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Dedicated to Full Member of the Russian Academy of Sciences
I.P. Beletskaya on occasion of her jubilee

Synthesis of Inulin Esters of Phenylcarboxylic Acids

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Abstract—Inulin esters were synthesized containing residues of ferulic, *p*-hydroxycinnamic, and vanillic acids with a maximum degree of substitution of 1.1. The inulin acylation was carried out with chlorides of 4-acetoxy derivatives of the corresponding hydroxyphenylcarboxylic acids in a heterogenic water-organic environment. The removal of the protecting acetyl group at the phenol hydroxyl was carried out by treating with pyrrolidine or ammonium acetate. The solubility of the esters obtained was evaluated.

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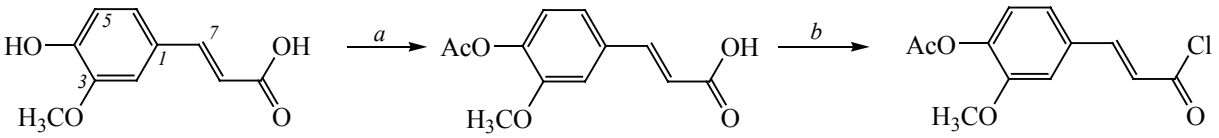
Inulin is contained in various types of plants, but it is most common obtained from succory (*Cichoriumin tybus*) or Jerusalem potato (*Helianthus tuberosus*). This polysaccharide of relatively low molecular weight is built commonly of several tens of fructose fragments connected by bonds β(2 → 1) and in some cases it may include glucose units. Various esters of this biopolymer were synthesized [1] and diverse applications were suggested for them in many fields, but the special attention is attracted by inulin as a promising component of the functional nutrition [2–4]. In this connection the most rational way of the chemical modification of inulin would be the synthesis of derivatives suitable for application in the food industry and in biomedical field, for instance, the preparation of derivatives possessing the antioxidant activity. It would be favorable to introduce into the inulin structure of vegetable phenols fragments, especially of those commonly present in the food and in the herbs. These phenol compounds are widely spread in the groups of hydroxycinnamic and hydroxybenzoic acids. Ferulic, caffeic, *p*-hydroxycinnamic, gallic acids exhibit a large antioxidant activity [5], are contained in the natural physiologically active compounds, in particular, in glycosides and poysaccaride–phenol compexes [6, 7]. Lately different researchers carried out the synthesis of esters of similar phenolic acids and polysaccarides. Publications are known on the synthesis of esters of xylane, starch, chitosan, dextran, and other polysaccharides with hydroxyphenylcinnamic acids, with the gallic acid through the acylation with the carboxylic acid chlorides or by the use of carbodiimide method in organic solvents [8–11].

The goal of our work was the preparation of inulin derivatives including the residues of ferulic (**Ia**), *p*-hydroxycinnamic (**IIa**), and also vanillic (**IIIa)** acids. The synthesis of the corresponding inulin esters was carried out by its acylation with 4-acetoxybenzenecarboxylic acids chlorides in a water-organic environment followed by the removal of the protective acetyl group from the phenol hydroxyl.

The preliminary synthesis of the acyl chlorides included the stage of the phenol hydroxyl acylation by treating with acetic anhydride solutions of acids **Ia–IIIa** in aqueous NaOH. Further the 4-acetoxy derivatives **Ib–IIIb** were treated with thionyl chloride and the obtained acyl chlorides **Ic–IIIc** without additional purification were used for inulin acylation (Scheme 1, synthesis of ferulic acid derivatives).

The acylation of inulin (**IV**) with the obtained

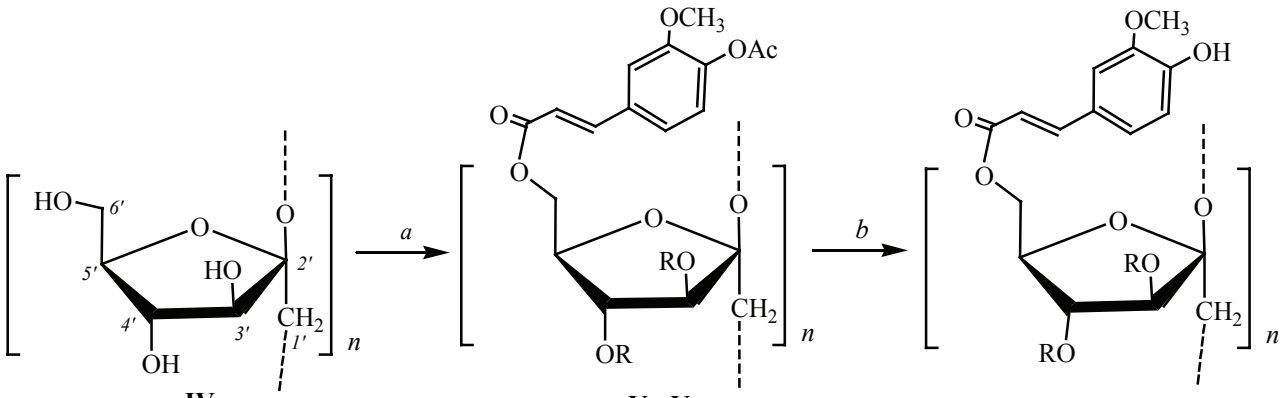
Scheme 1.



Ia **Ib** **Ib**

(*a*)– Ac_2O, NaOH, H_2O, 2 h, 0 → 25°C; (*b*) $SOCl_2$ in $CHCl_3$, boiling, 4 h.

Scheme 2.



IV **Va–Vc** **VIa–VIc**

(*a*) 4-acetoxyferulic acid chloride, NaOH, H_2O, 6 h, 0 → 25°C; (*b*) pyrrolidine or CH_3COONH_4 in DMAA–H_2O; R is the residue of ferulicoй acid (**a**); in positions 2O, 3O, 5O is the residue of *p*-hydroxycinnamic acid (**b**), the residue of vanillic acid (**c**)

chlorides of 4-acetoxy derivatives of the acids was carried out in a heterogenic environment water–$CHCl_3$ at room temperature for 6 h using NaOH as base (Scheme 2). The obtained acylated inulin derivatives **Va–Vc** contained from 5 to 56 wt% of the fragments of 4-acetoxy derivatives of phenolic acids. At the final stage of the synthesis (*b*) the protecting acetyl group was removed from the phenol hydroxyl.

The IR spectra of inulin esters **Va–Vc** contain an absorption band in the region 1760 cm^{-1} corresponding to the acetyl groups of the acetylated phenolic acids, which is absent in the spectra of compounds **VIa–VIc** obtained after the removal of the acetyl protection. In the region 1710–1730 cm^{-1} the bands characteristic of esters are observed, and at 1630 and 1510 cm^{-1} the bands appear corresponding to the skeletal vibrations of the aromatic ring. In the spectra of compounds **VIa–VIc** strong absorption bands in the region of 3400 cm^{-1} were assigned to the stretching vibrations of the "free" hydroxy groups and the hydroxy groups involved in hydrogen bonds, and the band with the maximum at 2930 cm^{-1} belonged to the stretching vibrations of the C–H bond.

In the ^{13}C NMR spectrum of inulin derivatives **Va–Vc** signals located at 162 ppm belong to the carbonyl of the ester group. In the region 115–151 ppm signals appear corresponding to the carbon atoms of the aromatic ring. In the spectra of polysaccarides **Vb,Vc, VIb, VIc** containing the residues of the vanillin and the ferulic acids the signals at δ 56 ppm belong to the methoxy group at the atom C^4 of the phenolic acid. The signals of carbon atoms of the polysaccaride part of compounds **V, VI** are located in the region of 60–100 ppm. In the spectra of inulin esters **Va–Vc** signals are additionally observed of the acetyl group at 168 (C=O) and 20 ppm (CH_3).

The data on the inulin acylation with the chlorides of 4-acetoxy derivatives of acid **Ia–IIIa** are compiled in the table. The degree of substitution was determined before the removal of the acetyl protection of the phenol hydroxy group. The esterification of inulin with acid chlorides **Ia–IIIa** in the developed system results in the

Conditions and results of inulin acylation with chlorides of 4-acetoxy derivatives of acids **Ia–IIIa**

Acid	Molar ratio phenolcarboxylic acid–monomer unit of insulin	Degree of substitution
Ia	1 : 2	1.02
	1 : 1	0.61
	2 : 1	0.42
IIa	1 : 2	1.13
	1 : 1	0.57
	2 : 1	0.37
IIIa	1 : 2	0.98
	1 : 1	0.55
	2 : 1	0.38

formation of heteropolysaccarides containing fractions of phenolic acids with the substitution degree γ from 0.1 to 1.1. In the table results are presented corresponding to the inulin acylation at the relatively high ratios acetoxyphenylcarboxylic acid chloride–monomer unit of inulin. At all studied ratios it was found that in the main reaction about a half of the acyl chloride brought into the process was consumed.

The acetyl deprotection was carried out under the action of pyrrolidine or CH_3COONH_4. These both compounds are known to be deacetylating agents in the syntheses of phenol derivatives [12, 13]. However at the use of pyrrolidine beside the removal of the acetyl group at the phenol hydroxyl the hydrolysis was observed of the ester bonds with the carbon leading to a significant (up to 20%) decrease in the substitution in inulin. The reduction of the temperature at the acetyl group removal with pyrrolidine from 25 to 0°C (at the same process duration) only sightly decreased the hydrolysis of the ester bond polysaccaride–*p*-hydroxyphenylcarboxylic acid.

At the use for the deacylation of CH_3COONH_4 no decrease was observed in the degree of substitution in the inulin. Taking into account also the easier procedure of the deacylation at the use of this salt [13] the application of CH_3COONH_4 can be recommended in the preparation of the other polysaccaride-phenol compounds.

The solubility of the obtained esters in water decreases with the growing fraction of the residues of the phenolic acids in the polysaccaride and does not depend on the nature of the introduced fragments. The majority of inulin esters obtained having the substitution degree over 0.1, both containing the fragments of 4-acetoxy derivatives of the phenolic acids and the deacetylated fragments of these acids, are insoluble in water. The samples of obtained esters **VIa–VIc** of ferulic, *p*-hydroxycinnamic, and vanillic acids with the degree of substitution γ from 0.1 to 0.2 partially dissolve in water giving opalescent solutions, but, unlike the initial inulin, they are completely soluble in DMSO and DMF only at heating. Inulin esters **Va–Vc** containing the 4-acetoxy derivatives of the phenolic acids are well soluble in these organic solvents. The samples of acylated inulin with γ 0.08–0.1 are soluble in water at heating, and with lower content of the phenol residues are well soluble in water. Although these data show that the introduction of the residues of the phenolic acids make the polysaccaride molecule more hydrophobic, we have found inulin esters **VIa–VIc** with a high content of the phenolic acid residues, insoluble in water and water solutions with a neutral pH, to be well soluble in water at its cautious alkalization to pH 8–9. The esters can be precipitated from these solutions at their acidification to pH 2–3. These observations indicate the formation of intra- and intermolecular "cross-linking" resulting from the formation of the hydrogen bonds involving the phenol hydroxy groups which is the main cause of the decreased solubility of the obtained esters. These hydrogen bonds can be destroyed by adding bases resulting in the formation of the corresponding phenolates and in the dissolution of the obtained phenolic polysaccarides.

Thus we obtained for the first time inulin esters with the ferulic, *p*-hydroxycinnamic, and vanillic acids with a high substitution degree. The efficient synthesis of these esters was developed in a heterogenic system $CHCl_3$–H_2O. The developed method can be used in the synthesis of similar esters of the other water-soluble polysaccarides. The modified method of removal of the acetyl protection from the phenol hydroxy group using ammonium acetate is simple, efficient, and does not affect the ester bonds in the acylated polysaccaride.

EXPERIMENTAL

^{13}C NMR spectra were registered on a spectrometer Bruker Avance II 300 (operating frequency 75 MHz) in DMSO-d_6. IR spectra were recorded on an IR Fourier spectrophotometer Shimadzu IR Prestige 21. Inulin (MP Biomedicals, LLS, M 5 × 10^3); ferulic [3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic], *p*-hydroxy-cinnamic [3-(4-hydroxyphenyl)prop-2-enoic], and vanillic (4-hydroxy-3-methoxybenzoicyю) acids purchased from

Sigma-Aldrich were before use recrystallized from aqueous ethanol and dried in a vacuum at 80°C. The solvents before use were purified by standard procedures [14].

The content of phenolic acid in a polysaccaride (ω, wt%) was determined by spectrophotometry using Folin–Ciocalteu reagent by the method [10]. With inulin esters containing fragments of phenolic acids acetoxy derivatives this analysis was performed after preliminary hydrolysis of the ester bond and the extraction of phenolic acids with ethyl acetate by the method [7]. The degree of substitution γ in the esterified polysaccaride was calculated by formula (1).

$$\gamma = \frac{M_{mu}\,\omega_{pa}}{M_{fpa} \times 100 - (M_{fpa} - 1)\omega_{pa}} \qquad (1)$$

Here M_{mu} is the molecular weight of thrr monomer unit of the polysaccaride; ω_{pa} is the content of the corresponding phenolic acid, wt%; M_{fpa} is the molecular weight of the fragment of the phenolic acid (177 for ferulic, 147 for *p*-hydroxycinnamic, 151 g/mol for vanillic acid).

Acetylation of phenolic acids **Ia–IIIa** was carried ot by procedure [15].

4O-Acetylferulic acid [3-(4-acetoxy-3-methoxyphenyl)prop-2-enoic acid] (Ib). Yield 73%. ^{13}C NMR spectrum, δ, ppm: 20.84 (OAc), 56.44 (OCH_3), 112.32 (C^2), 120.00 (C^8), 121.79 (C^6), 123.66 (C^5), 133.73 (C^1), 141.29 (C^4), 143.80 (C^7), 151.62 (C^3), 168.06 (C=O), 168.48 (COOH).

4-Acetoxycinnamic acid [3-(4-acetoxy-phenyl) prop-2-enoic acida] (IIb). Yield 78%. ^{13}C NMR spectrum, δ, ppm: 21.32 (OAc), 119.77 (C^8), 122.82 ($C^{3,6}$), 129.89 ($C^{2,6}$), 132.39 (C^1), 143.42 (C^7), 152.29 (C^4), 167.99 (C=O), 169.48 (COOH).

4O-Acetylvanillic acid (4-acetoxy-3-methoxybenzoic acida) (IIIb). Yield 75%. ^{13}C NMR spectrum, δ, ppm: 20.83 (OAc), 56.35 (OCH_3), 113.62 (C^2), 122.60 (C^6), 123.52 (C^5), 130.02 (C^1), 143.42 (C^4), 151.27 (C^3), 167.11 (COOH), 168.67 (C=O).

The synthesis of acetoxyphenylcarboxylic acids chlorides was performed by the method [15]. The obtained acyl chlorides **Ic–IIIc** were used in subsequent syntheses without additional purification.

Inulin ester with 4O-acetylferulic acid (Va). In 12 ml of water was dissolved 500 mg (3.1 mmol) of inulin, and at stirring under nitrogen atmosphere was added 3.2 mmol of NaOH. To the water solution of inulin cooled to 1–2°C was added at vigorous stirring 3.1 mmol acyl chloride **Ic** in 10 ml of $CHCl_3$. Then within 1 h the temperature of the reaction mixture was raised to 25°C, and the mixture was stirred at this temperature for 5 h. In order to isolate the inulin ester the reaction mixture was poured into 200 ml of 2-propanol, the formed precipitate was separated by centrifuge, washed with ethanol, and dried in a vacuum at 60°C. Yield 78%, γ 0.61. IR spectrum, ν, cm^{-1}: 3363 (OH), 2933 (CH), 1765 (COOH), 1726 (C=O), 1633, 1510 (C–H, aromatic ring), 1033 (C–O, carbohydrate skeleton). ^{13}C NMR spectrum, δ, ppm: 20.78, 56.43 (OCH_3), 62.60–62.00 ($C^{6',1'}$), 74.57 ($C^{4'}$), 77.13 ($C^{3'}$), 82.00 ($C^{5'}$), 103.63 ($C^{2'}$), 112.90, 117.50, 119.73, 121.71, 122.78, 123.92, 132.98, 133.64, 141.29, 142.21, 143.97, 148.41, 151.71, 168.03, 169.09.

Inulin ester with 4-acetoxycinnamic acid (Vb). γ 0.57. IR spectrum, ν, cm^{-1}: 3408, 2899, 1765, 1707, 1598, 1514, 1066. ^{13}C NMR spectrum, δ, ppm: 21.34, 62.51–62.00 ($C^{6'\,1'}$), 74.82 ($C^{4'}$), 77.24 ($C^{3'}$), 89.11 ($C^{5'}$), 104.14 ($C^{2'}$), 117.44, 119.78, 122.82, 122.97, 129.29, 130.49, 130.83, 131.96, 132.39, 143.43, 144.15, 146.23, 146.37, 148.13, 152.30, 152.87, 153.22, 163.16, 165.10, 166.18, 167.99, 169.48.

Inulin ester with 4O-acetylvanillic acid (Vc). γ 0.60. IR spectrum, ν, cm^{-1}: 3361, 2933, 1764, 1708, 1653, 1514, 1031. ^{13}C, δ, ppm: 20.86, 56.63, 62.16–62.54 ($C^{6',1'}$), 74.32 ($C^{4'}$), 77.67 ($C^{3'}$), 82.53 ($C^{5'}$), 103.65 ($C^{2'}$), 113.77, 114.12, 122.33, 122.79, 123.53, 124.14, 127.37, 127.73, 128.07, 141.87, 144.42, 144.58, 150.77, 151.73, 163.45, 168.65.

Removal of protecting acetyl group. *a*. In 3 ml of DMAA was dissolved 200 mg of inulin ester **V**, 0.1 ml of water and 3 mol-equiv of pyrrolidine per 1 mol-equiv of acetyl group was added. The obtained mixture was stirred for 5 min at 22°C, acidified with 0.25 N water solution of hydrochloric acid to pH 4.0, and the polysaccaride was precipitated by adding 20 ml of 2-propanol. The obtained polymer was separated, washed with ethanol, and the solvent and moisture were removed by lyophilic drying**.**

b. In 3 ml of DMAA was dissolved 200 mg of inulin ester **V**, 3.0 ml of water solution of CH_3COONH_4 was added (8 mol-equiv of CH_3COONH_4 per 1 mol-equiv of acetyl group). The mixture was stirred for 2 h at 22°C, and afterwards the polysaccaride was precipitated by adding 20 ml of 2-propanol. The obtained polymer was separated, washed with ethanol, and the solvent and moisture were removed by lyophilic drying**.** Yield 78–86%.

Inulin ester with ferulic acid (VIa). γ 0.55. IR spectrum (KBr), ν, cm^{-1}: 3472 (OH), 2941 (CH), 1717 (C=O), 1633, 1510 (C–H, aromatic ring), 1032 (C–O, carbohydrate skeleton). ^{13}C NMR spectrum, δ, ppm: 56.60, 63.24–62.88 ($C^{6',1'}$), 75.64–72.02 ($C^{4'}$), 80.67–79.54 ($C^{3'}$), 82.59–82.13 ($C^{5'}$), 105.34–102.54 ($C^{2'}$), 116.70, 117.55, 125.81, 127.43, 146.30, 147.77, 166.25.

Inulin ester with 4-hydroxycinnamic acid (VIb). γ 0.63. IR spectrum, ν, cm^{-1}: 3494, 2949, 1710, 1602, 1510, 1166. ^{13}C NMR spectrum, δ, ppm: 66.50–60.88 ($C^{6',1'}$), 76.10–75.64 ($C^{4'}$), 80.67 ($C^{3'}$), 82.59–82.15 ($C^{5'}$), 104.43–102.54 ($C^{2'}$), 116.30, 117.32, 127.51, 133.75, 146.03, 160.39, 166.34.

Inulin ester with vanillic acid (VIc). γ 0.60. IR spectrum, ν, cm^{-1}: 3361, 2933, 1716, 1653, 1514, 1031. ^{13}C NMR spectrum, δ, ppm: 56.11, 62.51–60.9 ($C^{6',1'}$), 74.72–73.34 ($C^{4'}$), 78.98–77.16 ($C^{3'}$), 82.74–82.13 ($C^{5'}$), 103.70–104.43 ($C^{2'}$), 113.10, 115.65, 121.06, 124.11, 147.79, 151.94, 166.13.

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