CONVERSION OF MONOSACCHARIDES INTO THEIR CORRESPONDING 2-GLYCOSULOSES BY INTACT CELLS OF THE BASIDIOMYCETE *Oudemansiella mucida*

Jindřich VOLC, Petr SEDMERA and Vladimír MUSÍLEK

Institute of Microbiology,
Czechoslovak Academy of Sciences, 142 20 Prague 4

Received January 25th, 1979

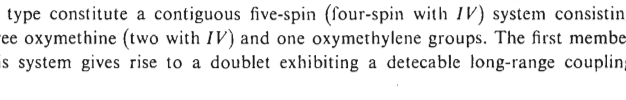
The production of D-*arabino*-, D-*lyxo*-, and D-*ribo*-2-hexosuloses and D-*threo*-2-pentosulose from their respective aldoses was demonstrated with washed mycelium suspensions of the basidiomycete *Oudemansiella mucida*. L-Sorbose was shown to be converted into D-*threo*-2,5-hexodiulose. The dicarbonyl sugar produced were isolated as their hydrazones, whose structures were inferred from spectroscopic evidence and the method of preparation.

Microbial oxidations of saccharides into their dicarbonyl derivatives have been shown to occur with some bacteria. *Acetobacter suboxydans* oxidizes methyl α- and β-D-*xylo*-pyranosides into respective glycosid-4-uloses[1]. The formation of glycosid-3-uloses from disaccharides was demonstrated with growing cells of *Agrobacterium tumeofaciens*[2,3]. Production of 2-hexosulose has been reported for the D-*arabino* compound by *Acetobacter roseus* grown on D-fructose[4] and for the L-*xylo* compound by *Gluconobacter melanogenus* grown on L-sorbose medium[5]. The diketose, D-*threo*-2,5-hexodiulose, is a known metabolite of numerous *Pseudomonas* and *Gluconobacter species*[6–8]. Previously we have shown that submerged cultures of the basidiomycete *Oudemansiella mucida*, growing on synthetic glucose medium, accumulate D-*arabino*-2-hexosulose[9]. In this paper, we report the oxidation capacities of resting cell suspension of this wood rotting fungus with regard to different monosaccharides. The purpose of our work was to establish a convenient method for biological conversion of some aldoses to 2-glycosuloses[10].

Dicarbonyl sugars were revealed by paper chromatography in the filtrate resulting from the biotransformation of D-allose, D-galactose, D-glucose, D-xylose, and L-sorbose. No oxidation was observed with D-arabinose, L-arabinose, D-lyxose, D-ribose, D-mannose, D-fructose, and D-tagatose.

To be isolated and identified, the oxidation products of aldoses were transformed into their peracetylated diphenylhydrazones which afforded good mass spectra (Table I). Oxidation products of D-allose, D-galactose, and D-glucose yielded mono-(diphenylhydrazones) $C_{26}H_{28}N_2O_9$ *I–III*; the oxidation product of D-xylose gave

analogically the hydrazone $C_{23}H_{24}N_2O_7$ *IV*. Infrared spectra of compounds *I*–*IV* contained absorption bands both of ester and conjugated ketone carbonyls. The ^{1}H-NMR spectra (Table II, III) confirmed the presence of two phenyl groups (ten aromatic protons), four acetoxy groups with compounds *I*–*III* (three with *IV*), one isolated proton and five protons of the CHO type with compounds *I*–*III* (four with *IV*). The one-proton singlet at approximately 6·6 ppm can be assigned to a CH=N— proton, whose vicinal atoms are devoid of protons. The protons of the CHO type constitute a contiguous five-spin (four-spin with *IV*) system consisting of three oxymethine (two with *IV*) and one oxymethylene groups. The first member of this system gives rise to a doublet exhibiting a detecable long-range coupling



to a δ 6·6 singlet indicating the proximity of both groups. The molecular ion, besides common loses of 42 and 60 mass units, decomposes according to a pathway M → → 223(a) → 195(b) → 168. The diagnostic ion *m/z* 223 has the elemental composition $C_{19}H_{11}N_2O$ and evidently contains the atoms $C_{(1)}$ and $C_{(2)}$. Taking all the facts into account, it can be stated that compounds *I*–*IV* are peracetylated 1-diphenyl-

TABLE I
Mass Spectroscopic Data of Acetylglycosulose Hydrazones *I*–*IV*[a]

Ion	Composition	*I*		*II*		*III*[a]		*IV*	
		m/z		*m/z*		*m/z*		*m/z*	
M^+	$C_{26}H_{28}N_2O_9$	512	21·2	512	12·0	512	8·1	440[b]	24·7
M−42		468	0·1	468	0·2	468	0·1	398	0·1
M−60		452	1·4	452	0·7	452	0·2	380	0·8
a	$C_{14}H_{11}N_2O$	223	81·8	223	69·9	223	71·9	223	60·2
b	$C_{13}H_{11}N_2$	195	10·3	195	11·4	195	11·9	195	8·4
c	$C_{12}H_{10}N$	168	92·4	168	100	168	83·1	168	100
c−1	$C_{12}H_9N$	167	40·9	167	49·4	167	34·8	167	37·6
d	C_2H_3O	43	100	43	100	43	100	43	96·7

[a] Values for *III* were taken from[9]. [b] Composition $C_{23}H_{24}N_2O_7$.

hydrazones of the corresponding 2-glycosuloses. Therefore, the enzymatic oxidation of the above aldoses by washed mycelium has occurred at the position 2, converting D-allose, D-galactose, D-glucose, and D-xylose into D-*ribo*, D-*lyxo*, and D-*arabino*--2-hexosulose, and D-*threo*-2-pentosulose, respectively. The physical constants

TABLE II

^{1}H-NMR Chemical Shifts of Acetylglycosulose Hydrazones *I*—*IV*

Deuteriochloroform as a solvent, hexamethyldisiloxane internal standard $\delta(H) = 0{\cdot}06$ ppm., chemical shifts expressed in the δ-scale; multiplicity: s singlet, d doublet, t triplet, mt multiplet.

Proton	*I*[a]	*II*[b]	*III*[b,c]	*IV*[b]
H—1	6·62 s	6·67 s	6·57 s	6·60 s
H—3	6·26 d	6·29 d	6·32 d	6·33 d
H—4	5·98 dd	6·72 dd	6·02 dd	5·91 mt
H—5d	5·26 mt	5·50 mt	5·40 mt	4·88 mt
H—5u	—	—	—	4·27 mt
H—6d	4·31 dd	4·35 dd	4·44 dd	—
H—6u	4·24 dd	4·06 dd	4·18 dd	—
CH_3CO	1·82 s	1·97 s	1·91 s	1·99 s
	1·91 s	2·02 s	2·04 s	2·07 s
	2·07 s	2·11 s	2·20 s	2·19 s
	2·13 s	2·12 s	2·20 s	—
C_6H_5	7·29 mt	7·32 mt	7·31 mt	7·32 mt

[a] 59·797 MHz; [b] 80 MHz; [c] Values for *III* taken from[9].

TABLE III

^{1}H-NMR Coupling Constants in Hz of Acetylglycosulose Hydrazones *I*—*IV*

J (i, j)[a]	*I*	*II*	*III*	*IV*
J (3, 4)	4·2	7·4	1·6	2·8
J (4, 5d)	6·8	3·0	9·2	5·8
J (4, 5u)	—	—	—	6·8
J (5d, 5u)	—	—	—	11·3
J (5, 6u)	2·2	5·0	2·3	—
J (5, 6d)	1·0	5·8	4·0	—
J (6u, 6d)	15·1	11·8	12·2	—

[a] *J* (1, 3) was in all compounds different from zero.

of hydrazones *II* and *III* agree well with the published data for the same derivatives of D-*lyxo*[11] and D-*arabino*-2-hexosulose[9,11], respectively.

The oxidation product of L-sorbose, which did not react with N,N-diphenylhydrazine under given conditions, gave a crystalline derivative *V* with phenylhydrazine, whose infrared spectrum showed no carbonyl band. According to the ratio of signal intensities of aromatic to CHO type protons in the ^{1}H NMR spectrum, the molecule of *V* contains two phenyl groups. One AB-system (2 H, δ 4·66 and 4·91, J_{AB} = 3·5 Hz) is due to two isolated vicinal protons; another AB-system (4 H, δ 4·37 and 4·54, J_{AB} = 13 Hz) represents two magnetically equivalent isolated terminal methylenes. The ^{13}C-NMR spectrum of *V* taken in hexadeuteriodimethyl sulfoxide was found to be symmetric: 147·1 s, 146·4 s, 113·6 d, 129·7 d(2C), 120·1 d(2C), 76·0 d, 58·1 t. The ion with the highest mass appeared at *m/z* 179 and accounted for one half of the molecule. Using the above data, *V* was identified as 2,5-bis(phenylhydrazono)-D-*threo*-2,5-hexodiulose. Therefore, D-fructose was oxidized at the position 5.

The yields of biotransformations were determined colorimetrically for D-*arabino*-2-hexosulose production. The conversion of D-glucose into the above hexosulose in shaked flask experiments amounted to 45–60%. On incubations in a laboratory fermentor, yields up to 73% were obtained. The remaining (unoxidized) glucose content amounted to 7·5% of its original value as estimated by the glucose oxidase method[12].

The production of 2-glycosuloses from aldoses by intact mycelial suspensions has not been so far reported. However, enzymatic activities responsible for the glycosulose formation by $C_{(2)}$ oxidation of D-glucose[13–15] and D-xylose[14] were observed in extracts and plasmolysed preparations of several higher fungi. Preparations of the green alga *Iridophycus flaccidum*[16] cazalyzed the conversion of D-galactose into D-*lyxo*-2-hexosulose while $C_{(6)}$ specific oxidation of the former sugar was accomplished by an extracellular oxidase from the basidiomycete *Polyporus circinatus*[17]. According to our knowledge, there is no mention in the literature about the biological formation of D-*ribo*-2-hexosulose.

The sugar biotransformations described above represent a preparatory useful route for rare naturally occurring glycosuloses, whose biochemical role has not been fully understood. In addition this dicarbonyl derivatives offer a convenient synthesis of sugars having specific $C_{(2)}$deuterium (or tritium) labelling by appropriate reduction or by enolization procedures.

EXPERIMENTAL

Evaporations were performed *in vacuo* at 35°C. Melting points (uncorrected) were taken on a Kofler hot stage apparatus. Infrared spectra were measured in KBr pellets with a Unicam SP-200 instrument; NMR spectra were measured at 80 MHz on a Tesla BS 487 spectrometer, at 59·797

and at 15·036 MHz on a Jeol FX-60 FT NMR spectrometer (^{1}H-NMR spectrum of *I* and ^{13}C-NMR spectrum of *V*) under conditions shown in Table II. Mass spectra were determined with a Varian MAT-311 spectrometer using the direct inlet, ionizing voltage 70 eV and ion source temperature 200°C. Metastable transitions were measured by the Direct Analysis of Daughter Ions (DADI) technique[18]. Optical rotations were determined with a Bendix–Ericsson 143A polarimeter. Thin-layer chromatography was performed on "ready-for-use" 20 Silufol UV_{254} (Kavalier Glassworks, Votice, Czechoslovakia) silica gel foils in the system chloroform–ethanol 20 : 3. Descending paper chromatography was made on paper Whatman No 1 using the system *n*-butyl acetate–acetic acid–acetone–water 140 : 100 : 33 : 80. Diphenylamine–aniline–phosphoric acid reagent was used for detection[19].

Dicarbonyl Sugar Production

The basidiomycete *Oudemansiella mucida* (SCHRAD. ex FR.) HÖHNEL, strain III (obtained from the fungal collection of the Institute of Microbiology, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) was cultured in an 18-litre laboratory fermentor on a chemically defined glucose medium[9] and harwested at the exponential growth phase (6th day). For transformations of sugars other than D-glucose, the mycelium was grown in the same medium with D-glucose replaced by D-mannitol. It was separated from the culture broth by filtration, washed extensively by tap water and centrifuged at 10 000 *g* for 10 min. Wet mycelium (250 mg of dry weight) was transferred to the 250 ml flasks containing 50 ml of 140 mM sugar and 50 mM sodium fluoride in distilled water. Suspensions were incubated on a rotatory shaker (3 Hz) for 24–32 h at 25°C during which time the sugar conversion was followed by paper chromatography. The broth filtrate was deionized on columns of ion-exchange resins (7 × 50 mm of Dowex 50W, H^+-cycle and Amberlite IRA-400, CH_3COO^--cycle) and concentrated to yield syrupy product. The large-scale preparations of D-*arabino*-2-hexosulose were performed in a laboratory fermentor by treating the sugar (140 mM in 50 mM sodium fluoride, 10 litres) with a suspension of washed mycelium (corresponding to 75 g of dry weight) for 28 h at 25°C and aeration of 8 litres air/min and a frequency of rotation of 6 Hz.

Yields of D-*arabino*-2-hexosulose were measured colorimetrically by determining the reduced 2,3,5-triphenyltetrazolium chloride[9]. Chromatographically pure hexosulose, quantified as a reducing sugar (ferricyanide method[20]) and expressed in glucose equivalents, was used for the construction of the calibration curve. The paper chromatographic mobilities of the sugar transformation products were: R_{allose} 0·76, blue spot; $E_{galactose}$ 0·85, blue violet; $R_{glucose}$ 0·72, blue; R_{xylose} 0·73 blue; $R_{sorbose}$ 0·57 and 0·77, two yellow spots.

Preparation of Glycosulose Derivatives

The treatment of syrupy oxidation products (0·5 g in 4 ml of 75% ethanol) obtained by the above described procedure with N,N-diphenylhydrazine (0·5 g) at room temperature for 24 h yielded a yellow crystalline derivative directly in the case of D-glucose and D-allose. With the oxidation product of D-galactose, the reaction mixture was extracted by ether, the extract was washed with water and evaporated to obtain a crystalline product. The hydrazon of the oxidation product of D-xylose was obtained in the crystalline form after purification by thin-layer chromatography. The resulting glycosulose hydrazones were acetylated by 1 : 1 acetanhydride–pyridine mixture 24 h at room temperature and then crystallized from 50% aqueous ethanol.

3,4,5,6-*Tetra*-O-*acetyl*-1-(*diphenylhydrazono*)-D-ribo-2-*hexosulose* (I): The oxidation product of D-allose yielded a hydrazone (206 mg) with m.p. 123–124°C, $[\alpha]_D^{23}$ −86·5° (*c* 0·85, chloroform);

IR spectrum: 1 750 (ester CO), and 1 675 cm^{-1} (ketone CO). For $C_{26}H_{28}N_2O_9$ (512·5) calculated: 60·93% C, 5·51% H, 5·47% N; found: 61·21% C, 5·45% H, 5·65% N.

3,4,5,6-*Tetra*-O-*acetyl*-1-(*diphenylhydrazono*)-D-*lyxo*-2-*hexosulose* (II): A derivative of the D-galactose oxidation product (172 mg) had m.p. 129–130°C, $[\alpha]_D^{23}$ +119·5° (*c* 0·86, chloroform); lit.[11]: m.p. 131°C, $[\alpha]_D^{20}$ +110° (*c* 1·0, chloroform); IR spectrum: 1 745 (ester CO) and 1 675 cm^{-1} (ketone CO).

3,4,5,6-*Tetra*-O-*acetyl*-1-(*diphenylhydrazono*)-D-arabino-2-*hexosulose* (III): A hydrazone of the D-glucose oxidation product (372 mg) had m.p. 129–130°C, $[\alpha]_D^{23}$ +38° (*c* 0·85, chloroform); lit.[11]: m.p. 133°C, $[\alpha]_D^{19}$ +27° (*c* 1·0, chloroform); IR spectrum: 1 750 (ester CO), and 1 680 cm^{-1} (ketone CO).

3,4,5-*Tri*-O-*acetyl*-1-(*diphenylhydrazono*)-D-threo-2-*pentosulose* (IV): Pale yellow crystals (185 mg) of the title compound, originating from D-xylose, had m.p. 111–112°C, $[\alpha]_D^{23}$ +83° (*c* 0·9, chloroform); IR spectrum: 1 745 cm^{-1} (ester CO) and 1 685 cm^{-1} (ketone CO). For $C_{23}H_{24}N_2O_7$ (440·5) calculated: 62·72% C, 5·49% H, 6·36% N; found: 63·06% C, 5·67% H, 6·26% N.

2,5-*Bis*(*phenylhydrazono*)-D-threo-2,5-*hexodiulose* (V): The syrupy product of biotransformed L-sorbose (0·5 g) was dissolved in water (2 ml) and mixed with a solution of phenylhydrazine hydrochloride (1 g) and sodium acetate (1·5 g) in 5 ml of water. Within 30 min, the mixture left at room temperature afforded yellow crystals (240 mg) with m.p. 133–135°C after recrystalization from 50% aqueous ethanol, $[\alpha]_D^{23}$ −163° (*c* 0·85, pyridine); lit.[21]: m.p. 133–135°C, $[\alpha]_D$ −164° (*c* 1·2, pyridine); IR spectrum: 3360 (OH), no carbonyl band present; mass spectrum: *m/e* 268 (19), 253 (8), 178 (69), 132 (38), 93 (100).

REFERENCES

1. Szarek W. A., Schnaar G. W.: Carbohyd. Res. *55*, C5 (1977).
2. Fukui S., Hochster R. M.: Can. J. Biochem. Physiol. *41*, 2363 (1963).
3. Hayano K., Fukui S.: J. Biochem. *64*, 901 (1968).
4. Ikeda K.: J. Gen. Appl. Microbiol. Tokyo *1*, 152 (1955).
5. Kitamura I., Perlman D.: Biotechnol. Bioeng. *17*, 349 (1975).
6. Sato K., Yamada Y., Aida K., Uemura T.: Agr. Biol. Chem. *33*, 1606 (1969).
7. Englard S., Avigad G.: J. Biol. Chem. *240*, 2297 (1965).
8. Kulhánek M., Ševčíková Z.: Folia Microbiol. (Prague) *10*, 362 (1965).
9. Volc J., Sedmera P., Musílek V.: Folia Microbiol. (Prague) *23*, 292 (1978).
10. Volc J., Sedmera P., Musílek V.: Czech. 175 897 (1976).
11. Henseke G., Liebenow W.: Chem. Ber. *87*, 1068 (1954).
12. Lloyd J. B., Whelan W. J.: Anal. Biochem. *30*, 467 (1969).
13. Bond C. R., Knight E. C., Walker T. K.: Biochem. J. *31*, 1033 (1937).
14. Ruelius H. W., Kerwin R. M., Janssen F. W.: Biochem. Biophys. Acta *167*, 493 (1968).
15. Baute M.-A., Baute R., Deffieux G., Filleau M.-J.: Phytochemistry *16*, 1895 (1977).
16. Bean R. C., Hassid W. Z.: Science *124*, 171 (1956).
17. Avigad G., Amaral D., Asensio C., Horecker B. L.: J. Biol. Chem. *237*, 2736 (1962).
18. Maurer K. H., Brunee C., Kappus G., Habfast K., Schroder U., Schulze P. 19th Conference on Mass Spectrometry, Atlanta, Ga., 1971, Paper K-9.
19. Kocourek J., Tichá M., Koštíř J.: J. Chromatogr. *24*, 117 (1966).
20. Hoffman W. S.: J. Biol. Chem. *120*, 51 (1937).
21. Avigad G., Englard S.: J. Biol. Chem. *240*, 2290 (1965).

Translated by the author (P. S.).