Phytochemistry Letters 10 (2014) 164–167

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

# Two new bufadienolides and one new pregnane from *Helleborus thibetanus*

Hui Zhang [a], Yanfang Su [a,*], Fengying Yang [a,b], Zeqing Zhao [a], Xiumei Gao [c]

[a] Tianjin Key Laboratory for Modern Drug Delivery and High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, PR China
[b] Pharmaceutical Engineering Department, School of Biological Science and Technology, University of Jinan, Jinan 250022, PR China
[c] Tianjin Key Laboratory of TCM Chemistry and Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China

ARTICLE INFO

*Article history:*
Received 16 May 2014
Received in revised form 27 August 2014
Accepted 29 August 2014
Available online 9 September 2014

*Keywords:*
*Helleborus*
Ranunculaceae
Bufadienolides
Pregnane

ABSTRACT

Two new bufadienolides, 3β,14β,16β-trihydroxy-5α-bufa-20,22-dienolide (**1**) and 14β-hydroxy-3β-[β-D-glucopyranosyl-(1→6)-(β-D-glucopyranosyl)oxy]-5α-bufa-20,22-dienolide (**2**), one new pregnane, 3β-hydroxypregna-5,16-diene-20-one-1β-yl sulfate (**3**), along with one known pregnane (**4**) were isolated from the dried roots and rhizomes of *Helleborus thibetanus*. Their structures were elucidated by the extensive use of 1D and 2D NMR experiments, together with IR and HRESIMS spectra and the results of enzymatic hydrolysis.



## 1. Introduction

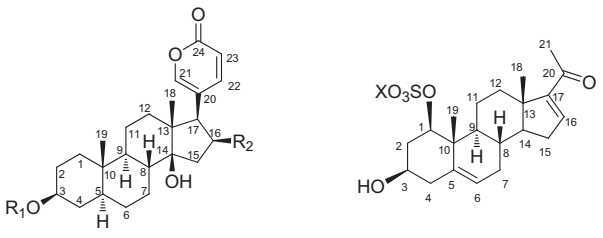
The genus of *Helleborus* is a member of the Ranunculaceae family. It comprises more than 20 species which are widely spread in Southeast Europe and West Asia. Previous phytochemical investigation on *Helleborus* illustrated that steroids including bufadienolides, phytoecdystones and steroidal saponins (Muzashvili et al., 2011; Yang et al., 2010a,b; Bassarello et al., 2008; Watanabe et al., 2003, 2005; Braca et al., 2004; Mimaki et al., 2003; Meng et al., 2001) were the main components. *H. thibetanus* Franch., an endemic plant of China, is mainly distributed in Sichuan, Gansu and Shaanxi. The roots and rhizomes of *H. thibetanus*, locally called "*XiaoTaoErQi*", have a wide use for the treatment of cystitis, urethritis, sores and traumatic injury (An et al., 2013; Yang et al., 2010a,b). One spirostanol sulfate, several bufadienolides and phytoecdystones had been isolated from *H. thibetanus* (Yang et al., 2010a,b). Herein, the isolation of two new bufadienolides (**1–2**), one new pregnane (**3**) (Fig. 1) and one known compound (**4**) from the title plant is reported. Their structures were elucidated by spectroscopic techniques including IR, MS, 1D and 2D NMR spectroscopy.

## 2. Results and discussion

Compound **1** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{24}H_{34}O_5$, deduced from the HRESIMS (*m/z* 425.2301 $[M+Na]^+$), as well as its $^{13}C$ NMR spectrum. IR absorptions at 3439 $cm^{-1}$ and 1725 $cm^{-1}$ supported the presence of hydroxyl and carbonyl groups. The assignments of **1** (Table 1) were established by a comprehensive analysis of $^1H$ and $^{13}C$ NMR, DEPT, COSY, HSQC, HMBC and NOESY spectra. Its $^1H$ and $^{13}C$ NMR spectroscopic data were similar to those of the known compound 14β,16β-dihydroxy-3β-[(β-D-glucopyranosyl)oxy]-5α-bufa-20,22-dienolide (Yang et al., 2010a), which has an α-pyrone ring at C-17 position and the A/B ring junction was *trans*. Comparison of the $^1H$ and $^{13}C$ NMR spectra of compound **1** and 14β,16β-dihydroxy-3β-[(β-D-glucopyranosyl)oxy]-5α-bufa-20,22-dienolide, allowed us to observe the absence of the signals for the β-D-glucose in **1**. The angular methyl carbon signal at $\delta_C$ 12.7 (Me-19) in **1** was very similar to the signal at $\delta_C$ 12.4 (Me-19) in both 5α-furostan and 5α-spirostan with 5α-H (Agrawal et al., 1985; Su et al., 2009), identifying an α-configuration of H-5, moreover, the correlations observed in the NOESY (Fig. 2) spectrum between Me-19 ($\delta_H$ 0.74) and Hax-2 ($\delta_H$ 1.65)/Hax-4 ($\delta_H$ 1.52)/Hax-6 ($\delta_H$ 1.14)/H-8 ($\delta_H$ 1.72)/Hax-11 ($\delta_H$ 1.17), between H-5 ($\delta_H$ 1.05) and H-3 ($\delta_H$ 3.84)/Hax-1 ($\delta_H$ 0.96)/H-9 ($\delta_H$ 0.85), and between H-3 ($\delta_H$ 3.84) and Hax-1 ($\delta_H$ 0.96) manifested the configuration of 5α-H and the A/B ring junction was *trans*. Therefore, the structure

* Corresponding author. Tel.: +86 22 27402885; fax: +86 22 27892025.
*E-mail addresses:* suyanfang@tju.edu.cn, suyfphd@sina.com (Y. Su).

http://dx.doi.org/10.1016/j.phytol.2014.08.024
1874-3900/




| | $R_1$ | $R_2$ |
|---|---|---|
| **1** | H | OH |
| **2** | Glc(1→6)Glc | H |

**3**

**Fig. 1.** Structures of compounds **1**–**3**, **3** given as salt (mostly $K^+$).

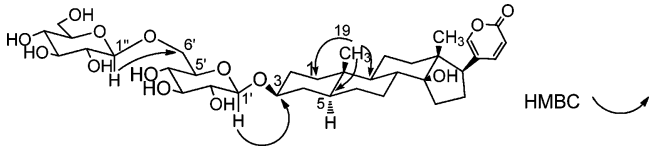



**Fig. 2.** Selected HMBC, NOE correlations for compound **1**.

of **1** was unambiguously identified as 3β,14β,16β-trihydroxy-5α-bufa-20,22-dienolide.

Compound **2** was isolated as a white amorphous powder. Its molecular formula was determined as $C_{36}H_{54}O_{15}$, deduced from the HRESIMS (*m/z* 733.3399 $[M + Na]^+$), as well as its $^{13}C$ NMR spectrum. The assignments of **2** (Table 1) were achieved by a comprehensive analysis of $^1H$ NMR, $^{13}C$ NMR, DEPT, COSY, HSQC, HMBC and NOESY spectra. The $^1H$ and $^{13}C$ NMR spectroscopic data disclosed that compound **2** was similar to compound **1**, significant differences in the chemical shifts of positions 15, 16 and 17 (Table 1), indicated **2** lacking the hydroxyl group at C-16. The two anomeric proton signals at $\delta_H$ 4.95 (d, $J$ = 7.5 Hz), 5.11 (d, $J$ = 7.5 Hz) in the $^1H$ NMR spectrum and two carbon signals at $\delta_C$ 102.3, 105.3 in the $^{13}C$ NMR spectrum were indicative of the presence of two hexose moieties. Enzymatic hydrolysis of **2** with snailase (Hu et al., 2004) afforded glucose (Glc), which was identified by TLC analysis. The β-orientation of the glucose was supported by the $J$ values of their anomeric H-atoms and the D configuration of the glucose was assumed from biogenetic consideration. The deshielded chemical shift observed for C-3 ($\delta_C$ 77.5) compared to C-3 ($\delta_C$ 70.9) of compound **1** provided the linkage of inner Glc to C-3, which were confirmed by HMBC (Fig. 3) correlation from H-1′ ($\delta_H$ 4.95) of inner Glc to C-3 ($\delta_C$ 77.5). The HMBC correlation between H-1″ ($\delta_H$ 5.11) of terminal Glc and C-6′ ($\delta_C$ 70.1) of inner Glc demonstrated the linkage of the two glucosyl at C-6′ ($\delta_C$ 70.1) of inner Glc, which could also be deduced by the chemical shift of C-6′ of inner Glc. Thus, the structure of **2** was characterized as 14β-hydroxy-3β-[β-D-glucopyranosyl-(1→6)-(β-D-glucopyranosyl)oxy]-5α-bufa-20,22-dienolide. Compound **3** was isolated as an amorphous solid, its molecular formula was determined as $C_{21}H_{30}O_6S$, deduced from the HRESIMS (*m/z* 409.1692 $[M - H]^-$), as well as its $^{13}C$ NMR spectrum. The presence of the sulfate functional group was further confirmed by a series of characteristic strong absorption bands at 1237, 1061 and 955 $cm^{-1}$ in its IR (KBr) spectrum (Yang et al., 2010b;

**Table 1**
$^1H$ (500 MHz), $^{13}C$ (125 MHz) NMR spectroscopic data of compounds **1** (pyridine-$d_5$) and **2** (pyridine-$d_5$)[a]

| No. | 1 | | 2 | | No. | 2 | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ | $\delta_C$ | | $\delta_H$ | $\delta_C$ |
| 1ax[b] | 0.96 | 38.0 | 0.97, m | 37.4 | 3-*O*-Glc | | |
| 1eq[c] | 1.68 | – | 1.62 | – | 1′ | 4.95, d (7.5) | 102.3 |
| 2ax | 1.65 | 32.7 | 1.64 | 30.0 | 2′ | 3.95, dd (8.5, 8.0) | 75.13 |
| 2eq | 2.02, m | – | 2.16, m | – | 3′ | 4.20 | 78.43 |
| 3 | 3.84, dddd (5.0, 5.5, 10.5, 11.0) | 70.9 | 4.02, dddd (5.5, 5.5, 10.5, 10.5) | 77.5 | 4′ | 4.12 | 71.65 |
| 4ax | 1.52 | 39.5 | 1.36 | 34.8 | 5′ | 4.11 | 77.2 |
| 4eq | 1.78, m | – | 1.82 | – | 6′ | 4.83, brd (11.5) | 70.1 |
| 5 | 1.05, m | 45.1 | 0.90 | 44.2 | | 4.33 | – |
| 6ax | 1.14 | 29.6 | 1.12 | 29.2 | Glc | | |
| 6eq | 1.24 | – | 1.22 | – | 1″ | 5.11, d (7.5) | 105.3 |
| 7ax | 1.16 | 28.6 | 1.08 | 28.0 | 2″ | 4.00 | 75.13 |
| 7eq | 2.39, m | – | 2.30, m | – | 3″ | 4.19 | 78.43 |
| 8 | 1.72 | 42.3 | 1.63 | 42.0 | 4″ | 4.18 | 71.65 |
| 9 | 0.85, td (15.5, 3.5) | 50.3 | 0.83 | 50.0 | 5″ | 3.89, m | 78.43 |
| 10 | – | 36.4 | – | 36.0 | 6″ | 4.47, dd (12.0, 2.0) | 62.7 |
| 11ax | 1.17 | 22.0 | 1.11 | 21.7 | | 4.32 | – |
| 11eq | 1.41, m | – | 1.34 | – | | | |
| 12ax | 1.25 | 41.5 | 1.20 | 40.7 | | | |
| 12eq | 1.48 | – | 1.35 | – | | | |
| 13 | – | 50.0 | – | 48.8 | | | |
| 14 | – | 84.9 | – | 84.3 | | | |
| 15 | 2.49, dd (14.5, 7.5) | 43.5 | 1.91 | 32.9 | | | |
| | 2.14, brd (14.5) | – | 1.81 | – | | | |
| 16 | 4.77, dd (7.5, 7.0) | 73.0 | 2.11, m; 1.83 | 29.4 | | | |
| 17 | 2.76, d (7.5) | 59.4 | 2.44 | 51.4 | | | |
| 18 | 0.98, s | 17.6 | 0.84, s | 17.2 | | | |
| 19 | 0.74, s | 12.7 | 0.63, s | 12.2 | | | |
| 20 | – | 119.7 | – | 123.3 | | | |
| 21 | 7.47, d (2.0) | 150.9 | 7.44, brs | 149.4 | | | |
| 22 | 8.48, dd (9.5, 2.5) | 151.7 | 8.19, dd (10.0, 2.0) | 147.6 | | | |
| 23 | 6.27, d (9.5) | 112.9 | 6.33, d (10.0) | 115.2 | | | |
| 24 | – | 162.6 | – | 162.1 | | | |

[a] Full assignments of the protons and carbons were accomplished by analysis of COSY, HSQC and HMBC spectra, and coupling pattern and coupling constants ($J$ in Hz) are in parentheses. Overlapped signals were given without designating multiplicity.
[b] ax = axial.
[c] eq = equatorial.