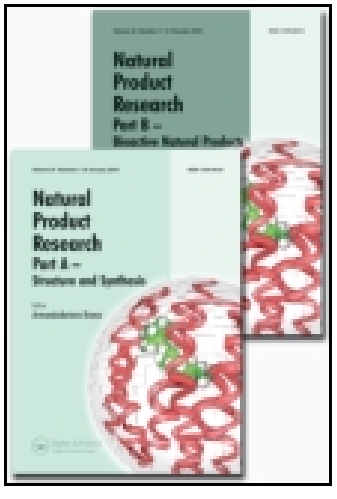

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

A new cytotoxic steroidal saponin from the rhizomes and roots of Smilax scobinicaulis

Cunli Zhang [a] , Shixiu Feng [b] , Lanxi Zhang [a] & Zhanjun Ren [c]
[a] College of Life Science , Northwest A&F University , Yangling , Shaanxi 712100 , China
[b] Laboratory of Southern Subtropical Plant Diversity , Shenzhen Fairy Lake Botanical Garden, Chinese Academy of Sciences , 160 Xianhu Road, Liantang, Shenzhen, 518004 , China
[c] College of Animal Science and Technology , Northwest A&F University , Yangling , Shaanxi 712100 , China
Published online: 17 Sep 2012.

To cite this article: Cunli Zhang , Shixiu Feng , Lanxi Zhang & Zhanjun Ren (2013) A new cytotoxic steroidal saponin from the rhizomes and roots of Smilax scobinicaulis , Natural Product Research: Formerly Natural Product Letters, 27:14, 1255-1260, DOI: 10.1080/14786419.2012.725396

To link to this article: http://dx.doi.org/10.1080/14786419.2012.725396

Natural Product Research, 2013
Vol. 27, No. 14, 1255–1260, http://dx.doi.org/10.1080/14786419.2012.725396

A new cytotoxic steroidal saponin from the rhizomes and roots of *Smilax scobinicaulis*

Cunli Zhang[a], Shixiu Feng[b], Lanxi Zhang[a] and Zhanjun Ren[c]*

[a]*College of Life Science, Northwest A&F University, Yangling, Shaanxi 712100, China;*
[b]*Laboratory of Southern Subtropical Plant Diversity, Shenzhen Fairy Lake Botanical Garden, Chinese Academy of Sciences, 160 Xianhu Road, Liantang, Shenzhen, 518004, China;*
[c]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China*

(*Received 19 January 2012; final version received 5 August 2012*)

A phytochemical investigation of the EtOH extract from the rhizomes and roots of *Smilax scobinicaulis* resulted in the isolation of a new isospirostanol-type steroidal saponin, namely (25 *R*)-5α-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**1**), along with four known steroidal saponins (**2**–**5**). The structures of these compounds were determined by 1D- and 2D-NMR spectroscopic analysis, FABMS and HR–ESI–MS as well as chemical degradation. The isolated saponins were evaluated for their *in vitro* cytotoxicity against A549, LAC and Hela human cancer cell lines, which demonstrated that only compound **1** possessed significant cytotoxic activity with IC_{50} values of 3.70, 5.70 and 3.64 μM, respectively.

Keywords: *Smilax scobinicaulis*; steroidal saponins; cytotoxicity

1. Introduction

The genus *Smilax* (family Liliaceae) comprises about 79 native species in China (Delectis florae reipublicae popularis sinicae agendae academiae sinicae edita, 1978), many of which have been used as herbal remedies for cardiac and cerebrovascular diseases for a long history. *Smilax scobinicaulis* (C.H.) Wright, also termed as *Hei Ci Ba Qia* in Chinese, is used in traditional medicine for the treatment of rheumatic arthritis, lumbago, gout and inflammatory diseases. Previous phytochemical investigations of this plant led to the isolation of a furostanol glycoside (Liu, Fu, Gao, & Qiu, 2002), seven steroidal saponins (Zhang, Li, Gao, & Fu, 2003; Zhang, Zhu, Cheng, & Li, 2003; Zhang et al., 2012). In our continuing research for new anti-cancer steroidal saponin, a new cytotoxic isospirostanol-type steroidal saponins namely (25 *R*)-5α-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**1**) was isolated from the 70% EtOH extract of the rhizomes and roots of *S. scobinicaulis*, along with four known compounds (**2**–**5**; Figure 1).

*Corresponding author. Email: renzhanjun@nwsuaf.edu.cn

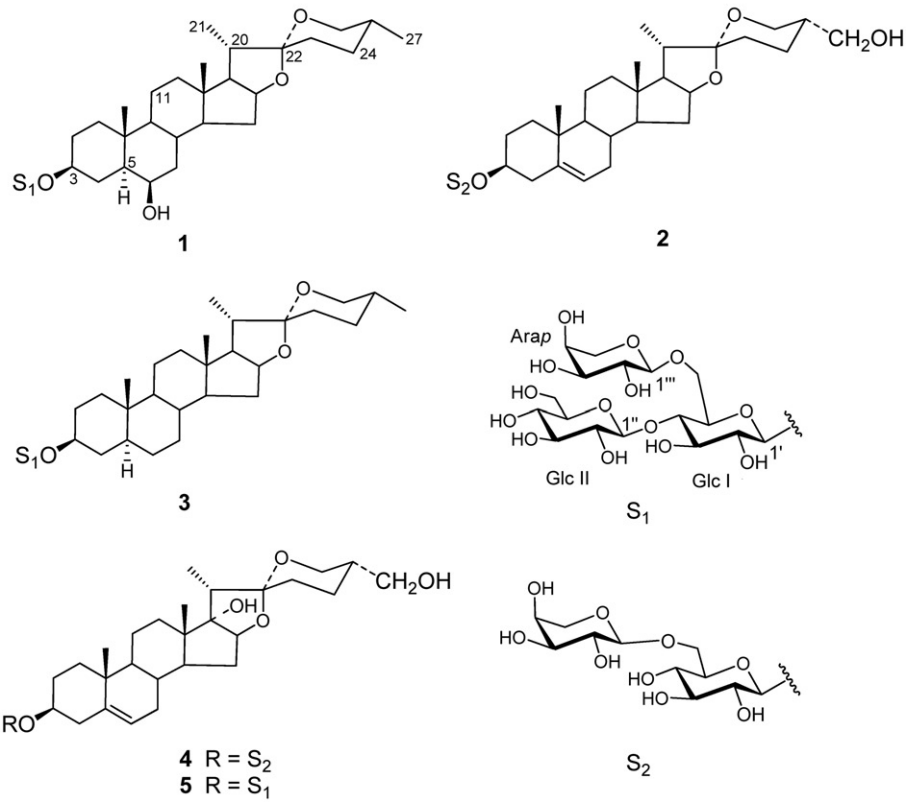

Figure 1. Chemical structure of compounds **1**–**5**.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder and showed a positive reaction in the Liebermann–Burchard test. Its molecular formula was deduced as $C_{44}H_{72}O_{18}$ from the quasi-molecular ion peak at *m/z* 887.4638 ($[M-H]^-$, $\Delta -0.2$ ppm) in the HR–ESI–MS, as well as the negative ion peak at *m/z* 887 $[M-H]^-$(1 0 0) in the FABMS. The IR spectrum showed strong absorptions for hydroxyl groups (3421 and 1049 cm^{-1}). In the ^{1}H-NMR and HSQC spectra, three anomeric protons at δ_H 4.93 (d, $J = 7.6$ Hz, H-1′), 5.54 (d, $J = 7.8$ Hz, H-1″) and 5.11 (d, $J = 7.5$ Hz, H-1‴) were observed, which were correlated with three anomeric carbon signals at δ_C 102.1 (C-1′), 104.9 (C-1″) and 105.6 (C-1‴), respectively. Acid hydrolysis of **1** resulted in the production of D-glucose and L-arabinose, which were confirmed by the combination of co-TLC comparison and GC analysis (Section 3). Furthermore, the ^{13}NMR and DEPT spectra exhibited 44 carbon signals, of which the distinctive quaternary carbon signal at δ_C 109.3 (C-22) led to the hypothesis that **1** was a spirostanol triglycosides (Zhang et al., 2012).

Comparison of the ^{1}H- and ^{13}C-NMR assignments of **1** with those of eruboside B reported from *Allium erubescens* previously (Ahmad & Basha, 2007), suggested the aglycone of **1** was (25*R*)-5α-spirostan-3β,6β-diol. Notably, the presence of a hydroxyl group at C-6 of the aglycone in **1** was disclosed by a significant downfield resonance at δ_C 71.9 (C-6), as well as by the HMBC correlations of H-6 (δ_H 4.04) with C-4 (δ_C 32.8), C-8 (δ_C 30.7) and C-10 (δ_C 35.9). The CH_3-27 was unambiguously attached at C-25 by the

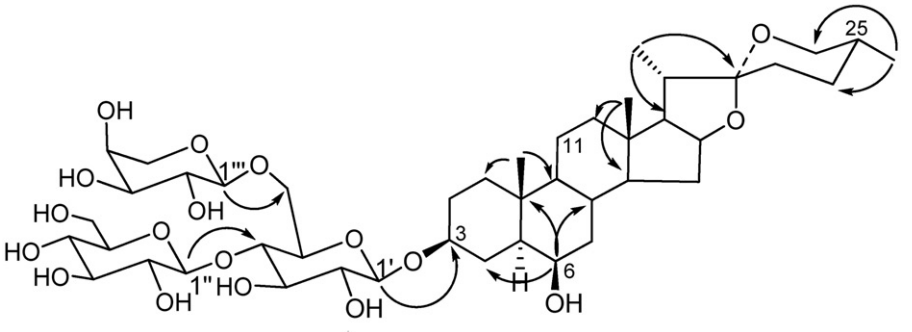

Figure 2. Key HMBC correlation (arrows) of compound **1**.

deduction of correlations form H-27 (δ_H 0.67) to C-24 (δ_C 28.8) and C-26 (δ_C 66.9) (Figure 1). The A/B and B/C *trans*-rings fusion were concluded by observation of the carbon signals at δ_C 49.0 (C-5), 54.5 (C-9) and 12.4 (C-19) in the ^{13}C-NMR spectrum, suggesting the 5-α configuration of H-5, respectively (Zhou & Chen, 2008). The β-configurations of 3-OH and 6-OH were further confirmed by comparison of its ^{1}H- and ^{13}C-NMR data with steroidal saponins from *Smilax officinalis* which demonstrated the same stereochemical configuration for the A and B rings, as well as the cross peaks between H-5 (δ_H 0.96)/H-3 (δ_H 3.83), and between H-5/H-6 in the NOESY spectrum (Bernardo, Pinto, & Parente, 1996). The 25 *R* configuration of the aglycone was confirmed by the two proton signals of H-26 at δ_H 3.59 (brd, $J = 10.5$ Hz, H-26ax) and 3.51 (dd, $J = 9.5$, 11.0 Hz, H-26eq) in the ^{1}H-NMR spectrum (Debella, Haslinger, Kunert, Michl, & Abebe, 1999). Moreover, comparison of the ^{13}C-NMR spectra of the aglycone portion of **1** with the known saponin from *S. officinalis* (25 *S*-spirostan-3β,6β-diol; no. 181375-68-8) showed the downfield shifts for the C-23 (+5.5 ppm), C-24 (+2.6 ppm), C-25 (+2.8 ppm) and C-26 (+1.8 ppm) due to the effect by the 25 *R* configuration of an methyl group at C-25 (Bernardo et al., 1996), which were consistent with the literature reported 25 *R*-spirostans (Acharya et al., 2010; Ahmad & Basha, 2007; Agrawal et al., 1995).

The attachment of the oligosaccharidic chain and the interglycosidic linkage was established by the analysis of HMBC experimental data of **1** as well as comparison to the previously isolated steroid saponins smilscobinoside A (Zhang et al., 2012), which demonstrated that they had the same sugar moieties. Briefly, long range correlation from H-1′ of the internal glucopyranose (Glc I) to C-3 (δ_C 77.7) of the aglycone, from H-1″ of glucopyranose (Glc II) to C-4′ (δ_C 81.1) of Glc-I, and from H-1‴ of the arabinopyranose to C-6′ (δ_C 68.4) of Glc-I proved the connections (Figure 2). Thus, the structure of **1** was elucidated as (25 *R*)-5α-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside.

The four known saponins (25 *S*)-spirostan-5-en-3β,27-diol-3-*O*-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**2**) (Ju & Jia, 1993), (25 *R*)-5α-spirostan-3β-ol (tigogenin) 3-*O*-β-D-glucopyrnosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**3**) (Kubo, Mimaki, Sashida, Nikaido, & Ohmoto, 1992), (25 *S*)-spirostan-5-en-3β,17α, 27-triol 3-*O*-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**4**) (Jia & Ju, 1992), (25 *S*)-spirostan-5-en-3β,17α,27-triol 3-*O*-β-D-glucopyrnosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranosid (**5**) (Ju, Peng, Jia, & Sun, 1994), which were determined by interpretation of their spectroscopic data as well as by comparison with reported data.

All the isolated compounds were evaluated for their cytotoxicity against A549, Hela and LAC human cancer cell lines following a standard MTT assay. Only compound **1** exhibited a significant cytotoxic activity against all the three cell lines, with IC_{50} values of 3.70, 3.64 and 5.70 μM, comparing to the positive control of doxorubicin with IC_{50} values of 1.08, 0.95 and 1.16 μM, respectively, while other saponins (**2**–**5**) were found to be inactive ($IC_{50} > 100$ μM).

3. Experimental

3.1. *General*

The ^{1}H- (500 MHz), ^{13}C- (125 MHz) and 2D-NMR spectra were determined on a Bruker DRX-500 instrument using TMS as internal standard. FAB-MS were collected on VG Auto Spec 3000 FAB/HRMS instrument. Melting points were detected on a Yanagimoto Seisakusho MD-S2 and uncorrected. Optical rotations were obtained on a Perkin Elmer 341 polarimeter with MeOH as solvent. The IR spectra were measured in KBr on an Equinox spectrophotometer. HRESI–MS data were obtained on an API QSTAR mass spectrometer. GC analysis was performed on a Thermoquest gas chromatograph using a CP-sil 5 CB capillary column (30 m × 0.25 mm^2, i.d.), with detection by FID.

Preparative HPLC was run with a Shimazu LC-6 A pump and a Shimazu RID-10 A refractive index detector using an XTerra prep MS C_{18} column (10 μm, 300 × 19 mm^2). For column chromatography (CC), silica gel (200 − 300 mesh; Qingdao Puke Isolation Material Co.), ODS-A (75 μm, YMC) and Sephadex LH-20 (Mitsubishi Chemical Holdings Co.) were used. Thin-layer chromatography was performed on $HSGF_{254}$ (Qingdao Puke Isolation Material Co.) plates.

3.2. *Plant material*

The rhizomes and roots of *S. scobinicaulis* C.H. were collected from Taibai Mountain of Shaanxi Province, People's Republic of China, in October 2008. The plant was identified by Zhenghai Wu of Northwest A&F University. An authenticated voucher specimen (XB 00045) was deposited at the Herbarium of Northwest A&F University.

3.3. *Extraction and isolation*

The air-dried and powdered plant material (30 kg) was extracted with 70% EtOH at room temperature and concentrated *in vacuo* to get a crude extract, which was suspended in H_2O and partitioned successively with petroleum ether, EtOAc and *n*-BuOH. The *n*-BuOH soluble portion (800 g) was chromatographed on a D_{101} macroporous adsorption resin and eluted successively with 40% EtOH, 50% EtOH, 60% EtOH, 70% EtOH and 80% EtOH, respectively, to afford five fractions Fr. 1−5. Fr.2 (42 g) was chromatographed over silica gel CC and eluted with $CHCl_3$: MeOH : H_2O (7 : 3 : 0.5) to yield two subfractions Fr. 2a and 2b. Fr. 2a was purified by a reversed-phase (RP-C_{18}) CC and eluted with 70% MeOH–H_2O to give compounds **1** (17 mg) and **5** (26 mg). Fr.3 (83 g) was subjected to a silica gel CC using a gradient of $CHCl_3$: MeOH (from 9 : 1 to 8 : 2) to afford two subfractions Fr. 3a and 3b. Further purification of Fr.3b by preparative HPLC with MeOH–H_2O (55%) gave compound **4** (17 mg). Fr.5 (54 g) was subjected to a silica gel CC using $CHCl_3$: MeOH : H_2O (8 : 2 : 0.2) to provide three subfractions Fr. 5a, 5b and 5c. Fr.5c was purified by preparative HPLC using 50% MeOH–H_2O to yield compounds **2** (45 mg) and **3** (27 mg).

(25 *R*)-5α-spirostan-3β,6β-diol 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-arabinopyranosyl-(1 → 6)]-β-D-glucopyranoside (**1**) was obtained as white, amorphous power; m.p. > 280°C; $[\alpha]_D^{20}$ −8° (*c* 0.5, MeOH).

^{1}H-NMR (500 MHz, pyridine-d_5): δ 0.88 (3H, s, CH_3-18), 1.13 (3H, s, CH_3-19), 1.01 (3H, d, $J = 7.5$ Hz, CH_3-21), 0.67 (3H, d, $J = 6.5$ Hz, CH_3-27), 3.83 (1H, overlapped, H-3), 4.04 (1H, brq, $J = 2.9$ Hz, H-6), 1.73 (1H, dd, $J = 7.5$ and 7.5 Hz, H-17), 1.84 (1H, dt, $J = 6.6$ and 6.6 Hz, H-20), 3.40 (1H, dd, $J = 9.5$ and 11.0 Hz, H-26a), 3.59 (1H, brd, $J = 10.5$ Hz, 1H, H-26b), 4.93(1H, d, $J = 7.6$ Hz, H-1′), 3.85 (1H, dd, $J = 7.5$ and 8.8 Hz, H-2′), 4.45 (1H, overlapped, H-3′) 4.45(1H, dd, $J = 9.2$ and 8.8 Hz, H-4′), 3.97 (1H, brd, $J = 8.2$ Hz, H-5′), 4.82 (1H, dd, $J = 8.0$ and 13.2 Hz, H-6′a), 4.68 (1H, dd, $J = 5.6$ and 8.0 Hz, H-6′b), 5.54 (1H, d, $J = 7.8$ Hz, H-1″), 4.12 (1H, dd, $J = 9.0$ and 7.8 Hz, H-2″), 4.37 (1H, dd, $J = 9.0$ and 9.2 Hz, H-3″), 4.25 (1H, overlapped, H-4″), 4.30 (1H, dd, $J = 5.5$ and 11.5 Hz, H-6″), 4.20 (1H, dd, $J = 9.0$ and 10.0 Hz, H-6″b), 5.11 (1H, d, $J = 7.5$ Hz, H-1‴), 4.56 (1H, dd, $J = 10.0$ and 7.5 Hz, H-2‴), 4.05 (1H, dd, $J = 8.0$ and 4.5 Hz, H-C3‴), 4.25 (1H, overlapped, H-4‴), 4.24 (1H, overlapped, H-5‴a) and 3.73 (1H, brd, $J = 11.5$ Hz, H-5‴b).

^{13}C-NMR (125 MHz, pyridine-d_5): δ 35.3 (C-1), 30.3 (C-2), 77.7 (C-3), 32.8 (C-4), 49.0 (C-5), 71.9 (C-6), 41.0 (C-7), 30.7 (C-8), 54.5 (C-9), 35.9 (C-10), 21.2 (C-11), 40.3 (C-12), 40.8 (C-13), 56.5 (C-14), 21.1 (C-15), 81.2 (C-16), 63.1 (C-17), 16.7 (C-18), 12.4 (C-19), 42.0 (C-20), 15.1 (C-21), 109.3 (C-22), 31.8 (C-23), 28.8 (C-24), 30.6 (C-25), 66.9 (C-26), 17.4 (C-27), 102.1 (C-1′), 74.8 (C-2′), 76.4 (C-3′), 81.1 (C-4′), 74.8 (C-5′), 68.4 (C-6′), 104.9 (C-1″), 75.2 (C-2″), 78.5 (C-3″), 71.8 (C-4″), 78.4 (C-5″), 62.5 (C-6″), 105.6 (C-1‴), 72.6 (C-2‴), 74.6 (C-3‴), 69.8 (C-4‴) and 67.3 (C-5‴).

Negative FABMS: m/z 887 $[M-H]^-$(1 0 0); HR–ESI–MS (negative-ion mode) m/z 887.4638 $[M-H]^-$ (Calcd $C_{44}H_{71}O_{18}$, 887.4640).

IR ν_{max} (KBr) cm^{-1}: 3421, 2933, 1049, 952, 898 and 863.

3.4. *Acid hydrolysis and sugar analysis*

Acid hydrolysis and sugar analysis was performed strictly as previously reported (Zhang, Gao, & Zhu, 2012). Briefly, a solution of compound **1** (5 mg) was hydrolysed with 2 M HCl in MeOH–H_2O (1 : 1) on boiling water at Ar atmosphere. The reaction mixture was determined on a TLC plate with *n*-BuOH–Me_2CO–H_2O (4 : 5 : 1) for sugar analysis. The aqueous residue was treated with pyridine and L-cysteine methyl ester (0.06 mol L^{-1}) hydrochloride for 60 min to obtain a thiazolidine carboxylates, and subsequently silylated by treatment with hexamethyldisilazane–trimethylchlorosilane (3 : 1). After centrifugation, the supernatant was concentrated and partitioned between *n*-hexane and water, and the hexane phase was subjected to GC. The absolute configurations of the monosaccharides were confirmed to be L-arabinose and D-glucose by comparison of the retention times of monosaccharide derivatives with those of standard samples: L-arabinose (RT: 10.23 ± 0.01 min) and D-glucose (RT: 14.24 ± 0.01 min), respectively.

3.5. *Cytotoxicity assay*

Cytotoxicity was determined against A549 (human lung cancer), LAC (human pulmonary carcinoma) and Hela (human cervical carcinoma) tumour cell lines based on the MTT assay method. The stock solution (10 mM) of **1** was prepared by dissolved in 0.5% dimethylsulphoxide (DMSO), and diluted with culture medium for experimentation. The tumuor cancer cell lines were cultured in the Dulbecco's Modified Eagle's Medium. The assay procedure was carried out as previously described (Mosmann, 1983), and doxorubicin was used as the positive control.

Acknowledgements

This project was jointly financially supported by the National Natural Science Foundation of China (30840063, 30770237) and Startup Foundation for Doctors of Northwest A&F University (01140506), as well as the Programme for Changjiang Scholars and Innovative Research Team in University (IRT0749).

References

Acharya, D., Mitaine-Offer, A.C., Kaushik, N., Miyamoto, T., Paululat, T., Mirjolet, J.F., … Lacaille-Dubois, M.A. (2010). Steroidal saponins from *Chlorophytum orchidastrum*. *Journal of Natural Products, 73*, 7–11.

Agrawal, P.K., Jain, D.C., & Pathak, A.K. (1995). NMR spectroscopy of steroidal sapogenins and steroidal saponins: an update. *Magnetic Resonance in Chemistry, 33*, 923–953.

Ahmad, V.U., & Basha, A. (2007). *Spectroscopic data of steroid glycosides: Spirostanes, bufanolides, cardendides* (Vol. 3, Part 1, p. 1751). New York, NY: Springer.

Bernardo, R.R., Pinto, A.V., & Parente, J.P. (1996). Steroidal saponins from *Smilax officinalis*. *Phytochemistry, 43*, 465–469.

Debella, A., Haslinger, E., Kunert, O., Michl, G., & Abebe, D. (1999). Steroidal saponins from *Asparagus africanus*. *Phytochemistry, 51*, 1069–1075.

Delectis florae reipublicae popularis sinicae agendae academiae sinicae edita (1978). *Flora Reipublicae Popolaris Sinicae* (Vol. 15, p. 180). Beijing: Science Press.

Jia, Z.H., & Ju, Y. (1992). Steroidal saponins form *Smilax lebrunii*. *Phytochemistry, 31*, 3173–3175.

Ju, Y., & Jia, Z.J. (1993). Minor steroidal glycosides from the roots of *Smilax lebrunii*. *Phytochemistry, 33*, 1193–1195.

Ju, Y., Peng, H., Jia, Z.J., & Sun, X.J. (1994). Studies on steroidal glycosides of *Smilax nigrescens*. *Journal of Lanzhou University (Natural Sciences), 30*, 64–67.

Kubo, S., Mimaki, Y., Sashida, Y., Nikaido, T., & Ohmoto, T. (1992). Steroidal saponins from the rhizomes of *Smilax sieboldii*. *Phytochemistry, 31*, 2445–2450.

Liu, J.Y., Fu, J.X., Gao, J.M., & Qiu, M.H. (2002). A new furostanol glycoside from the rhizomes of *Smilax scobinicaulis*. *Journal of Northwest Sci-tech University of Agriculature and Forestry, 30*, 222–224.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*, 55–63.

Zhang, C.L., Gao, J.M., & Zhu, W. (2012). Steroidal saponins from the rhizomes and roots of *Smilax scobinicaulis*. *Phytochemistry Letters, 5*, 49–52.

Zhang, C.L., Li, W.C., Gao, J.M., & Fu, J.X. (2003). Steroidal saponins from *Smilax scobinicaulis*. *Journal of Northwest Science-technology University of Agriculture and Forestry, 31*, 163–166.

Zhang, C.L., Zhu, W., Cheng, Y.M., & Li, W.C. (2003). Steroidal saponins from *smilax scobinicaulis*. *Acta Botanical Boreal–Occident Sinica, 23*, 1973–1976.

Zhou, L.B., & Chen, D.F. (2008). Steroidal saponins from the roots of *Asparagus filicinus*. *Steroids, 73*, 83–87.