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# Steroidal saponins from *Smilax china* and their anti-inflammatory activities

Bo Shao, Hongzhu Guo *, Yajun Cui, Min Ye, Jian Han, Dean Guo *

*The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, PR China*

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**Abstract**

Steroidal saponins, **1**, **2**, **3** and **4**, were isolated from the BuOH extract of *Smilax china* L., along with 13 known compounds, **5**–**17**. Their structures were elucidated on the basis of MS, 1D and 2D NMR spectroscopic analyses and chemical evidence. In the bioassay tests, all compounds showed inhibitory effects on cyclooxygenase-2 enzyme (COX-2) activities at final concentration of $10^{-5}$ M, and only compound **5** showed an inhibitory effect on production of TNFα (tumor necrosis factor α) in murine peritoneal macrophages at the same concentration.


*Keywords:* *Smilax china*; Liliaceae; Steroidal saponins; Smilaxchinoside A–D; Anti-inflammatory activities; Cyclooxygenase-2 enzyme; Tumor necrosis factor α

## 1. Introduction

*Smilax* (family Liliaceae; about 350 species) plants are distributed widely in tropical and temperate regions throughout the world, especially in East Asia and North America. Many of them have long been used as medicinal herbs. They are known to be rich in steroidal saponins (Bernardo et al., 1996; Ju and Jia, 1992, 1993, 1994; Jia and Ju, 1992; Woo et al., 1992; Kubo et al., 1992; Sautour et al., 2005). For example, the tuber of *Smilax china* L., known as "Ba Qia" (or "Jin Gang Teng") in Chinese, is used in traditional Chinese medicine (TCM) for treatment of diuretic, rheumatic arthritic, detoxication, lumbago, gout, tumor and inflammatory diseases (State Administration of Traditional Chinese Medicine of People's Republic of China, 1999). Recent pharmacological investigations showed that *S. china* has anti-inflammatory activity (Shu et al., 2006; Li and Zhou, 1996; Chen et al., 2000; Lu et al., 2003) and some steroidal saponins isolated from this plant exhibited significant cytotoxicity against several tumor cell lines (Hu and Yao, 2002; Hu et al., 1997). Previous phytochemical studies on this plant led to isolation of several saponins, including smilaxin, prosapogenin A of dioscin, gracillin, dioscin, pseudoprotodioscin, methylgracillin and methylprotodioscin (Sashida et al., 1992; Kim et al., 1989). However, a systematic phytochemical investigation of this plant has not been pursued, and the range of its bioactive compounds are unknown.

The present paper focusses on isolation and structural elucidation of saponins from the BuOH extract of *S. china*. In addition, their anti-inflammatory and cytotoxic activities are reported for the first time (see Fig. 1).

## 2. Results and discussion

The 95% EtOH and 50% EtOH extracts of *S. china* were suspended in water and extracted with petroleum ether, EtOAc and BuOH. The BuOH fraction was passed through a $D_{101}$ macropore resin eluted successively with 30% EtOH, 50% EtOH, 70% EtOH and 95% EtOH, respectively. The

* Corresponding authors. Tel./fax: +86 10 82805107 (H. Guo), tel./fax: +86 10 82802700 (D. Guo).
*E-mail addresses:* guohz@bjmu.edu.cn (H. Guo), gda@bjmu.edu.cn (D. Guo).

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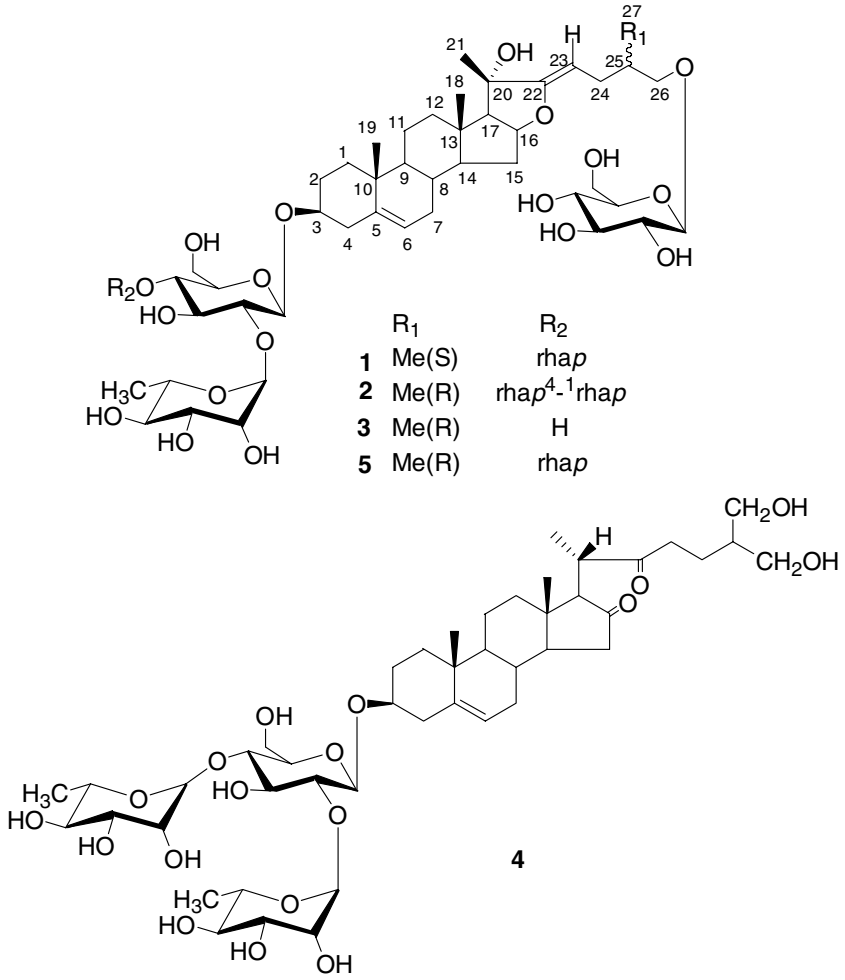


Fig. 1. The structures of compounds **1**–**5**.

concentrated 50% EtOH fraction was subjected to silica gel and Sephadex LH-20 column chromatography and finally purified by semi-preparative scale HPLC to afford four new compounds, **1**, **2**, **3** and **4**, along with 13 known compounds, **5**–**17**.

Compound **1** gave a pseudo-molecular ion peak $[M+Na]^+$ at *m/z* 1069.5184 (calculated for $C_{51}H_{82}O_{22}Na$, 1069.5195) in its high-resolution ESI-MS. Combined with the $^{13}C$ NMR spectroscopic data, its molecular formula was determined as $C_{51}H_{82}O_{22}$. Three tertiary methyl proton groups at $\delta$ 0.89, 1.04 and 1.70 (each *s*), a secondary methyl proton at $\delta$ 1.05 (1H, *d*, $J = 5.0$ Hz) and two trisubstituted olefinic protons at $\delta$ 5.29 (1H, *br s*, H-6) and $\delta$ 4.50 (1H, *m*, H-23), as well as protons attributable to an oxymethylene H-26 at $\delta$ 4.13 (1H, *dd*, $J = 9.5$, 6.0 Hz) and 3.50 (1 H, *dd*, $J = 9.5$, 7.5 Hz), observed in the $^1H$ NMR spectrum (Table 1). These data, when considered with the analysis of its $^{13}C$ NMR spectrum (three angular methyl groups at $\delta$ 13.5, 19.3 and 21.8, one secondary methyl group at $\delta$ 17.4, two trisubstituted double bonds at $\delta$ 140.8, 121.7 and $\delta$ 163.7, 91.3, and a methylene group linked to an oxygen atom at $\delta$ 75.2) (Table 2), indicated that the aglycone possessed a furost-5,22-diene skeleton. A comparison of the $^{13}C$ NMR spectroscopic signals of the aglycone moiety of **1** with the literature values (Chen et al., 2005), and an extensive gCOSY, HMQC and HMBC data analysis showed that the aglycone of **1** was 3β,20,26-trihydroxyfurost-5,22-diene. Its 25*S* configuration was deduced on the basis of differences in chemical shifts of the geminal protons at $H_2$-26 ($\delta a - \delta b = 0.63$ ppm), since the difference is usually >0.57 ppm for 25*S* compounds and <0.48 ppm for 25*R* compounds (Agrawal, 2004). The α-configuration of the C-20 hydroxyl group was also defined by the cross-peak between H-21 ($\delta$ 1.70) and H-18 ($\delta$ 0.89) in its NOESY spectrum. Hence, the aglycone of **1** was identified as (25*S*)-3β,20α,26-trihydroxy furost-5,22-diene.

Of the 51 carbon signals observed in the $^{13}C$ NMR spectrum of **1**, 27 were assigned to the aglycone part and the remaining 24 to the oligosaccharide moiety. The $^1H$ NMR and $^{13}C$ NMR spectra of **1** exhibited four sugar anomeric protons at $\delta$ 4.93 (1H, *d*, $J = 8.0$ Hz), 4.81 (1H, *d*, $J = 8.0$ Hz), 6.38 (1H, *brs*) and 5.83 (1H, *brs*) (Table 3), and carbon atoms at $\delta$ 100.2, 105.0, 102.0 and 102.8 (Table 2), respectively. Acid hydrolysis of **1** afforded D-glucose and L-rhamnose as revealed by HPLC analysis and comparison with authentic standards. The identity of the