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Structural Analysis of Glucan by Identification of Methylated Sugars by Proton Magnetic Resonance Spectra

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Methylation analysis is one of the most useful methods for the determination of polysaccharide structure. The key point of methylation analysis is the identification of partially methylated sugars obtained from the hydrolyzate of fully methylated polysaccharide, and its identification has been greatly simplified by the use of gas–liquid chromatography (GC). However, this analysis required authentic methylated sugars. Recently, studies on the mass spectra (MS) of partially methylated alditol acetate which were obtained from methylated sugars, and application of GC–MS for the identification of methylated sugars, has been reported.[2] The mass spectra of isomeric alditols having the same substitution pattern are not distinguishable from each other. While proton magnetic resonance (PMR) spectra of methylated sugars make it possible to discriminate the parent sugar by coupling constant of the anomeric proton. The chemical shifts of protons due to methoxyl groups of several methylated sugars[3,4] have been measured using their methyl glycosides in organic solvents ($CDCl_3$, C_6D_6 *etc.*). In the case of converting to the methyl glycoside, two anomers, methyl α- and methyl β-glycoside, are formed. Therefore, exact assignment of methoxyl proton is very complicated because the chemical shift of glycosidic methoxyl protons overlaps with those of another methoxyl protons.

In 1971, Gros[5] reported the chemical shifts due to methoxyl proton of mono-*O*-methyl derivatives of D-hexoses in D_2O, but these chemical shifts are not applicable for the identification of tri-*O*-methyl and di-*O*-methyl sugars derived from fully methylated polysaccharide. After that, Rathbone, *et al.*[6] reported the chemical shifts due to methoxyl proton of tri-*O*-

Table I. Chemical Shifts in δ (ppm) of Some Methylated D-Glucopyranose

Compound	Position of OMe				
	C-6	C-2(α)[a]	C-4	C-2(β)[b]	C-3
2,3-$(OMe)_2$		3.48		3.59	3.61
3,6-$(OMe)_2$	3.39				3.61
4,6-$(OMe)_2$	3.40		3.54		
3,4,6-$(OMe)_3$	3.39		3.54		3.62
2,3,6-$(OMe)_3$	3.39	3.48		3.59	3.61
2,4,6-$(OMe)_3$	3.40	3.48	3.54	3.59	
2,3,4,6-$(OMe)_4$	3.41	3.48	3.54	3.59	3.61

a) (α): α-anomer b) (β): β-anomer

1) Location: *20-1, Kitashinjuku 3-chome, Shinjuku-ku, Tokyo, 160, Japan.*
2) H. Björndal, B. Lindberg, and S. Svensson, *Carbohyd. Res.*, **5**, 433 (1967).
3) D. Gagnarire and L. Odier, *Carbohyd. Res.*, **11**, 33 (1969).
4) E.B. Rathbone and A.M. Stephen, *Tetrahedron Lett.*, 1339 (1970).
5) E.G. Gros, I.O. Mastronardi, and A.R. Frasca, *Carbohyd. Res.*, **16**, 232 (1971).
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methyl, di-*O*-methyl, and mono-*O*-methyl-D-galactopyranose in D_2O. Then, we examined the PMR spectra of three di-*O*-methyl, three tri-*O*-methyl, and a tetra-*O*-methyl derivatives of D-glucopyranose in D_2O and their applicability to glucans. As shown in Table I, the PMR spectra of methylated glucopyranoses having a methoxyl group at C-6 position such as 3,6-, 4,6-di-, 2,3,6-, 2,4,6-, 3,4,6-tri-, and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, showed a sharp signal at 3.39—3.41 ppm(s). All of methoxyl protons at C-3 position such as 2,3-, 3,6-di-, 2,3,6-, 3,4,6-tri-, and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose, showed a sharp signal at 3.61—3.62 ppm(s). Similarly, the signal of methoxyl group at C-4 position in glucopyranose appears at 3.54—3.55 ppm(s). On the other hand, the signal of methoxyl group at C-2 position appears at 3.48 and 3.59 ppm, reflecting the presence of two anomers. It is suggested that the former signal is due to methoxyl proton at C-2 in α-anomer and the latter signal is due to methoxyl proton at C-2 in β-anomer, because of the chemical shifts of methoxyl protons at C-2 position in methylglucosides of α- and β-anomer.[3]

From these data, chemical shifts at 3.48 and 3.59, 3.61—3.62, 3.54—3.55, and 3.39—3.41 ppm were assigned to methoxyl protons at C-2 in α- and β-anomer, C-3, C-4, and C-6 in glucopyranose, respectively, but chemical shift of methoxyl proton at C-2 only in β-anomer of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose differs from that of C-2 in β-anomer of tri- and di-*O*-methyl-D-glucopyranose.

Examination on the applicability of this method was carried out for structural investigation of two glucans, coriolan[7] consisting of (1—3)-D-glucopyranosyl main chain substituted at C-6 position with D-glucopyranosyl units and glycogen consisting of α-(1—4)-D-glucopyranosyl main chain substituted at C-6 position with α-D-glucopyranosyl units. These glucans were fully methylated with Hakomori reagents,[8] hydrolyzed, and separated into three fraction,

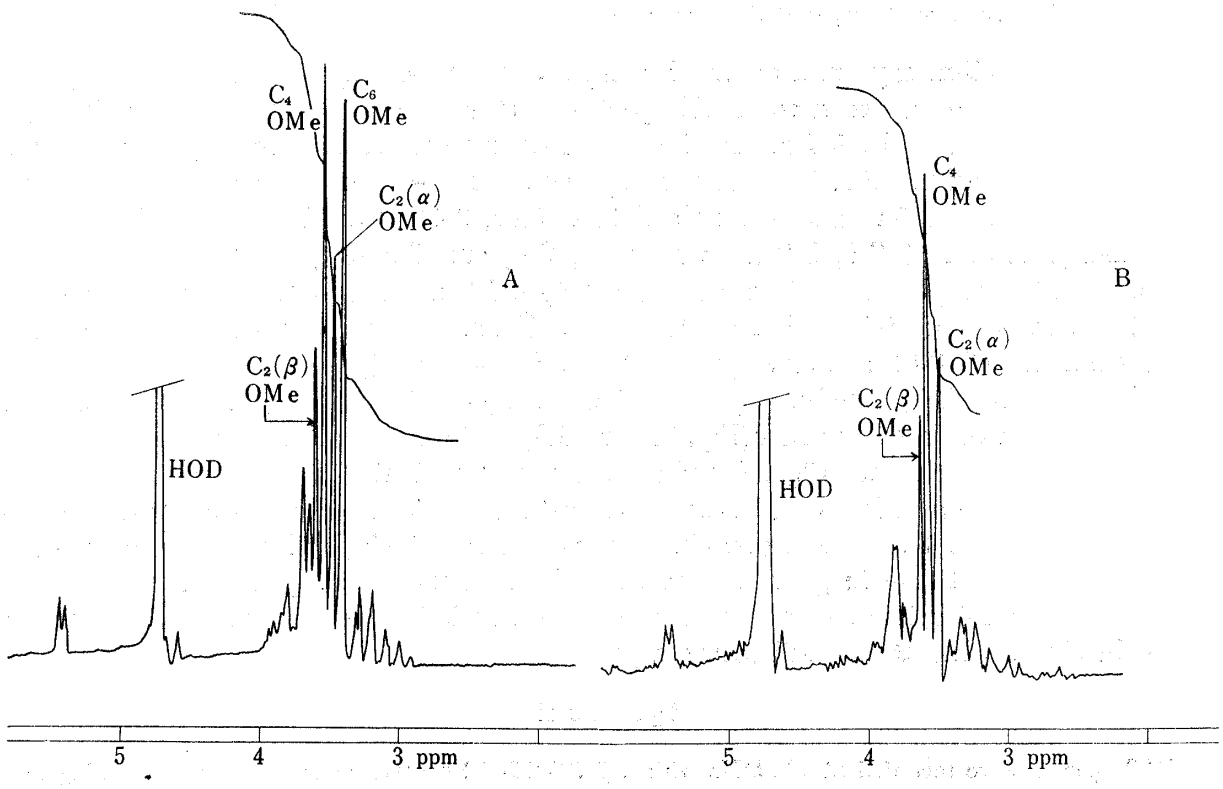

Fig. 1. PMR Spectra of Tri-*O*-methyl Fraction (A) and Di-*O*-methyl Fraction (B) derived from Coriolan (100 MHz: D_2O)

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8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

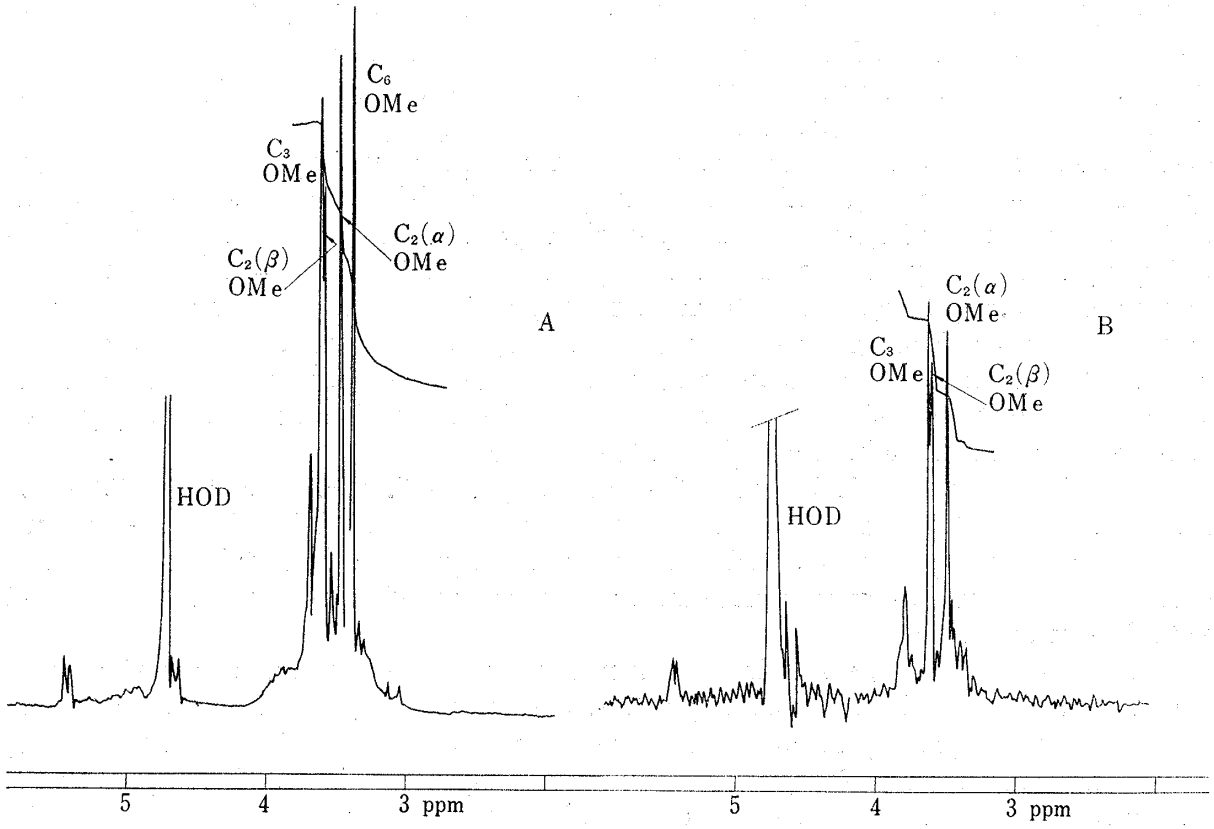

Fig. 2. PMR Spectra of Tri-*O*-methyl Fraction (A) and Di-*O*-methyl Fraction (B) derived from Glycogen (100 MHz: D_2O)

di-, tri-, and tetra-*O*-methyl fraction, by thin-layer chromatography using the solvent system of acetone–benzene,[9] and then the PMR spectra of these fractions were measured in D_2O. The spectra of the di- and tri-*O*-methyl fraction derived from coriolan and glycogen are illustrated in Fig. 1 and 2. Fig. 1A shows the spectrum of tri-*O*-methyl fraction derived from coriolan and illustrates four sharp signals at 3.40, 3.48, 3.54, and 3.59 ppm which is due to the methoxyl proton at C-6, C-2 (in α-anomer), C-4, and C-2 (in β-anomer), respectively. Therefore, tri-*O*-methyl fraction is identified as 2,4,6-tri-*O*-methyl-D-glucopyranose. Fig. 1B shows the spectrum of di-*O*-methyl fraction derived from coriolan and illustrates the three sharp signals at 3.48, 3.54, and 3.59 ppm due to C-2 (in α-anomer), C-4, and C-2 (in β-anomer), respectively. Therefore, di-*O*-methyl fraction is 2,4-di-*O*-methyl-D-glucopyranose. The results obtained from these spectra (Fig. 1A and 1B) are in good agreement with other data on the structure of coriolan. Fig. 2A and 2B show spectra of tri-*O*- and di-*O*-methyl fraction derived from glycogen. Similarly, tri- and di-*O*-methyl fraction should be 2,3,6-tri-*O*-methyl-D-glucopyranose and 2,3-di-*O*-methyl-D-glucopyranose, respectively. Also the results obtained from Fig. 2A and 2B are in good agreement with the structure of glycogen.

From these results, it is shown that the structure of hydrolyzate of fully methylated glucan can be elucidated without any authentic sample.

Experimental

PMR spectra were recorded at 100 MHz with a JNM-4H-100 spectrometer at normal operating temperature. Sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl) propionate was used as an internal standard in D_2O. Chemical shifts were expressed in δ (ppm). Sample concentration was about 5—10% (w/v).

Coriolan is a fungal polysaccharide isolated from *Coriolus versicolor*.[7] Glycogen was purchased from E. Merk Co. (Germany).

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Authentic samples of 2,3-,[10] 3,6-,[11] 4,6-di-,[12] 3,4,6-,[13] 2,3,6-,[14] 2,4,6-tri-,[15] and 2,3,4,6-tetra-*O*-methyl-D-glucopyranose[14] were prepared by authentic method.

The glucans, coriolan and glycogen, were methylated by the Hakomori's procedure[8] and fully methylated glucans were hydrolyzed with 90% HCOOH at 100° for 5 hr and then 1N H_2SO_4 at 100° for 5 hr. The hydrolyzates were neutralized with $BaCO_3$ and separated into three fractions, di-, tri-, and tetra-*O*-methyl fraction by preparative thin-layer chromatography (Wakogel B-5) using the solvent system acetone–C_6H_6 (1 : 1, v/v).[9]

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