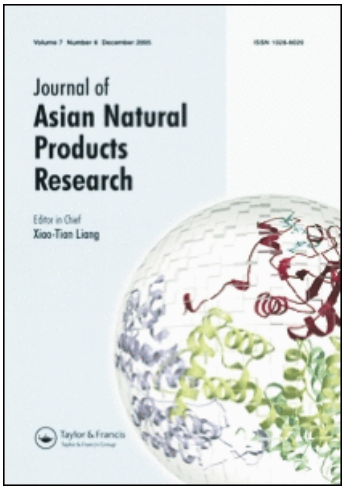

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ORIGINAL ARTICLE

Two new cytotoxic *ent*-clerodane diterpenoids from *Scutellaria barbata*

Gui-Wu Qu[a], Xi-Dian Yue[a], Gui-Sheng Li[b], Qun-Ying Yu[b] and Sheng-Jun Dai[b]*

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Two new *ent*-clerodane diterpenoids have been isolated from *Scutellaria barbata*, and their structures were established by detailed spectroscopic analyses as (13*R*)-6α,7β-dihydroxy-8β,13-epoxy-11β-nicotinyloxy-*ent*-clerodan-3-en-15,16-olide (scutelinquanine D, **1**) and (11*E*)-6α-acetoxy-7β,8β-dihydroxy-*ent*-clerodan-3,11,13-trien-15,16-olide (6-acetoxybarbatin C, **2**). *In vitro*, the isolated two new compounds showed significant cytotoxic activities against three human cancer cell lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells), and gave IC_{50} values in the range of 2.5–6.6 μM.

Keywords: *Scutellaria barbata*; *ent*-clerodane diterpenoid; scutelinquanine D; 6-acetoxybarbatin C; cytotoxic activity

1. Introduction

Scutellaria L. (Labiatae) is a large subcosmopolitan genus with 360 currently recognized species [1]. Recently, plants belonging to this genus have attracted attention owing to interesting biological activities observed for some of their *ent*-clerodane diterpenoids. *Scutellaria barbata* D. Don is a perennial herb which is natively distributed throughout Korea and southern China. This herb is known in traditional Chinese medicine as Ban-Zhi-Lian and traditional Korean medicine as Banjiryun, respectively, and has been used as both an anti-inflammatory and an anti-tumor agent [2–4]. In previous phytochemical studies on *S. barbata*, we reported the isolation of a series of *ent*-clerodane diterpenoids [5–8]. From a chemotaxonomic point of view, it is of interest to note that these *ent*-clerodane diterpenoids lack an oxygenated substituent at C-19 that is found in almost all of the *ent*-clerodane diterpenoids from European *Scutellaria* species [9].

As part of our ongoing search for new *ent*-clerodane diterpenoids, we investigated the aerial parts of *S. barbata* collected from the Linquan district, Anhui Province, China. This investigation led to the isolation of two new *ent*-clerodane diterpenoids, named scutelinquanine D (**1**) and 6-acetoxybarbatin C (**2**), the structures of which were elucidated by means of extensive spectroscopic analyses. In addition, compounds **1** and **2** were screened for cytotoxicity against three tumor cell lines (HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells), with IC_{50} values in the range of 2.5–6.6 μM. Herein, we report the isolation, structure elucidation, as well

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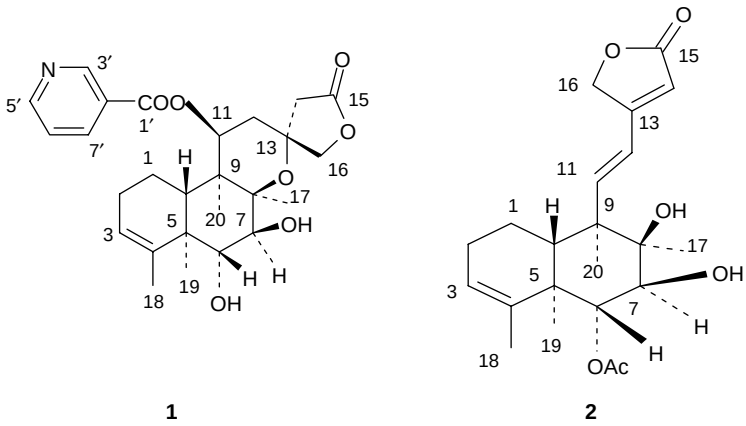

1 **2**

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

as the cytotoxic effects of the two new *ent*-clerodane diterpenoids (Figure 1).

2. Results and discussion

Compound **1** was obtained as white needles, and showed a positive response to Dragendorff's reagent. It had a molecular formula of $C_{26}H_{33}NO_7$ determined by HR-FAB mass spectrum at *m/z* 472.2341 $[M + H]^+$, which was the same as that of scutebarbatine G [8]. The IR, UV, ^1H and ^{13}C NMR spectra (Table 1) were closely related to those of scutebarbatine G, implying that they likely shared the same overall structure and were stereoisomers. This was confirmed by the analyses of the 2D NMR spectra. The key NOE signals (Figure 2) of **1** showed that its relative configuration except for C-13 was identical to those of scutebarbatine G. Irradiation of the proton at δ 1.32 (H_3-17) caused NOE enhancements of the H-7 (δ 3.64), H-11 (δ 5.80), H_a-14 (δ 2.54), H_b-14 (δ 2.91), and H_3-20 (δ 1.01) protons, whereas on irradiating the proton at δ 1.01 (H_3-20), NOE enhancements were observed for the H-7 (δ 3.64), H-11 (δ 5.80), H_b-14 (δ 2.91), H_3-17 (δ 1.32), and H_3-19 (δ 1.26) proton resonances. These facts, in addition to the NOESY cross-peaks from H-6 (δ 3.88) to H-10 (δ 2.55) and from H-11 (δ 5.80) to H_b-12 (δ 2.06), H_a-14 (δ 2.54), H_b-14 (δ 2.91), H_3-17 (δ 1.32), and H_3-20 (δ 1.01), indicated that H_3-17, H_3-19, H_3-20, H-7, H-11, and H_2-14 were cofacial and in α-configuration, while H-6 and H-10 were on the opposite side of the molecular plane and in β-configuration. Thus, compound **1** was elucidated as (13*R*)-6α,7β-dihydroxy-8β,13-epoxy-11β-nicotinyloxy-*ent*-clerodan-3-en-15,16-olide.

The molecular formula of compound **2** was determined to be $C_{22}H_{30}O_6$ by HR-FAB mass spectrum, which displayed a quasi-molecular ion at *m/z* 391.2128 $[M + H]^+$. The ^1H and ^{13}C NMR spectra of **2** (Table 1) indicated a substitution pattern in the octalin moiety similar to barbatin C [7]. The observed differences were due to the presence of an acetoxyl group (δ_H 2.14, 3H, s; δ_C 173.8 C; 21.8 CH_3) at C-6 in **2**. The location of the acetoxyl substituent in **2** was established from the HMBC spectrum. The HMBC spectrum showed a cross-peak of correlation between the signal at δ 173.8 (acetoxyl carbonyl) and the proton at δ 5.15 (1H, d, $J = 10.5$ Hz, H-6). This proton correlated with the singlet attributed to C-4 (δ 141.0), which in turn showed a cross-peak with H-10 (δ_H 2.28, 1H, dd, $J = 2.0$, 12.3 Hz). These facts established the acetoxyl group to be at C-6 position (Figure 3). The stereochemical assignments of the chiral centers in **2** were

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (in $CDCl_3$).

	1		**2**	
No.	δ_H	δ_C	δ_H	δ_C
1	1.60 (m, H_a-1) 2.18 (m, H_b-1)	28.2 CH_2	1.32 (m, H_a-1) 1.68 (m, H_b-1)	19.4 CH_2
2	2.75 (m, 2H)	33.9 CH_2	2.03 (m, 2H)	26.2 CH_2
3	5.23 (br s)	117.8 CH	5.28 (br s)	123.2 CH
4		144.7 C		141.0 C
5		44.8 C		43.5 C
6	3.88 (d, 10.0)	77.1 CH	5.15 (d, 10.5)	79.0 CH
7	3.64 (d, 10.0)	79.2 CH	3.73 (d, 10.5)	74.5 CH
8		78.4 C		77.2 C
9		39.7 C		47.7 C
10	2.55 (dd, 2.3, 12.3)	44.0 CH	2.28 (dd, 2.0, 12.3)	42.5 CH
11	5.80 (dd, 3.7, 11.8)	71.8 CH	6.39 (d, 16.7)	147.6 CH
12	1.79 (m, H_a-12) 2.06 (m, H_b-12)	29.1 CH_2	6.32 (d, 16.7)	121.6 CH
13		75.6 C		162.2 C
14	2.54 (d, 17.5, H_a-14) 2.91 (d, 17.5, H_b-14)	44.1 CH_2	5.92 (br s)	114.8 CH
15		173.7 C		173.6 C
16	4.21 (d, 8.7, H_a-16) 4.25 (d, 8.7, H_b-16)	79.5 CH_2	4.97 (dd, 1.4, 16.1, H_a-16) 5.01 (dd, 1.4, 16.1, H_b-16)	70.7 CH_2
17	1.32 (s, 3H)	20.0 CH_3	1.15 (s, 3H)	22.1 CH_3
18	1.81 (s, 3H)	20.6 CH_3	1.60 (s, 3H)	20.4 CH_3
19	1.26 (s, 3H)	15.2 CH_3	1.19 (s, 3H)	17.3 CH_3
20	1.01 (s, 3H)	21.4 CH_3	1.09 (s, 3H)	15.4 CH_3
1′		164.3 C		
2′		126.3 C		
3′	9.16 (br s)	150.4 CH		
5′	8.83 (br d, 4.6)	153.5 CH		
6′	7.46 (dd, 4.6, 7.7)	123.7 CH		
7′	8.26 (br d, 7.7)	137.2 CH		
OAc			2.14 (s, 3H)	173.8 C 21.8 CH_3

Notes: Coupling constants (in Hz) are presented in parentheses. The assignments were based on DEPT, HMQC, HMBC, and $^{1}H-^{1}H$ COSY experiments.

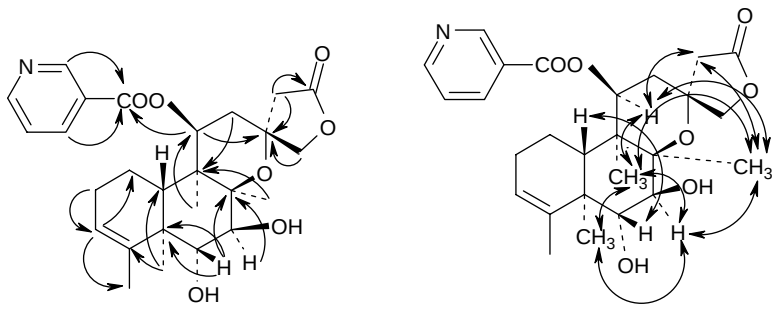

Figure 2. Key HMBC and ROESY correlations of **1**.

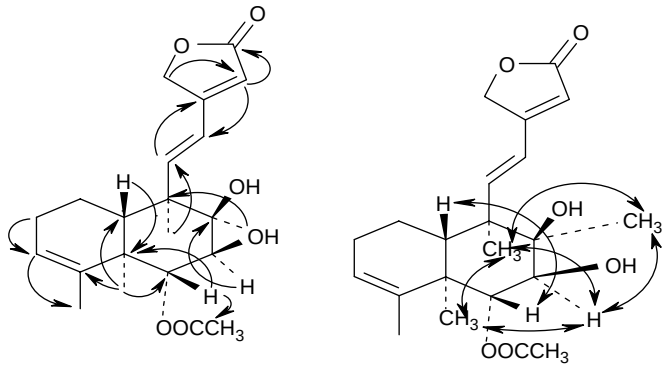

Figure 3. Key HMBC and ROESY correlations of **2**.

accomplished in the similar manner as described for barbatin C. Accordingly, it was assigned as (11*E*)-6α-acetoxy-7β,8β-dihydroxy-*ent*-clerodan-3,11,13-trien-15,16-olide.

Compounds **1** and **2** were evaluated for their cytotoxic activities against HONE-1, KB, and HT29 tumor cell lines by using methylene blue dye assay and anti-cancer drugs, etoposide and cisplatin as positive controls [10,11]. Two new compounds exhibited significant cytotoxicity as shown in Table 2.

3. Experimental

3.1 General experimental procedures

Melting points were measured on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. UV spectra were obtained on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 683 infrared spectrometer with KBr disks. FAB-MS and HR-FAB-MS were recorded on an Autospec-Ultima ETOF MS spectrometer. NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C), with TMS as the internal standard. Silica gel (200–300 mesh) for column chromatography and silica gel GF254 for preparative TLC were obtained from Qingdao Marine Chemical Factory (Qingdao, China).

Table 2. Cytotoxicity of compounds **1** and **2** against cultured HONE-1, KB, and HT29 cancer cell lines.

Compounds	Growth inhibition constant (IC_{50})[a] [μM]		
	HONE-1	KB	HT29
Etoposide[b]	0.7 ± 0.2 (0.48–0.91)	0.9 ± 0.3 (0.61–1.32)	2.1 ± 0.5 (1.74–3.48)
Cisplatin[b]	2.3 ± 0.6 (1.74–2.92)	3.4 ± 0.9 (2.47–4.41)	3.6 ± 1.8 (1.87–5.48)
1	3.9 ± 2.2 (1.72–6.08)	4.8 ± 1.8 (3.12–6.64)	6.6 ± 1.5 (5.08–8.23)
2	2.5 ± 1.9 (0.59–3.11)	4.5 ± 1.7 (2.96–6.71)	3.5 ± 1.9 (1.63–5.42)

Notes: [a]IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number and the results are means ± standard deviation of three independent replicates. An IC_{50} greater than 10 μM was considered to be no cytotoxicity. The 95% confidence limits are presented in parentheses.
[b]Positive controls.

3.2 Plant material

S. barbata D. Don was collected in Linquan district, Anhui Province, China, in September 2008, and identified by Prof. Yan-Yan Zhao, School of Pharmaceutical Science, Yantai University. The whole plants of *S. barbata* were harvested and air-dried at room temperature in the dark. A voucher specimen (YP08063) has been deposited at the Herbarium of the School of Pharmaceutical Science, Yantai University.

3.3 Extraction and isolation

The air-dried whole plants (38.2 kg) of *S. barbata* were finely cut and extracted three times (1 h × 3) with refluxing EtOH (120 liters × 3). Evaporation of the combined extracts under reduced pressure provided the ethanolic extract (1.7 kg). The extract was suspended in H_2O (10.0 liters), and partitioned with $CHCl_3$ (20 liters × 3) and EtOAc (20 liters × 3). The $CHCl_3$ fraction (367.1 g) was subjected to extraction with 3% HCl (3.0 liters × 3). Following this, the aqueous solution was adjusted with NH_4OH to pH 10 and extracted with $CHCl_3$ (2.5 liters × 3). The organic fractions were combined, and the solvent was evaporated under vacuum to yield the $CHCl_3$ alkaloidal fraction (78.0 g). The alkaloidal fraction was initially subjected to silica gel CC, eluted with cyclohexane–acetone (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, and 50:50) to give eight fractions. Fraction 5 (3.1 g) was further separated by reversed-phase silica gel (150 g, 40–50 μm) CC [eluted with MeOH–H_2O, 55:45, v/v], and subsequently purified on Sephadex LH-20 [100 g, eluted with EtOAc–CH_3OH, 50:50, v/v] to give **1** (72 mg) and **2** (113 mg).

3.3.1 Scutelinquanine D (*1*)

White needles, mp 154–155°C, $[\alpha]_D^{29}$ −92.6 ($c = 0.13$, MeOH). UV ($CHCl_3$) λ_{max}: 221, 256 nm. IR (KBr) ν_{max}: 3448, 1771, 1634, 1607, 1584, 1466, 1358 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. FAB-MS *m/z*: 472.5 $[M + H]^+$. HR-FAB-MS *m/z*: 472.2341 $[M + H]^+$ (calcd for $C_{26}H_{34}NO_7$, 472.2335).

3.3.2 6-Acetoxybarbatin C (*2*)

White needles, mp 157–158°C, $[\alpha]_D^{29}$ −103.4 ($c = 0.14$, MeOH). UV ($CHCl_3$) λ_{max}: 220, 256 nm. IR (KBr) ν_{max}: 3441, 1770, 1722, 1660, 1629, 1451, 1394, 1013 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. FAB-MS *m/z*: 391.4 $[M + H]^+$. HR-FAB-MS *m/z*: 391.2128 $[M + H]^+$ (calcd for $C_{22}H_{31}O_6$, 391.2121).

3.4 Anti-tumoral cytotoxic bioassays

Cytotoxic activities against HONE-1, KB, and HT29 cancer cell lines of the two new compounds were evaluated by a method as previously reported [5–8].

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