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Note

Electrosynthesis of oligosaccharide glycamines

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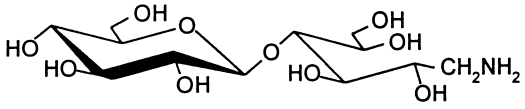
Abstract

1-Amino-1-deoxy-4-*O*-β-D-glucopyranosyl-D-glucitol and 1-amino-1-deoxy-4-*O*-β-D-galactopyranosyl-D-glucitol were obtained in high purity and almost quantitative yield by electro-reduction of the respective oximes of cellobiose and lactose. The methodology was extended to mixture of hepta-, octa- and nona-saccharides obtained by enzymatic degradation of xyloglucan. The oximes were prepared in situ from the above-mentioned oligosaccharides and a mixture of hydroxylammonium sulfate and ammonium acetate at pH 4–6 in aqueous solutions. Electrosynthesis of the glycamines was carried out at either constant current or constant potential in a divided electrolysis cell with a working mercury cathode and a platinum-coated titanium anode separated by a cation-selective membrane.

Keywords: Glycamines; Electrosynthesis; Electroreduction; Oligosaccharides; Xyloglucan

With the exception of 1-amino-1-deoxy-D-glucitol [1], glycamines are not easily accessible and their importance in organic chemistry and biochemistry has therefore been limited. This fact lead us to develop an electrochemical method for the preparation of monosaccharide glycamines on a bench-scale [2]. Glycamines of cellobiose, lactose and maltose have so far been prepared by chemical synthesis [3]. Since oligosacharide glycamines can find their place in various applications, e.g. as ligands in affinity chromatography [4], we decided to extend this method to the preparation of glycamines of the disaccharides cellobiose (**1**) and lactose (**2**) and to hitherto less accessible higher oligosaccharides derived from xyloglucan.



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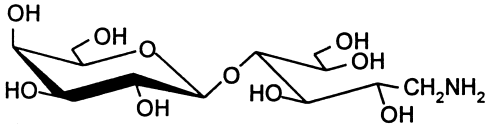

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In a preceding paper [2], we described optimal conditions for the reaction of monosaccharides with hydroxylamine and the subsequent electroreduction of the resulting oximes to the corresponding glycamines. In contrast to previous work in which acetate buffers or aqueous solutions of

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hydroxylammonium acetate were used for the preparation of oximes, a mixture of hydroxylammonium sulfate and ammonium acetate was the oximation reagent. This modification is advantageous because it avoids both the troublesome isolation of glycamines when employing acetate buffers and the need of preparing hydroxylammonium acetate, which is not commercially available. In addition, the ratio of hydroxylammonium sulfate to ammonium acetate can be changed arbitrarily in order to find optimal conditions for the formation of oximes.

The chemical synthesis of oximes of oligosaccharides and their subsequent electroreduction to the corresponding glycamines proceed according to Scheme 1. In the first step, addition of hydroxylamine to the carbonyl group of the saccharide (RCH=O) takes place. In the next step, generally acid-catalysed, the resulting carbinolamine is further dehydrated to the oxime [5,6]. Since only the protonated forms of oximes are reducible [7], the latter must be formed by protonation of the polarized azomethine group >C=N– of oximes in the strong electric field on the surface of the mercury cathode before the electroreduction step. Subsequently, the protonated form of the oxime undergoes a four-electron reduction to the protonated glycamine (Scheme 1):

$$R{-}CH{=}NOH + H^+ \longleftrightarrow (R{-}CH{=}NHOH)^+$$

$$(R{-}CH{=}NHOH)^+ + 4e^- + 4H^+ \longrightarrow R{-}CH_2NH_3^+ + H_2O$$

Scheme 1.

It is worth noting that the pH value of the solution decreases during formation of the oximes because the hydroxylammonium salt is replaced by the non-protonated oxime [2,8]. On the other hand, during electro-reduction of oximes, the pH value of the medium increases due to formation of the basic glycamine, consuming the liberated acid; moreover, electroreduction of the excess of hydroxylamine affords the more-basic ammonia [9]. The reaction conditions for the formation of oximes and their electroreduction to glycamines were chosen so as to keep the pH changes within the optimal pH 4–6 range.

Of the two main electro-reduction processes, involving constant current or potential, the more suitable and more advantageous one was applied in each case. Under the conditions used, the oxime was reduced preferential at constant current, as long as this was kept lower than that of the limit current value of the given oxime. It is not advantageous to start electroreduction from a pre-prepared oxime [10,11], because at pH 4 to 6 in water solution this entity decomposes by rapidly establishing the equilibrium with the starting compounds. Whereas both methods were found equally effective with cellobiose and with lactose, the constant current method showed better results with the higher xyloglucan-derived oligosaccharides. Good results with both electroreduction methods were ensured by a divided electrolysis cell equipped with a cation-selective, acid resistant, low electric-resistance membrane which allowed migration of hydrogen ions formed in the anodic compartment to the cathode where they are consumed in the 4-electron reduction of oximes into glycamines (Scheme 1).

The results presented here show that the electroreduction method can be employed as an useful alternative to methods hitherto used for the preparation of oligosaccharide glycamines. Its main advantage lies in that it is a simple one-pot procedure and yields products of high purity in almost theoretical yields.

Electro-reduction of the oligosaccharide oximes, and excess of hydroxylamine, produced the glycamine salts and ammonia. The freeze-dried reaction mixture, obtained from cellobiose or lactose, displayed the presence of 12 signals characteristic of the protonized forms of the disaccharide glycamines [3] and two singlets for the acetate anion in their ^{13}C NMR spectra. No signal indicative of the presence of starting disaccharides or those associated with the presence of impurities or by-products was found in the spectra of the products, thus providing evidence for their high purity. The solutions of glycamines were desalted by a strongly basic anion exanger (Dowex 1, OH^- cycle), while stirring the mixture vigorously by bubbling nitrogen through the solution during 30 min, which secured also the removal of the excess of ammonia. By evaporating the desalted solutions to dryness under reduced pressure at T_{max} 40°C, glycamines were obtained in almost theoretical yields (ca. 100 mg from 100 mg starting oligosaccharide) and high purity.

In the case of the electroreduction of the mixture of xyloglucan-derived oligosaccharides, the formation of glycamines was confirmed, besides results of elemental analysis (nitrogen contents), by the disappearance of signals of anomeric protons in the ^1H NMR spectrum.

1. Experimental

Chemicals.—Cellobiose was supplied by the Pilot plant of our Institute; lactose was from Lachema (Brno, Czech Republic), the mixture of hepta-, octa- and nona-saccharides Glc_4Xyl_3, Glc_4Xyl_3Gal, and $Glc_4Xyl_3Gal_2$ was obtained by partial degradation of xyloglucan with cellulase [12], and hydroxylammonium sulfate was purchased from Sigma (St. Louis, MO). Other chemicals used were of analytical grade.

Apparatus.—A universal mains-fed Lytron with adjustable voltage ranging from 3 to 6 V and current output up to 500 mA served as the DC power supply. A potentiostat PRT 40-5X (Tacussel, France) was employed for electroreduction under constant potential. Polarographic analysis was carried out with a polarograph LP 7 (Laboratorní Přístroje, Prague, Czech Republic). The quality of the starting disaccharides and the synthesized glycamines obtained was checked by ^{13}C NMR spectroscopy with a FT NMR Bruker AVANCE DPX 300 spectrometer.The one-dimensional ^1H and ^{13}C and two-dimensional homonuclear ^1H–^1H COSY and heteronuclear ^{13}C–^1H correlated NMR spectra (HSQC and HMBC) were measured in deuterated water at 25 °C. Chemical shifts were referenced to external acetone (δ 2.225 and 31.07 ppm for ^1H and ^{13}C, respectively). Electrosynthesis of glycamines was carried out in a divided electrolysis cell (Fig. 1), as described in, e.g. ref. [13], in which the cathodic compartment was separated from the anodic one by a cation-selective membrane, type RALEX CM purchased from Mega a. s., Stráž pod Ralskem, Czech Republic. A mercury layer of ca. 0.5 cm thickness on the bottom of 100 mL beaker of 5 cm in diameter connected through a fused platinium wire with the power supply was used as cathode. A glass cylinder about 8 cm in length and 2.5 cm in diameter closed at the bottom with a cation-selective membrane fixed through the hole in the cover was the anodic part of the electrolyzer. A platinum-coated titanium electrode in form of a cylinder (5×1.5 cm) served as the anode. In addition to the main hole for the anodic compartment, the cover contained further holes for a reference saturated calomel electrode (SCE) and sample withdrawal (Fig. 1). A soln of the oxime formed in situ served as the catholyte and 0.01 M aq H_2SO_4 was used as the anolyte. The soln in the electrolysis cell was agitated with a magnetic stirrer. When working at constant current, the electrolyzer was used as a two-electrode system with the mercury cathode and platinum-coated titanium anode linked to a stabilized DC source (Lytron). At constant-potential electro-reduction, a three-electrode system with a potentiostat and SCE as the reference electrode for maintaining the constant potential on the mercury cathode was used.

Analytical procedures.—Polarographic, paper-chromatographic, thin-layer chromatographic and NMR spectroscopy methods were used for monitoring

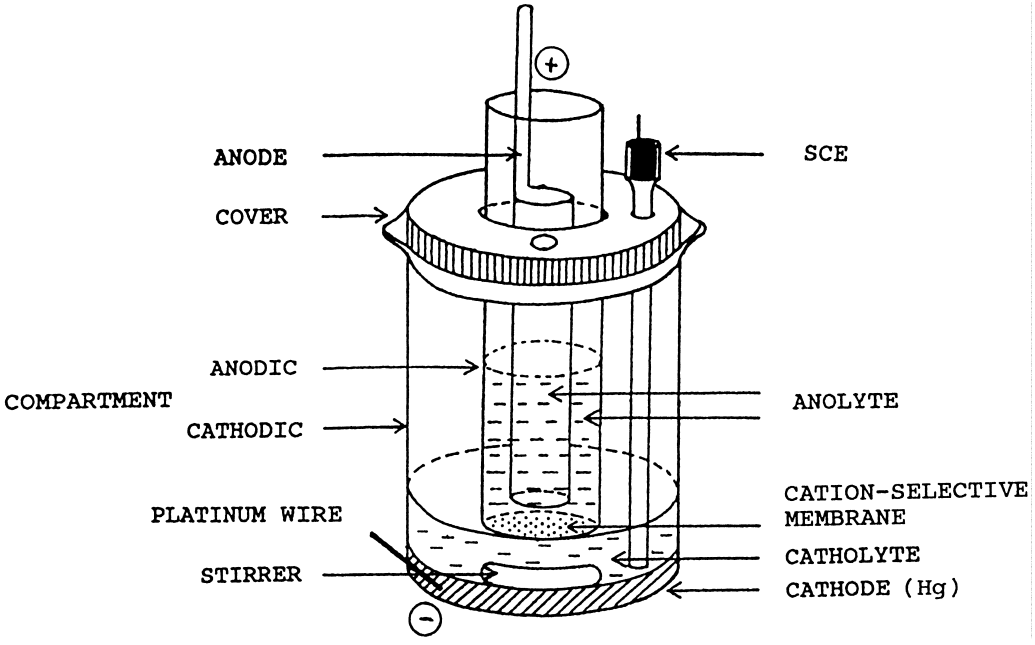

Fig. 1. Diagram of the divided two- and three-electrodes electrolyzer.

both the oxime formation during the reaction of oligosaccharides with hydroxylamine and the subsequent electro-reduction to glycamines. The starting oxime concentration and its decrease during the electro-reduction was monitored polarographically in pre-set time intervals in formate buffer solutions of pH 3.6. The concentration of hydroxylamine was also monitored by measuring polarographic waves associated with its anodic oxidation in a carbonate buffer of pH 10. The higher mobility of oximes and the slower one of glycaminium salts with relation to the mobility of the starting oligosaccharides during paper chromatography with 7:3 acetone-water and during TLC on silica gel 60 TLC with 2:1:1 n-propanol-MeOH-water made it possible to monitor the entire reaction course (i.e. the starting material, intermediates, final products). Components on paper chromatograms were visualized by alkaline silver nitrate. The sugars on TLC plates were detected by spraying with 1% orcinol in 10% (v/v) H_2SO_4 dissolved in ETOH and subsequent heating at 100°C for 10 min.

Electrosynthesis of disaccharide glycamines at constant current.—A mixture consisting of cellobiose or lactose (100 mg, 0.29 mmole), an aq soln of hydroxylammonium sulfate (1 M, 1 mL) and aq ammonium acetate (1 M, 0.5 mL) was stored overnight at room temperature and the oxime formed was then diluted with water to 10 mL; the final pH of the soln was 4.5. The cathodic compartment with a magnet bar, placed on a magnetic stirrer, was filled with mercury and the soln of oxime. The anodic section containing aq H_2SO_4 (0.01 M, 15 mL) and the platinum-coated titanium electrode was inserted into the cathodic compartment so that the surface of the cation-selective membrane was just immersed in the catholyte. To preserve its perfect function, the membrane, when out of action, was always maintained wet. Withdrawal of samples for analysis was performed periodically during the electro-reduction at a constant current (15 mA) with intensive mixing of the catholyte. The pre-set current intensity was kept constant by a rheostat connected in series to the electrolyser. Polarographic analysis revealed disappearance of the cellobiose or lactose oximes as well as the hydroxylamine within 7 h. Chromatographic analyses afforded the same result. Electrosynthesis of glycamines from xyloglucan-derived oligosaccharides was performed as described for the disaccharides cellobiose and lactose. A mixture of 100 mg of oligosaccharides with 1 mL 1 M hydroxylammonium sulfate and 0.5 mL of 1 M ammonium acetate in water was kept overnight at room temperature. The resulting solution of oximes was then diluted to 10 mL with water and 0.05 mL of concentrated acetic acid was added to bring the pH to ca. 4.3. The electroreduction conditions were the same as for the oximes of cellobiose and lactose except that the current was lowered to 5 mA. The reduction of the oximes was terminated within 8 h; the reduction of excess hydroxylamine took an additional 2 h at 20 mA.

1-amino-1-deoxy-4-O-β-D-glucopyranosyl-D-glucitol (**1**).—$[\alpha]_D$ −10.7° (*c* 4; H_2O). Anal. Calcd for $C_{12}H_{25}NO_{10}$ (343.33): C, 41.98; H, 7.34; N, 4.08. Found: C, 41.25; H, 7.55; N, 3.90. ^{13}C NMR (75 MHz, D_2O, pH 9.9) data: δ 103.21 (C-1′), 79.49 (C-4), 76.51 (C-5′), 76.37 (C-3′), 74.12 (C-2′), 73.40 (C-2), 71.82 (C-5), 71.44 (C-3), 70.08 (C-4′), 62.82 (C-6), 60.87 (C-6′), 43.68 (C-1); ^1H NMR (300 MHz, D_2O, pH 9.9) data: δ4.54 (d, 1 H, $J_{1'2'}$ 7.8 Hz, H-1′), 3.92 (H-5), 3.86 (H-6′a), 3.85 (H-6a), 3.84 (H-2), 3.82 (H-4), 3.76 (H-6′b), 3.73 (H-3), 3.72 (H-6b), 3.48 (H-3′), 3.42 (H-5′), 3.41 (H-4′), 3.31 (H-2′), 2.90 (dd, 1 H, $J_{1a,1b}$ 13.2 Hz, H-1a), 2.64 (dd, 1 H, $J_{1b,2}$ 8.4 Hz, H-1b).

1-amino-1-deoxy-4-O-β-D-galactopyranosyl-D-glucitol (**2**). $[\alpha]_D$ +10.9° (*c* 4; H_2O). Anal. Calcd for $C_{12}H_{25}NO_{10}$ (343.33): C, 41.98; H, 7.34; N, 4.08. Found: C, 41.68; H, 7.60; N, 3.85. ^{13}C NMR (D_2O, pH 9.9) data: δ 103.76 (C-1′), 79.72 (C-4), 75.92 (C-5′), 73.31 (C-3′), 73.06 (C-5), 71.88 (C-2′), 71.82 (C-2), 69.49 (C-4′), 62.84 (C-6), 61.57 (C-6′), 43.54 (C-1); ^1H NMR (D_2O, pH 9.9) data: δ 4.48 (d, 1 H, $J_{1'2'}$ 7.7 Hz, H-1′), 3.89 (H-4′), 3.89 (H-5), 3.87 (H-2), 3.83 (H-4), 3.83 (H-6a), 3.72 (H-3), 3.72 (H-6′a), 3.72 (H-6b), 3.72 (H-6′b), 3.66 (H-5′), 3.62 (H-3′), 3.52 (H-2′), 2.92 (dd, 1 H, $J_{1a,1b}$ 13.2 Hz $J_{1a,2}$ 3.5 Hz, H-1a), 2.68 (dd, 1 H, $J_{1b,2}$ 8.6 Hz, H-1b). The ^1H and ^{13}C NMR data for compounds (**1**) and (**2**) at pH 9.9 are in agreement with the literature values obtained at pH 12 except the differences in the resonances of carbon C-2 and C-5 atoms (lit. C-2:71.9 and C-5:73.9 [3]) for compound (**1**).

For the mixture of xyloglucan-derived oligosaccharides (DP 7-9), found: C, 39.20; H, 6.26; N, 0.0, and for their corresponding glycamines, found: C, 39.50; H, 6.27; N, 0.95. The ^1H NMR spectra of native and electro-reduced xyloglucan oligomers were measured in D_2O at 60 °C. While in the ^1H NMR spectrum of the native oligosaccharides H-1 anomeric signals for α and β reducing D-glucose

residues appeared at δ 5.28 ($J_{1,2}$ 4.21 Hz) and 4.72 ppm ($J_{1,2}$ 7.99 Hz) [14], they were absent in the spectrum of the corresponding glycamines. Since, in this case, the preparation originated from the mixture of oligomers, the complete NMR data is not presented.

Electrosynthesis of glycamines at constant potential.—For the preparation of glycamines of the disaccharides cellobiose and lactose, the potentiostatic method was also used. The experimental conditions were the same as for the reduction at constant current except for the constant power and consequent alterations in electrolysis. Increasing the potential of the working electrode from −1.3 V through −1.35 V to 1.4 V versus SCE shortened the time of electro-reduction from 10 h to 7.5 h and 5 h, respectively. During this time, oximes were completely reduced and reduction of the excess hydroxylamine required a further 30 min at −1.5 V.

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