Chemistry of Natural Compounds, Vol. 39, No. 6, 2003

WATER-SOLUBLE POLYSACCHARIDES FROM *Narcissus poeticus* AND THEIR BIOLOGICAL ACTIVITY

K. S. Zhauynbaeva, M. Kh. Malikova, D. A. Rakhimov, and Z. A. Khushbaktova

UDC 547.917

A homogeneous native acetylated glucomannan of molecular weight 32000 was isolated from bulbs of N. poeticus. *A linear chain polymer consisting of* β-*1*→*4-bound D-manno- and D-glucopyranoses was found using periodate and chromic oxidation and methylation. The glucomannan has low toxicity and exhibits distinct hypolipidemic activity.*

Key words: *Narcissus poeticus*, glucomannan, structure, oligosaccharides, biological activity.

In continuation of research on polysaccharides from plants of the *Narcissus* genus [1, 2], we studied water-soluble polysaccharides (WSPS) from *N. poeticus* bulbs. The air-dried raw material was treated with alcohol and extracted with water with subsequent evaporation and precipitation by alcohol [1, 2]. The yield of WSPS was 6%. Paper chromatography (PC) of the WSPS hydrolysate detected mainly mannose and glucose in addition to arabinose, rhamnose, and galactose. Gel chromatography over a column packed with Sephadex G-50 afforded several compounds [3]. The WSPS were fractionally precipitated from aqueous solution by alcohol to produce three fractions of homogeneous polysaccharides in yields of 16% (I), 52% (II), and 9.3% (III).

The fraction of greatest yield (II) was used for chemical investigations. Fraction II consisted of glucose and mannose in a 1:30 ratio according to GC. Therefore, this fraction was a glucomannan (GM).

The GM is a white amorphous powder that dissolves in water to form viscous solutions with η_{rel} = 50.7 (*c* 1.0, H_2O) and [α] -27° (*c* 1.0, H_2O).

The IR spectrum contains absorption bands at 820 (pyranose ring), 880 (*β*-glucoside bond), 1250 and 1740 (acetyl group), and 3300-3600 cm^{-1} (OH absorptions). We found 8.6% O-acetyl groups in GM using the literature method [4]. The acetyls were cleaved by precipitation with Fehling solution. This formed the deacetylated glucomannan (DAGM), which is insoluble in water and aqueous base and soluble in concentrated formic acid. Such behavior is characteristic of natural partially acetylated mannans and glucomannans [5]. Periodate oxidation of DAGM consumes 0.92 mole of $NaIO_4$ per anhydrohexose unit and releases 0.05 mole of formic acid. The products of Smith degradation [6] are mainly erythrite and traces of glycerine and mannose.

GM was methylated by the Hakomori method [7] to determine the substitution pattern of the monosaccharides in the GM chain.

The GM permethylate was formolyzed and hydrolyzed. The hydrolysis products were studied by TLC with authentic samples. We detected mainly 2,3,6-tri-O-methyl-D-mannopyranose in addition to traces of 2,3,6-tri-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-mannopyranose, and di-O-methylhexose.

The negative specific rotation of GM and its permethylate is consistent with *β*-glycoside bonds. This was confirmed by oxidation of acetylated GM with chromic anhydride [8]. The study of the product of Smith degradation and methylation led to the conclusion that GM is a linear polymer with *β*-1→4 bound D-manno- and D-glucopyranoses.

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 430-432, November-December, 2003. Original article submitted September 5, 2003.

0009-3130/03/3906-0520$25.00 ©2003 Plenum Publishing Corporation

TABLE 1. Effect of Glucomannan from *N. poeticus* on Development of Hyperlipidemia Caused by Administration to Animals of Triton WR-1339 (M ± m, n = 8)

Exptl. conditions	Blood serum content			
	cholesterol		triglycerides	
	M	% of control	M	% of control
Untreated animals	1.90±	-	0.88	-
Triton-1339 (control)	6.70±	252	5.98	579
Glucomannan, 50 mg/kg	5.16	23	5.53±	7.5
Glucomannan, 100 mg/kg	4.73	29.4	4.78±	20

Partial hydrolysis was used to determine the sequence of monosaccharide units in the chain and the structure of the main chain. PC of the hydrolysate using known standards identified the following oligosaccharides: 4-O-β-D-glucopyranosyl-D-mannopyranose, 4-O-β-D-mannopyranosyl-D-mannopyranose, 4O-β-D-mannopyranosyl-D-glucopyranose, O-β-D-manno-yranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose, and O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranosyl-(1→4)-O-β-D-mannopyranose.

Based on the results we conclude that GM from *N. poeticus* has a slightly branched linear structure with the repeating unit: Man*p*-Man*p*-Glc*p*-Man*p*-Man*p*-Man*p*-Man*p*-Man*p*-.

Thus, GM from *N. poeticus* bulbs has the structure of the most common type of plant GMs. However, it differs from known ones [9-11] in the ratio of sugars, molecular weight, content of acetates, and distribution of hexose units.

Next, certain aspects of the biological activity of WSPS from *N. poeticus* bulbs were studied. It was found during a determination of the acute toxicity that administering GM to white mice in doses of 500-1000 mg/kg had no effect; of 1500-2250 mg/kg, a certain sedative effect but no toxic or lethal effects.

Considering that one of the important biological effects of polysaccharides is the hypolipidemic one, we studied their ability to lower the levels of cholesterol, β-lipoproteides, and triglycerides in blood serum of control animals and those with experimental hyperlipidemia.

Administering GM once to control mice in doses of 10-25 mg/kg had no significant effect on the level of cholesterol and triglycerides in blood serum. Increasing the GM dose to 50-100 mg/kg lowered the cholesterol level by 15-18% ($p < 0.05$).

GM prevented rather markedly the development of hypercholesterolemia in experimental animals after administration to them of Triton WR-1339. A distinct reduction of the cholesterol level was observed. The triglyceride content was reduced to a lesser extent (Table 1).

EXPERIMENTAL

Chromatography was performed on Filtrak FN-11,16 paper by a descending method using the solvent system *n*-butanol:pyridine:water (6:4:3, 1); TLC, on Silufol UV-254 plates using MEK:NH_4OH (1%) (30:4, 2).

Spots were developed using anilinium acid phthalate (1) and periodate:$KMnO_4$:benzidine (2).

Total acid hydrolysis of polysaccharides was performed in H_2SO_4 (2 N) for 6 h on a boiling-water bath.

Hydrolysates were neutralized with $BaCO_3$, deionized over cation exchanger KU-2 (H^+), and analyzed by PC.

Monosaccharides and polyols were analyzed by GC as acetates of aldononitriles and polyols, respectively.

GC was carried out on a Chrom-5 instrument with a flame-ionization detector using a steel column (0.3 × 200 cm) packed with XE-60 (5%) on Chromaton NAW (0.200-0.250 mm), He carrier gas (60 mL/min), and 200°C.

IR spectra were recorded on a Perkin—Elmer Model 2000 IR-Fourier spectrometer in pressed KBr disks.

Viscosity was determined in an Ostwald viscometer with D = 0.77 mm. The acute toxicity of GM was determined using white mongrel mice of mass 18-20 g with internal administration of the studied preparation. Hypolipidemic properties of WSPS were studied using 60 mongrel white male rats of mass 180-220 g. Hyperlipidemia was induced in the rats by a single i.p. administration of Triton WR-1339 at a dose of 225 mg/kg. GM was administered once to control rats and twice to those with hyperlipidemia, once simultaneously with Triton WR-1339 and another time at 2 h before sacrificing. The contents of

cholesterol and triglycerides in blood serum were determined as before [12, 13].

WSPS Isolation. Air-dried raw material (20 g) was treated with boiling alcohol (200 mL, 1:10 and 1:5) for 2 h. The remaining raw material was dried and extracted with water (1:10 and 1:5) at room temperature. The extract was separated by centrifugation, evaporated, and precipitated with alcohol (1:3). The precipitate was separated and dried. Yield, 1.2 g.

WSPS Fractionation. WSPS (3 g) was dissolved in water (250 mL) and treated dropwise with alcohol while stirred vigorously. Fraction I was obtained after addition of 250 mL; fraction II, after 150 mL; fraction III, after 65 mL. The fraction yields were 16, 52, and 9.3%, respectively.

Amount of O-acetyls was determined as before [4]. Found: 8.6% O-acetyls.

DAGM was prepared by the literature method [5].

Periodate Oxidation and Smith Degradation. DAGM (100 mg) was oxidized with sodium periodate solution (0.05 M) at 15°C in the dark for 15 d. The excess of periodate was destroyed by adding ethyleneglycol (1 mL). The oxidation product was reduced by an excess of $NaBH_4$ and hydrolyzed by H_2SO_4 (5 mL, 0.5 N) for 6 h at 100°C. PC of the hydrolysate (system 1, developers 1 and 2) detected erythrite, glycerine, and mannose.

GM Methylation. Polysaccharide (100 mg) was methylated by the Hakomori method [7] to produce the fully methylated polysaccharide (80 mg). The IR spectrum lacked OH absorption bands.

Formolysis and Hydrolysis of the Permethylate. The permethylate (50 mg) was heated on a boiling-water bath with formic acid (5 mL, 90%) for 1 h, cooled, and evaporated. The solid was dissolved in H_2SO_4 (5 mL, 0.5 N) and hydrolyzed for 16 h at 100°C. The hydrolysate was worked up as usual. TLC (system 2, developer 1) detected 2,3,4,6-tetra-O-methyl-D-mannose, 2,3,6-tri-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-mannose, and di-O-methylhexose.

Partial Hydrolysis. GM (100 mg) was dissolved in formic acid (15 mL, 90%), reduced in concentration to 45%, and hydrolyzed for 4 h at 80°C. The solution was cooled, centrifuged, evaporated to dryness, and hydrolyzed by H_2SO_4 (0.5 N) for 10 min at 100°C. PC of the hydrolysate (system 1, developer 1) identified glucosylmannose, mannobiose, mannosylglucose, mannotriose, and mannotetraose by comparison with standards.

REFERENCES

1. D. A. Rakhimov and K. S. Zhauynbaeva, *Khim. Prir. Soedin.*, 277 (1997).
2. M. Kh. Malikova, K. S. Zhauynbaeva, and D. A. Rakhimov, *Khim. Prir. Soedin.*, 158 (2002).
3. H. Determann, *Gelchromatographie: Gelfiltration, Gelpermeation, Molekuelsiebe*, Springer-Verlag, Berlin (1967).
4. A. P. Kuznetsov and B. N. Stepanenko, *Dokl. Akad. Nauk SSSR*, 214 (1976).
5. M. Kh. Malikova and D. A. Rakhimov, *Khim. Prir. Soedin.*, 21 (1986).
6. B. N. Stepanenko, *Carbohydrate Chemistry* [in Russian], Vysshaya Shkola, Moscow (1978).
7. S. Hakomori, *Biochem. J.*, **55**, 205 (1964).
8. J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, **26**, 661 (1972).
9. K. Kato, Y. Kawacuchi, and T. Mizuno, *Carbohydr. Res.*, **29**, 469 (1973).
10. M. Tomoda, M. Yokoi, A. Torigoe, and K. Maru, *Chem. Pharm. Bull.*, **28**, 11, 3251 (1980).
11. K. S. Zhauynbaeva, M. Kh. Malikova, and D. A. Rakhimov, *Khim. Prir. Soedin.*, 186 (2003).
12. L. L. Abell, B. B. Lery, and B. B. Brodic, *Biol. Chem. J.*, **195**, 357 (1952).
13. B. P. Neri and C. S. Frings, *Clin. Chem.*, **19**, 1201 (1973).