Reactions of saccharides catalyzed by molybdate ions XXXI.* Oxidative degradation of 4-nitrophenylhydrazones of some 4-*O*-hexosylhexoses to 3-*O*-hexosylpentoses

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Received 13 March 1981

4-Nitrophenylhydrazones of maltose, cellobiose or lactose are degraded with hydrogen peroxide under catalytic action of molybdate ions to disaccharides shorter by one carbon atom — 3-*O*-α-D-glucopyranosyl-D-arabinose, 3-*O*-β-D-glucopyranosyl-D-arabinose and 3-*O*-β-D-galactopyranosyl-D-arabinose, respectively. The prepared hexosylpentoses were characterized by ^{13}C-n.m.r. spectroscopy.

4-Нитрофенилгидразоны мальтозы, целлобиозы и лактозы разлагаются перекисью водорода при каталитическом воздействии ионов молибдата на дисахариды, короче на один атом углерода, соответственно на 3-*O*-α-D-глюкопиранозил-D-арабинозы, 3-*O*-β-D-глюкопиранозил-D-арабинозы и 3-*O*-β-D-галактопиранозил-D-арабинозы. Полученные гексозилпентозы были характеризованы спектроскопией ^{13}C-ЯМР.

Phenylhydrazones of saccharides and their derivatives are often used for identification and characterization of reducing saccharides, and, in some instances, also for their isolation from reaction mixtures [1]. Recently we have explored 4-nitrophenylhydrazones of saccharides for determination of reducing mono- and disaccharides present in mixtures in natural and waste waters [2] as well as for determination of maltooligosaccharides from maltose to maltooctaose [3]. Further application of phenylhydrazones of aldohexoses or their derivatives is their degradation to aldopentoses with oxygen under catalysis with Pt/C in benzene or acetone [4] or, in aqueous solutions by hydrogen peroxide in the presence of molybdate ions [5, 6]. In the present paper we report on the oxidative degradation of reducing disaccharides, 4-*O*-hexosylhexoses, to the corresponding disaccharides shorter by one carbon atom, 3-*O*-hexosylpentoses.

The reaction of 4-nitrophenylhydrazine with a disaccharide (maltose, cellobiose, lactose) in aqueous methanol leads to the corresponding disaccharide 4-ni-

* For Part XXX see *Chem. Zvesti 34*, 518 (1980).

trophenylhydrazone. The hydrazone is without special isolation further treated with ammoniacal aqueous solution of hydrogen peroxide under catalytic action of molybdate ions to give the corresponding shorter disaccharide, 3-*O*-α-D-glucopyranosyl-D-arabinose (*I*), 3-*O*-β-D-glucopyranosyl-D-arabinose (*II*) or 3-*O*-β-D-galactopyranosyl-D-arabinose (*III*). The products are isolated from the reaction mixture by preparative paper chromatography in about 20% yield referred to the starting disaccharide. A similar reaction of 4-nitrophenylhydrazine with D-[U-^{14}C]glucose followed by oxidative degradation of the hydrazone afforded D-[U-^{14}C]arabinose in 44% yield [6]. The conversion to the lower disaccharides *I, II,* and *III* in the oxidative degradation of 4-nitrophenylhydrazones of 4-*O*-hexosylhexoses is not lower, however, final yields of products are strongly reduced during their isolation by paper chromatography due to not too high effectiveness of the separation (Table 1). As far as the conversion of the

Table 1

Separation of reducing disaccharides (*a*) and their 4-nitrophenylhydrazones (*b*) by paper chromatography (elution system 1-butanol—ethanol—water 5:1:4)

Disaccharide	*a* R_{Mal}	*b* $R_{NPhHMal}$
Maltose	1.00	1.00
3-*O*-α-D-Glucopyranosyl-D-arabinose	1.24	1.12
Cellobiose	0.93	0.94
3-*O*-β-D-Glucopyranosyl-D-arabinose	1.25	1.12
Lactose	0.77	0.91
3-*O*-β-D-Galactopyranosyl-D-arabinose	1.07	1.08

a) Development for 90—100 h; *b*) development for 18—20 h.

starting disaccharides to compounds *I, II,* and *III* is concerned, the described method is comparable with the methods of Ruff, Wohl, the degradation of *per-O*-acetylglycals by ionization [7] or degradation of disaccharides by hypochlorite oxidation [8]. The described procedure has to be carried out with a special care when applied to larger amount of material. The excess of hydrogen peroxide must be sufficiently destroyed before the concentration of the reaction mixtures to avoid the *explosive decomposition.* The described method is advantageous particularly for a low scale preparation of disaccharides *I, II,* and *III, e.g.* in the case of [U-^{14}C]-labelled products, because the whole sequence of reactions is carried out in one test tube by successive addition of reagents.

Disaccharides *I, II,* and *III* were characterized by ^{13}C-n.m.r. spectroscopy. From the intensity of C-1α and C-1β signals of the reducing end residue one may infer

that disaccharides form in aqueous media (25°C) an equilibrium mixture of α and β anomers in the ratio 2:1. The chemical shifts of the anomeric C-1 signals as well as the signals of C-2, C-4, and C-5 of the arabinopyranosyl ring in compounds *I*, *II*, and *III* are not significantly influenced by the 1→3 glycosidic bond. The signals were assigned to individual atoms in molecules of disaccharides *I*, *II*, and *III* (Table 2) by comparison with the ^{13}C-n.m.r. data for D-arabinose, methyl-α-D-glucopyranoside, methyl-β-D-glucopyranoside, and methyl-β-D-galactopyranoside, taking into consideration the rules valid for di- and oligosaccharides [9]. In disaccharides *I*, *II*, and *III* which contain identical reducing sugar unit (D-arabinose), the α effect of glycosylation is pronounced on carbon atom C-3 in that its signal is shifted to low magnetic field by 7.5 p.p.m. in average. As a generalization for disaccharides, the anomeric configuration of the glycosidic bond can be determined according to the position of the C-1′ signal of the nonreducing sugar unit [9, 10]. It is of interest that the C-1′ signals of disaccharides *I* and *II* are practically identical despite the fact that the disaccharides differ in the anomeric glycosidic bond. The C-1′ to C-6′ signals of disaccharide *I* are comparable with the C signals of methyl-α-D-glucopyranoside. Similarly, in disaccharide *II* the signals C-2′ to C-6′ (but not the C-1′ signal) are comparable with the signals of methyl-β-D-glucopyranoside [11]. It is known that steric interactions in molecules can affect the chemical shifts of C signals, which is consequently reflected in a signal shift to higher magnetic field [9, 10]. Regarding this fact the anomality of the shift of C-1′ signal in disaccharide *II* can be interpreted as a consequence of a steric

Table 2

^{13}C-NMR chemical shifts (p.p.m.) in hexosylpentoses

Atom	3-*O*-α-D-Glucopyranosyl-D-arabinose		3-*O*-β-D-Glucopyranosyl-D-arabinose		3-*O*-β-D-Galactopyranosyl-D-arabinose	
	α	β	α	β	α	β
C-1	97.5	93.8	97.3	93.7	97.9	93.7
C-2	73.0	68.8	74.1	68.1	72.0	69.1
C-3	81.6	78.1	80.7	77.5	80.6	76.6
C-4	69.5	69.5	70.7	71.4	69.3	69.9
C-5	67.5	61.7	67.1	61.9	67.3	62.3
C-1′	101.5			101.3		102.0
C-2′	73.0			74.1		71.6
C-3′	74.0			76.8		73.9
C-4′	70.8			70.7		69.9
C-5′	73.5			77.2		76.6
C-6′	61.7			61.9		62.3

C-1′—H_{ax} interaction with the oxygen atom of the axial hydroxyl group at C-4 of the reducing sugar residue. On the contrary, such an interaction is not allowed in disaccharide *I* (C-1′—H_{eq}). The α- and β-glycosidic bonds in disaccharides *I* and *II* can be distinguished on the basis of ^{1}H-n.m.r. spectra (100 MHz). The spectrum of disaccharide *I* shows a H-1′ doublet at 5.23 p.p.m. ($J_{1,2} = 4.0$ Hz) characteristic of an α-glycosidic bond. Doublet H-1′ at 4.60 p.p.m. ($J_{1,2} = 7.5$ Hz) in the spectrum of disaccharide *II* is characteristic of a β-glycosidic bond. The ^{1}H-n.m.r. spectra of disaccharides *I* and *II* also contain signals corresponding to α- and β-anomeric form of the reducing end sugar residue (H-1α 4.56 p.p.m., $J_{1,2} = 7.5$ Hz and H-1β 5.30 p.p.m., $J_{1,2} = 3.5$ Hz) which are in a good consonance with the ^{1}H-n.m.r. data for D-arabinose [12]. A conclusion may be drawn from the above results. The ^{13}C-n.m.r. spectral data are very useful for the determination of the position of glycosidic bond on D-arabinose while the ^{1}H-n.m.r. spectra are important for the ascertainment of the anomeric type of the nonreducing sugar residue in saccharides *I* and *II*.

Experimental

Composition of reaction mixtures and purity of saccharides was followed by chromatography on Whatman No. 1 paper in 1-butanol—ethanol—water 5:1:4 v/v (Table 1). The same chromatography was used for isolation of products.

^{13}C-N.m.r. spectra for D_2O solutions of the prepared saccharides at 25°C were recorded with a FT-NMR JEOL-FX-60 spectrometer under complete proton decoupling using methanol as an internal standard (Table 2). The chemical shift of the standard related to TMS was 50.1 p.p.m. Spectra were run under following parameters: pulse interval 1 s, pulse width 4 μs (45° flip angle), spectral width 4000 Hz, 8 K real data points, average number of accumulation 1000. ^{1}H-N.m.r. spectra were recorded for D_2O solutions at 40°C with a FT-NMR JEOL-FX-100 spectrometer using DSS as an internal standard.

Oxidative degradation of 4-nitrophenylhydrazones of disaccharides

A solution of disaccharide (2 g; maltose, cellobiose of lactose) in water (4 ml) was mixed with methanol (8 ml) and 4-nitrophenylhydrazine (0.9 g). The mixture was heated for 3 h at 60°C and, after dilution with methanol (4 ml) for another 3 h. After addition of 2.6% aqueous solution of ammonia (2 ml) and 5% solution of ammonium molybdate (2 ml) the mixture was cooled to 10—15°C; mixed with 30% solution of hydrogen peroxide (4 ml) and left to stand at room temperature for 20 h. The mixture was then treated with a small amount of palladium-coated charcoal (0.1 g) for another 48 h at room temperature. Finally the mixture was filtered, and the filtrate chromatographed on 6 sheets of Whatman No. 1 paper for 90—100 h in the 1-butanol—ethanol—water elution system. After detection of representative strips of the chromatograms with the aniline-hydrogen phthalate reagent (starting hexosylhexoses give brown colour and hexosylpentoses red) the zones of hexosylpentoses were eluted with water. The eluates (usually contaminated with the starting

hexosylhexoses) were concentrated and rechromatographed (3 sheets of the chromatographic paper) to give 300—370 mg of chromatographically homogeneous hexosylpentoses.

3-*O*-α-D-Glucopyranosyl-D-arabinose ($[\alpha]_D = +46°$) was obtained from maltose, 3-*O*-β-D-glucopyranosyl-D-arabinose ($[\alpha]_D = -79°$) from cellobiose and 3-*O*-β-D-galactopyranosyl-D-arabinose ($[\alpha]_D = -61°$) from lactose (optical rotations were measured at *c* 1 in water). Ref. [13] gives for the compounds $[\alpha]_D$ values in water $+47°$, $-90°$, and $-63°$, respectively.

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Translated by P. Biely