Fitoterapia 134 (2019) 404–410

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Semi-synthesis and structural elucidation of brevicanines A–D, four new C_{19}-diterpenoid alkaloids with rotameric phenomenon from *Aconitum brevicalcaratum*

Zhong-Sheng Wang[1], Wei Chen, Hai-Yue Jiang, Feng Gao*, Xian-Li Zhou*

Laboratory of Chemistry and Biodiversity, School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, PR China

ARTICLE INFO

Keywords:
Diterpenoid alkaloid
Rotameric phenomenon
Semi-synthesis
Cytotoxicity

ABSTRACT

Four new C_{19}-diterpenoid alkaloids brevicanines A–D (**1–4**) with rotameric phenomenon were isolated from *Aconitum brevicalcaratum*. They all possessed an unusual axial chiral phenyl-quinazoline side chain and their structures were elucidated by extensive spectroscopic analysis and chemical methods. Meanwhile, brevicanines A and B were semi-synthesized from their parent compound scaconine to further confirm their structures. Variable-temperature NMR spectroscopy was also used to investigate the atropisomers of brevicanine A, in which two sets of signals in ^{1}H NMR spectra were observed at room temperature and coalesced over 140 °C. It's the first time to determine the atropisomeric preference of diterpenoid alkaloids.

1. Introduction

Diterpenoid alkaloids were the main active constituents and characteristic components of the Chinese herbal medicine *Aconitum* and *Delphinium* species and showed promising cardiotonic, anti-arrhythmic analgesic, anti-tumor, anti-inflammatory and antioxidant activities [1–3]. The dynamic medicinal properties of these diterpenoid alkaloids have attracted considerable attention in natural product chemistry field for years and leaded to the isolation and identification of more than 1500 diterpenoid alkaloids with diverse structures. *Aconitum brevicalcaratum* (Finet et Gagnep.) Diels mainly distributed in Yunnan Province of China and has long been used for treating coughs, pains and cancer in Chinese traditional medicine [4]. Nine C_{19}-diterpenoid alkaloids were identified from *Aconitum brevicalcaratum* in early research and brevicanine exhibited anti-proliferative activity against MCF-7, HepG2 human cancer cell lines through inducing protective autophagy [5,6].

In our continuing research on the chemistry and biology of diterpenoid alkaloids [6], four novel diterpenoid alkaloids, brevicanines A–D (**1–4**) with rotameric phenomenon were isolated from the methylene dichloride extractions of the *A. brevicalcaratum* roots. They all featured a scarcely terminal linkage of phenyl-quinazoline which contained a chiral nonbiaryl axis [7]. In order to intensive study their configurations, brevicanine A and B were semi-synthesized from the available scaconine (**8**) isolated from *A. brevicalcaratum* [8]. Furthermore, the rotamers of compound **1** were investigated by variable-temperature NMR spectroscopy [9]. Therefore, we presented the structure elucidation, semi-synthesis and rotameric phenomenon of brevicanines A–D (**1–4**). Cytotoxic activity of these new compounds against several human cancer cell lines was also studied [10].

2. Experimental section

2.1. General experimental procedures

Optical rotations were measured in CH_3Cl using a PerkinElmer polarimeter with a sodium lamp operating at 598 nm and 20 °C. IR spectra were obtained using a Thermo Fisher Nicolet 6700 spectrometer. HRESIMS data were measured using a Q-TOF micro mass spectrometer (Waters). NMR spectra were recorded on a Bruker AV 600 spectrometer (Bruker). Thin layer chromatography (TLC) and silica gel (100–200 mesh) (Qingdao Marine Chemical Factory). Other chemicals and solvents were either purchased from commercial suppliers or purified by standard techniques.

2.2. Plant material

A. brevicalcaratum was collected from Lijiang, Yunnan, China, in August 2016 and was authenticated by Prof. Liangke Song at the School of life Science and Engineering at Southwest Jiaotong University,

* Corresponding authors.
E-mail addresses: gaof@swjtu.edu.cn (F. Gao), zhouxl@swjtu.edu.cn (X.-L. Zhou).
[1] Co-first author.

https://doi.org/10.1016/j.fitote.2019.03.012
Received 16 February 2019; Received in revised form 15 March 2019; Accepted 16 March 2019
Available online 18 March 2019
0367-326X/

Table 1
^{1}H NMR spectral data in $CDCl_3$ for compounds **1–4** (600 MHz, δ in ppm, J in Hz).

No.	1	2	3	4
1	2.86 m	2.85 m	2.77–2.87 m	2.83–2.88 m
2a	2.08 m	1.66 m	1.37 m	1.27 m
2b	2.14 m	2.08 m	1.75 m	1.73 m
3a	1.47 m	1.51 m	1.14 m	1.17 m
3b	1.12 m	1.57 m	1.50 m	1.57 m
5	1.34 m	1.23 m	1.35 m	1.34 m
6a	1.30 m	1.29 m	1.34 m	1.35 m
6b	1.76 m	1.66 m	1.71 m	1.79 m
7	1.96 d (7.8)	2.20 m	2.01 d (7.6)	2.16 m
9	2.08–2.14 m	2.25 m	2.16 m	2.27 m
10	2.31 m	2.18 m	1.58 m	2.22 m
12a	1.75 m	1.75 m	1.85 m	1.25 m
12b	2.27 m	2.31 m	2.07 m	2.31 m
13	1.59 m	1.65 m	2.58–2.62 m	2.24 m
14	3.62 t (4.8)	3.50 m	4.80 m	3.48–3.52 m
15a	1.85 m	2.13 m	1.82 m	1.96 m
15b	2.29 m	2.68 m	2.35 dd (9.6)	2.10 m
16	3.13 dd (9.6)	3.11 dd (8.4)	3.14 dd (8.4)	3.14–3.17 m
17	2.83 m	2.75 m	2.92 br s	2.71 br s
18a	3.80 ABq (10.8)	3.81 ABq (10.4)	3.84 ABq (10.6)	3.83 ABq (10.4)
18b	3.90 ABq (10.8)	3.90 ABq (10.4)	3.93 ABq (10.6)	3.90 ABq (10.4)
19a	1.88 m	1.86 m	1.92 m	1.90 m
19b	2.36 m	2.35 m	2.41 m	2.37 m
21a	2.27 m	2.28 m	2.32 m	2.29 m
21b	2.42 m	2.43 m	2.45 m	2.43 m
22	1.01 t (7.2)	0.99 t (7.2)	1.02 t (7.2)	1.01 t (7.2)
1-OMe	3.20 s	3.18 s	3.19 s	3.20 s
8-OCH_2CH_3				3.25 m
8-OCH_2CH_3				1.06 t (7.8)
8-OAc		1.91 s		
14-OAc			2.05 s	
14-OMe	3.38 s	3.39 s		3.38 s
16-OMe	3.28 s	3.29 s	3.21 s	3.33 s
3’	8.24 d (7.8)	8.17 d (7.8)	8.25 d (7.8)	8.24 t (7.8)
4’	7.60 d (7.2)	7.67 t (7.8)	7.70 t (7.8)	7.72 d (7.8)
5’	7.71 d (7.8)	7.75 t (7.2)	7.77 d (7.2)	7.76 t (7.8)
6’	7.25 t (7.8)	7.20 d (7.8)	7.27 d (7.8)	7.28 d (7.8)
5”	8.20 d (7.8)	8.10 d (7.8)	8.20 d (7.8)	8.20 d (7.8)
6”	7.45 t (7.8)	7.63 d (8.4)	7.47 t (7.2)	7.46 t (7.2)
7”	7.75 t (7.8)	7.54 t (7.8)	7.73 t (7.8)	7.70 t (7.8)
8”	7.69 t (7.8)	7.70 d (7.8)	7.64 t (7.8)	7.62 t (7.8)
2”-OMe	2.21 s	2.21 s	2.21 s	2.21 s

Sichuan Province, P.R. China, where a voucher specimen (No. ZN361520160821) was deposited.

2.3. Extraction and isolation

The powdered roots of *A. brevicalcaratum* (2.1 kg) were extracted with 95% EtOH (3 × 10 L) at room temperature for 3 days. The crude extract (300 g) was suspended in H_2O, the pH was adjusted to 3.0 using 10% HCl solution, and the suspension was extracted with petroleum ether (4 × 2 L) and EtOAc (4 × 2 L), successively. Then, the pH of the aqueous layer was adjusted to 9 using an aqueous ammonia solution and then extracted with CH_2Cl_2 (4 × 2 L) to obtain the alkaloid extract (40 g). By repeated column chromatography, the alkaloid extract was separated to afford compounds **1** (78 mg), **2** (56 mg), **3** (63 mg) and **4** (37 mg) (Scheme S1, Supporting information).

2.3.1. Brevicatine A (1)

White amorphous powder; $\alpha_D^{20} = -10.6$ (c = 0.40, $CHCl_3$). IR ν_{max}: 3444, 2924, 2818, 1718, 1571, 1471, 1379, 1292, 1046, 772 cm^{-1}. HRESIMS at m/z: 684.3619 $[M + H]^+$ (calcd for $C_{40}H_{50}N_3O_7$, 684.3649). ^{1}H NMR (600 MHz, $CDCl_3$) date, see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2.

2.3.2. Brevicatine B (2)

White, amorphous powder; $\alpha_D^{20} = +24.8$ (c = 0.40, $CHCl_3$). IR ν_{max}: 2926, 2855, 2819, 1723, 1687, 1571, 1451, 1292, 1091, 773 cm^{-1}. HRESIMS at m/z: 726.3749 $[M + H]^+$ (calcd for $C_{42}H_{52}N_3O_8$, 726.3754). ^{1}H NMR (600 MHz, $CDCl_3$) date, see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2.

2.3.3. Brevicatine C (3)

White, amorphous powder; $\alpha_D^{20} = +28.1$ (c = 0.40, $CHCl_3$). IR ν_{max}: 3442, 2926, 2817, 1724, 1687, 1571, 1471, 1248, 1090, 773 cm^{-1}. HRESIMS at m/z: 712.3607 $[M + H]^+$ (calcd for $C_{41}H_{50}N_3O_8$, 712.3598). ^{1}H NMR (600 MHz, $CDCl_3$) date, see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2.

2.3.4. Brevicatine D (4)

White, amorphous powder; $\alpha_D^{20} = +16.3$ (c = 0.40, $CHCl_3$). IR ν_{max}: 3441, 2925, 2819, 1688, 1571, 1471, 1291, 1269, 1196, 772 cm^{-1}. HRESIMS at m/z: 712.3894 $[M + H]^+$ (calcd for $C_{42}H_{54}N_3O_7$, 712.3962). ^{1}H NMR (600 MHz, $CDCl_3$) date, see Table 1; ^{13}C NMR (150 MHz, $CDCl_3$) data, see Table 2.

2.4. Synthesis

2.4.1. 2-methyl-4H-benzo[d][1,3]oxazin-4-one (6)

The anthranilic acid 137.1 mg (1.0 mmol) was suspended in acetic anhydride 2 mL. Upon heating all material was dissolved, and the mixture was refluxed until TLC indicated the reaction was finished (generally 2 h). Then, the solvent was removed in vacuo to give a solid. The crude material was recrystallized from hexane and ethyl acetate. The product was collected by filtration and washed with hexane. Yield = 90.3%, m.p. 82–83 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.86 (d, J = 7.6 Hz, 1H), 7.48 (td, J = 1.5 and 7.6 Hz, 1H), 7.20–7.16 (m, 2H), 2.15 (s, 3H).

2.4.2. 2-(2-Methyl-4-oxoquinazolin-3-yl) benzoic acid (7)

2-Methyl-4H-benzo[d][1,3]oxazin-4-one (**6**) 161.2 mg (1.0 mmol) and anthranilic acid 164.1 mg (1.2 mmol) were dissolved in glacial acetic acid 5 mL. The solution was refluxed until TLC indicated the reaction was finished (about 8 h). After cooling to room temperature, the mixture was concentrated in vacuo to give a solid. Then, the crude material was recrystallized and the product **7** was collected by filtration and washed with ethyl acetate. Yield = 75.5%, m.p. 256–258 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 13.11 (s, 1H), 8.10 (m, 2H), 7.82 (m, 2H), 7.67 (m, 2H), 7.57 (dd, J = 0.7 and 7.5 Hz, 1H), 7.50 (td, J = 0.9 and 8.3 Hz, 1H), 2.11 (s, 3H).

2.4.3. Brevicatine A (1)

A solution of the scaconine (82.3 mg, 0.194 mmol), 2-(2-methyl-4-oxoquinazolin-3-yl) benzoic acid (65.1 mg, 0.232 mmol), EDC·HCl (56.4 mg, 0.293 mmol), and DMAP (19.1 mg, 0.156 mmol) was added in dichloromethane (10 mL), and stirred at room temperature for 10 h. The solvent was removed by distillation under reduced pressure and concentrated to give a crude yellow solid. The crude material was separated by flash column chromatography using 7% methanol/dichloromethane as eluent to yield the desired product **1** as a white solid (100.2 mg, 76.7%).

2.4.4. Brevicatine B (2)

A solution of compound **1** (36.2 mg, 0.052 mmol) and TsOH (20.1 mg, 0.12 mmol) in acetic anhydrate (4 mL) was heated to reflux for 4 h. The acetic anhydrate was removed by simple distillation, and

Table 2
^{13}C NMR spectral data in $CDCl_3$ for compounds **1–4** (150 MHz, δ in ppm).

No.	1	2	3	4	No.	1	2	3	4
1	85.2 d	84.8 d	85.2 d	85.3 d	8-$COCH_3$		55.5 t		
2	26.1 t	26.2 t	25.9 t	26.4 t	8-OCH_2CH_3				22.5 q
3	32.4 t	32.1 t	32.4 t	32.4 t	8-OCH_2CH_3				16.3 s
4	37.7 s	37.6 s	37.8 s	37.7 s	14-OCH_3	57.8 q	57.7 q		57.8 s
5	46.0 d	45.5 d	45.7 d	45.6 d	14-$COCH_3$			170.9 s	
6	25.1 t	24.9 t	25.0 t	24.4 t	14-$COCH_3$			21.5 q	
7	45.7 d	41.6 d	46.3 d	41.0 d	16-OCH_3	56.3 q	56.6 q	56.2 q	56.5 q
8	73.9 s	86.0 s	73.6 s	77.4 s	1′	137.7 s	137.6 s	137.8 d	137.8 d
9	46.2 d	41.6 d	45.3 d	42.9 d	2′	129.8 s	129.8 s	129.9 s	129.8 d
10	36.9 d	39.8 d	35.5 d	38.9 d	3′	134.7 d	134.7 d	134.7 d	134.6 d
11	48.7 s	48.7 s	48.7 s	49.2 s	4′	130.1 d	129.9 d	130.0 d	130.1 d
12	29.4 t	29.0 t	28.5 t	29.9 t	5′	134.1 d	134.1 d	134.2 d	134.1 d
13	45.2 d	45.0 d	44.7 d	45.6 d	6′	132.6 d	132.7 d	132.7 d	132.6 d
14	84.4 d	83.3 d	77.0 d	84.1 t	7′	164.9 s	165.0 s	165.0 s	165.0 s
15	41.7 t	37.3 t	40.9 t	36.2 t	2″	154.0 s	154.1 s	154.1 s	154.0 s
16	82.6 d	83.2 d	81.7 d	83.9 d	4″	162.2 s	162.1 s	162.2 s	162.2 s
17	61.9 d	61.3 d	61.9 d	61.3 d	5″	127.1 d	127.0 d	127.1 d	127.2 d
18	71.4 t	71.1 t	71.2 t	71.5 t	6″	126.7 d	126.9 d	126.7 d	126.7 d
19	52.5 t	52.2 t	52.5 t	52.7 t	7″	128.7 d	128.6 d	128.7 d	128.8 d
21	49.2 t	49.1 t	49.3 t	49.2 t	8″	127.1 d	127.0 d	127.1 d	127.2 d
22	13.6 q	13.3 q	13.7 q	13.6 q	9″	147.7 s	147.6 s	147.8 s	147.8 s
1-OCH_3	56.2 q	56.2 q	56.1 q	56.3 q	10″	120.9 s	120.9 s	121.0 s	121.1 s
8-$COCH_3$		169.8 s			2″-CH_3	24.3 q	24.1 q	24.2 q	24.3 q

the residue was purified on silica gel using 6% methanol/dichloromethane as eluent to yield the desired product **2** as a white solid (28.3 mg, 74.5%).

2.5. Cell culture and cytotoxicity MTT assay

The MCF-7 and HepG2 were obtained from Peking Union Medical College, Beijing, China. The cells were cultured in Dulbecco's Modified Eagle's Medium-High (DMEM) medium supplemented with 10% FBS, 100 μg/mL penicillin, and 0.03% L-glutamine and maintained at 37 °C with 5% CO_2 in a humidified atmosphere. Cisplatin (Pt-amount 65%, Shanghai Aladdin Co., Ltd., China) was used as positive control. The cytotoxicity assay of all compounds was tested via the MTT method using breast cancer cells MCF-7 and liver cancer cells HepeG2.

3. Results and discussion

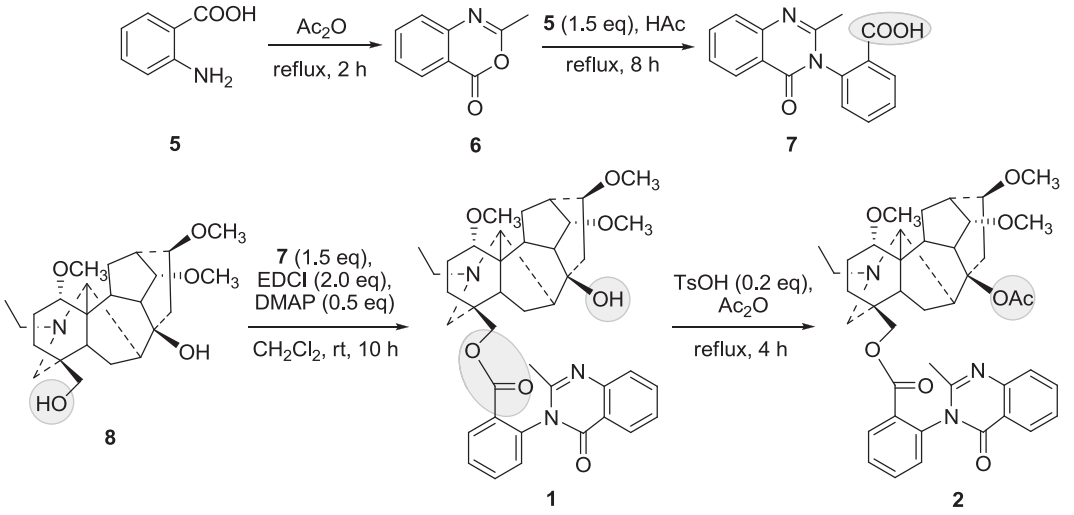
Four novel diterpenoid alkaloids, brevicanines A–D (**1–4**) were isolated from the EtOH extraction (methylene dichloride fraction) of air-dried and powered roots of *A. brevicalcaratum* by conventional chromatography. The ^1H NMR and ^{13}C NMR signals of these compounds in $CDCl_3$ appeared doubling phenomenon at room temperature which were unlike the previous diterpenoid alkaloids (For example, the ^1H NMR spectra of **1** exhibited two sets of signals in a ratio of nearly 1:2, Fig. 2) [11]. However, they were inseparable by various column chromatography (such as silica gel CC and semi-preparative HPLC) or repeated recrystallization. All these brevicanines were also subjected to crystallization for structures resolution, but any single crystals were obtained. Hence, we suspected that these natural isolates existed rotamers in solution.

Compared among the ^1H NMR spectra of compounds **1–4** that showed similarly peaks at the aromatic region (from 7.20 ppm to 8.30 ppm, Fig. S3, Supporting information). Furthermore, the IR spectra of brevicatines A–D indicated the absorption peaks for aromatic ring (1607, 1571, 1471 cm^{-1}) and carbonyl group (1718 cm^{-1}) (Fig. S5, S14, S23, S32, Supporting information). Considered with the ^{13}C NMR spectra, we inferred that they may possessed the same aromatic substituent and an ester group (δ_C 160–170 ppm) at least. Comparison of the NMR spectra of **2** with those of **1**, the chemical shifts of these two compounds were almost identical and indicated that they are possessed the same skeleton. The significant difference was that one more acetyl group in **2** (δ_H 1.90, 3H; δ_C 169.76 and 22.46).

Structurally, compound **1** was alkaline hydrolysised selectively and only two known compounds 2-(2-methyl-4-oxoquinazolin-3-yl) benzoic acid (**7**) and scaconine (**8**) as well as literature comparisons were produced (Table S1, Supporting information) [12]. However, no doubling signal was reported or observed in the NMR spectra of compounds **7**, **8** at room temperature. In order to further determine the configurations and investigate this rotameric phenomenon of these isolates that compounds **1** and **2** were semi-synthesized from the available scaconine (**8**) which was isolated from *A. brevicalcaratum* (Scheme 1).

(2-Methyl-4-oxoquinazolin-3-yl)benzoic acid (**7**) was synthesized according the literature method use anthranilic acid (**5**) as starting material, and then treated with scaconine (**8**) under EDCI/DMAP to yield compound **1** [13]. According to the literature, the esterification was regioselectivity at the primary hydroxyl (C_{18}-OH) due to the steric hindrance [14]. And then, compound **1** was generally acetylized with acetic anhydride for producing compound **2**. However, as was observed in the solubility in $CDCl_3$ between semi-synthetic compounds **1**, **2** with relatively isolated ones, the ^1H and ^{13}C NMR spectra were exactly consistent and still split as two sets (Fig. 3) at room temperature. Therefore, the isolate ones which were identical to the semi-synthetic compounds **1**, **2** and secured their configurations. Compounds **1**, **2** were further analyzed through extensive spectroscopic methods. It also confirmed that brevicatines **A–D** existed rotameric phenomenon. Additionally, the aromatic substituent group of compounds **1–4** was confirmed as (2-methyl-4-oxoquinazolin-3-yl)benzoyl.

In order to generate unambiguous NMR reference data, variable NMR spectroscopy was still used to investigate the atropisomeric phenomenon and conformation of brevicatine **A**. According to the relative reports [15], it suspected that atropisomers of compound **1** in solution arising from hindered rotation along the C—N bond of phenyl-quinazoline (the red-colored arrow along the aryl-amine bond in Fig. 4) [16], combined with the presence of bulk ester group at C-2′ position and C-2″ methyl should lead to the detectable occurrence in NMR spectroscopy. Indeed, it was confirmed by the ^1H NMR spectra in DMSO-d_6, which featured two sets of signals at room temperature (e.g., H-14 and H-14′, H-18A and H-18A′, H—18B and H-18B′, respectively). The doubling signals of hydrogen H-14, H-18A and H—18B were coalesced upon heating and gave coalescence temperature over 140 °C (Fig. 4) [17]. In view of literature examples describing rotamers, this experiment was the first determination of diterpenoid alkaloids with atropisomeric



Scheme 1. Semi-synthesis of brevicatines **A** (**1**) and **B** (**2**).

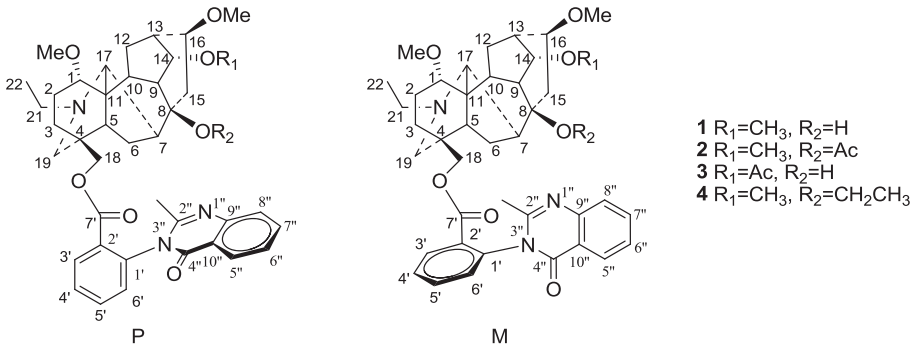


Fig. 1. Structures of compounds **1–4** (brevicanines A–D, P/M nomenclature was used to describe the absolute configurations of phenyl-quinazoline moiety).

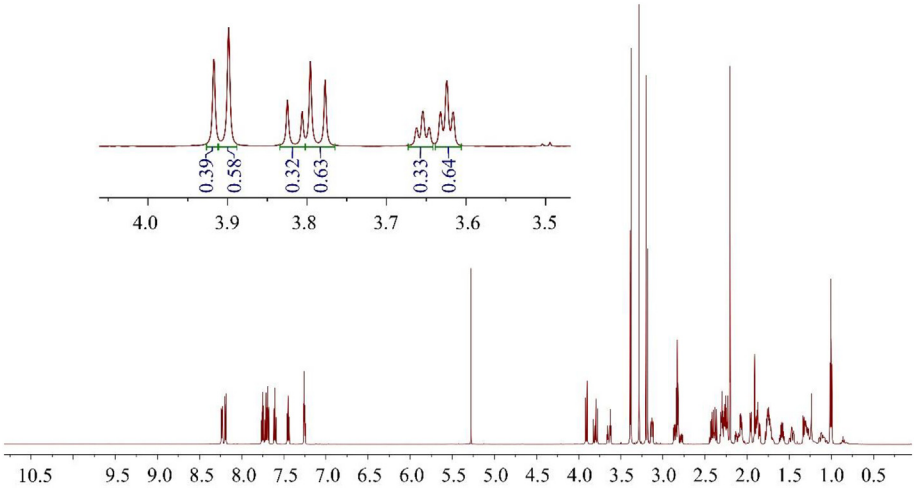


Fig. 2. ^{1}H NMR spectrum of compound 1 at room temperature.

phenomenon.

Brevicatine A (**1**) was isolated as a white powder, the molecular formula was determined as $C_{40}H_{49}N_3O_7$ based on HRESIMS at *m/z*: 684.3619 $[M + H]^+$, (calcd. 684.3649). The IR spectrum indicated the absorption peaks for a carbonyl group (1718 cm^{-1}), and aromatic ring (1607, 1571, 1471 cm^{-1}). The NMR spectra of it showed characteristic signals for an *N*-ethyl group [δ_H 1.01 (3H, t, J = 7.2 Hz); δ_C 49.2 t, 13.6 q) and three methoxyl groups (δ_H 3.20, 3.28, 3.38, each 3H, s; δ_C 56.2 q, 56.3 q, 57.8 q). Furthermore, the signals [δ_H 8.24 (1H, d, J = 7.8 Hz), 8.20 (1H, t, J = 7.8 Hz), 7.75 (1H, t, J = 7.8 Hz), 7.71 (1H, d, J = 7.8 Hz), 7.69 (1H, d, J = 7.8 Hz), 7.60 (1H, d, J = 7.2 Hz), 7.25 (1H, d, J = 7.8 Hz), 7.20 (1H, d, J = 7.8 Hz); δ_C 154.0, 147.7, 137.7, 134.7, 134.1, 132.6, 130.1, 129.8, 128.7, 127.1, 127.1, 126.7, 120.9], a methyl group (δ_H 2.21 s; δ_C 24.3 q) and two carbonyl groups (δ_C 164.9 s, 162.2 s) indicated the presence of a 2-(2-methyl-4-oxoquinazolin-3-yl)benzoate moiety. Analyses of the NMR and HRESIMS data revealed that **1** was an aconitine-type diterpenoid alkaloid. In the HMBC spectrum (Fig. 5), the long-range correlations between δ_H 3.20 s

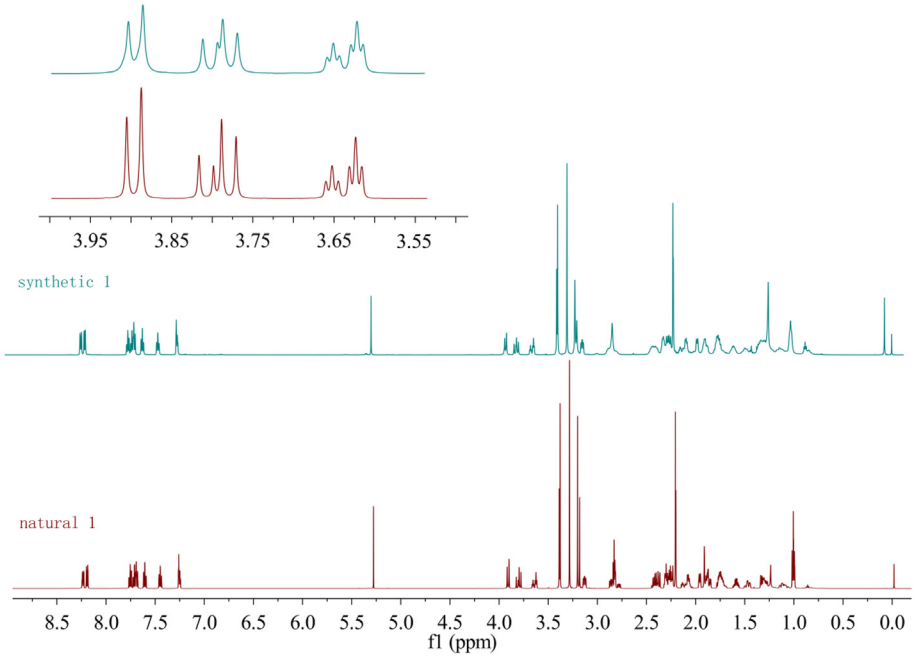


Fig. 3. ^{1}H NMR comparison of synthetic **1** and natural **1**.

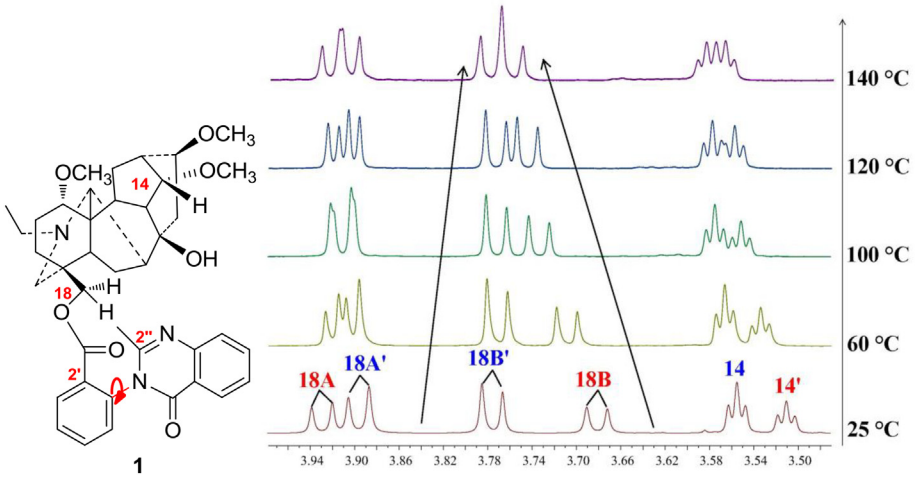


Fig. 4. Variable temperature ^{1}H NMR spectroscopy of compound **1** in DMSO-d_6.

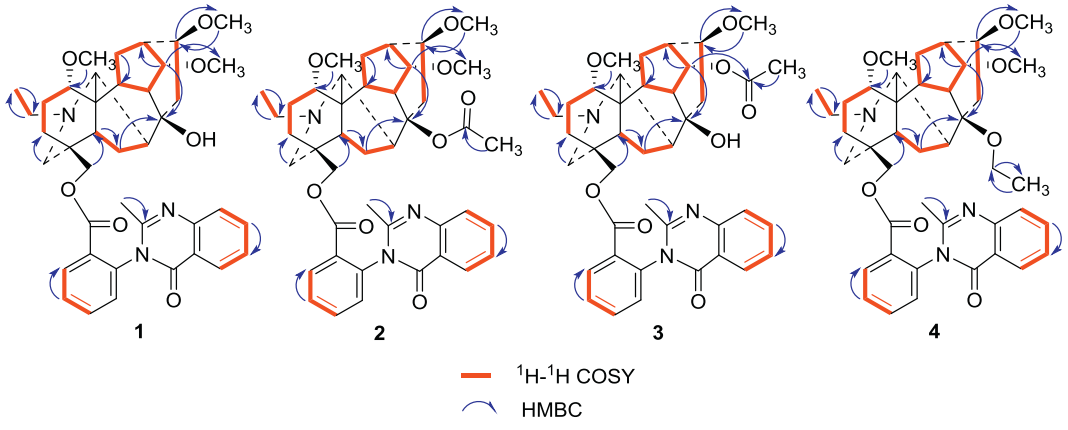


Fig. 5. Key HMBC and ^{1}H–^{1}H COSY correlations of compounds **1–4.**

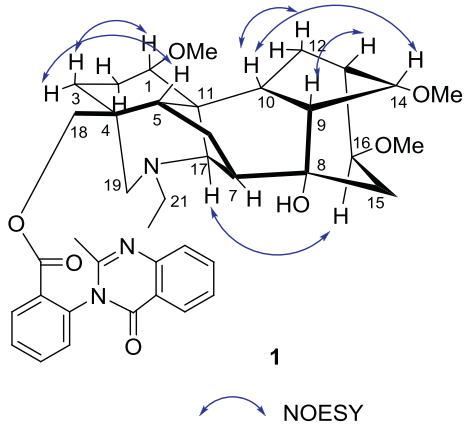


Fig. 6. Key NOESY correlations of compound **1**.

and δ_C 85.2, δ_H 3.28 s and δ_C 82.6, δ_H 3.38 s and δ_C 84.4 suggested three methoxy groups were assigned to C-1, C-16 and C-14, respectively. The correlations in the HMBC spectrum between H-18 [δ_H 3.80, 3.90 (each 1H, ABq, J = 10.8 Hz)] and C=O (δ_C 164.9) revealed that the 2-(2-methyl-4-oxoquinazolin-3-yl)benzoate moiety is installed at the C-18. In addition, five oxygen-bearing carbon signals were observed in ^{13}C NMR spectrum, apart from the above four ones, one hydroxyl groups were considered to exist. In the HMBC spectrum, one set of $^{1}H-^{13}C$ long-range correlations of H-17 (δ_H 2.83) with C-8 (δ_C 73.9) suggested that the hydroxyl group was installed at C-8.

The stereochemistry of compound **1** was deduced from the NOESY experiment, a correlation between H-1 and H-10 in the NOESY experiment (Fig. 6) suggested that the methoxy group at C-1 was α-oriented. According to the cross-peak between H-14 and H-10, H-16 and H-17 suggested the configuration of 14-OMe and 16-OMe as α and β respectively. Thus, the structure of **1** was determined as shown in Fig. 1.

Brevicatine B (**2**) was isolated as a white amorphous powder. Its molecular formula $C_{42}H_{51}N_3O_8$ was determined by HRESIMS (m/z 726.3749 $[M + H]^+$, calcd. 726.3754). Three methoxy groups (δ_H 3.18, 3.29, 3,39 each 3H, s), an *N*-ethyl group (δ_H 0.99, 3H, t, J = 7.2 Hz; δ_C 49.1 t, 13.3 q), an acetyl group (δ_H 1.91, 3H, s), and a 2-(2-methyl-4-oxoquinazolin-3-yl)benzoate moiety were observed in the NMR spectra. According to the HMBC, three methoxy groups were located at C-1, C-14 and C-16 positions respectively. Comparing the NMR spectra of **2** with those of **1**, the changes on chemical shift of C-7, C-8, C-9 and C-15 demonstrated that the hydroxyl group was replaced by the acetoxy group at C-8 position, which was supported by the the HMBC correlations of H-6, H-9, H-14 with C-8 (Fig. 5). Thus, the structure of compound **2** was determined as brevicatine B (**2**).

Brevicatine C (**3**) was isolated as a white amorphous powder. The molecular formula was determined as $C_{41}H_{50}N_3O_8$ based on the HRESIMS at m/z 712.3607 $[M + H]^+$ (calcd for 712.3598). By analyzing the molecular formula and NMR data of **3** with those of **1**, the major difference was that the methoxy group at C-14 was replaced by the acetoxy group in compound **3**, which was further supported by a set of $^{1}H-^{13}C$ long-range correlations of H-14 (δ_H 4.80) with C-13 (δ_C 44.7), C-9 (45.3), and the carbonyl carbon (δ_C 170.9). Therefore, the structure of compound **3** was determined as shown in Fig. 1, named as brevicatine C.

Bevicatine D (**4**) was obtained as a white amorphous powder, the molecular formula $C_{42}H_{54}N_3O_7$ was established on the basis of HRESIMS (m/z 712.3894 $[M + H]^+$, cacld. 712.3962). The NMR data revealed the presence of an ethoxy group [δ_H 1.06 (3H, t, J = 7.2 Hz); δ_C 55.5 t, 16.3 q]. The NMR data for **4** were similar to those for **1** except that the hydroxy group in **1** was replaced by the ethoxy group in **4** at C-8 position, which was supported by the HMBC correlations between H-6, H-9, H-14 and C-8. Thus, the structure of bevicatine D (**4**) was determined as shown in Fig. 1, and the full assignment of its spectroscopic data was achieved based on 1D and 2D NMR analyses.

All of the four compounds were evaluated for the cytotoxic activities against cancer cell lines MCF-7 and HepG2 in vitro. However, none of them showed potential cytotoxic activity against MCF-7 and HepG2 at 50 μM.

4. Conclusion

In summary, four new C_{19}-diterpenoid alkaloids, brevicanines A–D (**1–4**) with rotameric phenomenon were isolated from *A. brevicalcaratum*. The unique constructions of compounds **1** and **2** were semi-synthesized successfully. Their structures and configurations were elucidated on the basis of HRESIMS, IR, 1D, 2D NMR and chemical methods. Based on practical limitations, the rotamers cannot be isolated and tested separately due to such interconversion under room temperature. The cytotoxic activity against two cell lines also evaluated, but none of them exhibited potential inhibition against MCF-7 and HepG2 in vitro.

Conflict of interest

The authors declare no competing financial interests.

Acknowledgements

This project was supported by the National Natural Science Foundation of China (81773605 and 31570341), Science and Technology Project of Sichuan (2018JY0077), and the Interdisciplinary Frontier Basic Research Project of SWJTU (2682017QY04).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.03.012.

References

[1] P.G. Xiao, F.P. Wang, F. Gao, L.P. Yan, D.L. Chen, Y. Liu, A pharmacophylogenetic study of *Aconitum L.(Ranunculaceae)* from China, Acta Phytotaxon. Sin. 44 (2006) 1–46.
[2] J.F. Zhang, L. Chen, S. Huang, L.H. Shan, F. Gao, X.L. Zhou, Diterpenoid alkaloids from two *Aconitum* species with antifeedant activity against *Spodoptera exigua*, J. Nat. Prod. 80 (2017) 3136–3142.
[3] W.L. Xu, L. Chen, L.H. Shan, F. Gao, S. Huang, X.L. Zhou, Two new atisine-type C_{20}-diterpenoid alkaloids from *Aconitum Leucostomum*, Heterocycles 92 (2016) 2059–2065.
[4] L. Chen, Q. Wang, S. Huang, L.H. Shan, F. Gao, X.L. Zhou, Diterpenoid alkaloids from *Aconitum leucostomum* and their antifeedant activity, Chin. J. Org. Chem. 37 (2017) 1839.
[5] Y.H. Li, D.H. Chen, Two novel diterpenoid alkaloids isolated from the roots of *Aconitum Brevicalcaratum*, Acta Bot. Sin. 36 (1994) 148–152.
[6] H.Y. Jiang, S. Huang, F. Gao, Y.Q. Zhen, C. Li, X.L. Zhou, Diterpenoid alkaloids from *Aconitum brevicalcaratum* as autophagy inducers, Nat. Prod. Res. 20 (2018) 1–6.
[7] F. Su, Z. Zhao, S. Ma, R. Wang, Y. Li, Y. Liu, Y. Li, L. Li, J. Qu, S. Yu, Cnidimonins A-C, three types of hybrid dimer from *Cnidium monnieri*: structural elucidation and semisynthesis, Org. Lett. 19 (2017) 4920–4923.
[8] X.J. Hao, S.Y. Chen, J. Zhou, Three new diterpenoid alkaloids from *Aconitum Scaposum*, Acta Bot. Yunnanica 2 (1985) 217–224.
[9] S.R. Laplante, L.D. Fader, K.R. Fandrick, D.R. Fandrick, O. Hucke, R. Kemper, S.P. Miller, P.J. Edwards, Assessing atropisomer axial chirality in drug discovery and development, J. Med. Chem. 54 (2011) 7005–7022.
[10] S. Gao, P. Zhang, C. Zhang, F. Bao, H. Li, L. Chen, Meroterpenoids from *Ganoderma sinense* protect hepatocytes and cardiomyocytes from oxidative stress induced injuries, Fitoterapia 131 (2018) 73–79.
[11] X.X. Zong, G.Y. Yan, J.L. Wu, L.H. Leung, H. Zhou, N. Li, L. Liu, New C_{19}-diterpenoid alkaloids from the parent roots of *Aconitum carmichaelii*, Tetrahedron Lett. 58 (2017) 1622–1626.
[12] S.H. Shim, J.S. Kim, K.H. Son, K.H. Bae, S.S. Kang, Alkaloids from the roots of *Aconitum pseudo-laeve* var. *erectum*, J. Nat. Prod. 69 (2006) 400–402.
[13] L. Sun, J.M. Veith, P. Pera, R.J. Bernacki, I. Ojima, Design and synthesis of de novo cytotoxic alkaloids by mimicking the bioactive conformation of paclitaxel, Bioorg. Med. Chem. Lett. 18 (2010) 7101–7112.
[14] F.P. Wang, J.S. Yang, Q.H. Chen, L. Yu, B.G. LI, New access to 7, 17-seco norditerpenoid alkaloids *via* reduction of the corresponding 8-chloro derivatives, Chem. Pharm. Bull. 48 (2000) 1912–1916.
[15] C. Jonathan, Non-biaryl atropisomers: new classes of chiral reagents, auxiliaries,

and ligands? Angew. Chem. Int. Ed. 36 (1997) 949–951.
[16] A. Zask, J. Murphy, G.A. Ellestad, Biological stereoselectivity of atropisomeric natural products and drugs, Chirality 25 (2013) 265–274.
[17] S.R. LaPlante, P. Forgione, C. Boucher, R. Coulombe, J. Gillard, O. Hucke, A. Jakalian, M.A. Joly, G. Kukolj, C. Lemke, Enantiomeric atropisomers inhibit HCV polymerase and/or HIV matrix: characterizing hindered bond rotations and target selectivity, J. Med. Chem. 57 (2013) 1944–1951.