Iridoid Glucosides from Turkish *Phlomis tuberosa*

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From the aerial parts of *Phlomis tuberosa* a new iridoid glucoside, chlorotuberoside was isolated together with five known iridoid glucosides, lamalbide, shanzhiside methyl ester, 7-*epi*-phlomiol (= phloyoside I), sesamoside and 5-deoxysesamoside. Two known phenylethanoid glycosides, forsythoside B, and decaffeoylacteoside, three known neolignan glycosides, dehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside, dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside and dihydrodehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside, one flavone glycoside, luteolin 7-*O*-β-D-glucopyranoside, as well as β-sitosterol 3-*O*-β-D-glucopyranoside and 1-methyl-*O*-β-D-glucopyranoside were also obtained and characterized. The structures of the isolates were elucidated on the basis of spectroscopic data. The three known phenylethanoid glycosides, verbascoside, leucosceptoside A and martynoside were identified by TLC comparison with authentic compounds.

Key words: Phlomis tuberosa, Lamiaceae, Iridoid Glucosides, Chlorotuberoside

Introduction

Phlomis tuberosa L. is one of the 33 species growing in Turkey [1]. In Asian folk medicine *P. tuberosa* is used as a general roborant, for intoxications, tuberculosis, pulmonary and cardio-vascular diseases and rheumatoid arthritis [2]. Recent studies on this species from the flora of Bulgaria showed the presence of several iridoid and phenylethanoid glycosides [3, 4]. As a part of our ongoing phytochemical studies on Turkish *Phlomis* species, we have now investigated *P. tuberosa*. The present work describes the isolation and the structure elucidation of a new iridoid glucoside, chlorotuberoside (**1**), as well as 13 known metabolites.

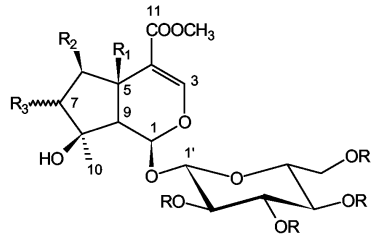
Experimental Section

General experimental procedures

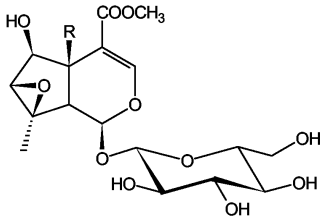
UV spectra were recorded on a Shimadzu UV-160A spectrophotometer. IR spectra (KBr) were measured on a Perkin Elmer 2000 FT-IR spectrometer. 1D-and 2D-NMR spectra were recorded on a Varian Unity Inova-500 MHz spectrometer in MeOD (glucosides) or $CDCl_3$ (acetate) using the solvent peak ($\delta_{H/C}$ 3.31/49.0 or 7.28/77.0, respectively) as internal standard. LC-HR ESIMS was performed on an Agilent HP 1100 Liquid Chromatograph equipped with a BDS-C18 reversed phase column running a water-acetonitrile (50 ppm TFA in water) gradient. The LC was coupled to a LCT of a TOF MS (Micromass, Manchester, UK) operated in the positive electrospray ion mode using 5-leucineenkephalin as lock mass. TLC analyses were carried out on silica gel 60 F254 precoated plates (Merck, Darmstadt), detection by 1% vanillin/H2SO4. For medium-pressure liquid chromatographic (MPLC) separations, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and Büchi columns (column dimensions 2.6×46 cm, and 1.8×35cm) were used. Silica gel 60 (0.063 – 0.200 mm; Merck, Darmstadt) and Polyamide (Fluka) were utilized for open column chromatography (CC). LiChroprep C_{18} (Merck) material was used for MPLC. Sephadex LH-20 (Fluka) was also used for further separations.

Plant material

Phlomis tuberosa L. (Lamiaceae) was collected from Sivas, Seyfebeli mountain pass, Central Anatolia, Turkey, in July 2001. The plant material was identified by one of us and the voucher specimen (AAD 9308) has been deposited at the Herbarium of the Department of Biology, Faculty of Science, Hacettepe University, Ankara, Turkey.



	R_1	R_2	R_3	R
1	H	OH	α-Cl	H
1a	H	OAc	α-Cl	Ac
2	H	OH	β-OH	H
3	H	H	β-OH	H
4	OH	OH	α-OH	H



	R
5	OH
6	H

Fig. 1. Iridoid glucosides (**1** – **6**) from *P. tuberosa.*

Extraction and isolation

The air-dried and powdered aerial parts of *P. tuberosa* (500 g) were extracted twice with MeOH (2 × 3 l, 5 h) at 45 °C. The combined methanolic extracts were evaporated to dryness *in vacuo* (108 g, yield 21%). The crude extract was suspended in H_2O and partitioned between $CHCl_3$ and *n*-BuOH respectively. An aliquot (11.5 g) of the lyophilised *n*-BuOH extract (21.7 g) was mounted on a column packed with Polyamide. Elution with H_2O and the increasing amounts of MeOH in H_2O (25 – 100%) yielded 11 main fractions, A – K. Fraction A (3.24 g) was subjected to C_{18}-Medium Pressure Liquid Chromatography (C_{18}-MPLC, column dimension: 2.6 × 46 cm) eluting with 0 to 50% MeOH in H_2O as eluent to obtain 14 fractions, A_{1-14}. Purification of fr. A_1 by SiO_2 column (EtOAc-MeOH-H_2O, 100:10:5 to 100:16.5:13.5) yielded methyl β-D-glucopyranoside (75 mg). Fraction A_5 (311 mg) was applied to SiO_2 CC using the mixtures of CH_2Cl_2-MeOH-H_2O (90:10:0 to 70:30:3) to give deoxysesamoside (**6**, 64 mg) in addition to three subfractions, A_{5b-d}. Fraction A_{5c} (58 mg) was rechromatographed on a SiO_2 column (EtOAc-MeOH-H_2O, 100:10:5) to afford decaffeoylacteoside (4 mg) and lamalbide (**2**, 37 mg). Likewise, fraction A_6 (61 mg) was subjected to SiO_2 CC utilising a CH_2Cl_2-MeOH-H_2O gradient (90:10:1 to 80:20:2) to obtain sesamoside (**5**, 19 mg) and lamalbide (**2**, 9 mg). Fraction A_7 (98 mg) was also applied to a SiO_2 column (CH_2Cl_2-MeOH-H_2O, 90:10:1 to 61:32:7) to yield shanzhiside methyl ester (**3**, 9.5 mg). Fraction A_8 (79 mg) afforded 7-*epi*-phlomiol (= phloyoside I) (**4**, 79 mg). Compound **1** (chlorotuberoside, 16 mg) was purified from fraction A_{11} (39 mg) by SiO_2 CC using an EtOAc-MeOH-H_2O mixture (100:8:2). Fraction C (274 mg) was subjected to C_{18}-MPLC (column dimensions: 1.8 × 35 cm), and eluted with MeOH-H_2O mixtures (40 – 100% MeOH) to yield five fractions, C_{1-5}. Repeated chromatography of fr. C_2 (53 mg) on a SiO_2 column (EtOAc-MeOH-H_2O, 100:8:1 to 100:8:2) gave a mixture of dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside and dihydrodehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside (13.5 mg). Fraction D (497 mg) was likewise applied to C_{18}-MPLC (column dimensions: 2.6 × 46 cm) using a MeOH gradient (30 – 100%) to obtain forsythoside B (226 mg) and dehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside (43 mg). Fraction I (418 mg) was chromatographed over SiO_2 column employing CH_2Cl_2-MeOH-H_2O mixtures (90:10:1 and 60:40:4) to give β-sitosterol 3-*O*-β-D-glucopyranoside (3 mg) as well as seven subfractions, I_{2-8}. Purification of fr. I_7 (55 mg) by Sephadex CC (MeOH) furnished luteolin 7-*O*-β-D-glucopyranoside (12.5 mg). Verbascoside was identified as the major compound in fr. G (1333 mg) by a TLC comparison (solvent systems: CH_2Cl_2-MeOH-H_2O, 61:32:7 and EtOAc-MeOH-H_2O, 100:17.5:13.5). Fraction F (1140 mg) was found to be rich in verbascoside, leucosceptoside A and martynoside and was not further studied due to their abundance in the genus *Phlomis*.

Chlorotuberoside (**1**): Amorphous powder; $[\alpha]_D^{25}$ −107.9 (*c* 0.1, MeOH); HRESI-MS: ($C_{17}H_{25}ClO_{11}$) found 441.1193 for $[M+H]^+$, calcd. 441.1163; UV λ_{max} (MeOH, nm): 234; IR ν_{max} (KBr, cm^{-1}) 3447, 1684, 1651, 1304, 1082; ^{1}H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 1.

Lamalbide (**2**): Amorphous powder; $[\alpha]_D^{25}$ −70.9 (*c* 0.1, MeOH); UV λ_{max} (MeOH, nm): 236; IR ν_{max} (KBr, cm^{-1}) 3448, 1702, 1650, 1294, 1081; ^{1}H NMR (500 MHz, CD_3OD): Table 1; ^{13}C NMR (125 MHz, CD_3OD): Table 1.

Acetylation of **1**

To a solution of **1** (4 mg) in pyridine (0.3 ml) was added acetic anhydride (0.15 ml) and the mixture was stirred at r.t. for 2 h. Work-up gave chlorotuberoside pentaacetate (**1a**), which was characterized by ^{1}H and ^{13}C NMR: Table 1.

Results and Discussion

Compound **1** was obtained as an optically active amorphous powder. Its molecular formula was determined as $C_{17}H_{25}ClO_{11}$ by HRESIMS and the spectrum

Table 1. The ^{13}C and ^{1}H NMR spectroscopic data for chlorotuberoside (**1**), lamalbide (**2**) (CD_3OD, ^{13}C: 125 MHz; ^{1}H: 500 MHz)* and the pentaacetate **1a** ($CDCl_3$, 500 MHz).

		1		**1a**‡		**2**	
C/H		δ_C ppm	δ_H ppm, *J* (Hz)	δ_C ppm	δ_H ppm, *J* (Hz)	δ_C ppm	δ_H ppm, *J* (Hz)
1	CH	93.0	5.66 br s	91.9	5.58 br d (1.0)	94.8	5.61 d (1.6)
3	CH	152.0	7.41 d (1.0)	150.0	7.36 br d (1.0)	152.8	7.40 s
4	C	111.3		110.3		111.7	
5	CH	35.8	2.83 ddd (11.5, 4.3,1.0)	31.9	2.99 ddd (11.5, 4.5, 1.0)	37.6	2.92 dd (10.8, 3.9)
6	CH	82.1	3.67†	78.9	5.14 dd (4.5, 8.9)	78.8	3.94 dd (4.4, 3.9)
7	CH	74.1	4.01 d (8.9)	71.5	4.13 d (8.9)	78.9	3.54 d (4.4)
8	C	77.3		76.7		78.5	
9	CH	47.5	2.66 d (11.5)	45.9	2.80 d (11.5)	49.3	2.80 dd (10.8, 1.6)
10	CH_3	18.6	1.19 s	18.9	1.31 s	22.1	1.20 s
11	C	169.1		165.7		169.5	
OCH_3	CH_3	51.6	3.74 s	51.5	3.65 s	51.9	3.72 s
1’	CH	99.3	4.61 d (7.9)	95.4	4.85 d (8.1)	99.7	4.60 d (7.9)
2’	CH	74.1	3.14 dd (7.9, 8.9)	70.4	4.96 dd (8.1, 9.5)	74.5	3.16 dd (7.9, 9.0)
3’	CH	77.5	3.35 t (8.9)	72.3	5.21 t (9.5)	77.9	3.35 t (9.0)
4’	CH	71.7	3.28 t (8.9)	67.8	5.11 t (9.5)	71.5	3.37 t (9.0)
5’	CH	77.9	3.30 m	72.1	3.75 ddd (10, 4, 2.5)	78.3	3.32 m
6’	CH_2	62.3	3.88 dd (12.0, 2.0)	61.4	4.15 m†	62.8	3.88 dd (11.9, 1.7)
			3.66 dd (12.0, 5.9)		4.31 dd (12.4, 4.2)		3.65 dd (11.9, 5.7)

* Proton and carbon assignments are based on 2D NMR (DQF-COSY, gHSQC, gHMBC and NOESY); † overlapped signal; ‡ additional signals for 5 acetyl groups at $\delta = 1.88 - 2.16$ (20.5 – 23.7). ^{13}C NMR data were obtained from gHSQC and gHMBC spectra.

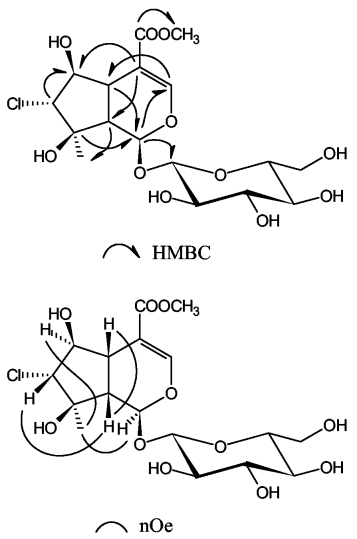


Fig. 2. Significant HMBC (C → H) and NOESY correlations for **1**.

displayed an additional signal two mass units above the $[M+H]^+$-peak. The UV spectrum of **1** revealed a maximum at 224 nm, typical for C-4 substituted iridoids. The ^{13}C NMR spectroscopic data (see Table 1) indicated the presence of 17 carbons resonances, six of which were assigned to a β-glucopyranosyl moiety. The remaining 11 resonances together with the corresponding proton signals were indicative of a C_{10} iridoid bearing a methoxycarbonyl group at C-4 position.

The ^{1}H and ^{13}C NMR chemical shifts of **1** were assigned by means of DQF-COSY, gHSQC and gHMBC (Fig. 2) experiments. The data of **1** were similar to those of lamalbide (**2**) (Table 1) [5]. However, the resonances attributed to C-7 ($\delta_C = 74.1$) and C-6 ($\delta_C = 82.1$) showed considerable upfield ($\Delta = 4.8$ ppm) and downfield ($\Delta = 3.3$ ppm) shifts, respectively. Additionally, the signals from H-6 ($\delta_H = 3.67$) and H-7 ($\delta_H = 4.01$) as well as the coupling constant between them ($J_{7,6} = 8.9$ Hz, *trans*-diaxial arrangement) were also different from those of lamalbide (**2**). These differences in chemical shift values and coupling pattern indicated (i) that the relative stereochemistry at C-7 was different from that found in lamalbide, and (ii) that the chlorine substituent was placed at this carbon atom. In the NOESY spectrum, the nOe interactions (Fig. 2) between H-1/H_3-10 and H_3-10/H-6 revealed that these protons were placed on the same side (α) of the molecule. Contrary, prominent nOe correlations were observed between H-9/H-5 and H-9/H-7, all positioned at the β-face of the cyclopentane ring. Thus, the substituent at C-7 had to be situated in the α-position. Changing the placement of the substituents at C-6 and C-7 from a *cis*- (as in **2**) to a *trans*-disposition (as in **1**) would be expected to cause a downfield shift for both carbon signals [6]. However, as noted above a downfield shift was only seen for the C-6 signal, while the C-7 signal was shifted upfield when compared to the spectrum of **2**. This is consistent with a chlorine atom in the 7α-position [7]. To further prove the pro-

posed structure, we acetylated **1** under mild conditions and obtained the pentaacetate (**1a**). The ^{1}H NMR spectrum (Table 1) was consistent with the given structure since a large downfield shift (+1.5 ppm) was seen for the H-6 signal when compared to the spectrum of **1** while the position of the remaining signals of the aglucone were virtually unchanged. Therefore, chlorotuberoside is proposed as trivial name for the new compound (**1**). Biosynthetically, **1** could be derived from 6-deoxysesamoside (**6**) by a nucleophilic attack of chloride from the α-face of the cyclopentane ring, which would take place on the least hindered carbon atom (C-7) of the epoxide. Chloro-substituted iridoids are not uncommon. The compound phloyoside II has a similar 7-chloro substituent and is known from four species of *Phlomis*, namely *P. rotata* [8], *P. younghusbandii* [7], *P. umbrosa* [9] and *P. mongolica* [10].

In addition to the new compound, the known compounds, lamalbide (**2**) [5], shanzhiside methyl ester (**3**) [11], 7-*epi*-phlomiol (= phloyoside I) (**4**) [7], sesamoside (**5**) [12], 5-deoxysesamoside (**6**) [3], forsythoside B [13], decaffeoylacteoside [14], dehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside [15], dihydrodehydrodiconiferyl alcohol 9-*O*-β-D-glucopyranoside and dihydrodehydrodiconiferyl alcohol 9'-*O*-β-D-glucopyranoside [16], luteolin 7-*O*-β-D-glucopyranoside [17], β-sitosterol 3-*O*-β-D-glucopyranoside [18] as well as 1-methyl-*O*-β-D-glucopyranoside [19] were isolated and identified by comparison of their spectroscopic (NMR and MS) data with those published in the literature.

According to the results of our project conducted on *Phlomis* species growing in Turkey, *P. tuberosa* seems to be the richest species concerning the iridoid glycosides among the 33 species. 7-*epi*-phlomiol (= phloyoside I) is new to the title species.

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