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A new cytotoxic cardenolide from the latex of *Antiaris toxicaria*

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Abstract

A new cardenolide, toxicarioside E (**1**), was isolated from the latex of *Antiaris toxicaria* (Pers.) Lesch (Moraceae). Its structure was elucidated on the basis of spectral data and chemical evidence. Compound **1** showed significant cytotoxicity against K562 and SGC-7901 cell lines *in vitro* by MTT method with the IC_{50} value of 0.026 and 0.027 μg/mL, respectively.

Keywords: *Antiaris toxicaria*; Moraceae; Toxicarioside E; Cardenolide; Cytotoxicity

The highly toxic latex from *Antiaris toxicaria* (Pers.) Lesch (Moraceae), is well known for its major components used as arrow and dart poisons in South East Asia [1,2]. The genus *Antiaris* comprises four species, of which only *A. toxicaria* is distributed in China, mainly in Guangxi, Guangdong, Yunnan and Hainan provinces. The latex from *A. toxicaria* was used as arrow poison for hunting by the indigenous people in Yunnan and Hainan provinces, especially the Li minority in Hainan. Previous studies of the toxicity of this plant in Indonesia or Malaysian led to the isolation of cardenolides from the latex and the seeds [3,4]. In our screening for cytotoxic agents from the Li folk medicine in Hainan Province, the ethanol extract of latex of *A. toxicaria* showed inhibitory effect towards human chronic myelogenous leukemia cell line (K562) and human gastric cancer cell line (SGC-7901). The solvent-solvent partition of ethanol extract combined with bioassay revealed the water-soluble fraction was the active one. Further bioassay-guided fractionation of the active fraction led to the isolation of a new cardenolide, its structure was unambiguously elucidated as antiarigenin 3-*O*-β-D-glucopyranosyl(1 → 4)-α-L-rhamnopyranoside (**1**) by extensive spectroscopic analysis and chemical evidence. Compound **1** showed cytotoxicity against K562 and SGC-7901 cell lines *in vitro* by MTT method with the IC_{50} value of 0.026 and 0.027 μg/mL, respectively.

Latex of *A. toxicaria* (Pers.) Lesch was collected in Lingshui county of Hainan Province, PR China in November 2005, and the plant was identified by Professor Zhu-Nian Wang. A voucher specimen (No. AN200511) was deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

4.0 L latex was extracted with 95% EtOH three times at room temperature and filtered. The combined extract was evaporated *in vacuo* to yield syrup (263.8 g), which was suspended in H_2O and partitioned with Petroleum ether and EtOAc successively. The H_2O layer was fractionated over a D-101 macroporous resin column eluting with H_2O, 50%

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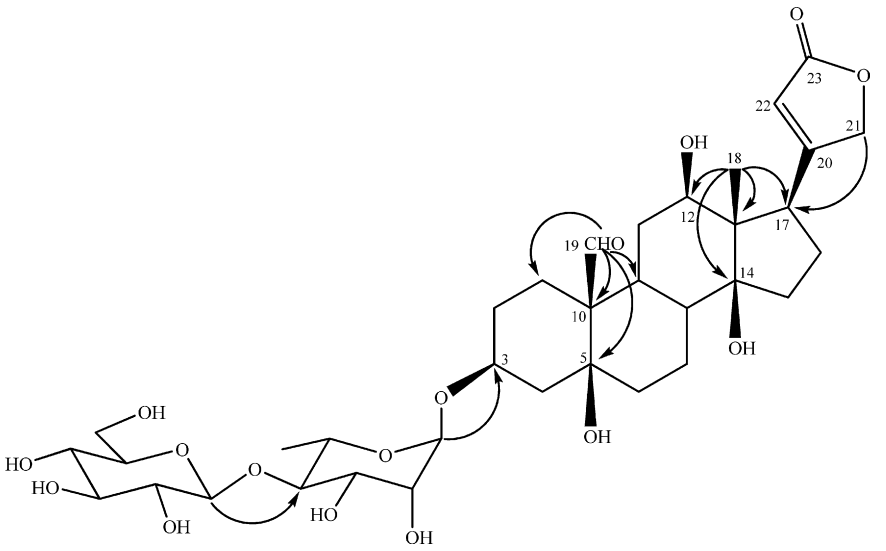

Fig. 1. Structure and selected HMBC correlations of compound **1**.

MeOH and 100% MeOH to yield three fractions. The 50% MeOH (16.4 g) fraction was subjected to chromatography on silica gel, eluted with $CHCl_3$–MeOH (8:1), to obtain fractions 1–11. After repeated silica gel column chromatography ($CHCl_3$–MeOH 5:1), fraction 8 (241.0 mg) afford **1** (70.6 mg).

Compound **1** was obtained as a white amorphous powder; m.p. 251–252 °C; $[\alpha]_D^{20}$ −18.5 (*c* 1.0, MeOH). The ion peak $[M+Cl]^+$ at *m/z* 763.2968 in the high-resolution ESI-mass spectrum corresponded to the molecular formula $C_{35}H_{52}O_{16}$ (calcd. for $[M+Cl]^+$ 763.2938). This formula can also be validated through ^1H NMR, ^{13}C NMR (DEPT) spectra. The IR spectrum displayed absorptions for free hydroxyl (3422 cm^{-1}), conjugated carbonyl (1732 cm^{-1}), and double bond (1625 cm^{-1}). In the ^1H NMR, signal at δ 5.91 (*s*, 1H, H-22) and signals at δ 4.90, 5.01 (each 1H, J_{AB} = 18.5 Hz, H-21a, H-21b) suggested the presence of the butenolide characteristic for cardenolide system. In addition, an extremely low-field signal at δ 10.06 (s, H-19) indicated an aldehyde group that is characteristic of the antiarigenin aglycone system. Other prominent signals included a high-field methyl singlet at δ 0.77 (H-18), also suggestive of a cardenolide nucleus; two anomeric proton signals at δ 4.84 (br. s, 1H, H-1′) and 4.59 (d, 1H, 7.8 Hz, H-1″) indicating that **1** was a glycoside incorporating two sugar units with β- and α-linkage, respectively. Comparison of the ^{13}C NMR data of **1** (Table 1) with those reported the aglycone was determined as antiarigenin [5] and the sugar chain was composed of a terminal β-glucose unit and an internal α-rhamnose unit [6]. On complete acid hydrolysis of

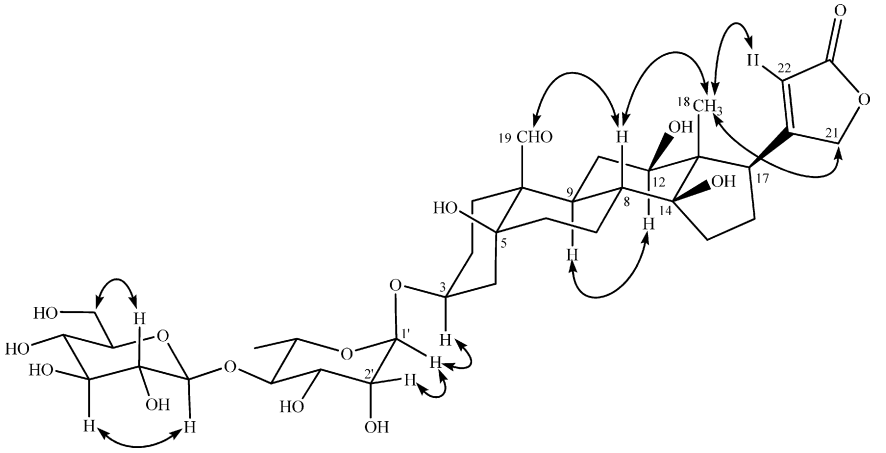

Fig. 2. Selected ROESY correlations of compound **1**.

Table 1
NMR data of **1** in CD_3OD (^1H: 400 MHz; ^{13}C: 100 MHz; δ ppm, J Hz).

Position	δ_H	δ_C	Position	δ_H	δ_C
1	1.74 (m), 2.07 (m)	19.2	19	10.06 (s)	209.6
2	1.71 (m), 1.78 (m)	25.9	20		177.3
3	4.14 (br. s)	75.3	21	5.01 (d, 18.5); 4.90 (d, 18.5)	75.4
4	1.69 (m), 2.08 (m)	37.3	22	5.91 (s)	117.8
5		75.2	23		178.3
6	2.16 (m), 1.61 (m)	36.2	1′	4.84 (br. s)	100.9
7	2.20 (m), 1.28 (m)	25.3	2′	3.29 (overlapped)	71.5
8	1.94 (m)	42.1	3′	3.80 (overlapped)	72.3
9	1.68 (m)	37.2	4′	3.64 (t, 9.2)	83.0
10		55.7	5′	3.68 (m)	69.4
11	1.73 (m), 1.26 (m)	31.5	6′	1.34 (d, 5.8)	18.1
12	3.34 (m)	75.1	1″	4.59 (d, 7.8)	105.6
13		56.9	2″	3.22 (t, 8.2)	76.0
14		86.2	3″	3.38 (t, 8.5)	78.0
15	1.93 (m), 1.72 (m)	32.6	4″	3.32 (overlapped)	72.5
16	2.09 (m), 1.96 (m)	28.2	5″	3.29 (overlapped)	78.1
17	3.32 (m)	46.8	6″	3.68 (d, 11.7); 3.85 (d, 11.7)	62.5
18	0.77 (s)	9.7			

1, D-glucose and L-rhamnose were determined by GLC analysis. From the 2D NMR (HMQC, ^1H–^1H COSY and HMBC) experiment, the chemical shifts of **1** were assigned, respectively. The HMBC correlations between H-3 (δ 4.14) and C-1′ (δ 100.9), and between H-1″ (δ 4.59) and C-4′ (δ 83.0) (Fig. 1), suggested that the rhamnosyl unit was linked to C-3, and the glucosyl unit linked to C-4′. The relative stereochemistry of **1** was determined by ROESY correlations (Fig. 2). Based on the above evidence, compound **1** was identified as antiarigenin 3-*O*-β-D-glucopyranosyl(1 → 4)-α-L-rhamnopyranoside, named toxicarioside E.

The cytotoxicity of compound **1** was evaluated *in vitro* using the MTT method [7]. Compound **1** showed significant cytotoxicity against the K562 and SGC-7901 cell lines with the IC_{50} values of 0.026 and 0.027 μg/mL, respectively. Mitomycin C was used as a positive control.

Acknowledgments

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