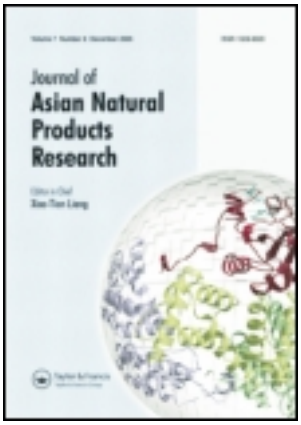

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A new drimane sesquiterpenoid glycoside from the seeds of *Antiaris toxicaria*

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A new drimane sesquiterpenoid glycoside, named 7-drimen-3β,11-diol 3-*O*-β-D-glucopyranoside, was isolated from the 95% EtOH extract of the seeds of *Antiaris toxicaria* (Pers.) Lesch. The chemical structure was completely elucidated using a combination of 1D and 2D NMR techniques (COSY, HMQC, HMBC, and ROESY) and HR-ESI-MS analysis. The compound showed inhibitory activities toward methicillin-resistant *Staphylococcus aureus* (MRSA), chronic myelogenous leukemia (K562), and human hepatoma (SMMC-7721) cell lines.

Keywords: *Antiaris toxicaria*; drimane sesquiterpenoid glycoside; 7-drimen-3β,11-diol 3-*O*-β-D-glucopyranoside; anti-MRSA; cytotoxicity

1. Introduction

Antiaris toxicaria (Pers.) Lesch. (Moraceae) is well known as 'upas tree' because it contains a complex mixture of cardenolide glycosides used as arrow and dart poisons prepared throughout southeastern Asia, from Burma to Indonesia [1–3]. The genus Antiaris (Moraceae) comprises four species, of which only *A. toxicaria* is distributed in China, mainly in Guangxi, Guangdong, Yunnan, and Hainan provinces [4]. Earlier studies of the toxicity of this plant in Indonesia, Malaysian, or China led to the isolation of cardenolides [5–11], flavonoids [12–14], and lignans [15]. Phytochemistry study of the seeds of *A. toxicaria* (Java, Indonesia) was only reported by Muhlradt *et al.* [9], and the chemical constituents isolated from the seeds are quite different from the latex of this plant. In our study of the seeds of *A. toxicaria* collected from Lingshui County of Hainan Province for the first time, a new drimane sesquiterpenoid glycoside (**1**) was isolated from the 95% EtOH extract of the seeds. Drimane sesquiterpenes showed attractive bioactive properties including antibacterial, antifungal, antifeedant, plant growth regulatory, cytotoxic, phytotoxic, piscicidal, and molluscicidal effects [16]. In this paper, we described the structural elucidation of compound **1** and its anti-methicillin-resistant *Staphylococcus aureus* (MRSA) and cytotoxic activities (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless oil and had a molecular formula $C_{21}H_{36}O_7$ based on its HR-ESI-MS at *m/z* 400.2459,

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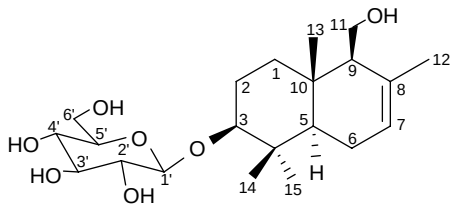

Figure 1. The structure of compound **1**.

which was supported by ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectral data (Table 1). The IR spectrum showed characteristic absorption for hydroxyl groups (3511 cm^{-1}) and for double bonds (1652 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 1) showed 21 carbon resonances, including four methyl, five methylene (two oxygenated), nine methine, and three quaternary carbons. The presence of a glucose moiety was determined by six characteristic carbon signals at δ_C 106.7, 78.3, 77.7, 75.7, 71.8, and 62.9 [17], which was further confirmed by its acid hydrolysis. The other 15 carbon signals at δ_C 135.0 (s), 123.8 (d), 90.8 (d), 61.3 (t), 58.3 (d), 51.4 (d), 40.1 (s), 38.9 (t), 36.6 (s), 28.6 (q), 27.3 (t), 24.3 (t), 22.2 (q), 16.7 (q), and 15.0 (q) in **1** indicated an aglycone of sesquiterpene, and these ^{13}C NMR spectral data were similar to a known drimane sesquiterpene, 3β-hydroxydrimenol [18,19]. The obvious differences were the significant down-fielded shift of C-3 from δ_C 79.6 to 90.8, as well as the presence of six sugar carbons in compound **1**. The key HMBC correlation from the anomeric proton signal at δ_H 4.30 (H-1′) to C-3 confirmed that the sugar moiety was linked to the drimane sesquiterpenoid aglycone at C-3 (Figure 2). The anomeric configuration of the glucose was determined as β on the basis of the coupling constant $J = 7.8$ Hz. The relative configuration of aglycone was same to the 3β-hydroxydrimenol and confirmed by the

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of compound **1** (in CD_3OD, δ).

No.	δ_H	δ_C	No.	δ_H	δ_C
1	2.02 (1H, m), 1.22 (1H, m)	38.9 (t)	12	1.72 (3H, s)	22.2 (q)
2	1.99 (1H, m), 1.98 (1H, m)	27.3 (t)	13	1.02 (3H, s)	28.6 (q)
3	3.18 (1H, dd, $J = 9.0$, 2.8 Hz)	90.8 (d)	14	0.90 (3H, s)	16.7 (q)
4		40.1 (s)	15	0.79 (3H, s)	15.0 (q)
5	1.19 (1H, m)	51.4 (d)	1′	4.30 (1H, d, $J = 7.8$ Hz)	106.7 (d)
6	1.95 (1H, m), 1.94 (1H, m)	24.3 (t)	2′	3.17 (1H, overlapped)	75.7 (d)
7	5.42 (1H, m)	123.8 (d)	3′	3.32 (1H, overlapped)	78.3 (d)
8		135.0 (s)	4′	3.27 (1H, overlapped)	71.8 (d)
9	1.78 (1H, m)	58.3 (d)	5′	3.25 (1H, m)	77.7 (d)
10		36.6 (s)	6′	3.69 (1H, dd, $J = 11.9$, 4.9 Hz) 3.82 (1H, d, $J = 11.9$ Hz)	62.9 (t)
11	3.77 (1H, dd, $J = 11.0$, 3.0 Hz) 3.53 (1H, dd, $J = 11.0$, 6.4 Hz)	61.3 (t)			

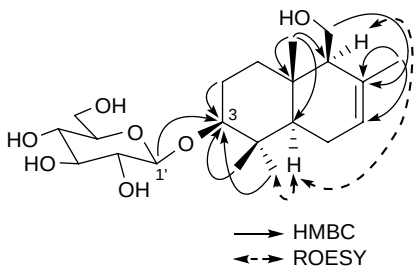

Figure 2. Key HMBC and ROESY correlations of compound **1**.

ROESY correlations (Figure 2). Based on the above evidence, compound **1** was determined as 7-drimen-3β,11-diol 3-*O*-β-D-glucopyranoside.

The antibacterial tests showed that compound **1** had inhibitory effects on MRSA, with a diameter of the inhibition zone of 10.0 mm. The diameter of the inhibition zone of the positive control, kanamycin sulfate, was 30.0 mm. The cytotoxicity of compound **1** was evaluated *in vitro* by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [20]. Compound **1** showed cytotoxicity against the K562 and SMMC-7721 cell lines with the IC_{50} values of 6.60 and 7.20 μg/ml, respectively, and mitomycin C was used as a positive control (Table 2).

3. Experimental

3.1 General experimental procedures

Optical rotation was recorded using a Rudolph Autopol III polarimeter (Rudolph Research Analytical, New Jersey, USA). The UV spectrum was measured with a Shimadzu UV-2550 spectrometer. The IR spectrum was obtained using a Nicolet 380 FT-IR instrument from KBr pellets. The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The HR-ESI-MS spectrum was measured with an API QSTAR Pulsar mass spectrometer. Column chromatography (CC) was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) was preformed with silica gel GF254 (Marine Chemical Industry Factory, Qingdao, China).

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

Compound	K562	SMMC-7721
1	6.60	7.20
Mitomycin C[a]	7.10	2.20

Note: [a]Positive control.

3.2 Plant material

Seeds of *A. toxicaria* (Pers.) Lesch were collected from Lingshui County of Hainan Province, China, in May 2009, and the plant was identified by Professor Zhu-Nian Wang. A voucher specimen (No. AN200905) has been deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

3.3 Extraction and isolation

The dried and crushed seeds (25.9 kg) of *A. toxicaria* were extracted three times with 95% of EtOH (50 L × 3) at room temperature. The combined extracts were concentrated under reduced pressure and suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc fraction (39.5 g), which showed inhibitory activity against MRSA and cytotoxic activities, was subjected to CC on silica gel with a gradient solvent system of $CHCl_3$–MeOH (1:0–0:1, v/v) to afford 10 fractions. Fraction 8 (4.42 g) was submitted to repeated CC on silica gel CC eluting with $CHCl_3$–MeOH (20:1 v/v) and Sephadex LH-20 ($CHCl_3$–MeOH 1:1 v/v) and finally yielded compound **1** (7.6 mg).

*3.3.1 7-Drimen-3β,11-diol 3-O-β-D-glucopyranoside (**1**)*

Colorless oil; $[\alpha]_D^{26}$ 22.2 ($c = 0.68$, MeOH); UV (MeOH): λ_{max} (log ε) 192 (5.00), 205 (2.82) nm; IR ν_{max}^{KBr} (cm^{-1}): 3512 (OH), 3044, 2978, 1652 (C=C), 1418, 1080, 728 cm^{-1}; for ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data, see Table 1. HR-ESI-MS: *m/z* 400.2459 $[M]^+$ (calcd for $C_{21}H_{36}O_7$, 400.2461).

3.4 Acid hydrolysis

Compound **1** (5 mg) was dissolved in MeOH (2.0 ml) and 5% of H_2SO_4 solution (2.0 ml) and hydrolyzed under reflux at 80°C for 3 h. The mixture was diluted twofold with distilled water and partitioned between $CHCl_3$ and H_2O. The aqueous layer was neutralized with aqueous $NaHCO_3$ solution (1.0 M) and evaporated to afford the residue. The sugar was determined as D-glucose by TLC ($CHCl_3$–MeOH–H_2O 7:3:0.5, $R_f = 0.45$) with an authentic sample and optical rotation dispersion ($[\alpha]_D^{22}$ +46.6 ($c = 0.2$, MeOH)).

3.5 Antimicrobial activity

The compound was tested for *in vitro* antimicrobial activity against MRSA (obtained from Hainan Institute of Drug Control, Haikou, China) by the filter-paper disk agar diffusion method [21]. The strains were cultured using nutrient agar (NA) in plates (90 mm in diameter). Fifty microliters of compound **1** (10 mg/ml) were impregnated on sterile filter paper disks (6 mm in diameter) and then applied aseptically to the surface of the agar plates. Ten microliters of kanamycin sulfate (0.08 mg/ml) were used as positive control. As an expression of the antimicrobial activities, the diameters of the inhibition zones including the 6 mm disk in diameter were measured after 24 h of incubation at room temperature. Experiments were carried out in triplicate and the results were presented as mean values.

3.6 Cell cultures and in vitro cytotoxicity assay

Compound **1** was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution with the concentration of 10 mM, which was then diluted to the required concentrations with RPMI 1640 medium (Beijing Xin Jing Ke Biotechnology Co. Ltd., Beijing, China). Cytotoxicity of compound **1** against two human tumor cell lines, K562 (human myeloid leukemia cell line) and SMMC-7721 (human hepatoma cell line), was measured. Cells were plated in 96-well plates for 24 h with initial density of 5000 cells/well and continuously exposed to different concentrations (0.1, 0.3, 0.9, 2.7, 8.1, and 24.3 μg/ml) of compound **1** for 72 h, with mitomycin C (Kyowa Hakko Kogyo Co. Ltd., Tokyo, Japan) as the positive control. Inhibition rates of cell proliferation after treatment were determined by the MTT method [20]. IC_{50} was calculated as the concentration (μg/ml) of samples causing 50% inhibition of cell viability. The IC_{50} values are listed in Table 2.

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