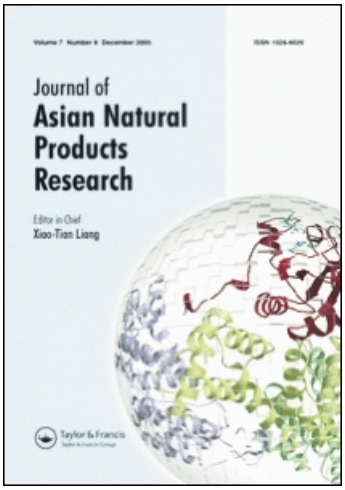


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Two new cytotoxic cardenolides from the latex of *Antiaris toxicaria*

Hao-Fu Dai[a]; Yu-Juan Gan[a]; Dong-Mei Que[a]; Jiao Wu[a]; Zhen-Chang Wen[b]; Wen-Li Mei[a]
[a] Key Laboratory of Tropical Crop Biotechnology, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China [b] Center of Analysis and Testing, Hainan University, Haikou, China

To cite this Article Dai, Hao-Fu , Gan, Yu-Juan , Que, Dong-Mei , Wu, Jiao , Wen, Zhen-Chang and Mei, Wen-Li(2009) 'Two new cytotoxic cardenolides from the latex of *Antiaris toxicaria*', Journal of Asian Natural Products Research, 11: 9, 832 — 837
To link to this Article: DOI: 10.1080/10286020903164285
URL: http://dx.doi.org/10.1080/10286020903164285

Journal of Asian Natural Products Research
Vol. 11, No. 9, September 2009, 832–837

Taylor & Francis
Taylor & Francis Group

Two new cytotoxic cardenolides from the latex of *Antiaris toxicaria*

Hao-Fu Dai[a], Yu-Juan Gan[a], Dong-Mei Que[a], Jiao Wu[a], Zhen-Chang Wen[b] and Wen-Li Mei[a]*

[a]*Key Laboratory of Tropical Crop Biotechnology, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;* [b]*Center of Analysis and Testing, Hainan University, Haikou 570228, China*

(*Received 4 May 2009; final version received 6 July 2009*)

Two new cardenolides, toxicarioside F (**1**) and toxicarioside G (**2**), were isolated from the latex of *Antiaris toxicaria* (Pers.) Lesch (Moraceae). Their structures were elucidated on the basis of spectral data and chemical evidence. Compounds **1** and **2** showed significant cytotoxicity against K562, SGC-7901, SMMC-7721, and HeLa cell lines *in vitro* by the MTT method.

Keywords: *Antiaris toxicaria*; toxicarioside F; toxicarioside G; cardenolide; cytotoxicity

1. Introduction

Antiaris toxicaria (Pers.) Lesch (Moraceae) is well known as 'upas tree' since it contains a complex mixture of cardenolide glycosides [1,2]. *A. toxicaria* is widespread over tropical areas in Southeast Asia and distributed in Guangxi, Guangdong, Yunnan, and Hainan Provinces of China. Previous studies of the toxicity of this plant in Indonesia or Malaysia led to the isolation of cardenolides from the latex, seeds, and stem [3,4]. In our previous screening for cytotoxic agents from tropical medicinal plants in Hainan Province, a new cardenolide named toxicarioside E possessing cytotoxicity has been isolated from the latex of *A. toxicaria* collected in Hainan Province of China [5]. In the continuous search for cytotoxic constituents, two new cardenolides, named toxicarioside F (**1**) and toxicarioside G (**2**), were obtained (Figure 1). Compounds **1** and **2** exhibited significant cytotoxicity against K562, SGC-7901, SMMC-7721, and HeLa cell lines. The present paperdiscusses their structural elucidation and cytotoxicity.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The ion peak $[M-H]^-$ at *m/z* 581.2597 in the high-resolution FAB-mass spectrum corresponded to the molecular formula $C_{29}H_{42}O_{12}$. This formula can also be validated through ^1H and ^{13}C NMR (DEPT) spectra. The IR spectrum displayed absorptions for free hydroxyl (3433 cm^{-1}), conjugated carbonyl (1737 cm^{-1}), and double bond (1625 cm^{-1}). In the ^1H NMR spectrum, the signal at δ 5.89 (s, 1H, H-22) and signals at δ 4.97 and 4.88 (each 1H, $J_{AB} = 18.1$ Hz, H-21a, H-21b) suggested the presence of the butenolide characteristic for the cardenolide system. In addition, an extremely

*Corresponding author. Email: meiwenli@yahoo.com.cn

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020903164285
http://www.informaworld.com

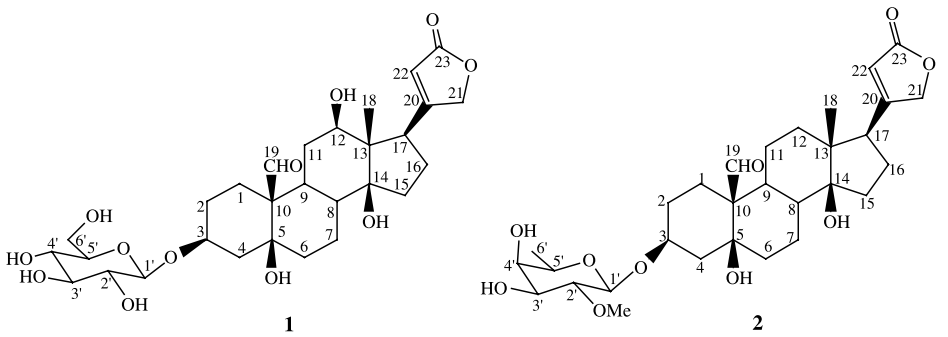


Figure 1. Structures of compounds **1** and **2**.

low-field signal at δ 10.03 (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; an anomeric proton signal at δ 4.33 (d, 1H, $J = 7.6$ Hz, H-1′) indicated that **1** was a glycoside incorporating a sugar unit with β-linkage. The aglycone was determined as antiarigenin by comparison of the ^{13}C NMR spectral data of **1** with those reported in the literature [6]. According to the vicinal coupling constants for the sugar proton signals and the ^{13}C NMR spectral data, the sugar moiety was determined as glucose, which agreed with the literature report [7]. This was further confirmed by complete acid hydrolysis of **1** followed by TLC analysis. The HMBC correlations between H-3 (δ 4.16) and C-1′ (δ 103.0) (Figure 2) suggested that the sugar moiety was linked to C-3. The relative stereochemistry of **1** was determined by ROESY correlations (Figure 2). Based on the above evidence, compound **1** was identified as antiarigenin 3-*O*-β-D-glucopyranoside, named toxicarioside F.

Compound **2** was obtained as a white amorphous powder. The ion peak $[M-H]^-$ at *m/z* 563.2851 in the high-resolution FAB-mass spectrum

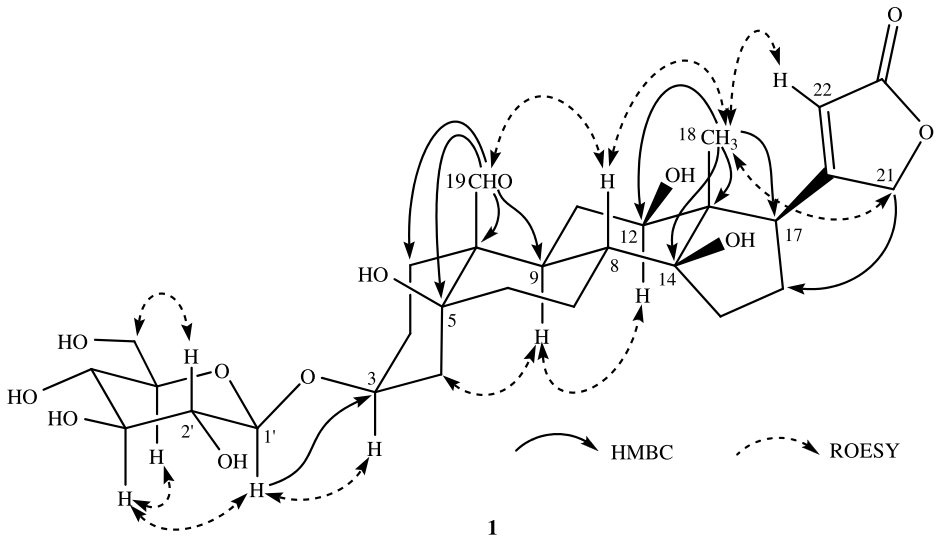


Figure 2. Key HMBC and ROESY interactions for compound **1**.

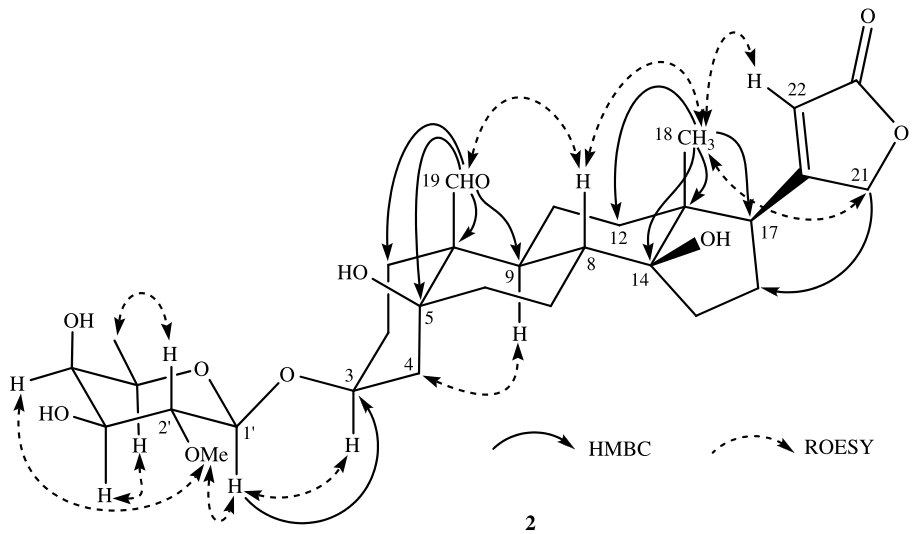
corresponded to the molecular formula $C_{30}H_{44}O_{10}$. This formula can also be validated through ^{1}H NMR and ^{13}C NMR (DEPT) spectra. The NMR spectral data of the aglycone of **2** were similar to those of **1** except for the appearance of a methylene group (δ_C 39.7), instead of a methine group in **1**. The aglycone of **2** was determined as strophanthidin by further comparison of the ^{13}C NMR spectral data of **2** with those reported in the literature [4]. In the ^{1}H NMR spectrum, an anomeric proton signal at δ 4.74 (d, 1H, $J = 7.8$ Hz, H-1′) indicated that **2** was a glycoside incorporating a sugar unit with β-linkage. According to the vicinal coupling constants for the sugar proton signals and the ^{13}C NMR spectral data, the sugar moiety was determined as 2-*O*-methyl-fucose, which agreed with the literature report [6]. The HMBC correlations between H-3 (δ 4.48) and C-1′ (δ 101.2) (Figure 1) suggested that the sugar moiety was linked to C-3. The relative stereochemistry of **2** was determined by ROESY correlations (Figure 3). Based on the above evidence, compound **2** was identified as strophanthidin 3-*O*-β-D-(2-*O*-methyl)-fucopyranoside, named toxicarioside G.

In addition to the effect of the cardenolides on the activity of the ubiquitous cell surface enzyme Na^+/K^+-ATPase, recent studies have demonstrated that this kind of compounds could inhibit the growth of cancer cells. We have, therefore, tested the isolated compounds for their cytotoxicity. The cytotoxicity of compounds **1** and **2** was evaluated *in vitro* using the MTT method [8]. Compounds **1** and **2** showed significant cytotoxicity against the K562, SGC-7901, SMMC-7721, and HeLa cell lines with the IC_{50} values of 0.001–0.044 μg/ml, and mitomycin C was used as a positive control.

3. Experimental

3.1 General experimental procedures

Melting points were obtained on a Beijing Taike X-5 stage apparatus and are uncorrected. Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rodolph Research Analytical, Hackettstown, NJ, USA). The UV spectra were measured on a Shimadzu UV-2550 spectrometer. The IR spectra were obtained on a Nicolet 380 FT-IR instrument, as KBr pellets. The NMR spectra were recorded



2

Figure 3. Key HMBC and ROESY interactions for compound **2**.

on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-FAB-MS spectra were measured with a VG Auto-Spec-3000 mass spectrometer. Column chromatography was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China). TLC was performed with silica gel GF254 (Marine Chemical Industry Factory).

3.2 Plant material

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

3.3 Extraction and isolation

With 95% EtOH, 4.0 liters of latex of *A. toxicaria* were extracted thrice 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

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of compounds **1** and **2** (δ, ppm; J, Hz).

	1 (CD_3OD)		**2** (C_5D_5N)	
Position	δ (H)	δ (C)	δ (H)	δ (C)
1	2.17, 1.67 (each 1H, m)	19.2	2.58, 1.93 (each 1H, m)	18.7
2	1.87, 1.71 (each 1H, m)	26.1	2.18, 1.74 (each 1H, m)	25.8
3	4.16 (br s)	74.8	4.48 (br s)	73.7
4	2.19, 1.65 (each 1H, m)	37.9	2.30, 1.81 (each 1H, m)	37.5
5		75.0		73.7
6	2.08, 1.61 (each 1H, m)	35.5	2.21, 1.89 (each 1H, m)	35.2
7	2.16, 1.31 (each 1H, m)	25.4	2.44, 1.45 (each 1H, m)	25.0
8	1.96 (m)	42.1	2.27 (m)	42.0
9	1.59 (m)	37.3	1.36 (m)	39.7
10		57.0		55.5
11	1.71, 1.26 (each 1H, m)	31.6	1.57, 1.33 (each 1H, m)	22.7
12	3.35 (dd, $J = 10.6, 4.1$)	75.2	1.77, 1.34 (each 1H, m)	39.7
13		55.9		50.0
14		86.2		84.5
15	1.98, 1.70 (each 1H, m)	32.7	2.03, 1.85 (each 1H, m)	32.2
16	2.08, 2.00 (each 1H, m)	28.3	2.04, 1.96 (each 1H, m)	27.3
17	3.29 (m)	46.8	2.78 (m)	51.2
18	0.77 (m)	9.8	0.87 (m)	16.1
19	10.03 (s)	209.8	10.40 (s)	208.6
20		178.3		175.8
21	4.97, 4.88 (d, $J = 18.1$)	75.5	5.29, 5.03 (d, $J = 18.1$)	73.8
22	5.89 (s)	117.9	6.13 (s)	117.8
23		177.3		174.5
1′	4.33 (d, $J = 7.6$)	103.0	4.74 (d, $J = 7.8$)	101.2
2′	3.19 (t, $J = 8.3$)	74.9	3.80 (t, $J = 8.0$)	82.5
3′	3.31 (t, $J = 9.4$)	78.1	4.01 (dd, $J = 9.4, 3.4$)	75.1
4′	3.25 (overlapped)	71.9	3.97 (dd, $J = <1, 3.4$)	73.0
5′	3.24 (m)	78.2	3.75 (m)	71.7
6′	3.85 (dd, $J = 11.2, 1.4$), 3.65 (overlapped)	63.0	1.51 (d, $J = 6.4$)	16.2
2-OCH_3			3.73 (s)	61.0

Table 2. *In vitro* cytotoxicities of compounds **1** and **2** (IC_{50} values, μg/ml).

Compounds	K562	SGC-7901	SMMC-7721	HeLa
1	0.020	0.006	0.001	0.002
2	0.044	0.010	0.009	0.013
Mitomycin C[a]	7.1	8.8	2.2	6.3

Note: [a]Positive control.

and EtOAc successively to afford petroleum ether extract and EtOAc extract. The H_2O layer was fractionated over a D-101 macroporous resin column eluting with H_2O, 50% MeOH and 100% MeOH to yield three fractions. The 100% MeOH (212.0 g) fraction was chromatographed over a silica gel column by gradient elution utilizing $CHCl_3$ and MeOH as the solvent system to give eight fractions. After repeated silica gel column chromatography ($CHCl_3$–MeOH 4:1), fraction 7 (4.76 g) afforded compound **1** (139.8 mg). The EtOAc extract was also chromatographed over a silica gel column by gradient elution utilizing $CHCl_3$ and MeOH as the solvent system to give 16 fractions, and fraction 12 (657.6 mg) was subjected to column chromatography on silica gel eluted with $CHCl_3$–MeOH (5:1) to obtain compound **2** (151.9 mg).

3.3.1 *Toxicarioside F (**1**)*

A white amorphous powder. Mp 245–247°C. $[\alpha]_D^{23}$ −15.8 (c = 0.57, MeOH). UV (MeOH) λ (log ε) nm: 218 (4.32). IR (KBr) ν_{max} (cm^{-1}): 3433, 2927, 1737, 1625, 1167, 1077, 1035. ^1H and ^{13}C NMR spectral data: see Table 1. HR-FAB-MS (neg.) m/z: 581.2597 $[M-H]^-$ (calcd for $C_{29}H_{41}O_{12}$, 581.2598).

3.3.2 *Toxicarioside G (**2**)*

A white amorphous powder. Mp 235–237°C. $[\alpha]_D^{20}$ −19.6 (c = 1.0, MeOH). UV (MeOH) λ (log ε) nm: 218 (4.30). IR (KBr) ν_{max} (cm^{-1}): 3448, 2942, 2867, 2353, 1735, 1619, 1065, 1012. ^1H and ^{13}C NMR spectral data: see Table 1. HR-FAB-MS (neg.) m/z: 563.2851 $[M-H]^-$ (calcd for $C_{30}H_{43}O_{10}$, 563.2856).

3.4 *Acid hydrolysis of 1*

Compound **1** (5 mg) was dissolved in MeOH (2.0 ml) and 5% H_2SO_4 solution (2.0 ml) and hydrolyzed under reflux for 3 h. The mixture was allowed to cool, diluted twofold with distilled water, and partitioned between EtOAc and H_2O. The aqueous layer was neutralized with aqueous $NaHCO_3$ solution (1.0 M) and evaporated to afford the residue. D-Glucose was identified by comparison on TLC ($CHCl_3$–MeOH–H_2O 7:3:0.5) with an authentic sample and optical rotation dispersion ($[\alpha]_D^{22}$ + 46.6 (c = 0.15, H_2O)).

3.5 *Cell cultures and* in vitro *cytotoxicity assay*

The human myeloid leukemia cell line (K562), human gastric cell line (SGC-7901), human hepatoma (SMMC-7721), and human cervical cancer (HeLa) cells were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology. Cells were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin sulfate at 37°C, 5% CO_2. The MTT assay was performed according to the method described in previous literature [8]. The IC_{50} values are listed in Table 2.

Acknowledgements

This research was financially supported by National Natural Science Foundation of China

(No. 30860345), National Nonprofit Institute Research Grant of CATAS-ITBB (No. ITBBZD0744), and National Programs for Science and Technology Development of China (2007B127B04).

References

[1] N.G. Bisset, *Lloydia* **29**, 1 (1966).
[2] T. Shrestha, B. Kopp, and N.G. Bisset, *J. Ethnopharmacol.* **37**, 129 (1992).
[3] W.L. Mei, Y.J. Gan, and H.F. Dai, *Chin. Tradit. Herb. Drugs* **39**, 151 (2007).
[4] M.M. Jiang, Y. Dai, H. Gao, X. Zhang, G.H. Yang, J.Y. He, Q.Y. Hu, J.Z. Zeng, X.K. Zhang, and X.S. Yao, *Chem. Pharm. Bull.* **56**, 1005 (2008).
[5] Y.J. Gan, W.L. Mei, Y.X. Zhao, and H.F. Dai, *Chin. Chem. Lett.* **20**, 450 (2009).
[6] C.A. Carter, R.W. Forney, E.A. Gray, A.M. Gehring, T.L. Schneider, D.B. Young, C.M. Lovett, J.L. Scott, A.C. Messer, and D.P. Richardson, *Tetrahedron* **53**, 13557 (1997).
[7] H.B. Chu and N.H. Tan, *J. Integr. Plant Biol.* **48**, 1250 (2006).
[8] T. Mosmann, *J. Immunol. Methods* **65**, 55 (1983).