Iridoid and Phenylethanoid Glycosides from *Phlomis tuberosa* L.

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A new iridoid glucoside, 8-*O*-acetylshanzhiside (**1**), was isolated from the aerial parts of *Phlomis tuberosa*, together with two known iridoid glucosides, shanzhiside methyl ester and lamalbide. The known phenylethanoid glycosides acteoside and forsythoside B were also obtained and characterized. The structure of **1** was determined by means of 1D- and 2D-NMR spectroscopic evidence.

Introduction

Phlomis tuberosa L. (Lamiaceae) is a widespread plant in Bulgaria (Stojanov *et al.*, 1967). There exist several reports on the flavonoids and polyphenolic compounds (Gella *et al.*, 1972; Glyzin *et al.*, 1972; Vavilova and Gella, 1973a and 1973b) and alkaloids (Khokhrina and Peshkova, 1974) from this plant. However, C₉ iridoids, harpagide and 8-*O*-acetyl harpagide have been described from *P. tuberosa* (Gella and Vavilova, 1972) but a recent study on the same plant species led to the isolation of 5-desoxyseamoside, seamoside, shanzhiside methyl ester and lamalbide, all C₁₀ iridoid glucosides with a C-4 methoxycarbonyl substituent (Alipieva *et al.*, 2000). The present study on *P. tuberosa*, has now resulted in the isolation of a new iridoid glucoside, 8-*O*-acetylshanzhiside (**1**), in addition to the known iridoid glucosides shanzhiside methyl ester (**2**) and lamalbide (**3**) as well as the known phenylethanoid glycosides acteoside (**4**) and forsythoside B (**5**). This paper deals with the structure elucidation of **1** by 1D- and 2D-NMR spectroscopy.

Material and Methods

General experimental procedures

Optical rotation was measured on a Perkin-Elmer 241&241 MC polarimeter using MeOH. UV spectra were recorded on a UVIKON 930 spectro-

photometer in MeOH. FTIR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer in KBr pellets. NMR measurements in CD₃OD at room temperature were performed on a Bruker DRX 500 spectrometer operating at 500 and 125 MHz for ¹H and ¹³C NMR, respectively. ¹H-¹H COSY, ¹H-¹³C HSQC, and ¹H-¹³C HMBC experiments were recorded by employing conventional pulse sequences. ESIMS were recorded in positive and negative ion modes on a Finnigan TSQ 7000 mass spectrometer. LiChroprep C₁₈ (Merck) was used for VLC (column 5.2 × 20 cm, i.d.). Silica gel 60 (0.063–0.200 mm, Merck) was used for open column CC. MPLC (medium pressure liquid chromatography) separations were performed on a Labomatic glass column (1.8 × 35.2 cm, i.d.) packed with LiChroprep C₁₈ (Merck), using a Lewa M5 peristaltic pump. TLC was carried out on pre-coated silica gel 60F₂₅₄ aluminum sheets (Merck). Compounds were detected by UV fluorescence absorption and/or spraying with vanillin-H₂SO₄ reagent followed by heating at 100 °C for 1–2 min.

Plant material

Phlomis tuberosa (Lamiaceae) was collected at florescence in West Stara Planina (West Balkan), Bulgaria in May 1998. A voucher specimen has been deposited in the herbarium of the Institute of Botany, Bulgarian Academy of Sciences [SOM 154959].

Extraction and isolation

The air-dried and powdered aerial parts of *P. tuberosa* (86 g) were extracted twice with MeOH (2×500 ml) at 40°C . The combined extracts were evaporated under reduced pressure and H_2O was added (500 ml). The H_2O -insoluble material was removed by filtration and the filtrate then partitioned with CHCl_3 . The CHCl_3 layer was removed. The aqueous layer was concentrated under reduced pressure to yield 6.59 g crude extract. The crude extract was then fractionated by reversed-phase vacuum liquid chromatography (RP-VLC, LiChroprep C_{18}) and eluted with H_2O , followed by increasing concentrations of MeOH (5–100%) to yield 6 main fractions (A–F) [Fr. A (2.282 g), Fr. B (65 mg), Fr. C (133.7 mg), Fr. D (114.6 mg), Fr. E (245 mg), Fr. F (314.4 mg)]. Fraction C was subjected to C_{18} medium pressure liquid chromatography (RP-MPLC) using gradient MeOH– H_2O (5–30%) mixtures to yield shanzhiside methyl ester (**2**) (4.85 mg) and lamalbide (**3**) (13.4 mg). CC of fraction D on silica gel (15 g) eluting with CHCl_3 –MeOH– H_2O (80:20:1, 80:20:2) mixtures afforded 6 fractions (Frs. D₁–D₆). Fraction D₅ (9 mg) was purified by Sephadex CC with MeOH to give **1** (3.0 mg). Fraction E was applied to RP-MPLC and fractionated with isopropanol– H_2O (5–20%) mixtures to yield acteoside (**4**) (37.7 mg) and forsythoside B (**5**) (115 mg).

8-O-acetylshanzhiside (1), white amorphous powder, 3.0 mg. $[\alpha]_{\text{D}}^{20} - 42^\circ$ (c 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (2.88); IR ν_{max} (KBr)

cm^{-1} : 3450 (OH), 1739 (ester), 1695 ($\text{C}=\text{C}-\text{COOH}$), 1400 (CH_3), 1250 ($=\text{C}-\text{O}-\text{C}$), 1100 ($\text{CH}-\text{OH}$). Positive-ion ESIMS: m/z $[\text{M}+\text{Na}]^+$ 457 (93); negative-ion ESIMS: m/z $[\text{M}-\text{H}]^-$ 433 (100). ^1H NMR (CD_3OD , 500 MHz): Table I. ^{13}C NMR (CD_3OD , 125 MHz): Table I.

Results and Discussion

The methanol extract of *P. tuberosa* was suspended in water and partitioned with chloroform. Removing the chloroform layer, the aqueous layer was fractionated over reversed-phase vacuum liquid chromatography (RP-VLC, LiChroprep C_{18}) followed by Si gel CC and C_{18} medium pressure liquid chromatography (RP-MPLC) and purification over Sephadex yielded pure compounds **1**–**5**.

The known compounds **2**–**5** were identified as shanzhiside methyl ester (**2**) (Achenbach *et al.*, 1981), lamalbide (**3**) (Brieskorn and Ahlborn, 1973; Ersöz *et al.*, 2001), acteoside (**4**) (Sticher and Lahloub, 1982), and forsythoside B (**5**) (Endo *et al.*, 1982), respectively, by comparing their ^1H and ^{13}C NMR data with previously published data.

Compound **1** was isolated as an amorphous powder, $[\alpha]_{\text{D}}^{20} - 42^\circ$ (c 0.1, MeOH). The molecular formula was established as $\text{C}_{18}\text{H}_{26}\text{O}_{12}$, on the basis of the ^1H and ^{13}C NMR data (*see* Table I), and of the pseudomolecular ions shown in positive ESI-MS (m/z 457 $[\text{M}+\text{Na}]^+$) and negative ESI-MS (m/z 433 $[\text{M}-\text{H}]^-$). The ^1H NMR spectrum of **1** exhibited signals for a conjugated iridoid structure with an acetoxy (δ_{H} 2.00, s) and a tertiary methyl

Table I. ^{13}C and ^1H NMR (CD_3OD , 125 MHz for ^{13}C and 500 MHz for ^1H NMR) data and HMBC correlations for **1**.*

C/H Atom		δ_{C}	δ_{H} J [Hz]	HMBC (from C to H)
Aglycone				
1	CH	94.9	5.62 <i>d</i> (3.8)	H-3
3	CH	147.9	7.10 <i>br.s</i>	
4	C	111.0	–	H-3, H-5
5	CH	43.6	3.01 <i>dd</i> (9.3/3.5)	
6	CH	77.6	4.13 <i>m</i>	
7	CH_2	46.9	2.24 <i>dd</i> (14.4/6.2) 2.17 <i>dd</i> (14.4/5.3)	H ₃ -10
8	C	90.1	–	H-9, H ₃ -10
9	CH	48.6	2.85 <i>dd</i> (9.3/3.8)	H ₃ -10
10	CH_3	22.1	1.55 <i>s</i>	
11	C	181.0		H-3
OCOCH ₃	C	172.9	2.00 <i>s</i>	H-9
	CH_3	22.3		H-7, H-8, H-9
Glucose				
1'	CH	100.6	4.65 <i>d</i> (7.9)	H-1
2'	CH	74.8	3.18 <i>dd</i> (7.9/9.0)	
3'	CH	77.9	3.38 <i>t</i> (9.0)	
4'	CH	71.7	3.30 <i>m</i> [†]	
5'	CH	78.3	3.32 <i>m</i> [†]	
6'	CH_2	62.9	3.90 <i>dd</i> (12.0/2.1) 3.66 <i>dd</i> (12.0/6.2)	

*The ^{13}C and ^1H NMR assignments were based on HSQC, HMBC and COSY experiments.[†] Coinciding with the solvent signal or pattern and therefore unclear due to signal overlapping.

(δ_{H} 1.55, *s*) function. In addition, the ^1H NMR signal at δ_{H} 4.65 (*d*, $J = 7.9$ Hz) was attributed to the anomeric proton signal of a β -glucose unit. In the ^{13}C NMR spectrum 18 carbon resonances, six of which could be assigned to a β -glucopyranosyl moiety, were observed. The complete assignments of all proton and carbon resonances were made by ^1H - ^1H COSY, ^1H - ^{13}C HSQC and HMBC experiments. Therefore, HMBC correlation observed between C-1' (δ_{C} 100.6, *d*) and H-1 revealed the attachment of the β -glucopyranose unit at the C-1 position of the iridoid aglycone. The ^1H NMR signal at δ_{H} 7.10 (*br.s*) was assigned to H-3, suggesting H-4 to be substituted, which was further verified by the typical ^{13}C NMR resonances associated with a C-4 carboxy-bearing iridoid in the region C-3 to C-5 (δ_{C} [C-3] 147.9, *d*; δ_{C} [C-4] 111.0, *s*; δ_{C} [C-5] 43.6, *d*), as well as by the HMBC correlation from COOH (δ_{C} 181.0, *s*) to H-3. In the ^1H - ^1H COSY spectrum, H-1 signal (δ_{H} 5.62, *d*, $J = 3.8$ Hz) was coupled to H-9 (δ_{H} 2.85, *dd*, $J = 9.3/3.8$ Hz), which in turn was correlated to H-5 (δ_{H} 3.01, *dd*, $J = 9.3/3.5$ Hz), indicating C-5 to be unsubstituted. H-5 exhibited a ^1H - ^1H COSY interaction with an oxymethine proton at δ_{H} 4.13 (*m*, H-6),

consistent with the hydroxyl group being attached to C-6 (δ_{C} 77.6, *d*). The secondary hydroxyl function at C-6 was assigned as β by comparing the ^{13}C NMR data of **1** with those of C-6 (OH) epimer iridoid glucosides (Bianco *et al.*, 1983). In the ^1H - ^1H COSY spectrum, H-6 proton showed an additional homonuclear coupling with the geminally coupled C-7 methylene protons (δ_{H} 2.24, *dd*, $J = 14.4/6.2$ Hz, H-7 α ; δ_{H} 2.17, *dd*, $J = 14.4/5.3$ Hz, H-7 β ; observed as AB part of an ABX system). HMBC correlation observed from C-8 (δ_{C} 90.1, *s*) to the tertiary methyl signal (δ_{H} 1.55, *s*), in addition to the heteronuclear long-range couplings between H₃-10/C-7 (δ_{C} 46.9, *t*) and H₃-10/C-9 (δ_{C} 48.6, *d*), showed the attachment of the methyl group at C-8. On the other hand, the chemical shift values of both C-8 and H₃-10 indicated an oxygen substitution at C-8 position. However, C-8 resonance showed a remarkable downfield shift compared with the iridoid analogues with a tertiary methyl and a hydroxyl group bearing at C-8 (Boros and Stermitz, 1990). Therefore, the acetoxy function in **1** was assigned to be positioned at the tertiary hydroxyl group at C-8 causing downfield shifts of both H₂-7 (δ_{H} 2.24, *dd* $J = 14.4/6.2$ Hz and

2.17, *dd J* = 14.4/5.3 Hz) and H-9 (δ_{H} 2.85, *dd J* = 9.3/3.8 Hz). Moreover, a significant HMBC cross-peak from OCOCH_3 (δ_{C} 172.9, *s*) to H-9 also supported this assumption. The complete NMR data of **1** based on ^1H - ^1H and ^1H - ^{13}C 2D-NMR measurements indicated that the structure of **1** was almost identical to that of 8-*O*-acetylshanzhiside methyl ester (Damtoft *et al.*, 1982; Nicoletti *et al.*, 1984), except for the substitution of a carboxyl group at C-4. Consequently, compound **1** was established as 8-*O*-acetylshanzhiside which was isolated for the first time from nature.

Concerning the iridoid glucosides of the genus *Phlomis*, only C_{10} iridoids, substituted with a methoxycarbonyl function at C-4 have been described up to now. To our knowledge, 8-*O*-acetylshanzhiside (**1**) is the first iridoid glucoside - substituted at C-4 by a COOH group- reported from *Phlomis* species, which may be of chemotaxonomic importance in the future. On the other

hand, iridoid composition of *P. tuberosa*, collected from West Stara Planina-West Balkan region was somewhat different from that of *P. tuberosa* collected from Barmuk bair-Sliven region (Alipieva *et al.*, 2000). This could illustrate a significant geographic variation of the iridoids in the same species.

Although *Phlomis* species are known to contain phenylethanoid glycosides (Jimenez and Riguera, 1994), no phenylethanoid glycoside has so far been reported from *P. tuberosa*. Therefore, acteoside (**4**) and forsythoside B (**5**) are the first phenylethanoid glycosides isolated from the title plant.

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