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Preparation of 2(3)-*O*-[2-(Hydroxyamino)-2-oxoethyl]- and 2(3)-*O*-[3-(Hydroxyamino)-3-oxopropyl]dextran

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Abstract—Hydroxame derivatives of carboxymethyl- and carboxyethyldextrans have been prepared and characterized by elemental analysis as well as by IR and UV spectroscopy. The effect of reaction conditions on the products yield has been studied.

Keywords: hydroxamic acid, carboxymethyldextran, carboxyethyldextran

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Polysaccharides modification is a promising trend in macromolecular chemistry. The so prepared derivatives can act as biologically active compounds, efficient chelating agents, and matrices for low-molecular drugs immobilization. The approaches to prepare polyligands and chemosorbents are of special interest. Previously, derivatives of crosslinked chitosan have been suggested as complex forming agents [1]; high chelating ability towards certain metal ions has been observed in the cases of derivatives of dioxime of dialedhydostarch [2] and dihydrazone of dialedhydostarch [3].

Hydroxamic acids and their derivatives have exhibited a wide spectrum of biological activity, and have been recognized as active complexing agents as well [4]. Biodegradable surfactants for polluted water treatment have been developed basing on D-glucose modified with carboxylic and hydroxamic groups [5]. *N*-Alkylhydroxamic derivatives of D-galacturonic acid also have been found to be efficient in this regard [6].

In view of the above, in this work we studied the modification of natural polysaccharide dextran with hydroxame-containing fragments. The preparation of the hydroxamic derivatives of carboxymethyl- and carboxyethyldextran is schematically shown below.

Carboxymethyldextran **Ib**, its ethyl ester **Ic**, and lactone **Ie** as well as carboxyethyldextran amide **IIa** were prepared as described elsewhere [7, 8]. The notation of functional groups contained in the so modified dextrans and numbers of these groups are given in the table.

Sodium salt of the hydroxamic acid **Id** was prepared by dissolution of ester **Ic** (C_{cm} 1.37, C_{est} 0.36) or lactone **Ie** in the freshly prepared solution of hydroxylamine hydrochloride in aqueous alkali, similarly to the procedure suggested for photometric determination of esterification degree of polysaccharides ester derivatives [9, 10]. The procedure was adopted in order to optimize the preparation conditions (the reactants ratio) and to maximize the product yield.

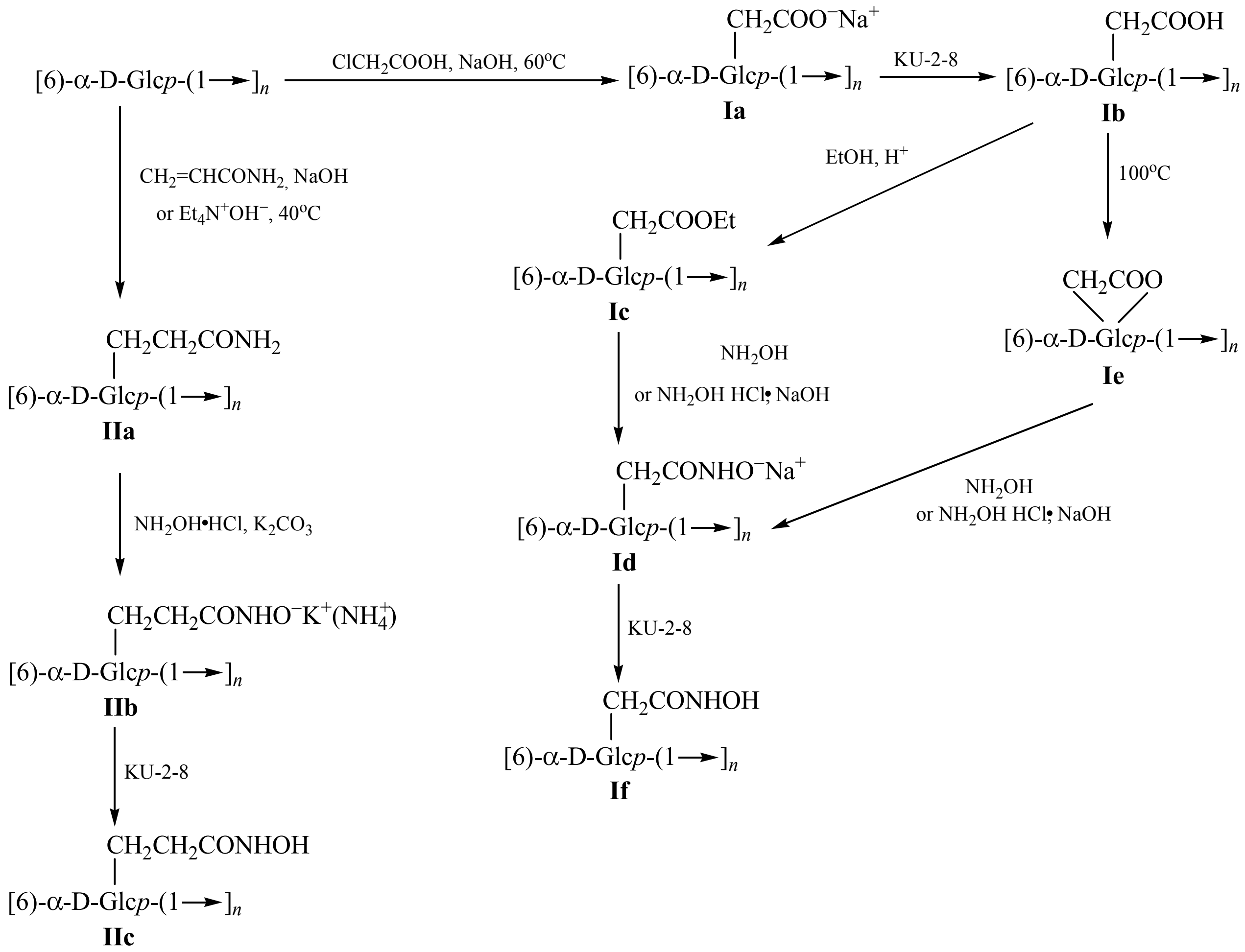
At varied molar ratio of alkali to hydroxylamine in the reaction mixture, the molar ratio of hydroxylamine to the ester groups of **Ic** (5 : 1), temperature (20°C), and the reaction duration (20 min) being the same, the highest yield of hydroxamic derivative **Id** (φ = 90%) was achieved at the alkali to hydroxylamine molar ratio of 1 : 2 (Scheme 1).

NaOH : NH_2OH (mol/mol)	0	0.5	1	2
φ, %	16	90	83	70

The same high yield of the hydroxamic derivative **Id** was obtained in the reaction with 10 wt % solution of hydroxylamine without alkali added, however, in that case the reaction duration was 16 h.

At constant molar ratio of alkali to hydroxylamine (1 : 2) and of hydroxylamine to the ester groups of **Ic** (5 : 1), the yield of hydroxamic derivative **Id** after 20 min of the reaction decreased upon heating.

Scheme 1.



Temperature, °C	20	40	60	80	95
φ, %	90	84	78	51	20

The observed decrease in the yield of hydroxamic derivative **Id** with increasing alkali concentration and temperature was likely to be due to the competition between the reactions of hydroxylation and alkaline hydrolysis of the $COOC_2H_5$ in **Ic**, as well as due to the low stability of hydroxame groups upon heating in alkaline medium.

We applied the photometric method [11] to study the effect of molar ratio of hydroxylamine to the ester groups on the product yield at other conditions being

Parameters of the chemically modified dextrans

Comp. no.	Modifying group	Notation for content of the modifying groups in dextran	Number of modifying groups per monomer unit
Ib	CH_2COOH	C_{cm}	1.20, 1.37, 1.47
Ic	$CH_2COOC_2H_5$	C_{est}	0.36–0.46
Id	CH_2CO	C_{est}	0.31
If	$CH_2CONHOH$	C_{ho}	0.34, 0.36, 0.42
IIa	$CH_2CH_2CONH_2$	C_{aop}	0.53
IIc	$CH_2CH_2CONHOH$	C_{ho}	0.27

the same: molar ratio of alkali to hydroxylamine 1 : 2, the reaction duration 20 min, and temperature 20°C. In that case the yield was expressed relative to the highest yield achieved under the above-mentioned conditions.

$NH_2OH : [COOC_2H_5]$ (mol/mol)	1.5	2	3	4	5	6	10
φ, %	52	55	65	82	100	92	58

From the obtained results it followed that with the ratio of hydroxylamine to the ester groups in the reaction mixture up to 5 the yield of hydroxamic derivative **Id** was improved; however, further increase of the studied ratio led to the decreased product yield, likely, due to enhanced alkaline hydrolysis (increase of the $NH_2OH : COOC_2H_5$ ratio at the fixed $NaOH : NH_2OH$ ratio meant increase of the $NaOH : COOC_2H_5$ ratio).

Sodium salt of the hydroxamic acid **Id** was prepared also from lactone **Ie** under the above-found optimal conditions of the **Ic**→**Id** transformation: the molar ratio of the alkali to hydroxylamine 1 : 2, the molar ratio of hydroxylamine to the lactone groups of **Ie** 5 : 1, the reaction duration 20 min, and temperature 20°C. According to elemental analysis of the product, the yield of hydroxame groups from lactone ones was 51%, significantly below the expected value. It was likely due to enhanced competing alkaline hydrolysis of lactone groups as compared to the case of ester ones.

The free hydroxamic acid **If** was prepared from sodium salt **Id** using the cation-exchange resin KU-2-8. The acid samples were characterized by numbers of hydroxamic CONHOH (C_{ho}) and carboxymethyl (C_{cm}) groups per a monosaccharide monomer unit. Hydroxamic group number was determined from the elemental analysis data, and the carboxylic group content was determined by conductometric titration. The C_{ho} values of 0.34, 0.36, and 0.42 were found for acid **If** samples prepared from carboxymethyldextran **Ib** with C_{cm} of 1.2, 1.37, and 1.47, respectively.

In order to prepare the hydroxamic acid **IIc** we applied the previously suggested [11] procedure designed for photometric determination of amide groups in polysaccharides; the preparation consisted in interaction of amide **IIa** (C_{ce} 0.24, C_{aop} 0.53; C_{ce} standing for the carboxyethyl groups content) with alkaline solution of hydroxylamine. The optimal conditions of the reaction were elaborated to be as follows: temperature 90°C and the reaction duration 40–60 min. By means of spectrophotometry it was demonstrated that the yield of hydroxamic derivative **IIb** was strongly affected by the alkali concentration in the reaction mixture. In particular, with more of NaOH in the mixture the target product yield decreased due to enhanced hydrolysis of amide groups of compound **IIa** as well as due to dealkylation and decay of the hydroxame groups. Attempting to decrease the side reactions contribution we used K_2CO_3 to liberate hydroxylamine from its hydrochloride.

With varied ratio of the hydroxylamine to K_2CO_3 in the reaction mixture, other conditions being the same (ratio of hydroxylamine to the amide groups of **IIa** 3 : 1, temperature 90°C, and reaction duration 60 min), the highest conversion of the amide groups, 51% as derived from the elemental analysis data, was reached at the $NH_2OH{\cdot}HCl : K_2CO_3$ ratio of 2.5 : 1.

$NH_2OH{\cdot}HCl : K_2CO_3$ (eq/eq)	1.3	1.6	2	2.5	3.3	6.7
φ, %	45	46	50	51	49	47

We further studied the effect of hydroxylamine excess during preparation of the derivative **IIb**, other conditions being the same: the ratio of hydroxylamine hydrochloride to potassium carbonate 2.5 : 1, the reaction duration 60 min, and temperature 90°C, by means of spectrophotometry [11]; the yield was expressed relative to the highest one under the studied conditions.

$NH_2OH{\cdot}HCl : [CONH_2]$ (mol/mol)	1.1	2	3	4	6	8
φ, %	90	98	100	95	83	67

The highest yield of hydroxame groups in the product was observed at the $NH_2OH{\cdot}HCl$ to amide molar ratio 3 : 1. The yields obtained in the presence of K_2CO_3 were by 25–30% higher than those in the presence of NaOH.

Using the KU-2-8 cation-exchange resin in the acidic form salt **IIb** was transformed into the hydroxamic acid **IIc** (C_{ho} 0.27).

The hydroxamic acids **If** and **IIc** were hygroscopic pale-pink powders, readily soluble in water and insoluble in acetone, ethanol, and other organic solvents.

IR spectrum of the prepared sodium salt **Id** contained absorption bands at 1600 cm^{-1} assigned to the carboxyl group stretching vibrations and at 1675 cm^{-1} assigned to ν[C=O(NH)]. The respective absorption bands were observed at 1575 and 1665 cm^{-1} in IR spectrum of the salt **IIb**. IR spectrum of the acid **If** contained the absorption bands at

1740 cm^{-1} (the carboxy group stretching vibrations) and at 1645 cm^{-1} (amide carbonyl stretching vibrations); the respective absorption bands were found at 1723 and 1655 cm^{-1} in the spectrum of the acid **IIc**.

Electron absorption spectrum of aqueous solution of the acid **If** contained the absorption band with λ_{max} of 212 nm, the sodium salt **Id** possessing the absorption band at λ_{max} of 225 nm. UV spectra of aqueous solutions of the acid **IIc** and the corresponding sodium salt **IIb** contained the absorption bands at λ_{max} of 215 and 235 nm, respectively.

EXPERIMENTAL

In this work we used commercial dextran [linear (1→6)-α-D-glucan] with *M* of 60000 produced by *Leuconostoc mesenteroides*. ^{13}C NMR spectra (solutions in D_2O) were registered on a Bruker AM-500 spectrometer at 50.32 MHz. IR spectra (KBr) were recorded on a FSM 1201 IR Fourier spectrometer. UV spectra (aqueous solutions, quartz cell, *l* 1 cm, and room temperature) of the hydroxamic derivatives were recorded on an Ocean Optics PX-1 spectrometer. Photometric measurements were performed with the KFK-3-01 electrocolorimeter.

2(3)-*O*-[2-(Hydroxyamino)-2-oxoethyl]-2(3)-*O*-carboxymethyl-(1→6)-α-D-glucan sodium salt (Id). *a*. 10 mL of NaOH aqueous solution (3 mol/L) was added at stirring to 10 mL of $NH_2OH·HCl$ aqueous solution (2 mol/L); then, 1 g of ethyl ester **Ic** or lactone **Ie** powder was added. The reaction mixture was stirred during 20 min at 20°C. Dialysis of the resulting mixture was performed against distilled water during 24 h, and then the solvent was evaporated off at the rotary evaporator till a film formation. The obtained film was triturated in acetone to get salt **Id** powder, it was then filtered off and dried at 20°C and residual pressure 20–25 mmHg to constant mass. Yield 1.04 g (95%), light-brown powder, C_{ho} 0.36.

b. 10 mL of 10% wt % hydroxylamine aqueous solution was added to 1 g of ester **Ic**. The reaction mixture was stirred during 16 h at 20°C. Salt **Id** was isolated as described in procedure *a*. Yield 0.98 g (90%). IR spectrum, ν, cm^{-1}: 3425, 2925, 1675 (CONH), 1600 (COO^-), 1418, 1323, 1150, 1100, 1015, 707, 588. UV spectrum (water): λ_{max} 225 nm. ^{13}C NMR spectrum (D_2O), δ_C, ppm: 177 (COO^-), 169 (CONH).

2(3)-*O*-[2-(Hydroxyamino)-2-oxoethyl]-2(3)-*O*-(carboxymethyl)-(1→6)-α-D-glucan (If). A solution containing 1 g of compound **Id** in 25 mL of water was applied to a chromatographic column filled with the KU-2-8 cationite. The eluate and the rinsate were united, and the solvent was evaporated to dryness in a vacuum evaporator. The acid **If** powder was triturated with acetone and dried at 20°C and residual pressure of 20–25 mmHg to constant mass. Yield 0.89 g (91%). Light pink powder. IR spectrum, ν, cm^{-1}: 3435, 2928, 1740 (COOH), 1645 (COOH), 1442, 1357, 1242, 1154, 1117, 1008, 762, 663. UV spectrum (water): λ_{max} 212 nm.

2(3)-*O*-[3-(Hydroxyamino)-3-oxopropyl]-2(3)-*O*-(2-carboxyethyl)-(1→6)-α-D-glucan ammonium-potassium salt (IIb). A solution prepared by mixing of 10 mL of $NH_2OH·HCl$ aqueous solution (2 mol/L) and 4 mL of K_2CO_3 aqueous solution (1 mol/L) was added to 1 g of amide Ia, and the reaction mixture was stirred during 1 h at 90°C. After cooling, the dialysis of the resulting mixture was performed against distilled water during 24 h, and then the solvent was evaporated off at the rotary evaporator till a film formation. The obtained film was triturated in acetone to get the powder that was then filtered off and dried at 20°C and residual pressure of 20–25 mm Hg to constant mass. Yield 0.87 g (90%), light yellow powder. IR spectrum, ν, cm^{-1}: 3425, 2928, 1665 (CONH), 1575 (COO^-), 1422, 1367, 1154, 1107, 1012, 765, 538. ^{13}C NMR spectrum (D_2O), δ_C, ppm: 177 (COO^-), 170 (CONH). UV spectrum (water): λ_{max} 235 nm.

2(3)-*O*-[3-(Hydroxyamino)-3-oxopropyl]-2(3)-*O*-(2-carboxyethyl)-(1→6)-α-D-glucan (IIc) was prepared similarly to compound **If** from compound **IIb**. Yield 0.94 g (89%). Light pink powder. IR spectrum, ν, cm^{-1}: 3435, 2928, 1723 (COOH), 1655 (COOH), 1438, 1357, 1272, 1201, 1157, 1114, 1012, 917, 843, 762. UV spectrum (water): λ_{max} 215 nm.

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