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# GLUCOMANNAN FROM *Narcissus poeticus* STUDIED BY PMR AND $^{13}$C NMR SPECTROSCOPY

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*Native acetylated glucomannan of molecular weight (MW) 32000 with a glucose:mannose ratio 1:30 was isolated from bulbs of* Narcissus poeticus. *Glucomannan was depolymerized to a fragment of MW 15000 with an unchanged primary structure and was studied using PMR and $^{13}$C NMR spectroscopy. It was found that the linear chain of the biopolymer consists of* β→*1-4-bound D-gluco- and D-mannopyranose units and the O-Ac groups are localized on C-2, C-3, and C-6 hydroxyls in certain anhydromannose units.*

**Key words:** *Narcissus poeticus*, glucomannan, isolation, PMR and $^{13}$C NMR spectroscopy, structure.

We previously reported on a study of carbohydrates isolated from bulbs of *Narcissus poeticus* L. (Amaryllidaceae) [1]. Our goal was to isolate glucomannans and establish their primary structure using PMR and $^{13}$C NMR spectroscopy.

Aqueous extracts of ground air-dried bulbs were precipitated with alcohol to produce water-soluble polysaccharides (WSPS) in 6.1% yield. Glucose and mannose in a 1:30 ratio were detected in the hydrolysate by PC and GC. Therefore, the WSPS is a glucomannan (GM). The IR spectrum of the GM exhibits absorption bands similar to those in spectra of known 1,4-β-manno-containing polysaccharides [2, 3]. The low-frequency region of the spectrum contains absorption bands at 810 and 865 cm$^{-1}$, which indicate the presence of mannose units and the β-configuration for the anomeric centers, respectively. In addition, the sugar ring is the pyranose isomer. Absorption bands at 1740 and 1250 cm$^{-1}$ are due to O-Ac groups in the GM. Treatment of GM with Fehling solution or base saponfies the acetyls (lack of the absorption bands noted above) and renders it insoluble in water. Therefore, narcissus GM in the plant material is partially acetylated, which explains its solubility in water.

The GM structure was studied by PMR and $^{13}$C NMR spectroscopy after the preliminary characterization.

Solutions of high viscosity were formed because of the high molecular weight (MW) of the GM. This interfered with the study of their high-resolution spectra. Therefore, we partially depolymerized GM with HCl (0.1 N) for 45 min at 85°C. The isolated depolymerized GM had an unchanged primary structure according to the practically identical ratio of monomers and identical IR spectrum although the MW was halved (Table 1).

Polysaccharide was studied using one-dimensional PMR and $^{13}$C NMR spectroscopy and two-dimensional (2D) homonuclear $^{1}$H/$^{1}$H COSY, TOCSY, and ROESY and heteronuclear $^{1}$H/$^{13}$C HSQC. The $^{13}$C NMR spectrum of the polysaccharide (Fig. 1*a*) contained in the resonance region of anomeric C atoms two strong (δ 101.4 and 103.7) and two weak (δ 100.1 and 101.0) signals. The strongest signals in the spectrum were peaks for $\underline{C}H_3\underline{C}O$ groups (δ 21.5 and 175.1, respectively). This indicates that the polymer is highly O-acetylated. A $^{13}$C NMR test for the number of bonded protons (APT [4]) showed that signals for most hydroxymethyls occur at δ 64.4 whereas only weak signals were observed in the resonance region of unsubstituted groups (δ 61.9-62.8). Thus, almost all sugars in the polymer are acetylated at C-6.

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TABLE 1. Properties of Starting and Depolymerized Glucomannan from *Narsiccus poeticus* L. Bulbs

| Parameters | Glucomannan | |
|---|---|---|
| | starting | depolymerized |
| Glc:Man ratio | 1:30 (GC) | 1:30 (GC)<br>1:30 (NMR) |
| MW* | 32000 | 15000 |
| $\eta_{char.}$ | 8.8 | 4.5 |
| IR spectra | Identical | |

*Sed. analysis.

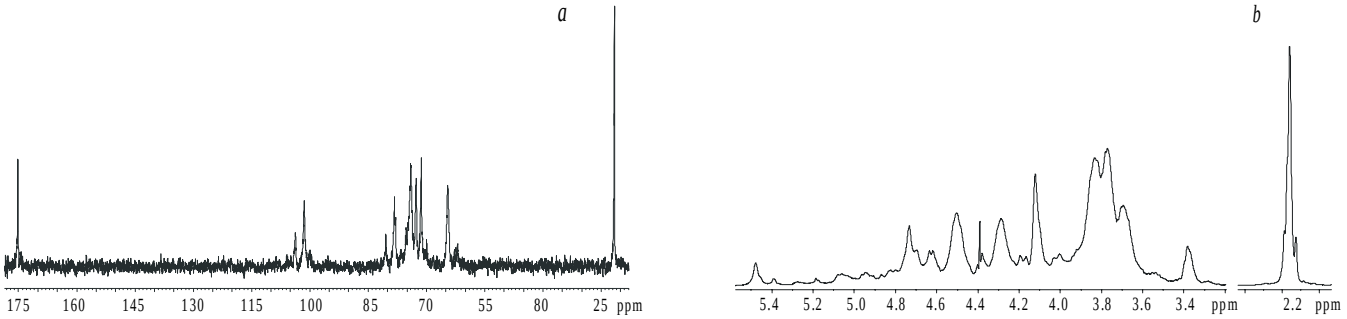


Fig. 1. $^{13}C$ NMR (1.75 MHz) (*a*) and PMR (500 MHz) (*b*) spectra of polysaccharide. Strong-field signals at $\delta_H$ 2.1-2.2 are diminished by a factor of four.

The strongest signals in the PMR (Fig. 1*b*) are also those for $CH_3CO$ groups at δ 2.16 (main signal) and 2.13 and 2.19 (weaker signals). The presence of three signals indicates that the polysaccharide is acetylated not only at the C-6 hydroxyls but also at others. This was confirmed by the presence in the weak-field region of the PMR spectrum of several signals, the chemical shifts of which did not correspond to those for anomeric protons of pyranoses with the β-configuration of the glycoside (below 5 ppm).

At first the use of 2D homonuclear $^1H/^1H$ COSY, TOCSY, and ROESY, which are usually used to decipher PMR spectra, was hindered by a lack of data for the location of signals of anomeric protons. Therefore, the 2D $^1H/^{13}C$ heteronuclear HSQC spectrum (Fig. 2) was analyzed before the proton spectra were deciphered. This revealed the positions for the resonances of the anomeric protons. The COSY, TOCSY, and ROESY spectra, in turn, were deciphered using the known signals for the anomeric protons as starting points for assigning the remaining signals. Based on the analysis of correlation peaks in the COSY, TOCSY, and ROESY spectra, it was found that all units in the polysaccharide have β-manno- or β-glucopyranose configurations. The spectrum contains, among others, correlation peaks for anomeric protons with H-4 protons of Man*p* or Glc*p* units. This confirms the presence of β-(1→4)-bonds between them. The position of the O-acetyls in the units was determined using the shift of the proton signals for the O-acetylated C atom to weak field compared with the weak field in the free units [5]. This feature revealed signals of units acetylated at the C-6 hydroxyl (Man*p* and Glc*p*, Table 2) and Man*p* units acetylated at the C-2 and C-3 hydroxyls. Signal assignments in the PMR enabled a more detailed assignment of the $^{13}C$ spectrum from correlation peaks in the 2D HSQC spectrum (Table 3). Independent assignment of signals in the $^{13}C$ spectrum confirmed the presence of O-acetyls in the positions noted above using shifts of the C signals belonging to the O-acetyls to weak field. Neighboring C signals shifted to strong field compared with their signals in nonacetylated units.

TABLE 2. PMR Chemical Shifts of Polysaccharide (δ, ppm)*

| Unit | H-1 | H-2 | H-3 | H-4 | H-5 | H-6 | H-6′ |
|---|---|---|---|---|---|---|---|
| →4)-*β*-D-Man*p*-(1 → | 4.73 | 4.12 | 3.78 | 3.84 | 3.53 | 3.80 | 3.80 |
| →4)-*β*-D-Glc*p*-(1 → | 4.65 | 3.38 | 3.69 | 3.69 | 3.50 | 3.90 | 3.73 |
| →4)-*β*-D-Man*p*-6-OAc-(1 → | 4.73 | 4.12 | 3.78 | 3.84 | 3.83 | 4.50 | 4.28 |
| →4)-*β*-D-Glc*p*-6-OAc-(1 → | 4.65 | 3.38 | 3.69 | 3.69 | 3.69 | 4.62 | 4.18 |
| →4)-*β*-D-Man*p*-2,6-OAc-(1 → | 4.94 | 5.48**; 5.39*** | 4.02 | 3.83 | 3.75 | 4.50 | 4.28 |
| →4)-*β*-D-Man*p*-3,6-OAc-(1 → | 4.81 | 4.19 | 5.06 | 4.10 | 3.75 | 4.50 | 4.28 |

*C$\underline{H}_3$CO at δ 2.16 (main signal) and 2.13 and 2.19 (minor signals); **strong signal; ***minor signal.

TABLE 3. $^{13}$C NMR Chemical Shifts of Polysaccharide (δ, ppm)*

| Unit | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 |
|---|---|---|---|---|---|---|
| →4)-*β*-D-Man*p*-(1 → | 101.4 | 71.3 | 72.6 | 78.2 | 76.5 | 62.3; 62.8 |
| →4)-*β*-D-Glc*p*-(1 → | 103.7 | 74.2 | 75.2 | 80.4 | 76.0 | 61.9 |
| →4)-*β*-D-Man*p*-6-OAc-(1 → | 101.4 | 71.3 | 72.6 | 78.2 | 74.0 | 64.4 |
| →4)-*β*-D-Glc*p*-6-OAc-(1 → | 103.7 | 74.2 | 75.2 | 80.4 | 74.0 | 64.4 |
| →4)-*β*-D-Man*p*-2,6-OAc-(1 → | 100.1 | 72.6 | 71.3 | 77.9 | 74.0 | 64.4 |
| →4)-*β*-D-Man*p*-3,6-OAc-(1 → | 101.0 | 69.9 | 74.5 | 74.4 | 74.0 | 64.4 |

*$\underline{C}H_3\underline{C}$O at δ 21.6 and 175.1, respectively.

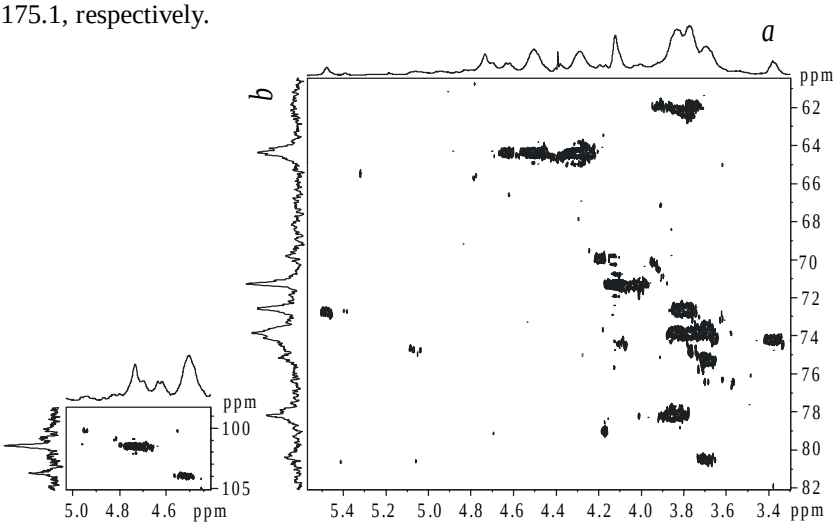


Fig. 2. Parts of the HSQC spectrum of polysaccharide. One-dimensional PMR (500 MHz) and $^{13}$C NMR (125 MHz) spectra are marked *a* and *b*, respectively.

Signal assignments in the $^{13}$C NMR spectrum enabled also a determination of the Man*p*:Glc*p* ratio in the polymer as 30:1. Comparison of integrated intensities of signals at 64.4 and 61.8-62.9 ppm determined the degree of acetylation of the units at the C-6 hydroxyl as 90%. The degree of substitution of the Man*p* units at the C-2 and C-3 hydroxyls that was found from the integrals of the relatively well resolved proton signals was estimated as 10-12%. Signals of Glc*p* units acetylated at the C-2 or C-3 hydroxyls were not found in the spectra. Apparently this was due to the low content of these units in the polymer compared with those of Man*p*.

## EXPERIMENTAL

IR spectra were recorded on a Perkin—Elmer System 2000 IR-Fourier spectrometer in pressed KBr disks. A total of 100 scans was used.

NMR spectra were recorded on Bruker AM-300 and DRX-500 spectrometers for $D_2O$ (99.95%) solutions at 60°C. Chemical shifts were calculated from the acetone signal as an internal standard ($\delta_H$ 2.225 and $\delta_C$ 31.45). Two-dimensional spectra were recorded on the Bruker DRX-500 instrument using the company's standard methods. The duration of mixing in the ROESY spectra was 30 ms.

Sedimentation analysis was performed on a MOM-3170 ultracentrifuge. Sedimentation curves were obtained at $C$ = 10 mg/mL in water, 50000 rpm, 20°C, and 10 min run time. The results were $S = 2.9 \cdot 10^{-13}$ and $D = 11.05 \cdot 10^{-7}$.

PC was performed on Filtrak FN-11 paper using butanol:pyridine:water (6:4:3) by a descending method. Spots were developed using anilinium acid phthalate. The quantitative composition of monosaccharides was determined as aldononitrile acetates. GC of sugar derivatives was recorded on a Chrom-5 instrument using a flame-ionization detector, steel column (0.200 × 0.3 cm), 5% Silicone XE-60 on Chromaton NAW (0.200-0.250 μm), column temperature 200°C, He carrier gas, and 60 mL/min. Solution viscosity of GM was measured on an Ostwald viscosimeter with capillary diameter 0.73 mm.

**GM** were isolated as before [1] and hydrolyzed in $H_2SO_4$ (2 N) for 5 h at 100°C.

**GM Depolymerization.** GM (0.5 g) was dissolved in HCl (50 mL, 0.1 N) and hydrolyzed at 85°C for 45 min. The hydrolysate was cooled to room temperature and precipitated by ethanol in a 1:3 ratio. The solid was separated by centrifugation, washed with alcohol, and dried in vacuum over $P_2O_5$. The yield of depolymerized GM was 56.8% (of starting WSPS).

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