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ISOLATION AND HYPOGLYCEMIC ACTIVITY OF ELEUTHERANS A, B, C, D, E, F, AND G: GLYCANS OF *ELEUTHEROCOCCUS SENTICOSUS* ROOTS[1]

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ABSTRACT.—Intraperitoneal injection of an aqueous extract of the crude drug "shigoka" (Siberian ginseng), *Eleutherococcus senticosus* roots, remarkably diminished plasma-sugar level in mice. Fractionation of the extract by monitoring the activity yielded seven glycans, eleutherans A, B, C, D, E, F, and G, which exerted marked hypoglycemic effects in normal and alloxan-induced hyperglycemic mice.

The crude drug "shigoka" (Siberian ginseng), the roots of *Eleutherococcus senticosus* Maximowicz (*Acanthopanax senticosus* Harms) (Araliaceae), has been utilized as a tonic and sedative remedy in Oriental medicine. In vitro, in vivo, and human studies in the Soviet Union have reported it to be a tonic and/or adaptogen alleged to increase nonspecific resistance of an organism to adverse influences. It has been employed as an abundant and inexpensive substitute for ginseng, the roots of *Panax ginseng* C. A. Meyer (Araliaceae), in the Soviet Union and now in other countries (1).

Although some research has been carried out on the physiological actions of Siberian ginseng, hypoglycemic activity has not been reported. Because hypoglycemic activity of this crude drug could be predicted if similar in action to ginseng (2-6), this research was set up to document any hypoglycemic activity that this Oriental medicine might possess.

This paper reports the isolation of seven hypoglycemic glycans from Siberian ginseng.

RESULTS AND DISCUSSION

The H_2O extract of Siberian ginseng exhibited significant hypoglycemic activity on ip administration to normal mice (Table 1). The extract was then fractionated by monitoring the hypoglycemic activity. The extract was dialyzed to afford an active nondialyzed portion which was chromatographed over DEAE-Toyopearl and Sephacryl S-200 and/or S-500 to isolate seven glycans which are named as eleutherans A, B, C, D, E, F, and G.

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

The molecular weights of eleutherans A, B, C, D, E, F, and G were estimated to be approximately 5.6×10^4, 3.7×10^4, 1.8×10^4, 7.8×10^3, 7.8×10^3, 3.3×10^3, and 7.0×10^4, respectively, by calibration of gel filtration over Sephacryl S-200 (in the cases of eleutherans B, C, D, E, and F) or S-500 (in the cases of eleutherans A and G).

Acid hydrolysis of these constituents resulted in formation of rhamnose, arabinose, xylose, mannose, galactose, and glucose (molar ratio, 0.3:0.1:0.2:3.6:1.0:1.4) for eleutheran A; rhamnose, arabinose, mannose, galactose, and glucose (0.6:0.1:0.1:1.0:0.8) for eleutheran B; rhamnose, arabinose, mannose, galactose, and glucose (0.9:0.6:0.3:1.0:2.7) for eleutheran C; rhamnose, arabinose, mannose, galactose, and glucose (0.1:0.5:0.2:1.0:0.3) for eleutheran D; rhamnose, arabinose, xylose, galactose, and glucose (2.0:1.5:0.1:1.0:0.1) for eleutheran E; arabinose, mannose,

[1]Antidiabetes drugs, Part 20. Also Part 107 in the validity of the Oriental medicines.

TABLE 1. Effect of Extract of Siberian Ginseng and Eleutherans A, B, C, D, E, F, and G on Plasma Glucose Level in Normal Mice (n=5)

Drug	Dose (mg/kg, ip)	Relative glucose level				
		0 h	7 h		24 h[a]	
		m[b]	m±SE	%	m±SE	%
control	—	100	95±3	100	98±6	100
extract	10^{4c}	100	62±3**	65	76±7*	78
control	—	100	101±6	100	96±5	100
eleutheran A	10	100	95±7	94	92±7	96
	30	100	84±5*	83	101±10	105
	100	100	73±8*	72	78±2*	81
control	—	100	96±7	100	103±7	100
eleutheran B	10	100	98±13	102	114±10	111
	30	100	119±16	124	113±8	110
	100	100	96±6	100	111±7	108
	300	100	61±5**	64	85±4*	83
control	—	100	96±6	100	101±4	100
eleutheran C	10	100	90±11	94	94±7	93
	30	100	70±9*	73	104±10	103
	100	100	61±6**	64	84±10	83
control	—	100	99±4	100	106±5	100
eleutheran D	10	100	106±6	107	102±8	96
	30	100	90±7	91	98±10	92
	100	100	80±6*	81	100±6	94
control	—	100	101±6	100	96±5	100
eleutheran E	10	100	97±5	96	99±12	103
	30	100	102±4	101	89±2	93
	100	100	89±6	88	91±4	95
control	—	100	99±5	100	102±4	100
eleutheran F	10	100	72±4**	73	92±6	90
	30	100	74±4**	75	97±3	95
	100	100	55±5**	56	75±6**	74
control	—	100	122±7	100	103±6	100
eleutheran G	3	100	74±3**	61	100±7	97
	10	100	61±4**	50	87±5	84
	30	100	54±4**	44	81±3**	79

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

galactose, and glucose (0.6:0.2:1.0:0.7) for eleutheran F; and rhamnose, arabinose, mannose, galactose, and glucose (0.3:0.1:4.0:1.0:0.9) for eleutheran G.

The one containing a fairly large amount of acidic sugar components was eleutheran E which, on acid hydrolysis, yielded galacturonic acid and glucuronic acid (2.2:1.0).

Eleutherans A, B, C, E, F, and G were also found to contain small amounts of *O*-acetyl groups (1.1, 2.2, 1.1, 1.7, 4.2, and 1.9%, respectively).

Elemental analysis indicated that some peptide moieties were present in these glycans which was confirmed by determination by the Lowry method (2.7, 3.2, 5.4, 5.9, 0.9, 6.2, and 5.8, respectively).

When eleutherans A-G were injected ip to normal mice, significant dose-related hypoglycemic activity was observed in all the eleutherans except for eleutheran E. Among them, eleutheran G exhibited the most intense effect (Table 1). Although the difference in the potencies of these glycans is of interest, their structure-activity re-

lationships have not yet been defined. Ip administration of the main glycan, eleutheran C, to alloxan-evoked hyperglycemic mice also lowered blood glucose level (Table 2).

Several papers have been published on polysaccharides from Siberian ginseng (9-15). Thus, Xu *et al.* (10,11,14) reported the isolation of two glycans, PES-A (heteroglucan, mol. wt. 7000) and PES-B (mol. wt. 76000) and Wagner *et al.* (15) recorded the isolation of two glucans (mol. wt. 15000 and 150000) and two heteroxylans (mol. wt. 30000 and 200000). These polysaccharides apparently are not identical to the present eleutherans A-G. The diverseness of the polysaccharide components of Siberian ginseng thus found indicates their variability depending on source, growth conditions, climate, and/or nature of soil as in the polysaccharide components of *Panax ginseng* (2-6).

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

Drug	Dose (mg/kg, ip)	Relative glucose level				
		0 h	7 h		24 h[a]	
		m[b]	m±SE	%	m±SE	%
control	—	100	85±7	100	83±9	100
eleutheran C	10	100	69±4	81	80±5	96
	30	100	76±9	89	65±7	78
	100	100	51±7**	60	67±9	81

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

Among the known physiological properties of Siberian ginseng polysaccharides, perhaps the most significant are the immune stimulant effects (11,13-15). Their ability to lessen thioacetamide, phytohemagglutinin, and X-ray toxicity and to exhibit an antitumor effect have also been reported (11). The present finding of hypoglycemic activity adds another activity to the variety of the physiological properties of Siberian ginseng polysaccharides.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were taken in D_2O. Chemical shifts (δ) are given in ppm downfield from TMS as internal standard (abbreviations: d=doublet, br=broad peak). Determination of molecular weight, sugar components, peptide contents, and *O*-acetyl contents were conducted as in a previous paper (7).

ISOLATION OF ELEUTHERANS A, B, C, D, E, F, AND G.—The crude drug "shigoka" (*E. senticosus* roots collected in Japan; voucher specimen deposited in Pharm. Inst., Tohoku University) (crushed, 100 kg) was extracted three times (200 liters) with H_2O for 2 days (each extraction) at room temperature. The combined solution was concentrated with polyamide ultrafiltration membrane (8) (Nitto, NTU-3508) to furnish a concentrate (30 liters). EtOH (10 liters) was added to the concentrate (2.4 liters) to yield a precipitate (38 g). The precipitate was chromatographed over DEAE-Toyopearl (2.2 id×45 cm) with H_2O, 1 M NaCl, and 0.1 N NaOH to give three fractions E-1, E-2, and E-3.

The fraction E-1 was successively 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, Sephacryl S-500 (4.0 id×95 cm) with 0.1 M Tris-HCl buffer (pH 7.0) containing 0.5 M NaCl (in the case of eleutherans A and B), DEAE-Toyopearl (2.2 id×45 cm) with 0.05 M Tris-HCl buffer (pH 9.0) containing NaCl (0 0.3 M) and Sephadex G-50 (4.0 id×95 cm) with 0.05 M phosphate buffer (in the case of eleutheran F) to yield eleutherans A-F.

Eleutheran A.—$[\alpha]_D$ +57.1° (c 0.413, H_2O); ir ν max (KBr) cm^{-1} 3340, 1027; ^1H nmr δ 1.14 (br), 1.89 (br), 4.83 (br), 4.89 (br), 5.13 (br); ^{13}C nmr δ 61.2, 67.0, 69.7, 70.3, 71.6, 73.5, 78.5, 97.8, 100.7, 102.3, 119.1, 134.0, 174.5. *Anal.* Found: C, 41.45; H, 6.37; N, 0.51%.

Eleutheran B.—$[\alpha]_D$ −21.5° (c 0.354, H_2O); ir ν max (KBr) cm^{-1} 3360, 1045; ^1H nmr δ 1.18 (br),

1.90 (br), 4.85 (br); ^{13}C nmr δ 16.7, 17.1, 22.7, 61.2, 63.1, 69.7, 70.8, 72.3, 75.8, 81.7, 101.3, 102.7, 103.6, 174.4. *Anal.* Found: C, 35.00; H, 5.27; N, 0.98%.

Eleutheran C.—[α]D −38.8° (c 0.364, H_2O); ir ν max (KBr) cm^{-1} 3355, 1043; ^{1}H nmr δ 1.09 (br), 1.86 (br), 4.89 (br), 5.03 (br), 5.24 (br); ^{13}C nmr δ 16.9, 60.7, 61.2, 62.3, 62.9, 64.2, 68.8, 69.6, 70.9, 72.9, 73.1, 75.3, 75.7, 76.1, 76.9, 81.6, 98.1, 101.9, 103.6, 108.0, 177.3. *Anal.* Found: C, 39.35; H, 6.24; N, 0.90%.

Eleutheran D.—[α]D −13.5° (c 0.327, H_2O); ir ν max (KBr) cm^{-1} 3390, 1016; ^{1}H nmr δ 1.07 (br), 4.80 (br), 4.89 (br), 5.06 (br), 5.14 (br); ^{13}C nmr δ 61.3, 68.8, 69.6, 70.3, 70.9, 71.5, 73.6, 76.7, 81.5, 84.1, 97.9, 103.5, 109.0. *Anal.* Found: C, 40.50; H, 6.42; N, 0.63%.

Eleutheran E.—[α]D +30.3° (c 0.402, H_2O); ir ν max (KBr) cm^{-1} 3290, 1018; ^{1}H nmr δ 1.05 (br), 2.06 (br), 4.93 (br); ^{13}C nmr δ 17.1, 60.9, 68.3, 70.1, 71.6, 72.3, 75.9, 78.9, 81.5, 86.7, 99.5, 102.4, 176.3. *Anal.* Found: C, 37.19; H, 5.76; N, 0.17%.

Eleutheran F.—[α]D +16.9° (c 0.308, H_2O); ir ν max (KBr) cm^{-1} 3380, 1040; ^{1}H nmr δ 1.24 (br), 1.92 (br), 4.94 (brd, J=9 Hz), 5.14 (br), 5.26 (br); ^{13}C nmr δ 16.7, 59.3, 61.3, 68.8, 70.9, 73.5, 78.4, 81.5, 84.0, 98.0, 99.8, 103.5, 171.3. *Anal.* Found: C, 30.79; H, 4.85; N, 0.86%.

Sephacryl S-200 (4.0 id×95 cm, with 0.1 M Tris-HCl (pH 7.0) containing 0.5 M NaCl) and Sephacryl S-500 (4.0 ID×95 cm, with 0.1 M Tris-HCl (pH 7.0) containing 0.5 M NaCl) chromatography of the fraction E-3 gave eleutheran G.

Eleutheran G.—[α] D +41.7° (c 0.097, H_2O); ir ν max (KBr) cm^{-1} 3350, 1033; ^{1}H nmr δ 1.28 (br), 1.96 (br), 5.00 (br); ^{13}C nmr δ 16.9, 61.1, 67.0, 69.6, 70.2, 70.5, 73.2, 75.7, 77.7, 78.1, 98.5, 100.7, 102.2, 172.5, 174.6. *Anal.* Found: C, 35.55; H, 5.62; N, 0.97%.

Eleutherans A-G 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. The sample was visualized with *p*-anisidine-H_2SO_4 reagent. Moving distances: 12.3 (eleutheran A), 10.1 (eleutheran B), 13.0 (eleutheran C), 10.0 (eleutheran D), 12.1 (eleutheran E), 15.7 (eleutheran F), 12.2 (eleutheran G), and 10.0 cm (glucose) toward the anode.

POLYACRYLAMIDE GEL ELECTROPHORESIS.—This was conducted on 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): 0.8 (5%, eleutheran A), 1.7 (5%, eleutheran B), 1.3 (10%, eleutheran C), 0.6 (30%, eleutheran D), 2.2 (30%, eleutheran E), 0.4 (30%, eleutheran F), 0.1 (10%, eleutheran G), 3.5 cm (5, 10, 30%, bromophenol blue).

DETERMINATION OF SUGAR COMPONENTS.—The neutral sugar contents (calculated as glucose) were determined as follows: phenol-H_2SO_4: 79.3 (eleutheran A), 48.3 (eleutheran B), 61.9 (eleutheran C), 64.5 (eleutheran D), 34.4 (eleutheran E), 56.4 (eleutheran F), 77.0% (eleutheran G); chromotropic acid-H_2SO_4 method: 61.7 (eleutheran A), 47.8 (eleutheran B), 58.8 (eleutheran C), 65.8 (eleutheran D), 50.6 (eleutheran E), 56.9 (eleutheran F), 50.9% (eleutheran G); anthrone-H_2SO_4 method: 40.5 (eleutheran A), 36.2 (eleutheran B), 46.1 (eleutheran C), 43.0 (eleutheran D), 18.2 (eleutheran E), 44.9 (eleutheran F), 42.6% (eleutheran G).

The hexauronic acid contents (calculated as glucuronic acid) were determined by the modified carbazole-H_2SO_4 method: 6.3 (eleutheran A), 11.8 (eleutheran B), 12.2 (eleutheran C), 9.0 (eleutheran D), 31.7 (eleutheran E), 18.3 (eleutheran F), 7.0% (eleutheran G).

MEASUREMENT OF HYPOGLYCEMIC ACTIVITY.—Male mice (Std:ddY strain, 25-30 g) were employed in groups of five and given food and drinking water freely. The eleutherans were dissolved in physiological saline solution and injected ip to normal mice or to 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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