Iridoid Glucosides from Turkish \textit{Phlomis tuberosa}

Ihsan Calisa, Hasan Kirmizibekmez\textsuperscript{a}, Tayfun Ersoz\textsuperscript{a}, Ali A. D"onmezb, Charlotte H. Gotfredsenc, and Søren R. Jensenc

\textsuperscript{a} Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, TR-06100 Ankara, Turkey
\textsuperscript{b} Department of Biology, Faculty of Science, Hacettepe University, 06532 Ankara, Turkey
\textsuperscript{c} Department of Chemistry, The Technical University of Denmark, Building 201, DK-2800 Lyngby, Denmark

Reprint requests to Dr. Hasan Kirmizibekmez. Fax: +90 312 311 4777. E-mail: hasankbekmez@yahoo.com

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From the aerial parts of \textit{Phlomis tuberosa} a new iridoid glucoside, chlorotuberoside was isolated together with five known iridoid glucosides, lamalbide, shanzhiside methyl ester, 7-\textit{epi}-phlomiol (\textit{=} phloyoside I), sesamoside and 5-deoxysesamoside. Two known phenylethanoid glycosides, forsythoside B, and decaffeoylacteoside, three known neolignan glycosides, dehydrodiconiferyl alcohol 9'-\textit{O}-\textit{β}-D-glucopyranoside, dihydrodehydrodiconiferyl alcohol 9-\textit{O}-\textit{β}-D-glucopyranoside and dihydrodehydrodiconiferyl alcohol 9'-\textit{O}-\textit{β}-D-glucopyranoside, one flavone glycoside, luteolin 7-\textit{O}-\textit{β}-D-glucopyranoside, as well as \textit{β}-sitosterol 3-\textit{O}-\textit{β}-D-glucopyranoside and 1-methyl-\textit{O}-\textit{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.

\textbf{Key words}: \textit{Phlomis tuberosa}, Lamiaceae, Iridoid Glucosides, Chlorotuberoside

\section*{Introduction}

\textit{Phlomis tuberosa} L. is one of the 33 species growing in Turkey [1]. In Asian folk medicine \textit{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 \textit{Phlomis} species, we have now investigated \textit{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.

\section*{Experimental Section}

\textbf{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\textsubscript{3} (acetate) using the solvent peak (δ\textsubscript{H} 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/H\textsubscript{2}SO\textsubscript{4}. For medium-pressure liquid chromatographic (MPLC) separations, a Lewa M5 pump, a LKB 17000 Minirac fraction collector, a Rheodyne injector, and B"uchi columns (column dimensions 2.6 × 46 cm, and 1.8 × 35 cm) were used. Silica gel 60 (0.063 – 0.200 mm; Merck, Darmstadt) and Polyamide (Fluka) were utilized for open column chromatography (CC). LiChroprep C\textsubscript{18} (Merck) material was used for MPLC. Sephadex LH-20 (Fluka) was also used for further separations.

\textbf{Plant material}

\textit{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.
increasing amounts of MeOH in H2O (25–100%) yielded addition to three subfractions, A5b (90:10:0 to 70:30:3) to give deoxysesamoside (6). MeOH-H2O, 100:10:5 to 100:16.5:13.5) yielded methyl CHCl3 and subjected to SiO2 CC utilising a CH2Cl2-MeOH-H2O gradient to give lamalbide (2). Likewise, fraction A5c (58 mg) was rechromatographed on a SiO2 column (EtOAc-MeOH-H2O, 100:10:5) to afford decaffeoylacteoside (4 mg) and lamalbide (2, 37 mg). Fraction A6 (79 mg) afforded 7-epi-phloymoside (D) (4, 79 mg). Compound I (chlorotuberoside, 16 mg) was purified from fraction A11 (39 mg) by SiO2 CC using an EtOAc-MeOH-H2O mixture (100:8.2). Fraction C (274 mg) was subjected to C18-MPLC (column dimensions: 1.8 × 35 cm), and eluted with MeOH-H2O mixtures (40–100% MeOH) to yield five fractions, C1–5. Repeated chromatography of fr. C5 (53 mg) on a SiO2 column (EtOAc-MeOH-H2O, 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 C18-MPLC (column dimensions: 2.6 × 46 cm) using a MeOH gradient (30–100%) to obtain forsythiside B (226 mg) and dehydrodiconiferyl alcohol 9'-O-β-D-glucopyranoside (43 mg). Fraction I (418 mg) was chromatographed over SiO2 column employing CH2Cl2-MeOH-H2O mixtures (90:10:1 and 60:40:4) to give β-sitosterol 3-O-β-D-glucopyranoside (3 mg) as well as seven subfractions, I2–8. Purification of fr. I7 (55 mg) by Sephadex CC (MeOH) furnished luteolin 7-β-D-glucopyranoside (12.5 mg). Verbascoside was identified as the major compound in fr. G (133 mg) by a TLC comparison (solvent systems: CH2Cl2-MeOH-H2O, 61:32:7 and EtOAc-MeOH-H2O, 100:8:2). Fraction G (133 mg) was subjected to C18-MPLC (column dimensions: 1 × 46 cm) eluting with a MeOH gradient (30–100%) to obtain sesamoside (5, 19 mg) and lamalbide (2, 9 mg). Fraction A7 (98 mg) was also applied to a SiO2 column (CH2Cl2-MeOH-H2O, 90:10:1 to 61:32:7) to yield shanzhiside methyl ester (3, 9.5 mg). Fraction A8 (79 mg) afforded 7-epi-phloymoside (= phloymoside D) (4, 79 mg).

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 H2O and partitioned between CHCl3 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 H2O and the increasing amounts of MeOH in H2O (25–100%) yielded 11 main fractions, A–K. Fraction A (3.24 g) was subjected to C18-Medium Pressure Liquid Chromatography (C18-MPLC, column dimension: 2 × 46 cm) eluting with a MeOH gradient (30–100%) to obtain 14 fractions, A1–14. Purification of fr. A1 by SiO2 column (EtOAc-MeOH-H2O, 100:10:5 to 100:16.5:13.5) yielded methyl β-D-glucopyranoside (75 mg). Fraction A5 (311 mg) was applied to SiO2 CC using the mixtures of CH2Cl2-MeOH-H2O (90:10:0 to 70:30:3) to give deoxysesamoside (6, 64 mg) in addition to three subfractions, A5b–d. Fraction A5c (58 mg) was rechromatographed on a SiO2 column (EtOAc-MeOH-H2O, 100:10:5) to afford decaffeoylacteoside (4 mg) and lamalbide (2, 37 mg). Likewise, fraction A6 (61 mg) was subjected to SiO2 CC utilising a CH2Cl2-MeOH-H2O gradient (90:10:1 to 80:20:2) to obtain sesamoside (5, 19 mg) and lamalbide (2, 9 mg). Fraction A7 (98 mg) was also applied to a SiO2 column (CH2Cl2-MeOH-H2O, 90:10:1 to 61:32:7) to yield shanzhiside methyl ester (3, 9.5 mg). Fraction A8 (79 mg) afforded 7-epi-phloymoside (= phloymoside D) (4, 79 mg). Compound I (chlorotuberoside, 16 mg) was purified from fraction A11 (39 mg) by SiO2 CC using an EtOAc-MeOH-H2O mixture (100:8.2). Fraction C (274 mg) was subjected to C18-MPLC (column dimensions: 1.8 × 35 cm), and eluted with MeOH-H2O mixtures (40–100% MeOH) to yield five fractions, C1–5. Repeated chromatography of fr. C5 (53 mg) on a SiO2 column (EtOAc-MeOH-H2O, 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 C18-MPLC (column dimensions: 2.6 × 46 cm) using a MeOH gradient (30–100%) to obtain forsythiside B (226 mg) and dehydrodiconiferyl alcohol 9'-O-β-D-glucopyranoside (43 mg). Fraction I (418 mg) was chromatographed over SiO2 column employing CH2Cl2-MeOH-H2O mixtures (90:10:1 and 60:40:4) to give β-sitosterol 3-O-β-D-glucopyranoside (3 mg) as well as seven subfractions, I2–8. Purification of fr. I7 (55 mg) by Sephadex CC (MeOH) furnished luteolin 7-β-D-glucopyranoside (12.5 mg). Verbascoside was identified as the major compound in fr. G (133 mg) by a TLC comparison (solvent systems: CH2Cl2-MeOH-H2O, 61:32:7 and EtOAc-MeOH-H2O, 100:17.5:13.5). Fraction F (1140 mg) was found to be amorphous powder. Its molecular formula was determined as C17H25ClO11 by HRESIMS and the spectrum was not further studied due to their abundance in the genus Phlomis.

Chlorotuberoside (1): Amorphous powder; [α]25D −107.9 (c 0.1, MeOH); HRESI-MS: (C17H25ClO11) found 441.1193 for [M+H]+, calcld. 441.1163; UV λmax (MeOH, nm): 234; IR νmax (KBr, cm−1) 3447, 1684, 1651, 1304, 1082; 1H NMR (500 MHz, CD3OD): Table 1; 13C NMR (125 MHz, CD3OD): Table 1.

Lamalbide (2): Amorphous powder; [α]25D −70.9 (c 0.1, MeOH); UV λmax (MeOH, nm): 236; IR νmax (KBr, cm−1) 3448, 1702, 1650, 1294, 1081; 1H NMR (500 MHz, CD3OD): Table 1; 13C NMR (125 MHz, CD3OD): 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 1H and 13C NMR: Table 1.

Results and Discussion

Compound I was obtained as an optically active amorphous powder. Its molecular formula was determined as C17H25ClO11 by HRESIMS and the spectrum...
Table 1. The $^{13}$C and $^1$H NMR spectroscopic data for chlorotuberoside (1), lamalbide (2) (CD$_3$OD, $^{13}$C: 125 MHz; $^1$H: 500 MHz)* and the pentaacetate 1a (CDCl$_3$, 500 MHz).

<table>
<thead>
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<th>C/H</th>
<th>$\delta$C ppm</th>
<th>$\delta$H ppm, J (Hz)</th>
<th>$\delta$C ppm</th>
<th>$\delta$H ppm, J (Hz)</th>
<th>1a‡</th>
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<td>2.99 ddd (11.5, 4.5, 1.0)</td>
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<td>3.65 dd (11.9, 5.7)</td>
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* 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.

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 = 4.01) and H-7 ($\delta$H = 4.13) as well as the coupling constant between them ($J_{6,7}$ = 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/H3-10 and H-3-10/H-6 revealed that these protons were placed on the same side ($\alpha$) of the molecule. Contrary, prominent nOe correlations were observed between H-9/H-5 and H-9/H-7, all positioned at the $\beta$-face of the cyclopentane ring. Thus, the substituent at C-7 had to be situated in the $\alpha$-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 7$\beta$-position [7]. To further prove the pro-

Fig. 2. Significant HMBC (C $\rightarrow$ 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 $\beta$-glucopyranosyl moiety. The remaining 11 resonances together with the corresponding proton signals were indicative of a C10 iridoid bearing a methoxycarbonyl group at C-4 position.
posed structure, we acetylated 1 under mild conditions and obtained the pentaacetate (1a). The 1H 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. youngusbandii [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], decaffeoylacteose [14], dehydrodiconiferyl alcohol 9'-O-β-D-glucopyranoside [15], dihydrodiconiferyl alcohol 9-O-β-D-glucopyranoside and dihydrodiconiferyl alcohol 9'-O-β-D-glucopyranoside [16], luteolin 7-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.

Acknowledgements

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