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Secondary Metabolites from *Phlomis kotschyana*

Ü. Şebnem HARPUT[1*], İclal SARACOĞLU[1], İhsan ÇALIŞ[1], Ali A. DÖNMEZ[2], Akito NAGATSU[3]

[1] *Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, 06100 Ankara-TURKEY*
e-mail: sharput@hacettepe.edu.tr

[2] *Hacettepe University, Faculty of Science, Department of Biology, 06532 Ankara-TURKEY*

[3] *Nagoya City University, Graduate School of Pharmaceutical Sciences, Department of Pharmacognosy, 467-8603 Nagoya-JAPAN*

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A lignan glycoside, syringaresinol-4′,4″-*O*-di-β-D-glucopyranoside (**1**) (=liriodendrin), 4 phenylethanoid glycosides, acteoside (**2**), isoacteoside (**3**), forsythoside B (**4**) and leucosceptoside B (**5**), a caffeic acid ester, chlorogenic acid (**6**) and 2 iridoid glucosides, lamiide (**7**) and auroside (**8**), were isolated from the methanolic extracts of the aerial parts of *Phlomis kotschyana* along with an acetophenone glycoside, 4-hydroxyacetophenone 4-*O*-[6′-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside (**9**). The structures of the isolated compounds were identified on the basis of spectroscopic (UV, IR, 1D- and 2D-NMR, and FAB-MS) methods.

Key Words: *Phlomis kotschyana,* Lamiaceae, Lignan glucoside, Phenylethanoid glycosides, Iridoid glucosides, Acetophenone glycoside.

Introduction

The genus *Phlomis* L. (Lamiaceae) is represented by 34 species in the Turkish flora, 21 of which are endemic[1]. Some *Phlomis* species are used as a tonic, stimulant, antipyretic and antidiabetic and for the treatment of allergies in Turkey and several other countries[2−5]. During our systematic phytochemical investigations on *Phlomis* species, we have studied almost all the members of the genus growing in Turkey. Our investigations of the aerial parts of these plants have resulted in the isolation of iridoids, phenylethanoid glycosides, lignans, neolignans, monomeric phenylpropanoids, monoterpene glucosides and diterpenoids[6−10]. In a continuation of our studies on the secondary metabolites of *Phlomis* species, we have further investigated *Phlomis kotschyana* Hub.-Mor. The present communication deals with the isolation and characterization of 9 glycosidic compounds from the title plant.

*Corresponding author

Experimental

General Experimental Procedures: The UV (MeOH, λ_{max}, nm) and IR (KBr, υ_{max}, cm^{-1}) spectra were recorded on a Shimadzu UV-240 and a Perkin Elmer 2000 FTIR spectrophotometer, respectively. Optic rotation was measured on a Rudolph Autopol-4 automatic polarimeter. NMR measurements were performed on a JEOL JNM-A 500 spectrometer in methanol-d_4 (^{1}H: 500 MHz; ^{13}C: 125 MHz). Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard. FAB-MS was recorded in an NBA matrix in the positive ion mode on a JEOL JMS-DX300 spectrometer. 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 performed on Labomatic (18.5 x 352 mm) and Büchi (25 x 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 pre-coated, commercial silica gel (Merck, 60 F_{254}) plates with $CHCl_3$-MeOH-H_2O (61:32:7, 70:30:3, 80:20:2) as a developing solvent system. Compounds **1-9** 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 kotschyana* Hub.-Mor. (Lamiaceae) was collected from the surroundings of Hatay in Inner Anatolia. A voucher specimen has been deposited in the Herbarium of the Biology Department, Hacettepe University, Ankara, Turkey (AAD 9473).

Extraction and Isolation: The air-dried aerial parts of *P. kotschyana* (280 g) were extracted with MeOH at 40 °C for 12 h (x2, 2 L). The combined extracts were evaporated under vacuum to give 26 g of crude extract. The MeOH extract was dissolved in H_2O (0.1 L). H_2O-insoluble material was removed by filtration. The filtrate was partitioned with $CHCl_3$ (x5, 100 mL), and the water fraction was lyophilized to yield 19 g dry weight. The water fraction was subjected to polyamide column chromatography and eluted with H_2O, followed by increasing concentrations of MeOH to give 6 fractions: A-F. [Fr. A (H_2O), 6.74 g; Fr. B (20% MeOH), 0.8 g; Fr. C (40% MeOH), 0.7 g; Fr. D (60% MeOH), 0.6 g; Fr. E (80% MeOH), 0.1 g, Fr. F (MeOH) 0.6 g].

The fraction eluted with H_2O from the polyamide column (Fr. A) was subjected to vacuum liquid chromatography using reversed-phase silica gel. Eluting with increasing amounts of MeOH in H_2O (0→100%) yielded 5 main fractions: A1-A5. Fraction A2 (60.0 mg) was found to contain compound **7.** Fraction A3 (69 mg) was chromatographed over silica gel by stepwise elution with $CHCl_3$-MeOH (90:10→70:30) and purified by Sephadex LH 20 (MeOH) to yield compounds **6** (8.1 mg) and **8** (6.6 mg)**.** Silica gel column chromatography of A4 (56 mg) and A5 (87 mg) eluting with $CHCl_3$-MeOH (90:10→80:20) resulted in the isolation of compounds **1** (7.7 mg) and **9** (11.2 mg), separately. Fraction B (0.8 g) was subjected to RP-MPLC eluting with increasing concentrations of MeOH and rechromatographed over a silica gel column eluting with $CHCl_3$-MeOH-H_2O (85:15:0→70:30:3) to give compound **5** (1.6 mg). Fraction C (0.7 g) was found to contain compound **4** in pure form. Chromatography of fractions D (0.6 g) and E (0.1 g) over silica gel by stepwise elution with $CHCl_3$-MeOH (90:10→70:30) gave crude compounds **2** (23.0 mg) and **3** (10. 1 mg), respectively.

Liriodendrin (1): UV, IR, ^{1}H (500 MHz, C_5D_5N) and ^{13}C (125 MHz, C_5D_5N) NMR data were identical to those reported in the literature[11].

Acteoside (2): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were

identical to those reported in the literature[12].

Isoacteoside (3): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[13].

Forsythoside B (4): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[14].

Leucosceptoside B (5): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[15].

Chlorogenic acid (6): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[16].

Lamiide (7): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[17].

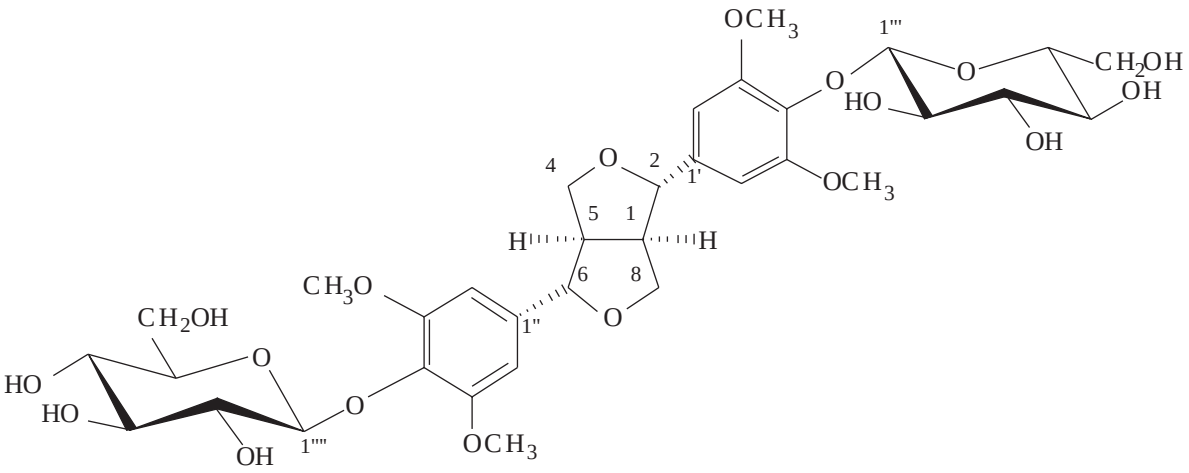
Auroside (8): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[18].

4-Hydroxyacetophenone 4-*O*-[6′-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside (9): UV, IR, ^{1}H (500 MHz, CD_3OD) and ^{13}C (125 MHz, CD_3OD) NMR data were identical to those reported in the literature[19].

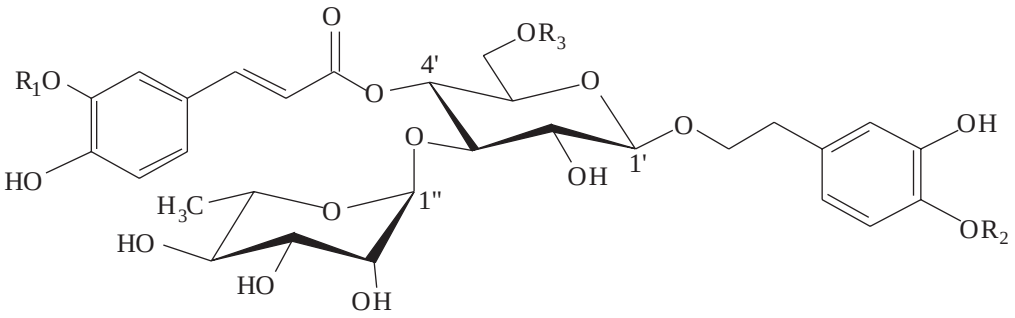
Results and Discussion

The methanol extract of *P. kotschyana* was suspended in water and partitioned with chloroform. The water fraction of the methanol extract was subjected to polyamide column chromatography and eluted with increasing concentrations of methanol to afford 6 main fractions. Repeated column chromatographies of the fractions resulted in the isolation of 9 compounds (**1-9**) in pure form (Figure). Compound **1** was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ – 42°, c = 0.1, DMSO). Its UV spectrum and IR characteristics confirmed its polyphenolic nature. The FAB-MS of compound **1** exhibited a pseudomolecular ion, $[M+Na]^+$, compatible with the molecular formula of $C_{34}H_{46}O_{18}$. Its ^{13}C NMR spectrum revealed 14 carbon signals. Corresponding protons in each of the carbon atoms were determined from a HMQC experiment indicating the 14 member structures. However, the molecular formula and ^{1}H and ^{13}C NMR spectra were in good agreement with the presence of a symmetrical diaryl-bicyclooctane structure (Table 1). Its ^{1}H NMR spectrum exhibited the characteristic signal at δ 6.93 (s, 4H) belonging to 1,3,4,5-tetrasubstituted benzene rings, a methoxy proton at δ 3.80 (s, 12H) and an anomeric proton signal at δ 5.82 (d, 2H, J = 7.1 Hz). Proton and carbon chemical shifts secured by 2D-NMR spectrum due to sugar moieties indicated the presence of 2 β-glucopyranose moieties in compound **1**. HMBC correlations between anomeric protons and the C-4′,4″ positions of the benzene rings confirmed the attachment of sugars to the aglycone. In addition, 2 oxymethylenes (δ 72.3, C-4,8), 2 oxymethines (δ 86.2, C-1,5) and 2 methines (δ 54.8, C-6,2) indicated the presence of a bis-tetrahydrofuran ring. HMBC correlations between C-1′,1″ (δ 135.1) and H-2,6 (δ 4.95 d) confirmed that compound **1** was a lignan glycoside with the 2,6-diaryl-3,7-dioxabicyclo-[3,3,0]-octane structure. The comparison of the signals of H-2/H-6, H-1/H-5 and H-2,4/H-2,8 with proton NMR data of diasteroisomeric compounds eudesmin, epieudesmin and diaeudesmin suggested that these positions were equatorial[20]. From these results, the structure of compound **1** was determined to be syringaresinol-4′,4″-*O*-di-β-D-glucopyranoside (=liriodendrin) by the comparison of its spectral data

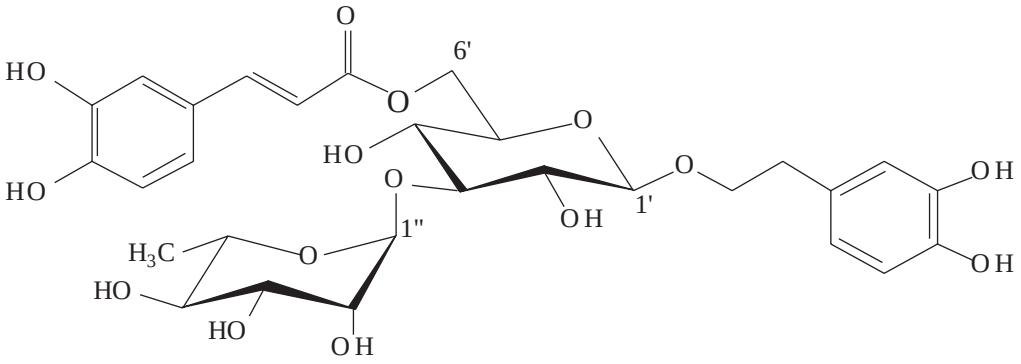
with those reported in the literature[11].



(1)

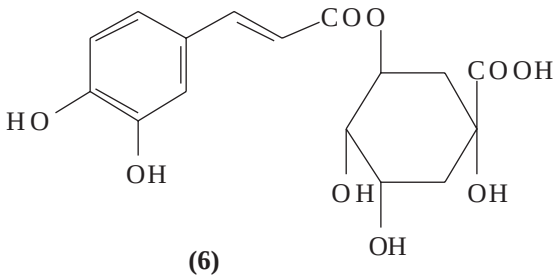


	R_1	R_2	R_3
(2)	H	H	H
(4)	H	H	Apiose
(5)	CH_3	CH_3	Apiose

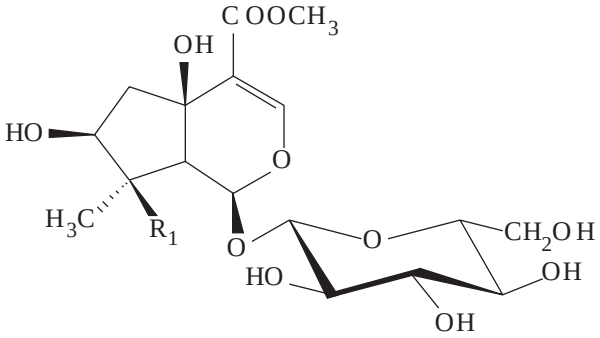


(3)

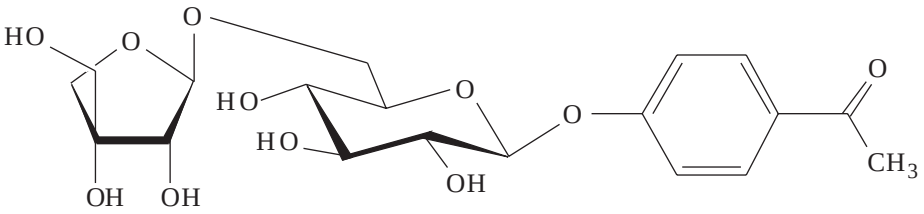
Figure. Isolated compounds from *Phlomis kotschyana.*



(6)



	R_1
(7)	OH
(8)	H



(9)

Figure. Contunied.

Table 1. ^{13}C and ^{1}H NMR spectral data and selected HMBC correlations for compound **1** (C_5D_5N; ^{13}C, 125 MHz; ^{1}H, 500 MHz).

C/H	DEPT	δ_C	δ_H	J(Hz)	HMBC (C→H)
1/5	CH	54.8	3.16 m		2/6, 4/8
2/6	CH	86.2	4.95 d	(4.0)	2′/2″/6′/6″
1′/1″	C	135.1			2/6
2′/2″/6′/6″	CH	104.9	6.93 s		2/6
3′/3″/5′/5″	C	154.0			OCH_3, 2′/2″/6′/6″
4′/4″	C	138.3			1‴/1⁗
4/8	CH_2	72.3	4.05 dd	(3.1/9.2)	4′/4″
			4.34 *		
OCH_3 x 4	CH_3	56.7	3.80 s		3′/3″/5′/5″
1‴/1⁗	CH	105.0	5.82 d	(7.1)	
2‴/2⁗	CH	76.1	4.27-4.35*		
3‴/3⁗	CH	78.4	4.27-4.35*		
4‴/4⁗	CH	71.7	4.27-4.35*		
5‴/5⁗	CH	78.7	3.94 m		
6‴/6⁗	CH_2	62.6	4.27 *		
			4.39 dd	(11.3/2.1)	

* Signal patterns are unclear due to overlapping.

Compounds **2**, **3, 4** and **5** were obtained as colorless, amorphous compounds. Their ^{1}H and ^{13}C NMR spectra exhibited signals belonging to caffeic acid (ferulic acid for **5**), 3,4-dihydroxyphenyl ethanol (3-hydroxy, 4-methoxyphenyl ethanol for **5**) and sugar moieties characteristic for the phenylethanoid glycosides. Their structures were determined as acteoside [**2**][12], isoacteoside [**3**][13], forsythoside B [**4**][14] and leucosceptoside B [**5**][15] by the complete interpretation of 1D and 2D NMR spectra and the comparison of their spectral data with those reported in the literature. The structure of compound **6** was found to be the same as that of chlorogenic acid, which was a very common compound in *Phlomis* species[16]. Compounds **7** and **8** were also obtained as colorless, amorphous powders. Their UV and IR absorptions were consistent with the presence of a 4-substituted iridoid structure conjugated with a methoxycarbonyl group. Based on a detailed examination of 1D and 2D NMR spectra, compounds **7** and **8** were identified as lamiide [**7**][17] and auroside [**8**][18].

Table 2. ^{13}C and ^{1}H NMR spectral data and selected HMBC correlations for compound **9** (CD_3OD; ^{13}C, 125 MHz; ^{1}H, 500 MHz).

C/H	DEPT	δ_C	δ_H	J (Hz)	HMBC (C→H)
1	C	132.7			1′
2	CH	117.4	7.16 d	(7.0)	
3	CH	131.8	7.98 d	(7.0)	1
4	C	163.1			2, 3, 5, 6
5	CH	131.8	7.98 d	(7.0)	1
6	CH	117.4	7.16 d	(7.0)	
7	C	199.8			3, 5
8	CH_3	26.6	2.56 s		1
1′	CH	101.6	4.98 d	(7.6)	2′, 3′
2′	CH	74.8	3.46 dd	(9.4/7.0)	
3′	CH	78.1	3.65 t	(9.3)	
4′	CH	71.6	3.36 t	(8.8)	
5′	CH	78.0	3.62 m		
6′	CH_2	68.8	3.62 dd	(10.9/1.5)	1″
			4.03 dd	(10.9/3.5)	
1″	CH	111.1	4.96 d	(2.7)	6′
2″	CH	77.9	3.91 d	(2.4)	
3″	C	80.5			
4″	CH_2	75.0	3.74 d	(9.6)	
			3.97 d	(9.6)	
5″	CH_3	65.5	3.58 s		

Compound **9** was isolated as an amorphous powder. Its ^{1}H NMR spectrum displayed 4 aromatic protons at δ 7.16 and δ 7.98 (each 2H, d, J = 7.0 Hz), which were observed as an AA′BB′ system suggesting the presence of a *p*-disubstituted benzene ring (Table 2). These resonances together with 2 carbon signals at δ 163.1 (C) and δ 26.6 (CH_3) as well as a sharp singlet in the ^{1}H NMR spectrum at δ 2.56 (3H) indicated the presence of acetophenone moiety. The presence of 2 proton signals represented by 2 doublets at δ 4.98 (J = 7.6 Hz) and δ 4.96 (J = 2.7 Hz), respectively, characteristic for the anomeric protons of 1 β-glucopyranosyl and 1 β-apiofuranosyl moiety, confirmed the presence of 2 sugar units. All protons of the 2 sugar units were assigned unambiguously from COSY and HMQC experiments that correlated all proton resonances with those corresponding carbons in each of the sugar units. The C-6′ of the glucose was shifted downfield

at δ 68.8, suggesting that β-apiose was attached to the C-6′ of the glucose. HMBC correlations between H_3-8/C-1, H-1′/C-4 and H1″/C-6′ confirmed the location of the 2 sugar units and the aceto group in the *p*-disubstituted benzene ring. Thus, the structure of the compound **9** was identified as 4-hydroxyacetophenone 4-*O*-[6′-*O*-β-D-apiofuranosyl]-β-D-glucopyranoside[19]. Compounds **1** and **9** were isolated for the second time from a *Phlomis* species and the detailed structure determination of these compounds was given for the first time in this study. Examination of the *Phlomis* species with regard to their chemical contents will be of assistance in clarifying chemotaxonomic classifications of the genus *Phlomis*.

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