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Phenolic Constituents from *Phlomis lycia*

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From the aerial parts of *Phlomis lycia* L. (Labiatae) a lignan glucoside, (-)-dihydrodehyrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside **(1)**; a caffeic acid ester, chlorogenic acid **(2)**; three phenylethanoid glycosides, forsythoside B **(3)**, alyssonoside **(4)** and leucosceptoside B **(5)**; and two iridoid glucosides, lamiide **(6)** and auroside **(7)**, were isolated. The structure elucidation of the isolated compounds was carried out by spectroscopic (UV, IR, 1D- and 2D-NMR, FAB-MS) methods.

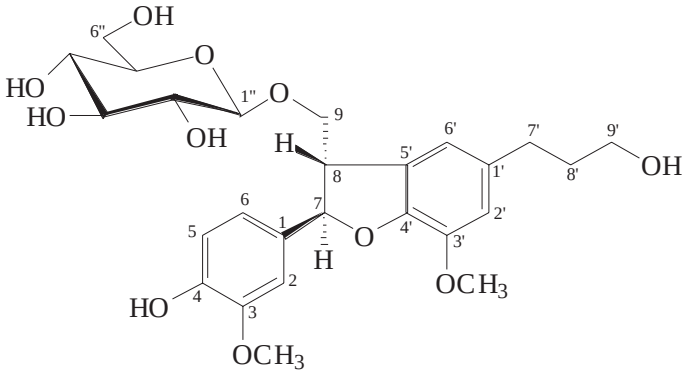
(-)-Dihydrodehyrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside **(1)** and chlorogenic acid **(2)** were isolated for the first time from *Phlomis* species in this study.

Key Words: *Phlomis lycia*, Labiatae, Lignans, Phenylethanoids, Iridoids.

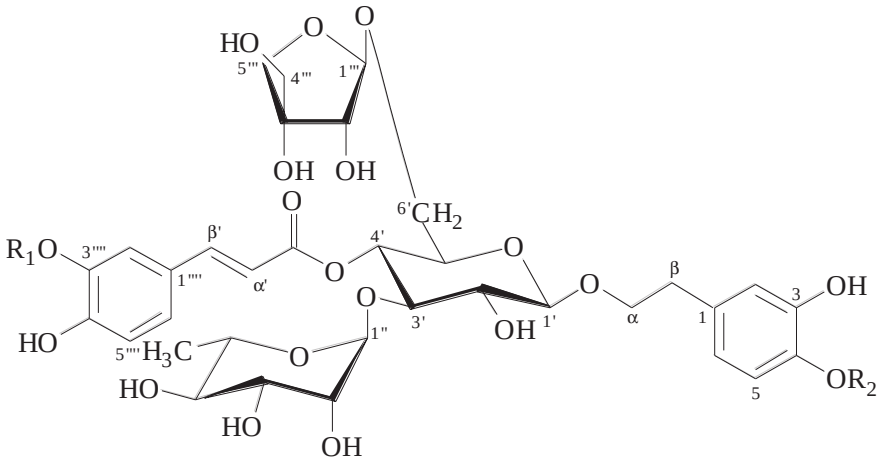
Introduction

There are 34 *Phlomis* L. species (Labiatae) growing in Turkey[1]. Some *Phlomis* species are used as tonics and stimulants in Anatolia[2] . During our systematic phytochemical investigations of *Phlomis* species we have studied *P. linearis* Boiss. & Bal.[3−6], *Phlomis armeniaca* Willd.[7], *Phlomis pungens* Willd. var. *pungens* [8−10], *Phlomis pungens* Willd. var. *hirta* Velen[11], *Phlomis bourgaei* Boiss.[12,13], and *Phlomis longifolia* var. *longifolia*[14].

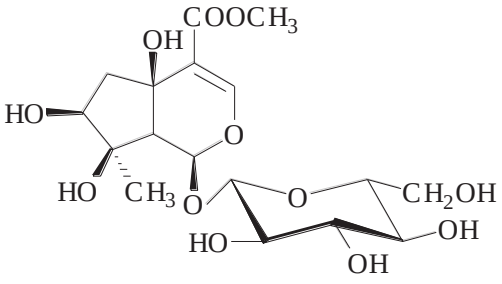
Investigations on the aerial parts of these plants have resulted in the isolation of phenylethanoid glycosides such as phlinosides A-E [3,4], verbascoside (=acteoside), leucosceptoside A, martynoside, forsythoside B[7−9,13,14], alyssonoside, leucosceptoside B, hattushoside[8−10,12] and iridoid glucosides such as lamiide, ipolamiide, auroside, shanzhiside methyl ester, 5-deoxypulchelloside, lamalbide and phlomiol[4−12,14] along with monoterpene glucosides, betulalbuside A and 8-hydroxylinaloyl-3-*O*-β-D-glucopyranoside[7] with diterpenoids such as jhanol and jhanol acetate[13]. In a continuation of our studies on the glycosidic constituents of *Phlomis* L. species, we investigated *Phlomis lycia* further and isolated a lignan glucoside (-)-dihydrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside **(1)**, and chlorogenic acid **(2)**, together with three phenylethanoid glycosides, forsythoside B **(3)**, alyssonoside **(4)** and leucosceptoside B **(5)**. Additionally, two iridoid glucosides, lamiide **(6)** and auroside **(7)**, were isolated from the same plant.



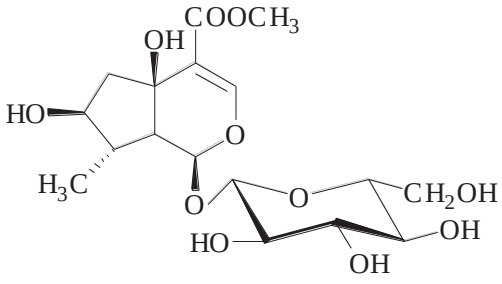
(-)- Dihydrodehydrodiconiferyl alcohol-9-O-β-D-glucopyranoside **(1)**



R_1	R_2	Compound
-H	-H	Forsythoside B **(3)**
$-CH_3$	-H	Alyssonoside **(4)**
$-CH_3$	$-CH_3$	Leucosceptoside B **(5)**



Lamiide **(6)**



Auroside **(7)**

Figure 1. Chemical Structures of the Isolated Compounds.

Experimental

General Procedures: UV (λ_{max}) and IR (cm^{-1}) were recorded on a Hitachi HP 8452 A and Perkin Elmer 257 spectrometers respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-A500 FT-NMR spectrometer in methanol -d_4, 500 MHz (^{1}H-NMR), 125.65 MHz (^{13}C-NMR). Chemical shifts were given in ppm with tetramethylsilane as an internal standard. FAB-MS was recorded on a JEOL JMS-DX 300 mass spectrometer. Optical rotation was measured with a Jasco DIP-4 digital polarimeter. Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 60-230 mesh), polyamide (Woelm) and sephadex LH-20 (Pharmacia). Medium pressure liquid chromatography (MPLC) was realized on a Labomatic (18.5x352 mm) glass column filled with Li Chroprep RP-18 using a Lewa M5 peristaltic pump. 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 and 80:20:2) as a developing solvent system. Compounds **1-7** were detected by UV fluorescence and/or spraying with 1% vanillin/H_2SO_4, followed by heating at 100^{o}C for 5 min.

Plant Material: *Phlomis lycia* D. (Labiatae) was collected from the hills near the highway between Antalya and Beldibi, Turkey, in April 1997. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 97046).

Extraction and Prepurification: Air-dried aerial parts of the plant (600 g) were extracted three times with methanol at 40^{o}C (x3, 3.0 l). The combined extracts were evaporated under vacuum to near dryness. H_2O (0.5 l) was added and the H_2O insoluble material removed by filtration. The filtrate was extracted with petroleum ether (4x0.2 l) and the petroleum ether phase rejected. The aqueous phase was evaporated under vacuum to near dryness and water extract was obtained (78 g). Half of it (39 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 three main fractions: Frs. A-C. (Fr.A: H_2O, Fr.B: 50 % MeOH, Fr.C: MeOH).

Isolation of the Compounds: The fraction eluted with H_2O from polyamide column (Fr.A) was rich in iridoid glucosides and, chromatographed over silica gel by stepwise elution with $CHCl_3$-MeOH-H_2O (80:20:2→ 60:40:4), yielded compounds **7** and **6**, respectively.

The fraction eluted with H_2O–MeOH (50:50) from the polyamide column (Fr.B), rich in phenolic compounds, was applied to a series of column chromatographies to yield compounds **1-5**. An aliquot (2.4 g) of Fr.B was subjected to Medium Pressure Liquid Chromatography (MPLC) by using a reversed-phase column. Eluting with increasing amounts of MeOH (20→70%) yielded four main fractions: Frs. B1-B4. Fr. B1 contained compound **2** (90 mg) while Fr. B2 contained compound **3** (340 mg) in pure form. Fr. B3 was subjected to MPLC, and eluting with increasing amounts of MeOH (25→50%) yielded two main fractions: Frs. $B3_A$ – $B3_B$. Fr. $B3_A$ was rechromatographed over silica gel by stepwise elution with $CHCl_3$-MeOH (95:5→80:20), yielding a fraction rich in **1**. This fraction was subjected to Sephadex LH-20 eluted with MeOH to give compound **1** (15 mg). Rechromatography of Fr. $B3_B$ over silica gel by stepwise elution with $CHCl_3$-MeOH (90:10→70:30) afforded compound **4** (50 mg). Fr. B4 was applied to the silica gel column and eluted with $CHCl_3$-MeOH (100:0→80:20) to yield compound **5**.

Structure elucidation of the isolated compounds was carried out by using ^{1}H,^{13}C-NMR, DEPT, ^{1}H,^{1}H-Correlation Spectroscopy (COSY), ^{1}H,^{13}C-Heteronuclear Multiple Quantum Coherence (HMQC) experiment, ^{1}H,^{13}C-Heteronuclear Multiple Bond Correlation Spectroscopy (HMBC) and FAB-MS as well

as UV and IR spectroscopy.

Results

(-)- Dihidrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside (1): $C_{26}H_{34}O_{11}$
FAB-MS: m/z 522 $[M]^+$, 523 $[M+H]^+$, 545 $[M+ Na]^+$
UV λ_{max} (MeOH) nm: 279, 227, 203
$[\alpha]_D^{24}$ (MeOH, *c.* 0.4): -5.95^o
^{1}H-NMR (CD_3OD, 500 MHz): Table 1
^{13}C-NMR (CD_3OD, 125.65 MHz): Table 1

Chlorogenic acid (2): $C_{16}H_{18}O_9$
FAB-MS: m/z 354 $[M]^+$, 355 $[M+H]^+$, 163 $[caffeoyl]^+$
UV λ_{max} (MeOH) nm: 328, 297sh, 245 sh, 218
IR υ_{max} (KBr) cm^{-1}: 3367 (OH), 1690 (C=O), 1630 (C=C), 1600 and 1524 (Arom. ring)
^{1}H-NMR (CD_3OD, 500 MHz): Table 2
^{13}C-NMR (CD_3OD, 125.65 MHz): Table 2

Forsythoside B (3): $C_{34}H_{44}O_{19}$
FAB-MS: m/z 779 $[M+Na]^+$
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-NMR (CD_3OD, 500 MHz): Table 3
^{13}C-NMR (CD_3OD, 125.65 MHz): Table 3

Alyssonoside (4): $C_{35}H_{46}O_{19}$
FAB-MS: m/z 793 $[M+Na]^+$, 177 $[feruloyl]^+$,
461 [M-apiose- feruloyl]$^+$
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)
^{1}H-NMR (CD_3OD, 500 MHz): Table 3
^{13}C-NMR (CD_3OD, 125.65 MHz): Table 3

Leucosceptoside B (5): $C_{36}H_{48}O_{19}$
FAB-MS: m/z 807 $[M+Na]^+$, 785 $[M+H]^+$, 177 $[feruloyl]^+$
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)
^{1}H-NMR (CD_3OD, 500 MHz): Table 3

^{13}C-NMR (CD_3OD, 125.65 MHz): Table 3

Lamiide (6)

UV λ_{max} (MeOH) nm: 232

IR υ_{max} (KBr) cm^{-1}: 3400 (OH), 1700 (C=O), 1640 (C=C)

Auroside (7)

UV λ_{max} (MeOH) nm: 229

IR υ_{max} (KBr) cm^{-1}: 3500 (OH), 1690 (C=O), 1630 (C=C)

Table 1. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125.65 MHz, CD_3OD) Data of (-)-Dihidrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside **(1)**.

Position	C $_{atom}$	δ $_C$ (ppm)	δ $_H$ (ppm)	J (Hz)	HMBC (C to H)
1	C	134.71			H-5
2	CH	110.89	7.00 d	(1.8)	H-6, H-7
3	C	149.08			O$\underline{CH}_3$, H-5
4	C	147.49			H-2, H-6
5	CH	116.13	6.77 d	(8.0)	
6	CH	119.83	6.85 dd	(8.0/1.8)	H-2, H-7
7	CH	89.26	5.58 d	(6.1)	H-2, H_2-9
8	CH	52.98	3.67 m		
9	CH_2	72.39	3.87*		H-1", H-7
			4.10 t	(7.9)	
3-OMe	CH_3	56.44	3.82 s		
1'	C	137.03			H_2-8'
2'	CH	114.25	6.72 d	(1.8)	H-6', H_2-7'
3'	C	145.26			O$\underline{CH}_3$
4'	C	147.53			H-2', H-6'
5'	C	129.75			H_2-9
6'	CH	118.27	6.83 d	(1.8)	H-2', H_2-7'
7'	CH_2	32.92	2.62 t	(7.6)	H-2', H-6', H_2-9'
8'	CH_2	35.84	1.81 m		
9'	CH_2	62.28	3.56 t	(6.4)	H_2-7'
3'-OMe	CH_3	56.52	3.85 s		
1"	CH	104.32	4.35 d	(7.6)	
2"	CH	75.21	3.22 dd	(9.0/7.9)	
3"	CH	78.26	3.32 t	(8.6)	H-5"
4"	CH	71.18	3.28 t	(8.6)	
5"	CH	78.11	3.26 m		H-3"
6"	CH_2	62.81	3.68 dd	(11.9/6.1)	
			3.87*		

* Signal pattern unclear due to overlapping

Discussion

Compound **1** was obtained as a liquid substance. The FAB-mass spectrum indicated the molecular formula $C_{26}H_{34}O_{11}$, which was confirmed by the NMR and DEPT data. The UV spectrum of compound **1** showed its phenolic nature. It seemed to be a phenolic monoglucoside, on the basis of the anomeric proton peak at

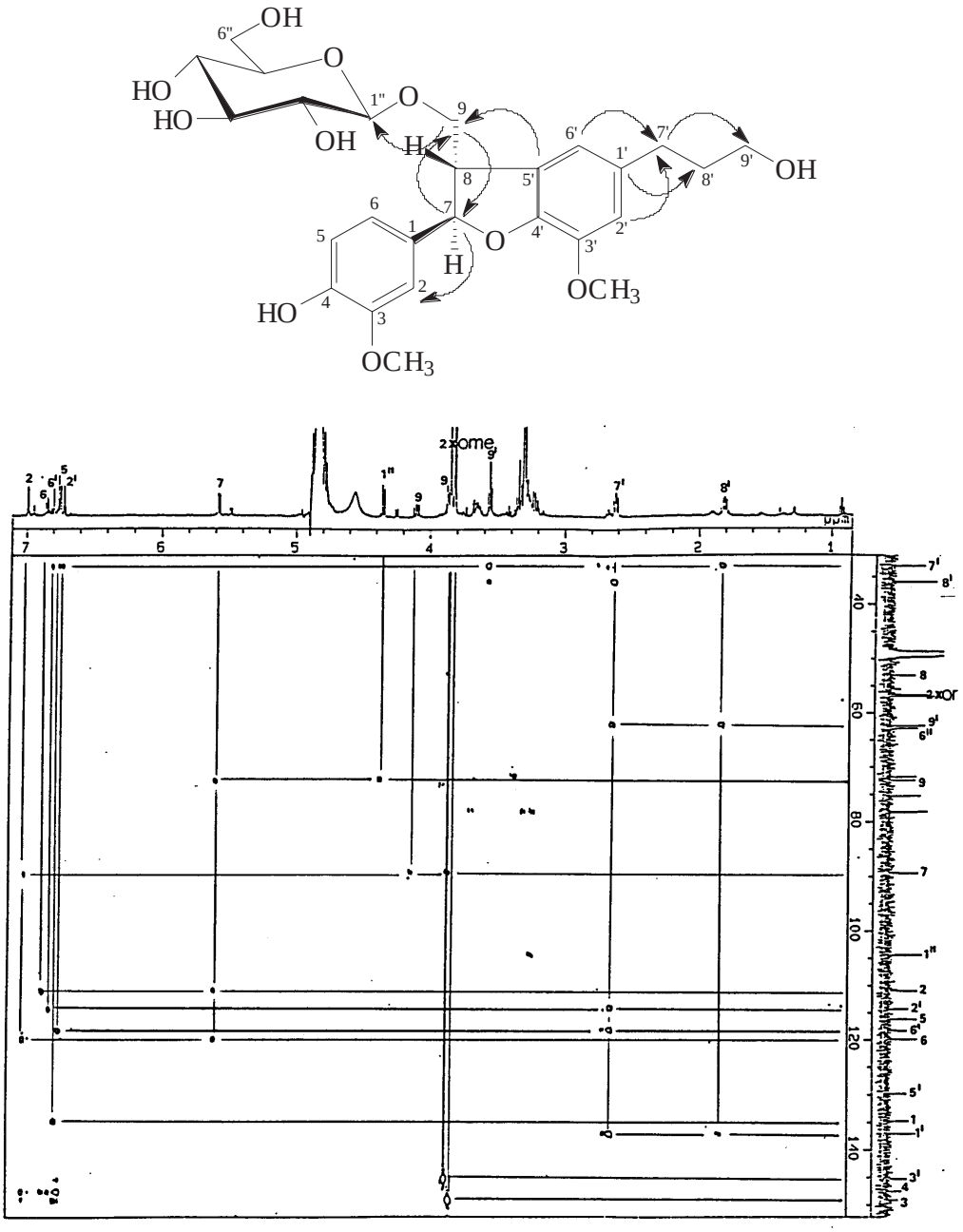


Figure 2. The Significant HMBC Correlations for (-)-Dihidrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside **(1)**. Arrows point from C to H.

δ_H 4.35 d (J=7.6 Hz) and the anomeric carbon resonance at δ_C 104.32, 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 a dihydroconiferyl alcohol side-chain and a dihydrobenzofuran ring (Table 1). In the ^{1}H- and^{13}C-NMR spectra, signals at δ_H 6.72 (d, J=1.8 Hz) and 6.83 (d, J=1.8 Hz), together with the corresponding carbon resonances at δ_C 114.25 d and 118.27 d, respectively, showed the presence of one of the aromatic moieties to be tetrasubstituted. A 2H triplet peak observed at δ_H 3.56 (J=6.4 Hz); δ_C 62.28 t was coupled with a 2H multiplet peak at δ_H 1.81; δ_C 35.84

t, which was further coupled with benzylic methylene protons at δ_H 2.62 (t, J=7.6 Hz); δ_C 32.92 t and attributed to the side chain of dihydroconiferyl alcohol moiety. The methylene protons (δ_H 3.87, 4.10) were coupled with a proton at δ_H 3.67 m, which was also coupled with a methine proton at δ_H 5.58 (d, J=6.1 Hz). These proton signals together with the corresponding carbon resonances at δ_C 72.39 t, 52.98 d and 89.26 d, 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 6.77, 6.85 and 7.00, indicating the second aromatic moiety to be trisubstitued. In the ^{13}C-NMR spectrum of **1**, the downfield shift of the signal C-9 (δ_C 72.39 t) belonging to the methylene group of the side-chain of dihydrofuran compared to those of C-9' (δ_C 62.28 t) revealed that the glycosidation was at this location. The connectivities between the glucose, coniferyl alcohol, dihydrobenzofuran ring and the remaining trisubstitued aromatic ring were confirmed by the heteronuclear multiple bond correlation (HMBC) experiment, which showed cross-peaks for the following pairs: H-1"/C-9, 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', H_2-9'/C-7' (Table 1, Figure 2).

Table 2. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125.65 MHz, CD_3OD) Data of Chlorogenic acid **(2)**.

Position	C $_{atom}$	δ_C (ppm)	δ_H (ppm)	J (Hz)	HMBC (C to H)
1	C	127.77			H-5, H-8
2	CH	115.68	7.04 d	(1.8)	H-6, H-7
3	C	147.08			H-5
4	C	149.55			H-2, H-6
5	CH	116.46	6.77 d	(8.2)	
6	CH	122.96	6.95 dd	(8.2/1.8)	H-2, H-7
7	CH	146.77	7.55 d	(15.8)	H-2, H-6
8	CH	115.24	6.26 d	(15.8)	
9	C	168.66			H-7, H-5'
1'	C	76.15			H-3', H-5'
2'	CH_2	38.20	2.04 d	(8.2)	H-4', H_2-6'
3'	CH	71.50	4.17 d	(3.0)	H-5'
4'	CH	73.48	3.72 dd	(8.2/3.0)	H_2-2', H_2-6'
5'	CH	71.90	5.33 m		H-3'
6'	CH_2	38.80	2.23 d	(11.5)	H_2-2'
			2.11 d	(11.3)	
7'	C	177.02			

The negative optical rotation value ($[\alpha]_D^{24}$ -5.95^o) suggested that **1** may have the same stereoisomeric structure as that obtained by Abe and Yamauchi ($[\alpha]_D$ -3.2^o)[15] rather than that obtained by Changzeng and Zhongjian ($[\alpha]_D^{15}$ +35.6^o)[16].

These results indicated that compound **1** was a (-)-dihidrodehydrodiconiferyl alcohol-9-*O*-β-D-glucopyranoside (Table 1, Figures 1 and 2)[15,16].

Compound **2** was obtained as pale yellow amorphous powder. It exhibited UV and IR absorptions confirming its phenolic nature. The ^{1}H-NMR spectrum of **2** exhibited three aromatic protons as an ABX system at δ_H 6.77-7.04, and two trans olefinic protons as an AB system at δ_H 6.26 and 7.55 (d, J= 15.8 Hz), indicating the presence of an (*E*)-caffeic acid moiety. On the other hand, two methylene protons δ_H 2.04 (2H, d, J=8.2 Hz) with 2.11 (1H, d, J=11.3 Hz) and 2.23 (1H, d, J=11.5 Hz) with three methine protons δ_H 3.72 (dd, J=8.2/3.0 Hz), 4.17 (d, J=3.0 Hz), and 5.33 m together with the corresponding carbon resonances

at δ_C 38.20 t, 38.80 t, 73.48 d, 71.50 d, and 71.90 d, respectively, showed the presence of a quinic acid moiety. The location of the (*E*)-caffeoyl moiety and the quinic acid was confirmed by the heteronuclear multiple bond correlation (HMBC) experiment showing the correlation peak between H-5' (δ_H 5.33 m) of quinic acid and the carbonyl carbon resonance (δ_C 168.66, C-9) of caffeic acid (Table 2). Therefore, the structure of **2** was identified as 5'-*O*-caffeoyl quinic acid (chlorogenic acid)[17].

Compounds **3**, **4** and **5** were obtained as colourless, amorphous powders. The UV spectra of **3-5** confirmed their polyphenolic natures. IR bands for hydroxyl groups and α,β-unsaturated ester and aromatic rings were observed.

The ^{1}H-NMR spectrum of compound **3** (Table 3) exhibited six aromatic protons as two ABX systems, at δ_H 6.56-7.04, and two trans olefinic protons as an AB system at δ_H 6.26 and 7.58 (d, J=15.9 Hz) belonging to the acyl and aglycone moieties. Additionally, a benzylic methylene (a triplet at 2.80 ppm, β-CH_2 of aromatic side-chain) and two nonequivalent protons at δ_H 3.73 and 4.03 were observed. Moreover, three doublets of the anomeric protons of **3** indicated its trisaccharidic structure. ^{1}H- and ^{13}C-NMR signals assigned to the sugar moiety showed that **3** should be composed of one β-D-glucose, one α-L-rhamnose and one β-D-apiose unit on the basis of its 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 4.35 (d, J=7.9 Hz; H-1'), 5.16 (d, J=1.5 Hz; H-1") and 4.89 (d, J=2.1 Hz; H-1"'), respectively. The acylation at C-4 hydroxyl of glucose was deduced from the downfield shift of H-4 of glucose (δ_H 4.93, t, J=9.4 Hz). The respective ^{13}C-NMR values are given in Table. The signal at δ_C 81.4 d due to the C-3 of glucose showed a downfield shift approximately 4 ppm, while the signal at δ_C 68.3 t due to the C-6 of glucose showed a downfield shift by approximately 7 ppm. These data showed that glycosidations should be at these locations. The connectivities between the acyl moiety, glucose, rhamnose and apiose were confirmed by the heteronuclear multiple bond correlation (HMBC) experiment which long range correlations were observed between the following protons and carbons: H-4' (δ_H 4.93 t) and CO (δ_C 167.9 s), H-1' (δ_H 4.35 d) and C-α (δ_C 72.2 t), H-1"'(δ_H 4.89 d) and C-6' (δ_C 68.3 t), H-6' (δ_H 3.49-3.69 dd) and C-1"' (δ_C 110.9 d), H-3' (δ_H 3.78 t) and C-1" (δ_C 102.8 d), H-1" (δ_H 5.16 d) and C-3' (δ_C 81.4 d). From the above results, compound **3** was assumed to be 3,4-dihydroxy-β-phenylethoxy-*O*-[α-L-rhamnopyranosyl-(1→3)]-*O*-[β-D-apiofuranosyl-(1→6)]-4-*O*-caffeoyl-β-D-glucopyranoside (forsythoside B)[8,9,12].

The ^{1}H- and ^{13}C-NMR spectra of **4** exhibited similar signals to those of forsythoside B **(3)**. The signals due to the sugar moiety were superimposable on those of **3**, the major difference being the presence of a resonance for a methoxyl group in **3** (δ_H 3.89, 3H, s and δ_C 56.4 q) (Table 3). The assigments of the chemical shifts for the methoxyl group and related aromatic protons (δ_H 7.19, 6.80 and 7.08, ABX system; 6.36 and 7.65, AB system) were established by comparison with those of phlinosides D and E[4], which contain ferulic acid as acyl moiety. The correlations were also confirmed by the HMBC spectrum of **4**. All other ^{1}H- and ^{13}C-NMR spectral data were very similar to those of forsythoside B **(3)**. Thus, compound **4** was identified as 3,4-dihydroxy-β-phenylethoxy-*O*-[α-L-rhamnopyranosyl-(1→3)]-*O*-[β-D-apiofuranosyl-(1→6)]-4-*O*-feruloyl-β-D-glucopyranoside (alyssonoside)[8,9,12].

Table 3. ^{1}H-NMR (500 MHz, CD_3OD) and ^{13}C-NMR (125.65 MHz, CD_3OD) Data of Forsythoside B **(3)**, Alyssonoside **(4)** and Leucosceptoside B **(5)**.

		3			**4**			**5**		
Position	C $_{Atom}$	δ_C (ppm)	δ_H	J (Hz) (ppm)	δ_C	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)
Aglycone										
1	C	131.2			131.3			132.3		
2	CH	116.9	6.68 d	(2.1)	117.0	6.68 dd	(7.9/2.1)	117.1	6.73 d	(1.8)
3	C	145.9			146.0			147.4		
4	C	144.5			144.5			147.5		
5	CH	116.2	6.67 d	(8.0)	116.2	6.66 d	(8.2)	112.9	6.81 d	(8.0)
6	CH	121.1	6.56 dd	(8.0/2.0)	121.2	6.56 dd	(8.2/2.1)	121.2	6.69 dd	(8.0/1.8)
α	CH_2	72.2	4.02 m		72.3	4.16 m		72.2 m	4.01 m	
			3.73 m			3.69*			3.74*	
β	CH_2	36.5	2.80 t	(7.3)	36.5	2.79 t	(7.3)	36.6	2.83 t	(7.6)
OMe	CH_3							56.4	3.81 s	
Acyl moiety										
1""	C	127.5			127.5			127.7		
2""	CH	115.1	7.04 d	(1.8)	117.7	7.19 d	(1.8)	111.8	7.20 d	(1.8)
3""	C	146.6			149.3			149.4		
4""	C	149.6			150.7			150.8		
5""	CH	116.3	6.77 d	(8.2)	116.4	6.80 d	(8.2)	116.5	6.82 d	(8.0)
6""	CH	123.0	6.95 dd	(8.2/1.8)	124.3	7.08 dd	(8.5/1.8)	124.3	7.08 dd	(8.2/1.8)
α '	CH	114.6	6.26 d	(15.9)	115.3	6.36 d	(15.8)	115.2	6.37 d	(15.8)
β '	CH	147.9	7.58 d	(15.9)	148.7	7.65 d	(15.8)	147.9	7.66 d	(15.8)
C=O	C	167.9			168.0			168.1		
OMe	CH_3				56.4	3.89 s		56.5	3.89 s	
$\beta-$ D-Glucose										
1'	CH	104.0	4.35 d	(7.9)	104.1	4.35 d	(7.9)	104.2	4.37 d	(7.9)
2'	CH	75.9	3.41 t	(9.1)	76.0	3.38 t	(9.1)	76.1	3.38 t	(9.2)
3'	CH	81.4	3.78 t	(9.1)	81.4	3.79 t	(9.1)	81.4	3.80 t	(9.4)
4'	CH	70.6	4.93 t	(9.4)	70.8	4.93 t	(9.4)	70.9	4.93 t	(9.7)
5'	CH	74.5	3.73*		74.5	3.69*		74.6	3.74*	
6'	CH_2	68.3	3.68 dd	(12.0/6.0)	68.4	3.70 dd	(12.0/6.1)	68.5	3.74*	
			3.49 dd	(12.0/2.0)		3.49 dd	(12.0/2.1)		3.51 dd	(12.0/1.8)
$\alpha-$ L-Rhamnose										
1"	CH	102.8	5.16 d	(1.5)	102.8	5.18 d	(1.5)	103.0	5.19 d	(1.5)
2"	CH	72.1	3.90 dd	(3.4/1.8)	72.2	3.89 dd	(3.4/1.8)	72.3	3.90 dd	(3.0/2.0)
3"	CH	71.9	3.55 dd	(9.4/3.4)	72.0	3.57 dd	(9.4/3.3)	72.05	3.56 dd	(9.4/3.0)
4"	CH	73.6	3.27 t	(9.4)	73.7	3.27 t	(9.4)	73.7	3.28 t	(9.7)
5"	CH	70.2	3.56 m		70.3	3.55 m		70.4	3.64 m	
6"	CH_3	18.3	1.08 d	(6.1)	18.4	1.09 d	(6.3)	18.4	1.09 d	(6.1)
$\beta-$ D-Apiose										
1"'	CH	110.9	4.89 d	(2.1)	110.9	4.89 d	(2.4)	111.0	4.90 d	(2.4)
2"'	CH	77.9	3.85 d	(2.1)	78.0	3.86 d	(2.4)	78.1	3.86 d	(2.1)
3"'	C	80.4			80.5			80.6		
4"'	CH_2	75.0	3.72 d	(9.7)	75.0	3.73 d	(9.4)	75.1	3.74 d	(9.7)
			3.91 d	(9.7)		3.91 d	(9.4)		3.92 d	(9.77)
5"'	CH_2	65.6	3.53 s		65.6	3.53 s		65.7	3.53 s	

*unclear due to overlapping

The ^{1}H- and ^{13}C-NMR spectra of compound **5** were almost identical to those of **3** and **4**, except that the signals of two methoxyl groups were found at δ_H 3.81 and 3.89 (each 3H, s) and δ_C 56.4 and 56.5 q, respectively (Table 3). HMQC and HMBC correlations confirmed that compound **5** has ferulic acid as acyl moiety and 3-hydroxy,4-methoxy phenylethyl alcohol as aglycone moiety. Thus compound **5** was established to be 3-hydroxy-4-methoxy-β-phenylethoxy-*O*-[α-L-rhamnopyranosyl-(1→3)]-*O*-[β-D-apiofuranosyl-(1→6)]-4-*O*-feruloyl-β-D-glucopyranoside (leucosceptoside B) [8,9,12].

Compounds **6-7** were obtained as colourless, amorphous powders. They were determined by direct Co-TLC methods and comparing their UV and IR spectra with the authentic substances obtained from earlier studies carried out in our department, and identified as the known iridoid glucosides, lamiide and auroside, respectively (Figure 1)[4−9,12].

However, the above-mentioned phenylethanoid and iridoid glycosides were previously isolated from several *Phlomis* species in our studies[1−14] . This is the first report of the isolation of a lignan glycoside and chlorogenic acid from the genus *Phlomis*.

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References

1. P.H. Davis, "Flora of Turkey and the East Aegean Islands", Vol. 7, University Press, Edinburgh, 1982.
2. T. Baytop, "Therapy with Medicinal Plants in Turkey (Past and Present), 2^{nd} ed. P.193, Nobel Tıp Kitapevleri, İstanbul, 1999.
3. İ. Çalış, A.A. Başaran, İ. Saracoğlu, O. Sticher and P. Rüedi, **Phytochemistry 29,** 1253-1257 (1990).
4. İ. Çalış, A.A. Başaran, İ. Saracoğlu, O. Sticher and P. Rüedi, **Phytochemistry 30,** 3073-3075 (1991).
5. A.A. Başaran, İ. Saracoğlu and İ. Çalış, **Hacettepe University, Journal of the Faculty of Pharmacy 11,** 81-88 (1991).
6. A.A. Başaran, İ. Saracoğlu, P. Rüedi, O. Sticher and İ. Çalış, "Phytochemical Investigations of *Phlomis linearis* Boiss. & Bal." in Proceedings Book of $VIII^{th}$ Symposium on the Plant Originated Pharmaceutical Raw Materials, eds. B. Çubukcu, G. Sarıyar, A. Mat, pp. 125-130, İstanbul, 1993.
7. İ. Saracoğlu, M. Inoue, İ. Çalış and Y. Ogihara, **Biol. Pharm. Bull. 18,** 1396-1400 (1995).
8. Ü. Ş. Harput, "The Comparative Pharmacognostical Studies on *Phlomis pungens* Willd.var. *pungens* and *Phlomis pungens* Willd. var. *hirta* Velen" M.Sc. Thesis, Hacettepe University, Ankara, Turkey, 1997.
9. İ. Saracoğlu, Ü.Ş. Harput, K. Kojima and Y. Ogihara, **Hacettepe University, Journal of the Faculty of Pharmacy 17,** 63-72 (1997).
10. İ. Saracoğlu, K. Kojima, Ü.Ş. Harput and Y. Ogihara, **Chem. Pharm. Bull. 46,** 726-727 (1998).
11. Ü.Ş. Harput, İ. Saracoğlu, F. Tülemiş, C. Akay and Ş. Cevheroğlu, **Hacettepe University, Journal of the Faculty of Pharmacy 18,** 1- 7 (1998).
12. Ü.Ş., Harput, İ. Saracoğlu and İ. Çalış, **Hacettepe University, Journal of the Faculty of Pharmacy 19,** 1- 11 (1999).
13. Ü.Ş., Harput, İ. Saracoğlu, İ. Çalış, K. Kojima and Y. Ogihara, "Diterpenoids from *Phlomis bourgaei*" in New Trends and Methods in Natural Products' Research, Posters, (Proceedings Book of XII^{th} Symposium on the Plant Originated Pharmaceutical Raw Materials) eds. İ. Çalış, A. Başaran, T. Ersöz, pp. 127-131, Rekmay Press, Ankara, 2000.
14. T. Ersöz, W. Schühly, S. Popov, N. Handjieva, O. Sticher and İ. Çalış, **Nat. Prod. Lett.**, 2001 in press.
15. F. Abe and T. Yamauchi, **Chem. Pharm. Bull. 34,** 4340-4345 (1986).
16. W. Changzeng and J. Zhongjian, **Phytochemistry 45,** 159-166 (1997).
17. A. Cheminat, R. Zawatzky, H. Becker and R. Brouillard, **Phytochemistry 27,** 2787-2794 (1988).