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New steroidal saponins with L-arabinose moiety from the rhizomes of *Smilax scobinicaulis*

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ABSTRACT

Phytochemical investigations of the rhizomes of *Smilax scobinicaulis* led to the isolation of seven steroidal saponins (**1**–**7**) of which four (**1**, **3, 4** and **6)**, nameed, Smilscobinosides C-F, respectively) are new. Five of these steroidal saponins with L-arabinose moiety are reported here for the first time in the genus *Smilax*. The structures were elucidated by spectroscopic analysis of the isolates and their hydrolysis products. The isolated compounds were evaluated for their cytotoxicity against four human tumor cell lines (SH-SY5Y, SGC-7901, HCT-116 and Lovo). Compounds **3** and **4** exhibited significant inhibition on HCT-116 (with IC_{50} values of 10.5 and 7.8 μM) together with inhibition on SGC-7901 (with IC_{50} values of 21.4 and 15.8 μM), respectively.

1. Introduction

The genus *Smilax* (Smilacaceae) comprises about 370 species, which are mainly distributed in the tropical and temperate zones throughout the world, especially in East Asia and North America (Tsukamoto, 1988). The rhizomes of *Smilax scobinicaulis* C.H. Wright, known as “Duan gen ba qia”, “Hei ci ba qia” or “Wei ling xian” in Chinese, are used in Chinese traditional medicine for the treatment of rheumatic arthritis and dispelling wind-evil (Flora of China, 2004). The genus *Smilax* is rich in steroidal saponins (Belhouchet et al., 2008; Shao et al., 2007; Jia and Ju, 1992), a group of natural products known to exhibit a range of bioactivities, such as anti-inflammatory, cytotoxic and anti-tumor effects (Shao et al., 2007; Ivanova et al., 2011; Lacaille-Dubois, 2005). From *S. scobinicaulis*, only two new spirostane-type steroidal saponins (named smilscobinosides A and B) and several flavonoids and phenolic compounds have been isolated (Zhang et al., 2012, 2014). As part of our continuous interests in steroidal saponins in he genus *Smilax* (Smilaceae) (Huang et al., 2012, 2009), a chemical investigation has been undertaken *S. scobinicaulis*. The current paper focuses on isolation and structural elucidation of four new steroidal saponins. All the new steroidal saponins with L-arabinose moiety were found for the first time in *Smilax* genus.

2. Results and discussion

2.1. Structure elucidation

The *n*-butanol fraction from 70% ethanol extract of *Smilax scobinicaulis* was successively chromatographed on macroporous resin, Sephadex LH-20, silica gel, ODS, and finally purified by semi-preparative HPLC to afford 4 new steroidal saponins (**1**, **3, 4** and **6)**, as well as three known compounds (Fig. 1).

Compound **1** was isolated as white amorphous powder. The molecular formula was inferred as $C_{44}H_{70}O_{19}$ based on the positive-ion HRESIMS peak at *m/z* 903.4511 $[M + H]^+$. The ^1H NMR data of **1** established the presence of four methyl groups at δ_H 0.54 (s, H-19), δ_H 0.95 (s, H-18), δ_H 1.19 (d, J = 7.0, H-21) and δ_H 0.69 (d, J = 6.1, H-27), respectively on a steroidal saponin skeleton. The DEPT and ^{13}C NMR spectra showed 44 signals comprising 4 methyls, 12 methylenes, 24 methines and 4 quaternary carbons, in which showed the presence of four methyl groups at δ_C 16.7 (C-18), δ_C 13.0 (C-19), δ_C 14.7 (C-21) and δ_C 16.9 (C-27), as well as one carbonyl group at δ_C 209.5 (C-6). The chemical shift values for the F-ring carbon atoms (δ_C 111.8, 67.3, 38.7, 31.7, 66, 16.9) indicated that compound **1** had one hydroxyl group at F-ring of the spirostanol skeleton. The HMBC correlations of δ_H 3.02 (H-

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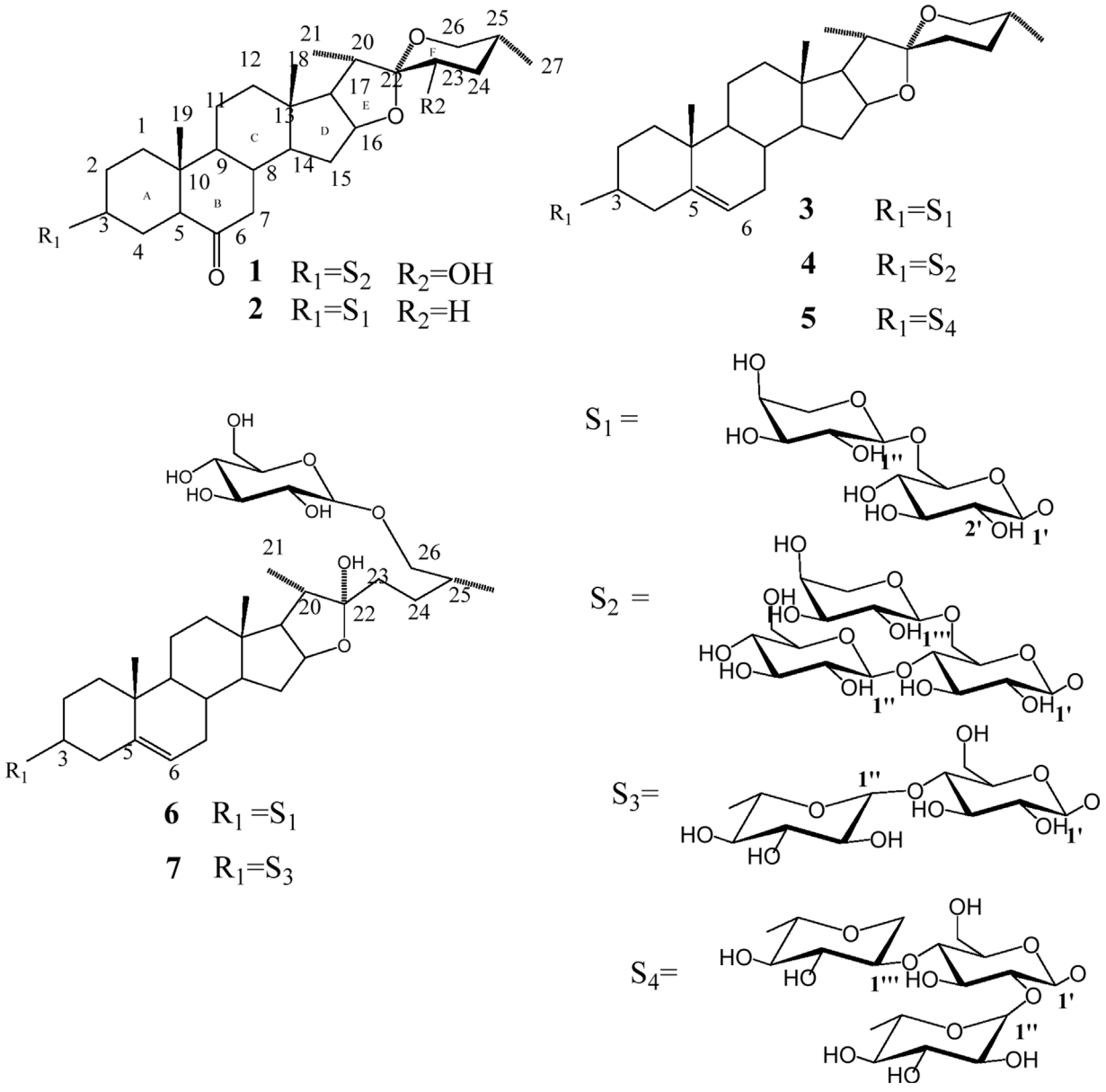

Fig. 1. Structures of isolated compounds **1–7**.

20) → δ_C 67.4(C-23); δ_H 1.78(H-25) → δ_C 67.4(C-23) and δ_H 3.84(H-23) → δ_C 35.8 (C-20); δ_H 3.84(H-23) → δ_C 31.7(C-25) together with the HMQC spectrum provided unequivocal evidence that the location of the hydroxyl group was at C-23. The difference in chemical shift positions of the germinal protons at δ_H 3.45 (H-26a) and δ_H 3.53 (H-26b) (δa − δb = 0.08 ppm), is consistent with *25R* configuration; the difference is usually > 0.57 ppm for *25S* compounds and < 0.48 ppm for *25R* compounds (Agrawal, 2004). Throufh comparison of the ^{1}H and ^{13}C NMR data with cantalasaponin I-B_1 (Kang et al., 2012), the aglycone of **1** was identified as (*25R*)-5α-spirostan-3β,23-diol-6-one (**2**).

The ^{1}H NMR spectrum of **1** further showed three anomeric protons corresponding to three sugar moieties at δ_H 4.90 (d, *J* = 7.76, H-1′), δ_H5.54 (d, *J* = 7.88, H-1″), δ_H 5.08(d, *J* = 7.44, H-1‴), which gave correlations with three anomeric carbon signals at δ_C 102 (C-1′), δ_C 105.0 (C-1″), δ_C 105.8 (C-1‴) in the HSQC spectrum, respectively. Acid hydrolysis and HPLC analysis revealed that compound **1** has two D-glucose and one L-arabinose sugar units. Moreover, the connectivity of each sugar units from C-1 to C-6 was determined from HSQC and HMBC spectra. The sequence of sugar chains and their linkage sites to the aglycone moiety were deduced from the HMBC spectrum, in which long-range correlations were observed from δ_H 4.90 (H-1′) to δ_C 76.9 (C-3), from δ_H 5.54 (H-1″) to δ_C 81.1 (C-4′), from δ_H 5.08 (H-1‴) to δ_C 68.4 (C-6′), respectively. Thus, the structure of **1** was identified as (*25R*)spirost-3β,23-diol-6-one-3-*O*-β-D-glucopyranosyl-(l → 4)-[α-L-arabinopyranosyl-(l → 6)]-β-D-glucopyranoside, and given the trivial name smilscobinoside C.

Compound **3**, white amorphous powder, its molecular formula was inferred as $C_{38}H_{60}O_{12}$ from the positive-ion HRESIMS $[M + H]^+$ peak at *m/z* 709.4085. The ^{1}H NMR spectrum displayed signals for two tertiary methyl groups at δ_H 0.8 (s) and δ_H 0.89 (s), two secondary methyl groups (δ_H 0.67, d, *J* = 5.4 Hz; δ_H 1.12, d, *J* = 6.9 Hz) and one olefinic proton δ_H 5.3 (brs, J = 4.6, H-6) that are characteristic of a steroidal sapogenin. The aglycone was identified as as diosgenin from analysis of the HMQC and HMBC spectra of **3**. The configuration at C-25 was deduced as *R* by the difference in chemical shifts of the geminal protons at δ_H 3.48 (H-26a) and δ_H 3.56 (H-26b) (δa − δb = 0.08 ppm). Two sugar anomeric protons at δ_H 4.94 (d, *J* = 7.76, H-1') and δ_H 4.95 (d, *J* = 6.64, H-1″) with the corresponding anomeric carbon signals at δ_C 102.9 (C-1'), δ_C 105.5 (C-1″) revealed that compound **3** has one D-glucose and one L-arabinose sugar units. The sequence of the sugar chain was determined from the ^{13}C NMR and ^{1}H NMR spectra. The deshielded value, of δ_C 69.5, of C-6' indicated