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ISOLATION AND HYPOGLYCEMIC ACTIVITY OF QUINQUEFOLANS A, B, AND C, GLYCANS OF *PANAX QUINQUEFOLIUM* ROOTS[1]

YOSHITERU OSHIMA, KUNIKO SATO, and HIROSHI HIKINO*

Pharmaceutical Institute, Tohoku University, Aoba-yama, Sendai 980, Japan

ABSTRACT.—An H_2O extract of the American crude drug "Amerika-ninjin" (American ginseng), *Panax quinquefolium* roots, exhibited significant hypoglycemic activity in mice. Activity-guided fractionation of the extract led to isolation of three glycans, quinquefolans A, B, and C, which displayed hypoglycemic effects in normal and alloxan-induced hyperglycemic mice.

We have recently reported isolation and hypoglycemic activity of the polysaccharides of the Oriental crude drug "ninjin" (ginseng), the roots of *Panax ginseng* C.A. Meyer (Araliaceae) collected from Korea, China, and Japan (1-6). In continuation to this work we now examined *Panax quinquefolium* L., another species of *Panax* in North America. The crude drug prepared from the roots of this species is known as "Amerika-ninjin" (American ginseng), which has been used clinically as a substitute for Oriental ginseng (a tonic and sedative agent) mainly in China. Although physiological actions of the constituents from this crude drug were mentioned in the literature, no report on hypoglycemic activity was found. Preliminary hypoglycemic results exhibited by the H_2O extract of American ginseng prompted us to investigate the active principles which resulted in the isolation of three hypoglycemic glycans.

RESULTS AND DISCUSSION

American ginseng was first extracted with MeOH and then with H_2O. When the H_2O extract was administered ip to normal mice, a significant lowering of blood sugar level was observed (Table 1). The crude polysaccharide fraction was obtained from the extract by usual methods, and this fraction was chromatographed over DEAE-Toyopearl, Sephacryl S-200 and S-500 to furnish three glycans which are now named as quinquefolans A, B, and C.

Homogeneity of these glycans was substantiated by electrophoresis, gel filtration, and DEAE-Toyopearl chromatography.

The molecular weights of these quinquefolans were estimated to be ca. $>2.0\times10^6$ by gel chromatography over Sephacryl S-500.

Acid hydrolysis, reduction, and acetylation followed by glc of these glycans showed that the neutral sugar components were mannose and glucose (molar ratio, 1.0:2.3) for quinquefolan A, mannose and glucose (1.0:5.5) for quinquefolan B, and xylose for quinquefolan C.

By using the modified carbasole-H_2SO_4 method, the acidic sugar components in quinquefolans A, B, and C were found to be 10.8, 11.7, and 7.1%, respectively.

Elemental analysis showed the presence of some peptide moieties in these glycans which was confirmed by the Lowry method (2.7, 2.9, and 2.3%, respectively).

The property of these glycans which was mentioned above was different from that of earlier reported glycans from ginseng (1-6).

When the quinquefolans were injected ip to normal mice, they showed significant hypoglycemic activity without changing the food intake of the treated-mice. Among them, quinquefolan A exhibited the most intense effect (Table 1). Ip injection of quin-

[1]Antidiabetes drugs, Part 26. Also Part 116 in the validity of the Oriental medicines.

TABLE 1. Effect of H_2O Extract of *Panax quinquefolium* Roots, and Quinquefolans A, B, and C on Plasma Glucose Level in Normal Mice (n=5)

Drug	Dose (mg/kg, ip)	Relative glucose level				
		0	7		24 (h[a])	
		m[b]	m±SE	%	m±SE	%
control	—	100	107±2	100	107±3	100
extract	10^4 [c]	100	77±6**[d]	72	90±9	84
control	—	100	97±2	100	94±4	100
quinquefolan A . . .	10	100	46±3**	47	70±3**	75
	30	100	45±2**	47	69±5**	74
	100	100	43±2**	45	52±4**	55
control	—	100	97±2	100	94±4	100
quinquefolan B . . .	10	100	90±6	92	100±4	106
	30	100	94±3	97	95±5	101
	100	100	83±4*	85	90±1	96
control	—	100	116±4	100	125±6	100
quinquefolan C . . .	10	100	67±6**	58	86±6**	69
	30	100	68±3**	58	86±5**	69
	100	100	65±1**	56	69±3**	55

[a]Time after administration.
[b]Plasma glucose level at 0 h: 140-170 mg/dl.
[c]Crude drug equivalent.
[d]Significantly different from the control, $*p<0.05$ or $**p<0.01$.

quefolan A to alloxan-induced hyperglycemic mice also lowered blood glucose level (Table 2). Although the difference in the potencies of these glycans is of interest, their structure-activity relationships have not yet been defined.

TABLE 2. Effect of Quinquefolan A on Plasma Glucose Level in Alloxan-induced Hyperglycemic Mice (n=5)

Drug	Dose (mg/kg, ip)	Relative glucose level				
		0	7		24 (h[a])	
		m[b]	m±SE	%	m±SE	%
control	—	100	85±5	100	84±5	100
quinquefolan A . . .	10	100	32±4**[c]	38	50±5**	60
	30	100	33±6**	39	44±6**	52
	100	100	25±3**	29	19±5**	23

[a]Time after administration.
[b]Plasma glucose level at 0 h: 250-450 mg/dl.
[c]Significantly different from the control, $**p<0.01$.

EXPERIMENTAL

Nmr spectra were measured in D_2O. Chemical shifts (δ) are expressed in ppm downfield from TMS as external standard (abbreviation: br=broad peak). Determination of molecular weight, sugar components, and peptide contents were carried out as described previously (7).

ISOLATION OF QUINQUEFOLANS A, B, AND C.—The crude drug "Amerika-ninjin" (*P. quinquefolium* roots collected in Wisconsin, USA) (crushed, 10 kg) was extracted with MeOH (20 liters×3) and then with H_2O (20 liters×3) for 1 day (each extraction) at room temperature. The combined aqueous solution

was concentrated under the reduced pressure. Treatment of the concentrated solution (1 liter) with EtOH (4 liters) yielded a precipitate (100 g). Chromatography of the precipitate (30 g) over DEAE-Toyopearl 650M (2.2 ID×45 cm) with 0.05 M Tris-HCl buffer (pH 8.0, NaCl concentration: 0-1 M) gave two fractions, P-1 and P-2.

The fraction P-1 was chromatographed over Sephacryl S-200 (4.0 ID×95 cm) with 0.1 M Tris-HCl buffer (pH 7.0) containing 0.5 M NaCl to give two fractions, P-3 and P-4. P-3 was further applied to chromatography over Sephacryl S-500 (4.0 ID×90 cm) with the same solvent to yield quinquefolans A, B, and C.

Quinquefolan A.—$[\alpha]_D -19.3°$ (*c* 0.06, H_2O); ir ν max (KBr) cm^{-1} 3350, 1000; ^1H nmr δ 3.30-4.10 (br). Anal. Found: C, 35.71; H, 5.82; N, 0.79%.

Quinquefolan B.—$[\alpha]_D +59.4°$ (*c* 0.11, H_2O); ir ν max (KBr) cm^{-1} 3250, 1000; ^1H nmr δ 3.20-4.20 (br). Anal. Found: C, 40.05; H, 6.26; N, 0.62%.

Quinquefolan C.—$[\alpha]_D -82.1°$ (*c* 0.04, H_2O); ir ν max (KBr) cm^{-1} 3320, 1015; ^1H nmr δ 3.40-4.20 (br). Anal. Found: C, 38.47; H, 6.39; N, 0.76%.

Quinquefolans A-C were dialyzed and chromatographed over Sephadex G-10 with H_2O before analysis.

GLASS-FIBER PAPER ELECTROPHORESIS.—This was performed with glass-fiber paper (Whatman,GF/C, 15×40 cm) and alkaline borate (pH 9.3, 0.025 M borax-0.1 M NaOH, 10:1) at 450 V for 2 h. Visualization was made with *p*-anisidine-H_2SO_4 reagent. Moving distances: 14.0 (quinquefolan A), 13.0 (quinquefolan B), 12.0 (quinquefolan C), and 5.3 cm (glucose) toward the anode.

POLYACRYLAMIDE GEL ELECTROPHORESIS.—This was conducted on a 5% polyacrylamide gel column (0.5 ID× 10 cm) with borate buffer (pH 9.3) at 2 mA/tube for 2 h. Visualization was performed by the thymol-H_2SO_4 method. Moving distances (concentration of gel): 0.5 (quinquefolan A), 0.9 (quinquefolan B), 0 (quinquefolan C), and 3.6 cm (bromophenol blue).

DETERMINATION OF SUGAR COMPONENTS.—The neutral sugar contents (calculated as glucose) were determined as follows: phenol-H_2SO_4 method: 54.3 (quinquefolan A), 61.6 (quinquefolan B), 32.1% (quinquefolan C); chromotropic acid-H_2SO_4 method: 94.0 (quinquefolan A), 81.3 (quinquefolan B), 52.5% (quinquefolan C); anthrone-H_2SO_4 method: 78.3 (quinquefolan A), 70.7 (quinquefolan B), 27.2% (quinquefolan C).

The hexauronic acid contents (calculated as glucuronic acid) were determined by the modified carbazole-H_2SO_4 method: 10.8 (quinquefolan A), 11.7 (quinquefolan B), 7.1% (quinquefolan C).

MEASUREMENT OF HYPOGLYCEMIC ACTIVITY.—Male mice (Std:ddY strain, 25-30 g) were employed in groups of five and given food and drinking H_2O freely. The quinquefolans were dissolved in physiological saline solution and injected ip into normal mice or into alloxan-induced hyperglycemic mice, pretreated with alloxan (35 mg/kg) 5 days prior to sample administration. Blood was drawn from the orbital sinus by micro-hematocrit tubes periodically. The glucose level of plasma obtained by centrifugation of blood was measured with a glucose analyzer by the glucose oxidase method. Data are expressed as mean ± S.E. One-way analysis of variance was used to evaluate the results.

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