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Cytotoxic Sesquiterpene Lactones from Aerial Parts of *Xanthium sibiricum*

Authors Lun Wang[1,2], Jing Wang[1,2], Fu Li[1], Xin Liu[3], Bin Chen[1], Ya-Xiong Tang[1], Ming-Kui Wang[1]

Affiliations
[1] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China
[2] Graduate University of Chinese Academy of Sciences, Beijing, China
[3] Beijing Entry-Exit Inspection and Quarantine Bureau, Beijing, China

Key words
- *Xanthium sibiricum*
- Compositae
- sesquiterpene lactones
- xanthanolides
- cytotoxicity

Abstract

Chemical investigation of the aerial parts of *Xanthium sibiricum* led to the isolation of four new xanthanolide-type sesquiterpene lactones, including two xanthanolide dimers, pungiolide D (**1**) and pungiolide E (**2**), and two xanthanolide monomers, 8-epi-xanthatin-1*α*,5*α*-epoxide (**3**) and 1*β*-hydroxyl-5*α*-chloro-8-epi-xanthatin (**4**), together with four known compounds, pungiolide A (**5**), 8-epi-xanthatin-1*β*,5*β*-epoxide (**6**), xanthatin (**7**), and 11*α*,13-dihydro-8-epi-xanthatin (**8**). The structures of these compounds were elucidated on the basis of spectroscopic data analysis. Pungiolide D (**1**) displayed an unusual structure featuring a 5/5/6-fused tricyclic system in the unit B. Compound **4** was shown to be a rare sesquiterpene lactone containing halogen, and its absolute configuration was determined by X-ray crystallographic analysis. The evaluation of the cytotoxic activities of the isolated new compounds against the SNU387 liver and A-549 lung human cancer cell lines showed that compound **4** possessed significant *in vitro* cytotoxicity with an IC_{50} value of 5.1 μM against SNU387 liver cells.

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Xanthium is a genus of the plant family Compositae consisting of about 25 species and distributed in nearly all parts of the world. The fruits and aerial parts of certain species in this genus have been used as a traditional medicine for a long time [1]. *Xanthium sibiricum* Patr. is the principal species widely distributed throughout China. It has been utilized in traditional Chinese medicine for the treatment of sinusitis, headache, arthritis, skin pruritus, and cancer [2]. Previous phytochemical studies on different parts of *X. sibiricum* indicated the presence of sesquiterpene lactones [3–5], ent-kaurane diterpenoids [6], thiazinediones, and caffeoylquinic acids [7–11]. Sesquiterpene lactones from *X. sibiricum* are mainly xanthanolides, which seem to be characteristic for this genus. Xanthanolides are bicyclic sesquiterpene lactones, in which a five-membered γ-butyrolactone ring is fused to a seven-membered carbocycle. Some of these xanthanolide-type sesquiterpene lactones have shown cytotoxic, antiprotozoal, and antimicrobial activities [12–14]. As a part of our continuing studies on the biologically active metabolites from traditional Chinese medicines, a methanolic extract of the aerial parts of *X. sibiricum* was investigated, and four new xanthanolide-type sesquiterpene lactones (**1**–**4**) together with four known compounds (**5**–**8**) were isolated. Herein, we describe the isolation and structural elucidation of the four new compounds. All of the isolated new compounds were evaluated for cytotoxic activities against SNU387 and A-549 tumor cells.

Materials and Methods

General experimental procedures

The melting point was measured without correction on an X-6 precise melting point instrument. Optical rotations were determined by using a Perkin-Elmer 341 polarimeter at room temperature. IR spectra were measured on a Perkin-Elmer 1725X-FT spectrometer with KBr disks. NMR spectra were recorded on a Bruker Avance-600 spectrometer. The chemical shift (*δ*) values are given in ppm with TMS as the internal standard, and coupling constants (*J*) are in Hz. HRESIMS

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Bibliography
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Correspondence
Prof. Dr. Ming-Kui Wang
Chengdu Institute of Biology
Chinese Academy of Sciences,
No. 9, Section 4
Renmin South Road
Chengdu 610041
China
Phone: + 86 28 85 22 90 73
Fax: + 86 28 85 22 90 73
wangmk@cib.ac.cn

were measured on an LTQ Orbitrap XL mass spectrometer. Sephadex LH-20 (Amersham Pharmacia Biotech), silica gel (100–200 mesh and 200–300 mesh; Qingdao Marine Chemical Factory), and silica gel 60 (40–63 µm; Merck) were used for column chromatography (CC). Preparative HPLC was performed using Ultimate ® XB-C_{18} (250 mm × 21.2 mm, 10 µm) preparative column. The ratios of solvent are described as a mixture by v/v. TLC was carried out on precoated silica gel GF_{254} (10–40 µm; Qingdao Marine Chemical Factory) plates; spots were visualized under UV light and by spraying with 5% H_2SO_4 in C_2H_5OH (v/v) followed by heating.

Plant material

The aerial parts of *Xanthium sibiricum* were collected from Kaixian in Chongqing city, People's Republic of China, in September 2011, and authenticated by Prof. W.K. Bao of Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (A-388) was deposited at the Laboratory of Phytochemistry, Chengdu Institute of Biology, Chinese Academy of Sciences. Paclitaxel (≥ 98%) was purchased from Sigma-Aldrich. The purity (> 96%) of compounds **1**–**4**, used for the biological assay, was determined by HPLC.

Extraction and isolation

The air-dried and powdered aerial parts of *X. sibiricum* (9.8 kg) were extracted with MeOH (3 × 15 L, 1 d, each) at room temperature and concentrated *in vacuo* to give a crude extract (712 g). The crude extract was suspended in H_2O and then extracted with petroleum ether (3 × 4 L), ethyl acetate (3 × 4 L), and *n*-butanol (3 × 4 L), successively. The EtOAc extract (152 g) was subjected to silica gel (10 × 120 cm, 100–200 mesh, 3.5 kg) column eluting with a petroleum ether/EtOAc (10 : 1, 5 : 1, 5 : 2, 2 : 1, 5 : 3, 5 : 4, 1 : 1, 0 : 1, each 18 L) gradient system to give fractions 1–9. Fraction 7 (10.4 g) was chromatographed on a silica gel column (5 × 55 cm, 200–300 mesh, 450 g) eluted with CH_2Cl_2/Me_2CO (25 : 1, 20 : 1, 25 : 2, 10 : 1, each 2 L) to afford six subfractions, 7a-7 f. Subfraction 7b (4.0 g) was divided into five major fractions (7b1–7b5) by silica gel CC (4 × 45 cm, 40–63 µm, 215 g), eluted with CH_2Cl_2/Me_2CO (25 : 1, 20 : 1, 25 : 2, 10 : 1, each 1 L), then fraction 7b3 was purified by reversed-phase preparative HPLC using a gradient of increasing MeOH (50–70%) in water at 18 mL/min for 40 min to yield **1** (t_R = 21.2 min, 70 mg) and **2** (t_R = 23.5 min, 90 mg). Subfraction 7c was purified by reversed-phase preparative HPLC with a gradient solvent system of MeOH/H_2O (from 50% to 80% MeOH) at 18 mL/min for 40 min to obtain **5** (t_R = 17.1 min, 420 mg). Fraction 5 (7.2 g) was applied to silica gel (5 × 55 cm, 200–300 mesh, 420 g) eluting with CH_2Cl_2/Me_2CO (25 : 1, 20 : 1, 25 : 2, 10 : 1, each 1.5 L) to provide four subfractions (5a-5 d). Fraction 5a (1.1 g) was fractionated using silica gel CC (4 × 45 cm, 40–63 µm, 210 g) eluted with CH_2Cl_2/Me_2CO (25 : 1, 20 : 1 each 800 mL) to give fractions 5a1–5a4. Fraction 5a2 was purified by reversed phase preparative HPLC using a gradient of increasing MeOH (40–65%) in water at 18 mL/min for 60 min to produce **3** (t_R = 21.4 min, 20 mg), **6** (t_R = 25.2 min, 26 mg), and **8** (t_R = 36.1 min, 30 mg). Fraction 5b (1.2 g) was subjected to Sephadex LH-20 CC (3 × 45 cm, 70 g) eluted with MeOH/H_2O (40%, 50%, 60%, 70%, 80% MeOH, each 500 mL) to give four subfractions (5b1–5b4). Subfraction 5b1 (0.7 g) was chromatographed over silica gel column (4 × 45 cm, 40–63 µm, 203 g) eluted with CH_2Cl_2/Me_2CO (30 : 1, 25 : 1, 20 : 1 each 800 mL) to afford four subfractions, 5b1a-5b1 d. Subfraction 5b1b was further purified by reversed-phase preparative HPLC using a gradient of increasing MeOH (40–70%) in water at 18 mL/min for 50 min to yield **4** (t_R = 30.6 min, 33 mg). Fraction 4 (22 g) was chromatographed on silica gel (5 × 55 cm, 200–300 mesh, 430 g) eluting with a CH_2Cl_2/Me_2CO (20 : 1, 25 : 2, 10 : 1, each 2 L) gradient to four fractions 4a-4 d. Fraction 4a was recrystallized from petroleum ether/EtOAc to give pure **7** (16 g).

Pungiolide D (**1**): white, amorphous powder, $[\alpha]_D^{20}$ − 91.0 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 277 (4.29), 232 (4.39) nm; IR (KBr) ν_{max} 2932, 1766, 1668, 1360, 1263, 1067 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; HRESIMS *m/z* 493.2574 $[M + H]^+$ (calcd. for $C_{30}H_{37}O_6$, 493.2585).

Pungiolide E (**2**): white, amorphous powder, $[\alpha]_D^{20}$ − 71.4 (c 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 277 (4.13), 219 (4.37) nm; IR (KBr) ν_{max} 2929, 1760, 1667, 1360, 1261, 1202 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 1**; HRESIMS *m/z* 493.2576 $[M + H]^+$ (calcd. for $C_{30}H_{37}O_6$ 493.2585).

8-epi-Xanthatin-1α,5α-epoxide (**3**): colorless gum, $[\alpha]_D^{20}$ + 57.4 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 221 (4.17) nm; IR (KBr) ν_{max} 2964, 1766, 1673, 1362, 1262, 1099, 803 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 2**; HRESIMS *m/z* 263.1279 $[M + H]^+$ (calcd. for $C_{15}H_{19}O_4$, 263.1278).

1β-Hydroxyl-5α-chloro-8-epi-xanthatin (**4**): colorless crystal, mp 112.5–113.5 °C; $[\alpha]_D^{20}$ + 95.5 (c 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.24) nm; IR (KBr) ν_{max} 3401, 2937, 1731, 1682, 1284, 1264, 1134, 985 cm^{-1}; ^1H and ^{13}C NMR data, see **Table 2**; HRESIMS *m/z* 299.1042 $[M + H]^+$ (calcd. for $C_{15}H_{20}ClO_4$, 299.1045).

X-ray crystallographic analysis of 1β-hydroxyl-5α-chloro-8-epi-xanthatin (**4**): Crystal data were collected from a colorless prism (0.40 × 0.18 × 0.18 mm^3) at 293(2) K: $C_{15}H_{19}ClO_4$, MW = 298.75, orthorhombic, space group $P2_12_12_1$, unit cell dimensions a = 7.2183(2) Å, b = 13.1818(6) Å, c = 15.7159(6) Å, V = 1495.38 (10) $Å^3$; α = 90.00°, β = 90.00°, γ = 90.00°, Z = 4, ρ_{calc} = 1.327 mg/mm^3, *F*(000) = 632. Data collection was performed on an Xcalibur Eos diffractometer with graphite monochromator, Mo Kα radiation. A total of 6488 reflections were measured, 2961 were unique (R_{int} = 0.0267), which were used in all calculations. The structure was solved by direct method using SHELXS (G.M. Sheldrick) and refined by a full-matrix least-squares method on F^2 by means of SHELXL (G.M. Sheldrick). The final R indices [$I > 2\sigma(I)$] were R_1 = 0.0422, wR_2 = 0.0820; R indices [all data], R_1 = 0.0573, wR_2 = 0.0904. Crystallographic data for the structure of **4** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 905733). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: + 44-(0)1223–336033 or e-mail: deposit@ccdc.cam.ac.uk].

Cytotoxicity assay

Compounds **1**–**4** were tested for cytotoxicity against SNU387 and A-549 cell lines by means of the MTT assay [15]. Briefly, cells were placed into 96-well plates at a density of 5000 cells per well and incubated at 37 °C for 24 h in 5% CO_2. The test compounds were added into triplicate wells at different concentrations and incubated for 48 h at 37 °C. After the incubation period, MTT solution (100 µL, 1 mg/mL) was added into each well, and the plate was incubated for another 4 h. The resulting formazan crystals were dissolved in DMSO (100 µL). The absorbance was determined at 570 nm using a plate reader. Cell viability (%) was measured, and a cell growth curve was plotted. IC_{50} values were calculated by the Reed and Muench method [16]. Paclitaxel (Sigma-

Table 1 ^{1}H and ^{13}C NMR data for compounds **1** and **2**.[a]

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
Unit B				
1		52.6 s	1.77, m[b]	50.8 d
2	7.16, s	145.1 d	7.20, d (1.2)	147.9 d
3		141.2 s		139.7 s
4		197.2 s		197.5 s
5	2.20, m[b]	42.5 d	1.39, m	35.0 d
6a	2.22, m[b]	31.9 t	1.93, dd (11.5, 5.3)	36.7 t
6b	1.56, m		1.66, m[b]	
7	2.83,t (6.5)	53.3 d	3.36, m	41.8 d
8	4.74, m	80.0 d	4.71, ddd (14.4, 9.0, 2.4)	80.3 d
9a	2.08, m[b]	30.5 t	2.00, br d (12.4)	40.1 t
9b	1.88, dd (15.0, 2.4)		1.79, m[b]	
10	1.78, t (7.8)	43.6 d	1.51, m	35.8 d
11		51.4 s		138.7 s
12		179.4 s		169.5 s
13a	1.16, s	18.5 q	6.30, d (2.9)	122.5 t
13b			5.58, d (2.9)	
14	1.30, d (7.7)	18.6 q	1.27, d (6.5)	21.7 q
15	2.37, s	25.9 q	2.33, s	25.8 q
Unit A				
1′		142.7 s		142.7 s
2′	6.91, d (16.1)	146.1 d	6.92, d (16.2)	146.1 d
3′	6.12, d (16.2)	125.7 d	6.12, d (16.2)	125.7 d
4′		198.4 s		198.4 s
5′	6.00, dd (9.0, 6.0)	135.6 d	6.03,dd (9.1, 5.8)	135.8 d
6′a	2.69, ddd (13.1, 13.1, 5.8)	24.1 t	2.68, ddd (13.6, 13.6, 6.1)	23.4 t
6′b	2.06, m[b]		2.20, ddd (13.2, 9.0, 3.6)	
7′	2.93, ddd (13.2, 9.6, 3.6)	44.3 d	2.96, ddd (13.2, 9.6, 3.6)	44.1 d
8′	4.67, t (11.0)	78.2 d	4.71, ddd (14.4, 9.0, 2.4)	78.4 d
9′a	2.29, m[b]	36.6 t	2.29, m[b]	36.5 t
9′b	2.04, m[b]		2.08, m	
10′	2.81, m[b]	32.2 d	2.81, m	32.3 d
11′		47.3 s		47.6 s
12′		180.3 s		180.2 s
13′a	2.18, dd (13.8, 5.4)	38.7 t	2.00, br d (12.4)	40.0 t
13′b	1.83, dd (13.8, 13.8)		1.89, dd (13.8, 13.8)	
14′	1.20, d (6.8)	21.4 q	1.21, d (6.9)	21.4 q
15′	2.26, s	28.1 q	2.27, s	28.0 q

[a] Data were measured in $CDCl_3$ at 600 MHz (^{1}H) and 150 MHz (^{13}C). Assignments were based on DEPT, HSQC, ^{1}H-^{1}H COSY, and HMBC experiments. [b] Overlapped signal

Aldrich) was used as a positive control. IC_{50} values against SNU387 and A-549 cells were 0.21 and 2.9 µM, respectively.

Supporting information

The 1D and 2D NMR spectra for compounds **1**–**4** are available as Supporting Information.

Results and Discussion

Pungiolide D (**1**) was isolated as an amorphous powder. The molecular formula was established as $C_{30}H_{36}O_6$ by HRESIMS (*m/z* 493.2574 $[M + H]^+$, calcd. for $C_{30}H_{37}O_6$, 493.2585), requiring thirteen degrees of unsaturation. The IR spectrum indicated the presence of a γ-lactone moiety (1766 cm^{-1}) and an *α,β,γ,δ*-unsaturated dienone system (1668 cm^{-1}) [17,18]. In the ^{1}H-NMR spectrum (**Table 1**), five methyl group signals at δ_H 1.16 (3H, s, H-13), δ_H 1.20 (3H, d, J = 6.8 Hz, H-14′), δ_H 1.30 (3H, d, J = 7.7 Hz, H-14), δ_H 2.26 (3H, s, H-15′), and δ_H 2.37 (3H, s, H-15) were observed, together with *trans*-double bond signals at δ_H 6.12 (1H, d, J = 16.2 Hz, H-3′) and δ_H 6.91 (1H, d, J = 16.1 Hz, H-2′) and two olefinic proton signals at δ_H 6.00 (1H, dd, J = 9.0, 6.0 Hz, H-5′) and δ_H 7.16 (1H, s, H-2). The ^{13}C-NMR (**Table 1**), DEPT, and HSQC spectra revealed the presence of 30 carbon resonances, comprising five methyls, five methylenes, seven methines, three quaternary carbons, six olefinic, and four carbonyl carbons. The above NMR data clearly indicated that a dimeric sesquiterpene lactone was present. Further comparison of the ^{1}H and ^{13}C-NMR spectral data with those of dimeric xanthanolides isolated from *Xanthium* species indicated that **1** was similar to the known compound pungiolide A (**5**), previously isolated from *X. pungens* [19], and clearly showed the structure of **1** sharing the same unit A with **5**, but differing in the positions of C-1, C-11, and C-13 of unit B. In the ^{13}C-NMR spectrum of **1**, the signal of C-1 appeared upfield at δ 52.6 compared with that exhibited by **5** at δ 68.7, which indicated the absence of the hydroxyl group at C-1 in **1**. It was also supported by the molecular formula ($C_{30}H_{36}O_6$), showing 16 mass units less than that of **5**. Moreover, the proton signal of H-2 (δ 7.16 s) and the DEPT spectrum showed that C-1 was a quaternary carbon, which was established by the HMBC correlations from H-14, H-9, and H-6 to C-1. The exomethylene in **5** was replaced by a methyl group (δ 1.16 s) in **1**, and it was determined by the HMBC correlations between H-13 and C-12. Certainly, the methyl singlet nature of this signal required a quaternary carbon C-11 (δ 51.4) which was supported by DEPT. In view of the thirteen degrees of unsaturation, there had to be an additional ring in the structure of **1**. In the HMBC spectrum (**Fig. 2**) of **1**, the HMBC correlations between H-2/C-11 and H-7/C-1 suggested that the

Table 2 ^{1}H and ^{13}C NMR data for compounds **3** and **4**.[a]

No.	3		4	
	δ_H	δ_C	δ_H	δ_C
1		63.5 s		77.6 s
2	6.99, d (15.5)	143.3 d	7.08, d (16.1)	151.7 d
3	6.25, d (15.5)	129.4 d	6.33, d (16.1)	129.0 d
4		197.8 s		198.3 s
5	2.96, dd (7.0, 2.2)	63.1 d	4.05, d (7.4)	65.4 d
6a	2.41, ddd (15.6, 10.2, 2.4)	28.4 t	2.59, dd (14.9, 11.4)	30.5 t
6b	2.30, ddd (15.6, 7.2, 4.8)		2.27, m[b]	
7	3.40, m	38.4 d	3.65, m	37.5 d
8	4.78, ddd (11.3, 7.9, 3.6)	78.7 d	4.82, ddd (12.0, 8.8, 3.1)	80.3 d
9a	1.98, ddd (14.4, 3.7, 3.7)	36.6 t	2.01, ddd (14.2, 11.9, 11.9)	34.2 t
9b	1.75, ddd (14.2, 11.0,11.0)		1.69, dd (14.3, 2.0)	
10	2.20, m	32.5 d	2.22, m[b]	33.2 d
11		138.4 s		138.9 s
12		169.5 s		169.7 s
13a	6.29, d (2.7)	122.2 t	6.30, d (3.4)	122.4 t
13b	5.58, d (2.7)		5.57, d (2.9)	
14	1.19, d (7.3)	19.5 q	0.93, d (7.1)	17.6 q
15	2.25, s	28.2 q	2.31, s	27.6 q

[a] Data were measured in $CDCl_3$ at 600 MHz (^{1}H) and 150 MHz (^{13}C). Assignments were based on HSQC, HMBC, and NOESY experiments. [b] Overlapped signal

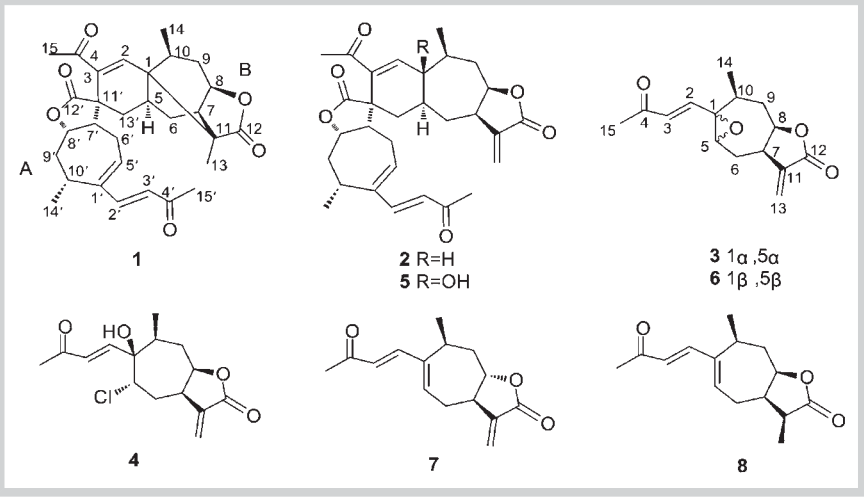

Fig. 1 Structures of compounds **1–8**.

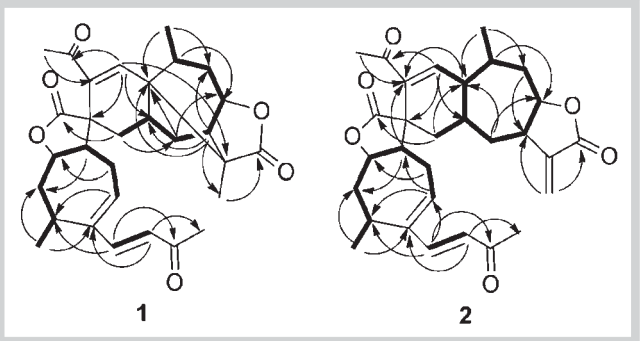

Fig. 2 Key ^{1}H-^{1}H COSY (bold) and HMBC (arrow) correlation of **1** and **2**.

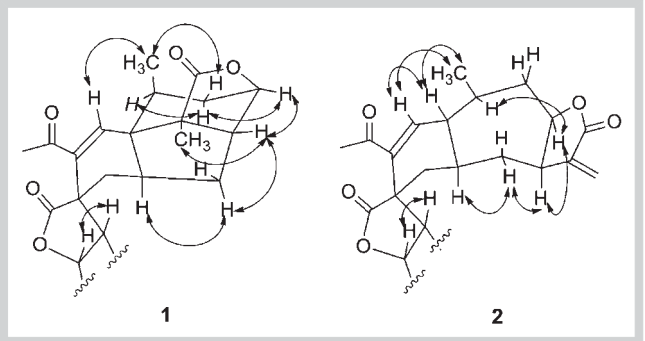

Fig. 3 Selected NOESY correlations of compounds **1** and **2**.

two aforementioned quaternary carbons (C-1 and C-11) were connected directly, and the additional ring was present. Accordingly, the planar structure of **1** was elucidated. The assignments of all proton and carbon signals could be established by 2D ^{1}H-^{1}H COSY, HSQC, and HMBC experiments. The stereochemistry of **1** was determined by a NOESY experiment (**Fig. 3**). The NOESY correlations H-7*α*/H-8*α*, H-7*α*/H-6*α*, H-6*α*/H-5, H-8*α*/H-9*α*, H-9*α*/H-10, H-14/H-9*β*, H-2/H-14, H-7′*β*/H-8′*β*, and H-14′/H-9′*β* indicated the same relative configurations at C-5, C-7, C-8, C-10, C-7′, C-8′, and C-10′ as those of **5**. Therefore, the structure of **1** was determined as shown (**Fig. 1**).

Pungiolide E (**2**) showed a pesudomolecular ion peak $[M + H]^+$ at *m/z* 493.2576 corresponding to the molecular formula $C_{30}H_{36}O_6$, implying 13 degrees of unsaturation. The IR bands at 1760 cm^{-1} and 1667 cm^{-1} revealed the presence of a *γ*-lactone moiety and an *α,β,γ,δ* doubly unsaturated carbonyl. The ^{1}H-NMR (**Table 1**) spectra showed four methyl groups (two singlets and two doublets). Two olefinic protons at δ_H 6.12 (1H, d, J = 16.2 Hz, H-3′) and δ_H 6.92 (1H, d, J = 16.2 Hz, H-2′) displayed the presence of a *trans* double bond, and a pair of olefinic protons at δ_H 5.58 (1H, d, J = 2.9 Hz, H-13a) and δ_H 6.30 (1H, d, J = 2.9 Hz, H-13b) at C-13 (δ 122.5) indicated the presence of one terminal double bond. All 30 carbon resonances were well-resolved in the ^{13}C-NMR (**Table 1**) spectrum. These data suggested that **2** was a dimeric xanthanolide-type sesquiterpene lactone. The NMR data of **2** were closely similar to those of **5** except for the absence of signal for 1-OH. In the ^{13}C-NMR and DEPT spectrum of **2**, the C-1 signal at δ_C 50.8 was observed as a methine resonance. At the same time, the olefinic proton of H-2 was present in doublet at δ_H 7.20 (d, J = 1.2 Hz) in the ^{1}H NMR spectrum. The above evidence indicated that **2** is the 1-dehydroxy derivative of **5**. This assumption was supported by its molecular weight and confirmed by the HMBC experiment (**Fig. 2**). The NOESY correlations H-7*α*/H-6*α*, H-5/H-6*α*, and H-1/H-14*β* displayed that H-1 and H-5 were *β* and *α*-oriented, respectively. The other stereocenters in **2** were determined as those in **1** by the NOESY experiment (**Fig. 3**). Thus, the structure of **2** was established as shown (**Fig. 1**).

Compound **3**, a colorless gum, was identified as an stereoisomer of the known compound 8-epi-xanthatin-1*β*,5*β*-epoxide (**6**) through its spectral data. It had the identical molecular formula of $C_{15}H_{18}O_4$ as determined by HRESIMS at *m/z* 263.1279 $[M + H]^+$ (calcd. for $C_{15}H_{19}O_4$, 263.1278). The ^{1}H-NMR data (**Table 2**) showed that **3** had features similar to those of **6** except for the marked difference in the couplings of H-5 and the chemical shift of H_2-6, indicating that they only differed in the configuration of the 1,5-epoxide group. The relative configurations at C-7, C-8, and C-10 were determined by a NOESY experiment. Though the stereochemistry at C-5 could not be established by the NOESY experiment, inspection of models favored an *α*-epoxide if the couplings $J_{5,6}$ were considered [17,20,21]. Thus, compound **3** was characterized as 8-epi-xanthatin-1*α*,5*α*-epoxide.

Compound **4**, a colorless crystal, gave a molecular formula of $C_{15}H_{19}ClO_4$ determined by HRESIMS at *m/z* 299.1042 $[M + H]^+$ (calcd. for $C_{15}H_{20}ClO_4$, 299.1045). Comparison of its ^{1}H and ^{13}C

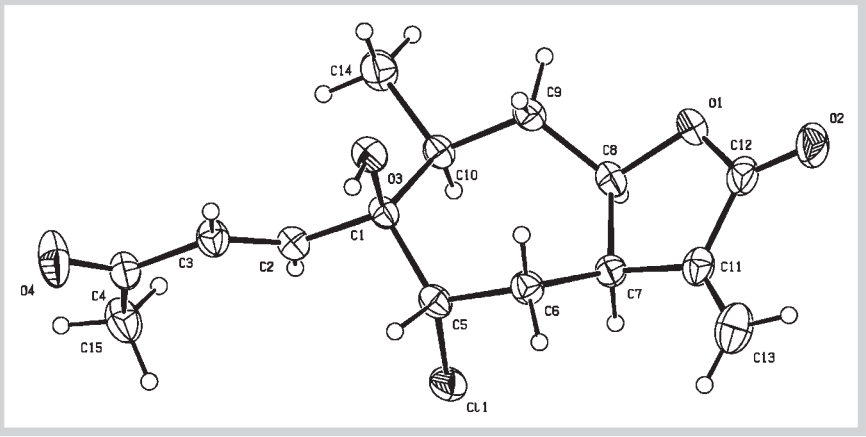

Fig. 4 X-ray structure of compound **4**.

NMR data (**Table 2**) with those of **3** suggested that they shared similar structures. The major differences were the carbon chemical shifts of C-1 and C-5 downfield to δ_C 77.6 and δ_C 65.4, respectively, and the significant downfield shift for H-5 (δ_H 4.05) in **4**. All these differences together with the molecular weight suggested that the functional group at C-1 was a hydroxyl group and a Cl atom was located at C-5. All signals of **4** were assigned from the 2D NMR experiments. The relative configurations at C-7, C-8, and C-10 were deduced from the NOESY experiments. However, the relative configurations at C-1 and C-5 could not be established by the NOESY spectrum. Fortunately, a single crystal of **4** was obtained after repeated recrystallization, and an X-ray diffraction analysis was realized, which clarified not only the planar structure but also the absolute configuration of **4** to be 1R, 5S, 7R, 8R, 10S (**Fig. 4**). Therefore, the structure was assigned as 1*β*-hydroxyl-5*α*-chloro-8-epi-xanthatin.

The known compounds were identified as pungiolide A (**5**) [19], 8-epi-xanthatin-1*β*,5*β*-epoxide (**6**) [21], xanthatin (**7**) [22], and 11*α*,13-dihydro-8-epi-xanthatin (**8**) [17], based on NMR data and mass spectrometric analysis, as well as by comparison of the spectral data with those reported.

The isolated four new compounds (**1**–**4**) were assayed for their *in vitro* cytotoxicity against SNU387 and A-549 human cancer cell lines using the MTT method. Compound **4** exhibited the most potent activity with an IC_{50} value of 5.1 µM against SNU387 cells. Compounds **1**–**3** showed moderate cell growth inhibitory activity against SNU387 cells, with IC_{50} values of 14.6, 11.7, and 9.6 µM, respectively. Compound **3** displayed moderate cytotoxic activity, and compound **4** weak cytotoxic activity against A-549 cells, with IC_{50} values of 9.5 and 20.7 µM, respectively. Compounds **1** and **2** showed no cytotoxicity against A-549 cells (IC_{50} > 40 µM).

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Conflict of Interest

The authors declare no competing financial interest.

References

1 *Chen YL. Xanthium L.* Flora Reipublicae Popularis Sinicae, Vol. 75. Beijing: Science Press; 1979
2 *Chinese Pharmacopoeia Committee.* Pharmacopoeia of China, Part 1. Beijing: Chemical Industry Press; 2005: 111–112
3 *Winters TE, Geissmen TA, Safir D.* Sesquiterpene lactones of *Xanthium* species. Xanthanol and isoxanthanol, and correlation of xanthinin with ivalbin. J Org Chem 1969; 34: 153–155
4 *Mahmoud AA.* Xanthanolides and xanthane epoxide derivatives from *Xanthium strumarium*. Planta Med 1998; 64: 724–727
5 *Bui VB, Liu ST, Zhu JJ, Xiong J, Zhao Y, Yang GX, Xia G.* Sesquiterpene lactones from the aerial parts of *Xanthium sibiricum* and their cytotoxic effects on human cancer cell lines. Phytochem Lett 2012; 5: 685–689
6 *Macleod JK, Moeller PD, Franke FP.* Two toxic kaurene glycosides from the burrs of *Xanthium pungens*. J Nat Prod 1990; 53: 451–455
7 *Ma YT, Huang MC, Hsu FL, Chang HF.* Thiazinedione from *Xanthium strumarium*. Phytochemistry 1998; 48: 1083–1085
8 *Qin LP, Han T, Li HL, Zhang QY, Zheng HC.* A new thiazinedione from *Xanthium strumarium*. Fitoterapia 2006; 77: 245–246
9 *Han T, Li HL, Zhang QY, Zheng HC, Qin LP.* New thiazinediones and other components from *Xanthium strumarium*. Chem Nat Compd 2006; 42: 567–570
10 *Dai YH, Cui Z, Li JL, Wang D.* A new thiaziedione from the fruits of *Xanthium sibiricum*. J Asian Nat Prod Res 2008; 10: 303–305
11 *Agata I, Goto S, Hatano T, Nishibe S, Okuda T.* 1,3,5-Tri-O-caffeoylquinic acid from *Xanthium strumarium*. Phytochemisty 1993; 33: 508–509
12 *Roussakis C, Chinou I, Vayas C, Harvala C, Verbist JF.* Cytotoxic activity of xanthatin and the crude extracts of *Xanthium strumarium*. Planta Med 1994; 60: 473–474
13 *Nour AMM, Khalid SA, Kaiser M, Brun R, Abdallah WE, Schmidt TJ.* The antiprotozoal activity of sixteen Asteraceae species native to Sudan and bioactivity guided isolation of xanthanolides from *Xanthium brasilicum*. Planta Med 2009; 75: 1363–1368
14 *Vasas A, Hohmann J.* Xanthane sesquiterpenoids: structure, synthesis and biological activity. Nat Prod Rep 2011; 28: 824–842
15 *Mosmann T.* Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63
16 *Reed LJ, Muench H.* A simple method of estimating fifty percent endpoints. Am J Hyg 1938; 27: 493–497
17 *Cumanda J, Marinoni G, Debernardi M, Vidari G, Finzi PV.* New Sesquiterpenes from *Xanthium catharticum*. J Nat Prod 1991; 54: 460–465
18 *Ahmed AA, Mahmoud AA, El-Gamal AA.* A xanthanolide diol and a dimeric xanthanolide from *Xanthium* species. Planta Med 1999; 65: 470–472
19 *Ahmed AA, Jakupovic J, Bohlmann F, Regaila HA, Ahmed AM.* Sesquiterpene lactones from *Xanthium pungens*. Phytochemistry 1990; 29: 2211–2215
20 *Omar AA, Elrashidy EM, Ghazy NA, Metwally AM, Ziesche J, Bohlmann F.* Xanthanolides from *Xanthium spinosum*. Phytochemistry 1984; 23: 915–916
21 *Bohlmann F, Singh P, Joshi KC, Singh CL.* Xanthanolides from *Xanthium indicum*. Phytochemistry 1982; 21: 1441–1443
22 *Deuel PG, Geissman TA.* Xanthinin. II. The structures of xanthinin and xanthatin. J Am Chem Soc 1957; 79: 3778–3783