Fitoterapia 130 (2018) 152–155

Contents lists available at ScienceDirect

Fitoterapia

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

Two novel terpenoids from the cultured *Perovskia atriplicifolia*

Wen-Xing Liu[a,b,1], Jia-Wen Zhao[a,1], Ai-Xue Zuo[c], Zhi Yang[d], Lu Gao[a], Min Zhou[a,*], Zhi-Yong Jiang[a,*]

[a] Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University, Chenggong New District, Kunming 650500, Yunnan, China
[b] School of Chemistry & Environment, Yunnan Minzu University, Chenggong New District, Kunming 650500, Yunnan, China
[c] Yunnan University of Traditional Chinese Medicine, Chenggong New District, Kunming 650500, Yunnan, China
[d] Yunnan Baiyao Group Co., Ltd. Chenggong New District, Kunming 650500, Yunnan, China

ARTICLE INFO

Keywords:
Perovskia atriplicifolia
Terpenoids
Dimer
Anti-HBV activity

ABSTRACT

Two new terpenoids, named biperovskatone B (**1**) and 1α- hydroxyl demethylsalvicanol quinine (**2**), were isolated from the cultured *Perovskia atriplicifolia*. Their structures were elucidated by comprehensive analyses of the MS, IR, 1D and 2D NMR spectra. Compound **1** was a novel diterpenoid dimer, containing two different rearranged 9(10 → 20)-abeoabietane type diterpenoid fragments. Compound **2** was a new icetexane diterpenoid with characteristic *ortho*-quinone carbonyl groups. Both compounds were assayed for their anti-HBV activity in vitro. Results suggested compounds **1** and **2** showed noticeable anti- anti-HBV activity, inhibiting the replication of HBV DNA with IC_{50} values of 10.78 and 8.61 μM, respectively.

1. Introduction

The *Perovskia* genus had attracted many scholars' attentions due to their broad-spectrum bioactivity such as leishmanicidal, antiplasmodial, and cytotoxic activity [1]. Our group also found the antivirus activity during the previous investigation and some bioactive chemicals had been isolated from *P. atriplicifolia* [2–6]. Furthermore, an interesting phenomenon had been found during the preceding research, that is, the chemical constituents in the cultured *P. atriplicifolia* were quite different from the wild one, which was confirmed by a detailed HPLC analysis. Our investigation on the cultured *P. atriplicifolia* during the last two years had led to the isolation of three novel terpenoids [2,3]. As a subsequent investigation on the cultured *P. atriplicifolia*, two new terpenoids, named biperovskatone B (**1**) and 1α- hydroxyl demethylsalvicanol quinine (**2**), were obtained from the titled plant. The structures of the two new compounds were elucidated by comprehensive analyses of the MS, IR, 1D and 2D NMR data. Compound **1** was a novel diterpenoid dimer, including two different rearranged 9 (10 → 20)-abeoabietane type diterpenoids. In vitro antivirus bioassay in HepG 2.2.15 cell line suggested compounds **1** and **2** possessed remarkable anti-HBV activity, inhibiting the replication of HBV DNA with IC_{50} values of 10.78 and 8.61 μM, respectively.

2. Experimental

2.1. General experimental procedures

Column chromatography (CC): silica gel (200–300 mesh; Qingdao Marine Chemical Inc., China); Lichrospher Rp-18 gel (40-63 μ; Merck, Germany). HPLC was performed on an Agilent 1260 liquid chromatograph (Agilent, America) equipped with a Venusil XBP C18 (10 × 250 mm, 5 μm) column. Melting points were obtained using a Beijing Tech X-5 micromelting point apparatus without correction. Optical rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter. UV spectra were obtained on a Shimadzu UV-2401A spectrophotometer. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm^{-1}. MS data were obtained on a Thermo Fisher LTQ XL LC/MS (Thermo Fisher, Palo Alto, CA, USA). NMR spectra were recorded on a Bruker AVANCE 400 spectrometer ($^1H/^{13}C$, 400 MHz/100 MHz), and chemical shifts were given in δ (ppm) with TMS as internal reference. The positive control lamivudine (3-TC, purity > 99%) was purchased from GlaxoSmithKline, Suzhou Co., Ltd. P. R. of China.

* Corresponding author at: Key Laboratory of Chemistry in Ethnic Medicinal Resources, Yunnan Minzu University, State Ethnic Affairs Commission & Ministry of Education, Jingming South Road, Kunming 650500, Yunnan, China.
E-mail address: jiangzy2010@163.com (Z.-Y. Jiang).
[1] Co-first authors, these authors contributed equally to this work.

https://doi.org/10.1016/j.fitote.2018.08.024
Received 23 June 2018; Received in revised form 27 August 2018; Accepted 29 August 2018
Available online 30 August 2018

2.2. Plant material

The whole plant of the cultured *P. atriplicifolia* was purchased from Sichuan Luxi Flower Gardening Co., Ltd. (Chengdu) in May 2013, and was identified as *Perovskia atriplicifolia* Benth. by Prof. Dr. Li-Gong Lei from Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (TSYJ-201358) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University.

2.3. Extraction and isolation

The air-dried of the whole cultured *P. atriplicifolia* (4.0 kg) were powdered and extracted with 90% ethanol under reflux for three times, 2 h each time. After concentrated in vacuo, the extract was suspended in water and successively partitioned with petroleum ether, chloroform and n-BuOH to give petroleum ether (A). chloroform (B), n-BuOH (C) and aqueous (D) fractions.

The petroleum ether (A) (103 g) fraction was then subjected to silica gel chromatography column (CC) and eluted with gradient petroleum ether/acetone (100:0, 98:2, 95:5, 90:10, 80:20) to afford six fractions (Frs.A.1–6). The Fr.A.5 (1.6 g, eluted by petroleum ether/acetone 90:10) was performed on silica gel CC (petroleum ether/acetone 95:5 to 90:10) to afford three sub-fractions (Frs. 5.1–5.3). Fr5.3 (0.65 g) was subjected to a MCI CC (MeOH/H_2O 70:30 to 100: 0) and further purified by HPLC with an eluent of CH_3CN/H_2O (40:60, flow rate: 2 mL/min) to yield compounds **1** (15 mg, Rt 26.3 min) and **2** (5 mg, Rt 18.7 min).

Compound **1**: White amorphous powder. M.p 113–115 °C; $[\alpha]_D^{18.9}$: -15.6 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε): 265 (2.42), 291 (3.13) nm.; IR (KBr) ν_{max} 3448, 2970, 1669, 1604, 1501, 1456 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 400 MHz and 100 MHz) data, see Table 1; ESIMS (pos.): *m/z* 653 $[M + Na]^+$; HRESIMS (pos.): *m/z* 653.3791 ($[M + Na]^+$, $C_{40}H_{54}O_6Na^+$; calc. 653.3818).

Compound **2**: Pale yellow amorphous powder. M.p 119–121.5 °C; $[\alpha]_D^{18.9}$: +26.2 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε): 270 (1.68), 240 (1.52) nm; IR (KBr) ν_{max} 3446, 2967, 1725, 1681, 1632, 1453, 1246, 1020 cm^{-1}; ^1H and ^{13}C NMR (CD_3OD, 400 MHz and 100 MHz) data, see **Table 2**; ESIMS (pos.): *m/z* 355 $[M + Na]^+$; HRESIMS (pos.): *m/z* 355.1879 ($[M + Na]^+$, $C_{20}H_{28}O_4Na^+$; calc. 355.1885).

Table 1
^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of compound **1** ($CDCl_3$, *J* in Hz).

No.	δ_C	δ_H	No.	δ_C (mult.)	δ_H
1	41.6 (CH_2)	1.94 (m); 1.60 (m)	1′	30.7 (CH_2)	1.99 (m); 1.82 (m)
2	19.4 (CH_2)	1.59 (m); 1.29 (m)	2′	16.2 (CH_2)	1.81 (m); 1.62 (m)
3	41.9 (CH_2)	1.33 (m); 1.16 (m)	3′	39.5 (CH_2)	1.88 (m); 2.12 (m)
4	33.7 (C)	–	4′	31.9 (C)	–
5	54.0 (CH)	1.08(dd,13.6,2.8)	5′	51.1 (CH)	1.75 (t, 8.0)
6	19.2 (CH_2)	1.59 (m); 1.42 (m)	6′	32.2 (CH_2)	2.09 (m)
					1.45 (m)
7	32.4 (CH_2)	2.10 (m); 1.49 (m)	7′	76.0 (CH)	4.85 (d, 6.4)
8	75.1 (C)	–	8′	137.6 (C)	–
9	52.3 (C)	2.85 (brd, 6.4)	9′	119.3 (C)	–
10	87.0 (C)	–	10′	80.0 (C)	–
11	100.1 (C)	–	11′	138.5 (C)	–
12	190.6 (C)	–	12′	136.6 (C)	–
13	123.7 (C)	–	13′	133.0 (C)	–
14	159.9 (C)	–	14′	113.8 (CH)	6.58 (s)
15	24.0 (CH)	3.43 (sept., 7.2)	15′	26.5 (CH)	3.35 (sept., 7.2)
16	20.1 (CH_3)	1.30 (d, 7.2)	16′	22.5 (CH_3)	1.29 (d, 7.2)
17	20.1 (CH_3)	1.27 (d, 6.8)	17′	22.9 (CH_3)	1.23 (d, 6.8)
18	31.7 (CH_3)	0.81 (s)	18′	30.6 (CH_3)	0.95 (s)
19	21.5 (CH_3)	0.76(s)	19′	26.5 (CH_3)	0.84 (s)
20	40.0 (CH_2)	2.38 (m, 2H)	20′	38.6 (CH_2)	2.42 (d, 17.6)
					2.66 (d, 17.6)

Table 2
^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) data of compound **2** (CD_3OD, *J* in Hz).

No.	δ_C	δ_H	No.	δ_C	δ_H
1	77.0 (CH)	3.58 (br.s)	10	73.5 (C)	–
2	26.4 (CH_2)	2.15 (m)	11	181.1 (C)	–
		1.45 (m)	12	181.6 (C)	–
3	35.9 (CH_2)	1.79 (m)	13	147.5 (C)	–
		1.10 (m)	14	140.2 (C)	6.78, s
4	34.8 (C)	–	15	28.6 (CH)	2.87, sept (6.8)
5	53.1 (CH)	1.68 (br.d, 12.4)	16	21.7 (CH_3)	1.13, d (6.8)
6	21.1 (CH_2)	1.88 (m)	17	21.9 (CH_3)	1.13, d (6.8)
		1.50 (m)	18	32.4 (CH_3)	0.93, s
7	36.9 (CH_2)	2.63 (m)	19	22.3 (CH_3)	0.88, s
		2.54 (m)	20	38.1 (CH_2)	3.00, d (14.4)
8	156.3 (C)	–			2.49, d (14.4)
9	135.7 (C)	–			

2.4. In vitro anti-HBV activity assay

The anti-HBV activity of the crude extract and pure compounds were assayed through Hep G2.2.15 cell line as our preceding report [3]. 3-TC (Lamivudine, commercial available), an approved clinical agent, was used as the positive control.

2.5. Cytotoxicity assay

The cytotoxicity assay was performed according to the MTT method [7] in 96-well microplates with 3-TC (Lamivudine) as a positive control. Briefly, the test samples were prepared at different concentrations. After Hep G2.2.15 cells seeding in 96-well microplate for 4 h, the samples (20 μL) were placed in each well and incubated for 3 days in 37 °C, then 0.1 mL MTT [3- (4, 5- dimethylthiazole −2 - yl) -2, 5- diphenyltetrazolium bromide, 400 μg/ mL] (GIBCO Invitrogen, Ohio, USA) was added for 4 h. After removal of the MTT medium, DMSO (100 μL/well) was added onto the microplate for 10 min. The formazan crystals were redissolved and the absorbance was measured on a microplate reader at 490 nm.

3. Results and discussion

3.1. Structure elucidation of new compounds

Compound **1** was isolated as a white amorphous powder. The molecular formula was concluded as $C_{40}H_{54}O_6$ by the HRESIMS at *m/z* 653.3791 $[M + Na]^+$ (calcd. For $C_{40}H_{54}O_6Na^+$ 653.3818), with fourteen unsaturation degrees. The IR spectrum showed the absorption bands at 3448, 1669, 1604, 1501, 1456 cm^{-1}, suggesting the presence of hydroxyl, carbonyl, and aromatic ring functions. In the ^1H NMR spectrum (Table 1), a singlet aromatic proton (δ_H 6.58, s, H-14′) and eight methyl signals were displayed. In addition, two pair of isopropyl protons were observed at δ_H 3.43 (1H, sept, *J* = 7.2 Hz, H-15), 1.30 (3H, d, *J* = 7.2 Hz, H-16), 1.27 (3H, d, *J* = 6.8 Hz, H-17), and δ_H 3.35 (1H, sept, *J* = 7.2 Hz, H-15′), 1.29 (3H, d, *J* = 7.2 Hz, H-16′), 1.23 (3H, d, *J* = 6.8 Hz, H-17′). The ^{13}C NMR spectrum (Table 1) exhibited forty carbons, including eight methyls, eleven methylenes, seven methines (including one oxygenated carbon) and fourteen quaternary carbons (including a carbonyl and seven oxygenated carbons). Detailed analyses of the NMR spectrum indicated that compound **1** contained two icetexane diterpenoid moieties. A comprehensive comparison of the ^1H and ^{13}C NMR spectra of compound **1** with those of biperovskatone [3], which had been isolated from the cultured *Perovskia atriplicifolia*, suggested that both compounds shared a similar skeleton. As shown in Table 1, the NMR data of unit A in compound **1** were almost the same as those of biperovskatone [3], illustrating that compound **1** also have the same unite A like biperovskatone. The structure of compound **1** differed