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Synthesis of Phenol-Containing Polysaccharides Basing on 6-Chloro-6-deoxy Cellulose and Phytogenous Phenolic Acids

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Abstract

Cellulose derivatives were synthesized containing up to 25 mass % of the fragments of ferulic or vanillic acid. Phenol-containing polysaccharides obtained by means of intermolecular nucleophilic substitution reaction between 6-chloro-6-deoxy cellulose and potassium or tetrabutylammonium salts of corresponding phenolic acids. The source halogenated deoxy polysaccharide was obtained *via* the action of sulphuryl chloride on cellulose in the presence of pyridine in *N*,*N*-dimethylformamide environment.

Key words: phenol-containing polysaccharides, ferulic acid, halogenated deoxy cellulose

INTRODUCTION

Phenol-containing polysaccharides such as xylans, arabinoxylans, pectins are widely distributed in the plant kingdom. The polysaccharide part of these polymers has, as a rule, irregular structure; the phenolic part represents the structural units of lignin, condensed phenols, hydroxybenzoic and hydroxycinnamic acids [1, 2]. Interest in natural and synthetic phenol-containing polysaccharides is caused by their role in the plant cell, presence in foods, the ability of neutralizing pathogenic free-radical processes, which is important for biomedicine [3, 4]. Moreover, from a fundamental and practical point of view, potentiality is attractive to obtain hybrid biodegradable polysaccharide-phenolic polymers based on such macromolecules via the methods of the oxidative condensation of phenolic fragments, or to use this method in order to control the physical and chemical properties of materials based on phenol-containing polysaccharides [5, 6].

For the synthesis of the esters of phenolic acids and polysaccharides researchers used the method of polysaccharide esterification by the chloroanhydrides of corresponding acids as well as the esterification by means of carbodiimide method. They obtained the esters of xylan, starch, dextran, cellulose with hydroxycinnamic acids, the conjugates of chitosan and gallic acid [7–9].

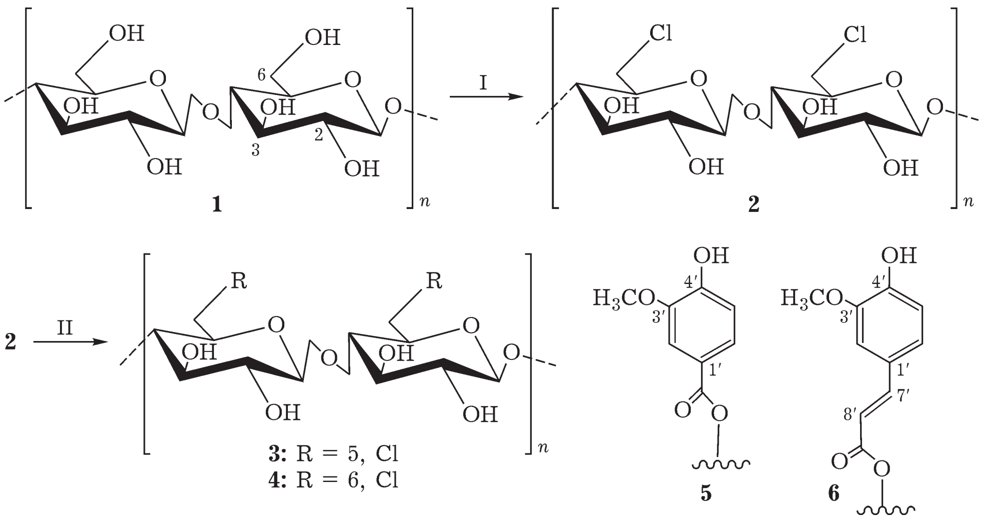
This work consisted in performing the synthesis of phenol-containing polysaccharides based on cellulose **1** *via* the reaction of intermolecular nucleophilic substitution involving cellulose chlorodeoxy derivatives and phenolic acid salts.

RESULTS AND DISCUSSION

As an intermediate for the synthesis of phenol-containing polysaccharides, we chose 6-chloro-6-deoxy cellulose **2** which was prepared by an original method *via* the action of SO_2Cl_2 on cellulose in DMFA environment in the presence of pyridine. This method for the synthesis of 6-chloro-6-deoxy cellulose is characterized by a high efficiency, relatively mild reaction conditions and it does not require preliminary dissolving the cellulose. The possibility of selective introducing the chlorine atom is connected with the mechanism of the reaction involving the stage of forming a reactive complex between DMFA and SO_2Cl_2 in a similar manner as Vilsmeier reagent. The complex formed substitutes selectively the primary hydroxyl groups at the chlorine atom [10]. The ^{13}C NMR spectra of chlorodeoxy cellulose having the substitution level with respect to chlorine atom (SL_{Cl}) equal to 0.95 exhibit a signal a 45.4 ppm, corresponding to the CH_2Cl group. The signal for C1 carbon atom at 102.17 ppm, according to [11], indicates the fact that 6-chloro-6-deoxy glucose residues are prevailing in the polymer. Within the region of 76–68 ppm there is a group of signals belonging to the unsubstituted C2–C5 atoms of the anhydroglucose unit. There is no signal at 61.4 ppm that corresponds to the C6 atom of unsubstituted anhydroglucose units in the spectrum.

The reaction between 6-chloro-6-deoxy cellulose with the salt forms of phenolic acids results in obtaining a mixed polysaccharide containing glucose units, esterified with corresponding phenolic acid at the C6 carbon atom of the elementary unit. The formation of the esters of phenolic acids and of the polysaccharide was confirmed by NMR and IR spectral data. The absorption band at 1705 cm^{-1} that is present in the IR spectra polysaccharide-phenol compounds obtained indicates the formation of an ester bond. The ^{13}C NMR spectra of phenol-containing polysaccharides **3** and **4** exhibit signals inherent in phenolic acid esters. At 165 ppm there are signals present of the carbon atom at the carbonyl group present, within the range of 152–145 ppm there are the signals of carbon atoms with methoxyl and hydroxyl groups in the aromatic ring. The ferulic acid ester is characterized by the signals of C8′ and C7′ carbon atoms at 146 and 115 ppm, respectively. At 56 ppm there are the signals of carbon atoms of the methoxy group observed for ferulic acid and vanillin. The main signals of the carbon atoms of the polysaccharide part are registered within the range of 100–60 ppm. Scheme 1 illustrates the synthesis of starting halogenodeoxy saccharide **2** and phenol-containing polysaccharides **3**, **4**.

The reaction of intermolecular nucleophilic substitution as carried out in two variants, whereby the solvent used was presented by DMSO. Although halogenodeoxy polysaccharide **2** is insoluble in DMSO, in the course of the interaction thereof with the tetrabutylammonium salts of phenolic acids (variant A of the synthesis), a rapid homogenization of the reaction mixture is observed. Rapid dissolving the



Scheme 1. Synthesis of 6-chloro-6-dezoxy cellulose **2** and phenol-containing polysaccharides based thereon. Reagents and conditions: I – SO_2Cl_2, Py in DMFA, 6 h, 25 °C; II – variant A: Tetrabutylammonium salt of phenolic acid, DMSO, 48 h, 80 °C; variant B: phenolic acid, K_2CO_3, DMSO, 48 h, 80 °C.

TABLE 1

Conditions and results of the synthesis of polysaccharide **3** containing vanillic acid fragments

Variants of synthesis	Reaction time, h	Fragment **5** content (ω), mass %
A	24	12.3
A	48	22.5
A	72	26.1
B	48	18.7
B	56	24.6

polysaccharide **2** in the presence of phenolic acids is observed also when using K_2CO_3 as a base (variant B of the synthesis). Thus, when using of both variants of the synthesis the reaction takes place in a homogeneous medium. From Table 1 one can see that under these conditions of the synthesis, both variants of the process allow obtaining the preparations of polysaccharides containing about 25 mass % of vanillic acid fragment.

Similar results were obtained in the synthesis of phenol-containing polysaccharide **4** (Table 2) containing the fragments of ferulic acid. Using the salts of phenolic acids and tetrabutylammonium (alternative synthesis) leads to slightly higher yields of the target compounds, however, the synthesis according to variant B with the use of K_2CO_3 is simpler because there is no preliminary stage of preparing and isolating the salt. In all the cases, an increase in reaction time leads to an increase in the content phenolic fragments in the polysaccharide. The greatest increase in phenolic fragments was observed within the first 24 h of reaction, thereafter the reaction rate decreases. Increasing the duration of the reaction exerts a positive effect on the content of phenolic acid fragments in the polysaccharide, but this leads to decreasing the yield of target compounds.

TABLE 2

Conditions and results of the synthesis of polysaccharide **4** containing ferulic acid fragments

Variants of synthesis	Reaction time, h	Fragment **6** content (ω), mass %
A	24	11.4
A	48	19.2
A	72	27.2
B	48	16.5
B	56	24.3

Polysaccharides **3** and **4** obtained represent light brown compounds slightly soluble in water. With increasing the content of phenolic acids, one observes increasing the solubility of phenol-containing polysaccharide in alcohols and ketones. Therefore, polysaccharides with the content of phenolic part greater than 20 mass % are partially soluble in ethyl alcohol and isopropyl alcohol, being completely soluble in acetone, DMSO, DMFA.

Thus, basing on chlorodeoxy cellulose derivative and phenolic acids we synthesized phenol-containing polysaccharides using the reaction of intermolecular nucleophilic substitution. Using this method one could obtain a mixed polysaccharide with a regioselective arrangement of phenolic moiety at C6 atom of elementary unit. Both variants of the synthesis using preliminary formed tetrabutylammonium salts of phenolic acids (variant A) and that using K_2CO_3 as a base (variant B) in a nucleophilic substitution reaction with 6-chloro-6-deoxy cellulose allow one to obtain polysaccharides containing about 25 mass % of phenolic acid fragments.

EXPERIMENTAL

Reagents

Microcrystalline cellulose (MCC) was obtained by means of acidic hydrolyzing a cotton fibre with an average polymerization level equal to 220 (viscosity determination in cadoxene) with the level of crystallinity amounting to 0.86 (XRD phase analysis). Before using, the MCC was dried under vacuum at 103 °C. Ferulic and vanillic acids (Sigma-Aldrich) were recrystallized from aqueous ethanol. Pyridine, DMFA, DMSO before using were dried and distilled as described by the authors of [12]. Other solvents were purified by means of distillation. Sulphuryl chloride (Sigma-Aldrich), tetrabutylammonium hydroxide (40 % aqueous solution, Alfa-Aesar) were used with no additional purification.

Methods of analysis

^{13}C NMR spectra were registered using a Bruker Advance II 300 (operating frequency 75 MHz) in DMSO-d_6 IR spectra were registered with the help of a Shimadzu IR Prestige 21FTIR spectrometer using tablets with KBr. The determination of chlorine and sulphur in the derivatives of cellulose was performed according a method described by the authors of [13], with a titrimetric termination.

The content of phenolic acids in the polysaccharide (ω) was measured spectrophotometrically after the hydrolysis of the ester bond and after the extraction of the phenolic acid with ethyl acetate by means of a method described by the authors of [7]. UV spectra were registered using a Shimadzu UV-1700 spectrophotometer.

Synthesis of 6-chloro-6-deoxy cellulose 2

A 5 g weighed sample portion of MCC (31 mmol) was added to 100 cm^3 of a mixture of DMF and pyridine to be held during 12 h at 25 °C. To the resulting heterogeneous mixture was added sulphuryl chloride, dropwise, upon cooling down to 5–7 °C and stirring for 15–20 min, whereby the reaction mixture was homogenized. The temperature was then increased up to 25 °C to hold the reaction mixture under stirring for 6 h. In order to isolate and purify the polysaccharide the homogenous solution was transferred to ice water, a precipitate formed was filtered, washed with water, ethanol with further transferring into a solution of concentrated aqueous ammonia in ethanol (1 : 5 by volume), wherein it was held during 1 day at 25 °C. The precipitate was separated, washed with water until obtaining neutral reaction of washing water, with further washing by ethanol. In order to remove sulphate groups, the polysaccharide was held in a 3 M aqueous solution of HCl at 65 °C for 5 h. The polymer was then separated, washed with water until obtaining neutral wash water pH, with further washing by ethanol and drying in vacuo at 60 °C. Residual sulphur content did not exceed 0.3 mass %, that of chlorine as less than 18.2 mass %.

For 6-chloro-6-deoxy cellulose (SL_{Cl} = 0.95): IR (KBr, ν, cm^{-1}): 3408 (OH), 2899 (CH_2), 752 (C–Cl). ^{13}C NMR (75 MHz DMSO-d_6/LiCl), δ, ppm: 102.17 (C1), 79.50 (C4), 76.18 (C3), 73.5–72.5 (C2, C5), 45.3 (CH_2Cl).

Synthesis of the ester of 6-chloro-6-deoxy cellulose and vanillic acid 3

Variant A. The tetrabutylammonium salt of vanillic acid was prepared *via* neutralizing an aqueous solution of vanillic acid by an aqueous solution of tetrabutylammonium hydroxide to obtain pH 8.2. The resulting salt was freeze-dried. There were mixed 500 mg (2.77 mmol) of polysaccharide **2** and 20 mL of DMSO to hold at a room temperature during 1 h, thereafter was added 3.29 g of a tetrabutylammonium salt of vanillic acid (3 mol equivalent excess per an elementary unit of polysaccharide). The reaction was carried out under argon at 80 °C during preset time. Upon completion, the homogeneous reaction mixture was cooled with further transferring into 600 mL of distilled water. The precipitate obtained was separated by means of centrifugation to wash then with water. The resulting polymer was reprecipitated from acetone *via* the addition of water; the precipitate was separated and dried in vacuo at 60 °C. We obtained 100–350 mg of powdered dark brown substance.

Variant B. A mixture of 500 mg (2.77 mmol) of polysaccharide **2** and 20 mL of DMSO was held during 1 h, thereafter were added 1.27 g of vanillic acid (3 mol equivalent excess per polysaccharide elementary unit) and 1.15 g (8.31 mmol) of K_2CO_3. The reaction was carried out under argon atmosphere at 80 °C during a preset time. Upon completion, the reaction mixture was cooled with further transferring into 600 mL of distilled water. The precipitate was separated by means of centrifugation being washed then with water. The resulting polymer was reprecipitated from acetone by the addition of water; the precipitate was separated to dry in vacuo at 60 °C. We obtained 100–350 mg of powdered dark brown substance.

IR spectrum (KBr, ν, cm^{-1}): 3442 (OH), 296, 2935 (CH), 1707 (C=O), 1599, 1514 (Ar).

^{13}C NMR (75 MHz, DMSO-d_6), δ, ppm: 165.86 (C=O), 151.98 (C4′), 147.79 (C3′), 124.14 (C6′) 120.89 (C1’), 115.66 (C5’), 113.18 (C2’), 102.66 (C1), 80.68 (C4), 74.05 (C3), 73.5–72.5 (C2, C5), 56.10 (OCH_3), 44.86 (CH_2–Cl).

Synthesis of the ester of 6-chloro-6-deoxy cellulose and ferulic acid 4

The synthesis was carried out in a similar manner as the synthesis of compound **3**.

IR spectrum (KBr, ν, cm^{-1}): 3346 (OH), 2960–2900 (CH, CH_2), 1705 (C=O), 1602 (Ar).

^{13}C NMR (75 MHz DMSO-d_6), δ, ppm: 162.77 (C=O), 148.19 (C3′), 147.96 (C4′), 146.55 (C7′), 128.06 (C1′), 119.58 (C6′), 115.92 (C8′), 110.08 (C2′), 102.13 (C1), 79.5–71.0 (C4, C3, C2, C5), 56.15 (OCH_3), 45.16 (CH_2–Cl).

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