Characterization of Two Polysaccharides Having Activity on the Reticuloendothelial System from the Root of *Glycyrrhiza uralensis*

Masashi TOMODA,* Noriko SHIMIZU, Mieko KANARI, Ryōko GONDA, Sumiko ARAI and Yūko OKUDA

Kyoritsu College of Pharmacy, Shibakōen, Minato-ku, Tokyo 105, Japan. *Received November 20, 1989*

Two polysaccharides, called glycyrrhizans UA and UB, were isolated from the root of *Glycyrrhiza uralensis* FISCHER. They were homogeneous on electrophoresis and gel chromatography, and showed reticuloendothelial system-potentiating activity in a carbon clearance test. Glycyrrhizan UA is composed of L-arabinose: D-galactose: L-rhamnose: D-galacturonic acid in the molar ratio of 20:14:1:3, and glycyrrhizan UB is composed of L-arabinose: D-galactose: D-glucose: L-rhamnose: D-galacturonic acid in the molar ratio of 12:10:1:10:20, in addition to small amounts of *O*-acetyl groups and peptide moiety, respectively. About 10% (glycyrrhizan UA) and 35% (glycyrrhizan UB) of the D-galacturonic acid residues exist as the methyl esters. Methylation analysis, carbon-13 nuclear magnetic resonance and periodate oxidation studies indicated their structural features.

Keywords *Glycyrrhiza uralensis*; root; licorice; polysaccharide structure; reticuloendothelial system; immunological activity; glycyrrhizan UA; glycyrrhizan UB; acidic arabinogalactan

The root of *Glycyrrhiza uralensis* FISCHER is a representative Chinese licorice with the trade name Tongpei licorice (Japanese name, Tohoku kanzo). The licorice root is a very important crude drug used in China and Japan as well as in European countries. Many components in this crude drug have been reported, but no pure polysaccharide with biological activity has so far been obtained. We have now isolated two polysaccharides from the root of *G. uralensis*, and these polysaccharides show significant activity on the reticuloendothelial system (RES). The present paper describes their isolation from this crude drug, their structural analysis and immunological activity.

Material and Methods

Isolation of Polysaccharides The material was imported from China. The sliced roots (1.5 kg) were extracted with hot water (15 l) under stirring for 1 h in a boiling water bath. After centrifugation, the supernatant was poured into two volumes of ethanol. After centrifugation and drying, the precipitate (28.3 g) was dissolved in 0.01% sodium sulfate (6.3 l) and centrifuged; 5% cetyltrimethylammonium bromide (850 ml) was then added to the supernatant. The precipitate was separated by centrifugation, then dissolved in 0.2 M sodium chloride (1420 ml). After centrifugation, the supernatant was poured into two volumes of ethanol. The resulting precipitate was dissolved in water, then dialyzed and lyophilized. The yield of this fraction (CTAB-ppt) was 841.5 mg. The supernatant obtained after addition of cetyltrimethylammonium bromide was poured into two volumes of ethanol, and after centrifugation, the precipitate was dissolved in water, then dialyzed and lyophilized. The yield of this fraction (CTAB-sup) was 3.5 g. Fraction CTAB-sup (300 mg) was dissolved in water and applied to a column (5 × 85 cm) of Sephacryl S-300. The column was equilibrated and eluted with 0.1 M Tris–HCl buffer (pH 7.0), and fractions of 20 ml were collected and analyzed by the phenol–sulfuric acid method.[1] Fraction 1 was obtained from tubes 27 to 29, fr. 2 from tubes 30 to 33, fr. 3 from tubes 34 to 44, and fr. 4 from tubes 45 to 61. Fractions 3 and 4 were dialyzed and rechromatographed using the same column of Sephacryl S-300. After dialysis and gel chromatography using a column (5 × 79 cm) of Sephadex G-25 with water, frs. A and B were obtained from frs. 3 and 4, respectively; their yields were 42 and 46 mg.

Fraction A (100 mg) was dissolved in 1/15 M phosphate buffer (pH 7.0) containing 0.15 M NaCl, 1 mM $MgCl_2$ and 1 mM $CaCl_2$, and applied to a column (1.5 × 40.5 cm) of Con A-Sepharose (Pharmacia Co.). The column was equilibrated and eluted with the same buffer at 4 °C, and fractions of 10 ml were collected. The eluates obtained from tubes 6 to 9 were combined, dialyzed and then concentrated. The solution from 250 mg of fr. A was applied to a column (5 × 77 cm) of Sephadex G-25. The column was eluted with water and fractions of 20 ml were collected. The eluates obtained from tubes 29 to 33 were combined, concentrated and lyophilized. Glycyrrhizan UA was obtained as a white powder. The yield from 3.5 g of fr. CTAB-sup was 261 mg. Fraction B was applied to affinity chromatography with Con A-Sepharose under the same conditions as those of fr. A, followed by dialysis and gel chromatography with Sephadex G-25. After concentration of the sugar-containing fraction, the solution obtained was treated with one-third volume of 10% trichloroacetic acid. Following centrifugation, the acid was removed from the supernatant by extraction with ether, then the aqueous layer was applied to a column (5 × 87 cm) of Sephadex G-25. The column was eluted with water, and fractions of 20 ml were collected. The eluates obtained from tubes 30 to 32 were combined, concentrated and lyophilized. Glcyrrhizan UB was obtained as a white powder from fr. B. The yield from 3.5 g of fr. CTAB-sup was 129 mg.

Polyacrylamide Gel Electrophoresis (PAGE) This was carried out in an apparatus with gel tubes (4 × 140 mm each) and 5 mM Tris–glycine buffer (pH 8.3) at 5 mA/tube for 40 min. Gels were stained by the periodate–Schiff (PAS) procedure and with Coomassie blue reagent. Glycyrrhizans UA and UB gave distinct bands at distances of 66 and 62 mm from the origin, respectively.

Gel Chromatography The sample (3 mg) was dissolved in 0.1 M Tris–HCl buffer (pH 7.0), and applied to a column (2.6 × 98 cm) of Sephacryl S-300, pre-equilibrated and developed with the same buffer. Fractions of 5 ml were collected and analyzed by the phenol–sulfuric acid method. Standard pullulans (Shōwa Denkō Co.) having known molecular weights were run on the column to obtain a calibration curve.

Phagocytic Activity This was measured as described in a previous report.[2] The samples and a positive control, zymosan (Tokyo Kasei Co.), were each dissolved in physiological saline and dosed i.p. (20 mg/kg body weight) once a day. The phagocytic index, K, was calculated by means of the following equation:

$$K = (\ln OD_1 - \ln OD_2)/(t_2 - t_1)$$

where OD_1 and OD_2 are the optical densities at times t_1 and t_2, respectively. Results were expressed as the arithmetic mean ± S.D. of five male mice (ICR-SPF).

Qualitative Analysis of Component Sugars Hydrolysis and cellulose thin-layer chromatography (TLC) of component sugars were performed as described in a previous report.[3] The configurations of component neutral sugars were identified by gas chromatography (GC) of trimethylsilylated α-methylbenzylaminoalditol derivatives.[4] GC was carried out on a Shimadzu GC-7AG gas chromatograph equipped with a hydrogen flame ionization detector.

Determination of Components Neutral sugars were analyzed by GC after conversion of the hydrolyzate into alditol acetates as described in a previous report.[2] Galacturonic acid was determined by a modification of the carbazole method.[5] Peptide determination was performed by the method of Lowry *et al.*[6]

Determination of *O*-Acetyl Groups The sample was hydrolyzed with 0.2 N hydrochloric acid and analyzed by GC using propionic acid as an internal standard as described in a previous report.[7]

Determination of *O*-Methyl Groups in Methyl Esters This was performed by GC after saponification using ethanol as an internal standard as described in a previous report.[8]

Nuclear Magnetic Resonance (NMR) NMR spectra were recorded on

TABLE I. Relative Retention Times on GC and Main Fragments in MS of Partially Methylated Alditol Acetates

	Relative retention time[a]	Main fragments (*m/z*)
1,4-Ac-2,3,5-Me-L-arabinitol	0.69	43, 45, 71, 87, 101, 117, 129, 161
1,5-Ac-2,3,4-Me-L-arabinitol	0.79	43, 101, 117, 161
1,3,5-Ac-2,4-Me-L-arabinitol	1.04	43, 87, 113, 117, 233
1,4,5-Ac-2,3-Me-L-arabinitol	1.13	43, 87, 101, 117, 129, 189
1,2,5-Ac-3,4-Me-L-rhamnitol	0.95	43, 89, 129, 131, 189
1,2,4,5-Ac-3-Me-L-rhamnitol	1.28	43, 87, 101, 129, 143, 189, 203
1,5-Ac-2,3,4,6-Me-D-glucitol	1.00	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,5-Ac-2,3,4,6-Me-D-galactitol	1.09	43, 45, 71, 87, 101, 117, 129, 145, 161, 205
1,3,5-Ac-2,4,6-Me-D-galactitol	1.36	43, 45, 87, 101, 117, 129, 161
1,4,5-Ac-2,3,6-Me-D-galactitol	1.44	43, 45, 87, 99, 101, 113, 117, 233
1,5,6-Ac-2,3,4-Me-D-galactitol	1.58	43, 87, 99, 101, 117, 129, 161, 189
1,2,4,5-Ac-3,6-Me-D-galactitol	1.75	43, 45, 87, 99, 113, 129, 189, 233
1,3,5,6-Ac-2,4-Me-D-galactitol	2.01	43, 87, 117, 129, 189

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. Abbreviations: Ac=acetyl; Me=methyl (*e.g.*, 1,4-Ac-2,3,5-Me-=1,4,-di-*O*-acetyl-2,3,5-tri-*O*-methyl-).

a JEOL JNM-GX 270 FT NMR spectrometer in heavy water containing sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard at 30 °C.

Reduction of Carboxyl Groups This was carried out with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate and sodium borohydride as described in a previous report.[9] The reduction was repeated four times under the same conditions. Yields were 27 mg from 52 mg of glycyrrhizan UA, and 21 mg from 50 mg of glycyrrhizan UB.

Methylation Analysis Methylation was performed with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide as described in a previous report.[10] The reaction was repeated three times under the same conditions. Yields were 4 mg from 10 mg of glycyrrhizans UA and UB, and 6 mg from 10 mg of their carboxyl-reduced derivatives, respectively. The products were hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated as described in a previous report.[11] The partially methylated alditol acetates obtained were analyzed by gas chromatography-mass spectrometry (GC-MS) using a fused silica capillary column (0.32 mm i.d. × 30 m) of SP-2330 (Supelco Co.) with a programmed temperature increase of 4 °C per min from 160 to 220 °C at a helium flow of 1 ml per min. GC-MS was performed with a JEOL JMS-GX mass spectrometer. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GC and the main fragments in MS are listed in Table I.

Periodate Oxidation The sample (5.0 mg) was oxidized with 0.05 M sodium metaperiodate (2.5 ml) at 5 °C in the dark. The periodate consumption was measured by a spectrophotometric method.[12] Oxidation was completed after 3 d. The reaction mixture was successively treated with ethylene glycol (0.04 ml) at 5 °C for 1 h and sodium borohydride (25 mg) at 5 °C for 16 h, then adjusted to pH 5.0 by addition of acetic acid. The solution was concentrated and applied to a column (2.6 × 98 cm) of Sephadex G-25. The column was eluted with water, and fractions of 10 ml were collected. The eluates obtaind from tubes 22 to 24 were combined, concentrated and lyophilized. Yields were 3.1 mg from glycyrrhizan UA and 2.6 mg from glycyrrhizan UB. Determination of the components was carried out as described above.

Results

The crude polysaccharide fraction was isolated from the root of *G. uralensis* by hot water extraction followed by precipitation with ethanol, then dissolved in dilute sodium sulfate. After treatment with cetyltrimethylammonium bromide, the supernatant obtained was poured into ethanol. The resulting precipitate was purified by gel chromatography with Sephacryl S-300 and Sephadex G-25, and fr. A and B were obtained. The solution of fr. A was subjected to affinity chromatography on Con A-Sepharose. A pure polysaccharide designated as glycyrrhizan UA was obtaind from the passed-through fraction with a phosphate buffer, followed by dialysis and gel chromatography with Sephadex G-25. The solution of fr. B was also subjected to affinity chromatography under the same conditions as those of fr. A. The passed-through fraction was dialyzed, concentrated and purified by gel chromatography with Sephadex G-25. The fraction obtained was treated with trichloroacetic acid followed by gel chromatography with Sephadex G-25, and another pure polysaccharide designated as glycyrrhizan UB was obtained. The isolation method of the two polysaccharides is summarized in Fig. 1. The Con A-adsorbed fractions obtained from frs. A and B by the affinity chromatography afforded the other RES-activating polysaccharides called glycyrrhizans UC and UE, respectively. Their properties and structural features will be reported in the near future.

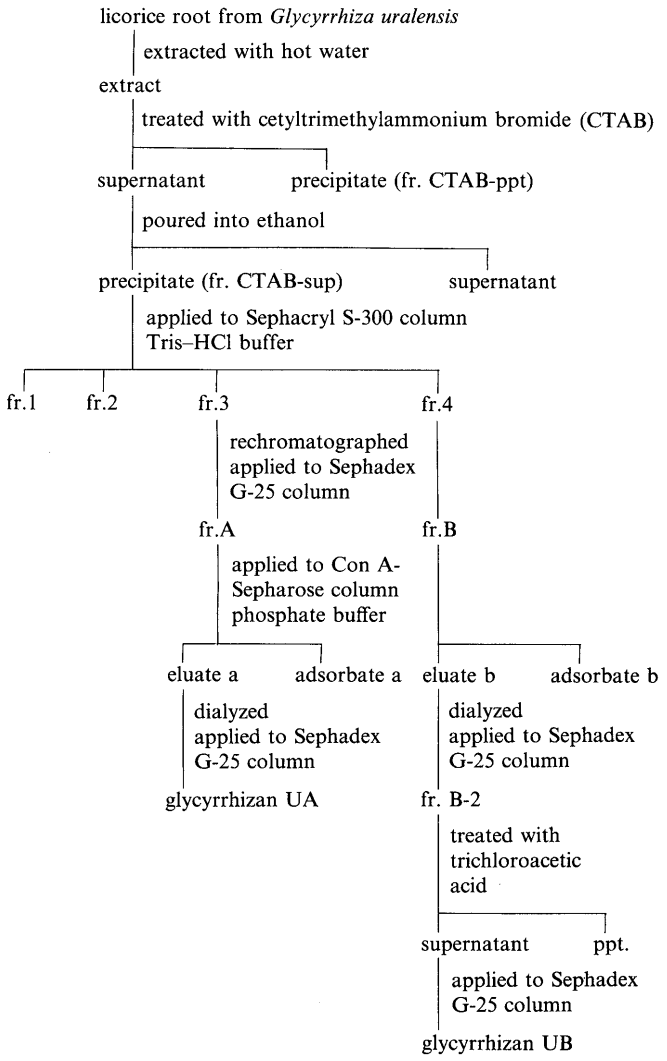

Fig. 1. Isolation of Glycyrrhizans UA and UB

Each polysaccharide gave a single band on PAGE, and gave a single peak on gel chromatography. Glycyrrhizan

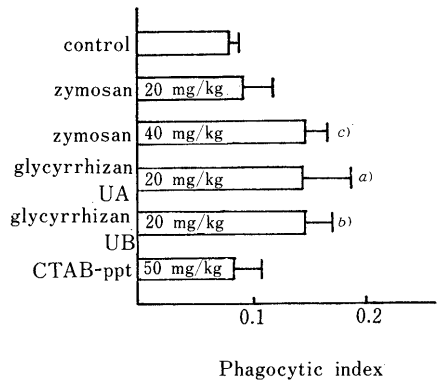

Fig. 2. Effects of Glycyrrhizans UA and UB, and Fr. CTAB-ppt on Carbon Clearance Index in ICR Mice

Significantly different from the control, *a*) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

UA had $[\alpha]_D^{24}$ $-56.3°$ (H_2O, $c=0.1$) and glycyrrhizan UB had $[\alpha]_D^{24}$ $+61.0°$ (H_2O, $c=0.1$). Gel chromatography gave values of 69.0×10^3 and 10.7×10^3 for the molecular masses of glycyrrhizans UA and UB, respectively.

The effects of the two polysaccharides and fr. CTAB-ppt on the RES were demonstrated by a modification[2] of the *in vivo* carbon clearance test[13] using zymosan as a positive control. As shown in Fig. 2, the phagocytic indices were significantly increased, suggesting the activation of RES by i.p. injection of the two polysaccharides. Fraction CTAB-ppt showed no RES activity.

Glycyrrhizan UA is composed of L-arabinose, D-galactose, L-rhamnose, D-galacturonic acid and a peptide moiety. Quantitative analyses showed that it contained 45.0% arabinose, 37.8% galactose, 2.5% rhamnose, 9.0% galacturonic acid and 3.2% peptide moiety. The molar ratio of these component sugars was 20 : 14 : 1 : 3. Glycyrrhizan UB is composed of L-arabinose, D-galactose, D-glucose, L-rhamnose, D-galacturonic acid and a peptide moiety. It contained 16.9% arabinose, 16.7% galactose, 1.9% glucose, 14.1% rhamnose, 37.3% galacturonic acid and 7.5% peptide moiety. The molar ratio of these component sugars was 12 : 10 : 1 : 10 : 20.

The carbon-13 NMR (^{13}C-NMR) spectrum of glycyrrhizan UA showed signals at δ 21.75 and 178.26 ppm, suggesting the presence of *O*-acetyl groups. In addition, the ^{13}C-NMR spectrum showed a signal at δ 57.01 ppm, suggesting the presence of *O*-methyl groups as carboxylic acid methyl esters. The presence of these groups was confirmed by GC of the hydrolyzate, and the polysaccharide contained 2.9% acetyl and 0.2% methoxyl groups. Thus about 10% of the galacturonic acid residues in the polysaccharide exist as methyl esters.

Further, the ^{13}C-NMR spectrum showed six signals due to anomeric carbons at δ 102.09, 102.44, 105.31, 105.85, 110.13 and 111.94 ppm. The first and the second were assigned to the anomeric carbons of α-D-galactopyranosyluronic acid and α-L-rhamnopyranose, respectively.[14] The signals at δ 105.31 and 105.85 ppm were assigned to the anomeric carbons of β-D-galactopyranose and α-L-arabinopyranose, respectively.[15,16] The signals at δ 110.13 and 111.94 ppm were assigned to the anomeric carbons of α-L-arabinofuranose.[16]

In the case of glycyrrhizan UB, its ^{13}C-NMR spectrum showed the signals of *O*-acetyl groups at δ 21.75 and 177.34 ppm and the signal of *O*-methyl groups as carboxylic acid methyl esters at δ 57.01 ppm. The presence of these groups was also confirmed by GC of the hydrolyzate, and quantitative analysis showed that the polysaccaride contained 2.5% acetyl and 2.3% methoxyl groups. About 35% of the galacturonic acid residues exist as methyl esters.

In addition, the ^{13}C-NMR spectrum of glycyrrhizan UB showed seven signals due to anomeric carbons at δ 100.31, 101.37, 102.18, 106.08, 107.06, 110.14 and 111.94 ppm. The first, the second and the third signals were assigned to the anomeric carbons of α-D-glucopyranose,[15] α-D-galactopyranosyluronic acid and α-L-rhamnopyranose, respectively. The signals at δ 106.08 and 107.06 ppm were assigned to the anomeric carbons of β-D-galactopyranose and α-L-arabinopyranose, respectively. The last two signals were assigned to the anomeric carbons of α-L-arabinofuranose.

The carboxyl groups of galacturonic acid residues in each polysaccharide were reduced to give the corresponding neutral sugar residues.[17] Both the original polysaccharides and the carboxyl-reduced derivatives were methylated with methylsulfinyl carbanion and methyl iodide in dimethyl sulfoxide.[18] The methylated products were hydrolyzed, then converted into the partially methylated alditol acetates. Hexuronic acid methyl ethers from the original samples were removed from the hydrolyzate by treatment with an anion-exchange resin. Analysis by GC-MS[19] gave the results shown in Tables II and III.

Table II. Methylation Analysis of Glycyrrhizan UA and Its Carboxyl-Reduced Derivative

Methylated sugars (as alditol acetates)	Molar ratios	
	Original	Carboxyl-reduced
2,3,5-Me-L-arabinose	22	22
2,3,4-Me-L-arabinose	4	4
2,3-Me-L-arabinose	26	26
2,4-Me-L-arabinose	8	8
3,4-Me-L-rhamnose	1	1
3-Me-L-rhamnose	2	2
2,3,4,6-Me-D-galactose	1	1
2,4,6-Me-D-galactose	8	8
2,3,6-Me-D-galactose	8	14
2,3,4-Me-D-galactose	3	3
3,6-Me-D-galactose	—	3
2,4-Me-D-galactose	22	22

Table III. Methylation Analysis of Glycyrrhizan UB and Its Carboxyl-Reduced Derivative

Methylated sugars (as alditol acetates)	Molar ratios	
	Original	Carboxyl-reduced
2,3,5-Me-L-arabinose	4	4
2,3,4-Me-L-arabinose	1	1
2,3-Me-L-arabinose	4	4
2,4-Me-L-arabinose	3	3
3,4-Me-L-rhamnose	6	6
3-Me-L-rhamnose	4	4
2,3,4,6-Me-D-galactose	3	3
2,4,6-Me-D-galactose	2	2
2,3,6-Me-D-galactose	1	19
2,3,4-Me-D-galactose	1	1
3,6-Me-D-galactose	1	3
2,4-Me-D-galactose	2	2
2,3,4,6-Me-D-glucose	1	1

(twenty-two)[a] α-L-Araf 1→ (one)[a] β-D-Galp 1→
(twenty-six)[a] →5 α-L-Araf 1→ (eight)[a] →3 β-D-Galp 1→
(four)[a] α-L-Arap 1→ (eight)[a] →4 β-D-Galp 1→
(eight)[a] →3 α-L-Arap 1→ (three)[a] →6 β-D-Galp 1→
6
(one)[a] →2 α-L-Rhap 1→ (twenty-two)[a] →3 β-D-Galp 1→
4
(two)[a] →2 α-L-Rhap 1→ (six)[a] →4 α-D-GalpA 1→
(three)[a] →4 α-D-GalpA 1→
2

Chart 1. Component Sugar Residues in the Minimal Unit in the Structure of Glycyrrhizan UA

a) Number of residues. Araf, arabinofuranose; Arap, arabinopyranose; Rhap, rhamnopyranose; Galp, galactopyranose; GalpA, galactopyranosyluronic acid.

(four)[a] α-L-Araf 1→ (three)[a] β-D-Galp 1→
(four)[a] →5 α-L-Araf 1→ (two)[a] →3 β-D-Galp 1→
(one)[a] α-L-Arap 1→ (one)[a] →4 β-D-Galp 1→
(three)[a] →3 α-L-Arap 1→ (one)[a] →6 β-D-Galp 1→
(one)[a] →4 β-D-Galp 1→
(six)[a] →2 α-L-Rhap 1→ 2
4 6
(four)[a] →2 α-L-Rhap 1→ (two)[a] →3 β-D-Galp 1→
(one)[a] α-D-Glcp 1→ (eighteen)[a] →4 α-D-GalpA 1→
(two)[a] →4 α-D-GalpA 1→
2

Chart 2. Component Sugar Residues in the Minimal Unit in the Structure of Glycyrrhizan UB

a) Number of residues. Glcp, glucopyranose; Araf, Arap, Rhap, Galp, GalpA, the same as those in Chart 1.

Each polysaccharide was subjected to periodate oxidation followed by reduction with sodium borohydride. The component sugar analysis of the product from glycyrrhizan UA showed that two-fifteenths of the arabinose units, five-sevenths of galactose units, two-thirds of rhamnose units and one-third of galacturonic acid units remained after periodate oxidation. In the product from glycyrrhizan UB, one-fourth of the arabinose units, a half of galactose units, two-fifths of rhamnose units and one-tenth of galacturonic acid units survived after periodate oxidation. No glucose was found in the product. Thus, these results were consistent with those of methylation analysis.

The accumulated evidence described above indicated that the minimal unit of glycyrrhizan UA is composed of thirteen kinds of component sugar units, and that the minimal unit of glycyrrhizan UB is composed of fifteen kinds of component sugar units, as shown in Charts 1 and 2.

Discussion

An RES-activating arabinogalactan called sanchinan-A has been obtained from the root of *Panax notoginseng*,[20] and it belongs to an arabino-3,6-galactan type polysaccharide. We have reported the RES activities and structural features of two acidic arabinogalactans called saposhnikovans A and C from the root and rhizome of *Saposhnikovia divaricata*,[2,21] two acidic arabinogalactans designated as MVS-IIIA and IVA from the seed of *Malva verticillata*,[22,23] two acidic arabinogalactans known as ukonans A and B from the rhizome of *Curcuma longa*[24,25] and an arabinoxylan called cinnaman AX from the bark of *Cinnamomum cassia*.[26] Saposhnikovan A has an α-1,4-linked D-galacturonan backbone bearing arabino-3,6-galactan type side chains, and saposhnikovan C possesses a rhamnogalacturonan backbone bearing α-3,5-branched L-arabinan and β-3,4-branched D-galactan side chains. MVS-IIIA has mainly arabino-3,6-galactan structure with α-1,3-linked L-arabinopyranosyl, β-1,4-linked D-xylosyl and α-1,4-linked D-galacturonan units. MVS-IVA belongs to a structural type similar to MVS-IIIA with additional α-1,2-linked L-rhamnose units. Further, ukonans A and B are basically arabino-3,6-galactans having a rhamnogalacturonan backbone. They belong to a type of polysaccharide similar to MVS-IVA. However, ukonans A and B have additional 3,4-branched D-xylosyl, 2,4-branched L-rhamnosyl and terminal and α-1,4-linked D-glucosyl residues. Cinnaman AX is a new structural type of RES-activating polysaccharide. It has a β-1,4-linked D-xylan backbone bearing terminal β-L-arabinopyranosyl units and α-L-arabinofuranosyl-(1→3)-β-L-arabinopyranose side chains.

As the other examples of acidic arabinogalactans having a phagocytosis-enhancing effect, polysaccharide F isolated from *Echinacea purpurea* cell culture[27] and polysaccharide Fb isolated from *Viscum album* berry[28] have been reported. Recently, the effect of the former polysaccharide in activating macrophages to cytotoxicity against tumor cells has been revealed.[29] Polysaccharide F has a β-1,3-linked D-galactan backbone with β-1,6-linked D-galactose side chains carrying terminal L-arabinose, and it also contains α-1,4-linked D-galacturonan residues. Fb possesses a rhamnogalacturonan backbone and arabino-3,6-galactan type side chains.

Evidently the main part of glycyrrhizan UA is occupied by the components of arabino-3,6-galactan type polysaccharide units. In addition, it has terminal and α-1,3-linked L-arabinopyranosyl, β-1,4-linked D-galactosyl residues and branched rhamnogalacturonan units. Glycyrrhizan UB is a basically similar type of polysaccharide to glycyrrhizan UA, though branched rhamnogalacturonan units occupy the major part of it. Further, glycyrrhizan UB possesses additional β-2,4-branched D-galactosyl and terminal α-D-glucosyl units. The presence of 1,3-linked L-arabinopyranosyl units in these polysaccharides may contribute to the RES activity.[22-26]

Acknowledgements We wish to thank Dr. M. Okada, Tsumura Laboratory, for the identification of material. This work was supported in part by The Science Research Promotion Fund of the Japan Private School Promotion Foundation.

References

1) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
2) N. Shimizu, M. Tomoda, R. Gonda, M. Kanari, N. Takanashi and N. Takahashi, *Chem. Pharm. Bull.*, **37**, 1329 (1989).
3) M. Tomoda, S. Kaneko, M. Ebashi and T. Nagakura, *Chem. Pharm. Bull.*, **25**, 1357 (1977).
4) R. Oshima, J. Kumanotani and C. Watanabe, *J. Chromatogr.*, **259**, 159 (1983).
5) T. Bitter and H. M. Muir, *Anal. Biochem.*, **4**, 330 (1962).
6) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
7) M. Tomoda, N. Shimizu, K. Shimada and M. Suga, *Chem. Pharm. Bull.*, **33**, 16 (1985).
8) M. Tomoda, M. Ichikawa and N. Shimizu, *Chem. Pharm. Bull.*, **34**,

4992 (1986).
9) M. Tomoda and M. Ichikawa, *Chem. Pharm. Bull.*, **35**, 2360 (1987).
10) N. Shimizu, M. Tomoda and M. Adachi, *Chem. Pharm. Bull.*, **34**, 4133 (1986).
11) M. Tomoda, K. Shimada, Y. Saito and M. Sugi, *Chem. Pharm. Bull.*, **28**, 2933 (1980).
12) G. O. Aspinall and R. J. Ferrier, *Chem. Ind.* (London), **1957**, 1216.
13) G. Biozzi, B. Benacerraf and B. N. Halpern, *Br. J. Exp. Pathol.*, **34**, 441 (1953).
14) N. Shimizu and M. Tomoda, *Chem. Pharm. Bull.*, **33**, 5539 (1985).
15) K. Bock, C. Pedersen and H. Pedersen, "Advances in Carbohydrate Chemistry and Biochemistry," Vol. 42, ed. by R. S. Tipson and D. Horton, Academic Press Inc., Orland, 1984, pp. 193—214.
16) J.-P. Joseleau, G. Chambat, M. Vignon and F. Barnoud, *Carbohydr. Res.*, **58**, 165 (1977).
17) R. L. Taylor and H. E. Conrad, *Biochemistry*, **11**, 1383 (1972).
18) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
19) H. Björndall, B. Lindberg and S. Svensson, *Carbohydr. Res.*, **5**, 433 (1967).
20) K. Ohtani, K. Mizutani, S. Hatono, R. Kasai, R. Sumino, T. Shiota, M. Ushijima, J. Zhou, T. Fuwa and O. Tanaka, *Planta Medica*, **53**, 166 (1987).
21) N. Shimizu, M. Tomoda, R. Gonda, M. Kanari, Aki. Kubota and Ake. Kubota, *Chem. Pharm. Bull.*, **37**, 3054 (1989).
22) M. Tomoda, M. Kanari, R. Gonda and N. Shimizu, *Phytochemistry*, **28**, 2609 (1989).
23) R. Gonda, M. Tomoda, N. Shimizu and M. Kanari, *Planta Medica*, **56**, 73 (1990).
24) M. Tomoda, R. Gonda, N. Shimizu, M. Kanari and M. Kimura, *Phytochemistry*, **29**, 1083 (1990).
25) R. Gonda, M. Tomoda, N. Shimizu and M. Kanari, *Chem. Pharm. Bull.*, **38**, 482 (1990).
26) M. Kanari, M. Tomoda, R. Gonda, N. Shimizu, M. Kimura, M. Kawaguchi and C. Kawabe, *Chem. Pharm. Bull.*, **37**, 3191 (1989).
27) H. Wagner, H. Stuppner, W. Schäfer and M. Zenk, *Phytochemistry*, **27**, 119 (1988).
28) H. Wagner and E. Jordan, *Phytochemistry*, **27**, 2511 (1988).
29) B. Luettig, C. Steinmüller, G. E. Gifford, H. Wagner and M.-L. Lohmann-Matthes, *J. Nat. Cancer Inst.*, **81**, 669 (1989).