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Reticuloendothelial System Potentiating of Polysaccharide from *Panax* Species

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Abstract

Polysaccharides which show reticuloendothelial system potentiating activity in carbon clearance tests have been examined in water extracts of *Panax* species. From the dried roots of *P. notoginseng*, an active polysaccharide called sanchinan-A was isolated. The molecular weight of sanchinan -A was estimated to be 1,500,000D and the structure was determined to be β-D-(1-3-galactan), possessing branch points at positions 0-6 at which (mainly-α-L-arabinofuranosyl and partly β-D-galactopyranosyl)-(1-6)-β-D-galacto-pyranosyl-(1-3)-β-D-galactopyranosyl side chains are attached on average, to two of three galactosyl units. From dried rhizomes of *P. japonicus*, several active polysaccharides were also isolated, and these structures were also determined. From the dried roots of *P. ginseng*, several polysaccharides which showed strong activity were isolated. The structures of these compounds are currently under investigation. The polysaccharide fraction (non-dialyzed fraction) of the water extract of red ginseng (steam-dried roots) did not exhibit activity, while the dialyzed fraction potentiated RES. Activity disappeared, however, during the process of separation due to the presence of a substance in the fraction which stabilizes an active substance.

Most of the chemical and pharmacological studies on Ginseng and the related medicinal plants (*Panax* species, Araliaceae), have been centered on the dammarane saponins which are characteristic of this species. However, so far as we know, biologically active polysaccharides of this genus have not been investigated until recently. In 1984, Hikino's group published the studies on glucans named panaxan A, B etc. from Ginseng which exhibit hypoglycaemic activity on administration to mice. Recently, physiological activities of polysacchrides of higher plants have attracted much attention from immunological view points. B.-X. Wang and his colleagues, Chang-Chun, China, reported the antitumor activity of Ginseng polysaccharides which contain PSG A-1 (consisting of galacturonic acid and arabinose, molecular mass : 4,500) and PSG A-2 (consisting of galactouronic acid, galactose, rhamnose and arabionose, molecular mass : 5,300). Y.-S. Zhang and his colleagues, Chang-Chun, China, isolated α-1,4-galacturonan (molecular mass : 720×10^3) and a pectin-like polysaccharide (molecular mass : $1,800 \times 10^3$) from Ginseng roots. They also isolated pectin-like polysaccharides from leaves and stems of Ginseng. Yamada and his co-workers have reported isolation and structure studies on anti-complementary polysaccharides from oriental traditional medicines including leaves of Ginseng.

We have conducted chemical studies on a reticuloendotherial system (RES) activating principle from the water extracts of *Panax* species. RES potentiation was followed by the *in vivo* carbon clearance test (in mice) according to the Halpern's method using zymosan as a positive control.

Sanchi-Ginseng (Roots of *Panax notoginseng*)

This plant cultivated in Yunnan, China, is a well-known Chinese traditional medicine which has been used as a hemostatic. Several Ginseng dammarane saponins, ginsenosides -Rb_1 -Re, -Rd and -Rg_1 as well as other dammarane saponins, notoginsenosides-R_1 and R_2 etc. which are characteristic of this plant, were isolated from this drug.

To an aqueous solution of the water extract was added an excess of ethanol. The resulting precipitate was subjected to chromatography on Sephadex G-50 and then on DEAE-Toyopearl 650M to give an active fraction named 2A, which was further separated by high performance gel permeation chromatography (HP-GPC) on TSKgel G-5000PW_{XL} and G-6000PW_{XL} to give a substance 2A-1 which is active on carbon clearance test . The following biological activities other than carbon clearance test were tested for 2A or 2A-1. 2A exhibited antitumor activity against Sarcoma-180 and increased the protective function against *Escherichia coli* infection in mice. 2A-1 enhanced the production of antibodies against sheep red blood cells and normalized delayed hypersensitivity reaction depressed by cyclophosphamide in mice. 2A-1 activated peritoneal exudate cells (PEC); increasing glucose consumption and cytostatic activity of PEC, though it showed no direct cytotoxicity against Swiss-3T3 and Sarcoma-180. No acute toxicity to mice was observed on single administration of 2A-1, 200mg/kg.

The analytical HP-GPC of 2A-1 appearently indicated the homogeneity. The results of complete acid hydrolysis and the ^{13}C-NMR spectrum suggested the formulation of 2A-1 as an amylopectin-like α-glucan having some β-galactopyranosyl and α-arabinofuranosyl units. On treatment with amyloglucosidase, 2A-1 gave a large amount of glucose. However, in the analytical HP-GPC of the hydrolysate, a peak of an unhydrolyzed substance was still observed at the same retention time (the same molecular mass) as that of 2A-1. This substance, named sanchinan-(A) (SA) was isolated from the hydrolysate by preparative HP-GPC and showed more remarkable activity than 2A-1 in the carbon clearance test.

The molecular mass of SA was estimated to be 1.5×10^6 by analytical HP-GPC. Mineral acid hydrolysis of SA afforded L-arabinose and D-galactose (approximately 1:3.3). By means of the methylation analysis and the repeated mild Smith's degradation as well as the NMR spectrometry, the structure SA was established to be composed

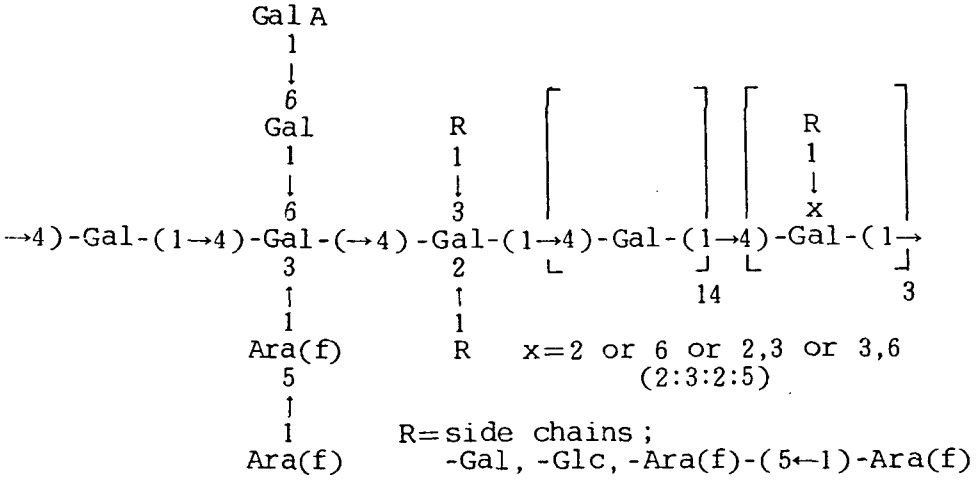

Fig. 1. Structure of PJN-3, a Reticuloendotherial System Activating Polysaccharide from Rhizomese of *Panax notoginseng*

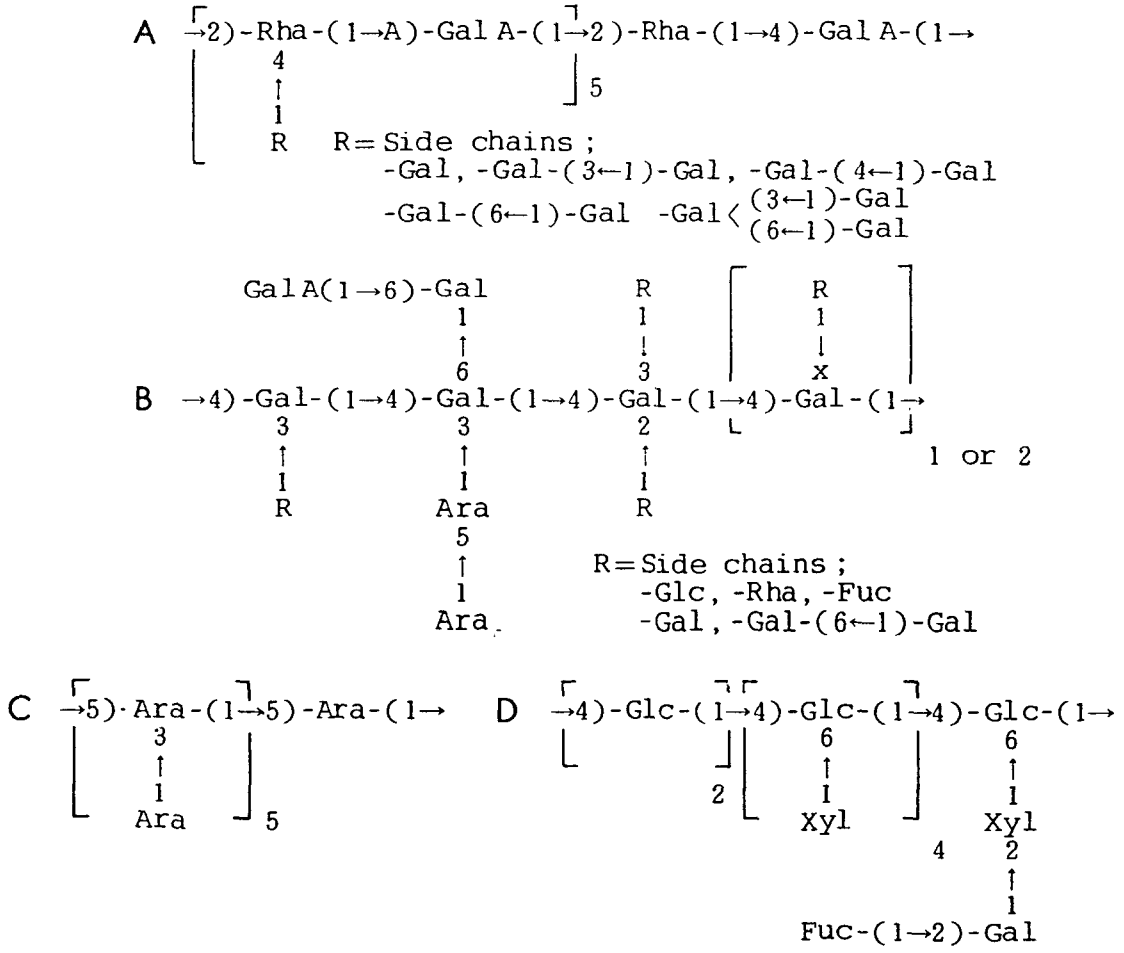

Fig. 2. Structure of PJ2-2 : A ; Rhamnogalacturonan unit, B ; Highly branched galactan unit, C ; Arabinan unit, D : Xyloglucan unit

of main units of β-D-(1→3)-linked D-galactopyranosyl residues having branching points at position 0-6 to which [mainly α-L-arabinofuranosyl and partly β-D-galactopyranosyl]-(1→6)-β-D-galactopyranosyl-(1→3)-β-D-galactopyranosyl side chains are attached on average to two of three galactose units. SA contains a small amount of protein (3.3%).

(K. Ohtani, K. Mizutani, S. Hatono, R. Kasai, R. Sumino, T. Shiota, M. Ushijima, J. Zhou, T. Fuwa and O. Tanaka : Planta Medica, 1987, 166)

Further investigations of other biological activities of SA are under progress.

Chikusetsu-Ninjin (Rhizomes of *P. japonicus*)

This plant grows wild throughout Japan. The medicinal use of rhizome is a stomachic, expectorant and antipyretic, being different from that of Ginseng. The saponin composition of this drug is also somewhat different from that of Ginseng.

The rhizomes were extracted with methanol and then with hot water. To the aqueous solution of the water extract, was added an excess of ethanol and the resulting precipitate was chromatographed on DEAE-Toyopearl 650M to give four active fractions, PJN, PJ1, PJ2, and PJ3.

PJN was further chromatographed on ion-exchange column to give three compounds, PJN-1, -2 and -3, which were homogeneous by HP-GPC. PJN-1 and -3 were active on carbon clearance test, while PJN-2 (α-glucan) was inactive.

PJ2 was also separated by ion-exchanges column chromatography to give one inactive compound, PJ2-1 (pectin-like polygalacturonan) and two active compounds, PJ2-2 and PJ2-3 which were homogeneous by HP-GPC.

The structure of PJN-1, (molecular mass : 23×10^3) was proved to be β-1,4-galactan.

PJN-3 (molecular mass: 40×10^3) consists of L-arabinose, D-galactose, D-glucose and D-galacturonic acid. By means of chemical and physical procedures, PJN-3 was formulated as shown in Fig 1.

PJ2-2 (molecular mass : 170×10^3) consists of L-arabinose, D-xylose, D-galactose, D-glucose, D-galacturonic acid, L-rhamnose and L-fucose. It was demonstrated that PJ2-2 is composed of the four well-defined components, A, B, C and D as shown in Fig 2.

PJ2-3 (molecular mass:40×10^3) afforded L-arabinose, D-xylose, D-galactose, D-galacturonic acid, D-glucose, L-rhamnose and L-fucose on acid hydrolysis. It was revealed that PJ2-3 is composed of four structural components, A, B, C and D as illustrated in Fig 3.

Dried Ginseng Roots :

The roots were extracted with methanol and then hot water. To the water extract, was added an excess of ethanol and a solution of the resulting precipitate in 0.5 M NaCl was dialyzed against water. The activity was observed not for dialyzed fraction but for non-dialyzed fraction, which was separated into seven fractions, PG-1 ~7 by chromatography on DEAE-Toyopearl 650M. The

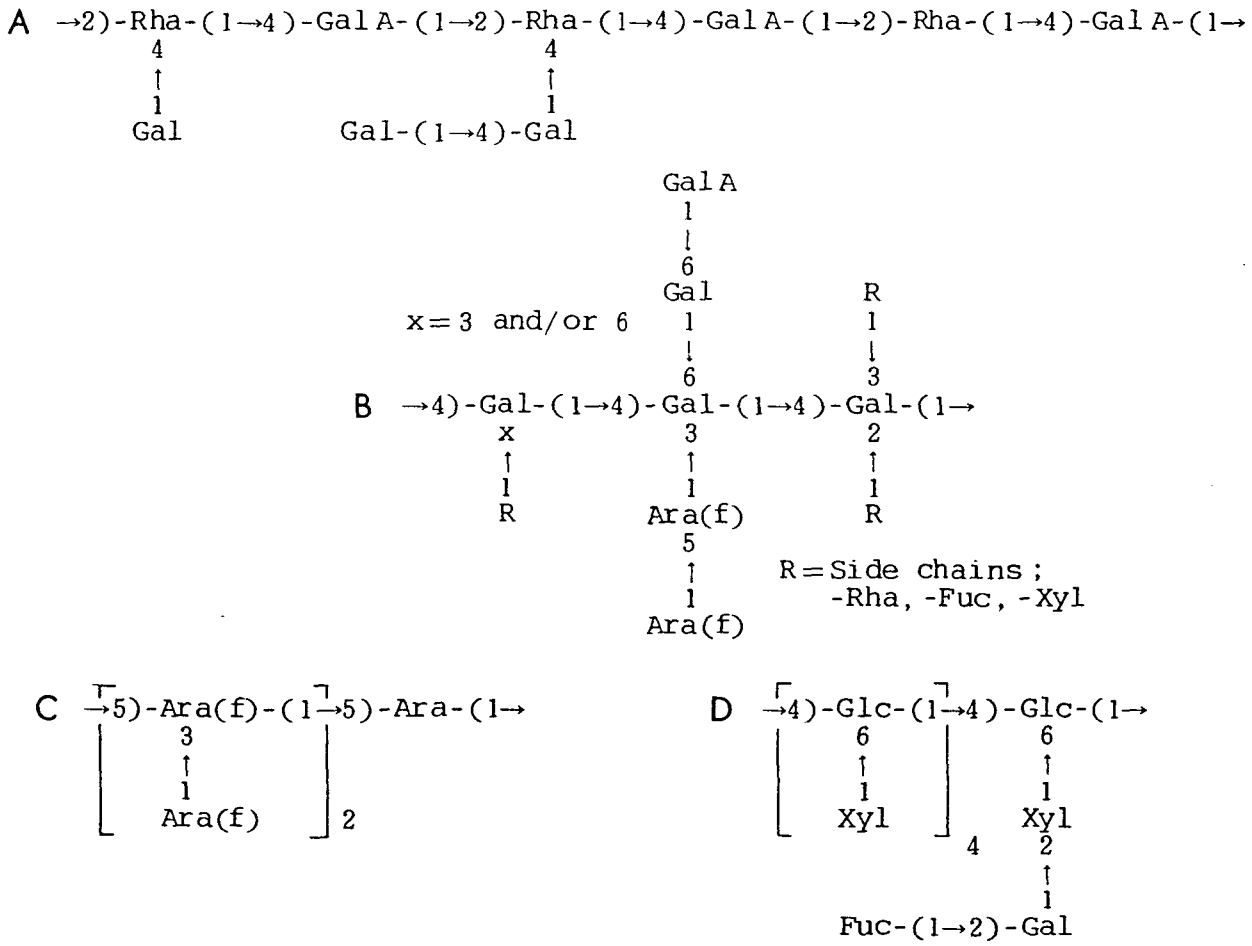

Fig. 3. Structure of PJ2-3 : A ; Rhamnogalacturonan unit, B ; Highly branched galactan unit, C ; Arabinan unit, D : Xyloglucan unit

most active fraction, PG-5 was further purified by chromatography on Toyopearl HW-55F, affording three highly active compounds, PG5-1, -2 and -3, which are complex cell wall carbohydrates. Both PG5-1 (molecular mass : 105×10^3) and -2 (29×10^3) consist of L-arabinose, L-rhamnose, D-mannose, D-galactose, D-galacturonic acid, D-glucose and D-glucuronic acid. PG5-1 and PG5-2 conatin 11.5 and 17.9% protein, respectively. The further structure elucidation of these polysaccharides are under progress.

Red Ginseng :

Red Ginseng powder supplied by Koera Ginseng and Tobacco Research Institute was extracted with methanol and then with hot water. To the aqueous solution of the water extract, was added an excess of ethanol and an aqueous solution of the resulting precipitate was dialyzed aganist water. In contrast to the above cases, the activity was observed not for non-dialyzed fraction but for dialyzed fraction. The active dialyzed fraction was chromatographed on highly porous polymer (Diaion HP-20) or on Toyopearl HW-40S. However, every fractions of both the chromatography exhibited no activity. Recombination of the fractions did not regenerate the activity. This suggested that during the process of preparation of Red Ginseng, high molecular active components of Ginseng are converted into low molecular active substances which are stable only in a crude state.

Acknowlegement

This study was supported by the Medical Society for Red Ginseng Research to which the authors' thanks are due.

Panax 속 식물의 다당류가 망내계 활성에 미치는 영향

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망내계에서 강한 탄소제거 활성을 나타내는 다당류를 *Panax*식물속에서 조사하였다. 건조한 *P. notoginseng* 에서 sanchinan-A라는 활성을 지닌 다당류를 분리하였다. Sanchinan-A의 분자량은 약 1,500,000 D 정도로 추정되며 6번 산소위치에 가지가 있는 β-D(1-3)-galactan 구조를 가지고 있다. 3개의 galactosyl units 에 2개 정도의 빈도로 6번산소위치에 주로 α-L-arabionfuranosyl 이 있으며 일부는 β-D-galactopyranosyl-(1-6)-β-D-galactopyranosyl-(1-3)-β-D-galactopyranosyl 가지가 있다. 건조한 *P. japonicus*의 근경에서도 활성을 지닌 몇가지 다당류가 분리되어 그 구조가 규명되었다. 건조한 *P. ginseng* 의 뿌리에서는 강한 활성을 지닌 몇가지 다당류가 분리되었다. 이들 화합물의 구조는 현재 구명중이다. 홍삼의 물추출물에서는 활성을 보이지 않았으나 투석한 분획에서는 현저히 망내계기능을 강화시켰다. 그러나 이러한 활성도는 분리과정에서 소실되었다. 이러한 사실로 보아 인삼중에서는 활성물질을 안정화시키는 어떤 물질이 그 분획중에 존개하기 때문인 것으로 사료된다.