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WATER-SOLUBLE GLYCOPROTEIN FROM *URTICA DIOICA* LEAVES

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Abstract—A neutral and an acidic carbohydrate–protein polymer were isolated from leaves of *Urtica dioica*. The neutral fraction was a glycoprotein, containing the serine-*O*-galactoside glycopeptide bond. Methylation analysis revealed a highly branched structure, arabinose constituting the exterior and mainly galactose the interior part of the carbohydrate moiety. Galacturonic acid was the major component of the acidic fraction.

INTRODUCTION

In several European countries, the stinging nettle has been in use as a folk-medicinal herb for the cure of various diseases, and also more generally as a health food. As part of a re-examination of the chemical constituents of the plant the water-soluble, non-dialysable fraction of *Urtica dioica* leaves was investigated.

RESULTS AND DISCUSSION

On chromatography of the crude aqueous extract on DEAE-Sephadex, the material was eluted in 3 fractions: A (neutral), B (acidic), and in addition, a fraction was eluted between A and B. The latter was not investigated further.

Gel chromatography of A and B on a calibrated Sepharose 4B column revealed for both fractions a very wide MW distribution, ranging continuously from *ca* 6000 to *ca* 100 000, the elution curve showing no distinct peaks. This large span in MW seemed to indicate physical rather than chemical heterogeneity and the two biopolymers were investigated without further fractionation.

The carbohydrate and protein content of A and B are given in Table 1 and the amino acid composition of A in Table 2. Due to its low protein content, fraction B was not subjected to amino acid analysis.

Attempted detection of any reducing sugar end group in A failed, indicating a glycoprotein. The commonly occurring glycopeptide bond in plant glycoproteins, the hydroxyproline-*O*-arabinoside bond [1] is hardly of any significance in the nettle biopolymer in view of the trace amount of hydroxyproline present. A serine-*O*-galactoside bond was recently demonstrated [2] in a glycoprotein from *Cannabis sativa* of the family Cannabinaceae which is closely related to the Urticaceae. In a glycoprotein containing such linkages between carbohydrate and protein the action of $NaOH–NaBH_4$ will cause bond cleavage by β-elimination, resulting in the liberation of the carbohydrate moieties terminating in a sugar alcohol. Of the unsaturated amino acid units formed in the β-elimination reaction, the α-aminopropenoic acid (from serine) will be hydrogenated to alanine, whereas the α-aminobutenoic acid residues (from threonine) will remain largely unchanged. Treatment of the nettle glycoprotein fraction A with $NaOH–NaBH_4$ gave an increase of *ca* 9% in alanine, a decrease of *ca* 13% in serine and a decrease of *ca* 16% in threonine. Only one alditol, galactitol, was detected after the reductive base-catalysed hydrolysis of the glycoprotein. These results strongly suggest that protein and carbohydrate are connected via serine- (and possibly threonine)-*O*-galactoside linkages.

Methylation analysis of fraction A (Table 3) shows that the nettle biopolymer molecules are branched. The

Table 1. Carbohydrate composition and protein content of neutral fraction A and acidic fraction B

	A	B
Arabinose	41.5	4.7
Rhamnose	3.8	19.3
Fucose	1.5	—
Xylose	3.0	—
Mannose	2.3	4.8
Galactose	23.9	12.8
Glucose	2.2	—
Galacturonic acid*	—	31.8
Hexosamine	trace	trace
Protein	10.0	3.5

* Estimated by the carbazol method [11].

Table 2. Amino acid composition of fraction A (μg/mg)

Hyp	Trace
Asp	11.5
Thr	9.0
Ser	9.4
Glu	9.2
Pro	5.5
Gly	7.8
Ala	8.6
Val	7.5
Met	0.6
Leu	6.1
Ile	4.0
His	1.5
Lys	4.6
Arg	4.5
Tyr	2.4
Phe	6.2

Table 3. Methylated sugars in hydrolysates of methylated fraction of material from stinging nettle

RTMG	Parent sugar	Primary mass fragments *m/e*	Area as % of total peak area
0.47	2,3,5-Tri-*O*-methylarabinose	45, 118, 161	33.0
0.58	2,3,4-Tri-*O*-methylarabinose	117, 118, 161, 162	6.4
0.81	3,5-Di-*O*-methylarabinose	45, 161, 190	8.2
0.86	3,4-Di-*O*-methylrhamnose	131, 190	11.7
0.86	2,5-Di-*O*-methylarabinose	45, 118, 234	
1.00	2,3,4,6-Tetra-*O*-methylglucose/mannose	45, 118, 161, 162, 205	trace
1.05	2,3-Di-*O*-methylarabinose	118, 189	5.8
1.14	2,3,4,6-Tetra-*O*-methylgalactose	45, 118, 161, 162, 205	3.8
1.28	2-Mono-*O*-methylrhamnose	118	trace
1.47	4-Mono-*O*-methylrhamnose	131, 261	trace
1.69	2,4,6-Tri-*O*-methylglucose	45, 118, 161	2.2
1.81	2,4,6-Tri-*O*-methylgalactose	45, 118, 161, 235	10.9
2.00	2,3,6-Tri-*O*-methylgalactose	45, 118, 234	1.7
2.44	2,3,4-Tri-*O*-methylgalactose	118, 162, 189, 234	2.7
4.08	2,3-Di-*O*-methylgalactose	118, 261	11.4

The sugars were determined as the peracetylated derivatives of the partially methylated deuterated glycitols of the respective sugars. Separation was achieved by GLC on a 200 × 0.25 cm column of OV-225, 3% on Varaport 30, with He as carrier gas and a column temperature of 190°

overall structure is evidently complex. Arabinofuranose residues occupy most of the end groups, together with some arabinopyranose and galactopyranose. The presence of 3,5-, 2,5- and 2,3-di-*O*-methyl-arabinose shows that arabinose is linked 1 → 2, 1 → 3 and 1 → 4/5. Unbranched galactose units are connected mainly 1 → 3, but some 1 → 4- and 1 → 6-linked residues are also present. The branch points are made up essentially by 4,6-di-*O*-substituted galactose units; however, trace amounts of 2- and 4-*O*-monomethylrhamnose indicate branching also on rhamnose.

Methylation analysis of the acidic fraction B proved partly unsuccessful, which was not surprising in view of the well known difficulties in obtaining fully methylated and undegraded uronic acid-containing polymers [3]. It was established, however, that the acidic fraction B contains arabinose end groups (furanose and pyranose). Rhamnose is linked 1 → 2 in the unbranched chain, and also constitutes branch points, since some of the rhamnose units are di-*O*-substituted at positions 2,4 and 3,4, respectively.

The results of two consecutive Smith-degradations of B were consistent with the methylation data. Arabinose was entirely destroyed after the 2nd degradation sequence, which is in keeping with its location as the end group and in an unbranched chain. Galactose and rhamnose showed no decrease from the 1st to the 2nd degradation, consistent with the occurrence of these sugars as branch points or as 1 → 3-linked residues. The Smith-alcohol fraction obtained after the 1st degradation contained propylene glycol derived from 1 → 2-linked rhamnose, and threitol originating from 1 → 4-linked galactose. As expected the Smith-alcohols from the 2nd degradation did not contain these compounds. Glycerol and ethylene glycol, resulting largely from arabinose (furanose and pyranose), were present in both Smith-alcohol fractions.

The present results indicate that the water-soluble stinging nettle biopolymer (fraction A) is cell wall glycoprotein [4, 5] of similar composition to that found for the corresponding *Cannabis sativa* glycoprotein [2, 6]. The glycoprotein nature of fraction B has not yet been confirmed.

EXPERIMENTAL

The general analytical methods used were the same as those employed previously [6]. *Urtica dioica* was collected in May–June in the Oslo area. After drying at room temp the leaves were milled to a powder and extracted by stirring with mixtures of $CHCl_3$ and MeOH to remove coloring matter and lipids. The dried residual powder (80 g) was stirred with H_2O (2 l.) for 4 hr at 40°. After filtration the plant material was re-extracted once with 1 l. of H_2O. The combined filtrates were dialysed against H_2O for 3 days followed by concn, filtration and lyophilization of the retentate fraction; yield of crude extract 2.4%.

The crude extract was purified by chromatography on a column of DEAE–Sephadex [6]. The following yields were obtained after dialysis and lyophilization of the respective fractions: Dilute buffer eluate (neutral fraction A) 4.8%; buffer containing 0.25 M NaCl eluate 11.5%; buffer containing 0.5 M NaCl eluate (acidic fraction B) 11.6%. Methylation [7, 8] and GC–MS of partially methylated alditol acetates [9] was performed as before [6] employing $NaBD_4$ to reduce the methylated sugars. The GLC was carried out on a 200 × 0.25 cm column of OV 225 (3%) on Varaport 30, with N_2 as carrier gas and a column temp. of 190°. In the GC–MS expts He was used as carrier gas.

Test for reducing end groups [10] was carried out by incubating the purified extract (20 mg) with $NaBH_4$ (40 mg) in H_2O (2 ml) for 18 hr at 20°. After acidification of the mixture with HOAc and removal of H_3BO_3 by repeated evapns with MeOH, the material was hydrolysed with 2 N H_2SO_4 (2 ml) at 100° for 5 hr. After neutralization the hydrolysate was passed through a Dowex 1 × 8 (OH^-) column which retains reducing sugars, but not alditols. The eluate was evapd to dryness, peracetylated with C_5H_5N and Ac_2O and subjected to GLC and GC–MS [6].

Smith-degradation and detection of serine-*O*-galactoside bond was carried out as described in ref. [2].

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