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Further Phenylethanoid Glycosides from *Phlomis physocalyx* Hub.-Mor.

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Introduction

The genus *Phlomis* (Lamiaceae) is represented by 34 species in Turkey1. 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[2-9]. In a previous paper, we reported a new phenylethanoid tetraglycoside, physocalycoside from the aerial parts of *Phlomis physocalyx* together with five known phenylethanoid glycosides, wiedemannioside C, verbascoside, leucosceptoside A, martynoside and forsythoside B as well as an iridoid glucoside, lamiide[10]. Further study on the *n*-BuOH fraction of the methanolic extract of the title plant was resulted in the isolation of two additional phenylethanoid glycosides, phlinosides C **(1)** and E **(2)**. In this paper we describe the isolation and structure elucidation of **1** and **2** by means of comprehensive 1D- and 2D-NMR datasets.

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Material and Methods

General Experimental Procedures

UV (MeOH) spectra were recorded on a Shimadzu UV-160A spectrophotometer. FT-IR spectra (KBr) were determined on a Perkin-Elmer 2000 FT-IR spectrophotometer. NMR measurements in CD_3OD were performed on a JEOL JNM-A500 FT-NMR spectrometer (^1H: 500 and ^{13}C: 125 MHz). Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standart. HRFABMS was recorded in positive mode on a JEOL JMS-DX 300 mass spectrometer with glycerol as matrix substance. For open column chromatography Sephadex LH-20 (Fluka) were used. TLC were carried out on pre-coated Kieselgel 60 F_{254} aluminium sheets (Merck). The compounds were detected by UV fluorescence and spraying 1% vanillin/H_2SO_4, followed by heating at 100 °C for 1-2 min.

Plant Material

Phlomis physocalyx Hub.-Mor. (Lamiaceae) was collected in July 2001 at Sivas near Gürün-Kangal-Kocakurt crossing at 1550 m. Voucher specimens have been deposited at the Herbarium of the Biology Department, Faculty of Science, Hacettepe University, Ankara, Turkey (AAD 9555)[10].

Extraction and Isolation

Dried and powdered aerial parts of *P. physocalyx* (600 g) were extracted with MeOH (3 x 2500 mL) at 40 °C and combined MeOH extracts were concentrated under reduced pressure (51.6 g). The resultant residue was then dissolved in H_2O (250 mL) and the water-soluble portion was partitioned between $CHCl_3$ (4 x 250 mL) and *n*-BuOH (4 x 250 mL). An aliquot of the *n*-BuOH extract (10 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 nine main fractions (Frs. E1-E9). Frs. E9 (50 mg) and E7 (64.4 mg) were subjected to column chromatography (CC) on Sephadex LH-20 (MeOH) individually to give **1** (22.8 mg) and **2** (23.4 mg), respectively. However, fractionation of fr. E7 over Sephadex LH-20 also yielded a subfraction rich in **2** (fr. E7e, 10 mg). Frs. E7e and E6 (39.5 mg)[10] were then combined and applied to CC over Sephadex-LH 20 (MeOH) to afford an additional amount of **2** (10.8 mg).

Phlinoside C (1): Amorphous powder. UV λ_{max} (MeOH) 203, 218, 246 (sh), 292, and 332 nm. IR ϑmax (KBr) 3398 (OH), 1700 (C=O), 1630 (C=C), 1595 and 1510 (aromatic rings) cm^{-1}. ^1H NMR (CD_3OD, 500 MHz): Table I. ^{13}C NMR (CD_3OD, 125 MHz): Table I. Positive-ion HRFABMS m/z 793.2558 $[M+Na]^+$ (calcd. for $C_{35}H_{46}O_{19}Na$: 793.2531).

Phlinoside E (2): Amorphous powder. UV λ_{max} (MeOH) 203, 218, 248 (sh), 290, and 330 nm. IR ϑmax (KBr) 3400 (OH), 1690 (C=O), 1630 (C=C), 1595 and 1510 (arom. rings) cm-1. 1H NMR (CD_3OD, 500 MHz): Table II. ^{13}C NMR (CD3OD, 125 MHz): Table II. Positive-ion HRFABMS m/z 785.2894 $[M+H]^+$ (calcd. for $C_{36}H_{48}O_{19}$: 785.2868).

Results and Discussion

Compounds **1** and **2** were obtained as amorphous substances. The UV spectra of both **1** and **2** indicated their polyphenolic nature and their IR spectra showed similar absorption bands for hydroxyl groups, α,β-unsaturated esters, and aromatic rings.

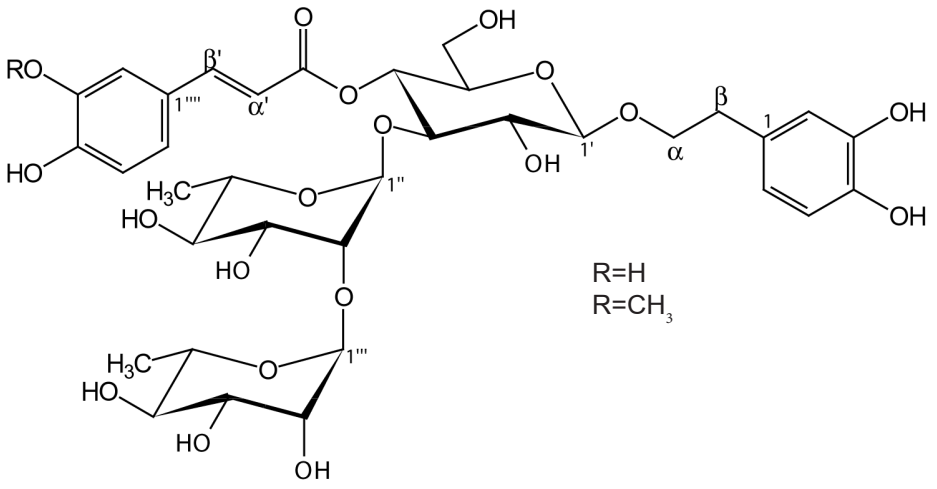

Figure 1

Phenylethanoid triglycosides isolated from *P. physocalyx.*

Molecular formula of compound 1 was determined as $C_{35}H_{46}O_{19}$ by the ^{13}C NMR spectrum and HRFABMS. The ^1H NMR spectrum of **1** (Table I) showed six aromatic protons as two ABX systems (δ_H 7.04-6.56 region) and two *trans*-olefinic protons as an AB system (δ_H 7.59 and 6.25; each,

d, *J* = 15.9 Hz) consistent with *(E)*-caffeic acid and 3,4-dihydroxyphenylethanol moieties[7]. Three anomeric proton signals appeared at δ_H 4.37 (d, *J* = 8.0 Hz), 5.34 (*d, J* = 1.7 Hz) and 4.90 (d, *J* = 1.7 Hz) were assigned to the anomeric protons of a β-glucose and two α-rhamnose units, respectively. The secondary methyl resonances appeared at δ_H 1.06 and 1.26 (each, d, *J* = 6.1 Hz) revealed the presence of two rhamnose units within **1.** The ^{13}C NMR data (Table I) confirmed the triglycosidic chain in 1, exhibiting three anomeric carbon resonances at δ_C 104.3, 101.7, and 103.8, which showed correlations with the anomeric protons of the glucose and two rhamnose units, respectively. The ^1H NMR data suggested that the caffeoyl moiety occupied the C-4^I position of the glucopyranose unit due to the downfield shift of H-4^I signal (δ_H 4.92, t, *J* = 9.6 Hz). An HMBC correlation observed between the carbonyl carbon (δ_C 168.3) of the caffeoyl moiety and the H-4^I confirmed that the caffeoyl group attached to the C-4^I position. Likewise, an HMBC cross-peak observed from C-α carbon (δ_C 72.3) of the phenylethyl alcohol unit to the anomeric proton of glucose (δ_H 4.37) showed that glucose was attached to the C-α position of the phenylethyl alcohol unit. The carbon signals arising from the glucose and first rhamnose units indicated that the glucose unit to be glycosylated at C-3^I (δ_C 81.7) and the first rhamnose unit at C-2^{II} (δ_C 80.1). Prominent heteronuclear long range couplings from C-1^{II} (δ_C 101.7) of first rhamnose to H-3^I (δ_H 3.79) of glucose as well as C-1^{III} (δ_C 103.8) of second rhamnose to H-2^{II} (δ_H 3.92) of first rhamnose supported this proposal. Consequently, the structure of compound **1** was established as 3,4-dihydroxy-β-phenylethoxy-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-glucopyranoside. Comparing its NMR data with those given in the literature, compound **1** was identified as phlinoside C, previously isolated from *Phlomis linearis*[11].

The HRFABMS data of compound **2** showed that the molecular formula of 2 to be $C_{36}H_{48}O_{19}$, which was in good agreement with the observation of 36 resonances in the ^{13}C NMR spectrum. The ^1H and ^{13}C NMR resonances attributed to the aglycon and sugar moieties were almost identical to those of phlinoside C **(1)** (Table II), indicating also the same aglycon and sugar units in **2.** Moreover, an HMBC experiment revealed the glycosidation pattern in **2** was identical to that of **1.** However, the NMR data of the acyl part of compound **2** were slightly different from that of **1**, exhibiting resonances attributed to a methoxy function (δ_H 3.88, s; δ_C 56.5, CH_3 q). A relevant HMBC coupling observed between C-3^{IIII} (δ_C 149.4) of the acyl moiety and the methoxy group unumbiguously

TABLE I

^{13}C and ^{1}H NMR (CD_3OD, ^{13}C: 125 MHz and ^{1}H: 500 MHz) data and HMBC correlations for **1***

	^{13}C NMR		^{1}H NMR	
C/H Atom	δ_C ppm**	DEPT	δ_H ppm, *J* (Hz)	HMBC (C→H)
Aglycon				
1	131.5	C	-	H-2, H_2-α, H_2-β
2	117.1	CH	6.68 d (2.0)	H-6, H_2-β
3	146.1	C	-	H-5
4	144.7	C	-	H-2, H-6
5	116.3	CH	6.67 d (8.0)	
6	121.3	CH	6.56 dd (8.0/2.0)	H-2, H_2-β
α	72.3	CH_2	4.04 m / 3.72 m	H-1^{I}, H_2-β
β	36.6	CH_2	2.79 t (7.9)	
Glucose				
1^{I}	104.3	CH	4.37 d (8.0)	H-2^{I}, H_2-α
2^{I}	76.0	CH	3.37 dd (8.0/9.0)	
3^{I}	81.7	CH	3.79†	H-1^{II}, H-4'
4^{I}	70.6	CH	4.92 t (9.6)	
5^{I}	76.1	CH	3.30†	
6^{I}	62.4	CH_2	3.64†/3.48†	
Rhamnose				
1^{II}	101.7	CH	5.34 d (1.7)	H-3^{I}
2^{II}	80.1	CH	3.92 dd (1.7/3.4)	
3^{II}	71.8	CH	3.65†	
4^{II}	74.0	CH	3.25†	
5^{II}	70.4	CH	3.52†	H_3-6^{II}
6^{II}	17.9	CH_3	1.06 d (6.1)	
Rhamnose (→C-2^{II})				
1^{III}	103.8	CH	4.90 d (1.7)	H-2^{II}
2^{III}	72.0	CH	3.92 dd (1.7/3.4)	
3^{III}	72.2	CH	3.62†	
4^{III}	72.4	CH	3.25†	
5^{III}	70.3	CH	3.68†	H_3-6^{III}
6^{III}	18.6	CH_3	1.26 d (6.1)	
Caffeoyl				
1^{IIII}	127.7	C	-	H-α^{I}, H-β^{I}, H-5^{IIII}
2^{IIII}	115.2	CH	7.04 d (2.0)	H-β^{I}, H-6^{IIII}
3^{IIII}	146.8	C	-	H-2^{IIII}, H-5^{IIII}
4^{IIII}	149.8	C	-	H-2^{IIII}, H-5^{IIII}, H-6^{IIII}
5^{IIII}	116.6	CH	6.77 d (8.3)	
6^{IIII}	123.2	CH	6.95 dd (8.3/2.0)	H-β^{I}, H-2^{IIII}
α^{I}	114.7	CH	6.25 d (15.9)	H-β^{I}
β^{I}	148.0	CH	7.59 d (15.9)	H-2^{IIII}, H-6^{IIII}
C=O	168.3	C	-	H-α^{I}, H-β^{I}, H-4^{I}

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

**Multiplicites are based on DEPT-135 experiment

†Signal patterns are unclear due to overlapping.

confirmed the attachment of the methoxyl grou p at C-3^{IIII}, indicating the presence of *(E)*-ferulic acid within **2** (Fig. 2).

Therefore, on the basis of its NMR data, the structure of **2** was determined as 3,4-dihydroxy-β-phenylethoxy-*O*-α-rhamnopyranosyl-(1→2)-α-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-glucopyranoside. The structure of **2** was identical to that of the phenylethanoid trisaccharide, phlinoside E, previously isolated from *Phlomis linearis*[12].

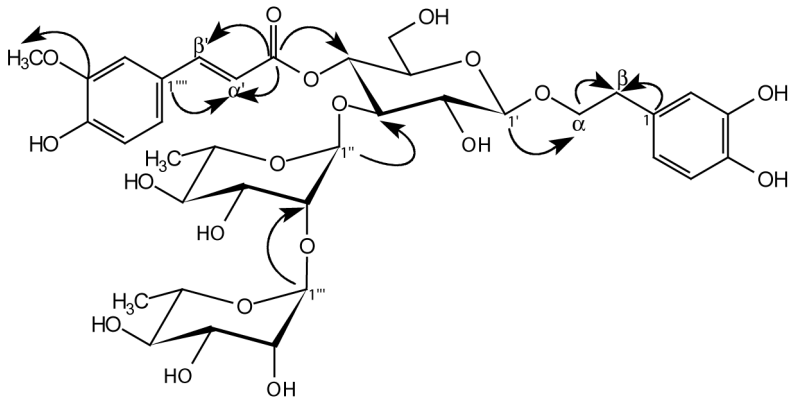

Figure 2

Selected HMBC for phlinoside E **(2)** (arrows point from C to H).

In a previous communication *P. physocalyx* was reported to contain two more phenylethanoid trisaccharides, wiedemannioside C and forsythoside B[10]. However, both the sugar units and the glycosidation pattern in phlinosides C **(1)** and E **(2)** were different from those of the former triglycosidic phenylethanoids. In phlinosides C **(1)** and E **(2)** the third sugar unit, which was rhamnose for both glycosides, linked to the C-2" position of the first rhamnose moiety, however, the third sugar unit in wiedemannioside C and forsythoside B (glucose and apiose, resp.) were attached to the C-6' position of the core sugar, glucose.

Previously, phenylethanoid triglycosides, named as phlinosides A-E from *Phlomis linearis*[11,12], and phlinoside F from *P. angustissima*[13] have been reported. Up to date, this is the second report for the isolation of two of these rare triglycosidic phenylethanoids from a member of the genus *Phlomis* and also nature. On the other hand, the structure elucidation of both **1** and **2** were performed and confirmed based on detailed

TABLE II

^{13}C and ^{1}H NMR (CD_3OD, ^{13}C: 125 MHz and ^{1}H: 500 MHz) data and HMBC correlations for **2***

	^{13}C NMR		^{1}H NMR	
C/H Atom	δ_C ppm**	DEPT	δ_H ppm, *J* (Hz)	HMBC (C→H)
Aglycon				
1	131.5	C	-	H-2, H_2-α, H_2-β
2	117.1	CH	6.70 d (2.2)	H-6, H_2-β
3	146.1	C	-	H-5
4	144.7	C	-	H-2, H-6
5	116.5	CH	6.69 d (8.2)	
6	121.3	CH	6.66 dd (8.2/2.2)	H-2, H_2-β
α	72.3	CH_2	4.03 m / 3.72 m	H-1^{I}, H_2-β
β	36.6	CH_2	2.79 t (7.9)	H-2, H-6, H_2-α
Glucose				
1^{I}	104.3	CH	4.37 d (7.7)	H-2^{I}, H_2-α
2^{I}	76.0	CH	3.38 dd (8.0/9.0)	
3^{I}	81.6	CH	3.79†	H-1^{II}, H-3^{I}, H-4^{I}
4^{I}	70.6	CH	4.92 t (9.6)	
5^{I}	76.2	CH	3.30†	
6^{I}	62.4	CH_2	3.65†/3.50†	
Rhamnose				
1^{II}	101.7	CH	5.35 d (1.7)	H-3^{I}
2^{II}	80.1	CH	3.92 dd (1.7/3.4)	
3^{II}	72.0	CH	3.65†	
4^{II}	74.1	CH	3.25†	
5^{II}	70.4	CH	3.52†	H_3-6^{II}
6^{II}	18.5	CH_3	1.07 d (6.1)	
Rhamnose (C-2^{II})				
1^{III}	103.8	CH	4.90 d (1.7)	H-2^{II}
2^{III}	71.8	CH	3.92 dd (1.7/3.4)	
3^{III}	72.2	CH	3.62†	
4^{III}	74.1	CH	3.25†	
5^{III}	70.3	CH	3.68†	H_3-6^{III}
6^{III}	17.9	CH_3	1.25 d (6.1)	
Feruloyl				
1^{IIII}	127.7	C	-	H-α^{I}, H-β^{I}, H-5^{IIII}
2^{IIII}	111.8	CH	7.19 d (2.0)	H-β^{I}, H-6^{IIII}
3^{IIII}	149.4	C	-	H-2^{IIII}, H-5^{IIII}, OCH_3
4^{IIII}	150.9	C	-	H-2^{IIII}, H-5^{IIII}, H-6^{IIII}
5^{IIII}	116.3	CH	6.80 d (8.2)	
6^{IIII}	124.4	CH	7.07 dd (8.2/2.0)	H-β^{I}, H-2^{IIII}
α′	115.1	CH	6.36 d (15.9)	H-β^{I}
β′	147.9	CH	7.66 d (15.9)	H-2^{IIII}, H-6^{IIII}
C=O	168.3	C	-	H-α^{I}, H-β^{I}, H-4^{I}
3^{IIII}-OCH_3	56.5	CH_3	3.88 s	

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

**Multiplicites are based on DEPT-135 experiment

†Signal patterns are unclear due to overlapping.

1D- and 2D-NMR experiments and the NMR data were given in full assignments in Tables I and II.

Acknowledgement

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Summary

Two phenylethanoid triglycosides, phlinosides C **(1)** and E **(2)** were isolated from the methanolic extract of the overground parts of *Phlomis physocalyx*. On the basis of spectroscopic evidence the structures of the isolated compounds were determined as 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside and 3,4-dihydroxy-β-phenylethoxy-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside, respectively. The structure elucidation of the phenylethanoid glycosides **1** and **2** are explained in detail. This is the second report for the isolation of these phenylethanoids from a member of the genus *Phlomis*.

Key Words: Phlomis physocalyx, Lamiaceae, phenylethanoid glycosides, phlinoside C, phlinoside E.

Özet

***Phlomis physocalyx*'den Elde Edilen Diğer Feniletanoit Glikozitleri**

Phlomis physocalyx'in toprak üstü kısımlarının MeOH ekstresinden iki feniletanoit triglikoziti olan filinozit C **(1)** ve filinozit E **(2)** izole edilmiştir. Bileşiklerin yapıları spektroskopik bulgulara dayanarak sırasıyla 3,4-dihidroksi-β-feniletoksi-*O*-α-L-ramnopiranozil-(1→2)-α-L-ramnopiranozil-(1→3)-4-*O*-kafeoil-β-D-glukopiranozit ve 3,4-dihidroksi-β-feniletoksi-*O*-α-L-ramnopiranozil-(1→2)-α-L-ramnopiranozil-(1→3)-4-*O*-feruloil-β-D-glukopiranozit olarak tayin edilmiştir. Feniletanoit glikozitleri **1** ve **2** nin yapı tayinleri ayrıntılı olarak açıklanmıştır. Bu

çalışma, bu feniletanoit bileşiklerinin *Phlomis* cinsine mensup bir bitkiden elde edilmeleri konusunda ikinci yayın olmaktadır.

Anahtar Kelimeler: Phlomis physocalyx, Lamiaceae, feniletanoit glikozitleri, filinozit C, filinozit E.

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