Phytochemistry Letters 16 (2016) 294–298

Contents lists available at ScienceDirect

Phytochemistry Letters

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

New steroidal saponins with cytotoxic activities from *Smilax trinervula*

Fang Liang, Jun-Wei He, Gen-Hua Zhu, Rong-Hua Liu, Ji-Cheng Shu, Feng Shao, Hui-Lian Huang*

Key Laboratory of Modern Preparation of TCM, Jiangxi University of Traditional Chinese Medicine, Ministry of Education, Nanchang 330004, China

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 19 May 2016
Accepted 20 May 2016
Available online xxx

Keywords:
Steroidal saponins
Cytotoxic activities
Smilax trinervula

ABSTRACT

Three new steroidal saponins, namely, trinervulosides A–C (**1–3**), were isolated from the rhizomes and roots of *Smilax trinervula*, together with four known compounds, **4–7**. Their structures were determined through chemical evidence, NMR spectroscopy, mass spectrometry and comparison with the literature. Compounds **1–7** were assayed for cytotoxic activities, and only trinervuloside B (**2**) showed activity against SGC-7901 and HCT-116 cell lines.

1. Introduction

Smilax (Smilaceae) comprises approximately 370 species of shrubs which grow in tropical and temperate areas around the world. The rhizomes of *S. china* ("Ba Qia" in Chinese) and *S. glabra* ("Tu Fu Ling" in Chinese) are commonly used as herbal materials in traditional Chinese medicine (TCM). Based on previous phytochemical investigation on *S. riparia*, *S. menispermoidea* and *S. china*, it can be concluded that the genus *Smilax* is rich in steroidal saponins (Sashida et al., 1992; Nikaidoa and Ohmotoa, 1992; Jia and Ju, 1992; Belhouchet et al., 2008; Zhang et al., 2012; Shao et al., 2007). Steroidal saponins from Smilaceae exhibit a range of bioactivities, such as anti-inflammatory, cytotoxicity and anti-tumor effects (Ivanova et al., 2011; Wu et al., 2010).

As part of our continued interest in steroidal saponins in *Smilax* (Smilaceae) plants (Huang et al., 2009; Lin et al., 2012), we have undertaken the chemical investigation of *Smilax trinervula*, an evergreen climbing shrub mainly distributed in the south of China (i.e., Zhejiang, Jiangxi, Hunan and Guizhou provinces). Following our previous work on this plant, which led to the isolation and characterization of phenylpropanoids and neolignans (Shu et al., 2015), the current article reports three new steroidal saponins and four known relatives from the rhizomes of *S. trinervula.*

2. Results and discussion

Seven compounds including three new steroidal saponins were identified from the extracts of the air-dried rhizomes of *S. trinervula*. Compound **1** (Fig. 1) was obtained as a white amorphous powder with a molecular formula of $C_{51}H_{84}O_{23}$, determined from the positive ion at *m/z* 1087.5265 $[M+Na]^+$ in the HR-ESI–MS. The structure of **1** was identified by comparison of its ^{1}H- and ^{13}C NMR (Tables 1 and 2), HSQC and HMBC data with those of congeners from *Solanum* genera plants (Zhu et al., 2001), *Dioscorea collettii* (Hu et al., 1999a, 1999b) and *Dioscorea anthaica* (Dong et al., 2001).

The ^{1}H NMR spectrum of **1** showed signals belonging to six methyl proton groups at δ_H 1.09(s, H-19), 1.22 (s, H-18), 1.45(d, J = 5.8, H-21), 0.95 (d, J = 6.6, H-27), 1.65(d, J = 6.2, H-6‴), 1.77 (d, J = 6.1, H-6″) and a olefinic proton at δ_H 5.29 (*br s*, H-6), as well as protons attributable to an oxymethylene H-26 at δ_H 3.56 (dd, J = 9.5, 6.0) and 3.95 (dd, J = 9.4, 6.5), observed in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR spectra of **1** showed 51 signals, 24 of which were assigned to the saccharide protons and 27 to the aglycone moiety. Among them, the aglycone moiety had three angular methyl groups at δ_C 13.4 (C-18), 19.5 (C-19), 24.2 (C-21), one secondary methyl group at d_C 17 (C-27), two olefinic carbons at d_C 141 (C-5), 121.8 (C-6), two hydroxyl carbon signals at d_C 75.2 (C-16), 62.3 (C-20) and a carbonyl carbon signal at d_C 173.2 (C-22). We determined that the aglycone possessed a pregnane-5-en skeleton through analysis of the ^{1}H- and ^{13}C NMR spectra. In addition, HMBC experiments revealed correlations between H-21/C-17, H-21/C-20; H-27/C-24, H-27/C-25, H-27/C-26, H-23/C-22, H-24/C-22, H-25/C-24, H-25/C-26 (Fig. 2). By comparison of the ^{13}C NMR spectroscopic signals of the aglycone moiety of **1** with values reported in the literature (Zhu et al., 2001; Hu et al., 1999a, 1999b; Dong et al.,

* Corresponding author.
E-mail address: huilianh@163.com (H.-L. Huang).

http://dx.doi.org/10.1016/j.phytol.2016.05.011
1874-3900/

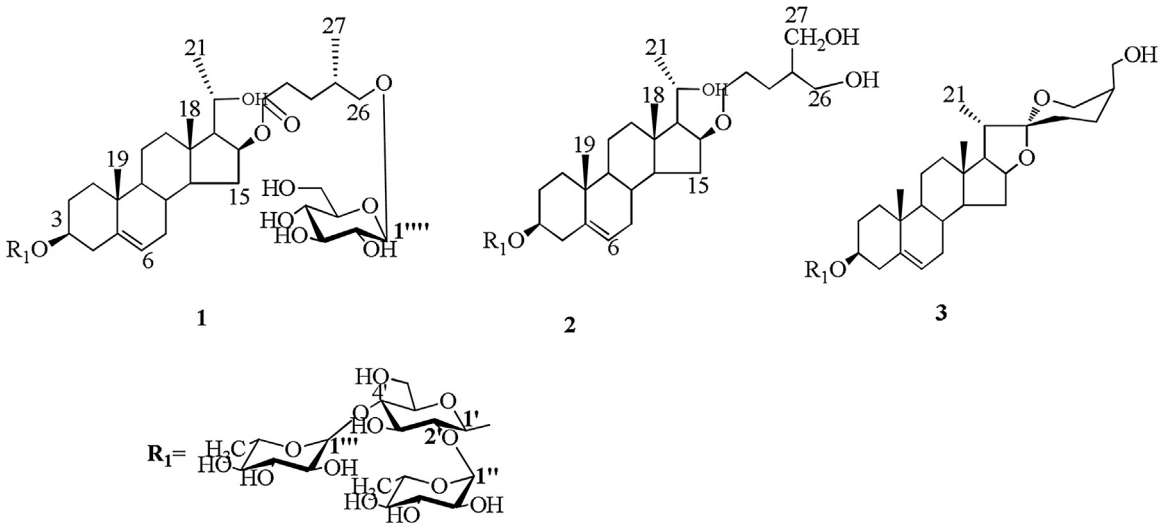

Fig. 1. Structures of compounds **1–3**.

2001), and extensive HMQC and HMBC data analyses, the aglycone of **1** was shown to be 3β, 20, 26-trihydroxy-20,22-seco-25(R)-furosta-5-en-22-one.

The assignments of the sugar moieties were similarly interpreted. The ^{1}H- and ^{13}C NMR spectra of **1** exhibited four sugar anomeric protons at d_H 4.97 (d, J = 7.5, H-1′), 6.44 (s, H-1″), 5.89 (s, H-1‴), 4.86 (d, J = 7.8, H-1⁗) (Table 2), and anomeric carbon atoms at d_C 100.3 (C-1′), 102.1 (C-1″), 102.9 (C-1‴) and 105 (C-1⁗), respectively. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose, as revealed by HPLC analysis and comparison with authentic standards. HSQC spectrum analysis showed signals for four sugar anomeric protons, which were correlated with four anomeric carbon signals, respectively. The connectivity of each sugar unit from C-1 to C-6 was determined using HSQC and HMBC. HMBC experiments revealed the following correlations: H-1′/C-3; H-1″/C-2′; H-1‴/C-4′, and H-1⁗/C-26 (Fig. 2). These analyses revealed the presence of 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D- glucopyranosyl and 26-*O*-β-D-glucopyranosyl groups in compound **1** (Fig. 2). Based on these data, it was established that the structure of compound **1** was 26-*O*-β-D-glucopyranosyl-3β,20,26-trihydroxy-20,22-seco-25(*R*)-furosta-5-en-22-one 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]- β-D-glucopyranoside, and given the rivial name trinervuloside A.

Compound **2** was isolated as a white amorphous powder and had the molecular formula of $C_{45}H_{76}O_{18}$ as determined by ^{13}C NMR and HR-ESI–MS. The HR-ESI–MS showed an $[M+Na]^+$ peak at m/z 927.6610, corresponding to $C_{45}H_{76}O_{18}Na$. The ^{1}H NMR spectrum of **2** showed the presence of five methyl groups at d_H 1.08 (s, H-18), 1.08 (s, H-19), 1.52 (d, J = 6.2, H-21), 1.79 (d, J = 6.1, H-6″) and 1.65 (d,

Table 1
^{1}H and ^{13}C NMR data of the aglycone moieties of **1–3** in pyridine.

Aglycone moiety position	**1**		**2**		**3**	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	37.1	1.00 m,1.71 m	37.5	0.98 m,1.75 m	37.5	0.99 m, 1.72 m
2	30.2	1.84 m,2.08 m	30.2	1.87 m,2.07 m	30.2	1.89 m, 2.08 m
3	78.1	3.89 m	78.1	3.89 m	78.1	3.90 m
4	39	2.8 m,2.77 m	39	2.76 m,2.8 m	39.0	2.74 (t, 11.3), 2.82 (dd, 13.2, 5.2)
5	141	–	140.8	–	140.8	–
6	121.8	5.29 br.s	121.9	5.33 br.s	121.8	5.32 (br.d 4.9)
7	32.1	1.90 m,2.02 m	32.3	1.90 m,2.03 m	32.3	1.86 m, 2.03 m
8	31.6	1.55 m	31.4	1.54 m	31.7	1.56 m
9	50.6	0.98 m	50.5	0.90 m	50.3	0.89 m
10	37.5	–	37.1	–	37.1	–
11	21	1.53 m	20.9	1.45 m	21.1	1.41 m
12	40.2	1.38 m,2.70(m	39.5	1.05 m,1.84 m	39.9	1.09 m, 1.67 m
13	43	–	41.3	–	40.5	–
14	54.3	0.91 m	54.5	0.89 m	56.6	1.03 m
15	36	1.26 m,2.46 m	36.5	1.57 m,2.29 m	32.2	1.39 m, 1.46 m
16	75.2	4.07 m	75.3	4.04 m	81.2	4.52 m
17	62.3	1.63 m	63.8	1.46 m	62.8	1.79 (br.d, 6.2)
18	13.4	1.22 s	14.2	1.08 s	16.4	0.83 s
19	19.5	1.09 s	19.4	1.08 s	19.4	1.06 s
20	65.2	4.47 m	66	4.5 m	42.5	1.90 m
21	24.2	1.45(d,5.8)	24.1	1.52(d, J = 6.2)	15.0	1.13 (d, 6.9)
22	173.2	–	71.7	4.27 m	109.9	–
23	32.5	2.48 m	31.1		27.3	1.51 m, 1.91 m
24	29.3	1.60 m,2.01 m	29.9	1.27 m,2.1 m	21.7	1.90m
25	33.5	1.93 m	33.4		36.3	1.90 m
26	74.8	3.56(dd, 9.5,6.0), 3.95(dd, 9.4,6.5)	64.9	4.25 m	60.7	4.02 (br.d,11.3), 4.15 (dd, 11.3, 2.6)
27	17	0.95(d, J = 6.6)	62.9	4.42 m,4.58 m	61.3	4.24 m