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Iridoids, flavonoids, and monoterpene diglycoside from the roots of *Triosteum himalayanum* Wall.

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ABSTRACT

The phytochemical investigation of the roots of *Triosteum himalayanum* Wall. led to the isolation of eleven iridoids (**1**–**6** and **8**–**12**), one monoterpene diglycoside (**7**), and four flavonoids (**13**–**16**), of which 4′,6′-di-*O*-isopropylidene sweroside (**1**) and secologanin diethylacetal (**2**) were new iridoid glucosides. The existence of iridoids and flavonoids provided further confirmation of the typical profile of the secondary metabolites found in the genus *Triosteum*, suggesting a close relationship among the species of Caprifoliaceae.

1. Subject and source

The genus *Triosteum* Linn. (Caprifoliaceae) consists of about eight species mainly distributed in East Asia and North America (Hsu et al., 1988), with 3 species in China (Xu et al., 1988). *Triosteum himalayanum* Wall., has been used in Chinese folk medicine for inducing diuresis and promoting blood circulation (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1999). The roots of *T. himalayanum* Wall. were collected from Tanchang County, Gansu Province of China in August 2005, and authenticated by Prof. Guo-Liang Zhang, the College of Life Science, Lanzhou University. A voucher specimen (No. 20050803) was deposited in the Institute of Organic Chemistry, Lanzhou University.

2. Previous work

Until now, only one paper has been published concerning the secondary metabolites of *T. himalayanum* Wall. indicating the presence of one known iridoids (triohima B) and two novel iridoids triohima A and C with an unusual δ-lactone-containing skeleton (Li et al., 2009).

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3. Present study

3.1. Extraction and isolation

Dried roots of *T. himalayanum* Wall. (5.1 kg) were extracted sequentially with acetone and MeOH at room temperature. The acetone extract (153.0 g) was fractioned by silica gel column chromatography (CC) using a step-gradient solvent system from 100% petroleum ether (PE) to 100% EtOAc with a 10% increment and a final MeOH wash to obtain nine fractions (Fr. AA–Fr. AI). Fr. AE was further purified by silica gel CC eluted with PE–EtOAc to yield compound **11** (30 mg) and Fr. AE-1. Fr. AE-1 was further purified by Sephadex LH-20 CC (MeOH) to afford a mixture of compounds **6** (60 mg) and **9** (60 mg). Fr. AH was subjected to macroporous absorption resin (D101) CC and eluted with H_2O, 30%, 50%, 70%, and 95% EtOH to yield six subfractions (AH1–AH6). AH2 was applied to silica gel CC using $CHCl_3$–MeOH (20:1–5:1) to give three subfractions (AH21–AH23). AH23 was further subjected to silica gel CC and then subjected to Sephadex LH-20 CC to afford compound **1** (55 mg). In the same manner, compounds **2** (60 mg) and **10** (65 mg) were obtained from AH5 and **3** (80 mg) was obtained from AH6. Fr. AG was separated on Sephadex LH-20 (MeOH–H_2O, 1:1) to afford compound **7** (75 mg).

The MeOH extract (249.6 g) was purified by Diaion HP-20 CC using H_2O–EtOH (0:1–1:0) to afford five fractions (Fr. BA–Fr. BE). Fr. BB was subjected to silica gel CC with EtOAc–MeOH (30:1–2:1) to give five subfractions (BB1–BB5). BB3 was separated by repeated silica gel CC with EtOAc–MeOH and then purified by Sephadex LH-20 CC with MeOH to yield compound **4** (100 mg). BB5 was subjected to silica gel MPLC with a gradient of $CHCl_3$–MeOH (1:0–40:1, 30:1–20:1, 10:1, 2:1, 1:1, 0:1) elution solvent to give six subfractions (BB51–BB56). BB53 was separated by silica gel CC with $CHCl_3$–MeOH (12:1) to afford compound **12** (12 mg). BB55 was applied to silica gel CC using $CHCl_3$–MeOH (12:1) to afford **5** (40 mg). BC was subjected to CC on silica gel ($CHCl_3$–MeOH, 20:1–0:1) to give four subfractions (BC1–BC4). Compound **8** (35 mg) was isolated from fraction BC2 by silica gel CC eluting with $CHCl_3$–MeOH (10:1). BC4 was subjected to Sephadex LH-20 CC, eluting with $CHCl_3$–MeOH (20:1, 10:1, 5:1, 3:1, 1:1), to afford compounds **13** (12 mg), **14** (12 mg), **15** (12 mg) and **16** (12 mg).

The structures of the isolated metabolites (compounds **1**–**16**, Fig. 1) were identified on the basis of spectroscopic data (MS, ^{1}H NMR, ^{13}C NMR and 2D NMR) and by comparison of these data with values in the literature. The compounds were identified as two new iridoid glucosides, 4′,6′-di-*O*-isopropylidene sweroside (**1**), and secologanin diethylacetal (**2**), together with fourteen known ones, *i.e.*, sweroside (**3**) (Cambie et al., 1990), secologanin dimethylacetal (**4**) (Kakuda et al., 2000), loganin (**5**) (Mpondo and Garcia, 1989), naucledal (**6**) (Mclean and Murray, 1972), urceolide (**7**) (Iwagawa and Hase, 1983), vincosamide (**8**) (Zhang et al., 2001), 3-epinaucledal (**9**) (Mclean and Murray, 1972), secologanin (**10**) (Markham et al., 1978), iso-secologaninaglycon (**11**) (Kinast and Tietze, 1976), 7-*O*-ethyl sweroside (**12**) (Chen et al., 2007), apigenin (**13**) (Sun et al., 2007), luteolin (**14**) (Markham et al., 1978), luteolin-7-*O*-*β*-D-glucoside (**15**) (Zhong et al., 2008), luteolin-4′-*O*-*β*-D-glucoside (**16**) (Bertrand et al., 2006).

The structure of compound **1** with molecular formula being established as $C_{19}H_{26}O_9$ by the molecular ion at *m*/*z* 399.1653 $[M+H]^+$ in the HR-ESI-MS, is consistent with the observation of 19 resonances in the ^{13}C NMR spectrum. ^{1}H and ^{13}C NMR spectra showed signals due to a vinyl group [δ_H 7.58 (1H, d, J = 2.4 Hz, H-3); δ_C 153.9 (C-3), 106.2 (C-4)], an oxymethylene [δ_H 4.47 (1H, m, H-7a) 4.34 (1H, m, H-7b); δ_C 69.8 (C-7)], and a sugar moiety [δ_H 4.75 (1H, d, J = 7.5 Hz, H-1′), 3.33 (1H, m, H-2′), 3.50 (1H, m, H-3′), 3.52 (1H, m, H-4′), 3.28 (1H, m, H-5′), 3.90 (1H, dd, J = 10.5, 5.4 Hz, H-6′a), 3.77 (1H, dd, J = 10.5, 10.2 Hz, H-6′b); δ_C 100.5 (C-1′), 75.6 (C-2′), 75.0 (C-3′), 74.8 (C-4′), 68.9 (C-5′), 63.2 (C-6′)]. The above observations along with signals at δ_C 168.4 (C-11), 153.9 (C-3), and 106.2 (C-4) suggested that **1** possessed an iridoid glucoside skeleton (Suyama et al., 2013).

The comparison of the NMR data between **1** and **3** reveals a similar structure of each other except for the appearance of two methyl groups [δ_H 1.37 (3H, s), 1.50 (3H, s); δ_C 19.4, 29.5] and a quaternary C-atom (δ_C 100.9) in **1** from an isopropylidene unit. On the basis of these data, **1** could be deduced as a sweroside acetonide derivative (Franzyk et al., 1997). The location of the isopropylidene moiety was assigned from the HMBC correlation of isopropylidene protons [δ_H 1.37 (3H, s), 1.50 (3H, s)] with C-4′ (δ_C 74.8) and C-6′ (δ_C 63.2)]. Therefore, the structure of compound **1** was determined as 4′,6′-di-*O*-isopropylidene sweroside (Fig. 1), and is described here for the first time.

Compound **2** was isolated as colorless oil. The molecular formula $C_{21}H_{34}O_{11}$ was determined by HR-ESI-MS (*m*/*z* 480.2441, $[M+NH_4]^+$, calcd for 480.2439). The ^{1}H NMR spectrum showed resonances corresponding to an iridoid glucoside at δ_H 7.42 (1H, d, J = 1.2 Hz, H-3), 5.51 (1H, d, J = 5.1 Hz, H-1), and 4.67(1H, d, J = 8.1 Hz, H-1′), as well as resonances from a vinyl group at δ_H 5.74 (1H, ddd, J = 17.1, 9.6, 8.4 Hz, H-8), 5.26 and 5.30 (H-10a and b), suggesting that **2** is a secoiridoid glucoside (Kakuda et al., 2000). The structural assignments of compound **2** were supported by comparison of the spectroscopic data of **2** with those of **4**. **2** showed almost the same NMR spectra as **4** (Table 2), except for ethoxy signals in **2** instead of methoxy signals in **4**. This is in accordance with the introduction of a diethyl acetal group at C-7, which was further verified by 2D NMR (HMBC correlations between H-1″/C-7 and C-2″; H-1‴/C-7 and C-2‴; and H-2″ and H-2‴/C-6) to derive the structure of **2** as secologanin diethylacetal that is reported here for the first time.

Tomassini et al. (Tomassini et al., 1995) have reported that compounds like secologanin dimethylacetal (**4**) and 7-*O*-ethyl sweroside (**10**) can be easily formed as artifacts by the reaction of secologanin (**8**) or secologanin acid with alcoholic solvents. Similarly, **2** was proposed to be formed by reaction of secologanin and ethanol.