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Structure of fomitellan A, a mannofucogalactan from the fruiting bodies of *Fomitella fraxinea*

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ABSTRACT

Chemical structure of fomitellan A, a polysaccharide with a mitogenic effect isolated from the fruiting bodies of *Fomitella fraxinea*, has been assigned as a mannofucogalactan with a repeating unit of penta-saccharide, which was composed of a (1→6)-linked D-galactopyranosyl backbone having a C-2 position substituted with disaccharide units of 3-*O*-D-mannopyranosyl-L-fucopyranosyl residue. The ^{1}H and ^{13}C NMR signals of fomitellan A have been completely assigned by extensive NMR experiments.

Mushrooms are a good source of food with high nutritional attributes, and some have been used as traditional medicines. In recent decades, due to their immunostimulating activity, polysaccharides from medicinal mushrooms have emerged as an important class of bioactive substances.[1–3] Several polysaccharides and protein-bound polysaccharides originated from medicinal mushrooms have been used clinically for treatment of cancer. Polysaccharopeptide Krestin (PSK) from *Coriolus versicolor*,[4] mesima from *Phellinus linteus*,[5] lentinan from *Lentinus edodes*,[6] and schizophyllan from *Schizophyllum commune*[7] have been used extensively in Asia as anticancer drugs. Most polysaccharides possessing antitumor and immunomodulating activities, such as schizophyllan, lentinan, and PSK, have been reported to have a β-glucan and heteroglucan skeleton. However, several cases of isolation of heterogalactans from *Lentinus edodes*, *Ganoderma applanatum*, and *Fomitopsis pinicola* have been reported.[8–10] Most of them have a backbone of (1→6)-α-D-galactopyranosyl residues, which are substituted at the C-2 position, either with L-fucopyranose or 3-*O*-α-D-mannopyranosyl-α-L-fucopyranosyl residues. In previous studies, we isolated a heterogalactan, fomitellan A, from the fruiting bodies of *Fomitella fraxinea* as an immunostimulating agent and its structure was proposed as a mannofucogalactan with a repeating unit of penta-saccharide, which was composed of a (1→6)-linked D-galactopyranosyl backbone having a C-2 position substituted with disaccharide units of 3-*O*-D-mannopyranosyl-L-fucopyranosyl residue.[11] In this Letter, we describe the detailed chemical structure and complete NMR assignments of a heterogalactan, fomitellan A, based on two-dimensional NMR studies.

Details of the extraction and isolation of fomitellan A have been previously described.[11] In brief, the fruiting bodies of *F. fraxinea* were extracted by 0.9% sodium chloride and, after elimination of salts by dialysis, the polysaccharide fraction was concentrated and separated by ethanol precipitation and DEAE-cellulose column chromatography. A polysaccharide fraction with a mitogenic effect exhibited a single peak when chromatographed on a Toyopearl HW-65F gel and was named fomitellan A.

Component sugars of fomitellan A were determined to be D-galactose, D-mannose, and L-fucose in the molar ratio 3:1:1 by acid hydrolysis. Using standard dextran molecules, the molecular weight of fomitellan A was established as about 15,000 by gel permeation–HPLC. In the ^{1}H NMR spectrum of fomitellan A in D_2O, five anomeric protons at δ 5.14 (2H), 5.07, 5.03, and 5.01, oxygenated methine and methylene protons at δ 4.30–3.60 derived from H-2 to H-6 of the component sugars, and methyl protons at δ 1.26 due to the L-fucopyranosyl residue were evident.

The ^{13}C NMR spectrum showed five anomeric carbon signals at δ 102.78, 101.83, 98.64, 98.47, and 98.43, suggesting that the repeating unit of fomitellan A was a penta-saccharide. In addition, signals due to a methyl (C-6) of the fucopyranosyl residue at δ

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16.17, four oxygenated methylenes (C-6 of four hexoses) at δ 61.66, 67.09, 67.22, and 67.67, and 20 oxygenated methines between δ 67.40 and δ 78.08 were evident from DEPT spectral data. Based on data previously reported, carbons at δ 102.8 and δ 101.83, observed at relatively low field, were suggested to be anomeric carbons of D-mannopyranose and L-fucopyranose, respectively.[9] Thus, carbons at δ 98.64, 98.47, and 98.43 were suggested to be anomeric carbons of three D-galactopyranosyl residues. Nine partial structures depicted as a bold-faced line on the proposed structure of fomitellan A in Figure 1 were elucidated by interpretation of ^{1}H-^{1}H DQF-COSY and TOCSY experiments, which showed connectivity from H-1 to H-6 of mannopyranose, and from H-1 to H-4, and between H-5 and H-6 of other component sugars, and could be connected by NOE correlations to the composition of each sugar unit. By analysis of HMQC experimental data, some overlapped proton signals were distinguishable, and assignments of ^{13}C signals were established.

Nine partial structures were unambiguously connected by analysis of HMBC (Fig. 2), HMQC-COSY, and HMQC-NOESY experimental data (Fig. 3); two experiments were later measured by the original PFG-HMQC-CONOESY pulse sequence, in which HMQC-COSY and HMQC-NOESY pulse sequences are combined with PFG (pulsed field gradient) for coherence selection and HMQC-COSY data acquisition time is placed in the mixing time of HMQC-NOESY, and each datum is separately accumulated and stored.[12] HMBC exhibited long-range correlations from H-1 of Gal-1 to C-5 of Gal-1 and C-6 of Gal-3, from H-1 of Gal-2 to C-6 of Gal-1, and from H-1 of Gal-3 to C-6 of Gal-2 and C-5 of Gal-3, as shown in Figure 2. HMBC correlations of H-2 of Gal-2 to C-1 of Fuc and of H-1 of Fuc to C-2 of Gal-2 revealed that L-fucopyranose was substituted at the C-2 position of Gal-2. Also, long-range correlations of H-3 of Fuc to C-1 of Man, and of H-1 of Man to C-3 of Fuc implied that D-mannopyranose should be substituted to C-3 of L-fucopyranose. Complete assignments of severely overlapped three proton signals of H-2 of Gal units were confirmed by HMQC-COSY analysis based on correlation between each C-2 and well-resolved H-1 signal of each galactopyranosyl unit. To confirm the sequential assignment of the (1→6)-α-D-galactopyranosyl backbone, NOEs between anomeric protons of each galactopyranosyl unit and H-6 of neighboring galactopyranosyl were confirmed by carbon editing of HMQC-NOESY data. In the NOESY spectrum, NOEs between H-1/H-2 of Gal-2 and H-1 of Fuc confirmed attachment of the anomeric carbon of L-fucopyranose to C-3 of Gal-2 by glycosyl linkage. Conjugation of D-mannopyranose to C-3 of L-fucopyranose was also ascertained by NOEs between H-3/H-4 of Fuc and H-1 of Man. HMQC-COSY and HMQC-NOESY data were also extremely useful in confirmation of the sequence of Man-(1→3)-Fuc-(1→2)-Gal, in spite of two overlapping anomeric proton signals of Fuc and Man units at δ

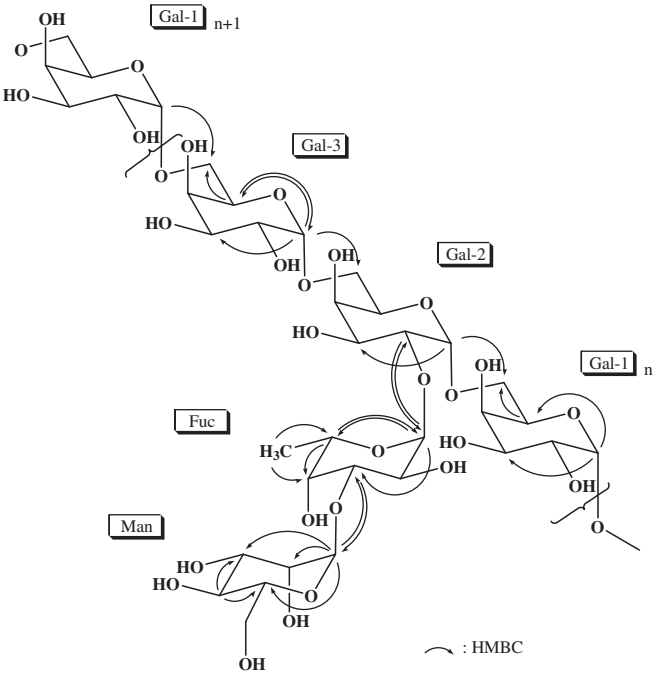

Figure 2. Long-range correlations obtained by HMBC data.

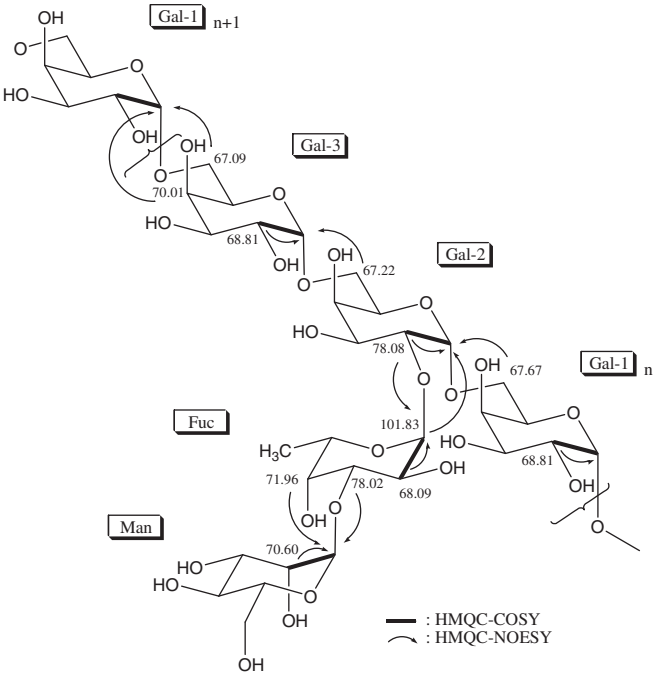

Figure 3. HMQC-COSY and HMQC-NOESY data focused on glycosyl linkage.

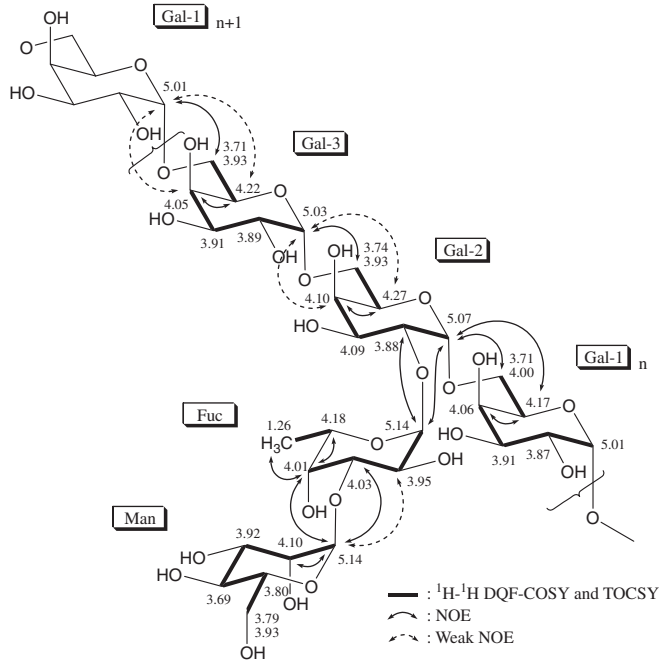

Figure 1. Partial structures by ^{1}H–^{1}H correlations and NOE data with ^{1}H NMR assignments.

Table 1
^{1}H and ^{13}C NMR spectral data for fomitellan A in D_2O at 40 °C

No.	δ_H	δ_C
Gal-1		
1	5.01	98.43
2	3.87	68.81
3	3.91	70.07
4	4.06	70.45
5	4.17	69.67
6	3.71, 4.00	67.67
Gal-2		
1	5.07	98.47
2	3.88	78.08
3	4.09	68.96
4	4.10	70.20
5	4.27	69.24
6	3.93, 3.74	67.22
Gal-3		
1	5.03	98.64
2	3.89	68.81
3	3.91	70.07
4	4.05	70.01
5	4.22	69.38
6	3.71, 3.93	67.09
Fuc		
1	5.14	101.83
2	3.95	68.09
3	4.03	78.02
4	4.01	71.96
5	4.18	67.67
6	1.26	16.17
Man		
1	5.14	102.78
2	4.10	70.60
3	3.92	70.95
4	3.69	67.40
5	3.80	73.91
6	3.79, 3.93	61.66

^{1}H and ^{13}C NMR spectra were recorded at 600 MHz and 150 MHz, respectively. Chemical shifts were referenced to a residual HOD signal at 4.65 ppm for ^{1}H NMR, and an external 1,4-dioxane signal at 67.4 ppm for ^{13}C NMR.

5.14. Remaining internal connectivity of two partial components of Gal-2 was confirmed by NOE correlation between H-4 and H-5 of Gal-2. All anomeric carbons were speculated as having the α-configuration from half-width of broad anomeric proton signals in ^{1}H NMR, and stereochemistry was established on the basis of their $^{1}J_{CH}$ coupling constants of 169.5 Hz, which were measured by an ^{1}H–^{13}C non-decoupling PFG-HMQC spectrum.[13]

Fomitellan A potently enhanced the proliferation of splenetic lymphocytes via the stimulation of DNA synthesis and synergistically induced the proliferation of splenetic lymphocytes when treated with lipopolysaccharide as a B-cell mitogen, concanavalin A as a T-cell mitogen, and pokeweed mitogen as a B- and T-cell mitogen.[11] The activity is comparable to schizophyllan, a fungal polysaccharide that is used as an immunomodulating anticancer agent. In this letter, the structure of fomitellan A was established as a mannofucogalactan by the above two-dimensional NMR studies, and the ^{1}H and ^{13}C NMR signals were completely assigned, as shown in Table 1.

Acknowledgments

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