Field Survey of *Glycyrrhiza* Plants in Central Asia (3).[1] Chemical Characterization of *G. glabra* Collected in Uzbekistan

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The chemical characteristics of *Glycyrrhiza glabra* L. were investigated at a habitat in Uzbekistan. HPLC analysis of the underground parts indicated that glycyrrhizin contents varied from 3.3 to 6.1% of dry weight, and that glabridin, a species-specific flavonoid for *G. glabra*, was detected in all underground samples (0.08—0.35% of dry weight). HPLC analysis of the leaves indicated that *G. glabra* plants collected in the present study could be divided into two types, RT-type and IQ-type, according to their major flavonol glycosides, rutin or isoquercitrin, respectively.

Key words *Glycyrrhiza glabra*; glycyrrhizin; flavonoid variation; rutin; isoquercitrin

Roots and stolons of *Glycyrrhiza* plants are important crude drugs, and their major sweet constituent, glycyrrhizin (GL), is used in large quantities as a well-known natural sweetener and as a pharmaceutical.[2,3] Extensive chemical studies revealed that roots and stolons of *Glycyrrhiza* plants contain not only GL but also many flavonoids,[4,5] including species-specific flavonoids,[5—9] such as glabridin (GB) for *G. glabra* L., glycycoumarin for *G. uralensis* FISCH. and licochalcone A for *G. inflata* BAT. Flavonoid variations in leaves of *Glycyrrhiza* plants have also been reported.[10,11] These variations in leaves and roots are very useful to identify the species and strains of *Glycyrrhiza* plants, but their relationships among geographical distribution, chemical and genetical characteristics have not yet been elucidated in detail.

G. glabra, one of the GL-producing species, is distributed in Spain, Italy, Turkey, Caucasus, Iran, Central Asia and the western part of China. To elucidate the variation of *G. glabra* in the world, we undertook field surveys of this species in Turkey,[12,13] Italy,[14] Spain[14] and Kazakhstan,[15] and found that it could be divided into two types according to their major flavonol glycosides in leaves.[11,15] The major leaf flavonol glycoside of *G. glabra* collected in Turkey, Italy and Spain was isoquercitrin (IQ), but that collected in Kazakhstan was rutin (RT).[14,15] Thus, in the present study, we investigated the chemical characterization of *G. glabra* at a habitat in Uzbekistan, one of the countries in Central Asia, and found that both IQ-type and RT-type of *G. glabra* grew together forming a mixed population at the collection site there.

Experimental

Plant Materials Underground parts, leaves and seeds of *G. glabra* L. were collected 10 km east of Yangiyer, Uzbekistan on 15 August, 2001. Leaves of *G. glabra* were collected 40 km north of Eskisehir, Turkey (90A05), 4 km northeast of Sant'Agata di Militello, Sicily, Italy (96A15), 8 km southeast of Calahorra, Spain (96B02) and 5 km northwest of Qasyk, Kazakhstan (01A19, 20) as reported previously.[13—15]

Chemicals Authentic samples of GL and GB were obtained from Maruzen Pharmaceuticals, Japan. IQ and RT were purchased from Extrasynthese, France. Pinocembrin (PN)[1,16,17] licoflavanone (LF)[1,17] and glabranin (GN)[1,16,18] were isolated from the leaves of *G. glabra* collected in Kazakhstan.[1]

HPLC Analysis of Underground Parts and Leaves HPLC analysis of underground parts and leaves was performed as reported elsewhere.[15] The quantities of constituents were determined on the basis of their peak area of UV absorption at 254 nm (GL), 280 nm (GB), 292 nm (PN, LF, GN) and 350 nm (IQ, RT). Each constituent was identified by comparison of its retention time and UV spectrum with the respective authentic sample.

Results and Discussion

A Field Survey of *Glycyrrhiza glabra* L. in Uzbekistan A field survey of *Glycyrrhiza* plants was carried out in Uzbekistan in August, 2001, and underground and aerial parts of *G. glabra* plants were collected at a site near Yangiyer, 100 km south of Tashkent, Uzbekistan. Morphological classification of plants was at first attempted based on phenotypic characteristics,[19,20] and all collected plants were identified as *G. glabra*. It is noteworthy that both *G. glabra* having eglandular fruits (var. *glabra*)[20] and *G. glabra* having echinulate fruits (var. *glandulifera* REG. *et* HERD.)[20] grew together forming a mixed population at the collection site in Uzbekistan (Fig. 1), as we observed in Turkey[12] and Kazakhstan.[15] The shapes of leaves of *G. glabra* collected in Uzbekistan, Kazakhstan, Spain, Italy and Turkey are shown

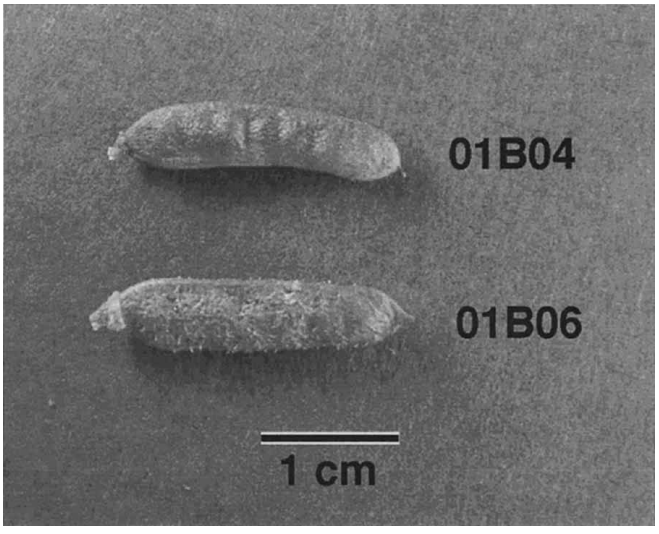

Fig. 1. Comparison of Fruits of *G. glabra* Collected in Uzbekistan, 01B04 (var. *glabra*) and 01B06 (var. *glandulifera*)

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in Fig. 2. Leaflets collected in Uzbekistan were from oblong to linear-oblong in shape, similar to those of *G. glabra* collected in Kazakhstan,[15] but differed from those collected in Spain, Italy and Turkey, which were from ovate to oblong.[13,14]

HPLC Analysis of GL and GB in Underground Parts Since GL, a sweet saponin, and GB, a species-specific flavonoid, are known to be important index compounds for underground parts of *G. glabra*,[4,7] HPLC analysis was performed to determine their content in the roots and stolons collected in the habitat (Table 1). GL contents were found to vary from 4.76% to 6.13% of dry weight in the roots, and from 3.33% to 5.98% of dry weight in the stolons, depending on the sample. It is noteworthy that GL content in the present study was higher than that of underground parts collected in our previous survey in Spain (0.7—4.4%)[14] and Italy (1.6—3.0%).[14] On the other hand, GB was detected in all the stolons and roots of *G. glabra* collected, and its content varied from 0.08% to 0.35%. The GB content was almost the same as that of underground parts collected in Spain (0.21—0.80%)[14] and Italy (0.07—0.27%).[14]

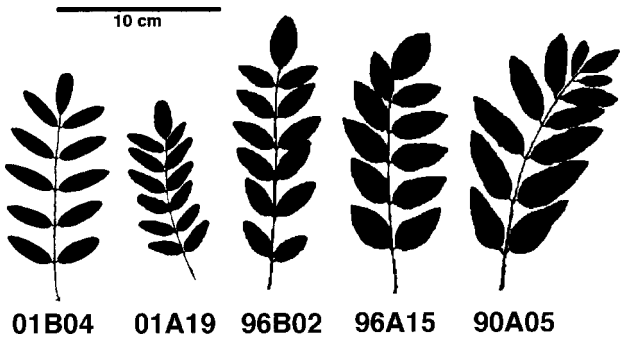

Fig. 2. Shapes of Leaves of *G. glabra* Plants Collected in Uzbekistan (01B04), Kazakhstan (01A19),[15] Spain (96B02),[14] Italy (96A15)[14] and Turky (90A05)[13]

Table 1. Contents of Glycyrrhizin (GL) and Glabridin (GB) in Underground Parts of *G. glabra* Collected in Uzbekistan

Root/Stolon	Diameter (mm)	Content (% of dry weight)	
		GL	GB
Root	12.3	6.13	0.12
Root	17.5	4.76	0.08
Root	20.5	5.87	0.15
Stolon	6.6	3.33	0.10
Stolon	10.4	3.35	0.20
Stolon	15.5	5.98	0.35

HPLC Analysis of Flavonoids in Leaves Flavonoid variations in the leaves of *Glycyrrhiza* plants were reported,[11] and *G. glabra* could be divided into two types, IQ type and RT type, according to their major flavonol glycosides, IQ or RT, respectively.[11,15] This might be a good marker by which to discriminate the origin of *G. glabra*,[11,15] which is distributed widely in the world from Spain to China. Thus, HPLC analysis was performed to examine the chemical compositions of the leaves collected in Uzbekistan. As shown in Table 2, the HPLC profiles of leaf extracts from the 10 plants collected in Uzbekistan were divided into the RT and IQ-types. Figure 3 shows typical HPLC profiles for the each type, and Fig. 4 shows structures of the two glycosides (IQ, RT) and three flavanones (PN, LF, GN), which were identified by comparison of their HPLC behaviour and UV spectra with those of the authentic samples. In the HPLC profile of seven RT-type leaves (01B01, 02, 05, 07, 08, 09, 10), RT was detected at 10.4 min as the major flavonol glycoside. RT contents in the RT-type leaves varied from 0.22% to 0.32% of dry weight, and their IQ contents were from 0.03% to 0.06% (Table 2). In the HPLC profile of three IQ-type leaves (01B03, 04, 06), IQ was detected at 11.5 min as the major flavonol glycoside. IQ contents in the IQ-type leaves varied from 0.39% to 0.57% of dry weight, and their RT contents were from 0.04% to 0.07% (Table 2). It is noteworthy, however, that there is no correlation between the HPLC profiles of leaf extracts (RT-type or IQ-type) and the morphological features of fruits (eglandular or echinulate).

As reported previously,[14,15] HPLC profiles of leaf extracts

Table 2. Contents of Rutin (RT), Isoquercitrin (IQ), Pinocembrin (PN), Licoflavanone (LF) and Glabranin (GN) in Leaves of *G. glabra* Collected in Uzbekistan, Kazakhstan, Italy, Spain and Turkey

Plant No.	Origin	Type of fruit	Type of HPLC profile	Content (% of dry weight)				
				RT	IQ	PN	LF	GN
01B01	Uzbekistan	*glabra*	RT-type	0.22	0.03	2.66	0.53	1.07
01B02	Uzbekistan	*glabra*	RT-type	0.25	0.05	1.53	0.21	0.25
01B03	Uzbekistan	*glandulifera*	IQ-type	0.05	0.57	0.45	0.00	0.09
01B04	Uzbekistan	*glabra*	IQ-type	0.04	0.39	1.79	0.16	0.42
01B05	Uzbekistan	*glabra*	RT-type	0.32	0.04	0.98	0.33	0.18
01B06	Uzbekistan	*glandulifera*	IQ-type	0.07	0.53	0.95	0.00	0.24
01B07	Uzbekistan	*glabra*	RT-type	0.29	0.03	1.74	0.12	0.39
01B08	Uzbekistan	*glabra*	RT-type	0.23	0.05	2.34	0.29	0.69
01B09	Uzbekistan	*glabra*	RT-type	0.24	0.05	1.53	0.22	0.22
01B10	Uzbekistan	—[a]	RT-type	0.25	0.06	2.30	0.28	0.33
01A19[15]	Kazakhstan	*glabra*	RT-type	0.26	0.03	1.39	0.21	0.52
01A20[15]	Kazakhstan	*glandulifera*	RT-type	0.27	0.09	0.86	0.23	0.32
96A15[14]	Italy	*glabra*	IQ-type	0.02	0.32	1.64	1.59	0.73
96B02[14]	Spain	*glabra*	IQ-type	0.03	0.47	1.21	1.13	0.29
90A05[13]	Turkey	—[a]	IQ-type	0.06	0.84	1.09	0.47	0.16

a) No fruit.

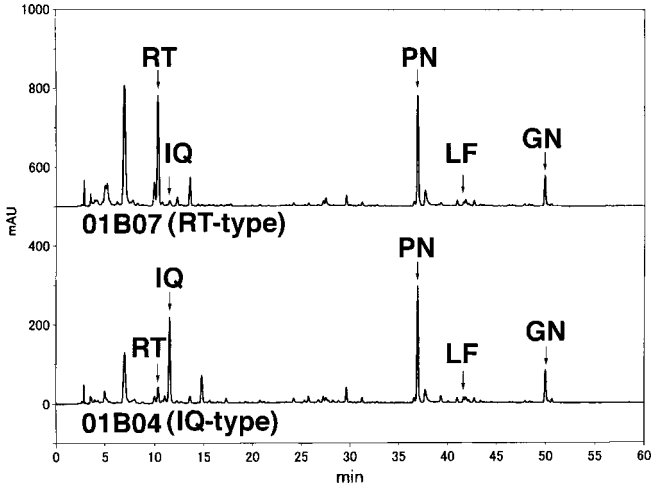

Fig. 3. HPLC Profiles of Methanol Extracts of Leaves of RT-type (01B07) and IQ-type (01B04) of *G. glabra* Collected in Uzbekistan

Absorbance at 350 nm. RT, rutin; IQ, isoquercitrin; PN, pinocembrin; LF, licoflavanone; GN, glabranin.

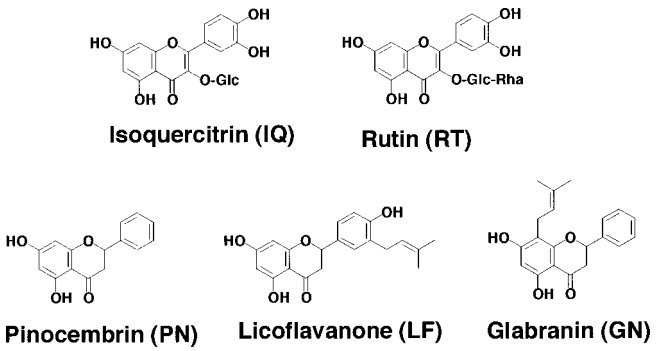

Fig. 4. Structures of Flavonol Glycosides (RT, IQ) and Flavanones (PN, LF, GN) in the Leaves of *Glycyrrhiza* Plants

of *G. glabra* plants collected in Spain, Italy and Turkey were the IQ-type, whereas those of the plants collected in Kazakhstan were the RT-type (Table 2). These results suggest that the former type of *G. glabra* is distributed from Spain to Uzbekistan, and the latter type from Uzbekistan to Kazakhstan,[15] probably as far as the western part of China. Since the contents of RT and IQ were relatively constant in the different leaves of *G. glabra*,[14,15,21] this variation will be a good marker to discriminate between eastern and western origins of this species worldwide. In addition to these, the three flavanones, PN, LF and GN, were recognized as major compounds common to both types. The contents of these flavanones were shown to vary greatly among different habitats in the world (Table 2),[10,14,15] suggesting that HPLC profiling of these flavanones in leaves can be useful to distinguish various *G. glabra* strains of different geographical origins.

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