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Some structural features of pectic polysaccharide from tansy, *Tanacetum vulgare* L.

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Abstract

The structure of polysaccharide isolated from tansy, *Tanacetum vulgare* L. referred to as tanacetan was elucidated by a combination of partial acid hydrolysis, Smith degradation, digestion by a commercial pectinase preparation and NMR spectral analysis of the fragments obtained. They consist of pectin built on a linear backbone of rhamnogalacturonan and the ramified 'hairy' regions. The backbone proved to consist of α-1,4-D-galactopyranosyluronan blocks interconnected by 1,2-linked rhamnose residues involved in the linear sugar chain. The hairy regions were shown to contain the following fragments of the backbone: → 4)-α-Gal*p*A-(1 → 2)-α-Rha*p*-(1 → 4)-α-Gal*p*A-(1 → and the side chains attached to the residues of rhamnopyranose of these fragments. The single and β-1,4-linked residues of galactopyranose were found to substitute 4-position of α-1,2-linked rhamnose residues of the backbone. In addition, the chains composed of β-1,4-, and 1,6-linked galactopyranose residues as well as the terminal residues of β-galactopyranose, α-arabinofuranose, and 2-mono-*O*-methyl-β-galactopyranose were detected. Some residues of α-arabinofuranose were shown to be substituted in 2-, 5-, 2,5-, and 3,5-positions, and residues of β-galactopyranose were found to be substituted in 4,6-positions. Some α-1,2-linked rhamnose residues appeared to occupy at the reducing end of the backbone. Smith degradation of the parent tanacetan TVF and of the product of its pectinase digestion led to polysaccharide fragments differed in high contents of the arabinose residues (42.5 and 48.6%, respectively) thus indicating to an occurrence of some ramified regions consisted of the arabinose residues.

Keywords: Plant polysaccharide; Tansy *Tanacetum vulgare* L; Pectins; Tanacetan; Structure; NMR spectroscopy of polysaccharides

1. Introduction

Using extraction with aqueous ammonium oxalate, pectic polysaccharide called tanacetan TVF has been isolated earlier from floscules of tansy, *Tanacetum vulgare* L. widespread at the European North of Russia (Polle, Ovodova, Shashkov, & Ovodov, 2001. Tanacetan TVF has been found to possess antiaterogenic activity (Polle, Gyunter, Popov, & Ovodova, 2000). The sugar chains of tanacetan TVF have been shown to consist of D-galacturonic acid, arabinose, galactose, and rhamnose as the main constituents. Trace amounts of glucose, xylose, mannose, apiose, and 2-*O*-methyl-xylose have been also detected in the hydrolysate of tanacetan TVF (Polle et al., 2001). A partial acid hydrolysis has been found to give rise to α-1,4-D-galacturonan as a constituent of the backbone. Digestion with pectinase was accompanied by a substantial cleavage of the galacturonan core of tanacetan TVF. Thus, tanacetan TVF proved to be a pectic polysaccharide (Polle et al., 2001) related to pectin isolated from sugar beat (Guillon & Thibault, 1990).

The present paper reports the fine chemical structure of tanacetan TVF using mild acid hydrolysis, Smith degradation and digestion by a commercial pectinase preparation followed by NMR spectral analysis of fragments obtained.

2. Experimental

2.1. Isolation of tanacetan TVF

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). Pectic polysaccharide (tanacetan) was isolated from floscules of *T. vulgare* L. using the procedure described earlier (Ovodova, Vaskovsky, & Ovodov, 1968) to yield 2.0% tanacetan TVF of the raw plant material.

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2.2. Enzymic hydrolysis

Tanacetan TVF (500 mg) was dissolved in distilled water (50 ml) and an aqueous solution of pectinase (10, 3.3 mg protein and 56.7 IU, Ferbak, Germany) was added. The resulting mixture was incubated in a dialysis bag with a simultaneous dialysis against distilled water for 3 h at 37 °C (Polle et al., 2001). Pectinase was inactivated by boiling at 100 °C and denaturated protein obtained was removed by centrifugation. The supernatant was concentrated and precipitated with four volumes of 96% ethanol. The precipitate was separated by centrifugation and washed with methanol up to the absence of free galacturonic acid, which was monitored by paper chromatography of the methanol washes.

The material obtained was subjected to molecular-sieve chromatography on Sephacryl S-500 column B to give a single peak with $K_{av} = 0$. Fractions corresponding to this peak were collected and lyophilized to furnish TVF-E fragment of tanacetan TVF (yield 135.7 mg).

2.3. Mild acid hydrolysis

Tanacetan TVF (110 mg) was treated with 0.01 M trifluoroacetic acid (TFA, 20 ml) for 4 h at 100 °C, the mixture obtained was subjected to centrifugation, the precipitate was washed with methanol up to disappearance of low molecular weight saccharides in washing solutions, which were monitored by paper chromatography. The residual material was dissolved in distilled water and lyophilized to give a crude fragment TFV-H1 which was purified by molecular-sieve chromatography on Sephacryl S-500 column A as the single peak eluted with distilled water with $K_{av} = 0$. Fraction corresponding to this peak were combined and lyophilized to give the purified TFV-H1 polysaccharide (yield 75 mg).

The supernatant was evaporated up to dryness to afford oligosaccharide fraction TFV-H2 (yield 34 mg). An absence of monosaccharides was confirmed by GLC of the corresponding alditol acetates.

Polysaccharide TFV-H1 (50 mg) was treated with 0.05 M TFA (5 ml) for 2 h at 75 °C, the mixture obtained was subjected to centrifugation, the precipitate was washed with methanol. The residual material was dissolved in distilled water and lyophilized to give a crude fragment TFV-H3 which was purified on Sephacryl S-500 column A as the single peak eluted with distilled water with $K_{av} = 0$. Fraction corresponding to this peak were combined and lyophilized to give the purified TFV-H3 (yield 39 mg).

Polysaccharide TFV-E (50 mg) was treated with 0.01 M TFA (10 ml) for 2 h at 100 °C. The mixture obtained was subjected to a treatment as in the case of TFV-H3. Molecular-sieve chromatography on Sephacryl S-500 column B afforded two polysaccharide fractions with $K_{av} = 0$ and $K_{av} = 0.4$ as follows: TFV-E1 (yield 18.9 mg) and TFV-E2 (yield 15.7 mg), respectively.

2.4. Smith degradation

Tanacetan TVF (500 mg) was dissolved in distilled water (50 ml) and 0.03 M aqueous sodium metaperiodate (10 ml) was added. The mixture obtained was kept for 24 h at 5 °C in the dark up to the point where the lowest optical density of the solution at 223 nm was observed. Ethylene glycol (0.5 ml) was added and the mixture was kept for 1 h at 5 °C followed by dialysis against changing distilled water for 3 days. The solution was concentrated up to 20 ml, the residual material was treated with sodium borohydride (500 mg) overnight at room temperature, and adding acetic acid up to pH 5 destroyed the excess of sodium borohydride. The mixture was dialyzed against changing distilled water for 2 days and lyophilized to afford the polyalcohol (yield 344.4 mg).

The polyalcohol (100 mg) was treated with 1% aqueous acetic acid (20 ml) for 2 h at 100 °C, the solution obtained was evaporated with diluted methanol. The residual material was dissolved in distilled water (5 ml), the solution was injected onto a Sephadex G-25 column and eluted by distilled water. Fractions corresponding to the single peak immediately with $K_{av} = 0$ were combined and the solution obtained was lyophilized to furnish the polysaccharide fraction TVF-S1 (yield 10.2 mg).

The fraction TVF-E (140 mg) in distilled water (14 ml) was treated with 0.02 M aqueous sodium metaperiodate (6 ml) for 24 h at 5 °C in the dark. The reaction mixture was subjected to a treatment as above to afford the polysaccharide fraction TVF-S2 (yield 50.7 mg).

2.5. General methods

The total contents of glycuronic acids were evaluated with 3,5-dimethylphenol in the presence of conc. H_2SO_4 (Usov, Bilan, & Klochkova, 1995) using the standard curve for galacturonic acid. The protein contents were determined according to Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951) using the standard curve for bovine serum albumin. The contents of methoxyl groups were estimated as described earlier (Wood & Siddiqui, 1971).

Spectrophotometric measurements were performed on an Ultrospec-3000 spectrophotometer (England).

NMR spectra were run on a Bruker DRX-500 instrument (Germany) for 3–5% solutions of polysaccharides in D_2O at 30 °C (aceton as an internal standard, δ_H 2.225 ppm, δ_C 31.45 ppm). Samples were exchanged by deuterium using two-fold evaporation with D_2O. Two-dimensional spectra were registered using the standard Bruker procedures. NOESY and ROESY spectra were performed using the mixing time of 500 and 200 ms, respectively. The 60 ms duration of MLEV17 spin-lock for TOCSY experiments was used.

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

The polysaccharide fractions (3–5 mg) were treated with

Table 1
Characteristics of tanacetan TVF and its fragments after partial acidic hydrolysis, pectinase digestion, and Smith degradation (n.d.: not determined, tr.: trace)

Tanacetans	Yield (%)	Protein (wt%)	GalA (wt%)	OMe (wt%)	Ara (mol%)	Gal (mol%)	Rha (mol%)	Glc (mol%)	Man (mol%)	Xyl (mol%)
TVF	2.0[a]	4.0	61.4	0.8	14.7	10.2	3.7	0.5	0.3	0.4
TVF-H1	68.2[b]	2.3	75.8	n.d.	6.0	9.3	3.6	0.6	0.3	0.3
TVF-H2	30.9[b]	Nil	Nil	n.d.	90.8	5.0	3.0	tr.	Nil	Nil
TVF-H3	78.0[c]	1.3	78.1	n.d.	2.4	12.6	2.6	0.9	0.3	0.3
TVF-E	27.1[b]	4.5	36.8	1.2	28.0	14.8	9.7	1.3	tr.	0.8
TVF-E1	37.8[d]	3.2	43.4	1.3	15.0	14.3	19.9	1.7	tr.	0.5
TVF-E2	31.4[d]	3.3	45.2	1.4	16.0	16.4	14.6	1.0	0.6	0.6
TVF-S1	7.0[b]	3.9	27.9	1.0	42.5	11.6	10.7	1.3	tr.	tr.
TVF-S2	10.6[b]	3.3	23.8	1.1	48.6	12.7	7.6	1.0	tr.	0.7

[a] Based on the crude plant raw material (Polle et al., 2001).
[b] Based on the parent tanacetan TVF.
[c] Based on the fraction TVF-H1.
[d] Based on the fraction TVF-E.

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 (York, Darvill, McNeil, Stevenson, & Albersheim, 1985).

Gas–liquid chromatography was performed on a Hewlett–Packard Model 4890 (US) chromatograph equipped with a flame-ionization detector and connected to a HP 3395A integrator. Neutral sugars as alditol acetates (York et al., 1985) were identified and quantified after chromatography through a capillary RTX-1 column (manufactured in the US, 0.25 mm × 30 m). Argon was the carrier gas and elution was realized by the temperature gradient: 175 °C (1 min) – 250 °C (2 min) with a slope of 3 °C/min.

The descending paper chromatography was run on the Filtrak FN-4 paper in a 6:4:3 *n*-butanol–pyridine–water system, the sugars were detected using aniline hydrogen phthalate at 105 °C.

Molecular-sieve chromatography was performed on Sephacryl S-500 column A and B (1.6 × 41 and 2.4 × 60 cm, respectively) or on Sephadex G-25 column (2.4 × 73 cm) with the void volume of 14, 88 and 109 ml, respectively. Elution was performed with distilled water at a flow rate of 28 ml/h. Fraction (3.5 and 6.8 ml each) were collected. Sugar content in fractions was determined by colorimetry (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

All aqueous solutions were concentrated under reduced pressure at 40–45 °C, centrifuged at 7000–8000 g for 10–20 min, and lyophilized.

3. Results and discussion

3.1. Preparation of polysaccharide

The pectic polysaccharide named tanacetan TVF has been isolated from the floscules of tansy *T. vulgare* L. as described earlier (Polle et al., 2001). The sugar composition of tanacetan is listed in Table 1.

3.2. Mild acid hydrolysis

Tanacetan TVF was found to be resistant to autohydrolysis with water for 24 h at 24 °C and to mild hydrolysis with 0.01 M TFA for 1–8 h at 50 °C. A cleavage of sugar chains of tanacetan TVF was observed at the treatment with 0.01 M TFA at 100 °C for 1–8 h. Arabinose was the single monosaccharide in the hydrolysate thus demonstrating that the residues of this sugar appeared to be in furanose form and/or some of these residues occupied the terminal positions of the ramified regions of the parent tanacetan TVF.

The optimum time of arabinose release was found to be 4 h. Mild acid hydrolysis of tanacetan TVF with 0.01 M TFA for 4 h at 100 °C resulted in the polysaccharide fraction

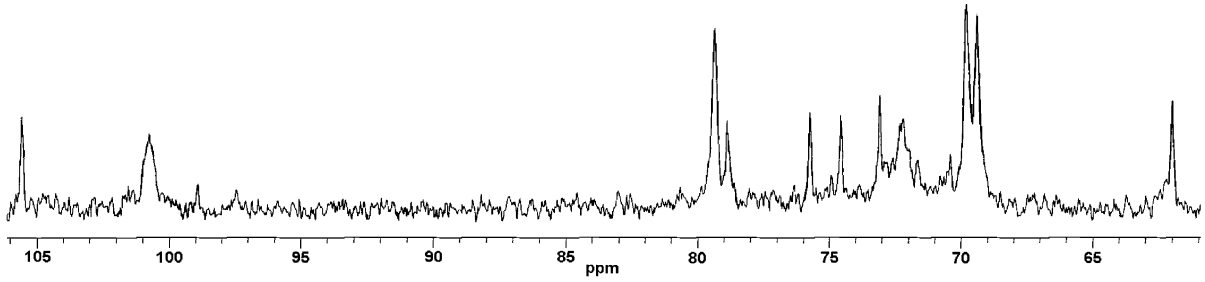


Fig. 1. ^{13}C-NMR spectrum of the polysaccharide fraction TVF-H3.

Table 2
The chemical shifts of signals in ^{13}C and ^{1}H-NMR spectra of tanacetan TVF and its fragments

Residue	Chemical shifts, δ (ppm), ^{13}C and ^{1}H						
	C1/H1	C2/H2	C3/H3	C4/H4	C5/H5;H5′	C6/H6;H6′	OMe
TVF-H2, TVF-E1, TVF-S2							
α-Ara*f*-(1 → 5	108.4/5.13	82.5/4.12	77.8/3.94	85.3/4.02	62.6/3.82;3.72		
α-Ara*f*-(1 → 3	110.3/5.23	82.7/4.22	77.8/3.94	85.3/4.12	62.6/3.82;3.69		
→ 5)-α-Ara*f*-(1 →	108.8/5.05	82.2/4.12	78.1/4.02	83.8/4.22	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		
→ 2)-α-Ara*f*-(1 →	108.4/5.16	85.2/4.13	77.8/3.96	85.2/4.05	62.4/3.84;3.73		
→ 2,5)-α-Ara*f*-(1 →	108.8/5.07	85.2/4.11	77.9/4.04	83.8/4.22	67.2/3.95;3.83		
TVF-H3, TVF-E1, TVF-S2							
→ 4)-β-Gal*p*-(1 →	105.5/4.63	73.0/3.68	74.5/3.77	78.4/4.17	75.7/3.71	62.2/3.87;3.82	
→ 4)-α-Gal*p*A-(1 →	99.7/5.25	69.1/3.87	69.9/3.95	78.5/4.41	72.5/4.66	174.8/-	
TVF-E1, TVF-S2							
β-Gal*p*-(1 → 4)-β-Gal*p*	104.8/4.47	72.2/3.55	74.0/3.68	69.9/3.97	76.4/3.68	62.1/3.78	
2-*O*-Me-β-Gal*p*-(1 →	104.0/4.50	83.3/3.29	74.3/3.36	69.9/3.93	77.5/3.70	62.2/3.87;3.82	61.2/3.48
→ 6)-β-Gal*p*-(1 →	104.4/4.52	73.0/3.53	74.0/3.71	71.5/4.12	75.0/3.92	70.5/4.05;3.92	
→ 4,6)-β-Gal*p*-(1 →	104.4/4.47	73.0/3.53	74.0/3.66	77.8/3.93	74.0/3.65	70.5/4.05;3.92	
TVF-E1							
→ 4)-α-Gal*p*A-(1 → 2)-α-Rha*p*-(1 →	98.8/5.0	69.1/3.92	69.7/4.10	77.4/4.43	72.3/4.62	174.3/-	
→ 2)-α-Rha*p*-(1 →	102.4/5.25	77.4/4.12	70.6/3.90	73.2/3.42	69.6/3.80	18.0/1.25	
→ 2)-α-Rha*p*	93.8/5.25	80.0/4.12	69.8/3.90	73.2/3.42	69.5/3.80	18.0/1.25	
→ 2,4)-α-Rha*p*-(1 →	99.7/5.25	77.4/4.12	70.6/3.90	81.3/3.74	67.6/3.68	18.0/1.30	
β-Gal*p*-(1 → 4)-α-Rha*p*	105.5/4.61	72.5/3.63	75.0/3.92	69.9/3.96	76.4/3.68	62.1/3.78	
→ 4)-β-Gal*p*-(1 → 4)-α-Rha*p*	104.4/4.54	73.0/3.51	74.0/3.66	78.6/4.16	75.0/3.92	61.7/3.78	

TVF-H1, yield 68.2%, $[\alpha]_D^{20}$ + 156.2° (*c* 0.1; H_2O) and the oligosaccharide fraction TVF-H2, yield 30.9%, in addition to arabinose as the main monosaccharide in the hydrolysate. Analytical data for TVF-H1 and TVF-H2 are given in Table 1.

A further hydrolysis of the polysaccharide fraction TVF-H1 with 0.05 M TFA for 4 h at 100 °C afforded the polysaccharide fraction TVF-H3, yield 78% of TVF-H1, $[\alpha]_D^{20}$ + 175.0° (*c* 0.1; H_2O). Characteristics of TVF-H3 are listed in Table 1.

From Table 1, fractions TVF-H1 and TVF-H3 have close sugar compositions. These data demonstrated that mild acid hydrolysis of tanacetan TVF led only to the release of arabinose at the first step of hydrolysis. The ratios of the other sugar constituents appeared to be constant in the two steps of the partial hydrolysis of tanacetan TVF. The conclusion is confirmed by simultaneous isolation of oligosaccharide TVF-H2 furnished arabinose mainly on a complete acid hydrolysis.

As follows from the ^{13}C NMR spectral data for the fraction TVF-H3 (Fig. 1), the sugar chain of the parent tanacetan contained regions consisted of α-1,4-linked D-galacturonic acid residues and β-1,4-linked galactopyranose residues (Table 2). The assignments of the carbon signals of the D-galacturonic acid residues coincided with those of the respective signals of the authentic α-1,4-D-galactopyranosyluronan (Polle et al., 2001). The assignments of the carbon signals from 4-linked galactan side chains was made as described earlier (Keenan, Belton, Matthew, & Howson, 1985; Pressy & Himmelsbach, 1984) and given in Table 2.

The assignments of signals in ^{13}C- and ^{1}H-NMR spectra of oligosaccharide TVF-H2 are listed in Table 2 and indicated an occurrence of the arabinose residues substituted in 2-, 5-, and 3,5- positions and the terminal residues of α-arabinofuranose.

An additional information concerning a sequence of the sugar residues in the oligosaccharide fraction TVF-H2 was obtained using ROESY spectra. Thus, the spectrum showed *trans*-glycosyl correlation signals of anomeric proton of the terminal residue of α-arabinofuranose with H5-atoms of α-arabinofuranose substituted in 5-position (H1/H5 5.13/3.87; H1/H5′ 5.13/3.79). These data indicated the presence of the following structural pattern in the oligosaccharide TVF-H2: α-Ara*f*-(1 → 5)-α-Ara*f*-(1 → …

The ROESY spectrum of TVF-H2 showed also trans-glycosyl correlation peaks of anomeric proton of α-arabinofuranose substituted in 5-position with H5-atoms of α-arabinofuranose substituted in 3,5-position (H1/H5 5.05/3.93; H1/H5′ 5.05/3.83) thus indicating an occurrence of the following fragment in the oligosaccharide TVF-H2:

…→5)-α-Ara*f*-(1→5)-α-Ara*f*-(1→…
3
↑

In addition, the ROESY spectrum of TVF-H2 showed *trans*-glycosyl correlation peaks of anomeric proton of

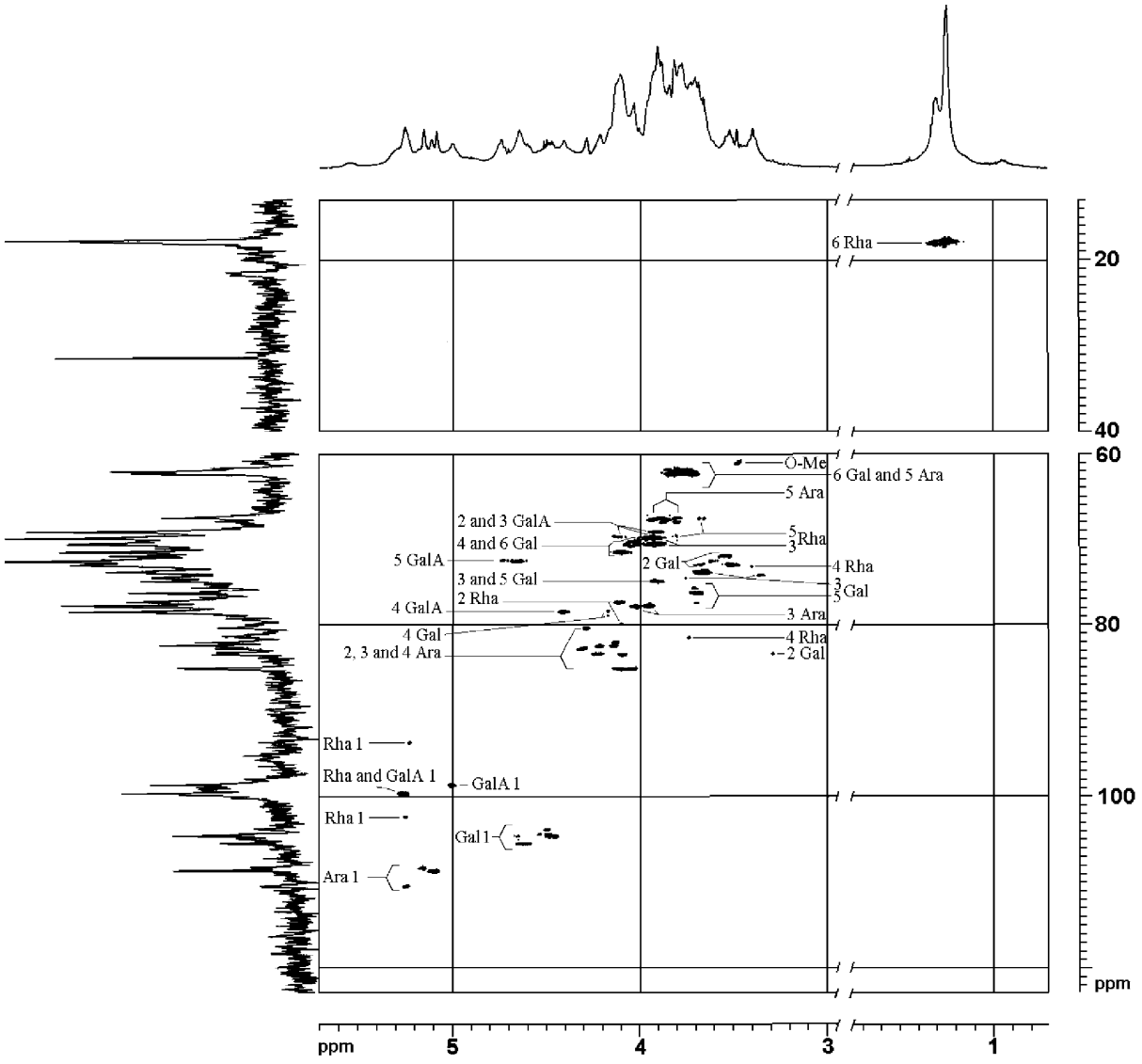


Fig. 2. $^{1}H/^{13}C$-HSQC spectrum of the polysaccharide fraction TVF-E1.

α-arabinofuranose substituted in 2-position with H3-atoms of α-arabinofuranose substituted in 3,5-position (H1/H5 5.16/4.08) confirming the presence of the following fragment:

…→5)-α-Ara*f*-(1→…
3
↑
…→2)-α-Ara*f*-1

On the basis of NMR-spectral data obtained, the possible structural pattern of the oligosaccharide TVF-H2 can be proposed:

α-Ara*f*-(1→5)-α-Ara*f*-(1→5)-α-Ara*f*-(1→5)-α-Ara*f*
3
↑
α-Ara*f*-(1→5)-α-Ara*f*-(1→2)-α-Ara*f*-1

3.3. Enzymic hydrolysis

The parent tanacetan TVF was subjected to digestion with α-1,4-D-polygalacturonase (pectinase) to furnish the digested polysaccharide TVF-E, yield 27.1%. Sugar composition of the polysaccharide fraction TVF-E is given in Table 1. Galacturonic acid substantially decreased to 36.8% while contents of the neutral sugars increased indicating a significant degradation of the galacturonan backbone. An increase in the methyl ester groups in fraction TVF-E in comparison with the parent tanacetan TVF appeared to be connected with preferable enzymic degradation of sugar chains composed of non-methoxylated galacturonic acid residues.

Partial acid hydrolysis of the polysaccharide fraction TVF-E with 0.01 M TFA for 2 h at 100 °C resulted in two homogeneous polysaccharides as follows: TVF-E1, $[\alpha]_D^{20}$ +

185.8° (*c* 0.1; H_2O) and TVF-E2, $[\alpha]_D^{20}$ + 228.4° (*c* 0.1; H_2O). Characteristics of the fractions TVF-E1 and TVF-E2 are given in Table 1.

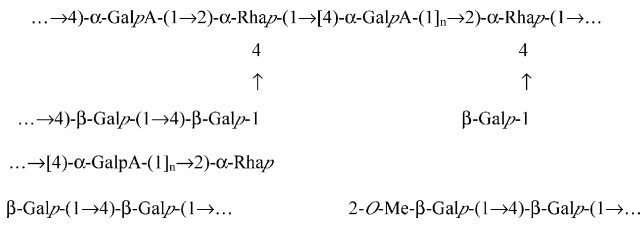
The two polysaccharides have close sugar compositions. Some changes were observed when compared to the polysaccharide TVF-E. Arabinose substantially decreased in both the polysaccharides thus indicating that the residues of arabinose are presented in furanose form and/or occupied the terminal positions of the polysaccharide TVF-E and, therefore, of the parent tanacetan TVF.

The polysaccharide fraction TVF-E1 was analyzed using NMR-spectroscopy. The $^1H/^{13}C$-HSQC spectral data (Fig. 2) indicated the presence of linear α-1,4-D-galacturonan (Table 2) as it was shown for the parent tanacetan and for the polysaccharide fragment TVF-H3. In addition, the data of heteronuclear $^1H/^{13}C$-HSQC and homonuclear TOCSY and ROESY spectroscopy demonstrated that the residues of rhamnopyranose are involved in the backbone by 1,2-linkages as it has been earlier shown for pectins of sugar beet (Renard, Lahaye, Mutter, Voragen, & Thibault, 1998) and for those of other origins (Ovodov, 1998). An up-field shift of the anomeric carbon and proton signals (93.8/5.25) in the $^1H/^{13}C$-HSQC and ROESY spectra indicated the occurrence of some residues of α-rhamnopyranose at the reducing end of the backbone.

The chemical shifts of signals in NMR spectra of the polysaccharide fragment TVF-E1 are given in Table 2.

The signals were confirmed by $^1H/^{13}C$-HSQC and ROESY spectral data. It must be noted that α-1,2-linked residues of rhamnopyranose are substituted in 4-position by single β-galactopyranose residues, which show a marked H6 shift of the methyl of the rhamnopyranose residue in $^1H/^{13}C$-HSQC spectrum, as described earlier (Vignon & Garcia-Jaldon, 1996). The other signals for such substitution of α-rhamnopyranose residues were also assigned from TOCSY, COSY, NOESY, ROESY, and $^1H/^{13}C$-HSQC spectra (Table 2). A down-field shift of the anomeric carbon and proton of the terminal β-galactopyranose residue attached to 4-position of rhamnopyranose was observed. This type of substitution is confirmed by *trans*-glycosyl correlation peaks in NOESY spectrum of anomeric proton of β-galactopyranose with H4 of α-rhamnopyranose, substituted in 2,4-position (H1/H4 4.61/3.74). An assignment of $^1H/^{13}C$-HSQC spectral data for signals of β-1,4-linked residues of galactopyranose attached to 4-position of α-rhamnopyranose residues was achieved as described earlier for soybean pectin (Fransen, Haseley, Huisman, Schols, Voragen, Kamerling et al., 2000). A confirmation of this bond is due to the *trans*-glycosyl correlation peaks in ROESY spectrum of the anomeric proton of β-galactopyranose with H4 of α-rhamnopyranose residues substituted in 2,4-positions (H1/H4 4.54/3.74).

^{13}C- and ^1H-NMR spectral data indicated the presence of β-1,4-linked galactopyranose residues in TVF-E1 (Table 2). A resonance of the anomeric atom at 104.8/4.47 in the $^1H/^{13}C$-HSQC spectrum indicated the presence of the terminal β-galactopyranose residues (Table 2) β-1,4-linked to another galactopyranose residue. The chemical shifts of C2–C6 of this terminal residue further confirmed this interpretation. In addition, the residues of 2-mono-*O*-methyl β-galactopyranose were detected (Table 2). An up-field shift of signal of C2 (83.3 ppm) in the $^1H/^{13}C$-HSQC spectrum (Fig. 2) and *trans*-glycosyl correlation peaks in ROESY spectrum of the *O*-methyl group with H2 of the terminal β-galactopyranose residue (OMe/H2 3.48/3.29) confirmed this interpretation. Thus, on the basis of the data obtained, the following fragments are present in the ramified regions of TVF-E1 and therefore in the parent tanacetan:



A down-field shift of signal of C6 (70.5 ppm) and moreover an assignment shift of signals of C4/H4 71.5/4.12 and 77.8/3.93 in the $^1H/^{13}C$-HSQC spectrum (Fig. 2) indicated the occurrence of the β-galactopyranose residues substituted in 6-, and 4,6-positions respectively. The other signals for such substituted residues were assigned from heteronuclear $^1H/^{13}C$-HSQC and homonuclear TOCSY spectra (Table 2).

The residues of α-arabinofuranose substituted in 2-, 3,5- and 5-positions as well as the terminal α-arabinofuranose residues were identified in the polysaccharide fragment TVF-E1 using the NMR spectral data (Table 2). *Trans*-glycosyl correlation peaks of the anomeric proton of α-arabinofuranose substituted in 2-position with H3-protons of α-arabinofuranose substituted in 3,5-positions are observed in the ROESY spectrum of TVF-E1.

3.4. Smith degradation

Further data concerning the structural features of tanacetan TVF were obtained after Smith degradation. The parent tanacetan TVF and fraction TVF-E were subjected to Smith degradation to give fractions: TVF-S1 and TVF-S2 respectively (yields 7.0 and 10.6% of the parent tanacetan, respectively). TVF-S1, $[\alpha]_D^{20}$ + 14.3° (*c* 0.1; H_2O) and TVF-S2 $[\alpha]_D^{20}$ + 8.8° (*c* 0.1; H_2O) were found to consist of the residues of D-galacturonic acid, arabinose, galactose, and rhamnose as the main constituents (Table 1). The residual amounts of galacturonic acid in both the fractions may be connected with an occurrence of some acetyl groups or with the ramified 'hairy' regions since some side chains appeared to be attached to the residues of galacturonic acid of the linear backbone. This interpretation needs confirmation.

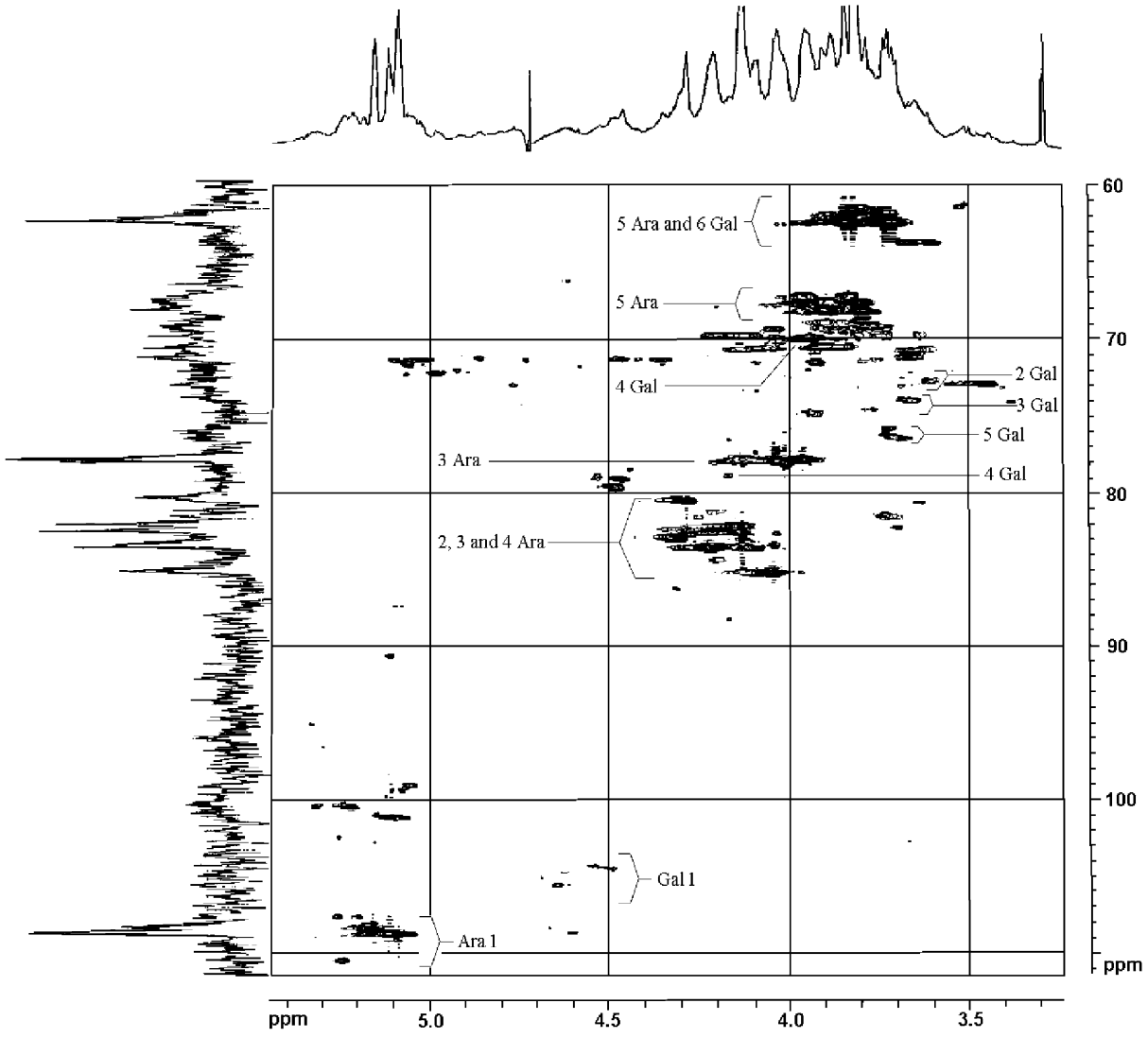


Fig. 3. $^{1}H/^{13}C$-HSQC spectrum of the polysaccharide fraction TVF-S2.

The content of arabinose in both polysaccharide fractions increased substantially in comparison with those in the parent polysaccharides TVF and TVF-E. These data demonstrated that the arabinose residues formed highly branched side chains in the ramified regions of tanacetan TVF. The $^{1}H/^{13}C$-HSQC spectral data for the polysaccharide fragment TVF-S2 (Fig. 3) confirm the occurrence of the α-arabinofuranose residues substituted in 2-, 3,5- and 5-positions as well as the terminal residues of α-arabinofuranose as constituent of branched sugar chains. These results confirm data obtained in TVF-H2 that the arabinose side chains are composed of 1,2-linked α-arabinofuranose residues attached by 1,3-linkeges to the chain that consisted of 1,5-linked α-arabinofuranose residues. All the signals of the α-arabinofuranose residues substituted in 2,5-positions, and of the terminal and β-1,4-linked residues of galactopyranose were assigned (Table 2).

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