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Iridoid and Phenylpropanoid Glycosides from *Phlomis grandiflora* var. *fimbrilligera* and *Phlomis fruticosa*

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From the aerial parts of *Phlomis grandiflora* var. *fimbrilligera*, two iridoid glucosides, 8-*epi*-loganin (**1**) and auroside (**2**), together with five phenylethanoid glycosides, forsythoside B (**3**), alyssonoside (**4**), verbascoside (**5**), hattushoside (**6**) and phlomisethanoside (**7**), were isolated. Iridoid glucosides auroside (**2**) and lamiide (**8**) and phenylethanoid glycosides, forsythoside B (**3**), alyssonoside (**4**), and verbascoside (**5**) as well as a lignan glucoside, syringaresinol-4′-*O*-β-D-glucoside (**9**), were characterized from the overground parts of *P. fruticosa*. The structures of the isolates were elucidated by means of one and two dimensional (COSY, HSQC and HMBC) NMR techniques and ESI MS.

Key Words: *Phlomis grandiflora, Phlomis fruticosa,* Lamiaceae, iridoid glucosides, phenylethanoid glycosides, lignan glucoside.

Introduction

The genus *Phlomis* L. is represented by thirty-four species in the flora of Turkey[1]. Among them, *P. grandiflora* is characterized by two varieties: *P. grandiflora* var. *grandiflora* and *P. grandiflora* var. *fimbrilligera*. *P. grandiflora* var. *fimbrilligera* is an endemic variety whereas *P. fruticosa* is a Mediterranean element[1]. To date, different classes of glycosides comprising the phenylpropanoids and phenylethanoids[2−13], iridoids[3−15], monoterpenoids[4,11] and diterpenoids[16] as well as a caffeic acid ester[10,12] have been isolated from Turkish *Phlomis* species. As a part of our continuing chemosystematic studies on the glycosidic constituents of the genus *Phlomis*, we investigated the aerial parts of *Phlomis grandiflora* var. *fimbrilligera* and *P. fruticosa*. In a previous report, the isolation of two iridoid glucosides, phlomoside A and 8-*epi*-loganin, together with phenylethanoid glycosides, verbascoside, hattushoside and phlomisethanoside, along with benzyl alcohol β-D-glucoside were described from *P. grandiflora* var. *grandiflora*[9]. Investigations on the European sister of *P. fruticosa* led to the identification of iridoid glucosides, phlomiol and lamiide from the leaves[17] and lamiidoside from the seeds[18]. However, no work has yet been reported on the phenylpropanoid constituents

of *P. fruticosa*. This paper deals with the study of the iridoid and the phenylpropanoid glycosides in *P. grandiflora* var. *fimbrilligera* and *P. fruticosa.*

Experimental

General Experimental Procedures: Optical rotations were measured on an Atropol IV polarimeter. UV (MeOH) spectra were recorded on a Shimadzu UV-160A spectrophotometer. IR (KBr) spectra were determined on a Perkin-Elmer FTIR 1720X spectrometer. NMR spectra were recorded on Bruker AMX 300 and Varian Unity 500 spectrometers. ESIMS were recorded on a Finnigan TSQ 7000 mass spectrometer. Polyamide (ICN), silica gel 60H (230-400 mesh), silica gel 60 (0.063-0.200 mm) (Merck) and Sephadex LH-20 were used for CC. Pre-coated silica gel 60 F_{254} aluminum sheets (Merck) were used for TLC. Plates were examined by UV fluorescence and sprayed with 1% vanillin in conc. H_2SO_4, followed by heating at 105°C for 1-2 min.

Plant Materials. *Phlomis grandiflora* H. S. Thompson var. *fimbrilligera* (Hub.-Mor.) Hub.-Mor. was collected from Antalya, Beldibi (S.W. Anatolia, Turkey), 10 km from Beldibi, in April 1997. *P. fruticosa* L. was collected from İzmir, Çeşme (W. Anatolia, Turkey), in the vicinity of Çiftlik village, in April 2000. Voucher specimens have been deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 97-045 and 00-028, resp.).

Extraction and Isolation. *P. grandiflora* var. *fimbrilligera* - Air-dried aerial parts of the plant (420 g) were extracted twice with MeOH-H_2O (4:1, each 4.5 l) at 60°C. After evaporation of the combined extract, H_2O (500 ml) was added and the insoluble material was removed by filtration. The filtrate was then extracted with a $CHCl_3$ and the water phase was concentrated to dryness and lyophilized (56 g, yield 13.3%). An aliquot of the aqueous extract (11.6 g) was fractionated over polyamide (200 g) employing H_2O (500 ml) and gradient MeOH-H_2O mixtures (25-100% , each 300 ml) to yield 7 main fractions (A-G). Fr. A was first extracted with *n*-BuOH (3x150 ml) to obtain a crude *n*-BuOH fraction (1610 mg). An aliquot of this fraction (880 mg) was subjected to silica gel CC (80 g), eluting with a CH_2Cl_2-MeOH-H_2O mixture (80:20:1, 80:20:2 and 70:30:3, each 500 ml) to yield **1** (110 mg) and **2** (357 mg). Fraction C (214 mg) was chromatographed over silica gel (35 g) using a CH_2Cl_2-MeOH-H_2O mixture (80:20:1, 500 ml) as eluent to afford **3** (28 mg), **4** (12 mg), **6** (46 mg) and **7** (20 mg). Compounds **4**, **6** and **7** were purified on Sephadex with MeOH. Fraction E (140 mg) was subjected to silica gel (20 g) CC employing a CH_2Cl_2-MeOH-H_2O mixture (80:20:1, 500 ml) to yield **5** (75 mg).

P. fruticosa - Dried and milled overground parts of *P. fruticosa* (512 g) were extracted twice with MeOH (each 2.5 l) at 60°C to give a crude MeOH extract (66.7 g, yield 13.2%). The MeOH extract was concentrated to dryness and the residue was taken up in H_2O. The water-soluble portion was partitioned between $CHCl_3$ (3x150 ml) and *n*-BuOH (4x150 ml), sequentially. The *n*-BuOH extract (34.7 g, yield 6.8%) was fractionated by polyamide CC (200 g) with H_2O (500 ml) and MeOH-H_2O mixtures (25-100% , 300 ml each). This yielded 8 main fractions (A-H). Fr. A (5.53 g) was subjected to silica gel CC (200 g) and eluted with a CH_2Cl_2-MeOH-H_2O mixture (90:10:1, 80:20:2, 70:30:3 and 60:40:4, each 500 ml) to yield frs. A_1-A_6. Fraction A_4 (305 mg), which was then subjected to silica gel CC (30 g) with CH_2Cl_2-MeOH-H_2O (80:20:2, 250 ml) as eluent, gave **2** (14.8 mg) and **8** (26 mg). Fraction D (814.5 mg) was chromatographed over silica gel (80 g) using a CH_2Cl_2-MeOH-H_2O mixture (80:20:2 and 70:30:3, each 500 ml) to afford **3**

and **4**, which were then purified by Sephadex CC with MeOH to give pure **3** (11.3 mg) and **4** (7.75 mg). Fraction G (715.7 mg) was subjected to silica gel (65 g) CC, employing CH_2Cl_2-MeOH (80:20, 250 ml) and CH_2Cl_2-MeOH-H_2O (80:20:2, 250 ml) mixtures to yield **5** (230.5 mg).

8-*epi*-loganin (1): $C_{17}H_{26}O_{10}$; UV λ_{max} (MeOH) nm: 236; IR υ_{max} (KBr) cm^{-1}: 3400, 1690, 1635 cm^{-1}; ^1H NMR (CD_3OD, 500 MHz): δ 7.39 (1H, s, H-3), 5.51 (1H, d, J= 4.0 Hz, H-1), 4.65 (1H, d, J= 7.9 Hz, H-1′), 3.90 (1H, dd, J= 11.9/2.0 Hz, H-6′$_b$), 3.82 (1H, m, H-7), 3.69 (3H, s, COOC<u>H</u>$_3$), 3.64 (1H, dd, J= 11.9/6.0 Hz, H-6′$_a$), 3.50 (1H, m, H-5′), 3.46 (1H, t, J = 9.2 Hz, H-3′), 3.42 (1H, t, J = 9.0 Hz, H-4′), 3.20 (1H, dd, J = 7.9/9.5 Hz, H-2′), 3.05 (1H. m, H-5), 2.60 (1H, dt, J= 8.5/8.5/4.0 Hz, H-9), 2.13 (1H, m, H-8), 2.05 (1H, m, H-6$_\beta$), 1.83 (1H, m, H-6$_\alpha$), 1.04 (3H, d, J= 7.4 Hz, H_3-10).

Auroside (2): UV, IR, and ^1H data were identical to those reported in the literature[8,19].

Forsythoside B (3): UV, IR, and ^1H data were identical to those reported in the literature[5,20].

Alyssonoside (4): UV, IR, and ^1H NMR data were identical to those reported in the literature[5,21].

Verbascoside (5): UV, IR, and ^1H data were identical to those reported in the literature[8,22].

Hattushoside (6): Positive ion ESI MS: m/z 635 $[M+Na]^+$; negative ion ESI MS m/z 611 $[M-H]^-$ (calc. for $C_{28}H_{36}O_{15}$); UV, IR, ^1H and ^{13}C NMR (Table) data were identical to those reported in the literature[6].

Phlomisethanoside (7): Pale yellow, amorphous powder. $[\alpha]_D^{25}$ –58.6°. UV λ_{max} (MeOH) nm: 222.5, 265, 286, 327; IR υ_{max} (KBr) cm^{-1}: 3400, 1699, 1620, 1518; positive ion ESI MS m/z 605 $[M+Na]^+$, negative ion ESI MS m/z 581 $[M-H]^-$ (calc. for $C_{27}H_{34}O_{14}$); ^1H and ^{13}C NMR (Table).

Lamiide (8): UV, IR, and ^1H data were identical to those reported in the literature[5,23].

Syringaresinol-4′-*O*-β-D-glucoside (9): UV, IR, ^1H and ^{13}C NMR data were identical to those reported in the literature[24].

Results and Discussion

From the aerial parts of *P. grandiflora* var. *fimbrilligera,* two iridoid glucosides, 8-*epi*-loganin (**1**) and auroside (**2**), and five phenylethanoid glycosides, forsythoside B (**3**), alyssonoside (**4**), verbascoside (**5**), hattushoside (**6**) and fimbrilloside (= phlomisethanoside) (**7**), were isolated by fractionation of the methanolic extract through a polyamide column, followed by silica gel CC. Compounds **2-6** were identified by comparing their spectral and physical data and by direct comparison with the authentic samples on a TLC plate, whereas the structures of **1** and **7** were identified based on the following evidence.

Compound **1** was obtained as an amorphous powder. Its UV and IR absorptions were typical of a conjugated enol-ether functional group. The ^1H NMR spectrum of **1** exhibited characteristic signals for an iridoid structure with a methoxycarbonyl (δ_H 3.69, s), an oxymethine (δ_H 3.81, m) and a secondary methyl group (δ_H 1.04, d, J = 7.4 Hz). Two hemiacetal proton signals at δ_H 5.51 (1H, d, J = 4.0 Hz) and δ_H 4.65 (1H, d, J = 7.9 Hz) were attributed to H-1 and the anomeric proton of a β-D-glucopyranose unit, respectively. The highly deshielded ^1H NMR signal at δ_H 7.39 (1H, s) was assigned to H-3, suggesting a carbomethoxy substitution at C-4. The chemical shifts and the multiplicities of both H-3 and H-9 (δ_H 2.60, dt, J = 8.5/8.5/4.0 Hz) indicated that the C-5 position of the iridoid aglycon is non-substituted. Therefore, the multiplet signal at δ_H 3.04 was assigned to H-5. Additionally, the H-7 proton signal showed a marked

downfield shift (δ_H 3.82, m), supporting the presence of oxygenation at C-7. The signals at δ_H 2.05 (m) and 1.83 (m) were readily attributed to the methylene protons at C-6. Moreover, the multiplet proton signal at δ_H 2.13 was assigned to H-8. The multiplicity of H-9 as well as the chemical shift value of H_3-10 (δ_H 1.04) indicated the presence of a secondary methyl group at C-8. Based on the above results and comparison with the published data, compound **1** was identified as 8-*epi*-loganin[19,25,26]. 8-*epi*-loganin (**1**) was previously reported from *Phlomis brachyodon*[19] and *P. grandiflora* var *grandiflora*[15].

Table ^{13}C and ^{1}H NMR Data of Phlomisethanoside (**7**) and Hattushoside (**6**)* (δ_C 75.5 MHz; δ_H 300 MHz, CD_3OD)

		7		**6**	
C/H atom		δ_C ppm	δ_H ppm J Hz	δ_C ppm	δ_H ppm J Hz
Aglycone					
1	C	130.45		130.4	
2	CH	130.87	6.90 d (8.5)	130.9	6.90 d (8.5)
3	CH	116.07	6.62 d (8.5)	116.1	6.62 d (8.5)
4	C	156.73		156.8	
5	CH	116.07	6.62 d (8.5)	116.1	6.62 d (8.5)
6	CH	130.87	6.90 d (8.5)	130.9	6.90 d (8.5)
α	CH_2	71.78	3.94 m, 3.52 m	71.8	3.93 m, 3.51 m
β	CH_2	36.45	2.71 t (7.6)	36.5	2.70 t (7.6)
Glucose					
1′	CH	103.05	4.29 d (7.6)	103.0	4.28 d (7.6)
2′	CH	77.98	3.40 dd (7.6/9.0)	77.9	3.40 dd (7.6/9.0)
3′	CH	78.97	3.48 t (9.0)	79.0	3.48 t (9.0)
4′	CH	71.78	3.27 t (9.0)	71.8	3.27 t (9.0)
5′	CH	77.85	3.22 m	77.9	3.22 m
6′	CH_2	62.73	3.84†	62.7	3.84†
			3.64 dd (12.0/5.5)		3.64 dd (12.0/5.4)
Apiose					
1″	CH	110.12	5.44 br s	110.1	5.44 d (0.4)
2″	CH	78.46	4.00 br s	78.5	4.00 d (0.4)
3″	C	79.27		79.3	
4″	CH_2	75.41	4.16 d (9.7)	75.5	4.17 d (9.7)
			3.78 d (9.7)		3.80 d (9.7)
5″	CH_2	68.50	4.45 d (11.5)	68.9	4.46 d (11.3)
			4.32 d (11.5)		4.34 d (11.3)
Acyl		Vanilloyl		Syringyl	
1‴	C	122.24		121.2	
2‴	CH	113.89	7.55 d (1.8)	108.6	7.35 s
3‴	C	149.05		149.0	
4‴	C	153.10		142.6	
5‴	CH (C)§	116.07	6.83 d (8.3)	149.0	
6‴	CH	125.47	7.61 dd (8.3/1.8)	108.6	7.35 s
C=O	C	167.93		168.0	
OCH_3	CH_3	56.47	3.85 s	57.0	3.84 s

*Data from ref. 6

†Signal pattern unclear due to overlapping

§C for hattushoside (**6**)

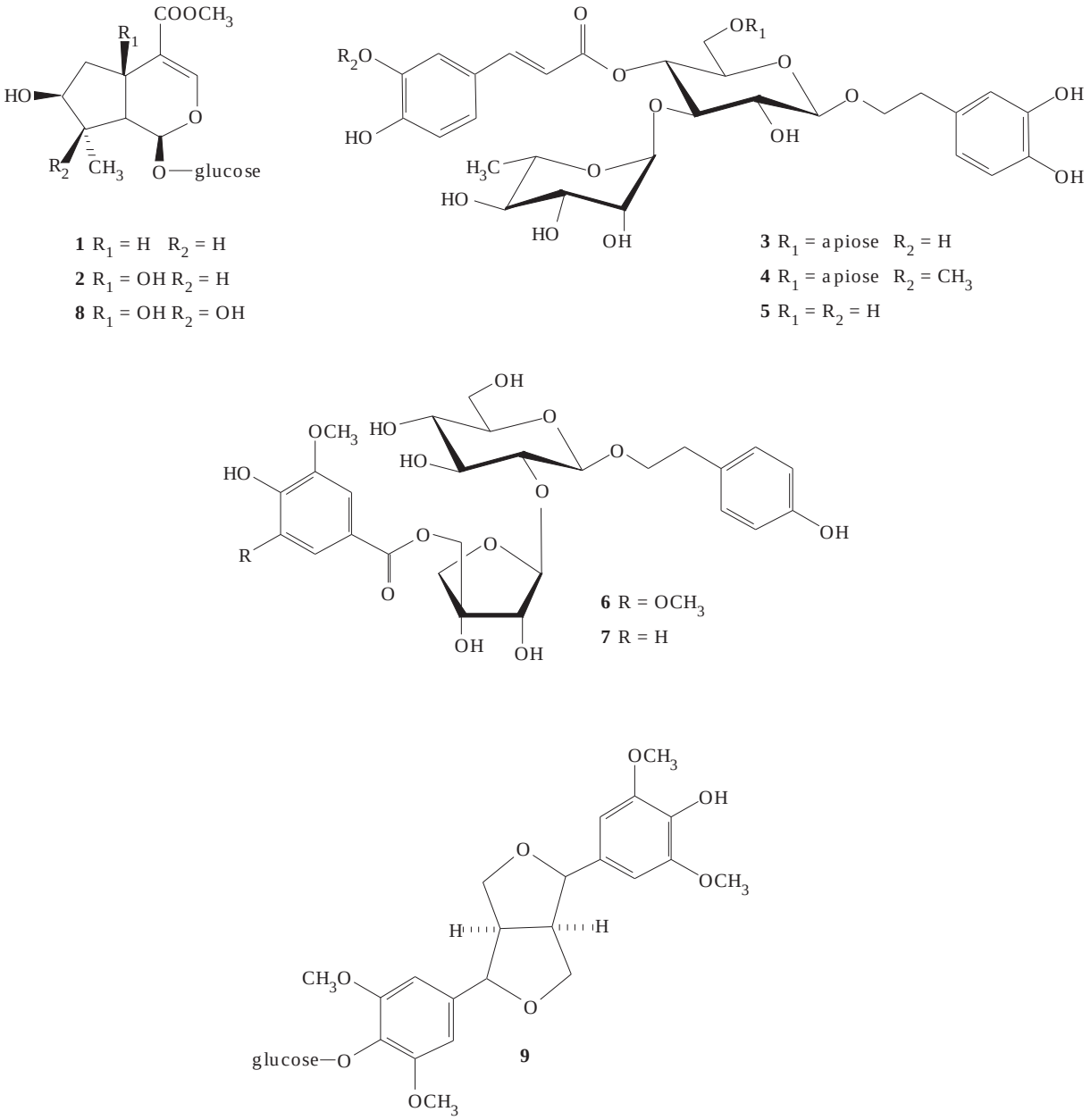

Figure Iridoid and phenylpropanoid glycosides from *P. grandiflora* var. *fimbrilligera*

The UV and IR spectroscopic data of **7** indicated its phenolic nature. The positive and negative ESI mass spectra exhibited quasimolecular ion peaks at m/z 605 $[M+Na]^+$ and 581 $[M-H]^-$, respectively. These data were compatible with the molecular formula $C_{27}H_{34}O_{14}$. The ^1H and ^{13}C NMR data of **7** were almost identical to those of hattushoside (**6**)[6] (Table), except for the resonances due to an acyl moiety. The proton resonances attributed to the acyl group observed as a set of three signals appearing as an ABX system at δ_H 7.61 (dd, J = 1.8/8.3 Hz, H-6‴), 7.55 (d, J = 1.8 Hz, H-2‴) and 6.83 (d, J = 8.3 Hz, H-5‴) were indicative of a trisubstituted phenyl moiety in the structure of **7**. Additionally, a methoxyl resonance was observed at δ_H 3.85 (3H, s, δ_C 56.47). These results together with the carbon resonances (Table) revealed the presence of a vanilloyl (3-hydroxy-4-methoxy-benzoic acid) moiety. Furthermore, in

the ^{1}H spectrum of **7** the resonances observed as A_2B_2 type aromatic groups (δ_H 6.90 and 6.62, each 2H, d, J_{AB} = 8.5 Hz, H-2/H-6 and H-3/H-5, respectively) and two methylene signals, which were coupled to each other (δ_H 3.94 and 3.52, each 1H, m, H_2-α; δ_H 2.71, 2H, t, J = 7.6 Hz, H_2-β), were consistent with the presence of a *p*-hydroxyphenethyl alcohol moiety. Further proof of this assumption was provided by the ^{13}C NMR data. Additionally, two proton signals at δ_H 4.29 (d, J = 7.6 Hz) and δ_H 5.44 (br s) were attributed to the anomeric protons of β-linked glucose and β-linked apiose moieties, respectively. The ^{13}C NMR data also confirmed the diglycosidic structure, exhibiting two anomeric carbon resonances at δ_C 103.05 and 110.12, which were readily assigned to the glucose and apiose units, respectively. All proton resonances for the sugar units were assigned unambiguously from a COSY experiment of **7**. The proton and carbon chemical shifts due to *p*-hydroxyphenethyl alcohol and the sugar moieties were in good agreement to those of hattushoside (**6**), indicating the same interglycosidic linkages and the site of acylation. However, the negative and positive ion ESI mass spectra of **7** indicated that the molecular weight of **7** was 28 mass units smaller than that of **6**, confirming the proposed structure. Consequently, the structure of **7** was established to be (4-hydroxyphenyl)ethyl-(5-*O*-vanilloyl)-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside. The trivial name fimbrilloside was proposed and reported for the glycoside[13]. However, Takeda *et al.* have also isolated a vanillic acid ester phenylethanoid glycoside, named phlomisethanoside, from *P. grandiflora* var. *grandiflora*[9]. We concluded that this compound must be identical to **7**. A comparison of the spectroscopic data proved the identity of the two compounds.

Likewise, the aerial parts of *P. fruticosa* afforded two iridoid glucosides, auroside (**2**) and lamiide (**8**), as well as three phenylethanoid glycosides, forsythoside B (**3**), alyssonoside (**4**), and verbascoside (**5**), together with a lignan glucoside, syringaresinol-4′-*O*-β-D-glucoside (**9**), by means of serial chromatographic separations. The identities of compounds **2**-**5**, **8** and **9** were established by chromatography (TLC) and from the spectroscopic data. Although lamiide (**8**) was previously reported from *P. fruticosa*[17], this is the first report on the isolation of auroside (**2**) from the same plant. However, we failed to detect the presence of phlomiol[17]. No phenylethanoid glycoside had been reported from *P. fruticosa*. Therefore, forsythoside B (**3**), alyssonoside (**4**) and verbascoside (**5**) are the first phenylethanoid glycosides isolated from the title plant. Similarly, syringaresinol-4′-*O*-β-D-glucopyranoside (**9**) was isolated for the first time from the genus *Phlomis*.

Conclusion

In this study, we investigated the glycosidic constituents of two *Phlomis* taxa, *P. grandiflora* var. *fimbrilligera* and *P. fruticosa*, which both belong to the section *Phlomis*, subsection *Dendrophlomis*[1]. The occurrence of the iridoid glucoside, auroside (**2**), and phenylethanoid glycosides, forsythoside B (**3**), alyssonoside (**4**) and verbascoside (**5**) in both specimens, indicates that the iridoid and phenylethanoid compositions of these two investigated plants are somewhat similar. However, in addition to auroside, 8-*epi*-loganin (**1**) was isolated from *P. grandiflora* var. *fimbrilligera*, while lamiide (**8**) was identified from *P. fruticosa*. Phenylethanoid glycosides, hattushoside (**6**) and fimbrilloside (= phlomisethanoside) (**7**) were the additional glycosides in *P. grandiflora* var. *fimbrilligera*.

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