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Studies on Index Compounds for HPLC Analysis of *Glycyrrhiza flavescens* Growing in Turkey

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HPLC of the EtOAc extracts of the subterranean parts of *Glycyrrhiza flavescens* BOISS. showed a characteristic chromatogram distinct from those of other species (*G. uralensis*, *G. glabra*, *G. echinata*, *G. macedonica*, *G. pallidiflora*). As index compounds for HPLC analysis of *G. flavescens*, 10 new stilbenic compounds were isolated and the structures were defined, in addition to identification of 4 known stilbenic compounds.

Keywords: *Glycyrrhiza flavescens*; index compound; stilbene; HPLC; LC/MS; structural elucidation

In our continuing studies on index compounds for HPLC analysis of *Glycyrrhiza* species,[1-3] *G. flavescens* BOISS.[4] proved to contain 14 stilbenic compounds, which are different from phenolic compounds (flavonoids, isoflavonoids, coumarins, chalcones, pterocarpenes, etc.) of many other *Glycyrrhiza* species.[5-8] The present report deals with the characterization of the index compounds for HPLC analysis of the subterranean parts of *G. flavescens*.

RESULTS AND DISCUSSION

The EtOAc extracts of the subterranean parts of *G. flavescens* provided an HPLC profile having 10 major peaks by 14 index compounds as shown in Fig. 1. These compounds were isolated by silica gel column chromatography, HPLC and TLC preparation of the extracts as described in the experimental.

Fourteen isolated compounds **1** — **14** were characterized and their structures were elucidated as shown at Fig. 2. Compounds **1**, **3**—**9**, **12**,and **14** were new and designated flavestins A—J. Compound **2** was identified as chiricanine A,[9] **10** as longistylin A,[10] **11** as longistylin C,[9] and **13** as longistylin B[10] by comparison of MS, ^{1}H-NMR and ^{13}C-NMR spectroscopic data with the reported data.

Flavestin A (**1**) was obtained as a yellowish powder, and showed a blue spot on TLC by Gibbs' reagent. The molecular formula was determined as $C_{20}H_{22}O_3$ by high resolution electron impact mass spectroscopy (HR-EI-MS) ($[M]^+$: *m/z* 310.1550). The UV spectrum [λ_{max} nm (log ε): 219.5 (4.32), 322.5 (4.38)] was similar to those of stilbene derivatives. The IR spectrum showed a hydroxy group, polysubstituted aromatic rings conjugated with a double bond, C-O bonds, and an alkane moiety as described in the experimental.

The ^{1}H-NMR spectrum of **1** suggested the presence of a 1,3,5-trisubstituted benzene ring (3 *meta*-coupled triplets at δ 6.64, 6.57, 6.33), a 1,3,4-trisubstituted

benzene ring (ABX type signals at δ 7.29, 7.27, 6.82), an *E*-double bond flanked with 2 benzenes (2 doublets at δ 7.01, 6.87 ppm), a prenyl group [δ 5.36 (1H, m), δ 3.40 (2H,br.d), δ 1.83 (3H, s), δ 1.82(3H, d, J=1Hz)], and a methoxy group [a singlet at δ 3.84 (3H, s)]. The ^{1}H- and ^{13}C-NMR signals were reasonably assigned on the basis of ^{1}H$-^{1}$H correlated spectroscopy (^{1}H$-^{1}$H COSY), and hetero nuclear signal quantum coherence (HSQC), and summarized in Tables 1 and 2.

The partial structures, 3-hydroxy-5-methoxy-phenyl (R_1 = R_2 = R_4 = H, R_3=OCH_3), 3'-prenyl-4'-hydroxy-phenyl(R_5 = prenyl, R_6 =OH), and *E*-double bond, and their connection were confirmed by the heteronuclear multiple bond correlations (HMBC) and nuclear Overhauser effects (NOE) [NOEs: OMe / H-6, 4 and H-1" / H-2']. Thus the structure was formulated as **1** in Fig. 2.

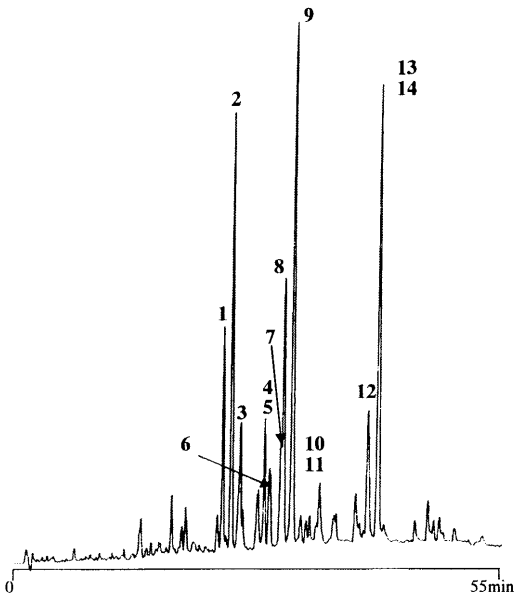
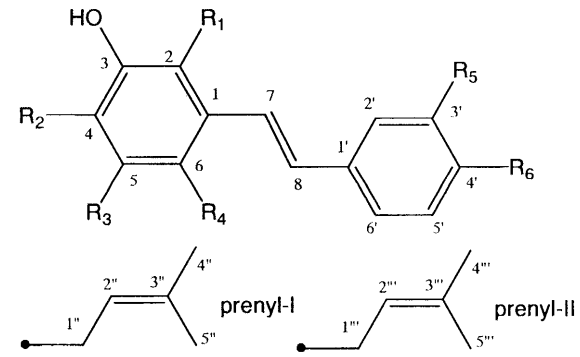
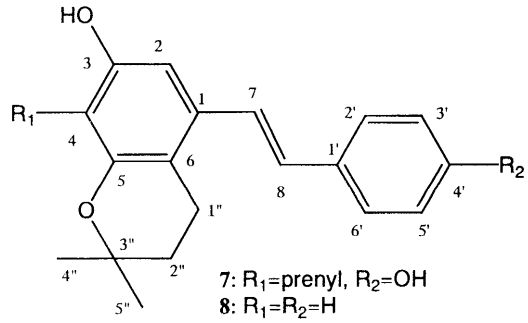


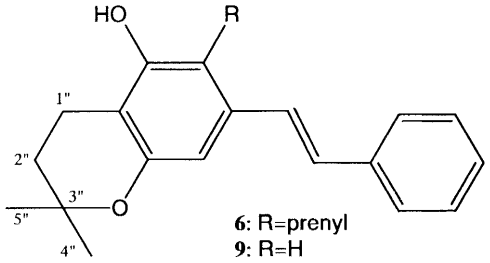
Fig. 1. HPLC Profile of EtOAc Extract from the Underground Parts of *G. flavescens*



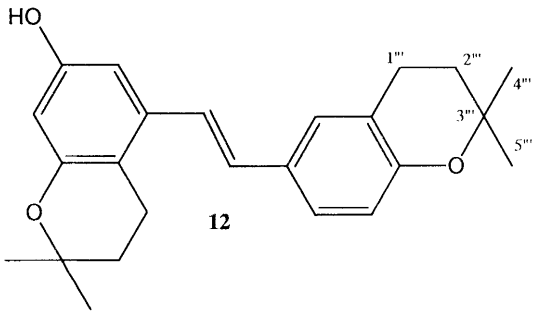
1: R_1=R_2=R_4=H, R_3=OMe, R_5=prenyl-I, R_6=OH
2*: R_1=R_4=R_5=R_6=H, R_2=prenyl-I, R_3=OH
3: R_1=prenyl-I, R_2=R_4=R_5=R_6=H, R_3=OH
4: R_1=prenyl-I, R_2=prenyl-II, R_3=R_6=OH, R_4=R_5=H
5: R_1=R_4=H, R_2=prenyl-I, R_3=R_6=OH, R_5=prenyl-II
10*: R_1=R_4=R_5=R_6=H, R_2=prenyl-I, R_3=OMe
11*: R_1=R_2=R_5=R_6=H, R_3=OMe, R_4=prenyl-I
13*: R_1=prenyl-I, R_2=prenyl-II, R_3=OH, R_4=R_5=R_6=H



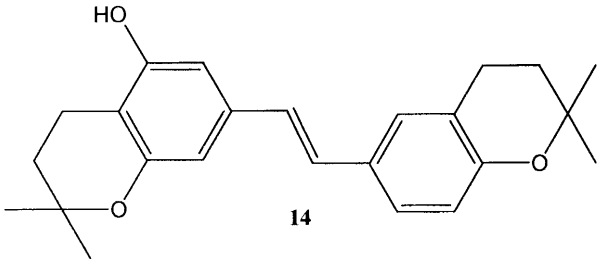
7: R_1=prenyl, R_2=OH
8: R_1=R_2=H



6: R=prenyl
9: R=H



12



14

Fig. 2. Structures of Isolated Compounds * means known compounds.

Flavestin B (**3**) was obtained as a yellowish powder, and showed a pale blue spot on TLC by Gibbs' reagent. The molecular formula was determined as $C_{19}H_{20}O_2$ by pos. HR-EI-MS ($[M]^+$: *m/z* 280.1460). The UV, IR, and ^{1}H-NMR spectra were similar with those of **2**, indicating the presence of a 1,2,3,5-tetrasubstituted benzene ring, a monosubstituted benzene, a conjugated *E*-double bond, two hydroxy groups, and a prenyl group.

The presence of the prenyl group at 2 in **3**, instead of 4 in **2**, was confirmed by HMBC between H-1"/C-3, H-4/C-3, and H-4/C-5, and by NOE between H-7/H-6, H-8/H-6, H-7/H-1", and H-8/H-1". Thus the structure was formulated as **3** in Fig. 2.

Flavestin C (**4**) was obtained as a yellowish powder showing a pale green spot on TLC by Gibbs' reagent, and gave the same HPLC peak with compound **5**. The molecular formula was determined as $C_{24}H_{28}O_3$ by HR-EI-MS ($[M]^+$: *m/z* 364.2023). The UV and IR spectra were similar with those of longistylin B(**13**). The ^{1}H-NMR spectrum of **4** showed the presence of a 1,4-disubstituted benzene ring (AA'BB' doublets of J=8.5 Hz at δ 7.36, 6.82), instead of monosubstituted benzene ring in **13**. The OH group at 4' was confirmed by HMBC between H-3'/C-4', and H-5'/C-4', and by NOEs between H-2', 6'/H-7, and H-2',6'/H-8. Thus the structure was formulated as **4** in Fig.2.

Flavestin D (**5**) was obtained as a yellowish powder showing a blue spot on TLC by Gibbs' reagent, and gave the same HPLC peak with **4**. The molecular formula was determined as $C_{24}H_{28}O_3$ by pos. HR-EI-MS ($[M]^+$: *m/z* 364.2039). The UV and IR spectra were similar with those of **1**, except for hypsochromic shifts in UV spectrum. The ^{1}H-NMR spectrum suggested the presence of a 1,3,4,5-tetrasubstituted benzene ring (2H, a singlet at δ 6.55), a 1,3,4-trisubstituted benzene ring (ABX type signals at δ 7.25, 7.23, 6.78), an *E*-double bond flanked with two benzenes (two doublets at δ 6.93, 6.77), two prenyl groups [δ 5.36, 5.27 (1H each, m), 3.37, 3.41 (2H each, br.d), 1.83, 1.81 (3H each, s), 1.79, 1.77 (3H each, d, J=1.0Hz)]. The partial structures, 3'-prenyl-4'-hydroxyphenyl (R_6=OH, R_5= prenyl), 3,5-dihydroxy-4-prenylphenyl (R_1=R_4=H, R_3=OH, R_2=prenyl) and an *E*-double bond, and their connection were confirmed by HMBC and NOE. Thus the structure was formulated as **5** in Fig. 2.

Flavestin E (**6**) was obtained as a yellowish powder showing a pale green spot on TLC by Gibbs' reagent, and the molecular formula was determined as $C_{24}H_{28}O_2$ by HR-EI-MS ($[M]^+$: *m/z*: 348.2093). The UV and IR spectra were similar with those of longistylin B (**13**). The ^{1}H-NMR spectrum suggested the presence of 1,2,3,4,5-pentasubstituted benzene ring (δ 6.72, 1H, s), a monosubstituted benzene ring, a prenyl group, a hydroxy group (δ 6.10, 1H, s), and a dimethyldihydropyran ring (δ 2.77, 2H, t, J=6.9 Hz; δ 1.82, 2H, t, J=6.9 Hz; δ 1.30, 6H, s) as summarized in Table 2. These partial structures were connected by HMBC and NOESY spectra [NOEs: H-6 / H-8 and H-1"'(prenyl) / H-7]. Thus the structure was formulated as **6** in Fig. 2.

Flavestin F (**7**) was obtained as a yellowish powder, showing a greenish blue spot on TLC by Gibbs' reagent, and the molecular formula was determined as $C_{24}H_{28}O_3$ by HR-EI-MS ($[M]^+$: *m/z* 364.2040). The ^{1}H-NMR spectrum was similar to that of **6**, except for the presence of *p*-hydroxyphenyl, instead of phenyl in **6**. The partial structures, a dimethyl- dihydropyran ring, a pentasubstituted benzene ring, a *p*-hydroxyphenyl group, a prenyl group, and an *E*-double bond, and their connection were confirmed by NOE. Thus **7** was formulated as shown in Fig. 2.

Flavestin G (**8**) was obtained as a yellowish powder, and Gibbs' reaction was negative on TLC. The molecular formula was determined as $C_{19}H_{20}O_2$ by HR-EI-MS ($[M]^+$: *m/z*: 280.1458). The UV and IR spectra were similar with those of **9**. The ^{1}H-NMR spectrum of **8** suggested the presence of the partial structures, a dimethyldihydropyran ring, a 1,2,3,5-tetraasubstituted benzene, a monosubstituted benzene ring, a prenyl group, and an *E*-double bond, and their connection were confirmed by HMBC and NOE. Thus the structure was formulated as **8** in Fig. 2.

Flavestin H (**9**) was obtained as a yellowish powder showing a blue spot on TLC with Gibbs' reagent, and the molecular formula was determined as $C_{19}H_{20}O_2$ by HR-EI-MS ($[M]^+$: *m/z*: 280.1456). The UV and IR spectra were similar to those of **8**. The ^{1}H-NMR spectrum of **9** was similar to that of **8**, except for the presence of 1,3,4,5-tetrasubstituted benzene ring (H-6: δ 6.61, d, J=1.5 Hz; H-2: δ 6.51, d, J=1.5 Hz), instead of 1, 3, 5, 6-tetrasubstituted benzene in **8**. The partial structures were connected by HMBC and NOESY spectra [NOEs: H-2 / H-7, H-2 / H-8, H-6 / H-7, H-6 / H8]. Thus the structure was formulated as **9** in Fig. 2.

Table 1. ^{1}H-NMR Spectral Data of Flavestins A (**1**) - K (**14**)

	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**14**
1	-	-	-	-	-	-	-	-	-	-
2	6.57 br.t (2.1)	-	-	6.55 s	-	6.69 s	6.69 d (2.5)	6.51 d (1.5)	6.65 d (2.5)	6.47 d (1.5)
3	[4.80 s (OH)]	[4.75 s (OH)]	[5.42 s (OH)]	[5.07 s (OH)]	[6.10 s (OH)]	[4.79 s (OH)]	[4.61 br.s (OH)]	[4.69 s (OH)]	[4.67 br.s (OH)]	[4.72 s (OH)]
4	6.33 t (2.1)	6.32 d (1.8)	-	-	-	-	6.26 d (2.5)	-	6.22 d (2.5)	-
5	-	[5.19* (OH)]	[5.01* s (OH)]	[5.07 s (OH)]	-	-	-	-	-	-
6	6.64 br.t (2.1)	6.68 d (1.8)	6.66 s	6.55 s	6.72 s	-	-	6.61 d (1.5)	-	6.57 d (1.5)
7	6.87 d (16.2)	7.29 d (16.0)	7.13 d (16.0)	6.77 d (16.1)	7.30 d (16.0)	7.14 d (16.0)	7.24 d (16.0)	6.94 d (16.0)	7.06 d (16.0)	6.93 d (16.2)
8	7.01 d (16.2)	6.29 d (16.0)	6.82* d (16.0)	6.93 d (16.1)	6.89 d (16.0)	6.86 d (16.0)	6.97 d (16.0)	7.02 d (16.0)	6.88 d (16.0)	6.77 d (16.2)
1'	-	-	-	-	-	-	-	-	-	-
2'	7.27 d (2.0)	7.48 m	7.36 d (8.5)	7.23 d (2.0)	7.47 m	7.35 d (8.5)	7.50 m	7.47 m	7.19 d (2.3)	7.17 d (2.0)
3'	-	7.36 t (7.5)	6.82* d (8.5)	-	7.34 t (7.5)	6.81 d (8.5)	7.36 t (8.5)	7.34 t (7.5)	-	-
4'	[5.20 s (OH)]	7.27 m	[5.01* s (OH)]	[5.16 s (OH)]	7.24 m	[5.21 s (OH)]	7.27 m	7.24 m	-	-
5'	6.82 d (8.5)	7.36 t (7.5)	6.82* d (8.5)	6.78 d (8.5)	7.34 t (7.5)	6.81 d (8.5)	7.36 t (8.5)	7.34 t (7.5)	6.77 d (8.5)	6.75 d (8.3)
6'	7.29 dd (8.5, 2.0)	7.48 m	7.36 d (8.5)	7.25 dd (8.5, 2.0)	7.47 m	7.35 d (8.5)	7.50 m	7.47 m	7.26 dd (8.5, 2.3)	7.23 dd (8.3, 2.0)
O-Me	3.84 s (3H)									
1"	3.40 br.d (7.1)	3.43 br.d (6.8)	3.42* br.d (6.8)	3.37 br.d (6.8)	2.77 t (6.9)	2.65 t (6.7)	2.77 t (6.8)	2.67 t (6.8)	2.76 t (6.9)	2.66 t (6.9)
2"	5.36 m	5.19 m*	5.18 m	5.36 m	1.82 t (6.9)	1.81 t (6.7)	1.83 t (6.8)	1.83 t (6.8)	1.82 t (6.9)	1.81 t (6.9)
3"	-	-	-	-	-	-	-	-	-	-
4"	1.82 d (1.0) (3H)	1.74 d (1.0) (3H)	1.74 d (1.0) (3H)	1.79 d (1.0) (3H)	1.30 s (3H)	1.34 s (3H)	1.33 s (3H)	1.35 s (3H)	1.32 s (3H)	1.34 s (3H)
5"	1.83 s (3H)	1.83 s (3H)	1.83* s (3H)	1.83 s (3H)	1.30 s (3H)	1.34 s (3H)	1.33 s (3H)	1.35 s (3H)	1.32 s (3H)	1.34 s (3H)
1'"			3.42* br.d (6.8)	3.41 br.d (6.8)	3.45 br.d (6.8)	3.43 br.d (6.8)			2.80 t (6.9)	2.79 t (6.9)
2'"			5.26 m	5.27 m	5.19 m	5.22 m			1.83 t (6.9)	1.82 t (6.9)
3'"			-	-	-	-			-	-
4'"			1.76 d (1.0) (3H)	1.77 d (1.0) (3H)	1.74 d (1.0) (3H)	1.75 d (1.0) (3H)			1.35 s (3H)	1.34 s (3H)
5'"			1.83* s (3H)	1.81 s (3H)	1.84 s (3H)	1.85 s (3H)			1.35 s (3H)	1.34 s (3H)

The spectra were taken with $CDCl_3$ in 500 MHz. Coupling constants (J) in Hz given in parentheses. (ppm)

*: overlapped

Table 2. ^{13}C-NMR Spectral Data of Flavestins A (**1**) - K (**14**)

	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**14**
1	140.1	138.6	135.7	137.3	135.2	134.2	137.9	136.7	138.3	137.3
2	105.6	117.9	117.9	106.2	118.1	106.6	104.4	104.3	104.1	104.0
3	156.8	155.5	153.6	155.1	153.4	155.1	154.6	154.0	154.5	153.1
4	100.5	102.8	113.3	112.7	116.6	115.6	103.7	108.1	103.1	107.6
5	161.1	154.5	152.9	155.1	152.8	152.9	155.1	155.2	155.0	155.1
6	104.7	105.4	105.2	106.2	105.5	124.5	111.8	108.1	111.5	107.7
7	129.2	126.4	124.4	125.8	126.7	122.6	125.6	128.3	122.9	125.6
8	126.0	131.2	129.9	128.4	130.2	127.9	130.7	128.4	130.5	128.3
1'	129.9	137.4	130.6	130.1	137.7	129.9	137.5	137.3	129.2	129.1
2'	128.4	126.6	127.9	128.3	126.5	127.9	126.6	126.5	127.9	127.7
3'	127.0	128.7	115.6	127.0	128.6	115.5	128.7	128.6	121.0	121.0
4'	154.3	127.8	155.2	154.2	127.5	152.5	127.7	127.5	154.1	153.9
5'	116.1	128.7	115.6	116.1	128.6	115.5	128.7	128.6	117.6	117.5
6'	125.9	126.6	127.9	125.7	126.5	127.9	126.6	126.5	125.6	125.7
O-Me	55.4									
1"	29.9	25.1	25.4	22.5	17.7	17.3	19.9	17.0	19.9	17.0
2"	121.6	122.4	122.7	121.4	41.7	32.3	32.8	32.1	32.8	32.2
3"	135.1	133.9	133.7	135.5	72.1	73.8	73.8	74.1	73.7	74.0
4"	25.8	25.8	25.8	25.8	29.7	27.6	26.6	26.7	26.6	26.6
5"	17.9	18.0	18.0	17.9	29.7	27.6	26.6	26.7	26.6	26.6
1"'			22.8	30.0	25.5	25.4			22.5	22.5
2"'			121.7	121.6	122.8	122.6			32.9	32.8
3"'			135.2	135.1	133.6	130.8			74.6	74.5
4"'			25.8	25.8	25.8	25.8			26.9	26.9
5"'			17.9	17.9	18.0	18.1			26.9	26.9

The spectra were taken with $CDCl_3$ in 125 MHz.

Flavestin I (**12**) was obtained as yellowish powder, and Gibbs' reaction was negative on TLC. The molecular formula was determined as $C_{24}H_{28}O_3$ by HR-EI-MS ([M]$^+$: *m/z* 364.2054). Flavestin J (**14**) was obtained as a yellowish powder showing a pale blue spot on TLC with Gibbs' reagent, and the molecular formula was determined as $C_{24}H_{28}O_3$ by HR-EI-MS ([M]$^+$: *m/z* 364.2021). The ^{1}H-NMR, UV and IR spectra of **12** and **14** were strikingly similar each other. The ^{1}H-NMR spectrum of **14** indicated the presence of 1,3,4,5-tetrasubstituted benzene ring, instead of 1,3,5,6-tetrasubstituted benzene ring in **12**. The fact was supported from NOESY spectrum : NOEs observed H-2 / H-7, H-2 / H-8, H-1" / H-7, H-1" / H-8, and H-1"' / H-2' in **12**, while H-2 / H-7, H-2 / H8, H-6 / H-7, H-6 / H8, and H-1"' / H-2' in **14**. Thus **12** and **14** were formulated as shown in Fig. 2.

Fourteen index compounds (10 new compounds and 4 known compounds) were stilbenes, which were supposed to be biosynthesized by malonyl CoA and cinnamyl CoA (3:1) catalyzed with stilbene synthase to produce a precursor, and followed by prenylation at 2, 4 and the structure /or 6, 11 of the precursor, followed by methylation of phenolic OH or cyclization through addition of phenolic OH(s) to double bond(s) of prenyl group(s).[12)]

These compounds were identified as index ones by LC/MS analysis.

EXPERIMENTAL

General The instruments used in the work were a Shimadzu spectrophotometer UV 1200 (for UV spectra); a Perkin-Elmer 1720X-FTIR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); a Hitachi M-8000 spectrometer (for LC/MS); a Varian Mercury 300, unity Inova-500 (for NMR spectra, measured in $CDCl_3$, on the δ scale using tetramethylsilane as an internal standard).

Column chromatography was carried out on silica gel (Wakogel C-200 Wako Pure Chemical Industries Ltd.). HPLC was conducted on a JASCO- PU 980 equipped with a UV-970 as a detector. Silica gel $60F_{254}$ (Merck) precoated TLC plates were used,

developed with *n*-hexane-EtOAc solvent system, and detection was carried out by irradiation of UV lamp, followed by spray of Gibbs' reagent .

Isolation of compounds 1—14 Dried barks of the underground parts (40.7 g) from *G. flavescens*, which were collected in Turkey, were refluxed with EtOAc (400 ml) for 3 hrs. three times. The solution was concentrated under reduced pressure to give the extract (11.5 g). The extract was chromatographed on a silica gel (60 g) column, and eluted with *n*-hexane : EtOAc (1:0→0:1) to give 5 fractions. Each fraction was subjected to HPLC separation. From frs. 1-2, compounds **2** (35.6 mg), **8** (6.5 mg), and **13** (5.7 mg) were obtained. From fr. 4, **1** (10.9 mg), **3** (8.7 mg), **4** (7.0 mg), **5** (6.3 mg), **7** (2.4 mg), **9** (3.5 mg), **10** (10.7 mg), **11** (11.0 mg), **12** (6.3 mg), and **14** (5.5 mg). From fr. 5, **6** (5.5 mg) was obtained.

Compound **1** was obtained as a yellowish powder, showing positive Gibbs' reaction (blue), and named flavestin A. $C_{20}H_{22}O_3$, HR-EI-MS *m/z*: $[M]^+$, 310.1550, error –1.7 mmu. UV λ_{max}(MeOH)nm (log ε): 322.5(4.38), 219.5(4.32). IR ν ($CHCl_3$)cm^{-1}: 3375(OH), 2916(CH), 1591(conjugated double bond), 805, 684(Ar.). HMBC: H-2 / C-7, H-6 / C-7, 3-OH / C-3, OMe / C-5, H-7 / C-2, 6, H-8 / C-2', 6', H-2' / C-8, 1", H-6' / C-8, H-1" / C-2', 4', H-2" / C-4", 5". NOE: OMe / H-4, 6, H-1" / H-2', H-2 / H-7, 8, H-6 / H-7, 8, H-2' / H-7, 8, H-6' / H-7, 8.

Compound **3** was obtained as a yellowish powder showing positive Gibbs' reaction (pale blue), and named as flavestin B. $C_{19}H_{20}O_2$, HR-EI-MS *m/z*: $[M]^+$, 280.1460, error –0.2 mmu. UV λ_{max}(MeOH)nm (log ε): 301.5(4.57), 213.5(4.71). IR ν ($CHCl_3$)cm^{-1}: 3351(OH), 2921(CH), 1599 (conjugated double bond), 834, 774, 754(Ar.). HMBC: H-1" / C-1, 3, H-4 / C-3, 5, H-6 / C-5, 7, H-8 / C-2', 6', H-2', 6' / C-8. NOE: H-7 / H-6, H-8 / H-6, H-7 / H-1", H-8 / H-1", H-2', 6' / H-7, H-2', 6' /H-8.

Compound **4** was obtained as yellowish powder, showing positive Gibbs' reaction (pale green), and named flavestin C. $C_{24}H_{28}O_3$, HR-EI-MS *m/z*: $[M]^+$, 364.2023, error –1.3 mmu. UV λ_{max}(MeOH)nm (log ε): 307.0(3.97), 209.0(4.24). IR ν ($CHCl_3$)cm^{-1}: 3370(OH), 2928(CH), 1603(Ar.), 1577(conjugated double bond), 835, 774(Ar.). HMBC: H-1" / C-1, 3, H-1"' / C-3, 5, 3-OH / C-3, H-3', 5' / C-4', H-2', 6' / C-8. NOE: 3-OH / H-1", 1"', H-6 / H-7, 8, H-1" / H-7, 8, H-2', 6' / H-7, H-2', 6' / H-8.

Compound **5** was obtained as a yellowish powder, showing positive Gibbs' reaction (blue), and named flavestin D. $C_{24}H_{28}O_3$, HR-EI-MS *m/z*: $[M]^+$, 364.2039, error 0.3 mmu. UV λ_{max}(MeOH)nm (log ε): 317.5(4.37), 207.0(4.52). IR ν ($CHCl_3$)cm^{-1}: 3382(OH), 2971(CH), 1603(Ar.), 829, 750(Ar.). HMBC: H-2, 6 / C-3, 5, H-1" / C-3, 5, H-2' / C-8, H-6' / C-8, H-2' / C-1"', H-5' / C-4', H-1"' / C-2', 4'. NOE: H-2, 6 / 3, 5-OH, H-1" / 3, 5-OH, H-2, 6 / H-7, 8, H-2' 6' / H-7, 8, H-2' / H-1"'

Compound **6** was obtained as a yellowish powder showing positive Gibbs' reaction (pale green), and named flavestin E. $C_{24}H_{28}O_2$, HR-EI-MS *m/z*: $[M]^+$, 348.2093, error 0.6 mmu. UV λ_{max}(MeOH)nm (log ε): 306.0(4.64), 208.0(4.83). IR ν ($CHCl_3$)cm^{-1}: 3365(OH), 2971(CH), 1610(Ar.), 829, 750(Ar.). HMBC: H-6 / C-4, 5, 7, H-1" / C-3, 5, 3", H-2" / C-4, 4", 5", H-1"' / C-3, 6. NOE: H-6 / H-7, 8, H-1"' / H-7, 8, H-2', 6' / H-7, 8.

Compound **7** was obtained as a yellowish powder, showing positive Gibbs' reaction (greenish blue), and named flavestin F. $C_{24}H_{28}O_3$, HR-EI-MS *m/z*: $[M]^+$, 364.2040, error 0.4 mmu. UV λ_{max}(MeOH)nm (log ε): 307.0(4.61), 213.0(4.80). IR ν ($CHCl_3$)cm^{-1}: 3396(OH), 2922(CH), 1607(Ar.), 1514(conjugated double bond), 965, 838, 773(Ar.). NOE: H-2 / H-7, 8, H-1" / H-7, 8, H-2" / 3-OH, H-2', 6' / H-7, 8.

Compound **8** was obtained as a yellowish powder, showing negative Gibbs' reaction, and named flavestin G. $C_{19}H_{20}O_2$, HR-EI-MS *m/z*: $[M]^+$, 280.1458, error -0.4 mmu. UV λ_{max}(MeOH)nm (log ε): 306.5(4.23), 209.5(4.41). IR ν ($CHCl_3$)cm^{-1}: 3370(OH), 2974(CH), 1588(Ar.), 837(Ar.). HMBC: H-2 / C-7, 3-OH / C-3, H-1" / C-1, 5, H-7 / C-2, 6, H-8 / C-2', 6'. NOE: H-2 / 3-OH, H-2 / H-7, 8, H-4 / 3-OH, H-1" / H-7, 8, H-2', 6' / H-7, 8.

Compound **9** was obtained as a yellowish powder showing positive Gibbs' reaction (blue), and named flavestin H. $C_{19}H_{20}O_2$, HR-EI-MS *m/z*: $[M]^+$, 280.1456, error -0.6 mmu. UV λ_{max}(MeOH)nm (log ε): 306.5(4.42), 206.0(4.45). IR ν ($CHCl_3$)cm^{-1}: 3378(OH), 2974(CH), 1618(Ar.), 822(Ar.). HMBC: H-2 / C-7, H-6 / C-7, H-7 / C-2, 6, 3-OH / C-3, H-1" / C-3, 5, H-2" / C-4, H-8 / C-2', 6'. NOE: H-2 / H-7, 8, H-6 / H-7, 8, H-1" / 3-OH.

Compound **12** was obtained as a yellowish powder, showing negative Gibbs' reaction, and named flavestin I. $C_{24}H_{28}O_3$, HR-EI-MS *m/z*: $[M]^+$, 364.2054,

error 1.8 mmu. UV λ_{max}(MeOH)nm (log ε): 302.0(4.30), 210.0(4.52). IR ν ($CHCl_3$)cm^{-1}: 3387(OH), 2975(CH), 1587(Ar.), 838, 756(Ar.). HMBC: H-2 / C-7, H-7 / C-2, H-1" / C-5, H-2" / C-6, H-2' / C-8, H-6' / C-8, H-1"' / C-2', 5', H-2"' / C-3'. NOE: H-2 / H-7, 8, H-1" / H-7, 8, H-1"' / H-2', H-2' / H-7, 8, H-6' / H-7, 8.

Compound **14** was obtained as a yellowish powder showing positive Gibbs' reaction (pale blue), and named flavestin J. $C_{24}H_{28}O_3$, HR-EI-MS *m/z*: $[M]^+$, 364.2021, error -1.5 mmu. UV λ_{max}(MeOH)nm (log ε): 306.0(3.99), 206.0(4.27). IR ν ($CHCl_3$)cm^{-1}: 3389(OH), 2975(CH), 1581(Ar.), 838, 756(Ar.). HMBC: H-2 / C-7, H-6 / C-5, H-6 / C-7, H-1" / C-3, H-2" / C-4, H-2' / C-8, H-6' / C-8, H-1"' / C-2' 4', H-2"' / C-3'. NOE: H-2 / H-7, 8, H-6 / H-7, 8, 3-OH / H-2, 1", H-2' / H-7, 8, H-6' / H-7, 8, H-1"' / H-2'.

LC/MS Analysis of 1－14 Dried bark powder (1.0 g) of the underground parts of *G. flavescens* was refluxed with EtOAc (20 ml) twice for 1 hr. After concentrating the solution, the residue was dissolved in MeOH to prepare a 10 ml solution. The passed solution (10 μ l) of Sep-pak C18 cartridge was injected to LC/MS. [column: Crestpak C18S(4.6 i.d.× 150 mm), solvent: CH_3CN – H_2O (30 : 70→80 : 20), gradient time: 70 min, flow rate: 0.8 ml/min, column temperature: 40℃, detection: 254 nm, MS: positive atmospheric pressure chemical ionization (APCI), drift voltage of 50 V, thermal nebulizing temperature: 350℃] Compound **1**: *m/z* 310 at 27.23 min; **2**: *m/z* 280 at 28.03 min; **3**: *m/z* 280 at 29.12 min; **4**: *m/z* 364 at 35.25 min; **5**: *m/z* 364 at 35.00 min; **6**: *m/z* 348 at 35.62 min; **7**: *m/z* 364 at 36.22 min; **8**: *m/z* 280 at 36.68 min; **9**: *m/z* 280 at 37.89 min; **10**: *m/z* 294 at 41.10 min; **11**: *m/z* 294 at 41.35 min; **12**: *m/z* 364 at 49.20 min; **13**: *m/z* 348 at 50.70 min; **14**: *m/z* 364 at 50.70 min.

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