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, C_9 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-desoxysesamoside, sesamoside, shanzhiside methyl ester and lamalbide, all C_{10} 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 spectrophotometer in MeOH. FTIR spectra were recorded on a Perkin-Elmer 2000 FT-IR spectrometer in KBr pellets. NMR measurements in CD_3OD at room temperature were performed on a Bruker DRX 500 spectrometer operating at 500 and 125 MHz for ^1H and ^{13}C NMR, respectively. ^1H-^1H COSY, ^1H-^{13}C HSQC, and ^1H-^{13}C 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 C_{18} (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 C_{18} (Merck), using a Lewa M5 peristaltic pump. TLC was carried out on precoated silica gel $60F_{254}$ aluminum sheets (Merck). Compounds were detected by UV fluorescence absorption and/or spraying with vanillin-H_2SO_4 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].

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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. D_1-D_6). Fraction D_5 (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).

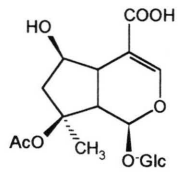
8-O-acetylshanzhiside **(1)**, white amorphous powder, 3.0 mg. $[\alpha]_D^{20}$ – 42° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 205 (2.88); IR υ_{max} (KBr) cm^{-1}: 3450 (OH), 1739 (ester), 1695 (C=C-COOH), 1400 (CH_3), 1250 (=C-O-C), 1100 (CH-OH). Positive-ion ESIMS: *m/z* $[M+Na]^+$ 457 (93); negative-ion ESIMS: *m/z* $[M-H]^-$ 433 (100). ^1H NMR (CD_3OD, 500 MHz): Table I. ^{13}C NMR (CD_3OD, 125 MHz): Table I.

Results and Discussion

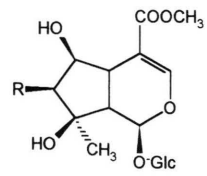
The methanol extract of *P. tuberosa* was suspended in water and partitioned with chloroform. Removing the chloroform layer, the aqueous layer was fractionated over reversed-phase vacuum liquid chromatography (RP-VLC, LiChroprep C_{18}) followed by Si gel CC and C_{18} medium pressure liquid chromatography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

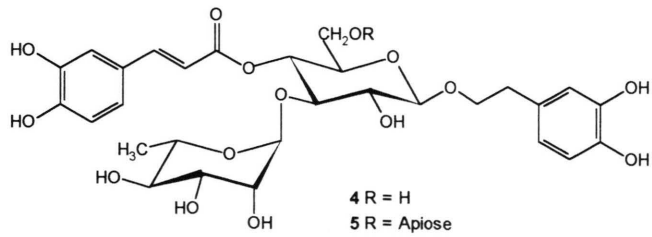
Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{20}$ – 42° (*c* 0.1, MeOH). The molecular formula was established as $C_{18}H_{26}O_{12}$, on the basis of the ^1H and ^{13}C NMR data (*see* Table I), and of the pseudomolecular ions shown in positive ESI-MS (*m/z* 457 $[M+Na]^+$) and negative ESI-MS (*m/z* 433 $[M-H]^-$). 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



1



2 R = H
3 R = OH



4 R = H
5 R = Apiose

Table I. ^{13}C and ^{1}H NMR (CD_3OD, 125 MHz for ^{13}C and 500 MHz for ^{1}H 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 *d* (3.8)	H-3
	3	CH	147.9	7.10 *br.s*	
	4	C	111.0	–	H-3, H-5
	5	CH	43.6	3.01 *dd* (9.3/3.5)	
	6	CH	77.6	4.13 *m*	
	7	CH_2	46.9	2.24 *dd* (14.4/6.2)	H_3-10
				2.17 *dd* (14.4/5.3)	
	8	C	90.1	–	H-9, H_3-10
	9	CH	48.6	2.85 *dd* (9.3/3.8)	H_3-10
	10	CH_3	22.1	1.55 *s*	
	11	C	181.0		H-3
	$OCOCH_3$	C	172.9	2.00 *s*	H-9
		CH_3	22.3		H-7, H-8, H-9
Glucose					
	1′	CH	100.6	4.65 *d* (7.9)	H-1
	2′	CH	74.8	3.18 *dd* (7.9/9.0)	
	3′	CH	77.9	3.38 *t* (9.0)	
	4′	CH	71.7	3.30 *m* †	
	5′	CH	78.3	3.32 *m* †	
	6′	CH_2	62.9	3.90 *dd* (12.0/2.1)	
				3.66 *dd* (12.0/6.2)	

*The ^{13}C and ^{1}H 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 ^{1}H 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 ^{1}H-^{1}H COSY, ^{1}H-^{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 ^{1}H 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 **C**OOH (δ_C 181.0, *s*) to H-3. In the ^{1}H-^{1}H 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 ^{1}H-^{1}H 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 ^{1}H-^{1}H 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-7α; δ_H 2.17, *dd*, *J* = 14.4/5.3 Hz, H-7$_\beta$; 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 H_3-10/C-7 (δ_C 46.9, *t*) and H_3-10/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 H_3-10 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 H_2-7 (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 O**C**OCH$_3$ (δ_C 172.9, *s*) to H-9 also supported this assumption. The complete NMR data of **1** based on ^{1}H-^{1}H and ^{1}H-^{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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