Table II. A comparison of the twelve NH_2-terminal amino acids in α-momorcharin and trichosanthin

α-Momorcharin	*Asp*	*Val*	Ala	*Phe*	*Arg*	*Leu*	Ala	*Gly*	*Ala*	Asp	Pro	Arg
Trichosanthin	*Asp*	*Val*	Ser	*Phe*	*Arg*	*Leu*	Ser	*Gly*	*Ala*	Thr	Ser	Ser

Identical residues are in italics.

used and β-M showed only one band after BrCN digestion, it seems that β-M was resistant to BrCN digestion and this fact suggests that it has no methionine residues. On the other hand, α-M yielded one major band and two minor bands, and T yielded two major bands and one minor band after BrCN digestion. Results of the BrCN digestion experiment are consistent with the number of methionine residues of the proteins revealed by amino acid analysis. The patterns of peptic digest for the three proteins were distinct, although there were probably some fragments with similar electrophoretic mobilities. Essentially the same results were obtained when the time period of peptic digestion was increased to 24 hours. Dissimilar electrophoretic patterns were also obtained after chymotryptic digestion of the proteins. β-M was most susceptible to digestion with chymotrypsin and was degraded into small peptides. Treatment of the three proteins with other proteases such as subtilisin, thermolysin and trypsin also resulted in dissimilar electrophoretic patterns of fragments. In immunodiffussion the immunoprecipitin arcs formed by interaction of the proteins with their antisera intersected each other.

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Sanchinan-A, a Reticuloendothelial System Activating Arabinogalactan from Sanchi-Ginseng (Roots of *Panax notoginseng*)

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Abstract: A polysaccharide (molecular mass: $1.50 \cdot 10^6$) named sanchinan-A which shows remarkable reticuloendothelial system potentiating activity in carbon clearance test, was isolated from Sanchi-Ginseng (roots of *Panax notoginseng*), a Chinese traditional medicine. Chemical and spectroscopic studies led to the structure which is composed of a β-D-(1→3)-linked galactopyranosyl-backbone possessing branching points at position *O*-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 galactosyl units. Sanchinan-A contains a small amount of protein (3.27 %).

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Introduction

Sanchi-Ginseng [= Tienchi, roots of *Panax notoginseng* (Burk.) F. H. Chen, Araliaceae, cultivated in Yunnan, China] is a well-known Chinese traditional medicine. It has been used

as a tonic and a hemostatic agent. As far as the chemical constituents of this crude drug are concerned, several dammarane saponins which were identical or closely related with Ginseng saponins, have been isolated (1). The pharmacological studies on this drug have been also centered on these saponins. Since biological activities of polysaccharides of higher plants have attracted much attention from immunological view points, the present authors have conducted isolation of a reticuloendothelial system (RES) activating principle from the extract of this drug.

Results and Discussion

RES potentiation was followed by the *in vivo* carbon clearance test according to the Halpern's method (2) using zymosan as a positive control. Powdered Sanchi-Ginseng was extracted with methanol to remove saponins etc. A freeze dried water extact (yield: 39 %) of the residue was dissolved in water and to this solution was added an excess of ethanol. The resulting precipitate which exerted the significant activity, was subjected to chromatography on Sephadex G-50 and then on DEAE-Toyopearl 650M to give an active fraction (**2A**), which was further separated by preparative gel permeation HPLC (GPC) to afford an active substance (**2A-1**) in a 0.28 % yield.

On acid hydrolysis, **2A-1** yielded glucose, galactose, and arabinose in the ratio 189 : 10 : 1 and the ^{13}C-NMR spectrum suggested the formulation of **2A-1** as an amylopectin-like α-glucan having some galactopyranosyl and arabinofuranosyl units. For intraperitoneally administrated **2A-1**, the following biological activities were observed (3): Potentiation of RES in carbon clearance test for mice, enhancement of the production of antibodies against sheep red blood cells in mice, and normalization of delayed hypersensitivity reaction depressed by cyclophosphamide in mice. No acute toxicity to mice was observed on single administration of **2A-1**, 200 mg/kg.

The analytical GPC of **2A-1** apparently indicated the homogeneity of this polysaccharide. On treatment with amyloglucosidase, **2A-1** gave a large amount of glucose. However, in the analytical GPC of the hydrolysate, a peak of an unhydrolyzed substance was still observed at the same retention time as that of **2A-1**. This substance, named sanchinan-A (**SA**, yield from **2A-1**: 3.0 %), was isolated from the hydrolysate by preparative GPC, and showed more remarkable activity than **2A-1** in the carbon clearance test (Fig. 1). Acid hydrolysis of

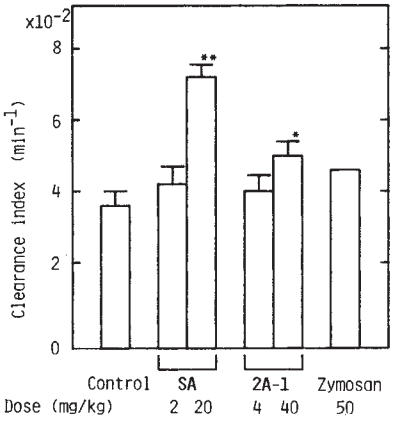

Fig. 1: Effect of **2A-1** and **SA** on carbon clearance index in ICR mice. The values are mean ± standard errors of 6 mice. Significantly different from the control, $^*p < 0.05$ or $^{**}p < 0.01$.

SA afforded L-arabinose and D-galactose (approximately 1 : 3.3) along with a negligible amount of glucose. Absence of uronic acid or its ester in the hydrolysate was revealed by the carbazole/H_2SO_4 method (4) using D-galacturonic acid as a standard. Total carbohydrate content determined by the phenol/H_2SO_4 method (5) (standard: D-galactose) was 93.6 %. As do some natural arabinogalactans, **SA** contained a small amount of protein (3.27 %), which was determined by the method of Lowry et al (6). It follows that **2A-1** is a mixture of amylopectin and an arabinogalactan (**SA**) with the similar molecular mass to each other and the activity observed for **2A-1** would be represented mainly by **SA**.

The molecular mass of **SA** was estimated to be $1.50 \cdot 10^6$ by comparison of the analytical GPC with those of authentic dextrans. The methylation analysis of **SA** (7) revealed the presence of terminal arabinofuranosyl, terminal galactopyranosyl, 3-linked galactopyranosyl, 6-linked galactopyranosyl, and 3,6-linked galactopyranosyl residues in the ratio 0.95 : 0.15 : 1.5 : 1.0 : 1.15, based on the E.c.r. theory (8). The ^{13}C-NMR signals at δ = 110.1, 84.6, 82.1, 77.3, 62.1 ppm indicated the presence of terminal α-arabinofuranosyl units and anomeric carbon signals at δ = 104.2 and 104.4 ppm revealed the β-anomeric configuration of galactopyranosyl residues.

The mild Smith's degradation (9) of **SA** yielded a polysaccharide (**SA-1**, molecular mass: $8.5 \cdot 10^5$) which consisted of D-galactose exclusively. The methylation analysis of **SA-1** revealed the presence of terminal galactopyranosyl, 3- and 3,6-linked galactopyranosyl residues in the ratio 2 : 1 : 2. A carbon signal of **SA-1** at δ = 104.1 ppm indicated the β-anomeric configuration. On further mild Smith's degradation, **SA-1** gave a polysaccharide (**SA-2**, molecular mass: $5.0 \cdot 10^5$). The methylation analysis of **SA-2** as well as its ^{13}C-NMR signals at δ = 104.6, 82.7, 75.5, 71.1, 69.2, and 61.7 ppm led to the formulation as a β-1,3-galactan.

Based on these results, it follows that **SA** is composed of main units of β-D-(1→3)-linked galactopyranosyl residues having branching points at position *O*-6 to which [mainly α-L-arabinofuranosyl and partly β-D-galactopyranosyl]-(1→6)-β-D-

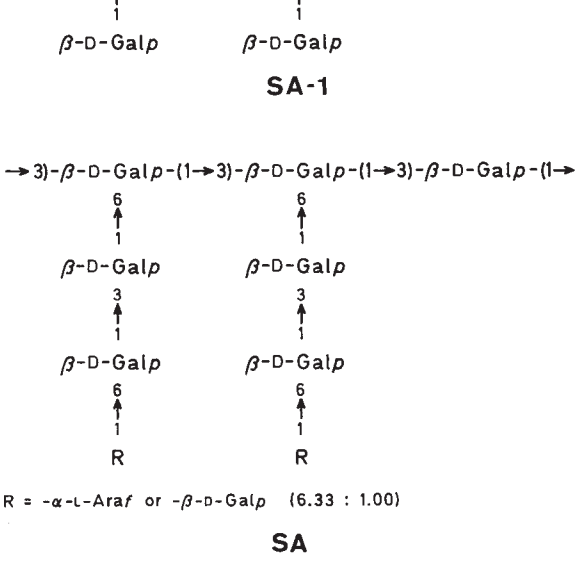

Chart 1: Structures of **SA** and **SA-1**

galactopyranosyl-(1→3)-β-D-galactopyranosyl side chains are attached on average to two of three galactose units (as shown in Chart 1). Recently, Yamada et al. reported the isolation of an arabinogalactan with anti-complementary activity from roots of *Angelica acutiloba* Kitagawa (10). This polysaccharide consist of β-D-(1→6)-linked galactopyranosyl backbone with branching points at position *O*-3, thus being different from **SA**.

Further investigations of other biological activities of **SA** are under progress and the details will be reported elsewhere.

Materials and Methods

The ^{13}C-NMR spectra were obtained with a JEOL FX-100 spectrometer at 25.00 MHz and a JEOL GX-270 spectrometer at 67.8 MHz at 85° C in D_2O (internal dioxane, 67.4 ppm relative to the signal for tetramethylsilane). Gas liquid chromatography-mass spectrometry (GC-MS) was carried out with a Shimadzu GC-MS 7000. HPLC was carried out with an HLC 803D (Toyo Soda Manuf. Co., Ltd., Japan). Detection was accomplished with a differential refractometer RI-8 (Toyo Soda Manuf. Co., Ltd.).

Determination of the molecular mass of polysaccharide

The polysaccharide (1.0 mg) was dissolved in 0.5 M NaCl (0.5 ml), and the solution was subjected to GPC on columns of TSKgel G-3000PW$_{XL}$, G-5000PW$_{XL}$ and G-6000PW$_{XL}$ (7.5 mm × 30 cm each) which were set one after the other; mobile phase: 0.5 M NaCl degassed by sonication with depression, flow-rate: 0.6 ml/min. The column was calibrated with standard dextrans of mol. mass ($\cdot 10^3$) = 2000, 500, 150, 70, and 40 (Sigma).

Extraction and Purification

Sanchi-Ginseng (1 kg) which was cultivated in Yunnan, China, was extracted with MeOH. The residue was decocted with hot H_2O (6 l) to give H_2O extracts (384 g) after evaporation. The H_2O extract was dissolved in H_2O (500 ml) and to this solution was added EtOH (1.5 l). The resulting precipitates were dissolved in H_2O. After removing insoluble substances by centrifugation, the aqueous solution was dialyzed against running water through seamless cellulose tubing for 24 hours. The non-dialysate, a brown powder (138 g) after lyophilization, was chromatographed on a column of Sephadex G-50 (eluted with H_2O) to be separated into two fractions, PN-1 (112 g) and PN-2 (26 g) in the order of elution. PN-1 was chromatographed on a DEAE-Toyopearl 650M column (HCO_3^- form) (eluted with 0.02 M NH_4HCO_3 and then with 0.3 M NaCl). The NH_4HCO_3 eluate was lyophilized repeatedly to give 2A as a white powder (65 g). 2A was further purified by GPC on TSKgel G-5000PWG (21.5 mm × 60 cm, mobile phase: H_2O), affording **2A-1** (28 g).

Enzymic Hydrolysis

A solution of **2A-1** (1 g) in 50 mM acetate buffer (pH 4.5, 50 ml) was incubated with amyloglucosidase (200 mg, 6 units/mg, from *Aspergillus niger*, Boehringer Mannheim GmbH, West Germany) at 37° C for 3 hours. The reaction mixture was heated at 100° C and then filtered. The filtrate was chromatographed on a column of Sepharose CL-4B (eluted with 0.5 M NaCl) to give glucose and **SA** (30 mg), a white powder, $[\alpha]_D^{24}$: −20.7° (*c* 0.37 H_2O). Nitrogen content in **SA** was found to be less than 0.89 % by elemental analysis. Content of protein determined by the method of Lowry et al. (6) was 3.27 %.

Analysis of component sugars of **SA**

SA was hydrolyzed with 1 N H_2SO_4 for 4 hours at 90° C. The hydrolysate was neutralized with $BaCO_3$ and then deionized with ion-exchange resin (Amberlite MB-3). To the solution, after concentration to 2 ml, $NaBH_4$ (10 mg) was added. After standing for 2 hours at room temperature, the solution was acidified to pH 3.5 with Dowex 50W (H^+ form) ion-exchange resin, filtered and concentrated to dryness. Boric acid in the reaction mixture was removed by repeated co-distillation with MeOH below 40° C. The resulting alditols were acetylated with acetic anhydride and pyridine (1 : 1, 1 ml) at 100° C for 1 hour. The acetylated alditol mixture was subjected to analysis by GLC [5 % ECNSS-M on Chromosorb W (2.6 mm × 2.0 m); column temperature 200° C; N_2 flow rate 40 ml/min; detection FID] showed the presence of arabinose and galactose (1 : 3.3) together with a trace of glucose. Total sugar content was determined by the phenol/H_2SO_4 method (5) using D-galactose as a standard; 93.6 %.

Absolute Configuration of Component Sugars (11)

A solution (50 µl) of the acid-hydrolysate of **SA** (*vide supra*) in H_2O was added to an EtOH solution (50 µl) of 1-α-L-benzylamine (10 mg) and $NaBCNH_3$ (5 mg) and the mixture was allowed to stand at 40° C for 4 hours. Acetic acid (10 µl) was added to this reaction mixture and the solution was concentrated to dryness. The residue was treated with BSA (20 µl) at 60° C for 1 hour and then subjected to GLC analysis [glass capillary column Carbowax 20M (0.25 mm × 25 m); column temperature 150° C; He flow rate 3 ml/min; spliting ratio 100; detection FID]. By comparison with respective peaks derived from authentic samples, L-arabinose and D-galactose were detected.

Methylation analysis of **SA**

A solution (0.5 ml) of methylsulfinyl carbanion was added to a solution of **SA** (15 mg) in dry DMSO and 1,1,3,3-tetramethylurea (1 : 1, 1 ml) (12, 13). The solution was treated in an ultra-sonic bath for 1 hour and then methyl iodide (0.5 ml) was added to the solution. After ultra-sonic agitation for 1 hour, ice-cooled water was added to the reaction mixture. The solution was dialyzed against running water for 24 hours and the non-dialysate was concentrated to dryness to give the permethylate of **SA** (12 mg) which showed no hydroxyl band in the IR spectrum. This permethylate was treated in 90 % formic acid at 90° C for 1 hour. The reaction mixture was concentrated to dryness and the residue was treated with 0.13 M H_2SO_4 for at 90° C for 16 hours. The solution was neutralized with $BaCO_3$, filtered and concentrated to 2 ml. The resulting mixture of methylated sugars was reduced and acetylated in the same way as sugar components analysis (*vide supra*) to give a mixture of methylated alditol acetates which was subjected to GC-MS analysis: 2.5 % Silicone GE XE-60 on Chromosorb W (2.6 mm × 2.0 m); He flow rate 30 ml/min; column temperature 155–245° C (1° C/min); ionization voltage 70 eV. 1,4-Di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol, 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactitol, and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol were detected in the ratio 0.95 : 0.15 : 1.50 : 1.00 : 1.15.

Mild Smith Degradation and Analysis of the Products

SA (100 mg) was dissolved in a 25 mM aqueous solution of $NaIO_4$ (25 ml) and the mixture was allowed to stand at 4° C for 6 days under shielding the light. Excess periodate was decomposed with ethyleneglycol (2 ml) and the solution was dialyzed against running water for 24 hours. The non-dialysate was lyophilized and dissolved in an aqueous solution (5 ml) of $NaBH_4$ (25 mg). After stirring at room temperature for 24 hours, the reaction mixture was acidified to pH 3.5 with Dowex 50W (H^+ form) ion-exchange resin, filtered, lyophilized and treated with 0.1 N H_2SO_4 at 25° C for 24 hours to give **SA-1** (28 mg). **SA-1** (20 mg) was treated in the same manner to give **SA-2** (13 mg).

Methylation analysis of **SA-1** and **SA-2** in the same procedure as **SA** (*vide supra*) revealed the formations of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol, and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylgalactitol in the ratio 2 : 1 : 2 from **SA-1** and only 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactitol from **SA-2**.

Carbon Clearance Test

Clearance of carbon particules from the blood circulation was assayed by the method of Halpern et al. (2) as follows. A solution of a sample in saline was addministrated intraperitoneally to six male mice (ICR strain, average body weight: 28 g). After 24 hours, a suspension of Peli-

kan carbon particles (drawing ink 17-black, Günther Wagner, Hannover, West Germany) in saline (1 : 3) (5 ml/kg body weight) was injected intravenously. Blood specimens of 25 µl were obtained by a puncture of retroorbital venous plexus at 2 and 10 min after the carbon injection. The blood specimens were suspended in 2 ml of 0.1 % Na_2CO_3 and relative amount of carbon was estimated with a UV-Visible spectrometer. The density readings were converted to semilogarithmic scale and plotted against time. The slope of the line was expressed as clearance index. Statistical analysis: The significance of the results obtained was evaluated by student's t test or the Aspin-Welch method.

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Eupatoriopicrin 19-*O*-Linolenoate and Other Constituents from *Eupatorium cannabium*

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Abstract: The investigation of the roots and aerial parts of *Eupatorium cannabium* afforded, in addition to the sesquiterpene lactones eupatolide, eupatoriopicrin, and eucannabinolide isolated previously, eight further lactones, three of which are new. Furthermore, in addition to known compounds, a new clerodane derivative and two further tremetone derivatives as well as 10-acetoxyneryl acetate were isolated.

Eupatorium cannabium L. (officinal: radix et herba Cunigundae) has been used in folk medicine. So far, in addition to triterpenes (1), the isolation of three germacranolides (2) and of pyrrolizidine alkaloids (3) have been reported. Also the essential oil has been investigated (4). We have now reinvestigated this species.

The extract of the aerial parts afforded, as reported previously (2), eupatoriopicrin, eucannabinolide, and eupatolide. Furthermore, 3β-hydroxyeupatoriopicrin (5), 20-deoxyeupatoriopicrin (6), eupatoriopicrin 19-*O*-acetate (7), the deoxy derivatives **3** (8) and **4** (7), sachalinin (**6**) (9), and three unknown lactones, the germacranolides **1** and **5** as well as the guaianolide **7** were present. In addition to these compounds, the clerodane derivative **12**, lutein, 4-hydroxy-β-ionone, (*E*)-hex-1-enoic acid, germacrene D, neryl acetate, and neryl isovalerate were isolated also.

The root extract gave sitosterol, neryl acetate, and the isobutyrate of nerol as well as 10-acetoxyneryl acetate (**11**), 6-hydroxytremetone (10), the 3β-angeloyloxy derivative **8** (11), the corresponding methacrylate **9**, and the isobutyrate **10**.

The ^{1}H-NMR spectrum of **1** was in part close to that of eupatoriopicrin. However, the 4′-H-signals were shifted downfield and the typical signals of the linolenoate residue were present. As no other ester signals could be detected, the linolenoate group only could be placed at C-4′. This was strongly supported by the mass spectrum which showed a molecular ion corresponding to $C_{38}H_{54}O_7$ and a fragment m/z = 392 ($C_{23}H_{36}O_5$) which agreed with the proposed acid ester. So far, no sesquiterpene lactone of this type has been reported. However, from an *Eupatorium* species, a β-hydroxystearate of a germacranolide-8β-[4-hydroxytiglate] was reported (12) and, from an *Acanthospermum* species, a mixture of C_{18}-esters of a melampolide was isolated (15).

The structure of **5** directly followed from the ^{1}H-NMR spectrum which was close to that of eupaserrin (14), the corresponding acetoxytiglate. Also, the structure of **7** clearly followed from the ^{1}H-NMR spectral data which were close to those of the known 2β-hydroxy derivative **7a** (15). The presence of a 2β-acetoxy group caused the expected differences. Spin decoupling allowed the assignment of all signals and the couplings indicated the configuration at C-2. The isolation of **7** together with related germacranolides is of interest as the latter surely are the precursors of the guaianolides which are common

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