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Phytochemistry 65 (2004) 2619–2625

PHYTOCHEMISTRY

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Glycosides from *Phlomis lunariifolia*

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Received 26 November 2003; received in revised form 14 April 2004
Available online 23 July 2004

Abstract

An aliphatic alcohol glycoside, lunaroside 1-octen-3-yl *O*-β-apiofuranosyl-(1 → 6)-*O*-[β-glucopyranosyl-(1 → 2)]-β-glucopyranoside, a phenylethanoid glycoside, lunariifolioside 2-(3,4-dihydroxyphenyl)ethyl *O*-β-apiofuranosyl-(1 → 6)-*O*-[*O*-β-apiofuranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 3)]-4-*O*-(*E*)-caffeoyl-β-glucopyranoside and a flavone glycoside, luteolin 7-*O*-[4-*O*-acetyl-α-rhamnopyranosyl-(1 → 2)]-β-glucuronopyranoside, were isolated from the aerial parts of *Phlomis lunariifolia*, in addition to 15 known glycosides. Their structures were elucidated on the basis of extensive 1D and 2D NMR spectroscopic interpretation and chemical degradation.

Keywords: *Phlomis lunariifolia*; Lamiaceae; Aliphatic alcohol glycoside; Lunaroside, Phenylethanoid glycoside; Lunariifolioside; Flavone glycoside

1. Introduction

The genus *Phlomis* is represented by 34 species in the flora of Turkey (Huber-Morath, 1982). Some *Phlomis* species are used as tonics and stimulants in Anatolian folk medicine (Baytop, 1999). During our systematic phytochemical examination of *Phlomis* species, we have now investigated *Phlomis lunariifolia* Sm. (Lamiaceae). This paper describes the isolation and characterization of three new compounds (**1**–**3**) as well as 15 known compounds.

2. Results and discussion

The crude methanolic extract of the aerial parts of the plant was suspended in H_2O and extracted with $CHCl_3$ and *n*-BuOH, respectively. A combination of size exclusion chromatography, vacuum liquid chromatography, column chromatography (CC), MPLC and HPLC of the *n*-BuOH phase led to the isolation of 18 glycosides. The structures of the known compounds were identified as syringin (Sano et al., 1991), phlomuroside (Kamel et al., 2000), betulalbuside A (Calış et al., 1993), verbascoside (Sticher and Lahloub, 1982), forsythoside B (Endo et al., 1982), alyssonoside (Calış et al., 1992), 5-deoxypulchelloside I (Ganapaty et al., 1988), shanzhiside methylester (Takeda et al., 1977), lamalbide (Eigtved et al., 1974), chrysoeriol 7-*O*-β-D-glucopyranoside (Markham and Chari, 1982; Markham and Greger, 1994), luteolin 7-*O*-β-D-glucopyranoside (Markham and Greger, 1994), dihydrodehydrodiconiferyl alcohol 9′-*O*-β-D-glucopyranoside (Takeda et al., 1998), dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside (Chanzeng and Zhongjian, 1997), dehydrodiconiferylalcohol 9′-*O*-β-D-glucopyranoside (Jiang et al., 2001) and chlorogenic acid (Cheminat et al., 1988).

The molecular formula of compound **1** was determined as $C_{25}H_{44}O_{15}$ by positive-ion HR-MALDIMS, exhibiting a pseudomolecular ion peak at *m/z* 607.2573 $[M+Na]^+$ (calcd. for $C_{25}H_{44}O_{15}Na$, 607.2577). The ^1H NMR spectrum (Table 1) showed three anomeric protons at δ_H 4.41 (d, $J = 7.8$ Hz, H-1′ of core sugar glucose), 4.63 (d, $J = 7.8$ Hz, H-1″ of terminal glucose) and 4.99 (d, $J = 2.3$ Hz, H-1‴ of apiose), indicating the presence of three sugar units in **1**. The corresponding anomeric carbon resonances were observed at δ_C 101.9, 104.9 and 110.8, respectively, in the ^{13}C NMR spectrum (Table 1). The relatively large J values of the anomeric protons indicated β-orientations for both glucopyranosyl

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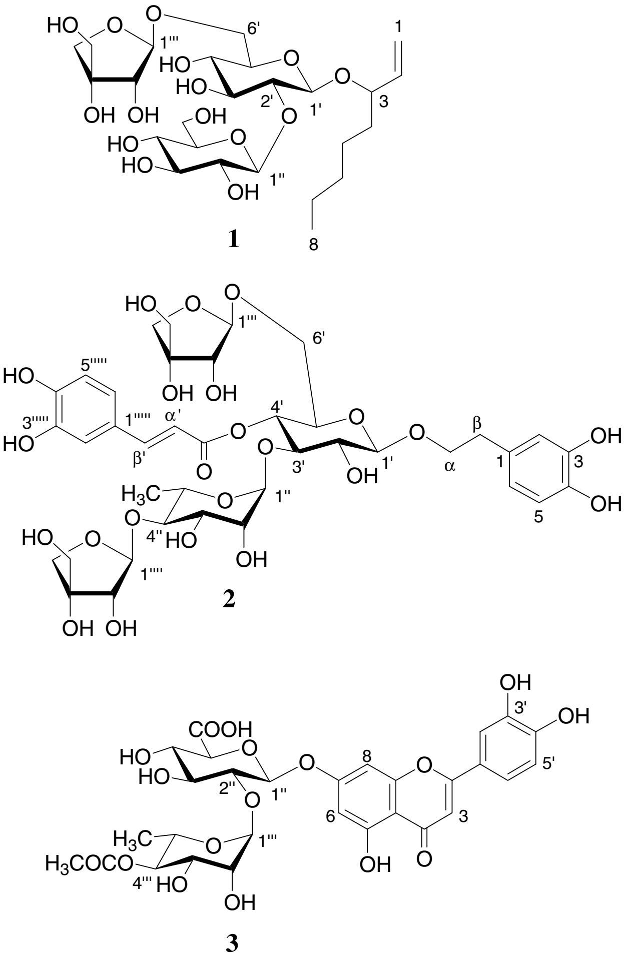
doi:10.1016/j.phytochem.2004.04.038

Table 1
^{1}H and ^{13}C NMR data, and HMBC correlations of compound **1** (δ_C: 75 MHz, δ_H: 500 MHz, CD_3OD)[a]

C/H Atom	1			
	δ_C	DEPT	δ_H [mult., J (Hz)]	HMBC ($C \rightarrow H$)
Aglycone				
1	117.0	CH_2	5.22 br d (17.7) 5.15 br d (10.4)	
2	140.7	CH	5.85 ddd (17.7, 10.4 7.4)	
3	84.2	CH	4.11 q like (7.4)	H-1′, H_2-1, H_2-5
4	35.9	CH_2	1.65[b] and 1.42[b]	
5	25.7	CH_2	1.35[b]	
6	33.1	CH_2	1.30[b]	H_3-8
7	23.8	CH_2	1.32[b]	H_3-8
8	14.5	CH_3	0.91 t (7.0)	
Glucose				
1′	101.9	CH	4.41 d (7.8)	H-3′
2′	82.3	CH	3.47 dd (7.8, 9.3)	H-1″
3′	77.7	CH	3.53 t (9.5)	
4′	71.5	CH	3.27[b]	
5′	76.8	CH	3.30[b]	
6′	68.3	CH_2	3.92 dd (12.0, 2.0) 3.56 dd (12.0, 5.2)	H-1‴
Glucose (T)				
1″	104.9	CH	4.63 d (7.8)	H-2″
2″	76.1	CH	3.22 dd (7.8, 9.3)	
3″	78.1	CH	3.57 t (9.5)	
4″	71.6	CH	3.27[b]	
5″	78.3	CH	3.25[b]	
6″	62.9	CH_2	3.84 dd (12.0, 2.0) 3.67 dd (12.0, 5.2)	
Apiose				
1‴	110.8	CH	4.99 d (2.3)	H_2-4‴
2‴	78.0	CH	3.88 d (2.3)	
3‴	80.6	C	–	H-4‴, H-5‴
4‴	75.0	CH_2	3.94 d (9.7) 3.75 d (9.7)	
5‴	65.7	CH_2	3.58 s	

T, terminal.
[a] Assignments confirmed by COSY, HMQC and HMBC spectra.
[b] Overlapped signal.



Scheme 1.

moieties. The ^{13}C NMR shifts of the anomeric carbon of the apiofuranosyl unit at δ_C 110.8 indicated also a β-orientation (Endo et al., 1982; Nicoletti et al., 1988). Acid hydrolysis gave glucose and apiose which were identified by TLC, confirming these deductions. The complete assignments of all proton and carbon resonances of the sugar moieties were based on the DQF-COSY, HSQC and HMBC (Table 1, Scheme 1) experiments. These experiments indicated that one of the two glucose units and apiose were terminal sugars, as shown by the absence of any glycosylation shift for their carbon resonances. On the other hand, C-2′ (δ_C 82.3) and C-6′ (δ_C 68.3) of the second glucose unit were suggested to be substituted by comparison with those of terminal glucose unit. In the HMBC spectrum, C-2′ and C-6′ of the core sugar glucose showed long-range correlations with the anomeric protons of terminal glucose (δ_H 4.63) and apiose (δ_H 4.99), respectively, revealing the sites of interglycosidic linkages in the triglycosidic sugar chain. The remaining eight carbon resonances and the corresponding protons were assignable to the aglycone. A DEPT-135 experiment sorted them into five methylenes (δ_C 117.0, 35.9, 33.1, 25.7 and 23.8), two methines (δ_C 140.7 and 84.2), and one methyl (δ_C 14.5) carbons. The COSY experiment showed that all corresponding proton resonances were observed in the same spin system. In the HMBC spectrum, the oxymethine carbon assigned as C-3 (δ_C 84.2) showed a long-range correlation with the anomeric proton of the core sugar glucose (δ_H 4.41) and vice versa supported the site of glycosidation. The signals assigned to the aglycone moiety were almost the same as those of ebractaetoside B (Kanchanapoom et al., 2001) and the mono and diglycosides of (3*R*)-1-octen-3-ol (matsutake alcohol) (Yamamura et al., 1998), previously isolated from *Acanthus ebracteatus* and *Mentha spicata*, respectively. The configuration at C-3 in **1** might be suggested to be in the *R*-form by comparing the ^{13}C NMR chemical shift of

C-3 with those reported for matsutake alcohol glycosides [δ_C 84.2 for **1**, 83.8 for ebractaetoside B (Kanchanapoom et al., 2001), 82.8 and 82.7 for (3*R*)-1-octen-3-yl 3-*O*-β-D-glucopyranoside and (3*R*)-1-octen-3-yl 3-*O*-β-D-primeveroside (Yamamura et al., 1998), respectively]. Based on these results, the structure of compound **1** was established as 1-octen-3-yl *O*-β-apiofuranosyl-(1 → 6)-*O*-[β-glucopyranosyl-(1 → 2)]-β-glucopyranoside, and named lunaroside.

Compound **2** was obtained as an amorphous powder with a molecular formula of $C_{39}H_{52}O_{23}$, as determined by data from positive-ion HR-MALDIMS, showing an $[M + Na]^+$ ion at *m/z* 911.2801 (calcd. for $C_{39}H_{52}O_{23}Na$, 911.2796) and ^{13}C NMR (Table 2). The ^1H NMR

Table 2
^1H and ^{13}C NMR data, and HMBC correlations of compound **2** (δ_C: 75 MHz, δ_H: 500 MHz, CD_3OD)[a]

C/H Atom	**2**			
	δ_C	DEPT	δ_H [mult., *J* (Hz)]	HMBC (C → H)
Aglycone				
1	131.4	C	–	H-α, H-β, H-5
2	117.1	CH	6.69 *d* (2.1)	
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	6.57 *dd* (8.0, 2.1)	H-β, H-2
α	72.4	CH_2	4.00 *m*, 3.71 *m*	H-1′
β	36.6	CH_2	2.79 *t* (7.3)	H-2, H-6
Glucose				
1′	104.1	CH	4.37 *d* (8.0)	H-α, H-2′
2′	76.1	CH	3.39 *dd* (8.0, 9.5)	
3′	81.7	CH	3.80 *t* (9.5)	H-1″
4′	70.8	CH	4.93 *t* (9.5)	
5′	74.6	CH	3.71[b]	
6′	68.4	CH_2	3.72[b], 3.48[b]	H-1‴
Rhamnose				
1″	102.9	CH	5.17 *d* (1.7)	H-3′
2″	72.3	CH	4.07 *dd* (1.7, 3.4)	
3″	71.9	CH	3.73 *dd* (3.4, 10.0)	
4″	80.3	CH	3.58[b]	H-1⁗, H-6″
5″	70.4	CH	3.54[b]	
6″	18.5	CH_3	1.09 *d* (6.2)	
Apiose (C-6′)				
1‴	111.0	CH	4.90[b]	H_2-6′, H-2‴
2‴	78.1	CH	3.73 *d* (3.0)	H_2-5‴
3‴	80.6	C	–	H-1‴, H_2-4‴, H_2-5‴
4‴	75.1	CH_2	3.92 *d* (9.6), 3.75 *d* (9.6)	H_2-5‴
5‴	65.6	CH_2	3.54 br *s*	H-2‴
Apiose (C-4″)				
1⁗	112.0	CH	5.15 *d* (3.0)	H-4″, H-2⁗
2⁗	78.1	CH	3.96 *d* (3.0)	H_2-5⁗
3⁗	80.2	C	–	H-1⁗, H_2-4⁗, H_2-5⁗
4⁗	74.9	CH_2	4.05 *d* (9.6), 3.92 *d* (9.6)	H_2-5⁗
5⁗	65.2	CH_2	3.59 br *s*	H-2⁗
Caffeoyl				
1′′′′′	127.6	C	–	H-α′, H-β′, H-5′′′′′
2′′′′′	115.2	CH	7.06 *d* (1.8)	
3′′′′′	146.8	C	–	H-2′′′′′, H-5′′′′′
4′′′′′	149.8	C	–	H-2′′′′′, H-5′′′′′, H-6′′′′′
5′′′′′	116.5	CH	6.78 *d* (8.2)	
6′′′′′	123.2	CH	6.96 *dd* (8.2, 1.8)	H-2′′′′′
α′	114.7	CH	6.28 *d* (15.8)	H-β′
β′	148.0	CH	7.60 *d* (15.8)	H-2′′′′′, H-6′′′′′
C=O	168.1	C	–	H-4′, H-α′, H-β′

[a] Assignments confirmed by COSY, HMQC and HMBC spectra.

[b] Overlapped signal.

spectrum of **2** (Table 2) exhibited the characteristic signals belonging to (*E*)-caffeoyl (three aromatic protons as an ABX system and two *trans*-olefinic protons) and 3,4-dihydroxyphenylethanol (three aromatic protons as an ABX system and two coupled methylenes) moieties. Additionally, four anomeric proton resonances appeared at δ_{H} 5.17 (*d*, $J = 1.7$ Hz, H-1″ of α-rhamnose), 5.15 (*d*, $J = 3.0$ Hz, H-1⁗ of β-apiose), 4.90 (H-1‴ of β-apiose) and 4.37 (*d*, $J = 8.0$ Hz, H-1′ of β-glucose), indicating its tetraglycosidic structure. The complete assingnments of all proton and carbon resonances were based on the DQF-COSY, HSQC and HMBC (Table 2, Scheme 1) experiments. The ^{13}C NMR spectroscopic data confirmed the tetraglycosidic sugar chain in **2**, exhibiting four anomeric carbon resonances at δ_{C} 112.0, 111.0, 104.1 and 102.9, which show correlations with the anomeric protons of two apiose units, glucose and rhamnose, respectively. These results were supported by the acidic hydrolysis of **2** yielding glucose, apiose and rhamnose. The proton and the carbon chemical shifts due to the apiose units indicated that both have to be in terminal positions. These results suggested that the structure of **2** is closely related to that of marruboside (Sahpaz et al., 2002), which contains the same molecular subunits, except for the glycosidic linkages. The carbon resonances arising from the glucose and rhamnose moieties revealed that the glucose unit is to be glycosylated at C-3′ (δ_{C} 81.7) and C-6′ (δ_{C} 68.4), while rhamnose unit at C-4″ (δ_{C} 80.3). The significant deshielding of H-4′ (δ_{H} 4.93) of glucose indicated that the caffeoyl unit was attached to C-4′(OH) position of glucose. Finally, HMBC experiment permitted the determination of all the relevant interfragmental connectivities. Thus, cross-peaks were observed between α-C atom (δ_{C} 72.4) of the phenethyl moiety and the H-1′ (δ_{H} 4.37) of the glucose unit, C-3′ (δ_{C} 81.7) of the glucose unit and H-1″ (δ_{H} 5.17) of the rhamnose, the carbonyl carbon (δ_{C} 168.1) of the caffeoyl moiety and H-4′ (δ_{H} 4.93) of the glucose, C-6′ (δ_{C} 68.4) of the glucose unit and H-1‴ (δ_{H} 4.90) of the first apiose, and C-4″ (δ_{C} 80.3) of the rhamnose unit and H-1⁗ (δ_{H} 5.15) of the second apiose. Therefore, the structure of **2** was established as 2-(3,4-dihydroxyphenyl)ethyl *O*-β-apiofuranosyl-(1 → 6)-*O*-[*O*-β-apiofuranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 3)]-4-*O*-(*E*)-caffeoyl-β-glucopyranoside, for which the trivial name lunariifolioside is proposed.

Table 3
^{1}H and ^{13}C NMR data, and HMBC correlations of compound **3** (δ_{C}: 75 MHz, δ_{H}: 500 MHz, CD_3OD)[a]

C/H Atom	3			
	δ_{C}	DEPT	δ_{H} [mult., *J* (Hz)]	HMBC (C → H)
Aglycone				
2	164.5	C	–	H-3, H-2′, H-6′
3	103.0	CH	6.73 *s*	
4	181.9	C	–	H-3
5	161.1	C	–	H-3, H-6
6	99.3	CH	6.36 *d* (2.0)	H-8
7	162.6	C	–	H-6, H-8, H-1″
8	94.2	CH	6.71 *d* (2.0)	H-6
9	157.0	C	–	H-8
10	105.4	C	–	H-3, H-6, H-8
1′	121.0	C	–	H-3, H-5′
2′	113.3	CH	7.40 *d* (2.1)	H-6′
3′	145.9	C	–	H-5′
4′	150.3	C	–	H-2′, H-5′, H-6′
5′	116.1	CH	6.86 *d* (8.7)	
6′	119.1	CH	7.39 *dd* (8.7, 2.1)	H-2′
Glucuronic acid				
1″	97.7	CH	5.23 *d* (7.5)	
2″	75.5	CH	3.50[b]	H-1‴, H-3″
3″	77.4	CH	3.48 *t* (9.0)	H-2″, H-4″, H-5″
4″	72.0	CH	3.19 *t* (9.0)	H-3″, H-5″
5″	73.4	CH	3.65 *d* (9.0)	
6″	171.6	C	–	H-4″, H-5″
Rhamnose				
1‴	99.8	CH	5.22 *d* (1.8)	H-2″
2‴	70.3	CH	3.70 br *s*	H-1‴
3‴	67.9	CH	3.50[b]	H-1‴, H-2‴, H-4‴, H-5‴
4‴	74.0	CH	4.77 *t* (9.7)	H-2‴, H-5‴, H_3-6‴
5‴	65.7	CH	3.99 *m*	H-1‴, H-4‴, H_3-6‴
6‴	17.5	CH_3	1.10 *d* (6.3)	H-4‴, H-5‴
CO*C*H$_3$	20.9	CH_3	1.98 *s*	
*C*OCH$_3$	170.0	C	–	H-4‴, CO*C*H$_3$

[a] Assignments confirmed by COSY, HMQC and HMBC spectra.
[b] Overlapped signal.

Compound **3** was obtained as an amorphous yellow powder. Its molecular formula was established as $C_{29}H_{30}O_{17}$ on the basis of pseudomolecular ion at *m/z* 651.1565 $[M+H]^+$ (calcd. for $C_{29}H_{31}O_{17}$, 651.1560) in the positive-ion HR-MALDIMS and NMR data (Table 3). The UV spectrum exhibited absorption maxima (218, 253, 268 sh, 345 and 356 sh nm) suggestive of a flavone structure. The IR spectrum of **3** showed hydroxyl (3413 cm^{-1}), ester carbonyl (1710 cm^{-1}), chelated carbonyl (1660 cm^{-1}) and aromatic (1609 and 1506 cm^{-1}) absorption bands. The ^{1}H NMR spectrum of **3** exhibited an ABX system at δ_{H} 7.40 (*d*, $J = 2.1$ Hz, H-2′), 7.39 (*dd*, $J = 8.7$, 2.1 Hz, H-6′) and 6.86 (*d*, $J = 8.7$ Hz, H-5′) due to a 3′,4′-disubstitution pattern of ring B. Moreover, two meta-coupled doublets at δ_{H} 6.71 ($J = 2.0$ Hz, H-8) and 6.36 ($J = 2.0$ Hz, H-6) were consistent with 5,7-dioxygenated ring A. These resonances together with the singlet at δ_{H} 6.73 (H-3) and the corresponding carbon signals revealed the presence of a luteolin moiety as aglycone (Markham and Chari, 1982). Additionally, the resonances of two anomeric protons, indicative of the presence of one β- and one α-linked sugar unit (δ_{H} 5.23 *d*, $J = 7.5$ Hz, δ_{H} 5.22 *d*, $J = 1.8$ Hz, respectively) and an acetate methyl at δ_{H} 1.98 were observed. Assignments for all proton and carbon resonances were achieved by DQF-COSY, HSQC, and HMBC (Table 3) experiments, which indicated the presence of β-glucuronic acid and α-rhamnose as sugar units. The appearance of a downfield signal at δ_C 75.5 for C-2″ of the glucuronic acid unit and the long-range correlations between this carbon and the anomeric proton of the α-rhamnose unit (δ_H 5.22) and vice versa in the HMBC spectrum, revealed the presence of a (1 → 2)-glycosidic linkage between the β-glucuronic acid and α-rhamnose moieties. The acetyl unit was found to be located at the C-4‴(OH) of the rhamnose moiety, on the basis of the strong deshielding of the H-4‴ (δ_H 4.77 *t*, $J = 9.7$ Hz) and C-4‴ (δ_C 74.0) signals of the rhamnose unit. This assumption was also supported by the upfield shift of the C-3‴ and C-5‴ signals of the rhamnose due to β-effect of the esterification as well as the long-range correlation between the carbonyl carbon of the acetyl group and H-4‴ of rhamnose. The other significant cross-peak in the HMBC spectrum was observed between the C-7 of the aglycone (δ_C 162.6) and the anomeric proton of the glucuronic acid which indicated that the disaccharide unit was attached to C-7(OH). Thus, the structure of this new compound was established as luteolin 7-*O*-[4-*O*-acetyl-α-rhamnopyranosyl-(1 → 2)]-β-glucuronopyranoside.

Lunaroside (**1**) is a rare type of glycoside and this is the first report from the genus *Phlomis*. Lunariifolioside (**2**) is the another representative of tetrasaccharidic phenylethanoid glycosides isolated from nature up to now; the previous examples are magnolioside C (Hasegawa et al., 1988), ballotetroside (Siedel et al., 1997), trichosantoside B (Calış et al., 1999), marruboside (Sahpaz et al., 2002) and physocalycoside (Ersöz et al., 2003). This compound is also the second tetraglycosidic phenylethanoid isolated from the genus *Phlomis*; the first example, physocalycoside (Ersöz et al., 2003), has recently been isolated from *Phlomis physocalyx*.

3. Experimental

3.1. General

Optical rotations were measured on a Rudolph autopol IV Polarimeter using a sodium lamp operating at 589 nm. UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. IR spectra (KBr) were measured on a Perkin–Elmer 2000 FT-IR spectrometer. The 1D- and 2D-NMR spectra were obtained on a Bruker® AMX 300 instrument (300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C) and DRX 500 FT spectrometer (500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C), at 295 K, for all 1D and 2D-NMR experiments. A Bruker with XWIN NMR software package was used to acquire NMR data. Positive mode HRMALDIMS were recorded on a Ionspec-Ultima-FTMS spectrometer, 2,5-dihydroxybenzoic acid (DHB) as matrix. TLC analyses were performed on silica gel 60 F-254 and cellulose F precoated plates (Merck, Darmstadt) for glycosides and sugar analysis, respectively; detection by 1% vanillin/H_2SO_4 (glycosides) and aniline phthalate (sugars). For medium-pressure liquid chromatographic separations, a Büchi 681 pump, a Büchi 684 fraction collector, a Rheodyne injector, and Büchi glass columns (column dimensions 2.6 × 46 cm, and 1.8 × 35 cm) were used. For HPLC, Merck-Hitachi L-6200 pump, UV detector L-4000 and Spherisorp S5 ODS 2 column (5 μ; 250 × 16 mm; Knauer) were used. Silica gel 60 (0.063–0.200 mm; Merck, Darmstadt) was utilized for open column chromatography (CC) and vacuum liquid chromatography (VLC). LiChroprep C-18 (Merck) material was used for MPLC and VLC. Sephadex LH-20 (Fluka) was also used for further separations. For TLC comparison, sugar standarts D(+)-glucose (Merck, Darmsdat), L(+)-rhamnose (Aldrich, Steinheim), and alkaline and acid hydrolysis products of a known glycoside, forsythoside B, were used.

3.2. Plant material

Phlomis lunariifolia Sm. (Lamiaceae) was collected from İçel, Anamur, South Anatolia, Turkey, in May 2000. Plant material was identified by Prof. Dr. Hayri Duman (Department of Biology, Faculty of Science, Gazi University, Ankara) and the voucher specimens (HUEF 00029) have been deposited at the herbarium of the Department of the Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

3.3. Extraction and isolation

The air-dried and powdered aerial parts of *P. lunariifolia* (250 g) were extracted twice with MeOH (2 × 2.5 l) at 45 °C. The combined methanolic extracts were evaporated to dryness in vacuo (34 g, yield 13.5%). The crude extract was dissolved in H_2O and partitioned between $CHCl_3$ and *n*-BuOH, respectively. The lyophilized *n*-BuOH phase (11 g) was fractionated over silica gel-VLC. Employment of CH_2Cl_2, CH_2Cl_2–MeOH–H_2O mixtures (100:10:0–60:40:4) and MeOH afforded 10 main fractions, A–J. Fraction D (1.200 g) was subjected to C_{18}-medium pressure liquid chromatography (C_{18}-MPLC) eluting with increasing amount of MeOH in H_2O (15–100%) to afford 11 fractions, D_1–D_{11}. Fraction D_2 (53 mg) was further applied to polyamide CC, using H_2O as eluent to yield phlomuroside (6 mg) and syringin (11 mg). Fraction D_6 (109 mg) was similarly subjected to polyamide column chromatography using stepwise gradient of MeOH (0–100%) in H_2O to obtain a mixture of dihydrodehydrodiconiferyl alcohol 9′-*O*-β-D-glucopyranoside (major), dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside (minor) (15 mg), and dehydrodiconiferylalcohol 9′-*O*-β-D-glucopyranoside (6 mg).

Fraction D_4 (42 mg) was subjected to silica gel (10 g) CC using CH_2Cl_2–MeOH–H_2O (85:15:1 and 80:20:1) mixtures to afford betulalbuside A. Purification of fractions D_7 (292 mg) and D_8 (77 mg) by Sephadex LH-20 CC (MeOH) furnished luteolin 7-*O*-β-D-glucopyranoside (22.5 mg) and chrysoeriol 7-*O*-β-D-glucopyranoside (3.5 mg), respectively. Fraction E (1.794 g) was subjected to C_{18}-MPLC employing increasing amount of *i*-PrOH in H_2O (10–40%) to afford nine main fractions, E_1–E_9. Fraction E_1 was almost pure lamalbide (67 mg). Fraction E_2 (59 mg) was subjected to silica gel CC eluting with CH_2Cl_2–MeOH–H_2O (90:10:1–80:20:1), a mixture of 5-deoxypulchelloside I and shanzhiside methylester (14 mg), and lamalbide (4 mg). 5-Deoxypulchelloside I and shanzhiside methylester were further purified by preparative HPLC using MeOH–H_2O (15–85; isocratic; flow rate: 6 ml/min). Fraction E_4 (79 mg) was also applied to a silica gel column (CH_2Cl_2–MeOH–H_2O, 80:20:2) to yield alyssonoside (8 mg) and forsythoside B (9 mg). Luteolin 7-*O*-β-D-glucopyranoside (10 mg) was purified by Sephadex LH-20 CC (MeOH) from fraction E_7 (110 mg). Fraction E_8 (45 mg) was separated by silica gel CC (EtOAc–MeOH–H_2O, 100:16.5:13.5) to obtain compound **1** (lunaroside, 19 mg). Fraction F (2.23 g) was applied to C_{18}-MPLC (15–20%, *i*-PrOH) to yield forsythoside B (577 mg) and a mixture of forsythoside B and verbascoside (1.08 g). Verbascoside was further purified by silica gel CC eluting with EtOAc–MeOH–H_2O (100:16.5:13.5) mixture. Fraction G (1.06 g) was likewise subjected to C_{18}-MPLC (10–100%, MeOH) to yield chlorogenic acid (138 mg) along with five fractions, G_1–G_5. Fraction G_2 (123 mg) was rechromatographed over silica gel eluting with EtOAc–MeOH–H_2O (100:10:5) mixture to give forsythoside B (50 mg) and compound **2** (lunariifolioside, 5 mg). Repeated chromatography of fraction G_4 (37 mg) on a Sephadex LH-20 column (MeOH) yielded compound **3** (luteolin 7-*O*-[2″-*O*-(4‴-*O*-acetyl-α-L-rhamnopyranosyl)]-β-D-glucuronopyranoside, 5 mg). As a main compound of fractions H (799 mg), I (170 mg) and J (53 mg), chlorogenic acid was identified by TLC comparison (solvent system, CH_2Cl_2–MeOH–H_2O, 61:32:7).

3.4. Lunaroside (*1*)

Amorphous colorless solid; $[\alpha]_D^{20}$ −54° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 214 nm; IR(KBr) ν_{max}: 3424 (OH), 1420 (C=C), and 1076 (C–O–C) cm^{-1}; ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectra, see Table 1; HR-MALDIMS *m*/*z*: 607.2573 $[M+Na]^+$ (calcd. for $C_{25}H_{44}O_{15}Na$, 607.2577).

3.5. Lunariifolioside (*2*)

Amorphous pale yellow solid; $[\alpha]_D^{20}$ −88° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 219, 246 (sh), 289, 328 nm; IR(KBr) ν_{max} 3430 (OH), 1698 (conjugated ester C=O), 1630 (C=C), 1604, 1524 and 1451 (aromatic rings) cm^{-1}; ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectra, see Table 2; HR-MALDIMS *m*/*z*: 911.2801 $[M+Na]^+$ (calcd. for $C_{39}H_{52}O_{23}Na$, 911.2796).

3.6. Luteolin 7-O-[2″-O-(4‴-O-acetyl-α-L-rhamnopyranosyl)]-β-D-glucuronopyranoside (*3*)

Amorphous yellow solid; $[\alpha]_D^{20}$ −87° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 218, 253, 268 (sh), 345, 356 (sh) nm; IR(KBr) ν_{max} 3413 (OH), 1710 (ester carbonyl), 1660 (chelated C=O), 1609 and 1506 (aromatic rings) cm^{-1}; ^1H (500 MHz, CD_3OD) and ^{13}C NMR (125 MHz, CD_3OD) spectra, see Table 3; HR-MALDIMS *m*/*z*: 651.1565 $[M+H]^+$ (calcd. for $C_{29}H_{31}O_{17}$, 651.1560).

3.7. Acid hydrolysis of forsythoside B

Forsythoside B (20 mg) was dissolved in 2N HCl (2 ml) and heated at 100° for 3 h, cooled and extracted with EtOAc two times (each 5 ml). The aqueous phase was neutralized passing through Dowex (Cl^- form) and evaporated to dryness. The residue was used for sugar standards (glucose, rhamnose and apiose; 1:1:1) in TLC using pyridine-EtOAc–AcOH–H_2O (36:36:7:21) as solvent mixture.

3.8. Acid hydrolysis of compounds *1* and *2*

Compounds **1** and **2** (each 5 mg) were separately dissolved in 1 ml 2N HCl and heated at 100° for 3 h. After usual workup as described for forsythoside B (3.7s), each residue was examined for sugars.

Acknowledgements

This work was supported in part by the Scientific and Technical Research Council of Turkey (TUBITAK, Project No.: SBAG-2304). The authors thank Dr. Walter Amrein (Institute of Organic Chemistry, ETH Zurich, Switzerland) for recording all mass spectra.

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