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Iridoids from *Phlomis aurea* Decne growing in Egypt

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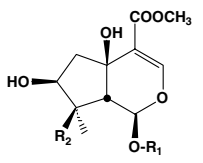
A new iridoid gentiobioside (**4**, assigned the name phlomiside) was isolated from the leaves of *Phlomis aurea* growing in Egypt, in addition to auroside (**1**), lamiide (**2**), 8-*epi*-loganin (**3**), forsythoside B (**5**), quercetin-3-*O*-β-D-glucopyranoside (**6**) and kaempferol-3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside (**7**). Structures of these compounds were elucidated by conventional methods of analysis as well as by different spectroscopic techniques.

1. Introduction

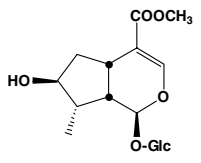
The family Lamiaceae is well-represented in Egypt, where several species belonging to different genera grow wildly. One of these species, *Phlomis aurea* Decne grows wildly in Sinai [1]. Chemical composition and antimicrobial activity of the essential oils from certain *Phlomis* species have been reported [2–4]. Several *Phlomis* species have been found to contain glycosides of iridoids [5–10], phenylpropanoids [11, 12], flavonoids [13, 14], diterpenes [15] and phenylethanoids [16, 17]. Certain flavonoids and iridoid glucosides have been reported from *P. aurea* and *P. floccosa* growing in Egypt [6, 10, 13]. Certain *Phlomis* species have been in use in folkmedicine in joints pain, bronchitis, cold and some other ailments [18]. Cytotoxic, cytostatic, anti-inflammatory, immunosuppressant and antimicrobial activities have been reported for phenylpropanoids from certain *Phlomis* species [19]. We report herein the isolation and structure elucidation of seven compounds including a new iridoid gentiobioside (**4**) from the leaves of *P. aurea* Decne growing in Egypt.

2. Investigations, results and discussion

Through repeated column chromatography of the acetone and methanol extracts of the leaves of *P. aurea*, seven compounds **1**-**7** were obtained and their structures were elucidated by different spectroscopic methods. Compounds **1**, **2**, **6** and **7** were obtained from the methanol extract, while **3**–**5** and an additional amount of **6** were obtained from the acetone extract. Compounds **1**–**5** were identified as auroside (**1**) [12], lamiide (**2**) [20], 8-*epi*-loganin (**3**) [21], forsythoside B (**5**) [16], while compound **4** was found to be a new iridoid gentiobioside.



1, R_1=Glc, R_2=H
2, R_1=Glc, R_2=OH
4, R_1=Glc^6-Glc, R_2=H



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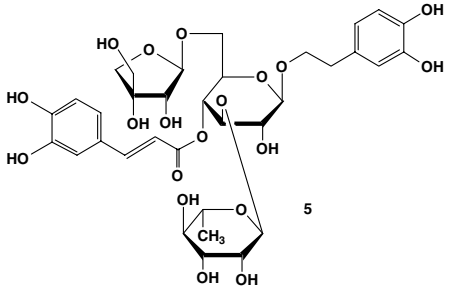

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Compound **4** was obtained as a white amorphous powder, $[\alpha]_D$ −36° (C 0.15, MeOH). The atmospheric pressure ionization mass (API-MS) and FAB MS (positive ion mode) of **4** showed a quasimolecular ion preak at m/z 591 $[M + Na]^+$ consistent with the molecular formula $C_{23}H_{36}O_{16}Na$ (HR-FAB-MS m/z 591.2846). The IR spectrum of **4** showed the presence of hydroxyl (3450 cm^{-1}, broad), carbonyl (1670 cm^{-1}) and olefinic (1070 cm^{-1}) functions. The ^1H NMR spectrum of **4** analyzed by the aid of ^1H-^1H COSY showed signals for two sugar units, signals for two anomeric protons at δ 4.45 and 4.56, and signals for a monoterpene aglycone. The large coupling constants of the anomeric protons ($J_{H1,2} = 7.5$ Hz) are typical of β-linked sugars. Signals for two β-glucopyranosyl units and an aglycone moiety were distinguished from the ^{13}C NMR and DEPT spectra (see experimental). Glucose was the only sugar component identified in the acid hydrolyzate of **4**. The ^{13}C chemical shifts of the sugar moiety and the downfield shift of C-6′ (+6 ppm) suggested a gentiobiose-type linkage (1 → 6) of the two glucose units. Long-range correlation observed between C-1″ and H-6′ confirmed this finding. Furthermore, long range correlation between C-1 and H-1′ confirmed acylation at C-1 of the aglycone with a gentiobiose unit. The compound was assigned the name "phlomiside".

This is the first report on the isolation of **4** from nature. In addition, forsythoside B previously reported from *P. pungens* Willd *var pungens* [16] is reported here for the first time from *P. aurea*. Moreover, the two flavonoid glycosides **6** and **7**, previously reported from *P. lychnitys* [14], are reported here for the first time in *P. aurea*.

3. Experimental

3.1. Plant material

P. aurea Decne (syn.: *P. angustifolia* Miller and *P. flavescens* Miller) was collected from Saint Katharine, Sinai, Egypt in April 1999. The plant was kindly identified by Prof. Abdelsalam El-Nuwaihy, Department of Botany, Faculty of Science, Ain-Shams University, Cairo, Egypt. A voucher specimen is deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The leaves were air-dried and ground to coarse powder for extraction.

3.2. Apparatus and methods

Optical rotations were measured in MeOH with a Jasco DIP-360 automatic polarimeter. IR spectra were measured in KBr with a Jasco FT/IR-230 infrared spectrometer. ^1H- and ^{13}C NMR spectra were measured with a Jeol JNA-LA 400WB-FT (^1H, 400 MHz; ^{13}C, 100 MHz) spectrometer, the chemical shifts being represented as ppm with TMS as an internal stand-

ard. Atmospheric pressure ionization mass (API-MS) spectra (positive mode) were measured with a Perkin-Elmer SCIEX API-*III* Biomolecular Mass Analyser. Fast atom bombardment (FAB) MS and high resolution FAB-MS were performed on a Jeol JMS-700T spectrometer and glycerol was used as a matrix.

3.3. Extraction, isolation and structure elucidation

The powdered air-dried leaves of *Phlomis aurea* (300 g) was exhaustively extracted by percolation with warm Me_2CO followed by warm MeOH. The MeOH extract was evaporated under reduced pressure to give 43.8 g of dry residue. The residue was suspended in H_2O (100 ml) and passed through a column of Diaion HP-20 (500 ml). After washing with H_2O, elution was started with 50% MeOH in H_2O and then MeOH. The 50% MeOH-eluted fraction (1.5g) was further fractionated by Sephadex LH-20 CC. Elution with 50% MeOH gave two subfractions, Fr. A (125 mg) and B (1.3 g). CC of Fr. B on a column of SiO_2 using $CHCl_3$-MeOH (7 : 3) afforded **1** (25 mg), **2** (79 mg), quercetin-3-*O*-β-D-glucoside **6** (6 mg) and kaempferol-3-*O*-β-D-glucopranosyl-(1-6)-β-glucoside **7** (5 mg).

The residue from Me_2CO extract (18 g) was similarly treated as the MeOH extract to give three subfractions: Fr. I (35 mg), Fr. II (1.5 g) and Fr. III (70 mg). Fr. I was chromatographed on a Sephadex LH-20 column using H_2O to give 10 fractions. Medium pressure liquid chromatography (MPLC) of Fr. 2-4 (23 mg) over RP-18, using 20% MeOH in water afforded a mixture of **4** and **5**. This mixture was separated by preparative layer chromatography (SiO_2, $CHCl_3$–MeOH–H_2O, 8 : 4 : 1, lower layer) to give **4** (10 mg) and **5** (5 mg). Fr. II furnished **3** (102 mg) after CC on SiO_2 using $CHCl_3$–MeOH (7 : 3), while Fr. III gave an additional amount of **6** (18 mg) after MPLC (RP-18) using 30% MeOH in H_2O.

Compound **1** was obtained as a white amorphous powder. API-MS m/z (rel. int.) 429 $[M + Na]^+$ (100). This compound was identified as auroside by direct comparison of its ^1H and ^{13}C NMR spectral data with those reported in literature [12].

Compound **2** was obtained as a white amorphous powder. API-MS m/z (rel. int.) 445 $[M + Na]^+$ (100). The ^1H- and ^{13}C NMR spectral data of this compound were identical to those reported for lamiide [20].

Compound **3** was obtained as a white amorphous powder. API-MS m/z (rel. int.) 413 $[M + Na]^+$ (100). This compound was identified as 8-*epi*-loganin by direct comparison of its spectral data with those reported [21].

Compound **4** was obtained as a white amorphous powder, $[\alpha]_D$ −36° (*c* 0.15, MeOH). API-MS m/z (rel. int.) 591 $[M + Na]^+$ (100), FAB-MS m/z 591 $[M + Na]^+$, HR-FAB-MS m/z 591.2846 (calculated for $C_{23}H_{36}O_{16}Na$: 591.2791). IR (KBr) ν_{max} cm^{-1}: 3450, 1760 and 1070. ^1H-NMR (400 MHz, CD_3OD): δ 0.94 (3H, d, J = 7 Hz), 2.02 (2H, m), 2.53 (1H, m), 3.10–4.15 (sugar protons), 3.73 (3H, s, $COOCH_3$), 4.47 (1H, d, J = 7.5 Hz, Glc-1′), 4.58 (1H, d, J = 7.5 Hz, Glc-1″), 5.73 (1H, brd, H-1), and 7.46 (1H, s, H-3). ^{13}C NMR (100 MHz, CD_3OD): δ 13.8 (C-10), 29.5 (C-5), 39.6 (C-6), 41.7 (C-9), 43.5 (C-8), 51.7 ($COO\underline{C}H_3$), 62.7 (Glc C-6′), 69.4 (Glc C-″), 70.4 (C-4′), 70.6 (C-4″), 73.2 (C-2′), 73.6 (C-2″), 76.2 (C-5′), 76.6 (C-5″), 76.8 (C-3″), 77.0 (C-3′), 78.3 (C-7), 95.6 (C-1), 99.6 (C-1′), 102.6 (C-1″), 115.9 (C-3), 153.5 (C-4), and 168.1 ($\underline{C}OOCH_3$). The sugar moiety proved to be gentiobioside. The compound was assigned the name phlomiside.

Compound **5** was obtained as a white amorphous powder. API-MS m/z (rel. int.) 795 $[M + K]^+$ (80) and 779 $[M + Na]^+$ (60). This compound was identified as forsythoside B by comparing its spectral data with those reported in the literature [12].

Compounds **6** and **7** were obtained as yellowish powders. Their physicochemical characters, chromatographic behaviour as well as UV, ^1H and ^{13}C NMR data were in agreement with those reported in literature [13, 14, 22, 23] for quercetin-3-*O*-β-D-glucopyranoside and kaempferol-3-*O*-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside, respectively.

3.4. Acid hydrolysis of **4**

A solution of **4** (2 mg) in 15% aqueous HCl–dioxane (1 : 1 v/v, 2 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was passed through a Sep-pak C18 cartridge eluting with H_2O and MeOH. The water eluate was concentrated *in vacuo* to give a residue in which glucose was identified by TLC (solvent system EtOAc–MeOh–AcOH–H_2O; 14 : 30 : 1.5 : 2). The MeOH eluate gave a resinous material which could not be identified.

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