Field Survey of *Glycyrrhiza* Plants in Central Asia (2).[1] Characterization of Phenolics and Their Variation in the Leaves of *Glycyrrhiza* Plants Collected in Kazakhstan

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A new prenylated flavanone, licoleafol, and a new prenylated dihydrostilbene, uralstilbene, together with four known compounds, 8-dimethylallyleriodictyol, sophoraflavanone B, gancaonin R, and 6-dimethylallyleriodictyol, were isolated from the leaves of *Glycyrrhiza uralensis* collected in Kazakhstan. HPLC analysis of the leaves of *Glycyrrhiza* plants collected in Kazakhstan showed that both *G. uralensis*-specific and *Glycyrrhiza glabra*-specific compounds were detected in the leaves of the morphologically intermediate-type plants, suggesting that the intermediate-type plant is a hybrid of *G. glabra* and *G. uralensis*. In addition, HPLC profiles of leaf extracts from offspring of intermediate-type plants were divided into the three types: the *G. uralensis* type, *G. glabra* type, and the intermediate type. From these results, it appears likely that the intermediate-type plant back-crosses with *G. glabra* and *G. uralensis* to generate a *G. glabra*-type plant and a *G. uralensis*-type plant, respectively.

Key words *Glycyrrhiza*; aerial part; chemical variation; species-specific constituent

Licorice is one of the most important crude drugs in the world, and its major triterpene saponin, glycyrrhizin, is a well-known natural sweetener and pharmaceutical.[2,3] *Glycyrrhiza glabra* and *Glycyrrhiza uralensis* are major glycyrrhizin-producing species and their distribution is different.[3—5] The former is distributed from southern Europe to the northwestern part of China, whereas the latter occurs from Central Asia to the northeastern part of China. Extensive chemical studies revealed that *Glycyrrhiza* plants produce not only glycyrrhizin but also many saponins and flavonoids,[3,6] and many species-specific flavonoids were also reported in the underground parts of respective *Glycyrrhiza* species.[6—10] It is also noteworthy that flavonoid variations in leaves of *Glycyrrhiza* plants were observed.[10—12] These variations in leaves might be a good marker to identify *Glycyrrhiza* plants and their plant specimens.

A field survey of *Glycyrrhiza* plants in Central Asia, where both *G. glabra* and *G. uralensis* are distributed,[4] was performed to compare the morphologic and chemical characteristics of *Glycyrrhiza* plants.[1] Intriguingly, *G. glabra* and *G. uralensis* grow together, forming a mixed population in the southeastern part of Kazakhstan, and morphologically intermediate-type plants between *G. glabra* and *G. uralensis* were also observed. HPLC analysis of their leaf extracts indicated a significant difference among *G. uralensis*, *G. glabra*, and the intermediate-type plants.[1] It is also noteworthy that both *G. glabra*-specific and *G. uralensis*-specific compounds were detected in the leaves of the intermediate type. This finding, coupled with Ashurmetov's results[13] showing that *G. glabra* and *G. uralensis* plants are capable of generating hybrids, suggests that these intermediate-type plants are natural hybrids between the two species.

In the present study, the characterization and variation of these index compounds from the leaves, which might be a good marker for revealing the differences among the three types of *Glycyrrhiza* plants, are discussed.

Results and Discussion

Isolation and Characterization of Flavonoids and Stilbenoids from Leaves of *Glycyrrhiza uralensis* and Intermediate-Type Plants Collected in Kazakhstan Air-dried leaves of *G. uralensis* collected in Kazakhstan were extracted with ethanol, and the ethyl acetate soluble fraction was subjected to a series of silica gel and reverse-phase silica gel (ODS) column chromatography to afford a new prenylated flavanone (**1**), and a new prenylated dihydrostilbene (**6**), together with four known compounds **2**—**5**. The known compounds were identified as 8-dimethylallyleriodictyol (8-prenyleriodictyol) (**2**),[14] sophoraflavanone B (**3**),[15,16] gancaonin R (**4**),[17] and 6-dimethylallyleriodictyol (6-prenyleriodictyol) (**5**),[14] by comparison of their spectral data with published values.

Compound **1**, called licoleafol, was obtained as a colorless amorphous solid and showed a quasimolecular ion $[M+H]^+$ at *m/z* 373.1282 in high-resolution positive FAB-MS, which corresponds to the molecular formula $C_{20}H_{21}O_7$. Compound **1** showed UV absorption at λ_{max} 291 nm and characteristic signals for a flavanone at δ_H 5.44 (dd, H-2), 3.08, and 2.81 (each dd, for H_2-3) in its ^{1}H-NMR spectrum, and δ_C 79.4 (C-2) and 43.2 (C-3) in its ^{13}C-NMR spectrum. In addition, the ^{1}H-NMR spectrum showed signals due to a hydrogen-bonded hydroxyl group (OH-5) at δ_H 12.11 (s), one isolated aromatic proton at δ_H 6.02 (s), and three aromatic protons at δ_H 6.85 (2H, s) and 7.08 (1H, s). These signal patterns were similar to those of 8-dimethylallyleriodictyol (**2**) except for the splitting patterns of the latter three protons. However, comparative analysis of ^{13}C-NMR spectra of **1** and **2** revealed that both compounds have an identical flavanone structure including the substitution pattern of the B ring (Table 1). The difference between proton signal patterns of B rings in **1** and **2** was presumably due to the different anisotropic effect of the substituents in both compounds. The remaining substituent C_5H_9O was deduced to be 3-hydroxymethyl-2-butenyl based

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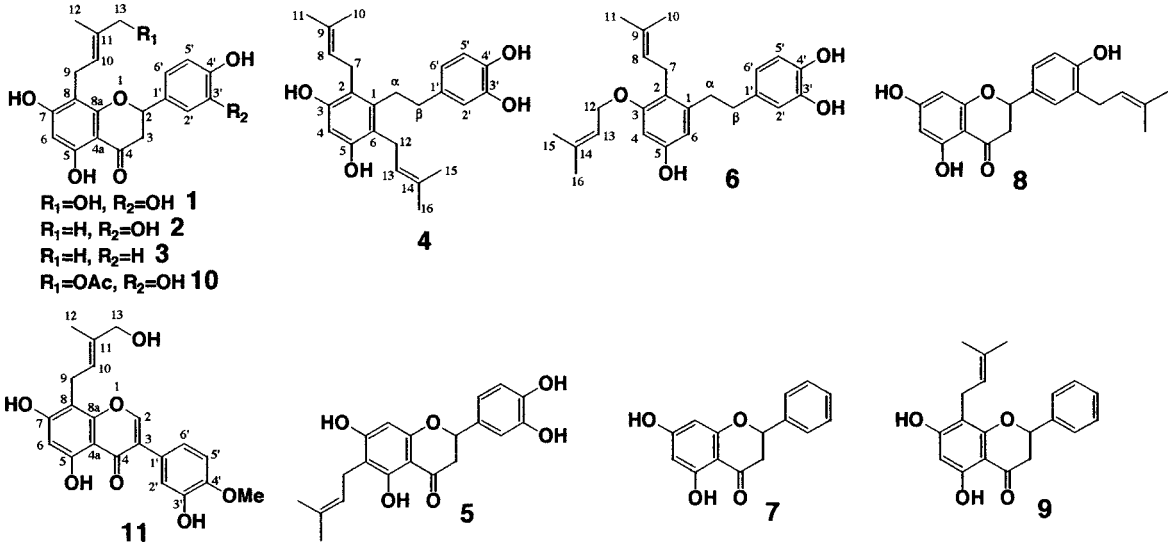

Chart 1

Table 1. ^{13}C-NMR Data (δ Values) of Licoleafol (**1**), 8-Dimethylallyleriodictyol (**2**), and Gancaonin D (**11**) in Acetone-d_6

C	1	2	11[18]
2	79.4	80.2	
3	43.2	44.0	
4	197.4	198.0	
4a	103.4	103.8	
5	163.0	163.4	
6	96.3	96.8	
7	165.0	165.4	
8	107.9	108.8	
8a	161.0	157.3	
9	21.8	22.7	21.6
10	124.0	124.1	122.6
11	135.6	132.4	136.6
12	13.8	18.3	13.8
13	68.6	26.4	68.3
1′	131.7	131.7	
2′	114.6	115.1	
3′	146.3	146.7	
4′	146.0	146.5	
5′	115.9	116.4	
6′	118.9	119.5	

on the comparison of ^{1}H- and ^{13}C-NMR spectra of C_5 units in **1** and **2**. Furthermore, the geometry of this substituent was concluded to be *E* by comparison of the ^{13}C-NMR spectrum of **1** with that of the prenyl group in gancaonin D {8-[(*E*)-3-hydroxymethyl-2-butenyl]-4′-methoxy-3′,5,7-trihydroxyisoflavone} (**11**).[18] The position of this side chain was suggested to be C-8 from the chemical shift value of the 5-hydroxy proton,[14] and this was confirmed by the correlation spectroscopy *via* long-range coupling (COLOC) spectrum of **1** (Fig. 1). The absolute configuration at C-2 of **1** was assigned to be *S* by comparison of its optical rotation ($[\alpha]_D$ −34°) with that ($[\alpha]_D$ −15°) of kanzonol S (**10**), a minor compound from the aerial parts of *Glycyrrhiza eurycarpa*.[19] Accordingly, licoleafol (**1**) was established to be (*S*)-8-[(*E*)-3-hydroxymethyl-2-butenyl]-3′,4′,5,7-tetrahydroxy-flavanone.

Compound **6**, called uralstilbene, showed a molecular ion

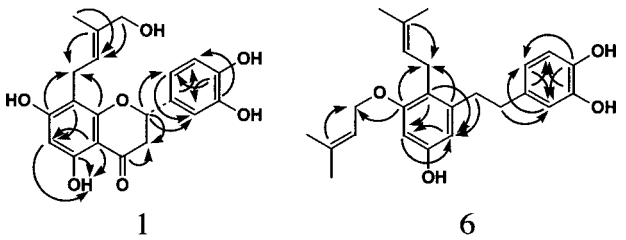

Fig. 1. COLOC Spectra of Compounds **1** and **6**

at *m/z* 382.2136 in the high resolution electron impact mass spectrum (HR-EI-MS), corresponding to the molecular formula $C_{24}H_{30}O_4$. The ^{1}H-NMR spectrum of **6** showed signals due to two pairs of benzylic methylene protons [δ_H 2.69 (2H, m) and 2.72 (2H, m)], two mutually meta-coupled protons [δ_H 6.32 and 6.34 (each 1H, d)], and ABC-type aromatic protons [δ_H 6.74 (1H, d), 6.73 (1H, d) and 6.56 (1H, dd)]. In addition, two prenyl groups [δ_H 1.64, 1.73 (each 3H, br s), 3.28 (2H, d) and 5.05 (1H, br t), and δ_H 1.73 and 1.76 (each 3H, br s), 4.47 (2H, br d) and 5.47 (1H, br t)], of which one was a *C*-prenyl group and the other an *O*-prenyl group, were also observed. These findings suggest that **6** is a diprenylated dihydrostilbene. Based on the two dimensional (2D) spectra (H–H COSY, C–H COSY, and COLOC spectra), two phenyl groups were assigned to be 2-dimethylallyl-3-dimethylallyloxy-5-hydroxyphenyl and 3,4-dihydroxyphenyl groups. In particular, the location of two prenyl moieties was established by the correlations between C-1/H_2-7, C-3/H_2-7, C-3/H_2-12, C-2/H-4, C-2/H-6, C-4/H-6, and C-6/H-4 in the COLOC spectrum (Fig. 1). The ^{1}H- and ^{13}C-NMR spectra of the latter aromatic ring were very similar to those of the corresponding ring in gancaonin R (**4**) (Table 2), which was isolated from the aerial parts of *G. uralensis*.[17] Thus the structure of uralstilbene was assigned as depicted in **6**.

Next, the leaves of the intermediate-type plants were extracted with ethanol, and the extracts were subjected to silica gel and ODS column chromatography to afford four known flavanones, pinocembrin (**7**),[20,21] sophoraflavanone B (**3**),[15,16,22] licoflavanone (**8**),[21] and glabranin (**9**),[20,23] which

Table 2. ^{13}C-NMR Data (δ Values) of Uralstilbene (**6**) and Gancaonin R (**4**) in Acetone-d_6

C	6	4
1	142.7	141.2
2	120.0	118.5
3	156.9	154.4
4	99.0	101.5
5	158.6	154.4
6	108.9	118.5
7	25.0	25.6
8	125.5	127.1
9	130.0	130.0
10	18.1[b)]	18.2
11	25.9[a)]	25.9
12	65.7	25.6
13	121.5	126.1
14	137.2	130.0
15	18.2[b)]	18.2
16	25.8[a)]	25.9
α	36.3	32.8
β	37.7	37.3
1′	134.7	135.1
2′	116.2	116.1
3′	145.7	145.8
4′	143.9	144.0
5′	115.9	116.0
6′	120.3	120.2

a, *b*) Assignments may be interchanged in each vertical column.

were also isolated from the leaves of *G. glabra*.[20—22)]

HPLC Analysis of *Glycyrrhiza* Leaves Collected in Kazakhstan *G. glabra* and *G. uralensis* grew together, forming a mixed population in the southeastern part of Kazakhstan, and the morphologically intermediate-type plants between *G. glabra* and *G. uralensis* were also observed.[1)] The contents of flavanones and dihydrostilbenes isolated in the present study might serve as a good marker to characterize these *Glycyrrhiza* plants. Thus the contents of phenolic constituents in the leaves of *Glycyrrhiza* plants collected in Kazakhstan were analyzed by HPLC. Figure 2 shows the HPLC chromatograms of leaf extracts from *Glycyrrhiza* plants, but the content of **3** was not determined because peak 3 was presumed to comprise compound **3** and an unidentified compound by comparison of the UV spectrum of **3** with that of peak 3. Table 3 shows the contents of eight compounds **1**, **2**, and **4**—**9** in the leaves. As reported previously,[1)] the HPLC profiles of leaf extracts from *Glycyrrhiza* plants collected in Kazakhstan were divided into the three types: the *G. uralensis* type, *G. glabra* type, and the intermediate type. In the HPLC profile of *G. uralensis* leaves, three flavanones (**1**, **2**, **5**) and two dihydrostilbenes (**4**, **6**) were detected as the major peaks. However, the HPLC profile of the *G. uralensis* leaves examined in the present study was different from that of *G. uralensis* leaves (from Chiba University, Japan) reported in our previous paper,[10)] in which compounds **1**, **2**, and **6** were not detected, suggesting that compositions of flavanones and dihydrostilbenes are different among the strains of *G. uralensis*. In the HPLC profile of the *G. glabra* leaves, three flavanones **7**, **8**, and **9** were detected as the major flavanones, these are consistent with those of *G. glabra* collected in Spain and Sicily.[24)] In the leaves of the intermediate-type plants, both *G. uralensis*-specific compounds (**1**, **2**, **5**, **6**) and *G. glabra*-specific compounds (**7**—**9**) were detected in different proportions.

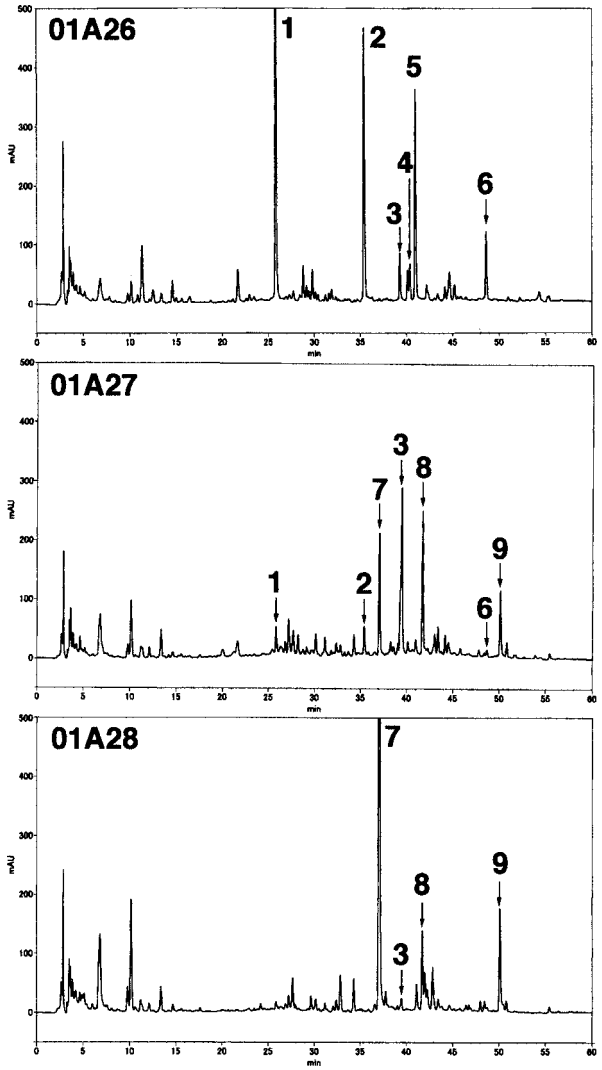

Fig. 2. HPLC Profiles of Methanol Extracts of Leaves of *G. uralensis* (01A26), the Intermediate-Type Plant (01A27), and *G. glabra* (01A28) Collected in Kazakhstan

Absorbance at 292 nm. **1**, licoleafol; **2**, 8-dimethylallyleriodictyol; **3**, sophoraflavanone B; **4**, gancaonin R; **5**, 6-dimethylallyleriodictyol; **6**, uralstilbene; **7**, pinocembrin; **8**, licoflavanone; **9**, glabranin.

Characterization of Offspring Derived from the Seeds of Intermediate Plants In the previous paper,[1)] we observed that the germination rates of seeds collected from an intermediate-type plant (01A27) were high (70%). This led us to elucidate the chemical characteristics of the offspring of this intermediate-type plant, which might be important sources for the breeding of *Glycyrrhiza* plants. Thus the seeds of the intermediate plant (01A27) together with those of *G. uralensis* (01A26) and *G. glabra* (01A28), which were also collected at the same collection site,[1)] were germinated, and the germinated seeds were planted in pots. These plants were cultivated indoors under artificial light for more than 1 year, and their leaves were harvested for analysis.

The leaf extracts from each of the 9 offspring were analyzed to compare their HPLC profiles, and the contents of compounds **1**, **2**, and **4**—**9** in their leaves are shown in Table 4. The relationship between the contents of compounds **1**, **2**, and **9**, of which the former two are index compounds for *G.*

Table 3. Contents of Licoleafol (**1**), 8-Dimethylallyleriodictyol (**2**), Gancaonin R (**4**), 6-Dimethylallyleriodictyol (**5**), Uralstilbene (**6**), Pinocembrin (**7**), Licoflavanone (**8**), and Glabranin (**9**) in the Leaves of *Glycyrrhiza* Plants Collected in Kazakhstan

Species	Collection site	Plant no.	Content (% of dry weight)							
			1	**2**	**4**	**5**	**6**	**7**	**8**	**9**
G. uralensis	A	01A05	0.35	0.21	1.82	0.57	0.56	0.00	n.i.[a)]	0.00
	B	01A07	0.10	0.04	0.46	0.08	0.06	0.00	0.00	0.00
	C	01A10	0.15	0.12	0.37	0.10	0.14	0.00	0.00	0.00
	D	01A14	0.16	0.07	0.96	0.19	0.55	0.00	0.00	0.00
	E	01A15	0.16	0.08	0.88	0.07	0.23	0.00	0.00	0.00
	F	01A17	0.16	0.07	0.97	0.15	0.65	0.00	0.00	0.00
	G	01A18	1.05	0.64	0.12	0.22	0.72	0.02	n.i.	0.00
	H	01A25	0.38	0.36	0.11	0.19	0.25	0.00	0.00	0.00
	I	01A26	0.71	0.50	0.25	0.39	0.69	0.00	n.i.	0.00
Intermediate plants	C	01A11	0.02	0.02	n.i.	n.i.	0.00	0.19	0.18	0.08
	C	01A13	0.14	0.12	n.i.	0.12	0.00	0.05	0.06	0.02
	H	01A23	0.03	0.00	0.00	0.04	0.00	0.11	0.08	0.03
	H	01A24	0.07	0.06	n.i.	0.10	0.15	0.15	0.17	0.10
	I	01A27	0.08	0.06	n.i.	n.i.	0.13	0.19	0.29	0.15
G. glabra	C	01A12	n.i.	0.00	n.i.	n.i.	n.i.	1.78	0.39	0.35
	F	01A16	0.00	0.00	n.i.	n.i.	n.i.	1.62	0.56	0.36
	G	01A19	0.00	0.00	0.00	n.i.	n.i.	1.39	0.28	0.48
	G	01A20	0.00	0.00	0.00	n.i.	0.00	0.85	0.28	0.34
	H	01A21	0.00	0.00	0.00	n.i.	0.00	1.13	0.38	0.56
	H	01A22	0.00	0.00	0.00	n.i.	0.00	2.10	0.28	0.19
	I	01A28	n.i.	0.00	n.i.	n.i.	n.i.	1.13	0.24	0.18
	J	01A29	n.i.	n.i.	n.i.	n.i.	n.i.	1.20	0.25	0.45
	K	01A30	0.00	0.00	0.00	n.i.	0.00	0.67	0.27	0.28
	L	01A31	0.00	0.00	0.00	n.i.	0.00	1.12	0.33	0.58

a) Not identified by UV spectrum.

Table 4. Contents of Licoleafol (**1**), 8-Dimethylallyleriodictyol (**2**), Gancaonin R (**4**), 6-Dimethylallyleriodictyol (**5**), Uralstilbene (**6**), Pinocembrin (**7**), Licoflavanone (**8**), and Glabranin (**9**) in the Leaves of *Glycyrrhiza* Plants Derived from Seeds of *G. uralensis* (01A26), Intermediate (01A27) and *G. glabra* (01A28)

Species of parent	Plant no.	Content (% of dry weight)								Type of HPLC profile
		1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	
G. uralensis	01A26-1	1.18	1.97	0.88	1.19	1.90	0.00	0.00	0.00	UR
(01A26)	01A26-2	1.25	0.70	0.52	0.33	1.08	0.00	0.00	0.00	UR
	01A26-3	1.36	0.97	0.40	0.66	0.63	0.00	0.00	0.00	UR
	01A26-4	0.77	0.46	0.00	0.15	0.97	0.00	0.00	0.00	UR
	01A26-5	0.83	0.35	0.00	0.60	2.00	0.00	0.00	0.00	UR
	01A26-6	1.31	0.68	3.25	1.70	1.23	0.00	0.00	0.00	UR
	01A26-7	0.88	0.75	0.00	0.22	0.71	0.00	0.00	0.00	UR
	01A26-8	0.64	0.56	0.33	0.26	0.83	0.00	0.00	0.00	UR
	01A26-9	0.76	0.40	2.28	1.02	1.30	0.00	0.00	0.00	UR
Intermediate	01A27-1	0.17	0.35	n.i.[a)]	0.11	0.32	0.15	0.08	1.42	HY
(01A27)	01A27-2	0.85	1.67	0.25	1.50	1.50	0.02	0.06	0.03	UR
	01A27-3	0.00	0.77	0.00	0.31	0.53	0.23	0.03	1.22	HY
	01A27-4	0.65	1.01	0.77	0.80	0.92	0.00	0.00	0.00	UR
	01A27-5	0.00	0.82	0.08	0.08	0.42	0.09	0.05	0.25	HY
	01A27-6	0.00	1.26	0.51	0.32	0.00	0.07	0.10	0.11	HY
	01A27-7	0.00	0.04	0.00	0.00	0.00	0.14	0.13	1.27	GL
	01A27-8	0.00	1.58	0.00	0.16	0.68	0.07	0.02	0.64	HY
	01A27-9	0.00	0.04	0.00	0.00	0.00	0.11	0.05	1.41	GL
G. glabra	01A28-1	0.00	0.03	n.i.	n.i.	0.00	0.28	0.14	3.03	GL
(01A28)	01A28-2	0.00	0.00	n.i.	0.00	0.00	0.13	0.05	3.10	GL
	01A28-3	0.00	0.11	0.00	n.i.	0.00	0.09	0.02	1.59	GL
	01A28-4	0.00	0.00	n.i.	0.00	n.i.	0.08	0.02	2.69	GL
	01A28-5	0.00	0.04	0.00	0.00	0.00	0.08	0.04	1.72	GL
	01A28-6	0.00	0.00	0.00	0.00	0.00	0.17	0.05	2.38	GL
	01A28-7	0.00	0.02	n.i.	0.00	0.00	0.16	0.03	2.28	GL
	01A28-8	0.00	0.00	0.00	0.00	n.i.	n.i.	0.00	0.03	GL?
	01A28-9	0.00	0.02	n.i.	0.00	0.00	0.20	0.08	2.97	GL

a) Not identified by UV spectrum. UR, *G. uralensis* type; HY, intermediate type; GL, *G. glabra* type.

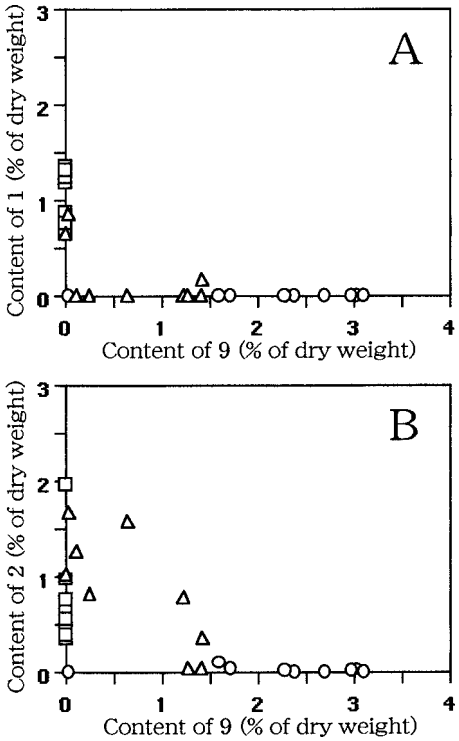

Fig. 3. Relationships between Contents of Compounds **1** and **9** (A), and of Compounds **2** and **9** (B) in the Leaves of Offspring of *G. uralensis* (□, 01A26), the Intermediate-Type Plant (△, 01A27), and *G. glabra* (○, 01A28) Collected in Kazakhstan

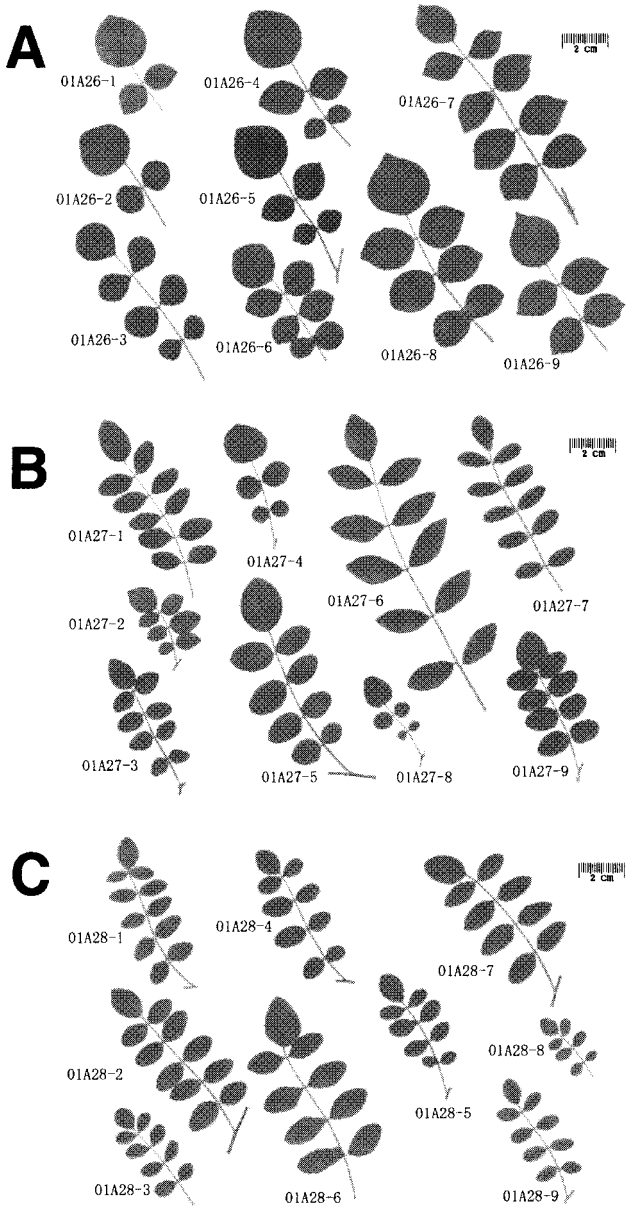

Fig. 4. Shapes of Leaves of Offspring of *G. uralensis* (A, 01A26), the Intermediate-Type Plant (B, 01A27), and *G. glabra* (C, 01A28) Collected in Kazakhstan

uralensis and the latter for *G. glabra*, in the leaves of these offspring is also shown in Fig. 3. The HPLC profiles of all offspring of *G. uralensis* (01A26) are almost identical to that of the parent plant. However, the contents of flavanones **1** and **2** in these offspring were higher than those in the parent plant, whereas the contents of dihydrostilbenes **4** and **6** varied among the 9 offspring. The HPLC profiles of offspring of *G. glabra* (01A28) were different from that of the parent plant. Compound **9** is a major flavanone in the HPLC profiles of these plants, and the contents of **9** in 8 offspring were much higher than that in the parent plant. This difference between the offspring and the parent is probably due to the conditions of cultivation, because the offspring plants were cultivated under artificial light. HPLC profiles of the leaves of *G. glabra* cultivated outdoors were similar to those of the parent *G. glabra* plant (data not shown). The HPLC profiles of the 9 offspring derived from the intermediate-type plant (01A27) were divided into the three types: *G. uralensis* type (01A27-2, -4), *G. glabra* type (01A27-7, -9), and their intermediate type (01A27-1, -3, -5, -6, -8), and both *G. glabra*-specific and *G. uralensis*-specific compounds were detected in the leaves of the intermediate-type plants. Figure 4 shows the leaf shapes of these offspring. The shapes of leaflets of offspring of plant 01A26 were ovate (Fig. 4A), typical of those of *G. uralensis*.[1] In contrast, those of offspring of plant 01A28 were oblong (Fig. 4C), characteristic of those of *G. glabra* in Kazakhstan.[1] The shapes of leaflets of offspring of the intermediate plant 01A27 varied from ovate to oblong (Fig. 4B), suggesting that the intermediate-type plant 01A27 is a hybrid of *G. glabra* and *G. uralensis*. It is noteworthy that the offspring of the *G. uralensis* type (01A27-2, -4) in the HPLC profile have ovate leaflets and those of the *G. glabra* type (01A27-7, -9) in the HPLC profile have oblong leaflets. From these findings, it seems likely that the back-crossing of the intermediate-type plant with *G. glabra* and *G. uralensis* generates *G. glabra*-type plants and *G. uralensis*-type plants, respectively. Further analysis based on their DNA sequences is underway to confirm this possibility.

Experimental

General Methods ^{1}H- and ^{13}C-NMR spectra were recorded using an EX-400 (JEOL) spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. FAB-MS and EI-MS were measured on a JMS-DX300 (JEOL) spectrometer. Silica gel 60 (70—230 mesh, Merck) and ODS (100—200 mesh, Fuji Silysia Chemical) were used for column chromatography. TLC plate Silica gel 60 F_{254} (Merck) was used for preparative TLC.

Plant Materials Leaves and seeds of *Glycyrrhiza* plants used in the present study were collected in Kazakhstan in August 2001.[1] Germinated seeds were planted in pots containing vermiculite, and these plants were fertilized

with liquid nutrients and were grown indoors under artificial light.

Isolation of Flavanones and Dihydrostilbenes from Leaves of *G. uralensis* Air-dried leaves (41 g) of *G. uralensis* collected at Almaty (site B) were extracted with ethanol at room temperature overnight. The ethanol extract was partitioned between water and ethyl acetate. The dried ethyl acetate-soluble fraction (4.4 g) was chromatographed on a silica gel (80 g) column using a series of mixtures of *n*-hexane and ethyl acetate (1 : 0, 9 : 1, 4 : 1, 1 : 1, 0 : 1, each 600 ml), and 600 ml of each fraction (fr. A—E) was collected and evaporated *in vacuo*. Fr. E (0.61 g) was separated by column chromatography on reverse-phase silica gel (ODS) (28 g) and eluted with 50% ethanol in 12-ml fractions. Frs. E15—20 were further purifed by preparative TLC (*n*-hexane : ethyl acetate, 1 : 2) to give licoleafol (**1**, 70 mg). Fr. D (2.3 g) was subjected to silica gel (150 g) column chromatography eluted successively with a series of mixtures of *n*-hexane and ethyl acetate (7.25 : 1, 6 : 1, 5 : 1, 4 : 1, 3 : 1, 2 : 1, 1 : 1, each 2 l). Each fraction (200 ml) was collected, and frs. D17—21 were purified by ODS column chromatography using 50% ethanol and preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give sophoraflavanone B[15,16] (**3**, 6 mg). Frs. D29—34 were further purified by ODS column chromatography using 50% ethanol and preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give uralstilbene (**6**, 60 mg). Frs. D35—38 were separated by another ODS column chromatography using 50% ethanol. Frs. D35/38-13—15 were purified by preparative TLC to give 8-dimethylallyleriodictyol[14] (**2**, 10 mg), and frs. D35/38-16—22 were purifed by preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give gancaonin R[17] (**4**, 56 mg). Likewise, frs. D44—53 were separated by ODS column chromatography using 50% ethanol, and eluted frs. D44/53-24—26 were purifed by preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give 6-dimethylallyleriodictyol[14] (**5**, 10 mg).

Licoleafol (**1**): An amorphous powder. $[\alpha]_D^{24}$ −34° (c=1.0, MeOH). UV λ_{max} (MeOH) nm (log ε): 291 (4.30), 335 (3.68). HR-positive FAB-MS $[M+H]^+$ m/z: 373.1282 (Calcd for $C_{20}H_{21}O_7$: 373.1287). ^{1}H-NMR (acetone-d_6) δ: 1.68 (3H, br s, H_3-13), 2.81 (1H, dd, J=3.4, 17.1 Hz, H-3A), 3.08 (1H, dd, J=11.7, 17.1 Hz, H-3B), 3.28 (2H, d, J=6.8 Hz, H_2-9), 3.92 (2H, br s, H_2-12), 5.44 (1H, dd, J=3.4, 12.0 Hz, H-2), 5.48 (1H, tm, J=6.8 Hz, H-10), 6.02 (1H, s, H-6), 6.85 (2H, s, H-5′, -6′), 7.08 (1H, s, H-2′), 12.11 (1H, s, OH-5). The ^{13}C-NMR spectral data are listed in Table 1.

Uralstilbene (**6**): An amorphous solid. UV λ_{max} (MeOH) nm (log ε): 283 (3.79). EI-MS $[M]^+$ m/z: 382.2136 (Calcd for $C_{24}H_{30}O_4$: 382.2115). ^{1}H-NMR (acetone-d_6) δ: 1.64 (3H, br s, H_3-11) 1.73 (6H, br s, H_3-10, -15) 1.76 (3H, br s, H_3-16), 2.69 (2H, m, H_2-β), 2.72 (2H, m, H_2-α), 3.28 (2H, br d, J=6.8 Hz, H_2-7), 4.47 (2H, br d, J=6.3 Hz, H_2-12), 5.05 (1H, br t, J=6.8 Hz, H-8), 5.47 (1H, br t, J=6.4 Hz, H-13), 6.32 (1H, d, J=2.5 Hz, H-6), 6.34 (1H, d, J=2.5 Hz, H-4), 6.56 (1H, dd, J=2.4, 8.3 Hz, H-6′), 6.73 (1H, d, J=2.0 Hz, H-2′), 6.74 (1H, d, J=8.3 Hz, H-5′). The ^{13}C-NMR spectral data are listed in Table 2.

Isolation of Flavanones from Leaves of Intermediate-Type Plants Air-dried leaves (14 g) from intermediate-type plants (01A11, 01A13, 01A24) collected at sites C and H were extracted with ethyl acetate overnight. The dried ethyl acetate extract (1.0 g) was chromatographed on an ODS (28 g) column using 50% ethanol, with 12-ml fractions collected. Frs. 10—13 were further purified on an ODS column using 40% ethanol and preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give pinocembrin[21] (**7**, 7 mg). Frs. 14—17 were further purified by preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give sophoraflavanone B[15,16] (**3**, 6 mg). Frs. 18—22 were further purified by ODS column chromatography using 50% ethanol and preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give licoflavanone[21] (**8**, 10 mg). Frs. 41—47 were further purified by ODS column chromatography using 50% ethanol and preparative TLC (*n*-hexane : ethyl acetate, 1 : 1) to give glabranin[23] (**9**, 9 mg). The known compounds **7**—**9** were identified by comparison with literature data.

HPLC Analysis of Leaves Dried leaves (40 mg) were extracted with 1 ml of 80% methanol at 60 °C for 2 h. An aliquot (10 μl) of the extract was analyzed by HPLC as previously reported.[1] Quantities of compounds were determined on the basis of their peak area of UV absorption at 292 nm (compounds **1**, **2**, **5**, **7**, **8**, **9**) or 282 nm (compounds **4**, **6**).

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