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Phlinoside F, a New Phenylethanoid Glycoside from *Phlomis angustissima*

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From the overground parts of *Phlomis angustissima*, a new phenylethanoid triglycoside, β-(3-hydroxy,4-methoxyphenyl)ethyl-*O*-[β-xylopyranosyl(1→2)-α-rhamnopyranosyl-(1→3)] −*O*-4-*O*-feruloyl-β-glucopyranoside, named phlinoside F, was isolated along with the known compounds alyssonoside, samioside, lamiide, auroside, naringenin, and syringaresinol-4-*O*-β-D-glucopyranoside. The structure of the new glycoside was elucidated by spectroscopic methods.

Key Words: *Phlomis angustissima*, Lamiaceae, phenylethanoid glycosides, phlinoside F, alyssonoside, samioside, lamiide, auroside, naringenin, syringaresinol-4-*O*-β-D-glucopyranoside.

Introduction

The genus *Phlomis* (Lamiaceae) is represented by 34 species in Turkey[1]. *Phlomis angustissima* is reported to be an endemic species. Previous investigations on Turkish *Phlomis* species by our research group led to the isolation and characterization of a number of secondary metabolites such as iridoids, phenylethanoid glycosides, lignans and flavonoids, and monoterpene glucosides[2−12]. As a part of our ongoing studies on the secondary metabolites of *Phlomis* species, we have now investigated the overground parts of *P. angustissima* and isolated a new phenylethanoid triglycoside, phlinoside F (**1**), together with the known compounds alyssonoside (**2**), samioside (**3**), lamiide (**4**), auroside (**5**), naringenin (**6**), and syringaresinol-4-

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O-β-D-glucopyranoside (**7**). The current paper deals with the isolation and structure elucidation of the new phenylethanoid glycoside, phlinoside F (**1**), as well as of the known compounds (**2-7**).

Experimental

General Experimental Procedures: The UV (MeOH) spectra were recorded on a Hitachi HP 8452 A spectrophotometer. The FTIR (KBr) spectra were determined on a Perkin-Elmer 2000 FTIR spectrophotometer. NMR measurements in CD_3OD at room temperature were taken using Bruker AMX 300 spectrometers (^{1}H: 300 MHz, ^{13}C: 75 MHz). FABMS were performed on a Finnigan 311 A spectrometer. Polyamide 6 (Fluka, 50-160 μm), silica gel 60 (0.063-0.200 mm, Merck) and Sephadex LH-20 (Fluka) were used for open column chromatographic separations. MPLC was performed on Labomatic (1.8 x 35.2 cm and 1.3 x 38 cm) and Büchi (2.5 x 46 cm) glass columns packed with LiChroprep RP-18 (Merck), using Lewa M5 (peristaltic) and Büchi B-684 pumps. VLC separation was realized on a small glass column (5.2 x 10 cm) packed with LiChroprep RP-18 (Merck). TLC analyses were carried out on pre-coated silica gel 60 F_{254} aluminum sheets (Merck). Compounds were detected by UV fluorescence and spraying with 1% vanillin/H_2SO_4, followed by heating at 100 °C for 1-2 min.

Plant Material. *P. angustissima* Hub.-Mor. (Lamiaceae) specimens were collected in June 2002 at the Denizli-Altınyayla crossing. Voucher specimens have been deposited at the Herbarium of the Biology Department, Faculty of Science, Hacettepe University, Ankara, Turkey (AAD 1097).

Extraction and Isolation. Dried and powdered aerial parts of *P. angustissima* (435 g) were extracted with MeOH (3 x 2500 mL) at 40 °C and combined MeOH extracts were concentrated under reduced pressure (60 g). The resultant residue was then dissolved in H_2O (200 mL) and the water-soluble portion was partitioned between $CHCl_3$(4 x 200 mL) and *n*-BuOH (4 x 200 mL). An aliquot of the *n*-BuOH extract (15.5 g) was chromatographed over polyamide (100 g) eluting with H_2O, followed by increasing concentrations of MeOH in H_2O (25%, 50%, 75% and 100%, each 250 mL) to yield 6 main fractions: A (0.25 g), B (5.89 g), C (3.68 g), D (3.19 g), E (1.44 g) and F (0.20 g). Fr. A (245 mg) was subjected to silica gel column chromatography with a gradient of $CHCl_3$-MeOH-H_2O (90:10:0→80:20:1) to give compound **4** (20 mg). Fr. B (5.89 g) was fractionated on RP_{18} using the VLC technique with MeOH-H_2O mixtures (0:100→45:55) as eluent to obtain 20 fractions (Frs. B_1-B_{20}). Fraction B_{16-17} (134 mg) was further purified by silica gel column chromatography eluting with a stepwise gradient of $CHCl_3$-MeOH mixture (95:5→90:10) to afford compounds **5** (11 mg) and **7** (9 mg). Fr. C (3.68 g) was chromatographed on LiChroprep RP-18 using the VLC technique eluting with MeOH-H_2O mixtures (0-50%, 100 mL each) to yield 36 fractions (Frs. C_1-C_{36}). Fr. C_{28-30} (1.6 g) was subjected to a silica gel column using CH_2Cl_2-MeOH-H_2O mixture (80:20:1→70:30:3) to afford compounds **2** (13 mg) and **3** (22 mg). Fr. C_{31-36} (332 mg) was chromatographed on a silica gel column using CH_2Cl_2-MeOH-H_2O (80:20:1→80:20:2) mixtures. Subfraction 14-21 obtained from this column (84 mg) was then subjected to silica gel column chromatography employing a CH_2Cl_2-MeOH (95:5→80:20) solvent system and repeated chromatographic separations on silica gel and Sephadex LH-20 yielded compound **1** (11 mg). Fr. F (202 mg) was first fractionated on a silica gel column eluting with $CHCl_3$-MeOH-H_2O (80:20:2→70:30:3) mixture and then Fr. F_{2-3} (44 mg) obtained from this fractionation was purified on a silica gel column using a CH_2Cl_2-MeOH mixture (99:1) to afford compound **6** (5 mg).

Phlinoside F (1): UV λ_{max} (MeOH) nm : 202, 218, 245 (sh), 292, and 335; IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1700 (C=O), 1630 (C=C), 1595 and 1510 (arom. rings); ^{13}C (75 MHz, CD_3OD) and ^{1}H (300 MHz, CD_3OD) NMR data are given in the Table; EIMS: m/z 785 $[M+H]^+$.

Table. ^{13}C (CD_3OD, 75 MHz) and ^{1}H (CD_3OD, 300 MHz) NMR data and HMBC correlations of Phlinoside F (**1**)*.

C/H atom	δ_C (ppm)	δ_H (ppm)	J (Hz)	HMBC (C→H)
Aglycone				
1	131.6			H-2, H_2-α,H_2-β
2	113.8	6.86 d	1.9	H-6, H_2-β
3	148.0			H-5
4	147.6			H-2, H-6, OC$\underline{H}_3$
5	117.1	6.80 d	8.4	
6	121.2	6.72 dd	8.4/1.9	H-2, H_2-β
α	72.1	4.00 m		H-1′, H_2-β
		3.71 m		
β	36.8	2.90 t	7.8	
OCH_3	56.4	3.82 s		
Glucose				
1’	104.2	4.38 d	7.8	H-2′, H_2-α
2’	75.3	3.30†		
3’	82.3	3.80 t	9.1	H-1″, H-4′
4’	70.5	4.92 t	9.4	
5′	76.0	3.54 m		
6′	62.4	3.81 dd	12.2/6.4	
		3.90†		
Rhamnose				
1″	102.0	5.40 d	1.8	H-3′
2″	83.0	3.93 dd	1.8/3.4	
3″	71.9	3.57 dd	9.8/3.4	
4″	74.2	3.28 t	9.8	
5″	71.1	3.56†		H-6″
6″	18.4	1.07 d	6.3	
Xylose				
1‴	107.6	4.55 d	7.8	H-2″
2‴	75.3	3.30†		
3‴	77.9	3.25†		
4‴	70.4	3.60†		
5‴	67.1	3.80† 3.20†		
Ester				
1⁗	127.5			H-α′, H-β′, H-5⁗
2⁗	111.8	7.19 d	1.7	H-β′, H-6⁗
3⁗	149.5			H-2⁗, H-5⁗
4⁗	150.7			H-2⁗, H-5⁗ H-6⁗, OC$\underline{H}_3$
5⁗	116.1	6.82 d	8.2	
6⁗	121.2	6.72 dd	8.2/1.9	H-β′, H-2⁗
α′	115.0	6.37 d	15.9	H-β′
β′	147.9	7.65 d	15.9	H-2⁗, H-6⁗
C=O	168.3			H-α′, H-β′, H-4′
OCH_3	56.4	3.89 s		

*All ^{1}H and ^{13}C assignments are based on 2D NMR (COSY, HMQC, and HMBC) experiments.

†Signal patterns are unclear due to overlapping

Alyssonoside (2): UV, IR, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[2,8].

Samioside (3): UV, IR, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[8,9,12].

Lamiide (4): UV, IR, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[2,8,14].

Auroside (5): UV, IR, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[15].

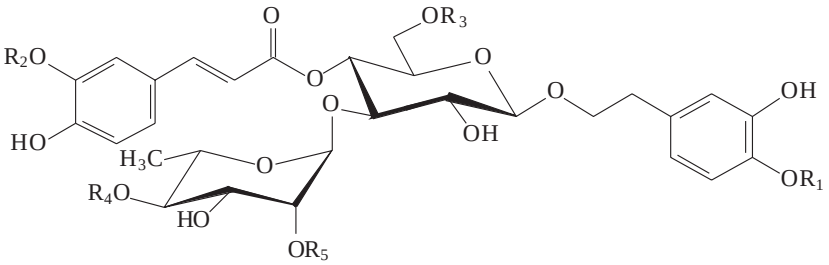
Naringenin (6): ^{1}H and ^{13}C NMR data were identical to those reported in the literature[16].

Syringaresinol-4-*O*-β-D-glucopyranoside (7): UV, IR, ^{1}H and ^{13}C NMR data were identical to those reported in the literature[8].

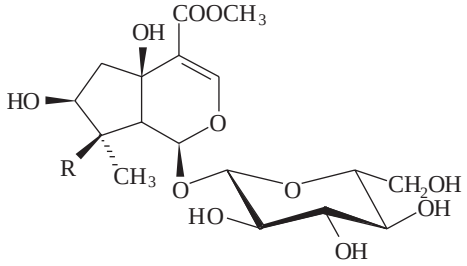
Results and Discussion

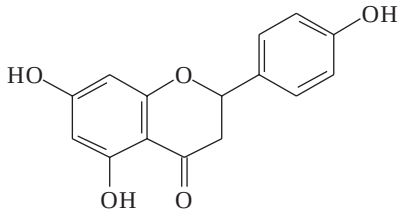
From the aerial parts of *P. angustissima* a new phenylethanoid triglycoside, phlinoside F (**1**) and 2 phenylethanoid glycosides, (**2**,**3**), 2 iridoid glucosides (**4**,**5**), a flavonoid (**6**), and a lignan glucoside (**7**) were isolated by fractionation of the methanolic extract through a polyamide column, followed by VLC, and open column chromatography on silica gel and Sephadex LH-20 (Figure 1). Compounds **2**-**7** were identified by comparing their spectroscopic data with those published in the literature, whereas the structure of compound **1** was identified based on the following evidence.

Phlinoside F (**1**) was isolated as an amorphous powder. The EIMS afforded the positive ion peak at m/z 785 $[M+H]^+$ implying a molecular formula of $C_{36}H_{49}O_{19}$. The UV absorption bands at λ_{max} 202, 218, 245 (sh), 292, and 335 nm indicated the polyphenolic nature of **1**. The IR spectrum showed absorption bands due to phenolic hydroxy groups (3400 cm^{-1}), α, β unsaturated esters (1700 cm^{-1}), olefinic double bonds (1630 cm^{-1}), and aromatic rings (1595 and 1510 cm^{-1}) in the molecule. The ^{1}H-NMR spectrum of compound **1** (Table) exhibited characteristic signals arising from (*E*)-ferulic acid and 3-hydroxy 4-methoxy phenylethanol moieties: 6 aromatic proton signals (2 x ABX systems, in the region of δ_H 7.19-6.72), 2 *trans*-olefinic proton signals (AB system, δ_H 7.65, d, J_{AB}= 15.9 Hz and δ_H 6.37, d, J_{AB}= 15.9 Hz), and a β-methylene proton signals (δ_H 2.90, 2H, t, J= 7.8 Hz) together with 2 non-equivalent proton signals (δ_H 4.00, 1H, m and 3.71, 1H, m) attributed to the side-chain of the phenethyl alcohol moiety. Additionally, 3 anomeric proton resonances at δ_H 5.40 (1H, d, J= 1.8 Hz, H-1$''$ of α-rhamnose), 4.38 (1H, d, J= 7.8 Hz, H-1$'$ of β-glucose), and 4.55 (1H, d, J= 7.8 Hz, H-1$'''$ of β-xylose) indicated the trisaccharidic nature of **1**. The ^{13}C NMR data (Table) also supported the triglycosidic structure of **1**, exhibiting 3 anomeric carbon resonances at δ_C 107.6 (C-1$'''$), 104.2 (C-1$'$), and 102.0 (C-1$''$), which showed correlations with the anomeric protons of the related sugar units. The complete assignments of all proton and carbon resonances were based on the DEPT, DQF-COSY, HSQC, and HMBC experiments. The ^{1}H NMR spectrum suggested that the feruloyl moiety occupied the C-4$'$ position of core glucose due to the downfield shift of the H-4$'$ proton resonance of the glucose unit (δ_H 4.92). This was also confirmed by a heteronuclear long-range coupling observed from the carbonyl carbon resonance (δ_C 168.3) of the acyl moiety to H-4$'$. On the other hand, an HMBC cross peak observed from the α-C carbon atom (δ_C 72.1) of the phenethyl moiety to the anomeric proton of glucose (δ_H 4.38, H-1$'$) indicated the attachment of the glucose unit at the C-α carbon atom of the aglycone. The highly deshielded carbon resonances arising from glucose and rhamnose units, which suggested that the glucose unit should be glycosylated at C-3$'$ (δ_C 82.3), and the rhamnose unit at C-2$''$ (δ_C 83.0). However, a prominent HMBC experiment allowed us to assign all the interglycosidic connectivities of the sugar sequence unambiguously. Thus, correlations were observed between C-3$'$ (δ_C 82.3) of glucose and the anomeric proton



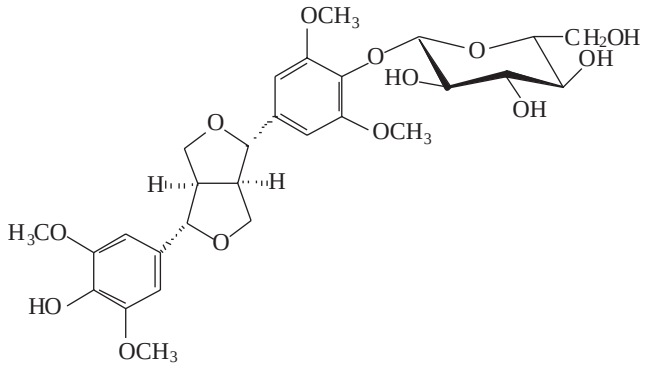
Compound	R_1	R_2	R_3	R_4	R_5
Phlinoside F (**1**)	CH3	CH3	H	H	Xylose
Alyssonoside (**2**)	H	CH3	Apiose	H	H
Samioside (**3**)	H	H	H	Apiose	H





Naringenin (**6**)

Compound	R
Lamiide (**4**)	OH
Auroside (**5**)	H



Syringaresinol-4-*O*-β-D-glucopyranoside (**7**)

Figure 1. Compounds isolated from *P. angustissima*.

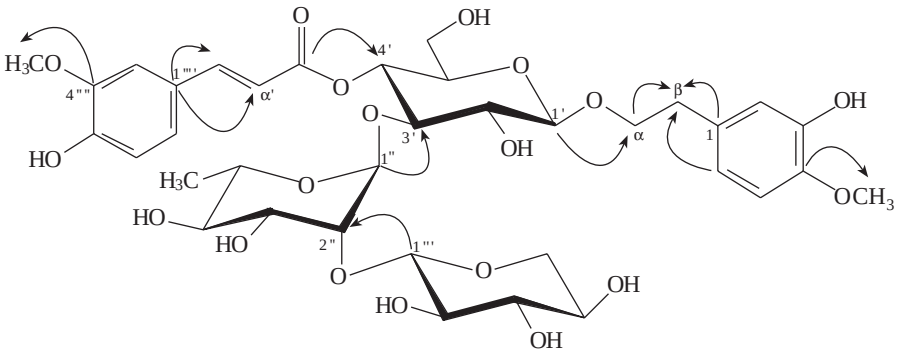


Figure 2. Selected heteronuclear multiple bond correlations (HMBC) for phlinoside F (**1**). Arrows point from carbon to proton.

of rhamnose (δ_H 5.40, H 1″), between C-1″ (δ_C 102.0) of rhamnose and H-3′ of glucose (δ_H 3.80), and between C-1‴ (δ_C 107.6) of xylose and H-2″ of rhamnose (δ_H 3.93). Some significant long-range couplings are given in Figure 2. Consequently, the structure of **1** was established as β-(3-hydroxy,4-methoxyphenyl)ethyl-*O*-[β-xylopyranosyl(1→2)-α-rhamnopyranosyl-(1→3)]-4-*O*-feruloyl-β-glucopyranoside. The structure of the new glycoside (**1**) was closely related to that of phlinosides B[13] and D[14] which have been isolated from *P. linearis*. The sugar sequence and the glucosidation pattern of phlinoside F (**1**) were identical to those of phlinosides B and D; on the other hand, there were differences from the acyl and aglycone moieties. Therefore, we proposed the trivial name phlinoside F for compound **1**.

The structures of the remaining isolates (**2-7**) were established as the known compounds allyssonoside[2,8], samioside[8,9,12], lamiide[2,8,14], auroside[15], naringenin[16], and syringaresinol-4-*O*-β-D-glucopyranoside[8], respectively, on the basis of comparison of their spectroscopic(UV, IR, ^{1}H and ^{13}C NMR) data with those reported in the literature.

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References

1. A. Huber-Morath, *Phlomis*, in "Flora of Turkey and the East Aegean Islands", Vol. 7, pp. 102-126, University Press, Edinburgh (1982), (edited by P.H. Davis).
2. T. Ersöz, İ. Saracoğlu, H. Kırmızıbekmez, F.N. Yalçın, Ü.Ş. Harput, A.A. Dönmez and İ. Çalış, **Hacettepe University, Journal of Faculty of Pharmacy 21**, 23-33 (2001).
3. T. Ersöz, O. Sticher and İ. Çalış, **Hacettepe University, Journal of Faculty of Pharmacy 21**, 35-45 (2001).
4. T. Ersöz, W. Schühly, S. Popov, N. Handjieva, O. Sticher and İ. Çalış, **Nat. Prod. Lett. 15**, 345-351 (2001).
5. T. Ersöz, Ü.Ş. Harput, İ. Çalış and A.A. Dönmez, **Turk. J. Chem. 26**, 1-8 (2002).
6. T. Ersöz, İ. Saracoğlu, Ü.Ş. Harput, İ. Çalış and A.A. Dönmez, **Turk. J. Chem. 26**, 171-178 (2002).
7. T. Ersöz, İ. Saracoğlu, D. Taşdemir, H. Kırmızıbekmez, A.A. Dönmez, C.M. Ireland and İ. Çalış, **Z. Naturforsch. 57c**, 221-225 (2002).
8. F.N. Yalçın, T. Ersöz, P. Akbay, İ. Çalış, A.A. Dönmez and O. Sticher, **Turk. J. Chem. 27**, 295-305 (2003).
9. F.N. Yalçın, T. Ersöz, P. Akbay, İ. Çalış, A.A. Dönmez and O. Sticher, **Turk. J. Chem. 27**, 703-711 (2003).
10. İ. Çalış, H. Kırmızıbekmez, T. Ersöz, İ. Saracoğlu, A.A. Dönmez, M. Mitova, N. Handjieva and S. Popov, **Acta Pharmaceutica Turcica 44**, 195-203 (2002).
11. T. Ersöz, K. Iv. Alipieva, F. N. Yalçın, P. Akbay, N. Handjieva, A.A. Dönmez, S. Popov and İ. Çalış, **Z. Naturforsch. 58c**, 471-476 (2003).

12. I. Kyriakopolou, P. Mgiatis, A.L. Skaltsounis, N. Aligiannis and C. Harvala, **J. Nat. Prod. 64**, 1095-1097 (2001).

13. İ. Çalış, A.A. Başaran, İ. Saracoğlu, O. Sticher and P. Ruedi, **Phytochemistry 30**, 1253-1257 (1991).

14. İ. Çalış, A.A. Başaran, İ. Saracoğlu, O. Sticher and P. Ruedi, **Phytochemistry 30**, 3073-3075 (1991).

15. Ü.Ş. Harput, İ. Saracoğlu and İ. Çalış, **Hacettepe University, Journal of Faculty of Pharmacy 19**, 1-11 (1999).

16. J.B. Harborne, "**The Flavonoids, Advances in Research Since 1986**", Chapman and Hall, London (1994).