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FLAVONOID VARIATION IN THE LEAVES OF *GLYCYRRHIZA GLABRA**

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Key Word Index—*Glycyrrhiza glabra*; Leguminosae; aerial parts; flavonoid; isoflavonoid.

Abstract—Genistein, pinocembrin, prunetin, 6-prenylnaringenin, licoflavanone and wighteone were isolated from the leaves of *Glycyrrhiza glabra* collected on the west coast of Anatolia, whereas lupiwighteone was found only in the leaves of *G. glabra* growing in middle or east Anatolia. The *G. glabra* plants growing in different areas of Turkey could be classified into two types according to the occurrence of lupiwighteone in the leaf.

INTRODUCTION

The roots and stolons of *Glycyrrhiza glabra* are used as a sweetener as well as a crude drug all over the world [1]. Although the chemical constituents of its roots and stolons have been extensively studied in detail [2, 3], those of the leaves have not received much attention [4–9].

We reported that HPLC analyses of the leaf extracts from *G. glabra* plants collected in various regions of Turkey indicated the existence of two types of peak patterns [10]. The present study deals with the isolation of these compounds from the leaves and the comparison of flavonoid constituents among the plants of different geographical origins in Turkey.

RESULTS

Leaves of *G. glabra* were collected in 11 regions (**A**–**K**) of Turkey in 1986 and 1990 (Fig. 1). The leaves of *G. glabra* collected in Fethiye (**D**) was extracted with methanol, and the ethylacetate soluble fraction was repeatedly subjected to silica gel and reverse phase silica gel column chromatography to isolate six known flavonoids and isoflavonoids, genistein (**1**) [8, 11], pinocembrin (**2**) [8, 9], prunetin (**3**) [12], 6-prenylnaringenin (**4**) [13], licoflavanone (**5**) [9] and wighteone (**6**) [14], which were identified by spectroscopic methods. Although **1**, **2** and **5** were already reported for the leaves of *G. glabra* [8, 9], **3**, **4** and **6** have been isolated from *G. glabra* for the first time. Compounds **1** and **3** were found in the roots of *Glycyrrhiza* spp. [15] and the aerial parts of *G. pallidiflora* [16], respectively, though in small amounts.

All these compounds (**1**–**6**) were detected as major components in HPLC profiles of the methanolic extracts prepared from the leaves collected at three sites (**B**, **C** and **D**) of west Anatolia (Fig. 2). By contrast, the leaves collected in the other regions (**A** and **E**–**K**) contained, in addition to **1**–**6**, a significant amount of an unidentified compound (**7**), which was hardly detectable in the leaves from sites **B**, **C** and **D** (Fig. 3). In order to isolate the latter compound, fresh leaves (300 g) collected from *G. glabra* plants (growing in Niigata from the seeds collected at site **A**), were washed with methanol for 20 sec, and the methanol wash was repeatedly subjected to reverse phase column chromatography to separate **7**. Compound **7** was identified as lupiwighteone [14] on the basis of its UV, ^{1}H NMR and mass spectra. This compound had been isolated also from the aerial parts of *G. uralensis* [17].

Table 1 shows the contents of flavonoids **1**–**7** in the leaves of *G. glabra* plants from various regions of Turkey. Quantitative analysis was performed by photodiode-array HPLC, and respective peaks were identified by their UV spectra. Plants growing in west Anatolia (**B**, **C** and **D**) could be distinguished from those in middle or east Anatolia (**A** and **E**–**K**) not only by the higher contents of five compounds (**1**, **3** and **4**–**6**) but also by the absence of **7**.

DISCUSSION

The seven free flavonoids (**1**–**7**) confirmed in the leaves of *G. glabra* seem to be localized largely on the surface of the leaf, since they could easily be extracted by washing the leaves with methanol for 20 sec (data not shown). Compounds **1**, **6** and **7** have been reported

*Part 3 in the series 'Field survey of licorice in Turkey'. For Part 2 see ref. [10].

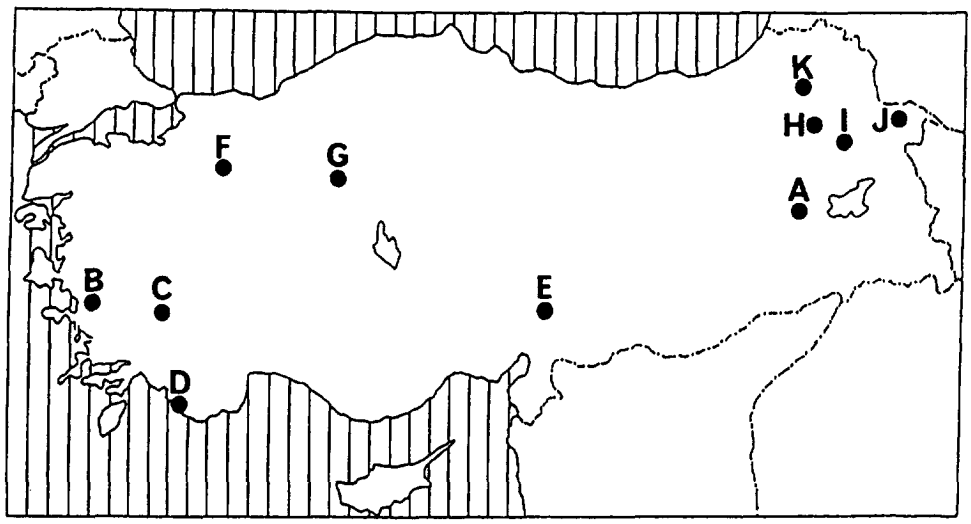


Fig. 1. Collection sites for *Glycyrrhia glabra* in Turkey. **A**: 55 km east of Muş; **B**: 20 km west of Aydın; **C**: 10 km north of Denizli; **D**: Fethiye; **E**: 20 km west of Kahramanmaraş; **F**: 40 km north of Eskişehir; **G**: 20 km west of Ankara; **H**: 60 km east of Erzurum; **I**: 20 km south of Ağrı; **J**: 50 km north of Doğubeyazıt; **K**: 60 km west of Kars.

to occur also on the leaf surface of *Lupinus* plants, showing an antifungal activity *in vitro* [18, 19]. It is possible, therefore, that they play a role in the chemical defence against fungal pathogens.

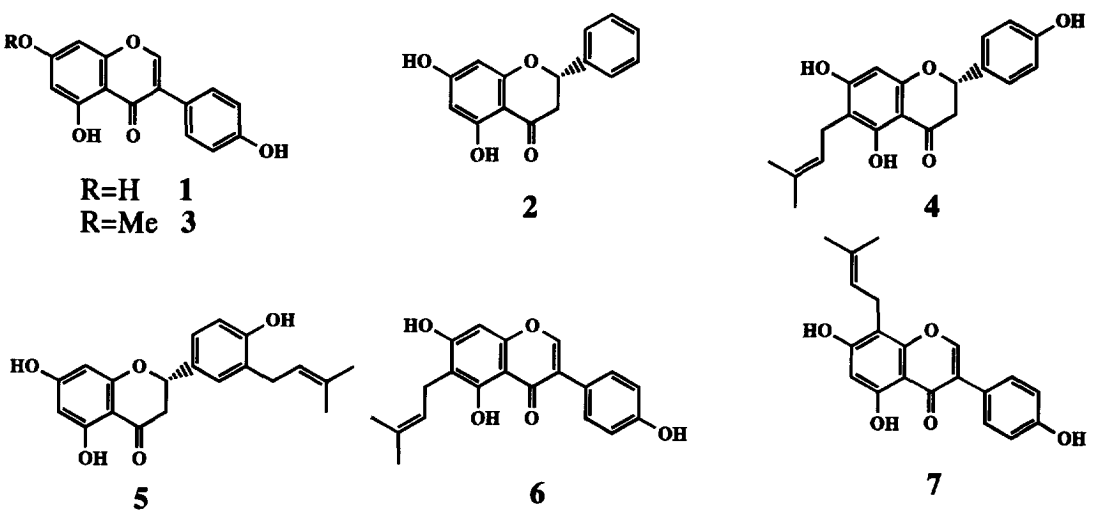
HPLC analyses of leaf extracts have demonstrated considerable differences in the content of flavonoids, in particular **7** between *G. glabra* plants growing on the west coast and those in other areas of Turkey. The structural difference between **6** and **7** is in the site of prenylation in **1**, which presumably is controlled by site-specific prenyltranferases. Although a number of flavonoid glycosides are known to exist in the aerial part of *G. glabra* [4, 5, 7], no significant differences were found by the HPLC analyses of these leaves.

G. glabra is widely distributed in the Old World, from Spain to China. *G. glabra* found in Turkey was classified into two varieties, *glabra* and *glandulifera*, according to the absence or presence of glands on the legume and ovary, respectively [20]. Since these varieties are difficult to distinguish from each other when not in fruit, the plants collected at sites **B**–**F** and **H**–**K** could not be identified at the variety level [10]. However, the plants collected at sites **A** [21] and **G** [10] were determined to be var. *glabra* and var. *glandulifera*, respectively, and the leaves from both sites **A** and **G** contain **7**. These data suggest that the difference in the flavonoid composition might serve as a marker for determining the habitat of *G. glabra*.

EXPERIMENTAL

Plant material. The leaves and seeds of *G. glabra* were collected in Turkey in 1986 (**A**) and 1990 (**B**–**K**), as reported previously [10, 21].

Extraction and separation. Air-dried leaves (50 g) of *G. glabra* collected in Fethiye in 1990 were extracted



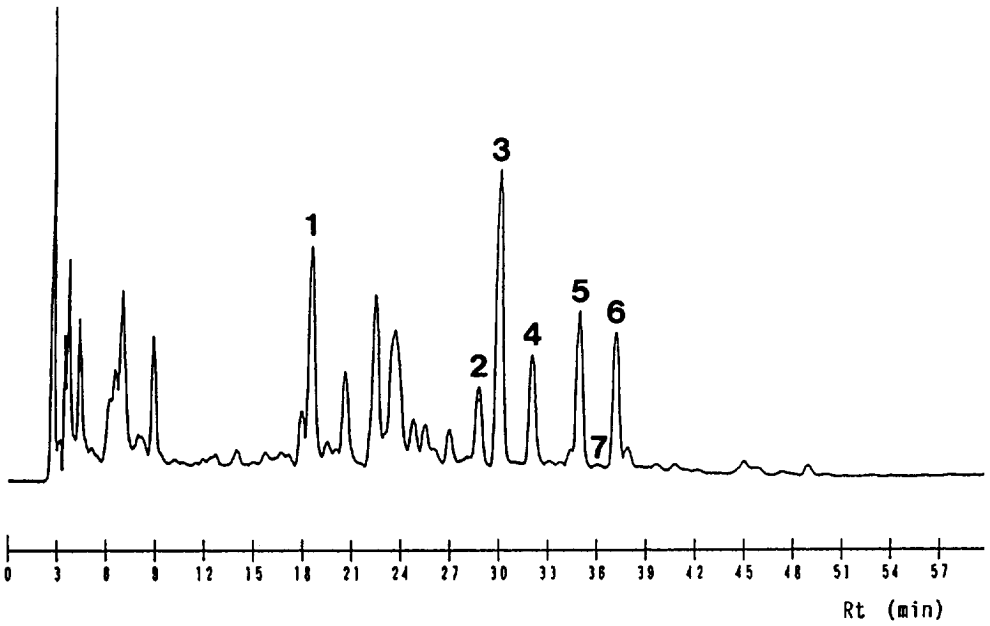


Fig. 2. HPLC profile of the MeOH extract of *G. glabra* leaves collected at site **B**; detection: UV 275 nm.

(× 2) overnight with 1 l of MeOH. The dried extract was suspended in 500 ml H_2O and extracted (× 3) with 500 ml EtOAc. The dried EtOAc extract (13.5 g) was subjected to silica gel CC (Wako-gel C-200, 470 g) and eluted successively with a series of mixts of *n*-hexane and EtOAc (1:0, 9:1, 4:1, 1:1, 0:1, each 1 l) and 500 ml of each fr. (1–10) were dried *in vacuo*. Fr. 7 gave **2**, which was identified by TLC and HPLC by comparison with the authentic sample [9]. Fr 9 (5.1 g) was subjected to silica gel CC (Wako-gel C-200, 230 g) and eluted with mixts of *n*-hexane–EtOAc–HOAc in proportions of 300:100:1 (2 l), 200:100:1 (1 l) and

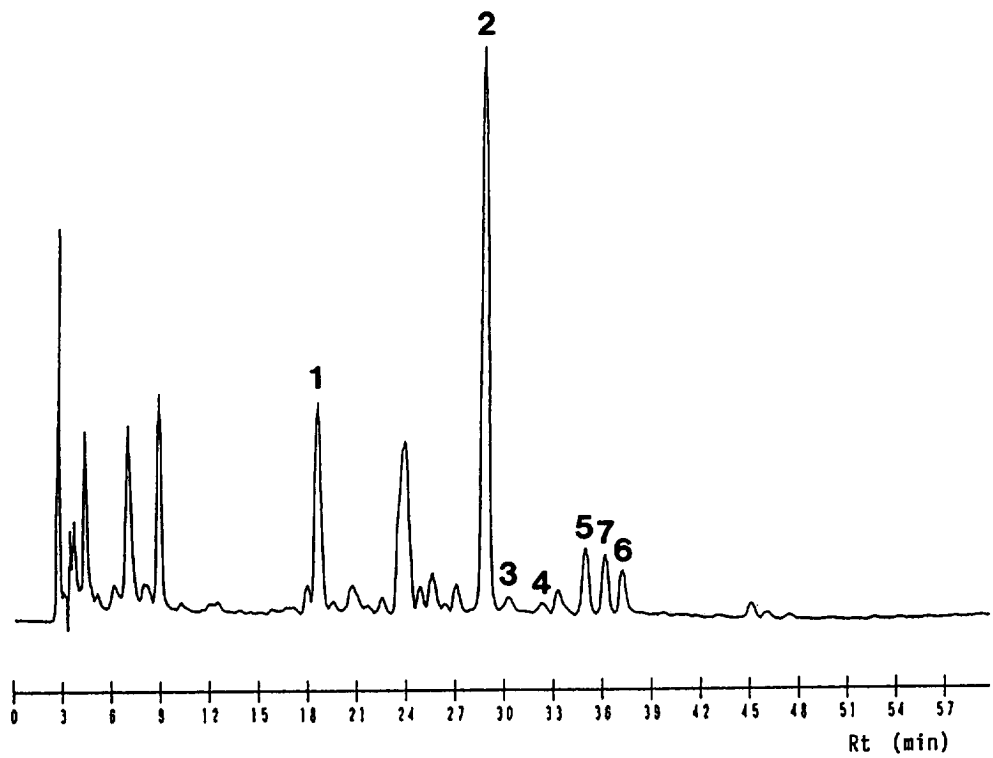


Fig. 3. HPLC profile of the MeOH extract of *G. glabra* leaves collected at site **F**; detection: UV 275 nm.

Table 1. Contents of **1**–**7** in *G. glabra* leaves collected from various sites in Turkey

Site	Contents (% dry wt) of flavonoids						
	1	**2**	**3**	**4**	**5**	**6**	**7**
A (var. *glabra*)	0.05	0.28	0.01	n.d.*	0.18	0.03	0.18
B	0.24	0.26	0.48	0.48	0.48	0.17	n.d.
C	0.20	0.21	0.43	0.36	0.53	0.20	n.d.
D	0.32	0.39	0.43	0.35	0.69	0.19	n.d.
E	0.06	0.25	0.05	0.29	0.17	0.03	0.04
F	0.19	1.22	0.02	0.07	0.20	0.05	0.14
G (var. *glandulifera*)	0.18	1.18	0.02	0.11	0.14	0.03	0.09
H	0.06	0.23	0.01	0.05	0.07	0.03	0.05
I	0.18	0.48	0.12	0.18	0.23	0.11	0.21
J	0.07	0.15	0.01	n.d.	0.07	0.04	0.07
K	0.20	0.15	0.01	0.12	0.10	0.04	0.11

*Not detected (<0.01%).

200:200:1 (2 l), successively. Each fr. (30 ml) was collected, and the combined fr. 52–57 was sepd by reverse phase silica gel CC (Cosmosil 75C18-OPN, Nacalai Tesque, 30 g) and eluted with 70% MeOH to yield **4** (37 mg) [13], **5** (40 mg) [9] and **6** (21 mg) [14]. Similarly, prunetin (9 mg, **3**) [12] was isolated from frs 58–62, and **1** (47 mg) [11] from frs 79–90, using reverse phase silica gel CC. These compounds were identified on the basis of UV, ^{1}H NMR and MS [9, 11–14].

For the isolation of **7**, the fresh leaves (100 g fr. wt) of *G. glabra* plants, derived from the seeds collected at site **A**, were washed with MeOH (1 l) for 20 sec, and the extract (2.1 g) was repeatedly subjected to reverse phase silica gel CC and eluted with 70% MeOH to isolate **7** (17 mg), which was identified on the basis of UV, ^{1}H NMR and MS [14].

Quantitative analysis of leaf flavonoids. Powdered samples (100 mg) were extracted with 10 ml MeOH at 60° for 2 hr. An aliquot (20 μl) of the extract was analysed by photodiode-array HPLC; column: Capcellpak C18 AG-120A (5 μm, 4.6 × 250 mm, Shiseido, Japan), solvent: MeCN–H_2O (1% HOAc) gradient of 20–80% MeCN in 60 min, flow rate: 0.9 ml min^{-1}, column temp.: 40°, detector: Shimadzu photodiode array SPDM-6A system (Shimadzu). The quantities of flavonoids were calculated from the peak height of UV absorption at 254 nm.

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