A new isoflavone glycoside from *Pueraria alopecuroides*

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A new isoflavone glycoside, (−)-tuberosin-3-O-β-D-glucopyranoside (1), along with 10 known compounds 1\textsubscript{a}-10, was isolated from *Pueraria alopecuroides*. Their structures were determined on the basis of spectral data including 1D and 2D NMR and HREIMS. These compounds were isolated from this plant for the first time.

Keywords: *Pueraria alopecuroides*; isoflavone glycoside; (−)-tuberosin-3-O-β-D-glucopyranoside

1. Introduction

*Pueraria alopecuroides* Craib is a valuable traditional Dai herb medicine, distributed in the south of Yunnan provinces of China. Dai people in that area of China use its tuberous roots to ease heart attack, improve blood circulation, treat hypertension and typhoid fever. It is a species close to *Pueraria mirifica*, which is a traditional health food in Thailand. The tuberous root of *P. mirifica* has estrogentic activity that can maintain vigour in postmenopausal women, and the major active compounds are several special isoflavonoids (Okamura et al. 2008). Therefore, it is expected that isoflavonoids with similar estrogentic activity will be discovered in *P. alopecuroides*, and *P. alopecuroides* could be developed as an alternative resource to *P. mirifica*.

Many isoflavonoids, isoflavone glycosides, such as krakhurin and miroestrol have been found previously in *P. mirifica* (Tahara et al. 1987). As a part of our ongoing search for bioactive secondary metabolites from Chinese tropical medicinal plants, a careful investigation on the chemical constituents of the tuberous roots of *P. alopecuroides*, a plant close to *P. mirifica*, led to the isolation and identification of one new isoflavone glycoside, (−)-tuberosin-3-O-β-D-glucopyranoside (1), together with 10 known compounds, tuberosin (1\textsubscript{a}), 4′,7-dihydroxyiso-
flavone (2) 4',7-hydroxyioflavone-7-O-β-d-glucopyranoside (3), ononin (4), wighteone (5), oroxylin-7-O-β-d-glucopyranoside (6), baicalin-7-O-β-d-glucopyranoside (7), kaempferol-7-O-β-d-glucopyranoside (8), chrysin-7-O-β-d-giuronate methyl ester (9), (Z)-7,11-dihydroxy-3-methoxy-10,11-dihydrodibenzo[b,f]oxepine-10-carbaldehyde (10).

2. Results and discussion

Compound 1 was isolated as a yellow amorphous powder. The molecular formula was determined as C_{26}H_{29}O_{10} on the basis of the [M]^+ peak in the HR-ESI-MS spectrum at m/z 500.49. The UV spectrum showed absorption maxima at 282.60 and 204.40 nm, while IR spectrum suggested the presence of OH groups (3442.79 cm^{-1}) and a conjugated olefinic bond (1623.09 cm^{-1}).

A group of δ = 4.13 (1H, t, J = 11.5 Hz), 3.99 (1H, t, J = 11.5 Hz) and 5.23 (1H, s) in the 1H-NMR spectrum (Table S1) suggested the presence of a O–CH$_2$–COH–CH–O unit forming the B and C rings of a pterocarpan nucleus. The 1H-NMR and 13C-NMR spectra of 1 were similar to those of (−)-tuberosin. A major difference was in the signals of 102.10(C-1″), 74.96(C-2″), 78.29(C-3″), 71.41(C-4″), 78.04(C-5″) and 62.54(C-6″). The 13C-NMR spectrum showed 26 signals, sorted by DEPT experiments into 2 CH, 2 CH$_2$, 13 CH, and 9 quaternary C. Based on the comparison of NMR spectral data with those reported in literature, the signals of seven O-bearing C at 102.10(C-1″), 74.96(C-2″), 78.29(C-3″), 71.41(C-4″), 78.04(C-5″) and 62.54(C-6″) were consistent with the β-d-glucopyranoside group (Woo & Piao 2004; Yan et al. 2014). Though the HMBC didn’t show the correlation signal from δ(H) 4.89 (overlap, 1H-1″) to δ(C) 160.39 (C-3), the ROESY showed the signal of δ(H) 4.89 (overlap, 1H-1″) was correlated to 6.80 (dd, J = 8.4, 2.4 Hz, H-C(2)) and 6.63 (d, J = 2.4 Hz, H-C(4)), which indicated that the β-d-glucopyranoside group was linked to the (−)-tuberosin group through C(1″)-O-C(3) (Figure S1). Thus, the structure of 1 was determined as (−)-tuberosin-3-O-β-d-glucopyranoside.

Ten known compounds isolated were identified as tuberosin (Ia) (Joshi & Kamat 1973), 4', 7-dihydroxyisoflavone (2) (Kinjo et al. 1987), 4',7-hydroxyioflavone-7-O-β-d-glucopyranoside (3) (Hirakura et al. 1997), ononin (4) (Lewis et al. 1998), wighteone (5) (Nomura et al. 1989), oroxylin-7-O-β-d-glucopyranoside (6) (Tomimori et al. 1982), baicalin-7-O-β-d-glucopyranoside (7) (Tomimori et al. 1984), kaempferol-7-O-β-d-glucopyranoside (8) (Markham et al. 1978), chrysin-7-O-β-d-giuronate methyl ester (9) (Rosa 1983) and (Z)-7,11-dihydroxy-3-methoxy-10,11-dihydrodibenzo[b,f]oxepine-10-carbaldehyde (10) (Hu et al. 2010) by the comparison with spectroscopic data in the literature.

3. Experimental

3.1. General experimental procedures

Optical rotations were taken on a Jasco DIP-370 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were measured on a Shimadzu-UV-2401A spectrophotometer (Shimadzu, Tokyo, Japan) with methanol as solvent. Infrared (IR) spectra were recorded on a Bio-Rad-FTS-135 spectrometer (Bio-Rad, Hercules, California) in KBr pellets. 1D- and 2D-NMR spectra were obtained on a Bruker-DRX-500 spectrometer (Bruker, Billerica, Massachusetts) (EI-MS) and a Micro Q-TOF MS (HE-RSI-MS), respectively. Column chromatography was run on silica gel (200–300 mesh; 10–40 mm) (Qingdao Marine Chemical Inc., Qingdao, P.R. China), RP-18 gel (40–63 mm) (Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia, Stockholm,
Sweden). Fractions were monitored by thin layer chromatography (TLC) and spots were visualised by heating silica gel plates sprayed with 10% H$_2$SO$_4$/H$_2$O.

3.2. Plant material
The tuberous roots of _P. alopecuroides_ were collected from Menglun and Mengyang, Xishuangbanna, Yunnan, China, in October 2012, and were authenticated by Professor Qi-Shi Song, Xishuangbanna Tropical Botanical Garden. A voucher specimen (no. 20121011) was deposited at the Research Group on Ethnomedicine of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

3.3. Extraction and isolation
The air-dried and powdered tuberous roots of _P. alopecuroides_ (40 kg) were extracted with 90% aqueous ethanol and filtered at room temperature. The filtrate was concentrated and extracted with petroleum ether, then extracted by CHCl$_3$ and BuOH. The BuOH extract (385.67 g) was subjected to silica gel column chromatography eluted with a MeOH/CHCl$_3$ from 1:100, up to 50:50 (by increasing MeOH and reducing CHCl$_3$), to generate six sub-fractions (Fr.1–Fr.6). All

![Figure 1. Structures of compounds 1–10.](image-url)
subfractions were collected and combined by TLC monitoring. Fr.2 (74 g) was further chromatographed over silica gel using MeOH/CHCl$_3$ (1:20) and RP-18 column (using a gradient of MeOH/H$_2$O (30:70) to reach (90:10)) affording compound 2 (21 mg) and compound 7 (15 mg). Fr.3 (121 g) was further chromatographed over silica gel using MeOH/CHCl$_3$ (1:10), RP-18 column (using a gradient of MeOH/H$_2$O (40:70) to reach (90:10)) and Sephadex LH-20 (MeOH) to yield compounds 1, 1a, 3–6, and 8. Compounds 9 and 10 were obtained from Fr.4 in a similar fashion (Figure 1).

(−)-Tuberosin-3-O-β-D-glucopyranoside (= 3-β-D-glucopyranoside-(6aS,13aS)-10,10-dimethyl-6H,10H-chromeno[6′,7′:4,5]furo[3,2-c]chromene-3,6a(13aH)-diol) (1): Yellow amorphous powder. [α]$_{23}^2$ = 119.333 (c = 0.0015 g/mL, MeOH). UV $\lambda_{max}$ (MeOH) (log ε): 282.60 (4.2312), 204.40 (4.9662) nm. IR (neat)max: 3442.79, 1623.09 cm$^{-1}$. $^1$H-NMR (600 MHz, CD$_3$OD) and $^{13}$C-NMR (150 MHz, CD$_3$OD) see Table S1. HR-ESI [M + Na]$^+$: 523.1576 m/z for C$_{26}$H$_{28}$O$_{10}$ (calcd. 523.1580).

4. Conclusions

P. alopecuroides is a valuable traditional Dai medicine. A new isoflavone glycoside, (−)-tuberosin-3-O-β-D-glucopyranoside (1), along with 10 known compounds 1a-10, was isolated from P. alopecuroides. These compounds were isolated from this plant for the first time.

Supplementary material

Supplementary material relating to this paper is available online at http://dx.doi.org/10.1080/14786419.2015.1038813.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Note

1. These authors contributed equally to this work.

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