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A cytotoxic cardenolide and a saponin from the rhizomes of *Tupistra chinensis*

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ABSTRACT

A new cardenolide tupichinolide (**1**) and a new steroidal saponin tupichinin A (**2**), together with seven known compounds, were isolated from the rhizomes of *Tupistra chinensis*. Their structures were established using spectroscopic analysis and chemical methods. Compound **1** was the first cardenolide isolated from *Tupistra chinensis* and exhibited potent cytotoxicity against five human cancer cell lines: HL-60, SMMC-7721, A-549, MCF-7 and SW480.

1. Introduction

Tupistra chinensis (Liliaceae), widely distributed in southwestern China, is commonly used in Chinese traditional medicine to treat throat irritation, rheumatic diseases and snake-bite [1,2]. According to the results of modern pharmacological experiments, the extracts of this species possessed significant antitumor activity [3,4]. In our screening for cytotoxic agents from Chinese medicinal plants, the ethanol extract from the rhizomes of *Tupistra chinensis* showed inhibitory effect towards several human cancer cell lines. Previous phytochemical investigations showed *Tupistra chinensis* is a rich source of steroidal sapogenins and saponins [5–14]. As a part of our search for new biologically active metabolites from traditional Chinese medicines, a new cardenolide tupichinolide (**1**) and a new steroidal saponin tupichinin A (**2**), together with seven known steroids, were isolated from the rhizomes of *Tupistra chinensis*. All compounds were also tested for cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7 and SW480 human cancer cell lines. This paper deals with the isolation, structure elucidation, and cytotoxic activity of these compounds (Fig. 1).

2. Experimental procedure

2.1. General

Optical rotations were measured with a Horiba SEPA-300 polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. IR spectroscopy was measured in a Bio-Rad FTS-135 spectrometer with KBr pellets. ESI-MS and HRESI-MS were recorded on an API QSTAR Pulsar 1 spectrometer. The NMR spectra were recorded on Bruker DRX-500 spectrometers with TMS as internal standard, and chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Silica gel (200−300 mesh; Qingdao Marine Chemical Inc., Qingdao, China), RP-18 gel (40−63 μm; Merck, Darmstadt, Germany), and Sephadex LH-20 (Amersham Pharmacia biotech, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. Solvents were distilled before use.

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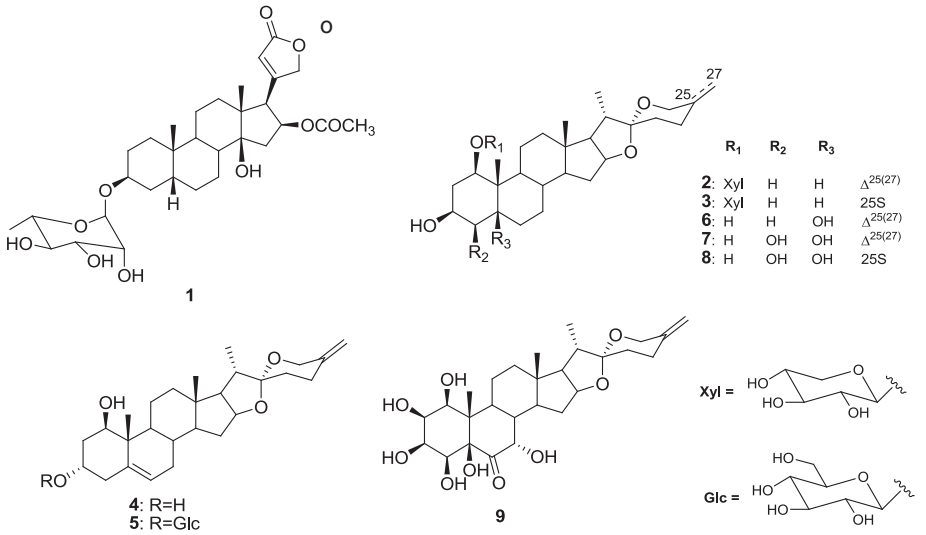

Fig. 1. Structures of compounds **1**–**9**.

2.2. Plant material

The rhizomes of *Tupistra chinensis* were purchased in Zhejiang province, China, in October 2010 and identified by one of the authors (Professor Yong-Xin Wen). A voucher specimen (CTM201001) was deposited at the Guangxi Key Laboratory of Functional Phytochemicals Research and Utilization, Guangxi Institute of Botany, Chinese Academy of Sciences, China.

2.3. Extraction and isolation

The air-dried and powdered sample (4.8 kg) was extracted with 95% EtOH (3×8 L) at room temperature. The EtOH extract was evaporated in a vacuum to yield a residue, which was partitioned between H_2O (2 L) and EtOAc (3×1 L). The EtOAc extract (300 g) was subjected to column chromatography over silica gel, eluting with a gradient of acetone in petroleum ether, to yield six fractions (Fr. 1—6). Fr. 3 (32 g) was further purified by CC (silica gel, petroleum ether:EtOAc, 9:1 to 0:1) and Sephadex LH-20 column (1 g; $CHCl_3$:MeOH, 1:1) to yield **4** (120 mg) and **6** (82 g). Fr. 4 (36 g) was repeatedly subjected to CC (silica gel, petroleum ether:acetone, 8:1 to 0:1) and Sephadex LH-20 column (1 g; MeOH:H_2O, 50:50 to 100:0) to afford **7** (60 mg), **8** (3 mg) and **9** (115 mg); Fr. 5 (26 g) was further purified by CC (silica gel, CCl_3:MeOH, 20:1 to 0:1) and RP-18 column (MeOH:H_2O, 0:100 to 100:0) to yield **1** (10 mg), **2** (30 mg), **3** (8 mg) and **5** (50 mg).

Tupichinolide (**1**): white needles; mp 242—244°C; $[\alpha]_D^{18.3}$ −59.58 (*c* 0.16, MeOH); IR (KBr) ν_{max}: 3431, 2932, 1738, 1736, 1632, 1048 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 601 $[M+Na]^+$; negative HRESIMS *m/z* 613.2779 $[M+Cl]^-$ (calcd for $C_{31}H_{46}O_{10}Cl$, 613.2779).

Tupichinin A (**2**): white amorphous powder; $[\alpha]_D^{19.3}$ −122.34 (*c* 0.15, MeOH); IR (KBr) ν_{max}: 3440, 2927, 1630, 1045 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; negative ESIMS *m/z* 561 $[M-H]^-$; negative HRESIMS *m/z* 561.3440 $[M-H]^-$ (calcd for $C_{32}H_{49}O_8$, 561.3427).

2.4. Acid hydrolysis of compounds **1** and **2**

Each compound (2 mg) was hydrolyzed with 1 M HCl–dioxane (1:1, 1 mL) at 80 °C for 4 h. The reaction mixture was partitioned between EtOAc and H_2O three times. The aqueous layer was neutralized with 2 M $NaHCO_3$ and then dried to in vacuo. The residue was dissolved in pyridine (0.5 mL), to which L-cysteine methyl ester hydrochloride in pyridine (0.1 M, 0.5 mL) was added. After reacting at 60 °C for 1 h, trimethylsilylimidazole (0.5 mL) was added to the reaction mixture and kept at 60 °C for another 30 min. The mixture was partitioned between *n*-hexane and H_2O, and the *n*-hexane extract was analyzed on Shimadzu GC-14C gas chromatograph equipped with a HP-5MS 30 m×0.32 mm column with the following conditions: column temperature, 210 °C; injector and detector temperature, 270 °C. By comparison of the retention times of the corresponding derivatives with that of standard sugar, the monosaccharides of compounds **1** and **2** were determined to be L-rhamnose, D-xylose, of which the retention time was 10.25, and 8.36 min, respectively.

2.5. Cytotoxicity assay

The cytotoxicity of the compounds against HL-60, SMMC-7721, A-549, MCF-7 and SW480 cell lines was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [15], with cisplatin as positive control. Cell was maintained in RPMI 1640 medium and seeded in 96-well plates. After 12 h incubation at 37 °C, the test compounds were added and the plate was further incubated. After 48 h, 20 μL of MTT solution was added to each well, which was incubated for a further 4 h. Then 20% SDS (100 μL) was added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The IC_{50} value was calculated by the Reed and Muench method [16].