THE STRUCTURE OF AN OLIGOSACCHARIDE AND ITS EFFECT ON CULTURED PLANT CELLS

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Abstract--The structure of an oligosaccharide and its effect on cultured plant cells are reported in this paper. The oligosacchride with different DP (degree of polymerization) were isolated from acid-hydrolyzed cell walls of cultured cells from *Panax ginseng*. The samples were passed through an active carbon column, a Dowex (H^+) column, and a Bio-Gel P-2 column and finally separated by HPLC. The oligosaccharide with different DP were obtained. The possible structure of an oligosaccharide DP-6 was characterized by TGC, GC-MS, FAB$^-$-MS and ^{13}C-NMR measurements.

Experiments showed that this oligosaccharide could increase the growth rate of *Taxus yunnanensis* cultured cells by 36.7% and promote the taxol yield by nearly 2 folds. Its effect was also found in other cultured plant cells such as panax notoginseng and *Carthamus tinctorius*.

Oligosaccharins are the oligosacchrides with regulatory activity derived from plant cell walls[1]. They have regulatory functions in growth, development, reproduction and defense against pathogenst[2], as a kind of elicitors, they also increase the rates of cell growth and the yields of secondary metabolites in cultured plant cells[3].

The structure of an oligosaccharide with DP-6 from the cell walls of cultured cells from *Panax ginseng* and its effect on cultured plant cells are reported in this paper. The possible structure of this oligosaccharide by characterized by GC, GC-MS, FAB$^-$-MS and ^{13}C-NMR measurements.

Materials and Methods

1. Cell culture of *Panax ginseng*

The callus of *P. ginseng* which had been subcultured in 50 ml flasks for 70 generations was incubated in MS medium supplemented with 2 mg/L 2,4-D and 0.1 mg/L KT and maintained at 26 ± 1 ^{0}C in darkness by subculturing at 25 days intervals. Suspension cultured cells were incubated on ratated shaker with 1/5 culture broth of the total capacity and rotative velocity of 120 rpm. The other cultured conditions were the same with the callus culture.

2.Extraction and isolation of oligosaccharedes

The broken cells from 4 repeating suspension culture were dissolved in 80% ethanol for 24 hours, and then their filtrates were disposed by Sevage method to remove the peptide portion. Oligosaccharides were obtained by partial acid-hydrolysis. Then the samples were neutralized with NaOH, filtered, treated, treated with active carbon and Dowex (H^+) to remove pigment and Na^+ concentrated, passed through a Bio-Gel P-2 column and three periods of oligosaccharides with different DP (degree of polymerization) were achieved. The second main peak fraction was concentrated and separated by Silica gel column. The 200ml eluate ($MeOH:H_2O=25:1$) was collected, concentrated. They were finally separated by HPLC

and three pure oligosaccharides with DP 6-8 which had the physiological effect [4] were obtained.

3.Identification of oligosaccharide DP-6

The structure of the oligosaccharide DP-6 was characterized by the methods of GC, GC-MS, FAB-MS and ^{13}C-NMR measurements described by the report.

4.Effect of oligosaccharide DP-6 on cultured plant cells

The cultured methods and conditions of cultured plant cells of *Taxus yunnaensis*, *Panax notoginseng* and *Carthamus tinctorius*, and the experiment methods of oligosaccharide DP-6 on these cultured plant cells were reported previously [6-8].

Results and Discussion

1. Extraction and isolation of oligosaccharides

Using the way of 80% alcohol extraction and Sevage method could remove lipid-soluble and protein fraction in cells, to ensure isolated oligosacchides be the hydrolysate of semi-cellulose in primary cell walls of cultured cells from *Panax genseng*. During the course of acid hydrolysation, the factors influenced must be controlled strictly. The quantity of products of oligosaccharide could be affected on the condition of more high or too low of temperature.

Using active carbon column and Dowex (H^+) column could remove pigment, salt and saponin etc. This was good for later isolation of oligosaccharides. The oligosaccharide preparation then be further separated and purified by Bio-Gel P-2 column. The majority fraction of oligosaccharide with DP 6-8 was obtained by this isolation method (Fig.1, II peak).

Shown in studies on oligosaccharide isolation nowadays, all the last isolation and purification have gone through HPLC method. During the course of most underivative oligosacchride HPLC isolation, purified water was used as elution solvent. But every base line of oligosaccharide's peak could not be still separated completely. Especially, when the quantity of input smple was large. So the collection should be with great care. Fig. 2 showed that the II peak part isolated by Bio-Gel P-2 column processed in HPLC. Among them, the peak 7(oligosaccharide DP-6) indicated a one point on TLC (Rf=0.95) collected from the HPLC isolation.

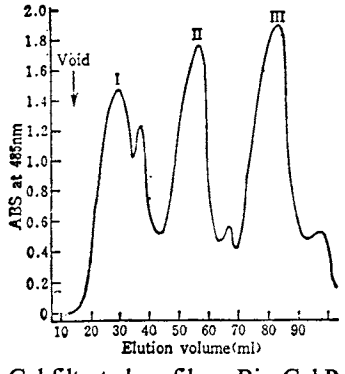

Fig. 1 Gel filtrated profileon Bio-Gel P-2 column Of the hydrolysats of oligosaccharides

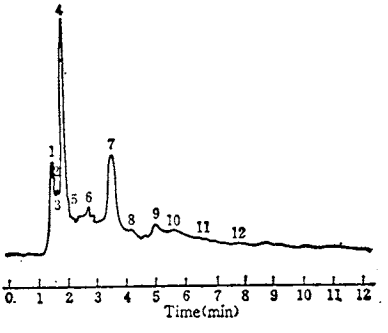

Fig. 2 HPLC of oligosaccharides

2. Identification of oligosaccharide DP-6

(1) FAB$^-$-MS

The FAB$^-$-MS spectra of oligosaccharide DP-6 (Fig. 3) appeared a fragment ion peak at *m/z* 989 (M-5 × H_2O-1)(losing 5 H_2O molecular), that showed it had not single saccharide which molecular weight was not *m/z* 180 (MW = 180), and did not appear a fragment ion peak decreasing by degree at *m/z* 162, that exhibited a branched chain in the molecular. The linked-scanning FAB$^-$-MS spectra of oligosaccharide DP-6 expressed branched chain at No. 4 single saccharide by signal at *m/z* 486(Fig.4).

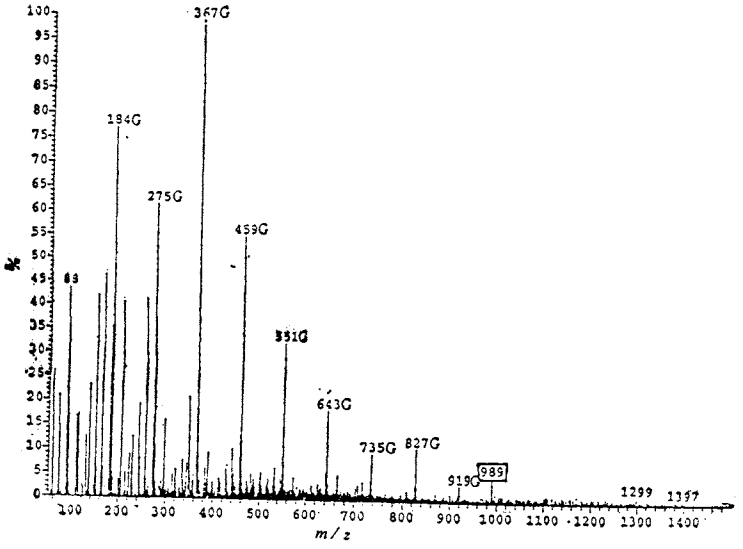

Fig. 3 FAB$^-$-MS spectrum of oligosccharide DP-6
G: mixed with the peaks of glycerin

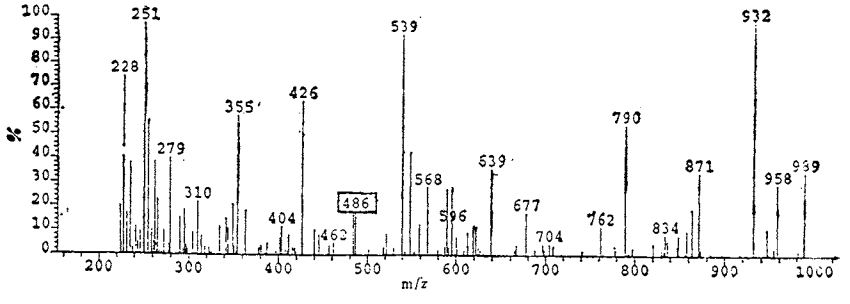

Fig. 4 Linked- scanning FAB$^-$-MS spectrum of oligosaccharide Dp-6

(2) Measurement of linked point among single saccharide of oligosaccharide DP-6

Acid-hydrolysis of hydrogenation of the whole methyl derivative oligosaccharide DP-6 yield acetyl derivatives, and tested by GC, GC-MS. The GC, GC-MS spectra of acetyl derivatives were determined by compared of standard spectra and index of computer. And the molar ratios of single saccharide were obtained by scanning integral of the peak area 9of chromatogram. The complete acid-hydrolyzed test showed that oligosaccharide DP-6 was consisted of Glc, Gal and Man (4:1:1)(Table 1).

Table 1 Methylation analysis of the linkage region of oligosaccharide DP-6

Methylation acetate derivative	Molar ratios
2,3,4,6-O-Me_4-Glc	0.9
2,3,6-O-Me_3-Glc	2.1
3,6-O-Me_2-Glc	1
2,3,4,6-O-Me_4-Gal	1.1
1,2,3,6-O-Me_4-Man	0.9

(3) ^{13}C-NMR

The ^{13}C-NMR spectra of oligosaccharide DP-6 (Fig. 5) appeared 6 beginning carbon signals at δ 95-107 ppm, the carbon signals at δ 107.41 ppm showed C_1 of α-D- galactocyl, at δ 103 ppm (4 × C) were C_1 carbon signals of β-glycosyl, at δ 98.87 ppm was C_1carbon signals of β-D- mannose by compared with the standard spectra of Glc, Gal, Man and their methyl derivatives. The other signals of oligosaccharide Dp-6 were showed in table 2.

By the analysis above, the structure of oligosaccharide DP-6 had been identified as in Fig.6

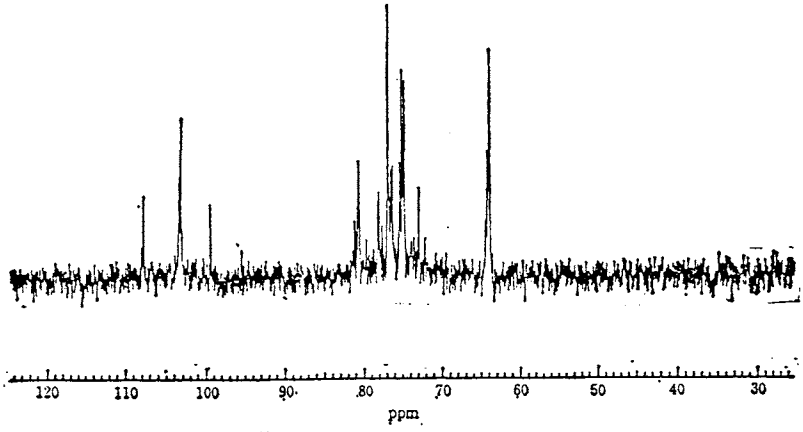

Fig. 5 ^{13}C-NMR data of oligosaccharide Dp-6

Table 2 ^{13}C-NMR data of oligosaccharide DP-6

δ (ppm)	107 (1 peak)	103 (4 peaks)	98 (1 peak)	80-79 (5 peaks)	77-75 (6 peaks)	74 (4 peaks)	73-71 (2 peaks)	64-63 (6 peaks)
Compd	Gal C_1	4×Glc C_1	Man C_1	3×Glc C_4 Man C_4 Glc C_2	Glc C_3, C_5 Man C_3, C_5 Gal C_3, C_5	3×Glc C_2 Gal C_4	Man C_2 Gal C_2	Glc C_6 Man C_6 Gal C_6

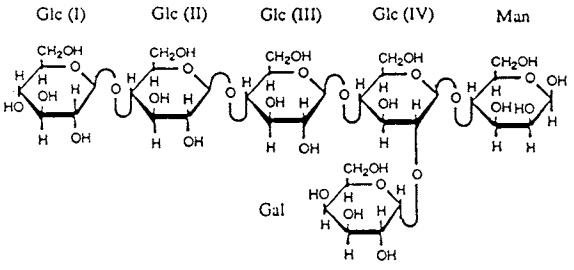

Fig. 6 Structure of oligosaccharide DP-6

3. Effect of oligosaccharide DP-6 on cultured plant cells

(1) Effect of oligosaccharide DP-6 on cultured cells of *Taxus yunnanesis*

The experiment result was showed in table 3. The 5.0mg/L of oligosaccharide DP-6 could increase the growth rate of *Taxus yunnanesis* suspension cells by 36.7% and promote the taxol yield by nearly 2 folds compared to the control.

Table 3 Effect of oligosaccharide DP-6 on cultured cells of *Taxus yunnanessis*

Oligosaccharide DP-6(mg/L)	Growth rate(g/L.d)	Taxol content(%)
0	0.26	0.026
1.0	0.32	0.047
5.0	0.35	0.079
10.0	0.27	0.025

(2) Effect of oligosaccharide DP-6 on cultured cells of *Carthamus tinctorius*

The previous study had showed that the 5.0 mg / L of oligosaccharide DP-6 could promote the α-tocopherol content by 1.1 folds and 20mg / L of oligosaccharide DP-6 increased the growth rate by 20.9% in the callus culture of *Carthamus tinctorius*. From a study of the effect of addition of oligosaccharide DP-6 on different days from the staring of cell growth, it was also seen that its effect on cell growth and α-tocopherol formation was evident the second day of its addition any time during the culture up to 20 days, but the total effect was the largest when it was added on the first day, because of the cumulative effect. When oligosaccharide DP-6 was added to a culture of suspended cells, the duration of exponential growth was shortened and the cells could be harvested earlier, because of the promotive effect on growth rate during exponential growth stage was increased by about 22% and the α-tocopherol content was promoted by about 1.6 folds by the addition of oligosaccharide DP-6

(3) Effect of oligosaccharide DP-6 on cultured cells of *Panax notoginseng*

In an appropriate concentration, the ologosaccharide DP-6 stimulated the saponin

formation and cell growth of *Panax notogensing* callus culture. The optimun concentration was about 15 mg/L, and promoted the growth rate and saponin yield by about 1 fold and 30% respectively.

In summary, the oligosaccharide DP-6 could increase the cell growth rate and the secondary metabolite yield in different cultured plant cells.

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