4 days.

Enzyme purification.—Partially purified P2O (5 U/mg protein) was prepared from the harvested mycelium of *O. mucida* using extraction and chromatographic procedures described earlier (hydrophobic interaction on phenyl-Sepharose CL 4B, Pharmacia-LKB, Uppsala, Sweden) [11]. Its activity was assayed by a coupled peroxidase reaction [12].

Bioconversion.—The aqueous reaction mixture for production of **2** (50 mL) contained D-glucose (1 g), P2O preparation (200 U), and catalase (8000 Sigma units, Reanal, Budapest, Hungary). Transformation of D-glucose to **2** via **1** proceeding for 18 h at 30 °C was monitored by TLC on cellulose coated foils (Lucefol, Kavalier, Votice, Czech Republic) in *n*-butyl acetate–acetic acid–acetone–water (140:100:33:80) using diphenylamine–aniline detection reagent [13] (**1** gives a characteristic blue spot, **2** gives a yellowish streak). Proteins were removed by passing the solution through a catex column in the H^+ cycle, the volume was reduced to 5 mL by vacuum evaporation at 20 °C, and the solution was allowed to stand at 4 °C for 3 weeks. White prisms of **2** (240 mg, 20%), mp 158–164 °C (decomp) were obtained.

^1H NMR [400 MHz, $(CD_3)_2SO$, 25 °C]: δ 3.223 (1 H, ddd, J 9.8, 6.3, 2.1 Hz, H-5), 3.322 (1 H, dd, J 9.8, 6.4 Hz, H-4), 3.374 (1 H, ddd, J 11.5, 6.3, 6.2 Hz, H-6u), 3.625 (1 H, ddd, J 11.5, 5.5, 2.1 Hz, H-6d), 4.395 (1 H, dd, J 6.2, 5.5 Hz, 6-OH), 4.513 (1 H, d, J 8.2 Hz, H-1), 4.593 (1 H, d, J 6.4 Hz, 4-OH), 4.813 (1 H, s, 2eq-OH), 4.891 (1 H, s, 2ax-OH), 5.179 (1 H, s, 3eq-OH), 5.193 (1 H, s, 3ax-OH), 6.073 (1 H, d, J 8.2 Hz, 1-OH); ^{13}C NMR [100 MHz, $(CD_3)_2SO$, 25 °C]: δ 61.77 (t, C-6), 69.44 (d, C-4), 75.68 (d, C-5), 93.46 (s, C-3), 94.31 (d, C-1), 94.70 (s, C-2); m/z (ESI^+) 235 (MNa^+), 249 (MK^+). Daughter spectrum of $[M + Na]^+$ ion: m/z 217 (25%, $[M + Na - H_2O]^+$), 203 (100%, $[M + Na - CH_3OH]^+$), 23 (35%, Na^+).

Additional NMR signals observed upon 1-day standing were ascribed to compound **3**. ^1H NMR [400 MHz, $(CD_3)_2SO$, 25 °C]: δ 3.151 (1 H, ddd, J 10.3, 5.4, 2.0 Hz, H-5), 3.546 (1 H, ddd, J 11.8, 6.3,

5.4 Hz, H-6u), 3.708 (1 H, ddd, *J* 11.8, 5.6, 2.0 Hz, H-6d), 4.297 (1 H, dd, *J* 10.3, 6.2 Hz, H-4), 4.365 (1 H, d, *J* 7.3 Hz, H-1), 4.768 (1 H, dd, *J* 6.3, 5.6 Hz, 6-OH), 5.297 (1 H, d, *J* 6.2 Hz, 4-OH), 6.186 (1 H, s, 3eq-OH), 6.224 (1 H, s, 3ax-OH), 6.872 (1 H, d, *J* 7.3 Hz, 1-OH). ^{13}C NMR [100 MHz, $(CD_3)_2SO$, 25 °C]: δ 61.19 (t, C-6), 71.31 (d, C-4), 76.23 (d, C-5), 94.33 (s, C-3), 97.42 (d, C-1), 205.85 (s, C-2).

Crystal structure analysis.—The X-ray diffraction data are given in Table 1. An Enraf–Nonius CAD4 diffractometer was used. The colourless prisms, unstable in air, were adjusted in a glass capillary containing the mother liquid. The structure was solved by direct methods and anisotropically refined by full-matrix least-squares methods. Positions of hydrogen atoms were found from difference synthesis and refined isotropically. Programs used were SDP [14], SHELXS86 [15], SHELXL93 [16], and PARST [17].

Acknowledgements

This work was supported by grants 204/94/1191 issued by Grant Agency of the Czech Republic and 620421 issued by Grant Agency of Academy of Sciences of the Czech Republic.

References

[1] J. Volc, P. Sedmera, and V. Musílek, *Collect. Czech. Chem. Commun.*, 45 (1980) 950–955.
[2] J. Volc, P. Sedmera, V. Havlíček, V. Přikrylová, and G. Daniel, *Carbohydr. Res.*, 278 (1995) 59–70.
[3] A. Bax and R. Freeman, *J. Magn. Reson.*, 44 (1981) 542–581.
[4] K. Bock, I. Lundt, and C. Pedersen, *Tetrahedron Lett.*, (1973) 1037–1040.
[5] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
[6] G.A. Jeffrey and H. Maluszynska, *Int. J. Quantum Chem.* (1981) 231–239.
[7] M. Schmidt, K. Albert, R. Brindle, C. Maichle-Mössmer, and K. Eger, *Z. Naturforsch. B*, 48 (1993) 1372–1380.
[8] A.D. Mighell and R.A. Jacobson, *Acta Crystallogr.*, 17 (1964) 1554–1560.
[9] G.V. Gridunova, V.E. Shklover, Y.T. Struchkov, R.V. Linko, A.N. Poplavskii, and A.M. Andievskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1990) 575–580.
[10] C.M. Bock, *J. Am. Chem. Soc.*, 90 (1968) 2748–2751.
[11] J. Volc, E. Kubátová, P. Sedmera, G. Daniel, and J. Gabriel, *Arch. Microbiol.*, 156 (1991) 297–301.
[12] J. Volc and K.-E. Eriksson, *Methods Enzymol.*, 161 (1988) 316–322.
[13] J. Kocourek, M. Tichá, and J. Koštíř, *J. Chromatogr.*, 24 (1966) 117–124.
[14] B.A. Frenz and Associates, Inc., *SDP, Structure Determination Package*, Enraf–Nonius, Delft, The Netherlands, 1985.
[15] G.M. Sheldrick, *SHELXS86, Program for Crystal Structure Solution*, University of Göttingen, Germany, 1986.
[16] G.M. Sheldrick, *SHELXL93, Program for Crystal Structure Determination*, University of Göttingen, Germany, 1993.
[17] M. Nardelli, *Comput. Chem.*, 7 (1983) 95–98.