Phenylethanoid Glycosides from *Phlomis integrifolia* Hub.-Mor.

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Two new phenylethanoid glycosides integrifoliosides A (**2**) and B (**3**), along with a known phenylethanoid glycoside alyssonoside (**1**) and a flavone glucoside chrysoeriol-7-*O*-*β*-D-glucopyranoside (**4**) were isolated from the aerial parts of *Phlomis integrifolia.* The structures of the new compounds were identified as 3,4-dihydroxy-*β*-phenylethoxy-*O*-*β*-D-apiofuranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-*β*-D-glucopyranoside (**2**) and 3-hydroxy-4-methoxy-*β*-phenylethoxy-*O*-*β*-D-apiofuranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-*β*-D-glucopyranoside (**3**), on the basis of spectroscopic (UV, IR, 1D- and 2D-NMR, and HR-FABMS) methods.

Key words: Phlomis integrifolia Hub.-Mor., Phenylethanoid Glycosides, Integrifoliosides A and B

Introduction

During our studies on the glycosidic constituents of *Phlomis* L. species (Lamiaceae) which is a part of the project "Chemotaxonomic Studies on *Phlomis* Genus Growing in Turkey", we have further investigated an endemic species, *Phlomis integrifolia.* Previously, the presence of a neolignan glucoside dehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside, an ester flavone glucoside chrysoeriol-7-*O*-(3″-*O*-*trans*-*p*-coumaroyl)-*β*-D-glucopyranoside, together with four phenylethanoid glycosides forsythoside B, verbascoside (= acteoside), leucosceptoside A, martynoside and additionally, an iridoid glucoside lamiide from the over-ground parts of the title plant have been described (Saracoglu *et al.*, 2003). In continuation of our investigation on the same plant we now report the isolation and structure elucidation of a known and two new phenylethanoid glycosides alyssonoside (**1**), integrifoliosides A (**2**) and B (**3**) as well as a flavone glucoside chrysoeriol-7-*O*-*β*-D-glucopyranoside (**4**).

Material and Methods

General experimental procedures

UV and IR spectra were recorded on a Shimadzu UV-160A and Perkin Elmer 2000 FTIR spectrophotometers respectively. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECP-600 FT-NMR spectrometer in methanol-d_4 (^{1}H: 600 MHz; ^{13}C: 150 MHz). Chemical shifts were given in ppm with tetramethylsilane as an internal standard. HRFAB-MS was performed on a JEOL JMS-700 spectrometer (Matrix: glycerol and NBA). Optical rotation was measured with a Rudolph Research Analytical Autopol IV polarimeter. Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 60–230 mesh), polyamide (Fluka, 50–160 μm) and sephadex LH-20 (Pharmacia). Medium pressure liquid chromatography (MPLC) was realized on Labomatic (18.5 mm × 352 mm) and Büchi (25 mm × 460 mm) glass columns filled with Li Chroprep RP-18 (Merck) using Lewa M5 peristaltic and Büchi B-684 pumps. Thin layer chromatography (TLC) was conducted on precoated, commercial silica gel (Merck, 60F_{254}) plates with $CHCl_3$/MeOH/H_2O (61:32:7 and

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80:20:2) as a developing solvent system. Compounds **1**–**4** were detected by UV fluorescence and/or spraying with 1% vanillin/H_2SO_4, followed by heating at 100 °C for 5 min.

Plant material

Phlomis integrifolia Hub.-Mor. (Labiatae) was collected from Malatya, between Darende and Akdag, steppe, 1460 m (East Anatolia, Turkey) in June 2001. A voucher specimen has been deposited in the Herbarium of the Biology Department, Faculty of Science, Hacettepe University, Ankara, Turkey (AA Donmez 9410).

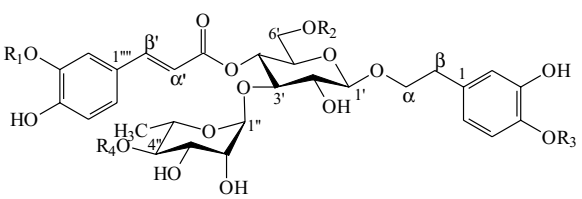
Extraction and isolation

Air-dried aerial parts of the plant (530 g) were extracted three times with methanol at 40 °C (3 × 2.0 l). The combined extracts were evaporated under vacuum nearly to dryness. H_2O (0.5 l) was added and the H_2O insoluble material removed by filtration. The filtrate was extracted with petroleum ether (3 × 0.25 l) and the petroleum ether phase rejected. The aqueous phase was extracted with *n*-butanol (4 × 0.2 l). *n*-Butanol extract (16 g) was dissolved in 40 ml of H_2O and chromatoghraphed over polyamide eluting with H_2O followed by increasing concentrations of MeOH to yield four main fractions: Fractions A–D. (fr. A: H_2O, fr. B: 25% MeOH, fr. C: 50% MeOH, fr. D: MeOH). The fractions eluted with H_2O (fr. A) and 50% MeOH (fr. C) from polyamide column were previously studied (Saracoglu *et al.*, 2003). The fraction eluted with H_2O/MeOH (75:25 v/v) from the polyamide column (fr. B) was applied to a series of column chromatographies to yield compounds **1**–**3**. An aliquot of fr. B (1.47 g) was applied to medium pressure liquid chromatography (MPLC) by using reversed-phase column. Eluting with increasing amounts of MeOH (25→100%) yielded two main fractions: frs. B1 and B2. Fraction B1 (137.0 mg) was rich in compound **3** and was rechromatographed over silica gel by stepwise elution with $CHCl_3$/MeOH (100:0→70:30 v/v) yielded compound **3** (7.4 mg) in a pure form. Fraction B2 was found to contain compounds **1** and **2**. Chromatography of fr. B2 (102.0 mg) over silica gel by stepwise elution with $CHCl_3$/MeOH (100:0→80:20 v/v) and then purification by Sephadex (MeOH) gave crude compounds **1** (4.0 mg) and **2** (5.0 mg). The fraction eluted with MeOH from the polyamide column (fr. D) was rich in the flavonoid glucosides. An aliquot of fr. D (350 mg) was applied to medium pressure liquid chromatography (MPLC) by using reversed-phase column. Eluting with increasing amounts of MeOH (30→100%) yielded two main fractions: Frs. D1 and D2. Fraction D1 was containing compound **4** (5.3 mg) as a pure form. Fraction D2 and the fraction eluted with 75% MeOH from polyamide column (fr. C) are still under the investigation.

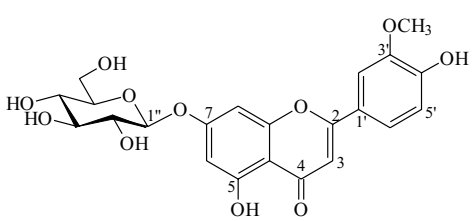
Results and Discussion

The methanol extract of *Phlomis integrifolia* was suspended in water and partitioned with petroleum ether. The aqueous phase was extracted with *n*-butanol and *n*-butanol extract was subjected to polyamide column chromatography eluting with H_2O followed by increasing concentrations of MeOH to afford four main fractions. Repeated chromatography of the polyamide fractions resulted in the isolation of four compounds (**1**–**4**) (Fig. 1).

Compounds **1**–**3** were obtained as amorphous powders. Their UV spectra (MeOH) showed λ_{max} at 330, 250sh, 235sh, and 220 nm indicating their polyphenolic nature. In the IR spectra (KBr) ab-



R_1	R_2	R_3	R_4	
$-CH_3$	-Apiose	-H	-H	Alyssonoside **(1)**
$-CH_3$	-H	-H	-Apiose	Integrifolioside A **(2)**
$-CH_3$	-H	$-CH_3$	-Apiose	Integrifolioside B **(3)**



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

Fig. 1. Structures of compounds **1**–**4**.

sorption bands typical for hydroxyls (3400 cm^{-1}), α,β-unsaturated ester (1695 cm^{-1}), olefinic double bond (1630 cm^{-1}) and aromatic rings (1610, 1520 cm^{-1}) were observed. The FAB-MS of **1** exhibited a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 793.3 suggesting the molecular formula $C_{35}H_{46}O_{19}$. The ^1H and ^{13}C NMR spectral data of **1** were identical to those reported for 3,4-dihydroxy-β-phenylethoxy-*O*-[α-L-rhamnopyranosyl-(1→3)]-*O*-[β-D-apiofuranosyl-(1→6)]-4-O-feruloylβ-D-glucopyranoside (alyssonoside) which was isolated from *Marrubium alysson* and *Verbascum thapsus* previously (Calis *et al.*, 1992; Warashina *et al.*, 1992).

Compound **2** was obtained as a colourless, amorphous powder with some inpurities. The HR FAB-MS of **2** exhibited a sodiated ion peak $[M+Na]^+$ at *m/z* 793.2531 (matrix: NBA) suggesting the molecular formula $C_{35}H_{46}O_{19}$ which was confirmed by the observation of two methyl, five methylene, twenty methine, and eight quaternary carbon resonances in its ^{13}C NMR spectrum (Table I). The ^1H NMR

Table I. ^{13}C and ^1H NMR (CD_3OD, 150 MHz for ^{13}C and 600 MHz for ^1H NMR) spectral data and selected HMBC correlations for compound **2***.

C/H	DEPT	δ_C (ppm)	δ_H (ppm)	*J* [Hz]	HMBC (H → C)
Aglycone					
1	C	131.46			
2	CH	117.11	6.69 d	(1.9)	C-3, C-6
3	C	146.13			
4	C	144.68			
5	CH	116.30	6.67 d	(7.9)	C-1, C-3, C-4
6	CH	121.24	6 56 dd	(7.9/2.0)	C-2, C-4
α	CH_2	72.36	4.00 m		C-1, C-1′
			3.70 m		
β	CH_2	36.57	2.79 t	(7.4)	C-1, C-2, C-6
Glucose					
1′	CH	104.20	4.36 d	(7.9)	C-α
2′	CH	76.42	3.39 dd	(9.1/7.9)	C-1′, C-3′
3′	CH	80.18	3.82 t	(9.1)	C-1″
4′	CH	70.42	4.91 t	(9.3)	C-3′, C-5′, C=O
5′	CH	76.00	3.55 m		
6′	CH_2	62.35	3.63 dd	(11.5/2.2)	
			3.55†		
Rhamnose					
1″	CH	102.18	5.26 d	(1.6)	C-3′, C-3″, C-5″
2″	CH	72.57	3.89 dd	(3.0/1.9)	C-1″
3″	CH	72.46	3.67 dd	(9.6/3.3)	
4″	CH	80.16	3.38 t	(9.1)	C-1‴, C-2″, C-3″, C-5″
5″	CH	68.79	3.62 m		
6″	CH_3	18.41	1.12 d	(6.0)	C-4″, C-5″
Apiose					
1‴	CH	111.37	5.19 d	(2.5)	C-4″, C-4‴
2‴	CH	78.42	3.60 δ	(2.5)	C-3‴, C-5‴
3‴	C	80.59			
4‴	CH_2	75.10	3.59†		C-1‴, C-2‴, C-3‴, C-5‴
5‴	CH_2	65.69	3.32 d	(11.5)	C-2‴, C-3‴, C-4‴
			3.22 d	(11.5)	
Acyl moiety					
1‴′	C	127.62			
2‴′	CH	111.92	7.19 d	(1.9)	C-3‴′, C-6‴′, C-β′
3‴′	C	150.93			
4‴′	C	149.41			
5‴′	CH	116.51	6.83 d	(8.2)	C-1‴′
6‴′	CH	124.34	7.10 dd	(7.9/2.0)	C-2‴′, C-3‴′, C-β′
α′	CH	115.21	6.34 d	(15.9)	C-1‴′, C=O
β′	CH	147.86	7.64 d	(15.9)	C-2‴′, C-6‴′, C=O
C=O	C	168.10			
OMe	CH_3	56.48	3.89 s		C-3‴′

* The ^{13}C and ^1H assignments were based on 2D-NMR (DQF-COSY, HMQC and HMBC) experiments.

† Signal patterns unclear due to overlapping.

spectrum of **2** showed characteristic signals arising from (*E*)-ferulic acid and 3,4-dihydroxyphenylethanol moieties: six aromatic proton signals (2 × ABX systems, in the region of δ_H 7.19–6.56), two *trans*-olefinic proton signals (AB system, δ_H 7.64 d, J = 15.9 Hz and 6.34 d, J = 15.9 Hz), β-methylene at δ_H 2.79 (2H, t, J = 7.4 Hz), and two non-equivalent protons at δ_H 4.00 and 3.70 (each 1H, m) of the side chain of the aglycone moiety. Additionally, three anomeric proton resonances appeared at δ_H 5.26 (d, J = 1.6 Hz), 5.19 (d, J = 2.5 Hz), and 4.36 (d, J = 7.9 Hz) indicated its trisaccharidic structure. ^{1}H and ^{13}C NMR signals assigned to the sugar moiety showed that **2** should be composed of one β-glucose, one α-rhamnose and one β-apiose unit on the basis of their chemical shift and coupling constants. Chemical shifts of protons due to glucose as well as those of the rhamnose and apiose moieties were assigned unambiguously from the homonuclear ^{1}H, ^{1}H-correlation (COSY) and a heteronuclear multiple quantum coherence (HMQC) experiment. The starting points for determining the chemical shifts of protons of glucose, rhamnose and apiose moieties are doublets at δ_H 4.36 (d, J = 7.9 Hz, H-1′), 5.26 (d, J = 1.6 Hz, H-1″) and 5.19 (d, J = 2.5 Hz, H-1‴), respectively. The ^{1}H NMR spectrum showed also the presence of a secondary methyl group at δ_H 1.12 (*d*, J = 6.0 Hz) which supported the presence of a rhamnose moiety in **2**. The ^{13}C NMR spectrum of **2** indicated the triglycosidic structure revealing the resonances at δ_C 111.37 (C-1‴ of a β-apiose), 104.20 (C-1′ of a β-glucose) and 102.18 (C-1″ of an α-rhamnose) (Table I). The feruloyl group was supposed to be positioned at C-4′ hydroxyl of glucose due to the strong deshielding of the resonance of the glucose unit (δ_H 4.91, t, J = 9.3 Hz). This assumption was supported by the heteronuclear long-range correlation observed between the H-4′ of glucose (δ_H 4.91) and the carbonyl carbon resonance (δ_C 168.10) of the acyl moiety (Fig. 2). On the other hand, a HMBC cross-peak observed from the anomeric proton of the glucose (δ_H 4.36, H-1′) to the C-α carbon resonance (δ_C 72.36) of the phenylethyl alcohol unit indicated the attachment of the glucose to be the C-α carbon atom of the aglycone. Although, the highly deshielded carbon signals arising from the glucose and the rhamnose units suggested that the glucose unit to be glycosylated only at C-3′ (δ_C 80.18), whereas the rhamnose unit at C-4″ (δ_C 80.16), however, a prominent HMBC experiment allowed us to assign unambiguously all the interglycosidic connectivities of the sugar sequence. Thus the correlations were observed between H-1″ (δ_H 5.26) of rhamnose and C-3′ (δ_C 80.18) of glucose, H-1‴ (δ_H 5.19) of apiose and C-4″ (δ_C 80.16) of rhamnose. These NMR data of sugar sequence were almost identical to those of samioside which was isolated from *Phlomis samia* (Kyriakopoulou *et al.*, 2001). The only difference was a feruloyl unit in the structure of **2** while samioside was containing a caffeoyl moiety. Some significant long-range correlations confirming the proposed structure were given in Fig. 2 and Table I. Thus, on the basis of its NMR data, the structure of compound **2** was established as 3,4-dihydroxy-β-phenylethoxy-*O*-β-D-apiofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside for which the trivial name integrifolioside A is proposed.

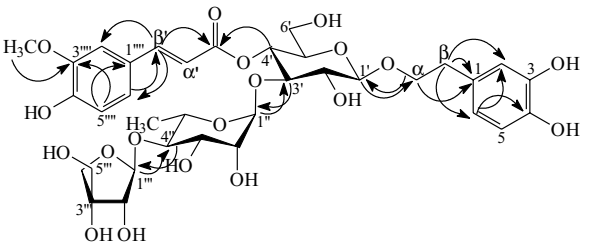


Fig. 2. Significant HMBC correlations for **2**. Arrows point from proton to carbon.

Compound **3** was obtained as a colourless, amorphous powder with negative optical rotation ($[\alpha]_D^{25}$ – 106°, c = 0.1; MeOH). The HR FAB-MS of **3** exhibited a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 807.2687 (matrix: glycerol) suggesting the molecular formula $C_{36}H_{48}O_{19}$ which was confirmed by the observation of three methyl, five methylene, twenty methine, and eight quaternary carbon resonances in its ^{13}C NMR spectrum (Table II). The ^{1}H NMR and ^{13}C NMR spectral data of compound **3** exhibited similar signals to compound **2**, the signals due to the sugar sequence were also superimposable on those of **2**. The major difference was the presence of a resonance for an additional methoxyl group in **3** (δ_H 3.81, 3H, s; δ_C 56.49). The molecular mass difference of 14 units also confirmed the existence of an extra methoxyl group in compound **3**.

Table II. ^{13}C and ^{1}H NMR (CD_3OD, 150 MHz for ^{13}C and 600 MHz for ^{1}H NMR) spectral data and selected HMBC correlations for compound **3***.

C/H	DEPT	δ_C (ppm)	δ_H (ppm)	J [Hz]	HMBC (H → C)
Aglycone					
1	C	132.93			
2	CH	117.07	6.72 d	(2.2)	C-3, C-6
3	C	147.40			
4	C	147.56			
5	CH	112.89	6.82 d	(8.2)	C-1, C-3, C-4
6	CH	121.15	6.68 dd	(8.2/2.2)	C-2, C-4
α	CH_2	72.12	4.5 m		C-1, C-1′
			3.67 m		
β	CH_2	36.55	2.82 t	(7.1)	C-1, C-2, C-6
OMe	CH_3	56.49	3.81 s		C-4
Glucose					
1′	CH	104.21	4.37 d	(7.9)	C-α
2′	CH	76.40	3.41 dd	(9.1/7.4)	C-1′, C-3′
3′	CH	80.16	3.84 t	(9.1)	C-1″
4′	CH	70.46	4.92 t	(9.6)	C-3′, C-5′, C=O
5′	CH	76.00	3.52†		
6′	CH_2	62.34	3.61 dd	(12.0/6.0)	
			3.52†		
Rhamnose					
1″	CH	102.18	5.27 d	(1.3)	C-3′, C-3″, C-5″
2″	CH	72.56	3.86 dd	(3.0/1.6)	C-1″
3″	CH	72.46	3.64 dd	(9.1/3.3)	
4″	CH	80.16	3.38 t	(9.6)	C-1‴, C-2″, C-3″, C-5″
5″	CH	68.78	3.61†		
6″	CH_3	18.72	1.12 d	(6.3)	C-4″, C-5″
Apiose					
1‴	CH	111.37	5.19 d	(2.7)	C-4″, C-4‴
2‴	CH	78.46	3.59†		C-3‴, C-5‴
3‴	C	80.62			
4‴	CH_2	74.82	3.59†		C-1‴, C-2‴, C-3‴, C-5‴
5‴	CH_2	65.83	3.33 d	(11.5)	C-2‴, C-3‴, C-4‴
			3.22 d	(11.5)	
Acyl moiety					
1‴′	C	127.55			
2‴′	CH	111.89	7.20 d	(1.6)	C-3‴′, C-6‴′, C-β′
3‴′	C	151.01			
4‴′	C	149.46			
5‴′	CH	117.07	6.83 d	(8.2)	C-1‴′
6‴′	CH	124.36	7.10 dd	(8.2/1.9)	C-2‴′, C-3‴′, C-β′
α′	CH	114.95	6.36 d	(15.9)	C-1‴′, C=O
β′	CH	147.88	7.65 d	(15.9)	C-2‴′, C-6‴′, C=O
C=O	C	168.14			
OMe	CH_3	56.49	3.89 s		C-3‴′

* The ^{13}C and ^{1}H assignments were based on 2D-NMR (DQF-COSY, HMQC and HMBC) experiments.
† Signal patterns unclear due to overlapping.

The assignments of the chemical shifts for the second methoxyl group and the related aromatic protons (δ_H 6.82, 6.72, 6.68, ABX system) showed that this group should be at C-4. This assumption was also supported by the cross peaks in the HMBC spectrum from the methoxyl group (δ_H 3.81) to C-4 (δ_C 147.56) of phenylethyl alcohol moiety (Table II). The complete assignments of all ^{1}H and ^{13}C signals of feruloyl and phenylethoxy moieties of **3** were based on the results of $^{1}H-^{1}H$ COSY, $^{1}H-^{13}C$ HMQC and HMBC experiments. Based on these data, compound **3** was established as 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-β-D-apiofuranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-4-*O*-feruloyl-β-D-glucopyranoside for which we proposed integrifolioside B as trivial name.

Compound **4** was isolated as a yellow, amorphous powder. It exhibited UV and IR absorptions confirming its phenolic nature. The UV spectroscopic data (λ_{max} 269, 344 nm) suggested that **4** was a fla-

vone. The IR spectrum was characterized by the absorption bands for hydroxyl (3384 cm^{-1}), ester carbonyl (1714 cm^{-1}), γ-pyrone carbonyl (1661 cm^{-1}), and aromatic rings (1608, 1508 cm^{-1}). The ^{1}H and ^{13}C NMR spectral data of **4** were identical to those reported for chrysoeriol-7-*O*-β-D-glucopyranoside (Markham and Chari, 1982; Markham and Greiger, 1986; Markham *et al.*, 1978).

During our previous study on the *Phlomis integrifolia*, we had isolated a disaccharide sugar moiety containing phenylethanoid glycosides such as, verbascoside (= acteoside), leucosceptoside A and martynoside along with a trisaccharidic phenylethanoid glycoside forsythoside B (Saracoglu *et al.*, 2003). Forsythoside B had a similar sugar sequence with alyssonoside (**1**). The above-mentioned phenylethanoid glycosides were previously isolated from several *Phlomis* species during our studies. Integrifoliosides A (**2**) and B (**3**) contained different sugar sequences from the above-mentioned phenylethanoid glycosides and they were isolated from nature for the first time.

Acknowledgements

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