Phytochemistry Letters 11 (2015) 209–214

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Six new polyhydroxy steroidal glycosides from *Dregea sinensis* Hemsl

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ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 12 December 2014
Accepted 12 December 2014
Available online 24 December 2014

Keywords:
Dregea sinensis Hemsl
Asclepiadaceae
Steroidal glycosides
Cytotoxic activity

ABSTRACT

The phytochemical investigation of the roots of *Dregea sinensis* Hemsl yielded six new polyhydroxy steroidal glycosides (1–6). The chemical structures have been elucidated on the basis of extensive one- and two-dimensional NMR spectroscopy, as well as by mass spectrometry. The cytotoxic activity of these compounds were evaluated *in vitro* while compounds 3 and 4 showed weak activity against human leukemia cells (HL-60) with IC_{50} values of 14.10 μM and 19.16 μM, respectively.

1. Introduction

Dregea sinensis Hemsl extensively distributed in the west of China and growth at an altitude of 500–3000 m of mountain woodland or bushes. It is well known that the decoction of roots is used in Dai ethnic minorities with detoxification, defervesce, detumescence and acesodyne (Editorial Committee of Flora of China, 2010). Various studies on this species have shown that C-21 steroids and steroidal glycosides were the main constituents (Liu et al., 2009, 2008, 2007; Shen et al., 1996; Shen and Mu, 1989; Jin et al., 1990; Jin and Mu, 1990, 1988) with minor products such as oligosaccharides (Shen and Mu, 1990) and phenylpropanoids (Chen et al., 2008). Anti-cancer, immunomodulatory (Juan et al., 2012) and anti-inflammatory (Liu et al., 2009) activities have been reported for this class of compounds. As part of our continuous effort to isolate bioactive C-21 steroidal compounds from *D. sinensis* Hemsl (Jia et al., 2014), we have examined the water precipitate of ethanol extract from the roots of *D. sinensis* Hemsl. In the present paper, we described the isolation and structural elucidation of six new polyhydroxy steroidal glycosides, those of which with benzoyl groups at C-11 and C-12 (**1–4**) were rare in C-21 steroidal glycosides. The chemical structures of compounds **1–6** were established by extensive analysis of one- and two-dimensional NMR spectroscopy and mass spectrometry combined the comparison with reported literature data. The isolated compounds were tested against HL-60 human leukemia cells.

2. Result and discussion

The isolated compounds (Fig. 1) were obtained from the water precipitate of ethanol extract of *D. sinensis* Hemsl by column chromatography (CC) and semi-preparative high performance liquid chromatography (HPLC). The analysis of ion fraction for the compounds was obtained by LC/MSD Trap mass spectrometer. The chemical structures were identified by one- and two-dimensional NMR analysis as arranged in Tables 1–4.

Compound **1** was obtained as white amorphous powder. The HR-ESIMS of **1** displayed a peak at *m/z* 1039.4554 $[M-H]^-$, corresponding to the molecular formula $C_{54}H_{72}O_{20}$. In the ^1H NMR spectrum, the singlet methyl signals of at δ_H1.73 (CH_3-18), 1.79 (CH_3-19) and 2.10 (CH_3-21) and the signal at δ_H 3.84 (H-3) corresponding to the secondary oxygenated carbon, and an olefinic proton signal at δ_H 5.45 (H-6) indicated the presence of a pregen-5-en-20-one skeleton (Juan et al., 2012). The proposed carbon skeleton was supported by two olefinic carbon signals at δ_C 140.1 (C-5) and 119.3 (C-6) and the HMBC correlations from the proton at δ_H 3.32 (H-17) to carbonyl carbon at δ_C 213.6 (–$COCH_3$). Through aromatic signals at δ_H 8.12 (2*H*, d, *J* = 7.5 Hz, Bz-3/7), 7.37 (1*H*, t, *J* = 7.5 Hz, Bz-5) and 7.27 (2*H*, t, *J* = 7.5 Hz, Bz-4/6) and the carbon signals at δ_C 167.3 (Bz-1), 133.8 (Bz-5), 131.0 (Bz-2), 130.4 (Bz-3/7) and 129.1 (Bz-4/6), one benzoyl group was identified (Tables 1 and

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http://dx.doi.org/10.1016/j.phytol.2014.12.016
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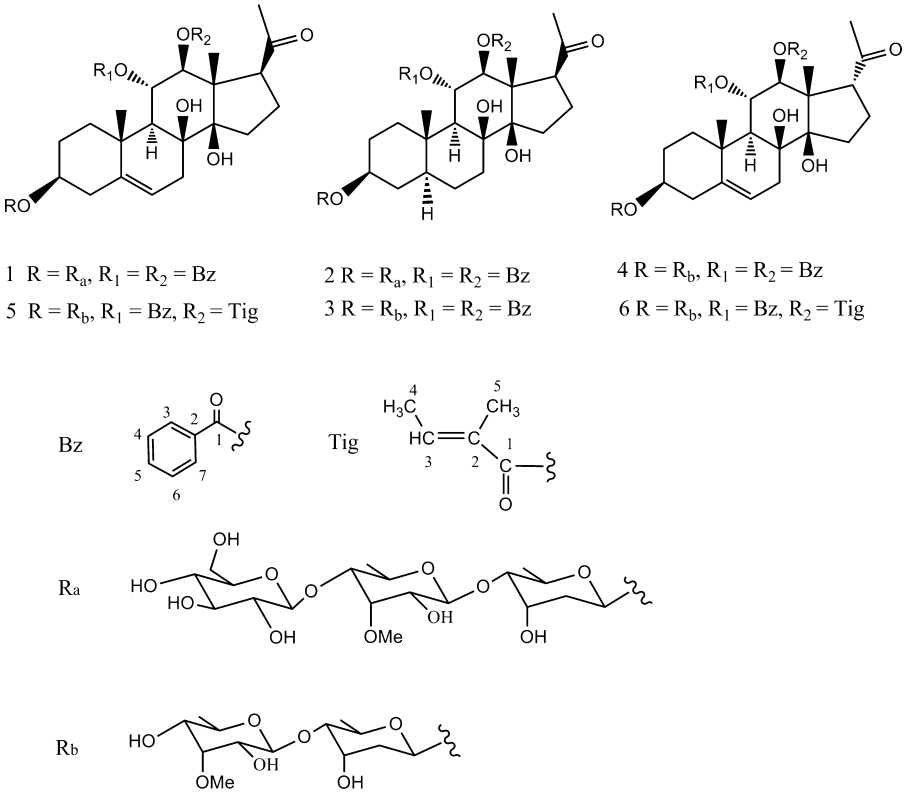

Fig. 1. Structures of compounds **1**–**6**.

3). This was supported by the correlations from H-3 to H-7 in the ^{1}H–^{1}H COSY spectrum (Fig. 2). The position of the benzoyl group was determined at C-12 based on the HMBC correlations from H-12 to its carbonyl carbon. In fact, another benzoyl group was simultaneously confirmed via aromatic signals at δ_H 8.04 (2*H*, d, *J* = 7.0 Hz, Bz-3/7), 7.32 (1*H*, d, *J* = 7.5 Hz, Bz-5) and 7.20 (2*H*, d, *J* = 7.5 Hz, Bz-4/6) and the carbon signals at δ_C 166.4 (Bz-1), 133.6 (Bz-5), 131.0 (Bz-2), 130.3 (Bz-3/7) and 129.0 (Bz-4/6). The long-rang correlation from H-11 to its carbonyl carbon suggested that the second benzoyl group was attached at C-11 (Fig. 2). The ion peaks at *m*/*z* 941.5 $[M + Na\text{-}122]^+$ and 819.5 $[M + Na\text{-}122\text{-}122]^+$ provided by ESIMS spectrum further confirmed the existence of two benzoyl groups.

The proton signals of two secondary methyl groups [δ_H 1.51 (3*H*, d, *J* = 6.0 Hz) and 1.58 (3*H*, d, *J* = 6.0 Hz)] and one methoxyl group [δ_H 3.80 (3*H*, s)] of deoxysugar and three anomeric protons [δ_H 4.85 (1*H*, d, *J* = 8.5 Hz), 5.08 (1*H*, d, *J* = 8.0 Hz) and 4.95 (1*H*, d, *J* = 7.5 Hz)] were observed in the sugar moiety. On the basis of the HMQC, HMBC and ^{1}H–^{1}H COSY spectra, the ^{1}H and ^{13}C NMR spectra of **1** suggested that three sugars were digitoxopyranosyl (Dig), 6-deoxy-3-*O*-methyl-allopyanose (Allme) and glucopyranose (Glc), respectively. Furthermore, the ESIMS ion peak at *m*/*z* 475.2 $[M + Na\text{-}588]^+$ confirmed the existence of three sugars. The linkage position and sequence of these sugars were ascertained by the HMBC spectrum, which showed distinct cross-peaks of correlations from δ_H 4.95 (H-1 of glucopyranose) to δ_C 83.3 (C-4 of 6-deoxy-3-*O*-methyl-allopyanose), from δ_H 5.08 (H-1 of 6-deoxy-3-*O*-methyl-allopyanose) to δ_C 88.7 (C-4 of digitoxose), from δ_H 4.85 (H-1 of digitoxose) to δ_C 78.2 (C-3 of aglycon) (Fig. 2). The β-linkage of monosacchalides were established by the large coupling constants (*J* = 7.5–10.0 Hz) observed for anomeric protons. Attempt to prove the absolute configurations of the sugars, the acid hydrolysis was carried out, however unsuccessful. According to the identification of monosacchalides in dregeoside A (Jin and Mu, 1988) and 12-*O*-benzyl-dihydrosarcostin 3-*O*-β-D-thevetopyranosyl-(1 → 4)-β-D-oleandropyranosyl-(1 → 4)-β-D-digitoxopyranosyl-(1 → 4)-β-D-cymaropyranoside (Liu et al., 2007) from a variant of *D. sinensis* Hemsl, sugar units in **1** were deduced as D-Dig, D-Allme and D-Glc, respectively.

The configurations of the aglycone in **1** were established by the analysis of the NOESY spectrum (Fig. 2). The NOESY correlations between CH_3-18, H-11 and CH_3-19; H-9, H-12 and H-17; H-3 and H-9 indicated β-configuration for H-11 and H-3 as well as α-configuration for H-17 and H-12, which was in agreement with the structures of 3-*O*-[β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyanosyl-(1 → 4)-β-D-canaropyranosyl]-11,12-di-*O*-tigloyl-17-β-marsdenin and 3-*O*-[β-D-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-D-allopyanosyl-(1 → 4)-β-D-oleandropyranosyl]-11,12-di-*O*-tigloyl-17β-marsdenin (Schneider et al., 1993). Thus, **1** was tentatively characterized as 3-*O*-[β-glucopyranosyl-(1 → 4)-6-deoxy-3-*O*-methyl-β-allopyanosyl-(1 → 4)-β-digitoxopyranoside]-11α,12β-di-*O*-benzoyl-17β-marsdenin.

Compound **2** was obtained as white amorphous powder. The molecular formula was established as $C_{54}H_{74}O_2$ by the HR-ESIMS ion peak at *m*/*z* 1041.4708 $[M - H]^-$. The assignment of ^{1}H and ^{13}C NMR signals showed that the chemical structure of **2** was very similar with that of **1** (Tables 1–4). The significant differences were that the signals of **1** at δ_H 5.45 and δ_C 119.3 disappeared in **2** and molecular weight of **2** in HR-ESIMS was less two units than that of **1**, which both indicated that there was not the double bond located at C-5 in **2**. Consequently, **2** was tentatively characterized as 3-*O*-[β-glucopyranosyl-(1 → 4)-6-deoxy -3-*O*-methyl-β-allopyanosyl-(1 → 4)-β-digitoxopyranoside]-11α,12β-di-*O*-benzoyl-17β-marsdenin-5,6-dihydrogen.

Compound **3** was obtained as white amorphous powder. The molecular formula of **3** was determined as $C_{48}H_{64}O_{15}$ on the basis of the HR-ESIMS ion peak at *m*/*z* 879.41780 $[M - H]^-$. The analysis of mass data and NMR values (Tables 1–4) suggested that the structure of **3** was similar with that of **2** while possessing two sugars**.** Single units of β-digitoxose and 6-deoxy-3-*O*-methyl-β-allopyanose were