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FUNDA NURAY YALÇIN

TAYFUN ERSÖZ

PINAR AKBAY

İHSAN ÇALIŞ

ALİ ARSLAN DÖNMEZ

See next page for additional authors

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Authors
FUNDA NURAY YALÇIN, TAYFUN ERSÖZ, PINAR AKBAY, İHSAN ÇALIŞ, ALİ ARSLAN DÖNMEZ, and OTTO STICHER

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Phenolic, Megastigmane, Nucleotide, Acetophenon and Monoterpene Glycosides from *Phlomis samia and P. carica*

Funda Nuray YALÇIN, Tayfun ERSÖZ, Pınar AKBAY, İhsan ÇALIŞ
Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy
TR-06100 Ankara, TURKEY
e-mail: funyal@hacettepe.edu.tr
Ali A. DÖNMEZ
Hacettepe University, Faculty of Science, Department of Biology,
TR-06532 Ankara, TURKEY
Otto STICHER
ETH-Zurich, Department of Applied BioSciences, Institute of Pharmaceutical Sciences,
Winterthurerstr. 190, CH-8057 Zürich, SWITZERLAND

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Phytochemical investigations on the aerial parts of *Phlomis samia* resulted in the isolation of a simple phenolic glucoside, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**); a megastigmane glucoside, phlomuroside (=3-hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside) (**2**); and a nucleotide glycoside, uridine (**3**). From the aerial parts of *P. carica*, the same phenolic glucoside, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**); as well as an acetophenon glucoside, picein (**4**); and 2 monoterpenoid glucosides, −betulalbuside A (**5**) and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**6**)− were isolated and identified. The structure elucidation of the isolates was based on spectroscopic evidence.

Key Words: Acetophenon glucoside, betulalbuside A, 2,6-dimethoxy-4-hydroxy- phenol-1-*O*-β-D-glucopyranoside, 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside, Lamiaceae, megastigmane glucoside, monoterpenoid glucosides, nucleotide glycoside, *Phlomis samia*, *P. carica*, phenolic glucoside, phlomuroside, picein, uridine.

Introduction

In a previous communication, we reported the isolation of a number of iridoid, phenylethanoid, lignan and monomeric phenylpropanoid glycosides from the overground parts of 2 *Phlomis* taxa, *P. samia*, and *P. carica*[1]. In continuing work on the same species, a simple phenolic glucoside, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**), together with a megastigmane glucoside, phlomuroside (=3-hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside) (**2**) and a nucleotide glycoside, uridine (**3**), from *P. samia.* In addition, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**), along with an acetophenon glucoside,

picein (**4**), as well as 2 monoterpenoid glucosides, betulalbuside A (**5**) and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**6**) from *P. carica,* were isolated by means of various chromatographic techniques. The current study describes the isolation and structure elucidation of isolates (**1-6**) from the title plants.

Experimental

General Experimental Procedures and Plant Materials: The general experimental procedures as well as the plant materials were the same as reported elsewhere[1].

Extraction and Isolation: The extraction and isolation procedure was reported previously[1]. Compounds **1-6** were isolated as given below:

P. samia: Fr B_7 (150 mg), obtained as reported previously[1], was subjected to C_{18}-MPLC (Lichroprep RP-18). Elution with a 5% stepwise gradient of MeOH in H_2O (0-40%) afforded compounds **1** (1.1 mg), **2** (8 mg), and **3** (3 mg).

P. carica: Fr B_1 (65 mg), as reported previously[1], was fractionated on C_{18}-MPLC (Lichroprep RP-18) eluting with a 5% stepwise gradient of MeOH in H_2O (10-20%) to yield compounds **1** (4 mg) and **4** (6 mg). Fr B_2 (200 mg), obtained as described previously[1], was rechromatographed on C_{18}-MPLC (Lichroprep RP-18) with a 5% stepwise gradient of MeOH in H_2O (5-40%) to give a mixture of **5** and **6** (8 mg). Despite all efforts, this mixture could not be separated by any chromatographic technique.

Results

2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (1): UV λ_{max} (MeOH) nm: 214, 225, 278; IR υ_{max} (KBr) cm^{-1}: 3400, 2935, 1580, 1632; ^1H NMR (CD_3OD, 300.13 MHz), data were identical to those reported in the literature[2–4].

Phlomuroside (2): UV λ_{max} (MeOH) nm: 210; IR υ_{max} (KBr) cm^{-1}: 3400, 2950, 1640, 1250; ^1H (CD_3OD, 300.13 MHz) and ^{13}C (CD_3OD, 75.5 MHz) NMR: Table 1.

Uridine (3): UV λ_{max} (MeOH) nm: 220; IR υ_{max} (KBr) cm^{-1}: 3400, 3200, 2950, 1650, 1250; ^1H (CD_3OD, 300.13 MHz) and ^{13}C (CD_3OD, 75.5 MHz) NMR: Table 2.

Picein (4): UV λ_{max} (MeOH) nm: 263; IR υ_{max} (KBr) cm^{-1}: 3371, 1661, 1605, 1590, 1511; ^1H NMR (CD_3OD, 300.13 MHz) data were identical to those reported in the literature[5,6].

Betulalbuside A (5): ^1H (CD_3OD, 300.13 MHz), and ^{13}C (CD_3OD, 75.5 MHz,) NMR: Table 3.

1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (6): ^1H (CD_3OD, 300.13 MHz), and ^{13}C (CD_3OD, 75.5 MHz,) NMR: Table 3.

Discussion

Some fractions previously obtained from the polyamide CC fractions of the *n*-BuOH extracts of *P. samia* and *P. carica*[1] were refractionated by RP-18 MPLC to yield compounds **1-6** (Figure 1). Compounds **1** and **4** were identified by comparing their spectroscopic data with those reported in the literature as 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**)[2–4] and picein (**4**)[5,6]. The structure elucidation of compounds **2**, **3**, **5** and **6** was based on the following evidence.

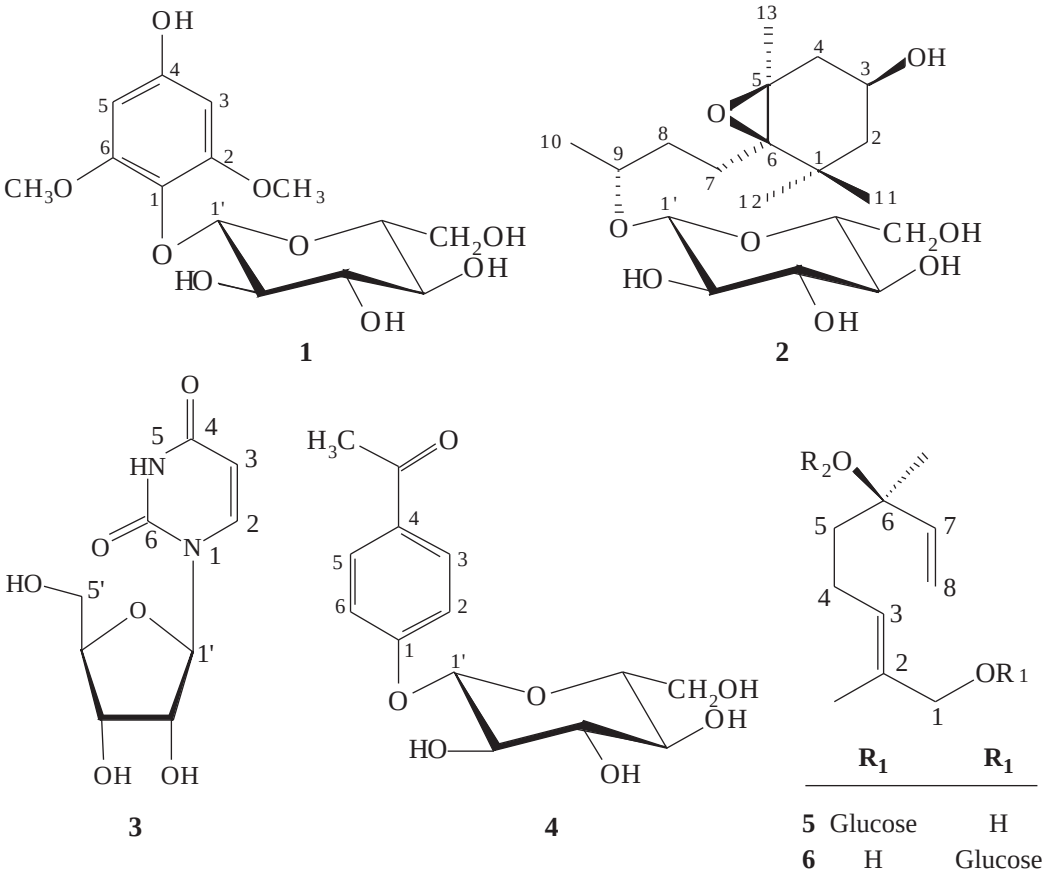

Figure 1. Compounds (**1-6**) isolated from *P. samia* and *P. carica.*

Compound **2** was obtained as a colorless amorphous powder. The UV spectrum of **2** showed a maximum at λ_{max} 210 nm and the IR spectrum exhibited absorption bands for OH (3400 cm^{-1}), C-H (2950 cm^{-1}), C=C (1640 cm^{-1}), and -C-O- (1250 cm^{-1}) functions. The ^{13}C and ^{1}H NMR spectra of **2** (Table 1) showed the presence of a β-glucopyranosyl moiety due to the signals at δ_C 102.6 and δ_H 4.35 (d, J = 7.8 Hz). The ^{13}C NMR spectrum exhibited 19 distinct carbon resonances, 6 of which were assigned for the β-glucopyranosyl unit. In the DEPT-135 spectrum, 4 methyl, 3 methylene, and 9 methine carbon resonances were assigned for **2**. The remaining quaternary carbons were ascribed to 3 quaternary cyclic carbons, 2 of which are oxygenated (δ_C 68.1, 71.2). Likewise, the carbon resonance at δ_C 64.5 (d) was assigned to an oxygen-bearing carbon atom at C-3. Moreover, the chemical shift values of δ_C 68.1 (s) (assigned as C-5) and δ_C 71.2 (s) (assigned as C-6), were characteristic for a 5,6-epoxy function. In the ^{1}H NMR spectrum, the singlet signals at δ_H 0.97, 1.12 and 1.19 were assigned to the tertiary methyl groups at C-13, C-12 and C-11, respectively, whereas a doublet signal at δ_H 1.28 (J= 6.4 Hz) was attributed to a secondary methyl function at C-10. The ^{13}C NMR resonances at δ_C 20.2 (C-13), 25.1 (C-12), 30.1 (C-11) and 21.0 (C-10) supported the presence of methyl groups. In addition, both the chemical shift values and the coupling constants of the proton resonances at δ_H 5.90 (d, J= 15.5 Hz) and 5.73 (dd, J = 15.5/6.5 Hz) indicated the presence of *trans*-olefinic protons in **2**. This assumption was based on the carbon resonances at δ_C 127.8 (d, C-7) and 137.2 (d, C-8). Therefore, the ^{13}C NMR, DEPT-135 and ^{1}H-^{1}H COSY spectra revealed that the aglycone

of compound **2** is a megastigmane of 3-hydroxy-5,6-epoxy-β-ionol structure[7,8]. The relative configuration of the epoxy function was determined based on the NOESY experiment. The nOe correlation observed between H-3 and H-13 suggested the β-configuration of the epoxy group at C-5 and C-6. The attachment of the glucose unit was assigned as C-9, due to the strong downfield shift of the C-9 signal (δ_C 76.9). The absolute configuration of C-9 was assigned as *R* by comparing the ^{13}C NMR data at C-9 (δ_C 76.9) and C-10 (δ_C 21.0) to those closely similar megastigmanes[7−9]. Consequently, the structure of **2** was identified as (3 *S*, 5 *S*, 6 *R*, 9 *R*)-3-hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside (=phlomuroside)[8,10].

Table 1. ^{13}C (CD_3OD, 75.5 MHz) and ^{1}H (CD_3OD, 300.15 MHz) NMR data of phlomuroside (**2**).

C/H atom	Mult.	δ_C (ppm)	δ_H (ppm) *J* (Hz)
Aglycon			
1	C	36.0	-
2	CH_2	48.0	1.22 dd (12.4/11.0)
			2.26 dd (14.2/3.1)
3	CH	64.5	3.73 m
4	CH_2	41.6	2.27 dd (14.1/6.5))
			1.61 dd (14.2/9.2)
5	C	68.1	-
6	C	71.2	-
7	CH	127.8	5.90 d (15.5)
8	CH	137.2	5.73 dd (15.5/6.5)
9	CH	76.9	4.42 t (6.2)
10	CH_3	21.0	1.28 d (6.4)
11	CH_3	30.1	0.97 s
12	CH_3	25.1	1.12 s
13	CH_3	20.2	1.19 s
Glucose			
1′	CH	102.6	4.35 d (7.8)
2′	CH	75.3	3.17 dd (7.8/10.2)
3′	CH	78.1	3.30 br s
4′	CH	71.3	3.33 d (7.6)
5′	CH	77.9	3.22 t (9.3)
6′	CH_2	62.5	3.68 dd (11.9/4.9)
			3.82 dd (11.9/2.4)

br s: broad singlet

Compound **3** was obtained as an amorphous powder. The IR spectrum showed absorption bands at 3400 (OH), 2950 (C-H), 1650 (C=O), 1250 (C-O) and 3200 (N-H) cm^{-1} and the UV spectrum exhibited a maximum at 220 nm. In the ^{1}H NMR (Table 2) spectrum of **3** the anomeric proton signal at δ_H 5.40 (d, J = 4.4 Hz) showed the presence of a sugar unit in **3**. However, the ^{13}C NMR (Table 2) spectrum of **3** exhibited 9 carbon resonances, 5 of which were assignable for the sugar unit, indicating the presence of a pentose moiety. A comparison of the ^{1}H and ^{13}C NMR data of the sugar residue with those given in the literature revealed the pentose unit in **3** to be an α-ribose[11]. On the other hand, the downfield shifted proton resonances, appearing as an AB system (J_{AB} = 8.1 Hz) at δ_H 5.69 and 8.02, were assigned to H-5 and H-6, respectively. Furthermore, the quaternary carbon resonances at δ_C 166.1 (C-5) and 152.0 (C-6) were attributable to 2 carbonyl functions. The complete interpretation of the NMR data together with the IR

Table 2. ^{13}C (CD_3OD, 75.5 MHz) and ^{1}H (CD_3OD, 300.15 MHz) NMR data of uridine (**3**).

C/H atom	DEPT	δ_C (ppm)	δ_H (ppm) J (Hz)
Aglycon			
1	-		-
2	C	152.0	-
3	-		-
4	C	166.1	-
5	CH	102.6	5.69 d (8.1)
6	CH	142.7	8.02 d (8.1)-
Ribose			
1′	CH	102.6	5.40 d (4.4)
2′	CH	75.4	4.16[(a)]
3′	CH	71.3	4.16[(a)]
4′	CH	86.4	4.01 m
5′	CH_2	62.3	3.85 dd (12.2/2.7)
			3.75 dd (12.5/2.7)

[(a)]Signal pattern unclear due to overlapping

Table 3. ^{13}C (CD_3OD, 75.5 MHz) and ^{1}H (CD_3OD, 300.13 MHz) NMR data and HMBC correlations of betulalbuside A (**5**) and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**6**).[(*)].

			5		**6**	
C/H Atom	DEPT-135	δ_C (ppm)	δ_H (ppm) J (Hz)	δ_C (ppm)	δ_H (ppm) J (Hz)	HMBC (H→C)
Aglycon						
1	CH_2	75.9	4.20 d (11.4)	69.0	3.90 br s	C-2, C-3, C-9, C-1′
			4.03 d (11.4)			**C-2, C-3, C-9**
2	C	132.9		135.8		
3	CH	130.1	5.48 dt (1.3/7.3)	127.0	5.40 dt (1.3/7.3)	C-9, C-1
						C-9,C-1
4	CH_2	23.5	2.10 m	23.2	2.20 m	C-5, C-2, C-3
						C-5, C-6, C-2, C-3
5	CH_2	42.9	1.51 m	41.2	1.65 m	C-3, C-4, C-6, C-7, C-10
						C-3, C-4, C-6, C-7, C-10
6	C	75.2		81.3		
7	CH	146.2	5.91 dd (17.7/11.0)	144.5	6.10 dd (17.7/11.0)	C-5, C-6, C-10
						C-5, C-6, C-10
8	CH_2	112.1	5.22 dd (17.7/1.5)	115.0	5.22 dd (17.7/1.5)	C-6, C-7
			5.03 dd (10.8/1.5)		5.16 dd (11.0/1.3)	**C-6, C-7**
9	CH_3	14.1	1.67 s	13.7	1.66 s	C-1, C-2
						C-1, C-2
10	CH_3	27.6	1.25 s	23.5	1.34 s	C-5, C-6, C-7
						C-5, C-6, C-7
Glucose						
1′	CH	102.6	4.24 d (7.8)	99.3	4.32 d (7.8)	C-1
2′	CH	75.1	3.10-3.35[(a)]	75.1	3.10-3.35[(a)]	
3′	CH	77.9	3.10-3.35[(a)]	77.9	3.10-3.35[(a)]	
4′	CH	71.7	3.10-3.35[(a)]	71.7	3.10-3.35[(a)]	
5′	CH	78.2	3.10-3.35[(a)]	78.2	3.10-3.35[(a)]	
6′	CH_2	62.8	3.66 dd (11.9/5.7)	62.8	3.79 dd (11.9/2.4)	
6′	CH_2	62.8	3.86 dd (11.9/2.3)	62.8	3.63 dd (11.9/5.6)	

[(*)]HMBC data: plain values for **5**, bold values for **6**.
[(a)]Signal pattern unclear due to overlapping
br s: broad singlet

absorption band observed at 3200 cm^{-1}, characteristic to the N-H function, revealed that **3** was a nucleotide glycoside[12]. Based on its NMR data and a comparison of the data given in the literature, compound **3** was identified as uridine[12–14].

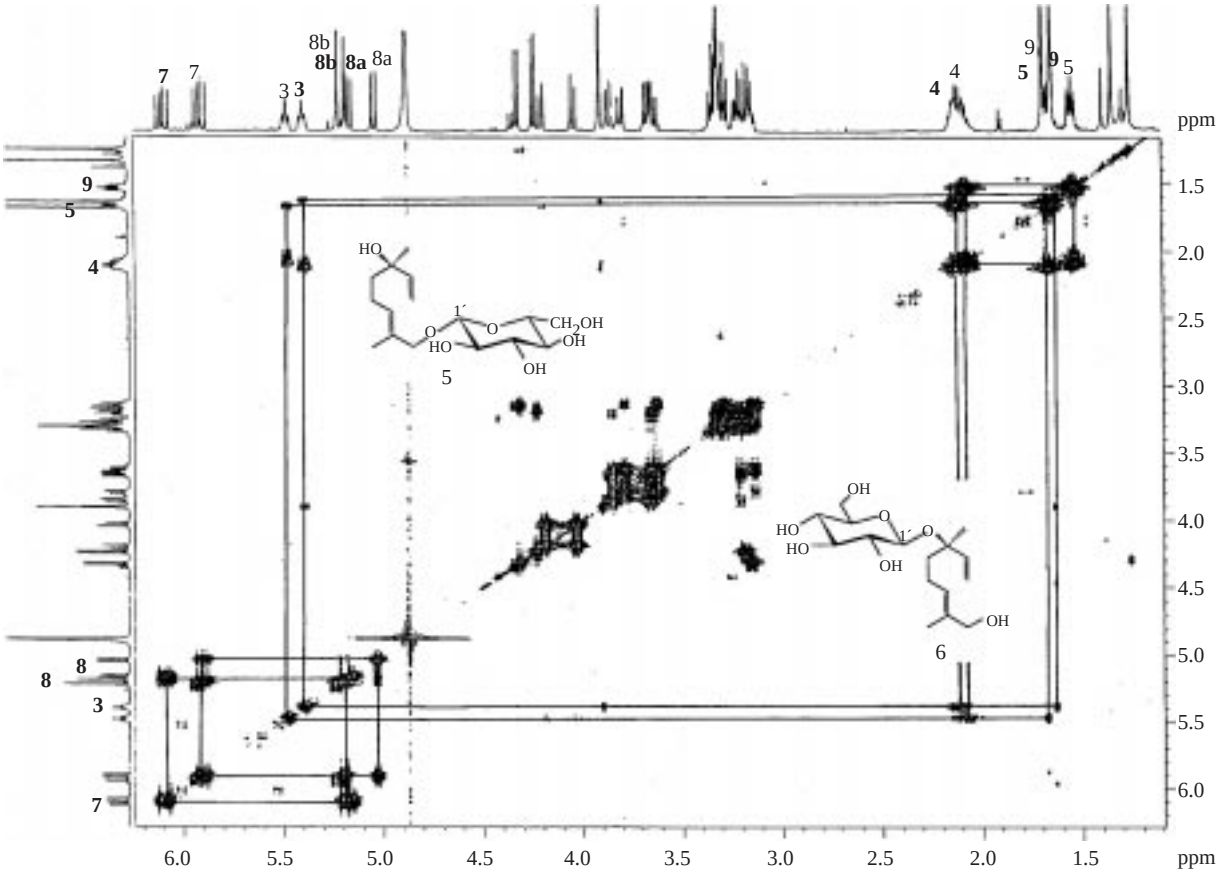

Figure 2. ^{1}H,^{1}H-DQF-COSY spectrum$^{(*)}$ of the mixture of **5** and **6** (CD_3OD, 500 MHz). $^{(*)}$**5**: plain numbers; **6**: bold numbers.

Compounds **5** and **6** were obtained as a mixture (1:1). Although this mixture could not be separated chromatographically, detailed 1D- and 2D-NMR experiments allowed the unambigious assignment of all carbon and proton resonances. In the ^{1}H NMR (Table 3) spectrum the singlet signals at δ_H1.25, 1.34, 1.66 and 1.67 were assigned to the tertiary methyl groups. A complete interpretation of the remaining NMR data relied on the results of DQF-COSY, HSQC and HMBC experiments. Anomeric proton signals appeared at δ_H 4.24 (d, J = 7.8 Hz) and 4.32 (d, J = 7.8 Hz) and the resonances in the region of δ_H 3.10-3.90 together with the corresponding carbon resonances inferred from the HSQC spectrum suggested the presence of 2 β-glucopyranose units. A phase-sensitive gradient double-quantum COSY experiment (Figure 2) allowed us to establish the spin system sequences for both the sugar residues and the aglycon part. A detailed interpretation of the NMR data showed the presence of 2 monoterpeneoid units (**5**, **6**). Thus, the proton resonances appearing as 2 sets of an ABX system at 5.22 (1H, dd, J = 17.7/1.5) and 5.03 (1H, dd, J = 10.8/1.5) as well as 5.22 (1H, dd, J = 17.7/1.5) and 5.16 (1H, dd, J = 11.0/1.3) were ascribed to the vinylic protons at C-8 for **5** and **6,** respectively. An additional 2 sets of proton resonances at δ_H 5.91 (1H, dd,

J = 17.7/11.0 Hz) and 6.10 (1H, dd, J = 17.7/11.0 Hz), which were vicinally coupled to H_2-8 protons, were assigned to H-7 of the monoterpenoid units (**5**, **6**), respectively. Furthermore, in the ^{1}H-^{1}H COSY spectrum, H-3 (δ_H 5.48 dt, J = 1.3/7.3 Hz) of **5** was correlated to the vicinally coupled C-4 methylene protons (δ_H 2.10, 2H, m), which in turn were coupled to the vicinally coupled C-5 methylene protons (δ_H 1.51, 2H, m). Similar COSY correlations were observed for compound **6**, where H-3 (δ_H 5.40 dt, J = 1.3/7.3 Hz) was correlated to the vicinally coupled C-4 methylene protons (δ_H 2.20, 2H, m), which were mutually coupled to the vicinally coupled C-5 methylene protons (δ_H 1.65, 2H, m) as in the case of **5**. However, the ^{1}H NMR resonances of **5** did not exhibit any ^{1}H-^{1}H COSY interactions with those of **6**, suggesting that these 2 monoterpenoid units are 2 distinct compounds. On the other hand, a prominent ^{1}H-^{13}C HMBC (Figure 3) experiment permitted the determination of the attachment of the glucopyranose units. Thus, a HBMC cross-peak observed from the anomeric proton of the first glucose unit (δ_H 4.24) to the C-1 (δ_C 75.9, t) carbon atom of **5** showed the attachment of the glucose unit at C-1 in compound **5**. Likewise, heteronuclear long-range coupling observed between the anomeric proton of the second glucose moiety (δ_H 4.32) and the C-6 (δ_C 81.3, s) carbon atom of compound **6** proposed the glucose unit to be glycosylated at C-6 in **6**. Therefore, the structures of the compounds in the mixture were identified as betulalbuside A (**5**)[15] and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**6**)[15].

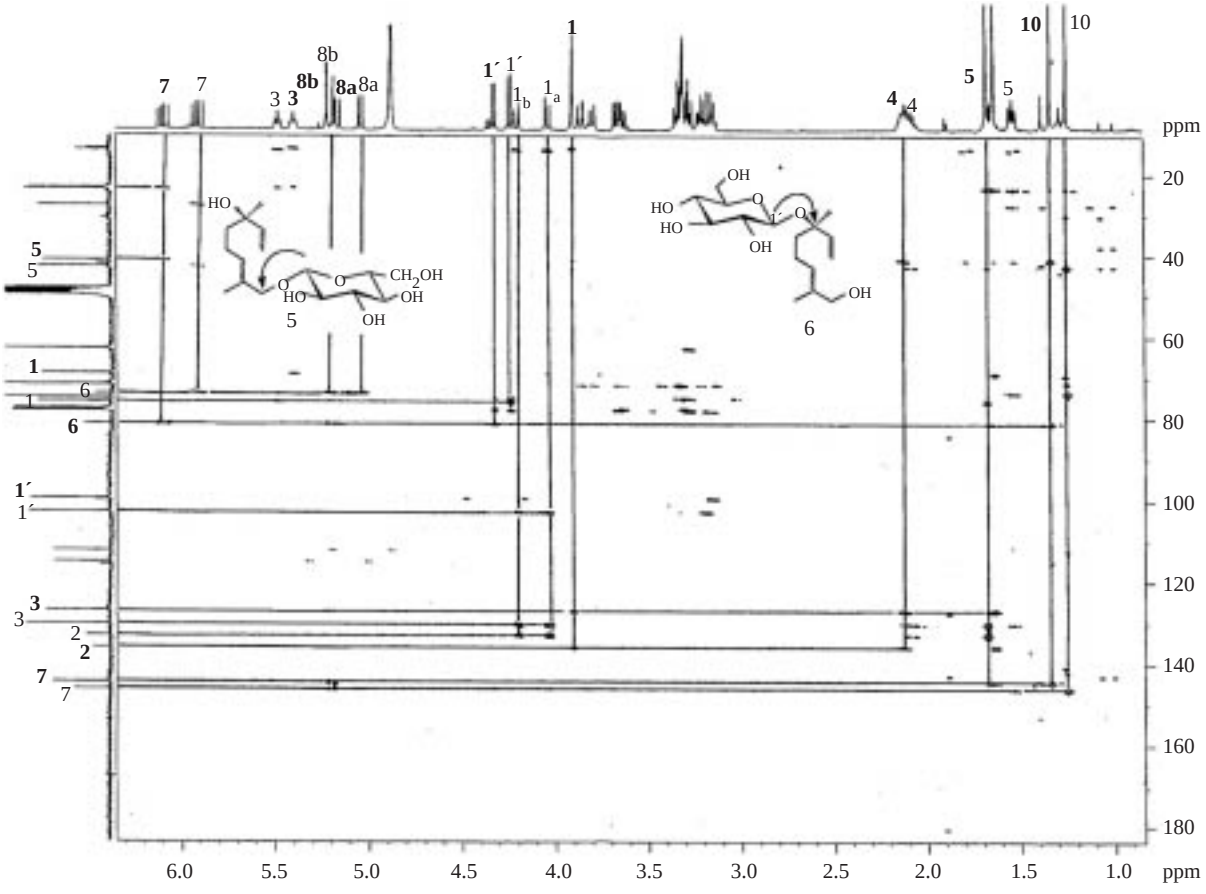

Figure 3. ^{1}H,^{13}C-HMBC spectrum$^{(*)}$ of the mixture of **5** and **6** (CD_3OD, 500 MHz). $^{(*)}$**5**: plain numbers; **6**: bold numbers.

Conclusion

Continuing our work on the previously investigated *Phlomis* species *P. samia* and *P. carica*, in addition to the previously isolated glycosides[1], we characterized a phenolic glucoside, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**), together with a megastigmane glucoside, phlomuroside (=3-hydroxy-5,6-epoxy-β-ionol-9-*O*-β-D-glucopyranoside) (**2**), and a nucleotide glycoside, uridine (**3**) from the aerial parts of *P. samia* by means of RP-18 MPLC. Chromatographic separations by RP-18 MPLC on *P. carica* resulted in the isolation of the same phenolic glucoside, 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**), along with an acetophenon glucoside, picein (**4**), and 2 monoterpenoid glucosides, betulalbuside A (**5**) and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**6**). Although, phlomuroside (**2**) was previously isolated from Egyptian *P. aurea*[10] samples, this is the first case of the isolation of a megastigmane glycoside from a Turkish *Phlomis* species. Previously, betulalbuside A (**5**) was reported from *P. armeniaca*[16] and *P. sieheana*[17]. However, this is the first case of the occurrence of 2,6-dimethoxy-4-hydroxyphenol-1-*O*-β-D-glucopyranoside (**1**), uridine (**3**), picein (**4**) and 1-hydroxylinaloyl-6-*O*-β-D-glucopyranoside (**5**) in the genus *Phlomis.* In our work, no additional glycoside could be detected in *P. monocephala.*

Acknowledgments

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