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New furostanol saponins with anti-inflammatory and cytotoxic activities from the rhizomes of *Smilax davidiana*

Meng Zhou[a], Liping Huang[b], Linyu Li[a], Yifei Wei[b], Jicheng Shu[a], Xing Liu[a], Huilian Huang[a,*]

[a] Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China
[b] College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China

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ABSTRACT

Seven new furostanol saponins have been isolated from the rhizomes of *Smilax davidiana*. Their structures were established by 2D NMR spectroscopic techniques (^{1}H,^{1}H-COSY, NOESY, HSQC and HMBC), mass spectrometry and comparison with the literature. The isolated compounds were subjected to evaluate anti-inflammatory and cytotoxic activities in vitro. Compounds **3**, **5** and **7** were found to have modest anti-inflammatory effects through suppression of IL-1β production and promote the expression of IL-10 in LPS-stimulated RAW 264.7 cells. Davidianoside F (**6**) showed activity against MCF-7 and HELA cell lines at the concentration of 10.2 μM and 4.3 μM, respectively.

1. Introduction

The saponins, classified as steroids or triterpenoid saponins depending upon the nature of the aglycon, are widely distributed in terrestrial plants. Steroid saponins often occur in Dioscoreaceae, Smilacaceae, Liliaceae and Scrophulariaceae, and can be subcategorized into spirostanol, isospirostanol, furostanol, pseudospirostanol and cholestanol saponins depending on the skeletal of aglycon. Some biological activities of many medicinal herbs, as cytotoxic, anti-inflammatory, hemolytic, anti-fungal, and anti-bacterial properties [1], are generally due to the secondary metabolites which often occur as steroid saponins.

Smilaceae comprises about 370 species that are mainly distributed in the tropical and temperate zones throughout the world, especially in East Asia and North America. Steroidal saponins are widely recognized as the 'marker compounds' in the plants of Smilacaceae [2]. In our continuing search for bioactive saponins from Smilaceae plants [3–5], we have undertaken the chemical investigation of *Smilax davidiana* (*S. davidiana*), an evergreen climbing shrub, mainly distributed in the south of China (i.e., Zhejiang, Jiangxi, Hunan and Guizhou provinces). To our best knowledge, until now, only two literatures were reported on *S. davidiana* [6,7]. Twelve phenolic compounds such as resveratrol and trans-resveratrol were isolated from this plant [6].

Steroidal saponins are widely recognized as the 'marker compounds' in the plants of Smilacaceae. In this paper, we describe the isolation and structure elucidation of seven new furostanol saponins (Fig. 1) from the *n*-BuOH fraction of the 70% ethanol extract of *S. davidiana* rhizomes. This study documents for the first time the steroidal saponins characters of *S. davidiana* in detail and contributes to the chemotaxonomy of *Smilax*. Moreover, the anti-inflammatory and cytotoxic activities of compounds **1–7** are also described.

2. Results and discussion

Seven new furostanol saponins were identified from the extracts of the rhizomes of *S. davidiana*. Compound **1** was isolated as a white, amorphous powder. Its molecular formula, $C_{57}H_{92}O_{26}$, was deduced by the positive-ion HRESIMS peak at *m/z* 1215.5694 $[M+Na]^+$. The structure of **1** was identified by comparison of its ^{1}H NMR and ^{13}C NMR (Tables 1 and 2), HSQC and HMBC data with those of congeners. The ^{1}H NMR spectrum of **1** showed signals belonging to seven methyl proton groups at δ_H 0.71 (3H, s, Me-18), δ_H 1.04 (3H, s, Me-19), δ_H 1.74 (3H, s, Me-21), δ_H 1.16 (3H, d, J = 6.48 Hz, Me-27), δ_H 1.59 (3H, d, J = 5.68 Hz, Rha Me-6‴), δ_H 1.59 (3H, d, J = 5.68 Hz, Rha Me-6‴), δ_H1.78 (3 H, d, J = 6.2 Hz, Rha Me-6″). Moreover, an olefinic proton at δ_H 5.3 (1H, br d, J = 4.04 Hz, H-6), as well as protons attributable to an oxymethylene H-26 at δ_H4.04 (H-26a) and δ_H3.78 (H-26b), were observed in the ^{1}H NMR spectrum (Table 1). The ^{13}C NMR spectra of **1** showed 57 signals, 27 of which were assigned to the aglycon moiety, and the other 30 were the saccharide carbons signals. Among them, the aglycon moiety had two angular methyl groups at δ_C14.3 (C-18), δ_C19.5 (C-19), two secondary methyl groups at δ_C11.7 (C-21), δ_C17.8 (C-27), four olefinic carbons at δ_C140.8 (C-5), δ_C121.8 (C-6), δ_C105 (C-20), δ_C154.5 (C-22), two hydroxyl carbon signals at δ_C84.6 (C-16), δ_C 39.6

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

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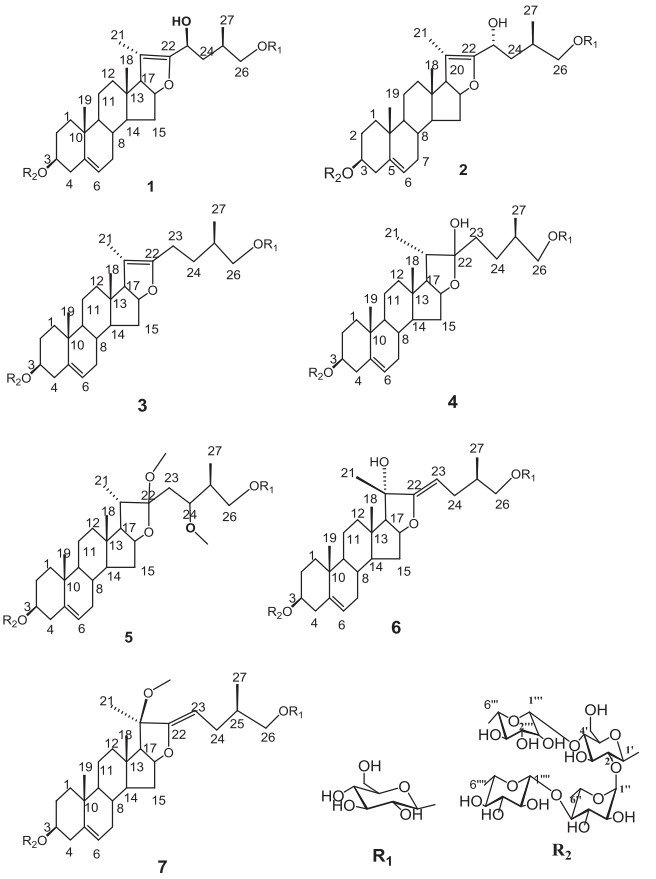

Fig. 1. The structures of compounds **1**–**7**.

(C-24). The DEPT spectrum of **1** showed the presence of 7 primary carbons, 11 secondary carbons, 34 tertiary carbons and 5 quaternary carbons. All of the spectral data showed **1** was the same aglycon as the known compound, dioscoreside C, except for the different substituent of C-23, **1** possessed a hydroxyl group at C-23 [8]. The absolute configuration of C-23 was assigned as *S* based on the NOESY spectrum. In the NOESY spectrum of 1, the protons at δ_H 1.74 (Me-21) correlated with the proton at δ_H 4.91 (H-23), which suggested the hydroxyl group of C-23 had β configuration. The configuration of C-25*R* was deduced on the basis of differences in chemical shifts of the geminalgeminal protons:the value of Δab (δ 26a–δ26b) is usually > 0.57 ppm for 25*S* compound and < 0.48 ppm for 25*R* compound [9]. In addition, HMBC experiments revealed the key correlations between H-18/C-17, C-14, H-27/C-25, C-26, H-23/C-25, C-24, H-24/C-23, H-19/C-9, C-1, H-25/C-24, C-23, C-27. Based on the inspection on the ^{1}H and ^{13}C NMR spectral data of **1**, the structure of the aglycon moiety was found to 3β, 23(*S*), 26-trihydroxy-25(*R*)-furosta-5,20(22)-diene.

The ^{1}H NMR and ^{13}C NMR spectra of compound **1** exhibited five sugar anomeric protons at δ_H4.98 (1H, d, J = 6.02 Hz, H-1′), δ_H5.85 (1H, br s, H-1″), δ_H6.41 (1H, br s, H-1‴), δ_H6.30 (1H, br s, H-1⁗) and δ_H4.86 (1H, d, J = 8.24 Hz, H-1⁗′), and five anomeric carbon atoms at δ_C100.3 (C-1′), δ_C102.2 (C-1″), δ_C103.4 (C-1‴), δ_C102.2 (C-1⁗) and δ_C105.1 (C-1⁗′), respectively. The identity of the monosaccharides and the sequence of the oligosaccharide chain were determined by the analysis of HSQC, HMBC, COSY spectra. HSQC spectrum analysis showed signals for five sugar anomeric protons, which were correlated with five anomeric carbon signals, respectively. All of the data showed that compound **1** possessed two glucoses and three rhamnoses. Acid hydrolysis of **1** yielded D-glucose and L-rhamnose, as revealed by HPLC analysis and comparison with authentic standards. The α-anomeric configuration for the rhamnose was determined by its C-5 data [10], and the β-anomeric configurations for the two glucoses were determined from their large $^3J_{1,2}$ coupling constants.

Table 1
^{1}H NMR data for the aglycon moieties of compounds **1–7** (400 MHz for ^{1}H in pyridine).

Aglycon moiety	1	2	3	4	5	6	7
1a	0.97m	0.98m	0.98m	0.98m	0.97m	0.97m	0.98m
1b	1.71m	1.71m	1.72m	1.72m	1.72m	1.70m	1.73m
2a	1.84m	1.82m	1.84m	1.84m	1.83m	1.80m	1.84m
2b	2.08m	2.06m	2.06m	2.06m	2.06m	2.05m	2.08m
3	3.86m	3.86m	3.87m	3.86m	3.86m	3.86m	3.86m
4a	2.72m	2.70m	2.71 br d (11.90)	2.69m	2.71m	2.70m	2.72m
4b	2.76m	2.76m	2.77dd (4.20,13.4)	2.76m	2.77m	2.78m	2.79m
5	–	–	–	–	–	–	–
6	5.30 br d (4.04)	5.27br d(4.2)	5.33 br d (4.2)	5.29br s	5.30 br d(4.04)	5.30br s	5.29brd (4.08)
7a	1.48m	1.48m	1.50m	1.48m	1.46m	1.45m	1.47m
7b	1.84m	1.83m	1.87m	1.85m	1.85m	1.84m	1.82m
8	1.48m	1.47m	1.48m	1.53m	1.47m	1.48m	1.48m
9	0.88m	0.88m	0.93m	088m	0.88m	0.86m	0.85m
10	–	–	–	–	–	–	–
11a	1.37m	1.37m	1.40m	1.40m	1.38m	1.37m	1.38m
11b	1.42m	1.41m	1.44m	1.44m	1.43m	1.43m	1.43m
12a	1.14m	1.17m	1.15m	1.12m	1.15m	1.14m	1.12m
12b	1.73m	1.75m	1.73m	1.73m	1.71m	1.73m	1.83m
13	–	–	–	–	–	–	–
14	0.84m	0.88m	0.88m	1.04 s	0.85m	0.95m	0.88m
15a	1.48m	1.55m	1.49m	1.48m	1.47m	1.51m	1.4m
15b	2.10m	2.12m	2.13m	2.01m	2.10m	2.04m	2.02m
16	4.81m	4.83m	4.78m	4.94m	4.84 br s	4.83m	4.95m
17	2.44 d (9.8)	2.48 d (10.12)	2.45 d (10.1)	2.02m	2.50 d (10.08)	2.35m	2.10m
18	0.71 s	0.82 s	0.71 s	0.88 s	0.69 s	0.90 s	0.84 s
19	1.04 s	1.00 s	1.05 s	1.04 s	1.04 s	1.04 s	1.03 s
20	–	–	–	2.22m	2.22m	–	–
21	1.74 s	1.79 s	1.63 s	1.33 d (6.6)	1.75 s	1.73 s	1.39 br s
22	–	–	–	–	–	–	–
23a	4.91m	4.91m	1.81m	2.05m	2.25m	4.54m	2.03m
23b	–	–	1.48m	2.03m	1.64m	–	–
24a	2.44m	2.14m	2.21m	1.67m	4.23m	2.25m	2.06m
24b	1.75m	1.97m	2.21m	2.03m	–	2.36m	2.48m
25	2.44m	2.27m	1.93m	1.91m	2.28m	2.05m	2.22m
26a	4.04m	4.06m	3.93m	3.94m	3.98m	3.95m	3.95m
26b	3.78m	3.78m	3.61m	3.61m	3.68m	3.69m	3.59m
27	1.16 d (6.48)	1.13 d (6.64)	1.01 d (6.08)	0.97 d (6.48)	1.09 d (6.44)	1.05 d (6.44)	1.07 d (6.6)
20-OCH_3							3.16 s
22-OCH_3					3.59 s		
24-OCH_3					3.33 s		

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′(δ_H4.98)/C-3(δ_C78.0);H-1″(δ_H5.85)/C-2′(δ_C 77.9);H-1‴(δ_H6.41)/C-4′(δ_C78.7);H-1⁗′(δ_H6.30)/C-4″(δ_C80.5); H-1⁗″(δ_H 4.86)/C-26(δ_C75.6). Therefore, the sugar unit was deduced as 3-O-[{α-L-rhamnopyranosyl-(l→4)-α-L-rhamnopyranosyl-(l→2)}]-α-L-rhamnopyranosyl-(l→4)-β-D-glucopyranoside and 26-O-β-D-glucopyranosyl. Based on above evidence, the structure of **1** was identified as 26-O-β-D-glucopyranosyl-3β,23(*S*),26-trihydroxy-25(*R*)-furosta-5,20(22)-diene-3-O-[{α-L-rhamnopyranosyl-(l→4)-α-L-rhamnopyranosyl-(l→2)}]-α-L-rhamnopyranosyl-(l→4)-β-D-glucopyranoside, and given the trivial name davidianoside A.

Compound **2** was obtained as a white amorphous powder with a molecular formula of $C_{57}H_{92}O_{26}$, determined by the positive-ion at *m/z* 1215.5736 $[M+Na]^+$ in the HR-ESI-MS. Comparison of the ^{1}H and ^{13}C NMR (Tables 1 and 2) and 2D-NMR data with **1** indicated that **2** has the same skeleton, except for the configuration of C-23. The C-23 *R* configuration was defined by the evidence of the NOESY spectrum and the