Phytochemistry 126 (2016) 47–58

Contents lists available at ScienceDirect

Phytochemistry

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

Polyoxypregnane steroids with an open-chain sugar moiety from *Marsdenia tenacissima* and their chemoresistance reversal activity

Sheng Yao [a,b,1], Kenneth Kin-Wah To [b,c,1], Liang Ma [a,b], Chun Yin [b,d], Chunping Tang [a,b], Stella Chai [b,d], Chang-Qiang Ke [a,b], Ge Lin [b,d,*], Yang Ye [a,b,e,*]

[a] State Key Laboratory of Drug Research and Natural Products Chemistry Department, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China
[b] Joint Research Laboratory of Promoting Globalization of Traditional Chinese Medicines between Shanghai Institute of Materia Medica, Chinese Academy of Sciences and The Chinese University of Hong Kong, China
[c] School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
[d] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China
[e] School of Life Science and Technology, ShanghaiTech University, Shanghai, China

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 29 February 2016
Accepted 8 March 2016
Available online 14 March 2016

Keywords:
Marsdenia tenacissima
Asclepiadaceae
C_{21} steroids
Open-chain sugars
Absolute configuration
X-ray crystallography diffraction
Chemoresistance reversal activity
Structure elucidation

ABSTRACT

A polyoxypregnane aglycone, 12β-*O*-acetyl-11α-*O*-isobutyryltenacigenin B, and four polyoxypregnane glycosides with a pachybionic acid ester moiety, 12β-*O*-acetyl-3-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandronyl)-11α-*O*-isobutyryltenacigenin B, 12β-*O*-acetyl-3-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandronyl)-11α-*O*-tigloyltenacigenin B, 12β-*O*-acetyl-3-*O*-(6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandronyl)-11α-*O*-2-methylbutyryltenacigenin B, and 12β-*O*-acetyl-3-*O*-(β-D-glucopyranosyl-(1→4)-6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-D-oleandronyl)-11α-*O*-tigloyltenacigenin B, were isolated from the canes of *Marsdenia tenacissima*, together with a disaccharide derivative. Their structures were elucidated by extensive spectroscopic analysis, and the absolute configurations were further determined by X-ray crystallographic analysis. With the exception of the disaccharide derivative, all five compounds are unusual naturally occurring polyoxypregnane glycosides bearing an open-chain sugar moiety. Two of these exhibit a wide spectrum of chemoresistance reversal activity, and potential mechanisms were studied accordingly.

1. Introduction

Marsdenia tenacissima (Roxb.) Wight et Arn., a plant belonging to the family Asclepiadaceae, is widely distributed from tropical to subtropical Asia. Its rhizomes and roots have long been used in China for the treatment of asthma, cancer, tracheitis, tonsillitis, pharyngitis, cystitis, and pneumonia (Jiangsu New College of Medicine, 1977). Previous chemical investigations resulted in the identification of more than 15 polyoxypregnane genins from *M. tenacissima*, classified into three types: tenacigenins A, B, and C (Yang et al., 1981) and their analogues (Deng et al., 2005a); 17β-tenacigenin B (Deng et al., 2005b) and the diester derivatives of tenacigenin B (Luo et al., 1993); and marstenacigenins A and B (Qiu et al., 1996). On the basis of the above aglycones, over 20 glycosides of tenacigenin B have been reported: from *M. tenacissima* tenacissosides A–N (Chen et al., 1999; Liu et al., 2008; Miyakawa et al., 1986; Wang et al., 2006; Xing et al., 2004) and marsdenosides A–K (Deng et al., 2005a, 2005c); seven glycosides of 17β-tenacigenin B named marsdenosides L and M (Huang et al., 2009) and tenacissimosides E–I (Yao et al., 2014), as well as four marstenacigenin glycosides named marstenacissides A–D (Xia et al., 2011). In addition, cissogenin (another type of compound) and its analogues, have been reported from seeds of the plant growing in India (Singhal et al., 1980a,b). Biological evaluations of extracts and isolated compounds from *M. tenacissima* also have antiasthmatic (Zhou et al., 1980), anticancer (Xue et al., 2012; Ye et al., 2014), and multidrug resistance (MDR) reversal (Han et al., 2012; Hu et al., 2008) activities. Furthermore, a new polyoxypregnane aglycone from this plant was able to circumvent P-glycoprotein (P-gp)-mediated MDR, as well as potentiating the activity of erlotinib and gefitinib in epidermal growth factor receptor tyrosine

* Corresponding authors at: Joint Research Laboratory of Promoting Globalization of Traditional Chinese Medicines between Shanghai Institute of Materia Medica, Chinese Academy of Sciences and The Chinese University of Hong Kong, China (G. Lin). State Key Laboratory of Drug Research and Natural Products Chemistry Department, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu-Chong-Zhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, China (Y. Ye).
E-mail addresses: linge@cuhk.edu.hk (G. Lin), yye@mail.shcnc.ac.cn (Y. Ye).
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.phytochem.2016.03.006
0031-9422/

kinase inhibitor (EGFR TKI)-resistant non-small-cell lung cancer cells (Yao et al., 2014).

In a continuation of efforts in the search for MDR-reversal agents from natural sources, the EtOAc fraction of the EtOH extract prepared from the stems of *M. tenacissima* was investigated. A polyoxypregnane aglycone (**1**) and four new C_{21} steroids with an open-chain sugar moiety (**2**–**5**), together with a known disaccharide derivative (**6**), were afforded. Here, the isolation and structural characterization of these new compounds, their chemoresistance reversal activity, and mechanistic studies are presented.

2. Results and discussion

2.1. Structure elucidation

A 95% EtOH extract of the air-dried stems of *M. tenacissima* (8.0 kg) was suspended in water and extracted with EtOAc. The EtOAc fraction was dissolved in MeOH, and partitioned into a MeOH and a petroleum ether extract. After repeated column chromatography over silica gel, Sephadex LH-20, and MCI gel, and preparative HPLC, compounds **1**–**6** were obtained (Fig. 1).

Compound **1** was obtained as a colorless, amorphous powder. Its HR-ESI-MS peak at *m*/*z* 499.2642 ([M+Na]$^+$, calculated for $C_{27}H_{40}O_7Na$ 499.2672) suggested a molecular formula of $C_{27}H_{40}O_7$ with eight degrees of unsaturation. Its IR spectrum showed absorption bands for hydroxy groups at 3435 cm^{-1} and carbonyl groups at 1736 and 1707 cm^{-1}. The ^{1}H NMR spectrum (Table 1) displayed proton signals for three methyls at δ_H 1.06 (s), 1.08 (s), and 2.18 (s), and two oxygenated methines at δ_H 4.98 (d, J = 10.1 Hz) and 5.35 (t, J = 10.1 Hz). The proton signals at δ_H 1.96 (s), and δ_H 1.09 (6H, d, J = 2.7 Hz) and 2.38 (m), along with carbon resonances at δ_C 170.6 (s) and 20.8 (q), and at δ_C 176.0 (s), 34.5 (d), 18.9 (q), and 18.4 (q), indicated the presence of acetyl and isobutyryl ester groups, respectively. By comparison with polyoxygenated pregnane steroids from the title plant, the NMR data (Tables 1 and 2) of **1** almost resembled those of 12*β*-*O*-acetyl-11α-*O*-2-methylbutyryltenacigenin B (**1a**) (Hu et al., 2008). The only difference found was that the NMR data assigned to a 2-methylbutyryl group in **1a** was replaced by those assigned to an isobutyryl unit. The long-range correlation of H-11 (δ_H 5.35)/C-1′ (δ_C 176.0) inferred that the isobutyryl ester group was located at C-11. Therefore, compound **1** was determined to be 12*β*-*O*-acetyl-11α-*O*-isobutyryltenacigenin B.

Compound **2** had a molecular formula of $C_{41}H_{64}O_{15}$ containing 10 degrees of unsaturation, as deduced by HR-ESI-MS. IR absorption bands at 3450, 1738, and 1707 cm^{-1} indicated the presence of hydroxy and carbonyl groups. The ^{1}H NMR spectrum of compound **2** (Tables 1 and 3) displayed signals for three singlet methyl groups at δ_H 1.06 (6H, s) and 2.19 (s), two doublet methyls at δ_H 1.07 (6H, d, J = 2.7 Hz), a multiplet methine at δ_H 2.37, and two oxygenated methines at δ_H 4.98 (d, J = 10.1 Hz) and 5.34 (t, J = 10.1 Hz). The anomeric proton signal at δ_H 4.62 (d, J = 8.0 Hz) and the corresponding carbon resonance at δ_C 100.4 are indicative of a sugar moiety with a β-linkage. Extensive analyses established that the NMR data of the aglycone of **2** closely resembled those of compound **1**, except for significant differences in the chemical shifts of C-2, C-3, and C-4. This suggested a linkage of sugar moieties to C-3 in **2**. Interpretation of the H-H COSY and HSQC spectra of compound **2** revealed a further two partial moieties –C(6′)H$_3$–C(5′)H–C(4′)H–C(3′)H–C(2′)H$_2$– and –C(6″)H$_3$–C(5″)H–C(4″)H–C(3″)H–C(2″)H–C(1″)H–. In the HMBC spectrum of **2**, the long-range correlations from the methoxy signal at δ_H 3.41 (s) to the oxygenated methine carbon at δ_C 78.3 (C-3′), and from the methoxy signal at δ_H 3.65 (s) to the oxygenated methine carbon at δ_C 80.6 (C-3″) indicated that the two methoxy groups were attached to C-3′ and C-3″, respectively. The HMBC correlation from H-3′ (δ_H 4.00) to the carbonyl at δ_C 171.2 suggested the presence of a hexanyl ester group. The HMBC correlations from H-5″ (δ_H 3.52 to C-1″ (δ_C 100.4), and

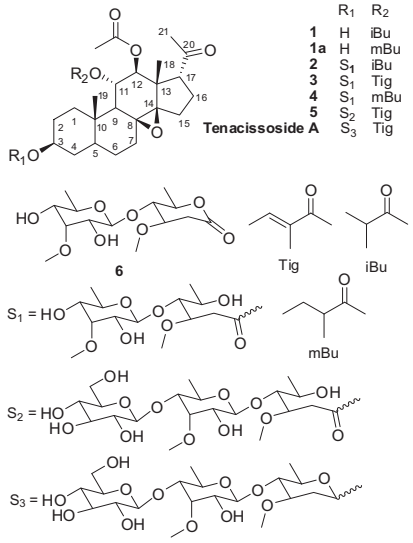

Fig. 1. Structures of the isolated compounds.

Table 1
^{1}H NMR spectroscopic data of the aglycone moieties of compounds **1**–**5** (δ in ppm, J in Hz).

Position	**1**[a,c]	**2**[b,c]	**3**[a,c]	**4**[a,c]	**5**[a,d]
1a	1.24, m	1.30, m	1.22, m	1.27, m	1.33, m
1b	1.48, m	1.51, m	1.52, m	1.50, m	1.54, m
2a	1.37, m	1.41, m	1.40, m	1.41, m	1.46, m
2b	1.69, m	1.72, m	1.66, m	1.70, m	1.67, m
3	3.58, m	4.68, m	4.66, m	4.66, m	4.68, m
4a	1.33, m	1.43, m	1.40, m	1.41, m	1.42, m
4b	1.63, m	1.66, m	1.66, m	1.67, m	1.72, m
5	1.35, m	1.43, m	1.40, m	1.41, m	1.52, m
6a	1.40, m	1.42, m	1.38, m	1.36, m	1.44, m
6b	1.57, m	1.60, m	1.52, m	1.52, m	1.58, m
7a	1.24, m	1.23, m	1.24, m	1.22, m	1.27, m
7b	1.87, m	1.87, m	1.86, m	1.87, m	1.90, m
9	1.98, d (10.1)	2.02, d (10.1)	2.00, d (10.3)	2.00, d (10.1)	2.02, d (10.2)
11	5.35, t (10.1)	5.34, t (10.1)	5.38, t (10.3)	5.33, t (10.1)	5.36, t (10.2)
12	4.98, d (10.1)	4.98, d (10.1)	4.90, d (10.3)	4.96, d (10.1)	5.01, d (10.2)
15a	1.56, m	1.55, m	1.58, m	1.57, m	1.52, m
15b	1.98, m	1.98, m	1.98, m	1.95, m	1.98, m
16a	1.58, m	1.61, m	1.60, m	1.59, m	1.66, m
16b	2.15, m	2.16, m	2.14, m	2.14, m	2.21, m
17	2.92, d (7.6)	2.92, d (7.4)	2.90, d (7.3)	2.90, d (7.4)	3.00, d (7.3)
18	1.08, s	1.06, s	1.07, s	1.04, s	1.09, s
19	1.06, s	1.06, s	1.04, s	1.04, s	1.05, s
21	2.18, s	2.19, s	2.19, s	2.18, s	2.13, s
C-11	iBu	iBu	Tig	mBu	Tig
2′	2.38, m	2.37, m		2.15, m	
3′	1.09, d (2.7)	1.07, d (2.7)	6.72, q (6.9)	1.30, m	6.80, q (7.6)
4′	1.09, d (2.7)	1.07, d (2.7)	1.72, d (6.9)	0.86, t (7.2)	1.76, d (7.6)
5′			1.72, s	1.01, d (7.2)	1.77, s
C-12	Ac	Ac	Ac	Ac	Ac
2″	1.96, s	1.96, s	1.85, s	1.94, s	1.82, s

[a] Measured at 300 MHz.
[b] Measured at 400 MHz.
[c] In $CDCl_3$.
[d] In CD_3OD.