Conversion of *N*-(4-nitrophenyl)glycosylamines of aldoses to aldohexuronic acids by nitrogen dioxide

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Oxidation of the primary hydroxyl group of *N*-(4-nitrophenyl)glycosylamines of aldoses by nitrogen dioxide followed by hydrolytic elimination of the aglycon leads to uronic acids which are isolated by chromatography. This simple procedure for preparation of aldohexuronic acids was successfully applied for preparation of D-[U-^{14}C]mannuronic, D-[U-^{14}C]glucuronic, and D-[U-^{14}C]galacturonic acid from the corresponding [U-^{14}C]-labelled aldohexoses.

Первичная гидроксильная группа *N*-(4-нитрофенил)гликозиламинов альдогексоз окисляется газообразной двуокисью азота, и после гидролитического отстранения агликона хроматографически выделяются образующиеся уроновые кислоты. Данная методика получения альдогексуроновых кислот проста и была успешно применена также для получения D-(U-^{14}C)маннуроновой, D-(U-^{14}C)глюкуроновой и D-(U-^{14}C)галактуроновой кислот из соответствующих (U-^{14}C)-меченных альдогексоз.

Selective oxidation of primary hydroxyl groups of saccharides has been in a number of examples effected by nitrogen dioxide. Secondary hydroxyl groups are relatively resistant against the oxidation. The hemiacetal group of the oxidized saccharides must be, however, protected. For these reasons, the oxidation was mostly applied to polysaccharides (starch [1], [U-^{14}C]-labelled starch [2], cellulose [3—5], mannan [3]) and, in the case of monosaccharides, to their *O*-alkyl glycosides [6, 7]. It should be noted that with polysaccharides the oxidation of secondary hydroxyl groups was also observed in a low extent [8]. Free uronic acids are then obtained upon hydrolysis of the oxidized *O*-glycosides or acidic polysaccharides formed.

It is generally known that *O*-glycosides of uronic acids as well as acidic polysaccharides are more resistant to hydrolysis than the corresponding *O*-glycosides of aldoses and neutral polysaccharides. In addition, uronic acids are rather unstable under conditions of acid hydrolysis [2]. In the present work we report on the oxidation of primary hydroxyl group of aldehexoses under the

protection of their hemiacetal groups in the form of *N*-arylglycosides from which the aglycon can be easily released by hydrolysis.

Under the conditions given in Experimental aldohexoses react with 4-nitroaniline to give the corresponding *N*-(4-nitrophenyl)glycosylamines in 65—80% yield. After drying, the reaction mixture is treated with gaseous nitrogen dioxide. *N*-Glycosidic bond is than cleaved by aqueous solution of formic acid and the resulting hexuronic acids are isolated by paper chromatography (Table 1). This procedure carried out with [U-^{14}C]-labelled D-mannose, D-glucose, and D-galactose gave D-[U-^{14}C]mannuronic acid, D-[U-^{14}C]glucuronic acid, and D-[U-^{14}C]galacturonic acid in 10—15% yields, referred to the starting [U-^{14}C]-labelled aldohexoses which can be partially recovered (D-mannose and D-galactose in 5—10%, D-glucose in 10—15%). A similar treatment of *N*-(4-nitrophenyl)-D-mannosylamine or *N*-phenyl-D-mannosylamine on a larger scale afforded D-mannuronic acid which was isolated by ion-exchange chromatography on Dowex 50 W (X-2, Ba^{2+} cycle), eluted with water, in 14 and 17% yield, respectively, referred to the starting *N*-aryl-D-mannosylamine.

Table 1

Paper chromatography of uronic acids and their lactones obtained by oxidation of *N*-arylglycosides of aldohexoses with nitrogen dioxide

Saccharide	Relative mobility in solvent					
	S_1[a]			S_2[b]		
Acid						
D-Glucuronic	0.50	3.17		1.06	3.18	
D-Mannuronic	0.54	2.01		2.41		
D-Galacturonic	0.54			1.09	2.38	
D-Taluronic	0.51	2.00		1.23		
D-Iduronic	0.64	3.30		1.17	4.50	
D-Guluronic	0.44	1.30	3.25	0.92	1.69	3.10
D-Altruronic	0.57			1.32		
D-Alluronic	0.36			1.13		
D-Glucose	1.00			1.00		
D-Mannose	1.48			1.17		
D-Galactose	0.96			0.90		
D-Talose	1.92			1.64		
D-Idose	1.95			1.56		
D-Gulose	1.45			1.15		
D-Altrose	1.26			1.35		
D-Allose	1.31			1.28		

a) Flow 18—20 h; *b*) flow 7—8 h.

In order to prevent the hydrolytic cleavage of the *N*-glycosidic linkage during oxidation of *N*-arylglycosides, it is necessary to work with dry starting compound and nitrogen dioxide in a dry reaction environment. However, the decomposition of nitrogen dioxide to nitric acid and nitric oxide is conditioned by certain humidity, water serving as a promoter of the oxidation of the primary hydroxyl group in the saccharide [1]. In the first reaction step nitric acid esterifies the primary hydroxyl group. The nitroester group is then converted to carboxyl group. The product of the esterification and oxidation is reaction water which plays a role in the hydrolysis of the glycosidic bonds. In the case of *N*-arylglycoside oxidation the reaction water brings about a partial hydrolysis of *N*-glycosidic linkage resulting in the liberation of starting aldoses and their oxidation products, uronic acids. Subsequent oxidation of these compounds at C-1 gives the corresponding aldonic and aldaric acids. In this connection it is interesting to note that under similar reaction conditions arylhydrazones of aldoses afford only trace amounts of uronic acids and the main oxidation products are represented by nonreducing saccharides — aldonic and aldaric acids [2]. The main difference in the behaviour of *N*-arylglycosides and arylhydrazones of aldoses during oxidation with nitrogen dioxide consists probably in their different structural arrangement. *N*-Arylglycosides are oxidized mainly as cyclic structures which predominate acyclic structures. Opposite is true for arylhydrazones. Due to parallel hydrolysis of *N*-glycosidic linkage, the oxidation of *N*-arylglycosides is effective only for a limited period (under the conditions used for 3—4 h) because a longer treatment leads to a decrease in the proportion of uronic acids in the mixture.

The described oxidation procedure is suitable to be carried out both on a semimicroscale and a larger scale. The fact that the whole procedure can be done in one test-tube by successive additions of reagents, makes it convenient particularly for preparation of ^{14}C-labelled hexuronic acids.

Experimental

Liquid nitrogen dioxide (Syntézia, Pardubice, Czechoslovakia), D-[U-^{14}C]glucose (Institute for Research, Production, and Use of Radioisotopes, Prague), and D-[U-^{14}C]galactose (Radiochemical Centre, Amersham) were commercial products. The following saccharides were synthesized according to the literature: D-[U-^{14}C]mannose [2], D-allose, D-altrose [9], D-gulose, D-idose, D-talose [10].

Saccharides were separated by chromatography on Whatman No. 1 paper in solvent system 1-butanol—ethanol—water 5:1:4 (v/v) (S_1) and ethyl acetate—acetic acid—water 3:1:1 (v/v) (S_2) [11] and detected with the aniliniumhydrogenphthalate reagent or sodium periodate. Isolation of D-mannuronic acid was done by ion-exchange chromatography on a column (2.5 × 90 cm) of Dowex 50 W (X-2, 75—150 μm, Ba^{2+} cycle), eluted with water at a rate of 30 ml h^{-1}. Radioactivity of saccharides was measured with a scintillation spectrome-

ter Packard 3330 (USA) using a toluene scintillation fluid (Tesla, Přemyšlení, Czechoslovakia). Optical rotation of saccharides was measured with a Perkin—Elmer polarimeter, type 141.

The oxidation of saccharides by nitrogen dioxide was done in a closed glass vessel (desiccator) in which the solid saccharides were present in a test-tube or a Petri dish in a 3—4 mm thick layer. The compounds were exposed to gaseous nitrogen dioxide at room temperature for a desired time. Gaseous nitrogen dioxide was either liberated by evaporation from an additional vessel filled with liquid nitrogen dioxide mixed with an appropriate amount of phosphorus pentoxide or, generated from sodium nitrite powder (dried *in vacuo* at 80°C) by adding 90% sulfuric acid in a separate apparatus connected with the desiccator.

Preparation and oxidation of N-(4-nitrophenyl)hexopyranosylamines

Aldohexose (100—120 mg), water (0.5 ml), and methanolic solution of 4-nitroaniline (2.5 ml of a solution containing 3 g of 4-nitroaniline and 3 ml of acetic acid in 95 ml of methanol) were mixed in a test-tube provided with a glass-ground stopper and heated at 65°C for 2 h. Further portion of the methanolic solution of 4-nitroaniline (4 ml) was added and the mixture heated again for 2 h. The solution was then evaporated under reduced pressure and the dry distillation residue oxidized with gaseous nitrogen dioxide for 3 1/2 h. The oxidized product was hydrolyzed in 10% aqueous solution of formic acid (2 ml) at 90°C for 6 h. After filtration the mixture was applied on one sheet of paper and chromatographed in the solvent system S_1. Isolated uronic acids or their lactones were then rechromatographed in the solvent system S_2. Uronic acids and their lactones give bright red colour on detection with the aniliniumhydrogenphthalate reagent already at room temperature within 5—10 min. Relative mobilities of aldohexoses and aldohexuronic acids are listed in Table 1.

[U-^{14}C]-*Labelled* D-*glucuronic*, D-*mannuronic, and* D-*galacturonic acid*

[U-^{14}C]-Labelled D-glucose, D-mannose, or D-galactose (10—15 mg, 130 kBq) in water (0.1 ml) was mixed with methanolic solution of 4-nitroaniline (1 ml, detail composition see above) and heated at 65°C for 2 h, and after addition of further methanolic solution of 4-nitroaniline (2 ml), for other 2 h. Measurements of the radioactivity distribution on paper chromatograms showed that the starting [U-^{14}C]aldohexoses were converted to the corresponding *N*-(4-nitrophenyl)glycosides in 65—80%. Their oxidation with nitrogen dioxide, subsequent hydrolysis, and chromatographic isolation and purification of products were done as described above. The isolated uronic acids contained 10—15% of the radioactivity of the starting aldohexoses.

D-*Mannuronic acid*

Dry *N*-(4-nitrophenyl)-D-mannosylamine (7.5 g) or *N*-phenyl-D-mannosylamine (6.3 g) was oxidized with gaseous nitrogen dioxide for 3 1/2 h at room temperature. The oxidized

product was hydrolyzed in 10% aqueous solution of formic acid (80 ml) at 90°C for 6 h. The mixture was then diluted with water (80 ml), treated with charcoal and the filtrate extracted with ethyl acetate (3 × 50 ml). The aqueous phase was concentrated and chromatographed on a column of Dowex 50 W and eluted with water. Fraction 1 (200—310 ml) contained D-mannose (3—4 g) and fraction 2 (310—390 ml) 0.6—0.8 g of amorphous D-mannuronolactone. Dry product (P_2O_5) showed $[\alpha]_D^{23} = +47°$ (5 min) → $+49°$ (30 min) → $+48°$ (24 h) (*c* 2, water) and $[\alpha]_D^{23} = +31°$ (2 min) → $+18°$ (5 min) → $+9°$ (30 min) → $+10°$ (24 h) (*c* 1, 4% aqueous solution of ammonium molybdate). Ref. [12] gives for D-mannuronolactone $[\alpha]_D^{22} = +79.9°$ (3 min) → $+93.8°$ (29 min) → $+92.7°$ (24 h) (*c* 1.9, water).

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