Note

Identification of *O*- α -D-Glucopyranosyluronate-(1 $\rightarrow$ 2)-D-galactose Isolated from the Acidic Polysaccharide of *Fusarium* sp. M7-1

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During the course of the our studies on the chemical structure of the acidic polysaccharide of *Fusarium* sp. M7-1, we have found that mild acid hydrolysis of the polysaccharide gave an acidic disaccharide which was identified as *O*- α -D-glucopyranosyluronate-(1 $\rightarrow$ 2)-D-galactose mainly by ^1H -NMR spectrometry. This paper describes the isolation and identification of this disaccharide.

The mycelium of *Fusarium* sp. M7-1 was obtained by the method described in our previous paper.¹⁾ The dried mycelium (100 g) was suspended in 2 l of deionized water and heated at 120°C for 1 hr and then the mycelium was removed by centrifugation. The supernatant was dialyzed and chromatographed on columns of Sephadex G 50 (3 cm $\times$ 80 cm) and Toyopearl HW 65 (3 cm $\times$ 90 cm). The acidic sugar-containing fractions were evaporated to dryness (yield about 10 g). This substance is an acidic polysaccharide consisting of 20% protein, 57% neutral sugar (D-galactose, D-mannose, D-glucose, and L-rhamnose in a molar ratio of about 14:10:2:1 and 23% uronic acid. The acidic polysaccharide (700 mg) was hydrolyzed with 0.1 M trifluoroacetic acid (TFA) for 5 hr at 100°C in sealed tubes. After removing TFA by extraction with ethyl acetate, the aqueous phase was passed through a column of Dowex 50 (H⁺, 1 cm $\times$ 30 cm) with deionized water. The eluate was put on a column of Dowex 1 $\times$ 2 (Cl⁻, 2.5 cm $\times$ 30 cm) and eluted with a linear gradient of 0 to 2 M NaCl. The acidic sugar-containing fractions were concentrated and chromatographed on a column of Bio-Gel P-2 (3 cm $\times$ 90 cm) equilibrated with 0.1 M NaCl eluting with the same solvent and then desalted by gel filtration on a column of Bio-Gel

P-2. This gave about 200 mg of white powder designated as compound A. Compounds B and C were obtained by reduction of the reducing terminus of compound A with NaBH₄ and NaBD₄ respectively. The carboxyl reduced compound (compound D) was obtained by reduction of compound B by the method of Taylor and Conrad.²⁾ Each compound was hydrolyzed with 2 M TFA at 100°C for 3 hr (for compound D) and for 24 hr (for compounds A and B). Each hydrolyzate of compounds A and B was separated into neutral and acidic fractions on a column of Dowex 1 $\times$ 2 (Cl⁻). The hydrolyzate of compound D was separated into two fractions (A and B) by HPLC on Hitachi GL-C611. The analytical data of these samples are shown in Table I. On acid hydrolysis, compound A gave D-galactose and D-glucuronic acid, compound B gave

Fig. 1. Partial ^1H -NMR Spectra of the Compound B (a) and the Compound C (b).

The spectra were recorded on a JEOL JNM-GSX 500 spectrometer at 500 MHz in deuterium oxide at 70°C. The chemical shifts were measured relative to an external acetone standard.

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Abbreviations: Glc α 1 $\rightarrow$ 2Gal, *O*- α -D-glucopyranosyl-(1 $\rightarrow$ 2)-D-galactose; GlcUA α 1 $\rightarrow$ 2Gal, *O*- α -D-glucopyranosyluronate-(1 $\rightarrow$ 2)-D-galactose; Man β 1 $\rightarrow$ 4GlcUA α 1 $\rightarrow$ 2Gal, *O*- β -mannopyranosyl-(1 $\rightarrow$ 4)-*O*- α -D-glucopyranosyluronate-(1 $\rightarrow$ 2)-D-galactose; Man β 1 $\rightarrow$ 2Man β 1 $\rightarrow$ 4GlcUA α 1 $\rightarrow$ 2Gal, *O*- β -D-mannopyranosyl-(1 $\rightarrow$ 2)-*O*- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-*O*- α -D-glucopyranosyluronate-(1 $\rightarrow$ 2)-D-galactose; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography.

Table I. TLC AND HPLC OF THE HYDROLYZATES OF COMPOUNDS A, B AND D

Material	<i>R_f</i> value on TLC		Retention time (min)		[α] _D (deg.) ^c 1% in water
	A	B	Hitachi 3013N ^a	Hitachi GL-C611 ^b	
D-Glucose	0.46	0.09	80.0	15.2	+54.4
D-Galactose	0.43	0.07	25.0	16.0	+81.8
D-Mannose	0.51	0.15	13.0	17.4	—
D-Galactitol	—	—	—	21.5	—
D-Sorbitol	—	—	—	23.7	—
D-Mannitol	—	—	—	19.4	—
D-Glucuronic acid	0.24	0.40	—	—	—
D-Galacturonic acid	0.17	0.20	—	—	—
Hydrolyzate of compound A					
Neutral fraction	0.42	0.07	25.1	16.0	+81.2
Acidic fraction	0.25	0.41	—	—	—
Hydrolyzate of compound B					
Neutral fraction	—	—	—	21.5	—
Acidic fraction	0.24	0.40	—	—	—
Hydrolyzate of compound D					
Fraction A	0.45	0.09	80.1	15.1	+54.5
Fraction A reduced with NaBH ₄	—	—	—	23.7	—
Fraction B	—	—	—	21.4	—

—, not measured.

A, *n*-Butanol-ethanol-water (2:1:1, v/v/v); B, *n*-Propanol-ethyl acetate-water (7:1:2, v/v/v).^a Eluted with 0.167 M borate buffer (pH 7.5) at a flow rate of 1 ml per min at 65°C.^b Eluted with deionized water at a flow rate of 1 ml per min at 60°C.^c Measured with a JASCO J-20C Automatic Recording Spectropolarimeter at room temperature. Fig. 2. ¹H-NMR Spectrum (a) and NOE Difference Spectrum (b) of the Compound D.

The spectrum was recorded with the same conditions as those in Fig. 1. The NOE difference spectrum was recorded with the irradiation at 5.23 ppm.

D-galactitol and D-glucuronic acid, and compound D yielded equimolar amounts of D-glucose and D-galactitol. Fab-mass spectra of compounds A and D gave the molecular weights of 356 and 344, respectively (data were not shown). The J values of the H-1 signal of glucuronic acid residue in compound A and of glucose in compound D were 3.9 and 3.7 Hz, respectively indicating α -linkage. This was further confirmed by the fact that compound D was hydrolyzed by an α -glucosidase from yeast.³⁾ The signal at 4.09 ppm in compound C was much simpler than that of compound B, indicating that the signal is the C-2 proton of galactitol (Fig. 1(B)). NOE was observed at the C-2 proton of galactitol with irradiation at the H-1 signal of the glucose residue in compound D (Fig. 2(b)). These data indicate that glucuronic acid is linked to C-2 of galactitol. The large vicinal coupling constants ($J=9$ –10 Hz) of four sequential protons (H-2 to H-5) indicate that the glucose residue in compound D should be in pyranose form. From these data, it can be concluded that compound A is GlcUA α 1 $\rightarrow$ 2Gal. On acid hydrolysis 90% of uronic acid and 67% of Galactose in the acidic polysaccharide were released as GlcUA α 1 $\rightarrow$ 2Gal.

Siddiqui and Adams reported the presence of an acidic polysaccharide in the culture filtrate of *Gibberella fujikuroi* (*Fusarium moniliforme*) and they suggested that glucuronic acid residues were mainly non-terminal and were attached to both D-galactose and D-mannose units.⁴⁾ In this study, we found that glucuronic acid and galactose residues in the acidic polysaccharide of *Fusarium* sp. M7-1 are mainly present as GlcUA α 1 $\rightarrow$ 2Gal. This was further confirmed by our recent findings⁵⁾ that acetolysis with mild acid

conditions of the acidic polysaccharide gave mainly Glc α 1 $\rightarrow$ 2Gal, Man β 1 $\rightarrow$ 4GlcUA α 1 $\rightarrow$ 2Gal and Man β 1 $\rightarrow$ 2Man β 1 $\rightarrow$ 4GlcUA α 1 $\rightarrow$ 2Gal units in a molar ratio of about 5:12:2 and traces of mannose, galactose, and unidentified acidic oligosaccharides. More recently we found that these oligosaccharide units are linked together through β 1 $\rightarrow$ 5 galactofranosidic linkages and the acidic polysaccharide is linked to protein through an O-glycosidic linkage (unpublished data). Together with the results of this study and our recent findings mentioned above, it can be concluded that GlcUA α 1 $\rightarrow$ 2Gal is present as the skeletal structure of the acidic polysaccharide. The detailed chemical structure and general properties of the acidic polysaccharide are under investigation.

References

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