Hypoglycemic Activity of Some Triterpenoid Glycosides

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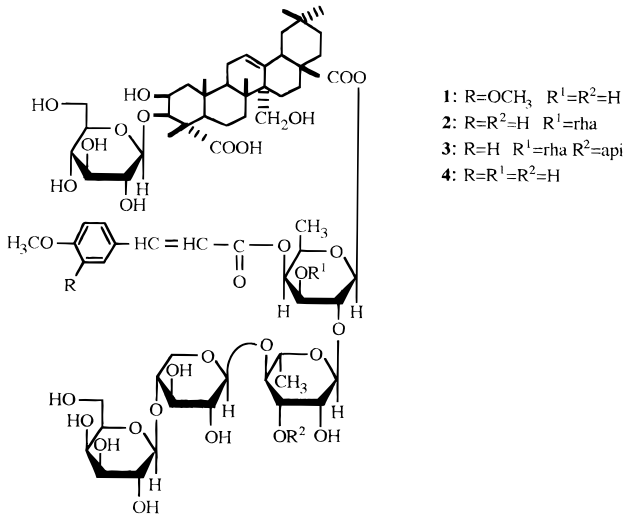
Four triterpenoid glycosides isolated from the rhizomes of *Polygala senega* var. *latifolia*, senegins II−IV (**1**−**3**) and desmethoxysenegin II (**4**), were tested for hypoglycemic activity in normal and KK-Ay mice. Compounds **1** and **2** reduced the blood glucose of normal mice 4 h after intraperitoneal administration and also significantly lowered the glucose level of KK-Ay mice under similar conditions. Compounds **3** and **4**, as well as senegose A (**5**), an oligosaccharide ester, were inactive when tested against normal mice.

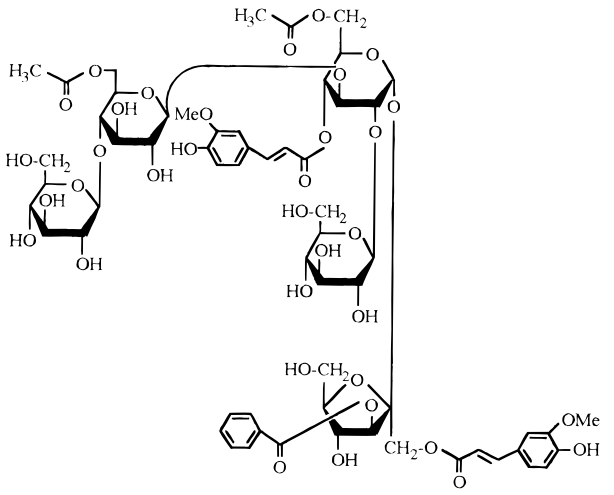
Polygala senega var. *latifolia* has been used traditionally for the treatment of cough. The constituents of this plant have been investigated chemically, with some glycosides being found to occur.[1−3] We have previously reported the hypoglycemic effect of "Polygonati Rhizoma" and "Officinalis Rhizoma" and identified as active components certain steroids and iridoid glycosides.[4,5] In the present paper, we describe the hypoglycemic activity of four triterpenoid glycosides (**1**−**4**) and one oligosaccharide ester isolated from the rhizomes of *Polygala senega* var. *latifolia*.

Results on normal mice are summarized in Table 1. Compounds **1** and **2** showed hypoglycemic activity at a dosage of 1 and 5 mg/kg, 4 h after administration. The hypoglycemic effects of **1** and **2** in normal mice were dose-dependent. No change in blood glucose levels were observed in mice treated with compound **3**, **4**, or **5**. In addition, the triterpenoid glycosides **1**−**4** decreased the blood glucose level of KK-Ay mice, an animal model of obese non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia (Table 2). The hypoglycemic effects of **1**−**3** were potent, and glucose levels were similar to the basal level of normal mice, indicating that those triterpene glycosides may affect insulin resistance of peripheral tissues. The compounds had a much more potent hypoglycemic activity than that of the positive control, tolbutamide.

Furthermore, **1** and **2** showed intense hypoglycemic effects compared with **4**. From these observations, the nature of the substitution at the R^1 and R^2 positions seems to be important for such action. Thus, the 1,3,4-trisubstituted benzene position of the cinnamic acid moiety may have an effect on hypoglycemic action. However, the blood glucose levels remained almost unchanged in senegose A (**5**)-treated normal and KK-Ay mice. From these findings, it seems that the presence of the aglycon part is essential for the hypoglycemic activity of compounds **1**−**4**. In addition, the hypoglycemic activity may be affected by the number of sugar units.

It is interesting that these triterpenoid glycosides produce a significant hypoglycemic effect, indicating that such compounds may be useful for treating NIDDM.





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Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were obtained using a VXR-500 instrument. SIMS were measured with a Hitachi M-4100 instrument using glycerol as matrix. HPLC was performed on an apparatus equipped with a 510 dual-pump (Waters), a model 680 gradient controller (Waters), and a 990 photodiode-array detector (Waters).

Plant Material. Rhizomes of *Polygala senega* L. var. *latifolia* Torrey and Gray (Polygalaceae) were cultivated

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Table 1. Effect of Compounds **1**−**5** on Blood Glucose in Normal Mice

treatment	dose (mg/kg)	n	blood glucose level[a] (mg/100 mL) 0 h	4 h	7 h
control		4	207 ± 1	196 ± 6	193 ± 1
1	1	5	211 ± 12	139 ± 8[b]	149 ± 7[b]
	5	5	213 ± 11	137 ± 13[b]	140 ± 11[b]
2	1	4	205 ± 4	167 ± 7[b]	172 ± 8[c]
	5	5	210 ± 8	151 ± 2	160 ± 10
3	1	5	193 ± 9	177 ± 10	171 ± 11
	5	5	184 ± 4	187 ± 14	169 ± 5[c]
4	5	5	215 ± 7	199 ± 6	195 ± 10
5	5	5	211 ± 8	197 ± 8	199 ± 9
tolbutamide	50	4	200 ± 7	160 ± 9[b]	170 ± 8[c]

[a] Blood glucose levels are expressed as means ± S.E. [b] Significance level is represented as $p < 0.01$. [c] Significance level is represented as $p < 0.05$.

Table 2. Effect of Compounds **1**−**5** on Blood Glucose in KK-Ay Mice

treatment	dose (mg/kg)	n	blood glucose level[a] (mg/100 mL) 0 h	4 h	7 h
control		6	468 ± 41	445 ± 30	487 ± 28
1	1	5	395 ± 52	226 ± 11[b]	305 ± 59
	5	5	446 ± 41	217 ± 19[c]	182 ± 9[c]
2	1	5	418 ± 61	195 ± 18[b]	210 ± 34[b]
	5	5	438 ± 43	204 ± 16[d]	146 ± 8[c]
3	1	4	419 ± 36	207 ± 27[c]	276 ± 29[b]
	5	5	428 ± 61	276 ± 56	388 ± 84
4	5	6	500 ± 45	306 ± 41[c]	325 ± 55[b]
5	5	6	461 ± 54	401 ± 64	368 ± 54
tolbutamide	50	4	537 ± 44	495 ± 66	370 ± 37[b]

[a] Blood glucose levels are expressed as means ± S.E. [b] Significance level is represented as $p < 0.05$. [c] Significance level is represented as $p < 0.01$. [d] Significance level is represented as $p < 0.001$.

in Sannan-cho, Hyogo, Japan, and collected in November 1992. A voucher specimen (herbarium no. 370) has been deposited at the herbarium of the Department of Science, Kyoto University.

Extraction and Isolation. The rhizomes (100 g) of *Polygala senega* L. var. *latifolia* were defatted with toluene and extracted three times with aliquots of 500 mL of MeOH. Next, the combined MeOH extract (42.5 g) was partitioned between *n*-BuOH and H_2O (1:1). The *n*-BuOH layer was evaporated to dryness (19.3 g) and separated by preparative HPLC [Cosmosil, 5C18-Ar (ODS-type), 250 mm × 20 mm i.d.; mobile phases A: 0.2 M $NaClO_4$ −60% $HClO_4$ (1000:0.2), and B: CH_3CN; flow rate, 9 mL/min; UV detector, 315 nm)]. The *n*-BuOH fraction (40 mg) was subjected to HPLC repeatedly (25 times) with solvents A/B (65:35→58:42) to yield compounds **1**−**4** (**1**, 109 mg; **2**, 93 mg; **3**, 57 mg; **4**, 104 mg). The same fraction (each 200 mg) was separated by preparative HPLC five times with solvents A/B (78:22→75:25→66:34) to give compound **5** (126 mg). Compounds **1**−**5** were identified by comparison their of ^{1}H-NMR, ^{13}C-NMR, and SIMS (**1**, $[M - H]^-$ *m/z* 1455; **2**, *m/z* 1571; **3**, *m/z* 1717; **4**, *m/z* 1425; **5**, *m/z* 1367) data.[1,6]

Bioassays. Male ddY strain (normal) mice (5 weeks old) (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) were kept in an experimental animal room for 7 days with free access to food and water. The mice were housed in an air-conditioned room (22 ± 2 °C with a 12 h light−12 h dark cycle). Male KK-Ay (diabetic) mice (12 weeks old; Clea, Tokyo, Japan) were used for blood glucose level determinations. Mice with a blood glucose level about 300 mg/dL, considered to be diabetic, were used in this study. The animals were divided into several groups, and four to six animals were used for each group. The triterpenoid glycosides (**1**−**4**) and compound **5** were dissolved in physiological saline and injected ip into the mice. As a control, the saline solution was also injected into the mice. Tolbutamide was used as a positive control. For the determination of blood glucose levels, blood samples were withdrawn from the cavernous sinus with a capillary syringe.

Determination of Blood Glucose. Blood glucose levels in both normal and diabetic animals were determined by the glucose oxidase method.[7] All the data were expressed as means ± S.E., and the Student's *t*-test was used for the statistical analysis. The values were considered to be significantly different when the *p* value was less than 0.05.

References and Notes

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