POLYSACCHARIDES OF *Eremurus*.

XIII. STUDY OF THE STRUCTURE OF THE PARTIALLY ACETYLATED GLUCOMANNANS BY THE ^{13}C NMR METHOD

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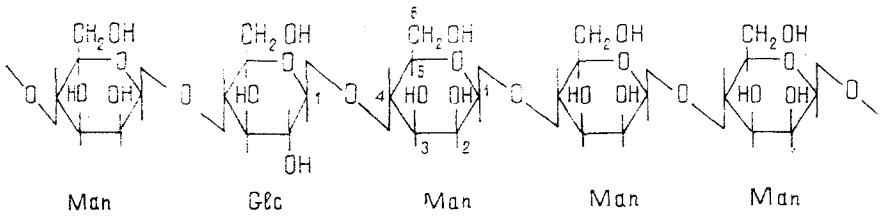
The ^{13}C spectra of the glucomannans from *Eremurus lactiflorus* and *E. luteus* are analyzed in comparison with the spectra of low-molecular-weight model compounds. It has been shown that the linear polymer chain consists of 1 → 4-bound glycosidic residues in the pyranose form, which confirms the results of earlier chemical investigations of these polysaccharides.

Previously, by the fractionation of a water-soluble polysaccharide from the tuberous roots of *Eremurus lactiflorus* and *E. luteus* [1-3] and the study of one of the fractions of the glucomannan by various chemical methods, a structure with repeating units was established.



The glucommannans isolated from plants of the genus *Eremurus* have not previously been studied by the ^{13}C NMR method. The interpretation of the spectra of glucomannans is necessary in order to determine their characteristic features, and, in the final account, it must assist in establishing the chemical structures of similar biopolymers.

Below we give the characteristics of the glucomannan:

Glucomannan from	Ratio of glucose and mannose		IR spectrum [1] cm^{-1}	Mol. wt.
	GLC [1-3]	^{13}C NMR		
E. lactiflorus	1:2.8	1:2.5	3600-3200 (OH) 1730 and 1250 (ester group)	79,000
E. luteus	1:3.4	1:3.3	880 (β-glucosidic bond) and 815 (hexapyranose ring)	150,000

Figure 1 gives the ^{13}C NMR spectrum of the glucomannan from *E. lactiflorus* in the region of resonance from 60 to 105 ppm. This region of the spectrum is almost identical for all the polysaccharides investigated, only the intensities of the two series of signals differing. The first series is formed by the stronger signals at 101.5, 77.85, 76.6, 72.9, and 71.5 ppm. According to the results of the analysis of the polymers for their monomeric compositions [1, 2], it is natural to assign this series of signals to the resonance of the carbon atoms of the mannose residues. The second series forms weaker signals at 103.8, 80.0, 76.4 ("shoulder" on the strong 77.6 ppm peak), 75.6, and 74.45 ppm, obviously relating to the

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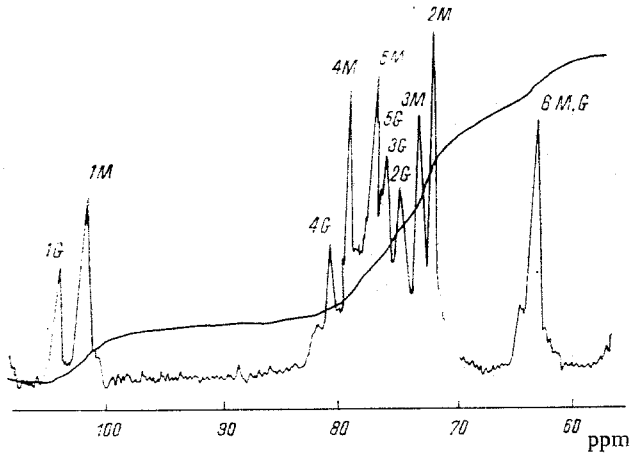

Fig. 1. ^{13}C NMR spectrum of the glucomannan from *E. lactiflorus* (M — mannose; G — glucose).

resonance of the carbon atoms in the glucose residues. So far as concerns the signal at 62.0 ppm, located in the region of the resonance of carbon atoms unsubstituted by hydroxymethylene groups [4], its integral intensity fairly accurately corresponds to the sum of the integral intensities of the two peaks in the region of the resonance of the anomeric carbon atoms (103.8 and 101.5 pm). From this fact two important conclusions concerning the structure of polysaccharides follow directly:

The polysaccharides studied contain no residues substituted at C_6, since in this case, as the result of a downfield shift of the C_6 signal through the α-effect of glycosylation [4], we should observe a decrease in the integral intensity of the peak at 62.0 ppm in comparison with the total intensity of the peaks from C_1; and

both residues in the polysaccharides exist in the pyranose form, since glucose and mannose furanosides are characterized by signals from C_6 at 64-65 ppm, regardless of the configurations of their glycosidic centers [5], while in the spectra of the polysaccharides investigated this region is free from signals.

An analysis of the region of resonance of the aromatic carbon atoms unambiguously determines the configuration of the glycosidic centers of the glucopyranose residues: The position of a signal of lower intensity (103.8 ppm) leaves no doubt that the glucopyranose residues have the β configuration, since for α-glucopyranoside residues the C_1 signals are never observed in a field less than that corresponding to a shift of 102 ppm [4].

The configuration of the glycosidic centers of the mannose residues cannot be determined from the position of the C_1 signal of these residues — the C_1 signals of the α and β anomers of mannopyranose and mannopyranosides have overlapping ranges of resonance [4]. However, from the positions in the spectrum of all the lines belonging to the mannose residues (the series with the greatest intensity) it is possible to determine not only the configuration of the glycosidic centers but also the type of substitution of these residues.

In actual fact, on comparing the ^{13}C NMR spectra of the polysaccharides under investigation with the spectra of model compounds — a series of monosubstituted methyl ethers of D-mannopyranose [6] — it becomes obvious that, in the polysaccharides, substitution at C_2 or C_3 is excluded (regardless of the configuration of the glycosidic centers of the mannopyranose residues), since with this type of substitutions the spectra should include signals from C_4 with an unsubstituted group in the resonance region of 66-68 ppm. In the spectra of the polysaccharides, this region contains no signals whatever.

Since substitution at C_6 is excluded (see above), the only remaining possibility is substitution at C_4. For mannopyranose residues with the α configuration of the glycosidic center substituted at C_4, three signals in the region of resonance between 72.5 and 71.0 ppm (from C_2, C_3, and C_5), are characteristic, and for those with the β configuration only one (from C_2) [6]. In the spectra of the polysaccharides there is, in each case, only one strong signal in this region (71.5 ppm). It follows from this that the glycosidic centers of the 4-O-substituted mannopyranose residues in the polysaccharides studied have the β configuration of the glycosidic center.

A definitive assignment of all the signals of the mannopyranose residues in the polysaccharides was made by comparing their spectra with the spectra of methyl β-D-mannopyranoside and 4-O-methyl-β-D-mannopyranose [6]. The first of the two model compounds makes it possible to determine the positions of the C_1 and C_2 signals in the mannopyranosyl residues of the polymer, and the second those of the C_3, C_4, C_5, and C_6 signals, since it is precisely these carbon atoms in the polymer residues and in the model compounds that have similar immediate environments.

The good agreement of the chemical shifts of the corresponding carbon atoms in the polysaccharides (what is in view here is the series of stronger signals) and in the model compounds referred to above definitively confirms the correctness of the determination of the configuration of the glycosidic centers and of the type of substitutions in the mannopyranose residues of the polysaccharides.

The ^{13}C NMR chemical shifts of the glucomannans and of some model compounds are given below (R indicates a "reducing" end, and N a "nonreducing" end; the chemical shifts of the carbon atoms having similar closest environments to the corresponding carbon atoms of the polysaccharides are underlined):

Compound	Residue	C_1	C_2	C_3	C_4	C_5	C_6	O-Me	Literature
Methyl-β-D-mannopyranoside		101,1	71,4	74,0	67,9	77,4	62,2	58,2	[6]
4-O-Methyl-β-D-mannopyranose		94,6	72,1	73,9	77,7	76,3	61,8		[6]
Methyl-β-cellobioside	R	104,5	74,2	75,9	80,3	76,4	61,8	59,9	[7,8]
	N	103,9	74,6	77,2	71,2	77,5	62,4		
Glucomannans	Man	101,5	71,5	72,9	77,85	77,6	62,0		
	Glc	103,8	74,45	76,4	80,0	75,6	62,0		

Analysis of the group of low-intensity signals belonging to the carbon atoms of the β-glucopyranoside residues permits the question of the type of substitution in these residues to be answered. A signal with a chemical shift of less that 77 ppm from C_5 is characteristic for unsubstituted β-glucopyranoside residues or such residues substituted at C_2 or C_3; in such residues substituted at C_4 or C_5 the signal of this carbon atom is shifted upfield (~76 ppm) because of the β-effect of glycosylation [7, 8]. In the series of low-intensity signals, two (at 103.8 and 80.0 ppm) must be due to the resonance of carbon atoms participating in the formation of the interunit bond [4, 7]. Then one of the remaining signals of the series must belong to C_5; but neither of them has a chemical shift below 77 ppm and therefore the β-glucopyranoside residues in the polysaccharides are substituted at C_6 or C_4. Since substitution at C_6 is excluded (see above), it remains to assume that they are substituted at C_4. A definitive confirmation of this hypothesis is the good agreement of the low-intensity signals in the spectra of the polysaccharides and of the model compound methyl β-D-cellobioside [7] (as in the preceding case, one must compare the C_1 and C_2 chemical shifts of the residue at the "nonreducing" end and the C_3-C_6 "reducing" end of the cellobioside with the chemical shifts of the corresponding carbon atoms of the β-D-glucopyranoside residues in the polymers).

According to the results of chemical analysis [1], the polysaccharides of *E. lactiflorus* and *E. luteus* are partially acetylated. In the ^{13}C NMR spectra of these polysaccharides there are in fact small signals in the 21.5-22 ppm and 174-175 ppm regions corresponding to the resonance of methyl and carboxyl groups of acetates [4]. However, the relatively low sensitivity and resolving capacity of the instrument did not permit us to determine the degree of acetylation and the positions of the acetate groups in the polysaccharides with a sufficient degree of accuracy.

Thus, according to the results of ^{13}C NMR spectroscopy, the polysaccharides of *E. lactiflorus* and of *E. luteus* are glucomannans in which D-glucopyranose and D-mannopyranose form a linear chain of β-1,4-linked residues. In all the polysaccharides, the mannose is the predominating component: some residues are partially acetylated. These facts are in complete harmony with the results of earlier investigations performed [1-3].

EXPERIMENTAL

^{13}C NMR spectra were recorded on a WR-60 instrument (Bruker) with a working frequency for carbon nuclei of 15.08 MHz using solutions of the polysaccharides in ^2H_2O at 80°C. Metha-

nol was used as internal standard (50.15 ppm). The volume of the memory was 8/4 K, the length of pulse 10 μsec (85°), the pulse repetition frequency 0.7 sec, the width of the spectrum 3750 Hz, and the number of accumulations 100-150 thousand.

SUMMARY

The ^{13}C NMR spectra of the glucomannans from *E. lactiflorus* and *E. luteus* have been analyzed in comparison with the spectra of low-molecular-weight model compounds. It has been shown that the linear polymeric chain consists of 1→4-β-linked glycoside residues in the pyranose form, which confirms the results obtained previously from chemical investigations of these polysaccharides.

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PARTIAL METHYLATION OF METHYL α-D-GLUCOPYRANOSIDE. I. LIQUID CHROMATOGRAPHY OF THE ACETATES OF THE TRI-O-METHYL ETHERS OF METHYL α-D-GLUCOPYRANOSIDE

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A method is described for obtaining the acetates of the tri-O-methyl ethers of methyl α-D-glucopyranoside via the partial methylation of methyl α-D-glucopyranoside and the subsequent preparative liquid chromatography of the acetates of the tri-O-methyl ethers.

At the present time, methods have been described for obtaining methyl ethers of monosaccharides which are based on the partial methylation of methyl glycosides followed by chromatography of the mixtures obtained [1-6]. For this purpose the preparative GLC of methyl ethers of methyl α-D-mannopyranoside [1], of methyl β-D-xylopyranoside [2], of methyl α-D-galactopyranoside [3], and of methyl α-L-rhamnopyranoside [4] have been used. Liquid chromatography on silica gel has been employed successfully for the separation of methyl ethers of methyl α-L-rhamnopyranoside [5]. An advantage of liquid chromatography on silica gel in comparison with GLC consists in the possibility of using larger amounts of substances. However, the slight differences in the chromatographic mobility of the methyl ethers do not permit the wide use of this method. With the aid of liquid chromatography on silica gel we have succeeded in achieving the separation of the acetates of the tri-O-methyl ethers of methyl α-D-glucopyranoside and of obtaining all the tri-O-methyl ethers in the individual state with good yields.

The partial methylation with dimethyl sulfate in alkali of 50 g of methyl α-D-glucopyranoside yielded 31 g of a mixture of methyl ethers soluble in the organic phase and 22 g soluble in the aqueous phase. It was shown by TLC that the organic phase (fraction A) contained tetra-, tri-, di-, and mono-O-methyl ethers, and the aqueous phase (fraction B) di- and mono-

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