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Neolignan, Flavonoid, Phenylethanoid and Iridoid Glycosides from *Phlomis integrifolia*

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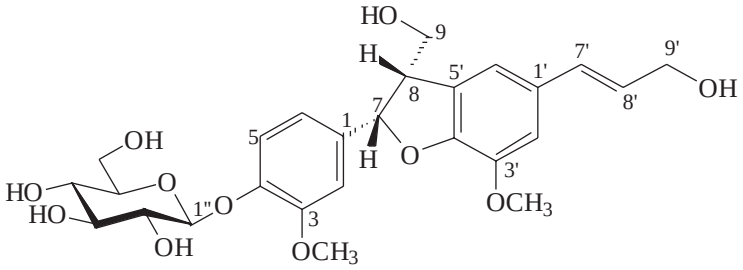
From the aerial parts of *Phlomis integrifolia* Hub.-Mor. (Lamiaceae) were isolated a neolignan glucoside, dehyrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside **(1)**, an ester flavone glycoside, chrysoeriol 7-*O*-(3$''$-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside **(2)**, four phenylethanoid glycosides, forsythoside B **(3)**, verbascoside (=acteoside) **(4)**, leucosceptoside A **(5)** and martynoside **(6)** along with an iridoid glucoside, lamiide **(7)**. The structure elucidation of the isolated compounds was carried out by spectroscopic (UV, IR, 1D- and 2D-NMR) methods. The isolation of neolignan glucoside dehyrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside **(1)** is reported for the first time from a *Phlomis* species as well as for the family Lamiaceae.

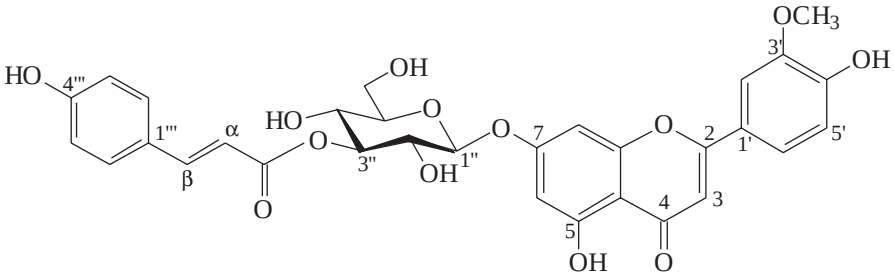
Key Words: *Phlomis integrifolia*, Lamiaceae, Neolignans, Phenylethanoids, Flavonoids, Iridoids.

Introduction

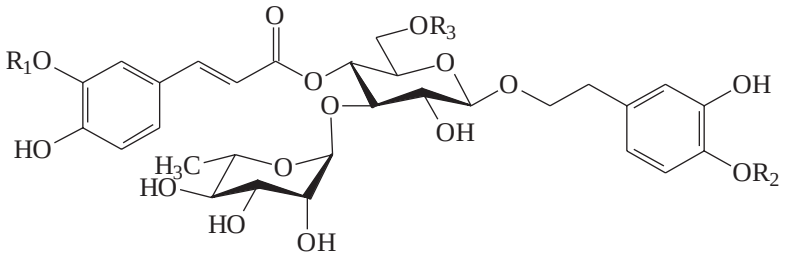
There are 34 *Phlomis* L. species (Lamiaceae) in the flora of Turkey[1]. Some *Phlomis* species are used as tonics and stimulants in phytotherapy[2]. During our systematic phytochemical investigations on *Phlomis* species, particular members of the genus growing in Turkey have been studied. Investigations on the aerial parts of these plants yielded in the isolation of iridoids, phenylethanoid glycosides, lignans, neolignans, monomeric phenylpropanoids, monoterpene glucosides and diterpenoids[3−6]. As part of our systematic studies on *Phlomis* species, the present study was conducted on an endemic species, *Phlomis integrifolia*, and a neolignan glucoside dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside **(1)**, and an ester flavone glycoside chrysoeriol 7-*O*-(3$''$-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside **(2)**, together with four phenylethanoid glycosides, forsythoside B **(3)**, verbascoside (=acteoside) **(4)** leucosceptoside A **(5)** and martynoside **(6)**, were isolated. Additionally, an iridoid glucoside lamiide **(7)** was isolated from the same plant.



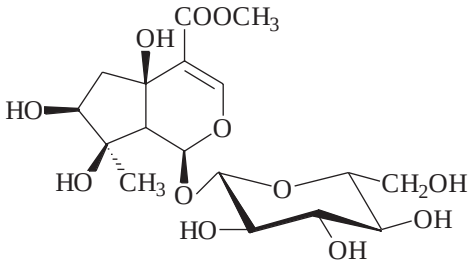
Dehydrodiconiferyl alcohol-4-O-β-D-glucopyranoside **(1)**



Chrysoeriol 7-O-(3''-O-trans-p-coumaroyl)-β-D-glucopyranoside **(2)**



R_1	R_2	R_3	
-H	-H	-Apiose	Forsythoside B **(3)**
-H	-H	-H	Verbascoside (=Acteoside) **(4)**
$-CH_3$	-H	-H	Leucosceptoside A **(5)**
$-CH_3$	$-CH_3$	-H	Martynoside **(6)**



Lamiide (7)

Figure. Compounds isolated from *Phlomis integrifolia.*

Experimental

General Procedures: UV (λ_{max}) and IR (cm^{-1}) were recorded on Hitachi HP 8452 A and Perkin Elmer 257 spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker AMX 300 and Bruker DRX 500 spectrometers in methanol-d_4, and DMSO-d_6. (^{1}H: 300.13 and 500 MHz; ^{13}C: 75.5 and 125 MHz). Chemical shifts were given in ppm with tetramethylsilane as an internal standard. FAB-MS was performed on a Finnigan 311 A spectrometer. 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 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, $60F_{254}$) plates with $CHCl_3$-MeOH-H_2O (61:32:7 or 80:20:2) as a developing solvent system. Compounds **1-7** were visualized by UV fluorescence and/or spraying with 1% vanillin/H_2SO_4, and followed by heating at 100 °C for 5 min.

Plant Material: *Phlomis integrifolia* Hub.-Mor. (Lamiaceae) was collected from Malatya, between Darende and Akdağ, steppe, 1460 m (E. Anatolia, Turkey) in June 2001. A voucher specimen has been deposited in the Herbarium of the Biology Department, Hacettepe University, Ankara, Turkey (AA Dönmez 9410).

Extraction and Prepurification: Air-dried aerial parts of the plant (530 g) were extracted three times with MeOH at 40 °C (x3, 2.0 L). The combined extracts were evaporated under vacuum to dryness (40 g). H_2O (0.5 L) was added and the H_2O insoluble material was removed by filtration. The filtrate was then extracted with petroleum ether (3 x 0.25 L) and the petroleum ether phase was discarded. The aqueous phase was further extracted with n-butanol (4 x 0.2 L), and the organic layer was evaporated to dryness (16 g). n-Butanol extract was redissolved in 40 mL of H_2O and chromatographed over polyamide column eluting with H_2O followed by increasing concentrations of MeOH to yield four main fractions: Frs. A-D. [Fr. A: H_2O (6 g), Fr. B: 25% MeOH (2 g), Fr. C: 50% MeOH (4 g), Fr. D: MeOH (2 g)].

Isolation of the Compounds: The fraction eluted with H_2O from the polyamide column (Fr. A) was chromatographed over silica gel with $CHCl_3$-MeOH (80:20) and yielded two main fractions: Frs. A1 and A2. Fr. A1 was subjected to Sephadex LH-20 and was eluted with MeOH to give compound **2** (13 mg). Fr. A2 was applied to MPLC on Li Chroprep RP-18 and was eluted with increasing concentrations of MeOH in H_2O (20→70%) to yield compound **7** (17 mg). The fraction eluted with H_2O – MeOH (75:25) from the polyamide column (Fr. B) was applied to a series of column chromatographies to yield compounds **1** and **3**. An aliquot of Fr. B was applied to the MPLC system on Li Chroprep RP-18 and was eluted with increasing amounts of MeOH (25→70%) to yield three main fractions: Frs. B1-B3. Fr. B1 was rich in compounds **1** and **3**, and was rechromatographed over silica gel by stepwise elution with $CHCl_3$-MeOH (100:0→70:30) to yield compounds **1** (2.5 mg) and **3** (32 mg). However, studies on Frs. B2 and B3 are still in progress. The fraction eluted with H_2O – MeOH (50:50) from the polyamide column (Fr. C) was found to contain compounds **4-6**. Rechromatography of Fr. C over silica gel by stepwise elution with $CHCl_3$-MeOH (90:10→60:40) yielded two main fractions: Frs. C1-C2. Fr. C2 was a single compound on TLC analysis and was identified as compound **4** (10 mg). Fr. C1 was rich in compounds **5** and **6** and was rechromatographed over silica gel by stepwise elution with $CHCl_3$-MeOH (100:0→80:20) to yield compounds **6** (2.5 mg) and **5**

(1.5 mg), respectively. The fraction eluted with MeOH from the polyamide column (Fr. D) is still under investigation.

Structure elucidation of the isolated compounds was carried out using ^{1}H-, ^{13}C-NMR, DEPT and 2D-NMR (DQF-COSY, HSQC, ROESY and HMBC) as well as UV and IR spectroscopy.

Results

Dehyrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside (1): $C_{26}H_{32}O_{11}$

$[\alpha]_D^{20}$ (*c* 0.05, MeOH): -61.97°; UV λ_{max} (MeOH) nm: 310, 276, 221, 203; IR υ_{max} (KBr) cm^1: 3320 (OH), 1647 (C=C), 1599, 1511 (Arom. rings); ^{1}H- (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): Table 1.

Chrysoeriol 7-*O*-(3″-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside (2): $C_{31}H_{28}O_{13}$

UV λ_{max} (MeOH) nm: 269, 344; IR υ_{max} (KBr) cm^{-1}: 3384 (OH), 1714 (ester C=O), 1661 (γ-pyrone C=O), 1608 and 1508 (Arom. rings); ^{1}H- (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): Table 2.

Forsythoside B (3): $C_{34}H_{44}O_{19}$

UV λ_{max} (MeOH) nm: 333, 291sh, 220, 203; IR υ_{max} (KBr) cm^{-1}: 3393 (OH), 1698 (C=O), 1630 (C=C), 1605 and 1522 (Arom. rings); ^{1}H- (CD_3OD, 300.13 MHz) and ^{13}C-NMR (CD_3OD, 75.5 MHz) data were identical to those reported in the literature[4,6].

Verbascoside (=Acteoside) (4): $C_{29}H_{36}O_{15}$

UV λ_{max} (MeOH) nm: 330, 288 sh, 235 sh, 206; IR υ_{max} (KBr) cm^{-1}: 3392 (OH), 1699 (C=O), 1630 (C=C), 1605 and 1517 (Arom. rings).

Leucosceptoside A (5): $C_{30}H_{38}O_{15}$

UV λ_{max} (MeOH) nm: 332, 285 sh, 246, 237 sh, 203; IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1699 (C=O), 1630 (C=C), 1600 and 1515 (Arom. rings).

Martynoside (6): $C_{31}H_{40}O_{15}$

UV λ_{max} (MeOH) nm: 330, 287 sh, 220, 203; IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1700 (C=O), 1625 (C=C), 1605 and 1515 (Arom. rings).

Lamiide (7): $C_{17}H_{26}O_{12}$

UV λ_{max} (MeOH) nm: 232; IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1700 (C=O), 1640 (C=C); ^{1}H- (CD_3OD, 300.13 MHz) and ^{13}C-NMR (CD_3OD, 75.5 MHz) data were identical to those reported in the literature[4,6].

Discussion

Compound **1** was obtained as an amorphous powder. The UV spectrum of compound **1** showed its phenolic nature. It might be a phenolic monoglucoside, on the basis of the anomeric proton peak at δ_H 4.89 d and the anomeric carbon resonance at δ_C 102.70, which was consistent for a β-linked glucose moiety. The ^{1}H-NMR spectrum of **1** indicated the presence of five aromatic protons due to two aromatic moieties, two methoxyl groups, protons of an (*E*)-coniferyl alcohol side-chain and a dihydrobenzofuran ring (Table 1). The above spectral data suggested that compound **1** was a dehydrodiconiferyl alcohol-type lignan[7–11]. In the ^{1}H- and

Table 1. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside **(1)**.

C/H	DEPT	δ_C(ppm)	δ_H(ppm)	J(Hz)	HMBC (H →C)
1	C	138.05			
2	CH	111.10	7.03 d	(1.8)	C-3, C-4, C-6, C-7
3	C	150.95			
4	C	147.69			
5	CH	117.96	7.15 d	(8.4)	C-1, C-2, C-3, C-4
6	CH	119.36	6.93 dd	(8.2/1.8)	C-2, C-4, C-7
7	CH	88.80	5.59 d	(5.9)	C-1, C-2, C-6, C-8, C-9, C-4′, C-5′
8	CH	55.42	3.50*		C-1, C-5′
9	CH_2	64.93	3.86*		C-7, C-8, C-5′
			3.78 dd	(11.0/7.3)	
3-OCH_3	CH_3	56.73	3.83 s		C-3
1′	C	130.02			
2′	CH	112.08	6.96*		C-3′, C-4′, C-6′, C-7′
3′	C	145.55			
4′	C	149.01			
5′	C	132.74			
6′	CH	116.50	6.96*		C-2′, C-3′, C-4′, C-7′
7′	CH	131.95	6.54 d	(15.9)	C-1′, C-2′, C-6′, C-9′
8′	CH	127.62	6.23 dt	(15.8/5.9)	C-1′, C-9′
9′	CH_2	63.86	4.20 dd	(5.9/1.1)	C-7′, C-8′
3′-OCH_3	CH_3	56.67	3.88 s		C-3′
1″	CH	102.70	4.89*		C-4
2″	CH	74.89	3.51 dd	(7.8/9.3)	
3″	CH	77.84	3.46*		
4″	CH	71.32	3.39*		
5″	CH	78.20	3.38 m		
6″	CH_2	62.47	3.86*		
			3.68 dd	(12.0/2.0)	

*Signal patterns unclear due to overlapping.

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

^{13}C-NMR spectra, the 2H resonance of two protons at δ_H 6.96 together with the corresponding carbon resonances at δ_C 116.50 (CH) and 112.08 (CH), showed the presence of one of the aromatic moieties to be tetrasubstituted. Two trans olefinic protons at δ_H 6.54 (1H, d, J = 15.9 Hz) and 6.23 (1H, dt, J = 15.8/5.9 Hz) were attributed to the side-chain of coniferyl alcohol moiety. The methylene protons (δ_H 3.86, 3.78) were coupled with a proton at δ_H 3.50, which was also coupled with a methine proton at δ_H 5.59 (1H, d, J = 5.9 Hz). These proton signals, together with the corresponding carbon resonances at δ_C 64.93 (CH_2), 55.42 (CH) and 88.80 (CH), respectively, were attributed to the dihydrofuran ring and its side-chain. Additionally, three aromatic proton signals were observed as an ABX system at δ_H 6.93 (1H, dd, J = 8.2/1.8), 7.03 (1H, d, J = 1.8) and 7.15 (1H, d, J = 8.4), indicating that the second aromatic moiety was trisubstituted. In the ^{13}C-NMR spectrum of **1**, there were no downfield shifts of the signal C-9 (δ_C 64.93; CH_2), belonging to the methylene group of the side-chain of dihydrofuran, and those of C-9′ (δ_C 63.86; CH_2) of the side-chain of coniferyl alcohol revealed that the glycosidation was not at these locations. The downfield shift

observed at C-4 (δ_C 147.69; C) indicated that the glycosidation might be at this location. The connectivities between the glucose, coniferyl alcohol, dihydrobenzofuran ring and the remaining trisubstituted aromatic ring were confirmed by the heteronuclear multiple bond correlation experiment (HMBC), which showed the cross-peaks for the following pairs: H-1″/C-4, H_2-9/C-7, H-7/C-9, H-2/C-7, H_2-9/C-5′, H_2-8′/C-1′, H_2-7′/C-2′, H_2-7′/C-6′, H_2-7′/C-9′ and H_2-9′/C-7′ (Table 1).

The complete analysis of the remaining ^{1}H- and ^{13}C-NMR signals, assigned by 2D-NMR experiments (DQF-COSY, HSQC and HMBC), revealed that compound **1** was dehyrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside. Although the stereochemistry at C-7 and C-8 could not be established from the available data, the similarity between all the NMR data and the negative optical rotation value of compound **1** ($[\alpha]_D^{20}$-61.97°) suggested that **1** may have the same stereoisomeric structure as that reported by Salama et al. ($[\alpha]_D^{15}$-71.2°)[10]rather than that reported by Changzeng and Zhongjian[9]. Consequently, compound **1** was identified as dehydrodiconiferyl alcohol-4-*O*-*β*-D-glucopyranoside (Table 1, Figure)[10,11].

Compound **2** was isolated as a yellow amorphous powder. It exhibited UV and IR absorptions confirming its phenolic nature. The UV spectroscopic data (UV λ_{max} 269, 344 nm) suggested that **2** was a flavone. The IR spectrum was characterized by 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}). In the ^{1}H-NMR spectrum of **2** (Table 2), three aromatic proton resonances at δ_H 7.59 (2H, H-2′/H-6′) and 6.95 (1H, d, J = 8.6, H-5′) indicated the 3′, 4′ disubstitution pattern of the B ring. Moreover, two signals in the aromatic region appeared as *meta*-coupled doublets at δ_H 6.48 (1H, d, J = 1.8 Hz, H-6) and 6.91 (1H, d, J = 1.8, H-8), consistent with a 5,7-disubstituted A ring of flavonoid. Singlet signals at δ_H 7.00 (1H) and 3.89 (3H) were readily attributed to H-3 and a methoxyl group, respectively. Additionally, two more aromatic proton signals at δ_H 7.59 and 6.80 (each 2H, d, J = 8.5 Hz) and two trans olefinic protons at δ_H 7.59 (d, J = 16.0 Hz) and 6.45 (1H, d, J = 16.0 Hz) indicated that compound **2** should consist of an additional *p*-substitued aromatic ring with a *trans*-side chain. Together with the corresponding carbon resonances of the mentioned additional protons and the presence of an *α, β*-unsaturated ester carbon at δ_C 166.18 (C) showed that the last substituent must be a *trans-p*-coumaric acid. An anomeric proton signal at δ_H 5.28 (1H, d, J = 7.7 Hz) indicated the presence of one sugar unit which, according to UV and ^{1}H-NMR data, was attached to C-7. The complete interpretation of the NMR data was based on the ^{1}H,^{1}H-DQF-COSY, ^{1}H,^{13}C-HSQC and HMBC experiments. Thus, aglycone was found to be chrysoeriol[12,13]. The structure of the sugar unit was elucidated using DQF-COSY and HSQC experiments as *β*-glucopyranosyl[13,14]. Although a 2.0 ppm downfield shift of C-3″ (δ_C 77.23; CH) along with the 4.0 ppm upfield shifts of the C-2″ and C-4″ (δ_C 71.25; CH and δ_C 67.49; CH, respectively) resonances of glucose suggested that the *trans-p*-coumaroyl moiety is placed at C-3″. The positions of sugar residue and *trans-p*-coumaroyl moiety were unambiguously determined by the HMBC experiment (Table 2). Thus, compound **2** was characterized as chrysoeriol 7-*O*-(3″-*O*-*trans-p*-coumaroyl)-*β*-D-glucopyranoside. Although chrysoeriol 7-*O*-(3″-*O*-*trans-p*-coumaroyl)-*β*-D-glucopyranoside was isolated from *P. aurea*[15], *P. floccosa*[15], *P. lychnitys*[16]and *P. purpurea*[17]previously, this is the first report of the occurrence of this compound in an endemic Turkish *Phlomis* species.

Compounds **3-6** were obtained as colorless, amorphous powders. The UV spectra of **3-6** confirmed their polyphenolic natures. IR bands for hydroxyl groups and *α, β*-unsaturated ester and aromatic rings were observed. The ^{1}H- (CD_3OD, 300.13 MHz) and ^{13}C-NMR (CD_3OD, 75.5 MHz) data of **3** were

identical to those reported for a phenylethanoid glycoside, forsythoside B, in the literature[4,6]. Compounds **4-6** were determined by direct Co-TLC methods and also by comparing their UV and IR spectra with those of authentic substances obtained from previous studies carried out in our department and identified as the known phenylethanoid glycosides, verbascoside (=acteoside), leucosceptoside A and martynoside, respectively (Figure)[6,18].

Table 2. ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (125 MHz, DMSO-d_6) data of chrysoeriol 7-*O*-(3″-*O*-*trans*-*p*-coumaroyl)-β-D-glucopyranoside **(2)**.

C/H	DEPT	δ_C(ppm)	δ_H (ppm)	J(Hz)	HMBC (H →C)
2	C	164.23			
3	CH	103.44	7.00 br.s		C-2, C-4, C-10, C-1′
4	C	182.08			
5	C	161.15			
6	CH	99.54	6.48 d	(1.8)	C-5, C-7, C-10
7	C	162.69			
8	CH	95.08	6.91 d	(1.8)	C-6, C-7, C-9, C-10
9	C	156.93			
10	C	105.46			
1′	C	121.19			
2′	CH	110.28	7.59*		C-3′, C-4′, C-6′, C-2
3′	C	148.10			
4′	C	151.12			
5′	CH	115.81	6.95 d	(8.6)	C-1′, C-3′
6′	CH	120.56	7.59*		C-2′, C-4′, C-2
3′-OCH_3	CH_3	55.97	3.89 s		C-3′
1″	CH	99.54	5.28 d	(7.7)	C-7
2″	CH	71.25	3.48*		
3″	CH	77.23	5.07 t	(9.3)	C=O
4″	CH	67.49	3.42*		
5″	CH	76.94	3.62 m		
6″	CH_2	60.24	3.73 br.d	(11.3)	
			3.57 br.d	(11.2)	
1‴	C	125.22			
2‴/6‴	CH	130.26	7.59 d	(8.5)	C-4‴, β
3‴/5‴	CH	115.81	6.80 d	(8.5)	C-1‴
4‴	C	159.78			
α	CH	114.82	6.45 d	(16.0)	C-1‴
β	CH	144.54	7.59 d	(16.0)	C=O
C=O	C	166.18			

*Signal patterns unclear due to overlapping.

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

Compound **7** was obtained as a colorless, amorphous powder. Its UV spectrum showed an absorption peak characteristic of a 4-substitute iridoid enol ether system. The IR absorption bonds were characteristic for hydroxy groups (3400 cm^{-1}), a carbonyl (1700 cm^{-1}) and an enolic double bond (1640 cm^{-1}). The ^{1}H-(CD_3OD, 300.13 MHz) and ^{13}C-NMR (CD_3OD, 75.5 MHz) data of **7** were identical to those reported for an iridoid glucoside, lamiide, in the literature[4,6].

The above-mentioned phenylethanoid and iridoid glycosides were previously isolated from several other *Phlomis* species during our studies[3−6].

Although there are numerous studies on flavonoid contents[15−17], reports on the isolation of lignan glucosides from the genus *Phlomis* are limited in number. So far, the neolignan glucosides (-)-dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside from *P. lycia*[4] and *P. chimerae*[5], (-)-4-*O*-methyl-dihydrodehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside and (-)-4-*O*-methyldehydrodiconiferyl alcohol-9′-*O*-β-D-glucopyranoside from *P. chimerae*[5] and a lignan glucoside syringaresinol-4′-*O*-β-D-glucopyranoside from *P. fruticosa*[3] and *P. monocephala*[6] have been reported. The mentioned neolignan glucosides showed a 9-*O*- or 9′-*O*-glycosidation pattern, whereas dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside isolated from *P. integrifolia* indicated a 4-*O*-glycosidation pattern. This is the first report of the isolation of dehydrodiconiferyl alcohol-4-*O*-β-D-glucopyranoside in the genus *Phlomis*.

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