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Isolation and General Characterization of Polysaccharides from Tansy *Tanacetum vulgare* L.

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Abstract—Using extraction with 0.75% aqueous ammonium oxalate, the following polysaccharide fractions were isolated: tanacetans TVF, TVS, and TVR from floscules, sprouts, and roots, respectively, of *Tanacetum vulgare* L., spread throughout the European North of Russia. The sugar chain of tanacetan TVF consists of *D*-galacturonic acid (61.4%), arabinose (14.7%), galactose (10.2%), and rhamnose (3.7%) as the main constituents as well as xylose, glucose, mannose, apiose, and 2-*O*-methylxylose in trace amounts. Tanacetans TVS and TVR were shown to differ in the sugar quantitative composition. They contain 67 and 28% galacturonic acid, respectively. A partial acid hydrolysis of the tanacetan TVF gave a polysaccharide fragment TVF1, α-1,4-*D*-galacturonan (GalA 98.2%). Digestion with pectinase (α-1,4-*D*-polygalacturonase) resulted in fragment TVF3, containing residues of arabinose (27.1%) and galactose (17.3%). NMR spectroscopy allowed detection of the terminal residues of α-Ara*f* and β-Gal*p* as well as of the residues of α-Ara*p* substituted in 3,5- and 5-positions. Thus, tanacetan TVF was proved to be a pectic polysaccharide.

Key words: polysaccharide, tansy Tanacetum vulgare L., pectins, arabinogalactans, tanacetans, NMR spectroscopy of polysaccharides

Genus *Tanacetum* comprises plants of approximately 200 various species, the majority of which are representatives of the European, in particular Russian, flora [1]. Tansy *T. vulgare* L. is widespread in various regions of Russia including Komi Republic. A great number of physiologically active low-molecular-mass compounds, especially flavonoids and terpenoids [1], have been isolated from various species of *T. vulgare* L. The great interest to *T. vulgare* is associated with the extensive use of this plant in the folk medicine for treating intestinal diseases, ulcer, and rheumatism in addition to a high antibacterial and antihelminthic activity of compounds from this plant [1, 2]. Very limited information is, however, available on the chemical structure and physiological activity of the *T. vulgare* polysaccharides. The bulk of polysaccharides of this plant were earlier found to possess a considerable antiulcerous activity [3]. As a result of a preliminary study of polysaccharides isolated from *T. vulgare* at the period of general flowering, it was shown [3] that galacturonic acid accounts for 49.3% weight of dried polysaccharide, thus suggesting the *T. vulgare* polysaccharides' being pectic substances. No further information concerning chemical structure and physiologic activity of the polysaccharides from *T. vulgare* has been found in the scientific literature.

Earlier we described the isolation of various fractions of pectic polysaccharides named tanacetans from the dried aerial part of *T. vulgare* using the consecutive extraction with water and aqueous solutions, and revealed an antiaterogenic activity of tanacetans, particularly, their ability to bind low-density lipoproteins [4].

This work is devoted to another approach to the isolation and general chemical characterization of polysaccharides from *T. vulgare* L.

RESULTS AND DISCUSSION

Tanacetans were isolated from *T. vulgare* according to the method [5], based on the aqueous ammonium oxalate extraction of fresh plant material previously treated with aqueous formalin and acidified water (pH 4). Using this procedure, tanacetans TVF, TVS, and TVR were isolated from the *T. vulgare* floscules, sprouts, and roots, respectively, to give 2.0, 1.3, and 0.7% of the polysaccharides of the raw plant material (Table 1). As is seen from Table 1, the carbohydrate chains of tanacetans TVF and TVS contain the residues of galacturonic acid, arabinose, galactose, and rhamnose as major constituents and the residues of xylose, glucose, mannose, apiose, and 2-*O*-methylxylose as traces.

Tanacetan TVR, isolated from roots, substantially differs from both other samples of polysaccharides in

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Table 1. Characterization of tanacetans from floscules (TVF), sprouts (TVS), and roots (TVR) of *T. vulgare*

Characterization	TVF	TVS	TVR
Yield*, %	2.0	1.3	0.7
Content, %			
Protein	4.0	12.9	34.9
GalA	61.4	67.1	28.2
Ara	14.7	3.9	1.1
Gal	10.2	3.0	1.5
Rha	3.7	1.5	0.6
Glc	0.5	0.8	2.2
Man	0.3	0.4	0.4
Xyl	0.4	0.6	0.1
Api	Traces	–	–
2MeXyl	Traces	–	–

* Based on the crude plant raw material.

Table 2. Characterization of fragments of tanacetan TVF after partial acidic hydrolysis (TVF1) and pectinase digestion (TVF2, TVF3)

Characterization	TVF1	TVF2	TVF3
Yield*, %	48.1	23.7	13.5
Content, %			
Protein	0.8	10.1	10.7
GalA	98.2	29.9	28.4
Ara	–	28.1	27.1
Gal	1.0	16.8	17.3
Rha	1.0	11.6	11.9
Glc	0.5	2.2	3.0
Man	–	0.9	1.3
Xyl	–	Traces	1.0
Api	–	Traces	Traces
2MeXyl	–	Traces	Traces

* Based on the parent tanacetan TVF.

the sugar quantitative composition and is characterized by a low level of galacturonic acid and an elevated level of the protein (Table 1).

Galacturonic acid was obtained as a result of the complete acidic hydrolysis of tanacetans TVF, TVS, and TVR with 2 M trifluoroacetic acid (TFA) and was identified by gas-liquid chromatography as the corresponding methyl glycoside peracetates [6].

The polysaccharide fragment TVF1, resulting from the partial acid hydrolysis of tanacetan TVF with diluted TFA (0.1 M, 100°C, 3 h), is a polygalacturonan containing 98.2% galacturonic acid and possessing a high positive specific rotation, $[\alpha]_D^{20}$ + 246.3° (*c* 0.1; H_2O), typical of α-1,4-*D*-galacturonan. These data demonstrated the *D*-configuration of the galacturonic acid as a constituent of tanacetan TVF.

As follows from the ^{13}C NMR spectral data (Fig. 1), the sugar chain contains regions composed of the α-1,4-linked *D*-galacturonic acid residues, displaying the anomeric C-atom signal at 100.6 ppm. The positions of signals of other atoms of the *D*-galacturonic acid residues (69.5, 70.0, 79.4, 72.3, and 175.9 ppm) coincided with those of the respective signals of the authentic α-1,4-*D*-galactopyranosyluronan [7].

Pectic polysaccharides, containing α-1,4-linked *D*-galacturonic acid residues, are well known to be digested with pectinase (α-1,4-*D*-polygalacturonase). A digestion of TVF with this enzyme (3 h) gives rise to the polysaccharide fragment TVF2, accounting for 23.7% of the par-

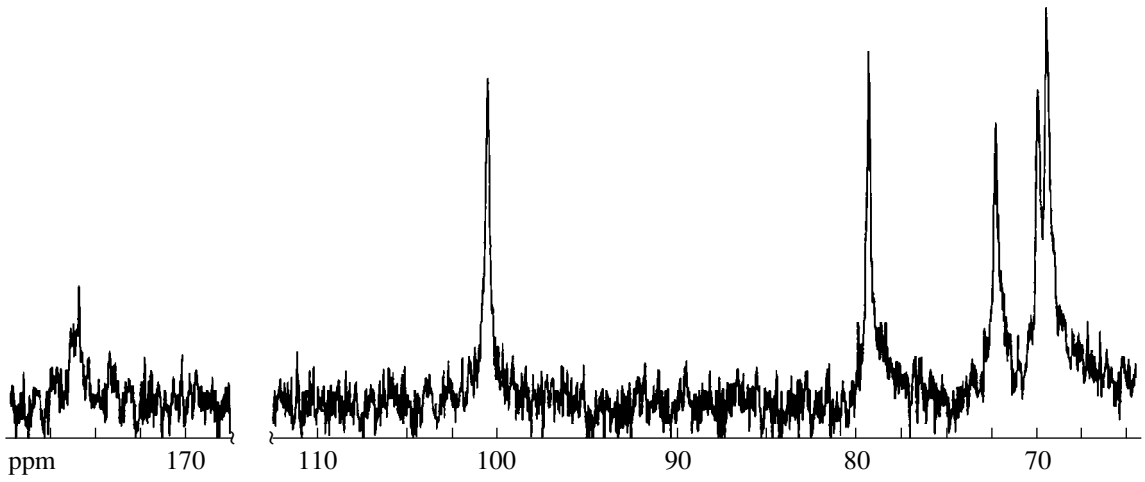

Fig. 1. ^{13}C NMR spectrum of α-1,4-*D*-galacturonan TVF1.

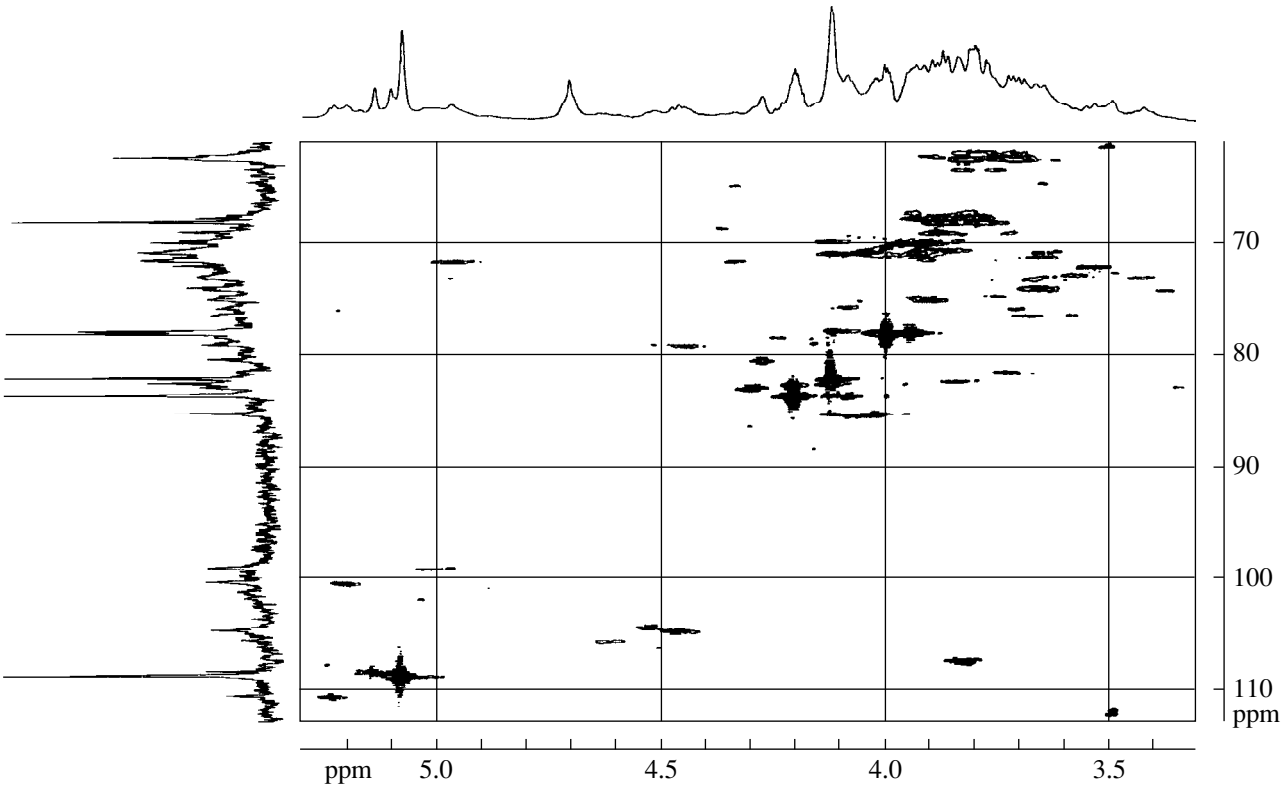

Fig. 2. $^{1}H/^{13}C$ HSQC spectrum of fragment TVF3.

ent tanacetan TVF; the backbone of tanacetan is therefore α-1,4-*D*-galacturonan. The sugar chain of TVF2 was shown to consist of residues of galacturonic acid, arabinose, galactose, and rhamnose as major constituents and residues of xylose, glucose, mannose, apiose, and 2-*O*-methylxylose as minor constituents (Table 2). Free galacturonic acid was detected by paper chromatography in the supernatant after the pectinase digestion.

The subsequent pectinase hydrolysis of the TVF2 fragment afforded polysaccharide fragment TVF3, having a positive specific rotation, $[\alpha]_D^{20}$ + 20°(*c* 0.1; H_2O).

The sugar chain of TVF3 contains residues of galacturonic acid, arabinose, galactose, and rhamnose as major constituents. As is seen from Table 3, fragments TVF2 and TVF3 do not virtually differ from each other, which indicates the completeness of the digestion even after a single treatment with the enzyme.

The signals in the heteronuclear $^{1}H/^{13}C$-HSQC spectrum (Fig. 2) of the polysaccharide fragment TVF3 demonstrated the presence of the α-arabinofuranose residues substituted in 3,5- and 5-positions, as well as the terminal residues of α-arabinofuranose and β-galactopyranose (Table 3) [8].

Table 3. The chemical shifts of signals in ^{13}C and ^{1}H NMR spectra of the polysaccharide fragment TVF3

Residue*	Chemical shifts; δ, ppm ($^{13}C/^{1}H$)					
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5	C6/H6
α-Ara*f*-(1 → 5	108.4/5.13	82.5/4.12	78.0/3.94	84.3/4.02	62.6/3.82; 3.71	–
α-Ara*f*-(1 → 3	110.3/5.22	82.7/4.20	78.0/3.94	84.3/4.12	62.6/3.82; 3.79	–
→5)-α-Ara*f*-(1 →	108.8/5.08	82.2/4.12	78.1/4.00	83.8/4.20	68.2/3.87; 3.79	–
→3,5)-α-Ara*f*-(1 →	108.7/5.11	80.5/4.28	84.3/4.08	83.0/4.30	67.9/3.93; 3.83	–
β-Gal*p*-(1 →	104.8/4.47	72.2/3.53	74.0/3.66	70.9/4.11	76.4/3.68	62.1/3.78

* Interpretation as described in [8].

The signals of rhamnopyranose were detected only in the proton spectrum, COSY and TOCSY (H1 5.03, H2 4.04, H3 3.82, H4 3.40, H5 3.60, H6 122). The H–C correlation failed to display peaks of the rhamnose residues so that the type of substitution of these residues could not be determined.

The signals of galacturonic acid were not found, possibly, due to their partial overlap with the signals of arabinose in the proton spectra, and their substantial broadening. The only reliable peaks in the ROESY spectrum are those of H1/H2, H4, H5a, and H5b of the arabinofuranose residues, confirming the 1,5-linkage.

The results of the partial acid hydrolysis and the enzymatic digestion of tanacetan TVF confirm it to be a pectic polysaccharide.

EXPERIMENTAL

Plant material. The harvest of the aerial part of *T. vulgare* was carried out at the period of general flowering in July near Syktyvkar (Komi Republic, Russia). The roots of *T. vulgare* were collected in October, after the plant fruiting was over.

General analytical methods. The overall content of glycuronic acids was evaluated with 3,5-dimethylphenol in the presence of conc. H_2SO_4 [9] using the standard curve for galacturonic acid. The protein content was determined according to Lowry's method [10] using the standard curve for bovine serum albumin.

Spectrophotometric measurements were performed on an Ultrospec-3000 spectrophotometer (England). NMR spectra were run on a Bruker DRX-500 instrument (Germany) for a 3–5% solution of polysaccharides in D_2O at 343 K (acetone as an internal standard, δ_h 2.225, δ_c 31.45). Two-dimensional spectra were registered using the standard Bruker procedures.

Optical rotations were measured at 20°C in aqueous solutions on a Polatronic MHZ polarimeter (Germany).

Gas-liquid chromatography was performed on a Hewlett-Packard Model 4890 (United States) chromatograph equipped with a flame-ionization detector and an HP 3395A integrator on a capillary column RTX-1 (0.25 mm × 30 m); argon was used as a carrier gas. Neutral sugars were detected and quantitated as the corresponding alditol acetates [10]. Galacturonic acid was identified as the corresponding methyl glycoside peracetates according to [6]. The temperature range was 175°C (1 min) ⟶ 250°C (2 min), Δ3°C/min for alditol acetates and 150°C (1 min) ⟶ 290°C (2 min), Δ5°/min for methylglycosides.

The descending paper chromatography was run on the Filtrak FN-4 paper in a 6 : 4 : 3 *n*-butanol–pyridine–water system; monosaccharides were detected using aniline hydrogen phthalate at 105°C. All aqueous solutions were concentrated under reduced pressure at 40–45°C, centrifuged at 5000–6000 rpm for 10–20 min, and lyophilized.

Isolation of polysaccharide fractions. Tanacetans TVF, TVS, and TVR were isolated from fresh floscules (1714 g), sprouts (970 g), and roots (260 g) in yields of 33.9, 13.1, and 1.9 g, respectively, according to the method described earlier [5].

Complete acid hydrolysis. The polysaccharide fraction (3–5 mg) was treated with 2 M TFA (1 ml) containing *myo*-inositol (0.5 mg/ml) as an internal standard. The mixture was heated for 4 h at 100°C, the acid was removed by multiple evaporations to dryness with methanol. The monosaccharides were identified by GLC [11].

Partial acid hydrolysis. A mixture of TFA (0.1 M, 20 ml) and tanacetan TVF (105.1 mg) was heated for 3 h at 100°C. The undissolved precipitate was centrifuged, repeatedly washed with methanol up to the absence of sugars in the supernatant, dissolved in water with addition of ammonia up to pH 5, and lyophilized. The polysaccharide fragment TVF1 was obtained (yield 50.6 mg).

Enzymatic hydrolysis. Tanacetan TVF (499.3 mg) was dissolved in water (50 ml), and an aqueous solution of pectinase (10 mg, Ferbak, Germany) was added. The resulting mixture was incubated in a dialysis bag with the simultaneous dialysis against distilled water for 3 h at 37°C. Pectinase was inactivated by boiling at 100°C and removed by centrifugation. The supernatant was concentrated and precipitated with 4 volumes of 96% ethanol. The precipitate was separated by centrifugation and washed with methanol up to the absence of free galacturonic acid. The residual material was dissolved in water and lyophilized to yield the polysaccharide fragment TVF2 (118.4 mg).

This material (100 mg) was dissolved in water (10 ml), and the solution was treated with pectinase (2 mg) as described above. The polysaccharide fragment TVF3 (67.4 mg) was obtained.

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