Carbohydrate Polymers 84 (2011) 173–179

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

A simple methylation method for obtaining water-soluble O-methyl glucomannan derivatives

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ARTICLE INFO

Article history:
Received 4 October 2010
Received in revised form 8 November 2010
Accepted 9 November 2010
Available online 18 November 2010

Keywords:
Amorphophallus
Glucomannan
Methylation

ABSTRACT

Water-soluble O-methyl glucomannan derivatives (O-MGs) with various degrees of substitution were obtained by direct alkylation reaction of an insoluble glucomannan with methyl iodide without any organic solvent including methanol and dimethyl sulfoxide. The structure of O-MGs was characterized by ^{1}H, ^{13}C, ^{1}H ^{13}C NMR-HSQC, and ^{1}H ^{13}C NMR-HMBC spectra. The reactive conditions for synthesizing of O-MG derivatives were also evaluated. The results shown that the optimal conditions for methylation of glucomannan were pH 10, temperature of 40 °C for 3 h. The degree of substitution ($\overline{DS}$) of O-substitution increased from 0.09 to 0.175 since the ratio (w/v) of glucomannan/methyl iodide changed from 1/3 to 1/15.

1. Introduction

Amorphophallus-sp. is grown in mountain or hilly areas in subtropical regions, mainly in the South East of Asia, including Vietnam. It has been used as food and food additives in China and Japan for more than 1000 years. Glucomannan (GM) is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods (hemicellulose), roots, tubers and many plants bulbs. Despite the variety of sources, the most common used type of GM is named konjac glucomannan (KGM), which is extracted from tubers of *Amorphophallus* plants. Irrespective of its origin, GM is composed of β-1,4-linked D-mannose and D-glucose monomers. However, the mannose/glucose monomer ratio may vary depending on the original source of GM (Gao & Nishinari, 2004; Ishrud, Zahid, Viqar, & Pan, 2001; Koroskenyi & Mc Carthy, 2001; Xiao, Gao, & Zhang, 2000). Glucomannan and its derivatives have been investigated and used in many fields, such as food, film-forming, biomedical... (Vuksan, Jenkins, et al., 1999; Vuksan, Stevenpiper, et al., 2000). Although, the applications of glucomannan were restricted for its viscosity and low solubility, so various studies were conducted to improve the ability of dissolving of glucomannan in organic solvents, polar solvents and especially in water by chemical modification techniques, such as methylation (Hakomori, 1964; Haworth & Percival, 1932), tosylation (Takechi & Furuhata, 1999) and carboxyalkylation (An, Dong, Dung, & Thien, 2010; An, Dong, Dung, Thien, & Du, 2010; Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002).

The methylation of glucomannan has been firstly done by Haworth et al., and up to now, this method has been used as an important method in characterization the structure of polysaccharides. This method has been done also for aiming at to increase the solubility of this polysaccharide in water and organic solvents (Hakomori, 1964; Haworth & Percival, 1932; Kishida, 1979; Shatwell, Sutherland, Ross-Murphy, & Dea, 1991). However, the method for methylation of glucomannan was quite complicated. For example, according to Hakomori et al. for obtaining methyl glucomannan derivative, the dry glucomannan was dissolved in dry dimethyl sulfoxide and then methylated with methyl iodide in the presence of methylsulfinyl carbanion (Hakomori, 1964). Perhaps, in our opinion, this method was only significant when it was used to investigate the structure of polysaccharides because the use toxic and expensive chemicals as methanol could provoke some disadvantages of processing and applying this product both in foodstuff and medicine. With the aim to make the water-soluble derivatives of glucomannan, the use of methanol was discarded because the structure of modified polymers should be not only as close as possible to that of initial polymers but also were water-soluble.

Glucomannan isolated from *Amorphophallus paeoniifolius* was almost insoluble in water at ambient temperature. For enlarging the application of glucomannan and simplifying its preparation, in this work, the glucomannan was methylated by methyl iodide only in aqueous medium without methanol as well as dimethyl sulfoxide for obtaining the water-soluble O-methyl glucomannan derivatives. The reactive conditions for synthesizing of O-CMs such as ratio (w/v) of GM/MI and pH were also evaluated. The structure of O-methyl glucomannan derivatives was characterized carefully by mean of NMR spectroscopy as evidences.

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doi:10.1016/j.carbpol.2010.11.021

2. Experimental

2.1. Materials

The glucomannan ($M_v = 1.15 \times 10^6$ Da) was isolated from the tubers of *A. paeoniifolius* according to the method mentioned in reference (An, Dong, Dung, & Thien, 2010; An, Dong, Dung, Thien, & Du, 2010). Methyl iodide and sodium hydroxide were commercial products of Merck Co., (Germany). All other chemicals and reagents used in experiments were of analytical grade.

2.2. Synthesis of O-methyl glucomannan derivatives

The O-methyl glucomannan derivatives were synthesized as follows: glucomannan (1 g) was dispersed in 100 ml of water by stirring, then methyl iodide (3; 5; 9; 15 ml marked as O-MG1; O-MG2; O-MG3; and O-MG4, respectively) was added and the mixture was stirred strongly for 30 min; then the pH was adjusted to 8 and 10, respectively, by slowly adding 10% NaOH aqueous solution, while stirring vigorously. The reactant system became quite transparent solution due to swelling of glucomannan as the pH was raised, but reverted to a completely transparent solution when the temperature of system was up to 40 °C, then maintaining at this temperature for 3 h. This solution was filtered and cooled to ambient temperature; the pH was adjusted to pH 7 by using 1% HCl solution. The O-MGs were precipitated with ethanol under stirring at room temperature, then filtered and redissolved in distilled water. This process was repeated three times. The final products obtained after lyophilization were used for further characterization.

2.3. Characterization of O-methyl glucomannan derivatives

^{1}H, ^{13}C, ^{1}H ^{13}C HSQC-NMR and ^{1}H ^{13}C HMBC-NMR spectra of the glucomannan and the O-methyl glucomannan derivatives were recorded on the 500 MHz Bruker Avance spectrometer, the sample concentrations being about 5 g/l for ^{1}H NMR and 70 g/l for ^{13}C NMR spectra in D_2O, at 353 K.

Degree of substitution at O-atom ($\overline{DS}$) of O-methyl glucomannan derivative could be evaluated by their ^{1}H NMR integrals using the following formula:

$$\overline{DS} = \frac{I_{H1'}}{I_{\sum H1}}$$

where $\overline{DS}$ is degree of substitution at O-atom of substituted derivative. $I_{H1'}$ and $I_{\Sigma H1}$ were the integrals of the hydrogen atom bonded at C1 of substituted glucomannan unit and C1 of all substituted and unsubstituted glucomannan unit, respectively.

The molecular weight of the O-MGs was determined by membrane osmometry (Osmomat 090, Gonotec) using membranes with a cut off of 20,000 Da; at 30 °C and sample concentration of 0.2–1%.

3. Results and discussion

3.1. Synthesis of O-methyl glucomannan

Due to both the glucomannan and methyl iodide were not soluble in water, so the methylation was occurred in the heterogeneous state. This reason might limit the ability of reaction of reactant and therefore, the yield of methylation was quite low. For this reason, many related papers have been reported to the use of methanol or dimethyl sulfoxide in the methylation of glucomannan (Haworth & Percival, 1932; Hakomori, 1964; Kishida, 1979; Shatwell et al., 1991). However, this problem could be overcome by doing as mentioned in the experimental part. Due to the hydration of glucomannan and with the high speed stirring, the mixture of glucomannan and methyl iodide was in the polydispersive state. At this time, sodium hydroxide was added to the mixture. The presence of sodium hydroxide would make glucomannan swell and lead the macromolecular chain of glucomannan to be more flexible, so that their hydroxyl groups became more active and readily reacted with the methyl iodide by the nucleophilic substitution reaction for forming the methoxyl groups. In this process, the hydrolysis reaction of methyl iodide by sodium hydroxide could occur as a side reaction for forming methanol. For reducing this unexpected reaction, the methylation was done at low temperature (40 °C). The success of methylation of glucomannan was proved by investigating its structure by the nuclear magnetic resonance spectrocopy.

3.2. NMR analysis

The ^{1}H, ^{13}C and ^{1}H ^{13}C HSQC-NMR spectra of GM were shown in Figs. 1A, 2A and 3A, respectively.

The NMR spectra of glucomannan were registered directly in state of polymer chain, dissolved transparently in D_2O at 353 K (the hydration of glucomannan took place well at this temperature) (no NaOH be used as previously). The signals of glucose and mannose units were overlapped, so that its spectrum was rather simple.

In the ^{1}H NMR spectrum of glucomannan, the signal at 5.84 ppm was assigned to H1 proton of both mannose and glucose units (overlapped); the signals of H2–H6 proton were overlapped in region 3.91–4.58 ppm. In the ^{13}C NMR spectrum of glucomannan, the signals were assigned as follow: C1 (δ 101.3 ppm); C2 (72.08); C3 (72.34); C4 (78.14); C5 (74.00) and C6 (61.43).

The NMR spectra of O-MGs in D_2O were recorded and shown in Figs. 1B,C, 2B,C, 3B,C and 4. The chemical shift data of O-MG derivatives were summarized in Tables 1 and 2.

Table 1
^{1}H NMR chemical shift data of O-MGs (δ ppm) (5g/l in D_2O at 353 K).

Signals	O-MG1	O-MG4
H1 of substituted units	6.088	6.084
H1 of initial units	5.821	5.823
H2 of unsubstituted units (overlapped with the signal of H6)	4.285	4.327
H2 of substituted units (overlapped with the signal of H6)	4.305	4.342
H3 of both un- and substituted units	4.090	4.096
H4 of both un- and substituted units	4.105	4.110
H5 of both un- and substituted units	4.400; 4.417	4.406; 4.422
H6a; H6b (overlapped with the signal of H2)	4.335; 4.261	4.342; 4.296; 4.292
H of (C2)OCH_3 group	4.021	4.028
H of (C3)OCH_3 group	3.867; 3.885; 3.905	3.875; 3.912
H of (C6)OCH_3 group	3.793; 3.807	3.800; 3.812
DOH	4.621; 4.700	4.618

Table 2
^{13}C NMR chemical shift data of O-MGs (δ ppm) (70 g/l in D_2O at 353 K).

Signals	O-MG1	O-MG4
C1 of initial units and substituted units	100.42	100.50
C2 of substituted units	72.59	73.07
C2 of unsubstituted units	72.09	72.13
C3 of both un- and substituted units	72.40	72.48
C4 of un-substituted units	78.26	78.37
C4 of substituted units	-	81.54
C5 of both un- and substituted units	74.03	74.09
C6 of both un- and substituted units	61.44	61.46
C signal of $-OCH_3$ groups	59.42	59.28

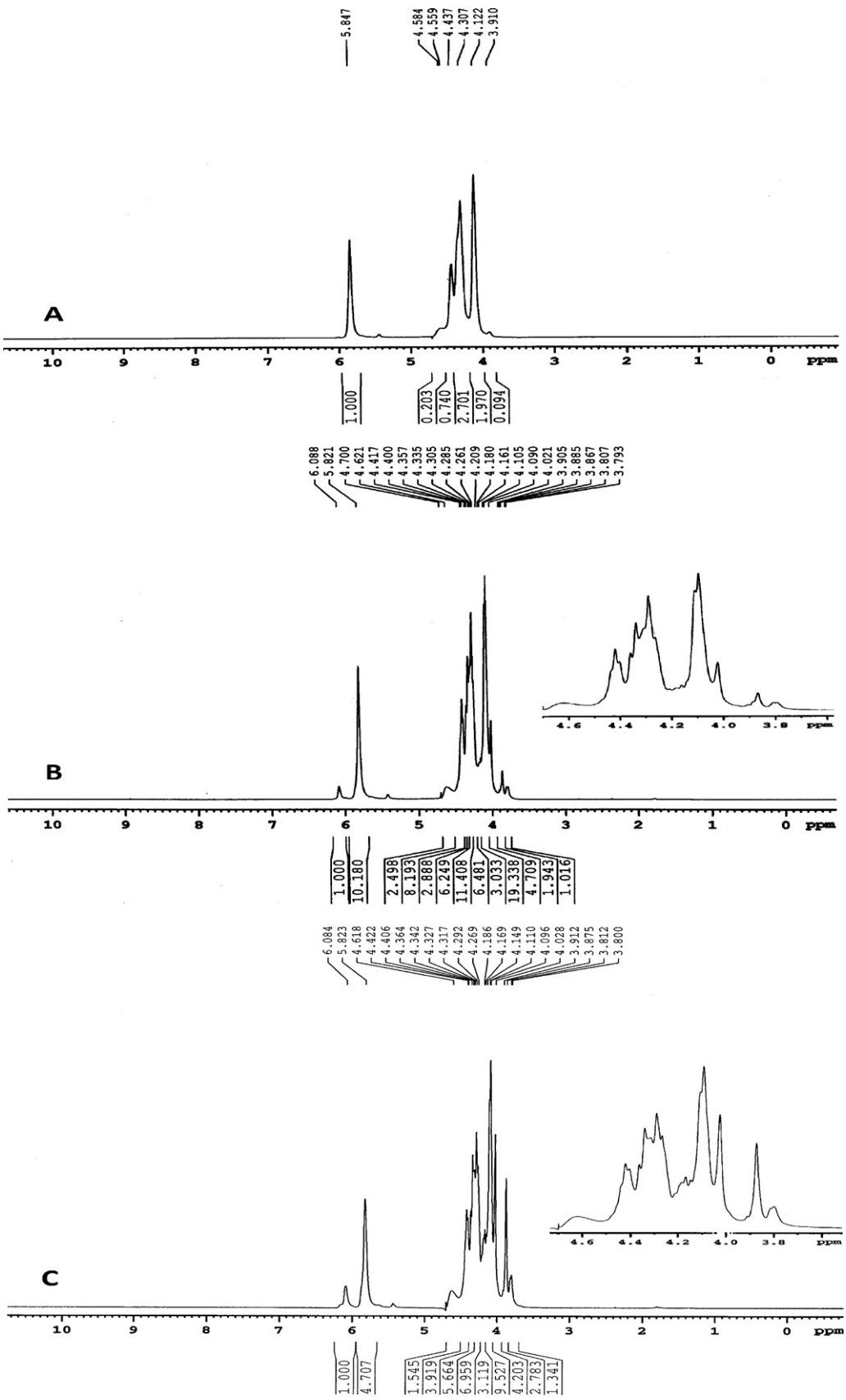

Fig. 1. The ^{1}H NMR spectra of GM (A) and O-MG1 (B) and O-MG4 (5 g/l in D_2O at 353 K).

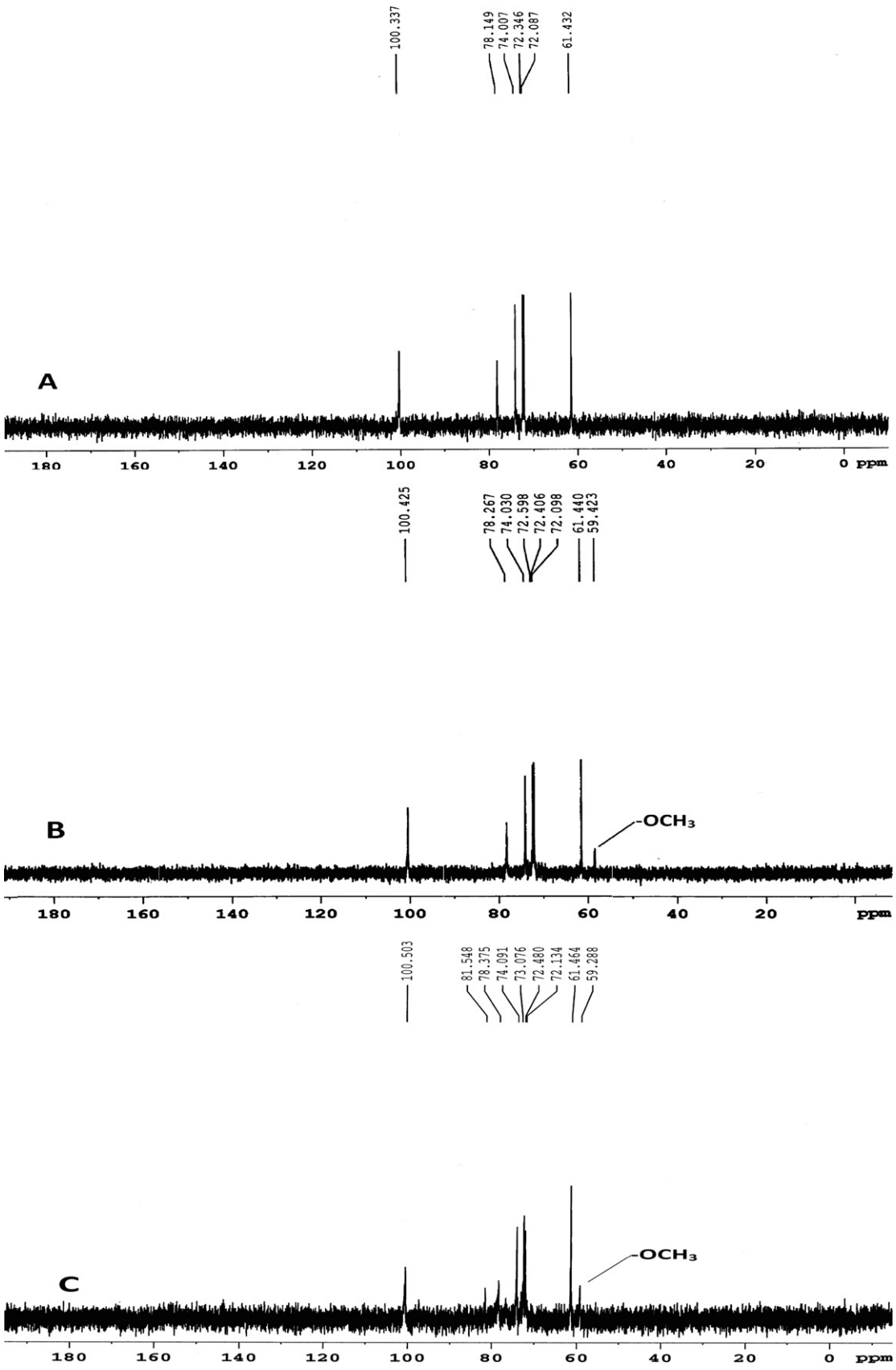

Fig. 2. The ^{13}C NMR spectra of GM (A); O-MG1 (B) and O-MG4 (C) (5 g/l in D_2O at 353 K).

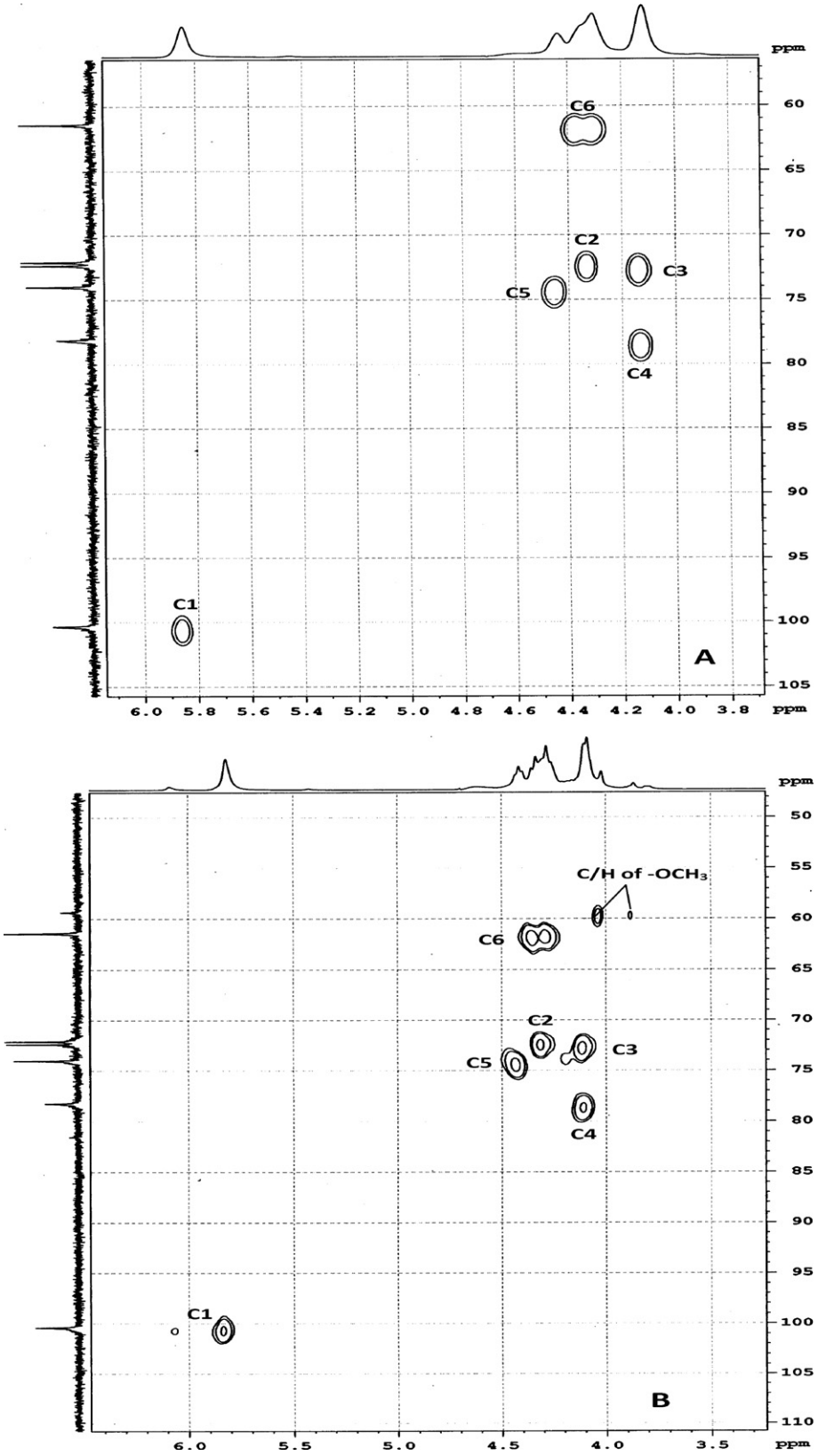

Fig. 3. The ^{1}H ^{13}C HSQC NMR spectra of GM (A) and O-MG1 (B).

The structural modifications introduced by the methylation could be observed by comparing the ^{1}H NMR and ^{13}C NMR spectra of glucomannan and that of the O-MG derivatives. In the ^{1}H NMR spectrum of O-MG1, the new signals observed at 3.793–4.021 ppm were assigned for proton of methyl groups, meanwhile, these signals were also observed at 3.800–4.096 ppm in the spectrum of O-MG4. In their ^{13}C NMR spectra, the new signal appeared at 59.42 ppm (in the spectrum of O-MG1) and at 59.28 ppm (in the

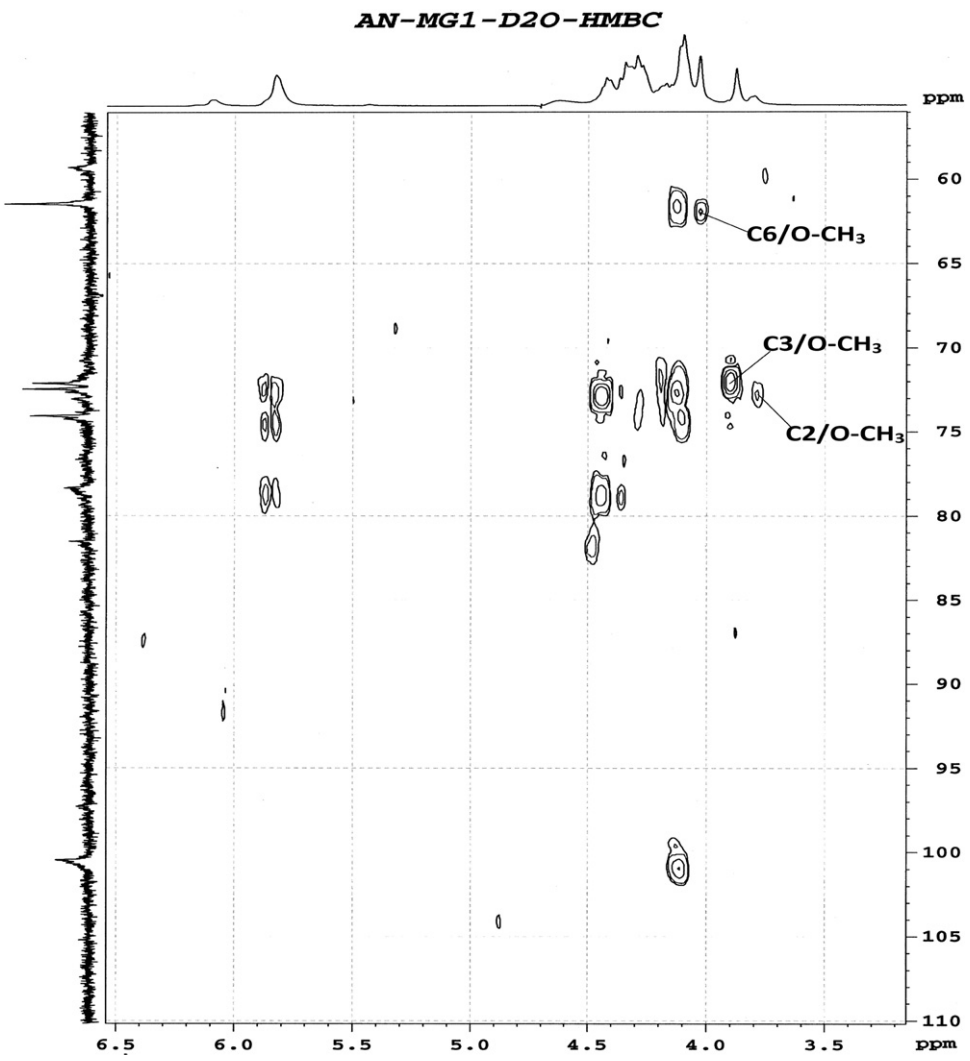

Fig. 4. The ^{1}H ^{13}C HMBC NMR spectrum of O-MG4.

spectrum of O-MG4) was assigned for carbon signals of substituted methyl groups. Thus, the appearance of the new signals in both ^{1}H and ^{13}C NMR spectra was taken as evidences proved that the methylation was taken place for forming the O-MG derivatives.

The above observations were affirmed by the crosspeaks in the ^{1}H ^{13}C NMR-HSQC spectra of glucomannan (Fig. 3A) and that of O-MG1 derivative (Fig. 3B). In the heteronuclear single quantum corehence (^{1}H ^{13}C NMR-HSQC) spectrum of O-MG1 (Fig. 3B), the new crosspeaks observed at δ 3.80/59.42 ppm; 3.88/59.42 ppm and 4.02/59.42 ppm were due to H/C signals of the substituted methoxyl groups (the C signal of substituted group was overlapped at 59.42 ppm). These were significant evidences for identifying the chemical structure of the methylated glucomannan.

In order to determine the position of substituted methoxyl groups in the glucopyranose ring, the heteronuclear multiple bond corehence (^{1}H ^{13}C NMR-HMBC) spectrum of O-MG4 was recorded (Fig. 4). As shown in Fig. 4, the crosspeaks observed at δ 73.07/4.028 ppm; 72.48/3.875 ppm and 61.46/3.8 ppm were assigned for C/H signals of C6/O-CH_3; C3/O-CH_3 and C2/O-CH_3. Therefore, the chemical shift of proton of methyl groups could be assigned as presented in Table 1.

3.3. Determination of degree of substitution

The degree of substitution ($\overline{DS}$) for a O-methyl glucomannan derivative was defined as number of substitutions of hydroxyl groups per monomer unit of O-MG.

In this work, the ^{1}H NMR spectrum of glucomannan were registered directly in state of polymer chain, dissolved transparently in D_2O at 353 K (no NaOH be used as previously), therefore their glucose and mannose signals were overlapped, so that the spectrum was rather simple. The $\overline{DS}$ of O-methyl glucomannan derivatives could be determined quite simply by the data of ^{1}H NMR spectra using the integrals of proton of substituted methyl groups or that of proton (H1) of substituted glucomannan. According to the first method, the $\overline{DS}$ could be determined using the integrals of proton of methoxyl groups as follows: $\overline{DS} = [(\sum I_{CH3}/3)/\sum I_{H1}]$. The resulting $\overline{DS}$ values of O-MG1 and O-MG4 were approximately of 0.228 and 0.486, respectively. However, as shown in the ^{1}H ^{13}C HSQC-NMR spectra (Fig. 3B), the H signals of the –OCH_3 groups were overlapped with the signals of H4 and H3, so that the results were calculated from the formula as above mentioned could be not so accurate. In the method that used the integrals of proton H1 of substituted glucomannan, the $\overline{DS}$ could be determined as mentioned in the experimental part. Also as shown in

Table 3
Effect of methyl iodide amount and pH on the extent of methylation.

Samples	GM (g)	MI (ml)	pH	R^a (%)	$\overline{DS}$	Mn (g/mol)
O-MG1	1	3	8	3.5	–	1.02×10^6
	1	3	10	125.5	0.09	1.13×10^5
O-MG2	1	5	8	5.2	–	1.08×10^6
	1	5	10	132.5	0.116	1.87×10^5
O-MG3	1	9	8	6.6	–	1.12×10^6
	1	9	10	139.6	0.124	4.15×10^5
O-MG4	1	15	8	7.5	–	1.14×10^6
	1	15	10	148.2	0.175	6.13×10^5

$\overline{DS}$ values were calculated using the formula $\overline{DS} = (I_{H1'})/(I_{\sum H1})$.

[a] R = water soluble O-MG product (g)/GM (g) × 100%.

Fig. 3B, it could be seen that the signals of proton H1 of substituted and unsubstituted units were not overlapped, so the resulting $\overline{DS}$ value of O-MG1 was 0.09 and that of O-MG4 was 0.175 could be more accurate than that of above mentioned one. Therefore, this method was used for determination the $\overline{DS}$ value of the O-MGs.

The degree of substitution of methoxyl group at C2, C3 and C6 could also determined using the following formula: $\overline{DS}_i = [(I_{C_i\text{–OCH3}}/3)/\sum I_{H1}]$, where $I_{C_i\text{–OCH3}}$ and ΣI_{H1} were the integrals of the hydrogen atom bonded at C_i of substituted glucomannan unit and C1 of all substituted and unsubstituted glucomannan unit, respectively. The $\overline{DS}_i$ values calculated using that formula to O-MG1 were 0.03; 0.058 and 0.14 corresponding to C2, C3 and C6, respectively; and that of O-MG4 were 0.078; 0.166 and 0.245. However, these results could be inaccurate because the overlap of the signals as above mentioned.

3.4. Various reaction conditions for obtaning O-methyl glucomannan

The effect of reaction conditions on synthesis of O-methyl glucomannan was summarized in Table 3. Every methylation was carried out at 40 °C. The ability of methylation of glucomannan was evaluated via the $\overline{DS}$; R (R was the percent ratio of water soluble O-MG (g) per GM) and molecular weight. It was shown that the methylation of glucomannan was hardly occurred at pH 8, but it run well at pH 10. In the range of these experiments, at the same amounts of MI, the more the increase of pH, the more $\overline{DS}$ value and R augment. The $\overline{DS}$ value was also increased when the ratio (v/w) of MI/GM increased. As also seen in Table 1, the molecular weight of O-MG derivatives was slightly decreased with increasing of pH from 8 to 10. This could be due to the depolymerization of glucomannan occurring in the methylation process. Thus, the pH of reactive medium was an important factor of the preparation of O-MG derivatives.

4. Conclusion

The methylation of glucomannan was done quite simply for forming the water-soluble O-methyl glucomannan derivatives by the direct alkylation of a glucomannan with methyl iodide at pH 10 and temperature of 40 °C without using methanol, dimethyl sulfoxide as well as methylsulfinyl carbanion. The chemical structure of O-MG derivatives was investigated quantitatively and qualitatively NMR spectroscopy. The appearance of the new signals in the ^{1}H NMR, ^{13}C NMR and also in the ^{1}H ^{13}C NMR HSQC of O-MG derivatives confirmed the presence of substituted methoxyl group on the methyl glucomannan macromolecule. Their degrees of substitution were calculated using the integrals of ^{1}H NMR signals and increased from 0.09 to 0.175 since the ratio (w/v) of glucomannan/methyl iodide changing from 1/3 to 1/15. The resulting O-MG derivatives have promising applications to film-forming for packaging, encapsulating or as a stabilizer for preparation of metal nanoparticles.

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