Fitoterapia 129 (2018) 126–132

Contents lists available at ScienceDirect

Fitoterapia

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

Iridal-type triterpenoids with anti-HBV activity from *Iris confusa*

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ARTICLE INFO

Keywords:
Iris confusa
Iridal-type triterpenoids
Anti-HBV activity

ABSTRACT

Five new iridal-type triterpenoids, named as spirioiridoconfal A-C (**1**–**3**), isobelamcandal (**4**) and 17-hydroxyl-27-ene-iridal (**5**), along with one known compound (**6**) were isolated from the whole plant of *Iris confusa*. Their structures were determined by extensive spectroscopic analyses. Compounds **3** and **6** showed moderate activity against HBV DNA replication in vitro with the IC_{50} value at 84.6 ± 24.9 (SI = 2.1) and 58.6 ± 6.1 (SI = 12.7) μM relatively.

1. Introduction

Iris confusa Iridaceae, is widely distributed in China, and its rhizomes have been used as a folk medicine to treat acute tonsillitis and bronchitis [1]. Iridal-type triterpenoids are the characteristic constituents of *Iris* genus which usually contain a highly substituted cyclohexane ring with an *α*, *β*-unsaturated aldehyde group and a non-cyclic homofarnesyl side chain [2–4]. Iridal-type triterpenoids were revealed with various bioactivities including cytotoxicity [5, 6], neuroprotection [7], antiplasmodial activity [8], ichthyotoxicity [9], and PKC activation [10, 11]. The only paper concerning to the chemical constituents of *I. confusa* reported a series of isoflavonoids [12]. From a biosynthetic point of view, *I. confusa* should be rich in iridal-type triterpenoids, although their presence is still unclear. The unique structures of iridals and the abundant resources of *I. confusa* encouraged us to study the chemical constituents of this species.

Hepatitis B virus (HBV) infection, the major cause of hepatitis, is a severe health problem around the world. Vaccines, interferons and nucleosides are the main treatment strategies for HBV infection. Nevertheless, they are still unsatisfactory due to the adverse side effect and drug-resistance [13]. Natural products have been used to treat hepatitis for a long time and have been the fascinating resources for anti-HBV drug discovery. A series of anti-HBV candidates have been found in our previous endeavors, such as protostane triterpenoids from *Alisma orientalis* [14], swerilactones from *Swertia mileensis* [15, 16], chlorogenic acid analogs from *Artemisia capillaris* [17], plolyacetylene glucosides from *Artemisia scoparia* [18] and sesquiterpenoids from *Cyperus rotundus* [19]. As a continuous search for anti-HBV substances from natural sources, five new iridal-type triterpenoids (**1**–**5**) along with a known compound (**6**) were isolated from *I. confusa*, of which compounds **3** and **6** showed moderate anti-HBV activities. The isolation, structure elucidation and anti-HBV activities of the isolates were discussed in this paper.

2. Experimental section

2.1. General experimental procedures

General experimental procedures were listed in the Supporting information.

2.2. Plant material

The whole plants of *Iris confusa* Sealy. were collected in Qujing County, Yunnan Province, China, in September 2016, and authenticated by Prof. Hua Peng, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2016-0902) has been deposited in the Group of Anti-virus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The fresh whole pants of *I. confusa* were cut into pieces (30 kg) and extracted with 70% EtOH (3 × 100 L) under reflux. The combined EtOH extracts were evaporated under reduced pressure and suspended in H_2O, which were partitioned with EtOAc and *n*-BuOH. The EtOAc

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https://doi.org/10.1016/j.fitote.2018.06.005
Received 27 April 2018; Received in revised form 7 June 2018; Accepted 8 June 2018
Available online 12 June 2018
0367-326X/

part (280 g) was fractionated by silica gel column (3.0 kg, 20 × 50 cm) using a petroleum ether-acetone gradient system (95:5, 90:10, 85:25 and 80:20) to give seven fractions, Fr. 1 - Fr. 7. Fr. 5 (27 g) was separated by MPLC on a CHP20P MCI gel column (490 g, 5 × 50 cm) eluted with 50%, 60%, 70%, 80%, 90% MeOH-H_2O and MeOH to provide five subfractions (Fr. 5-1 - Fr. 5–5). Fr. 5–3 (4.0 g) was subjected to aluminium oxide column (60 g, 2 × 10 cm) eluted with $CHCl_3$-MeOH (95:5) to obtain five fractions (Fr. 5-3-1 - Fr. 5–3-5). Fr. 5–3-2 (300 mg) was purified using Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) followed by semi-preparative HPLC (MeCN-H_2O, 25:75, *v*/v, 3 mL/min) to afford compound **5** (10 mg). Fr. 5–3-3 (600 mg) was fractioned over a silica gel column ($CHCl_3$-Acetone, 80:20) and further purified by semi-preparative HPLC (MeCN-H_2O, 40:60, *v*/v, 3 mL/min) to give compounds **4** (10 mg) and **6** (6 mg). Fr. 5-3-4 (800 mg) was submitted to silica gel column ($CHCl_3$-Acetone, 70:30) to afford three fractions, Fr. 5-3-4-1 - Fr. 5–3–4-3. The second fraction Fr. 5-3-4-2 (80 mg) purified by semi-preparative HPLC (MeCN-H_2O, 50:50, *v*/v, 3 mL/min) to give compounds **1** (5 mg), **2** (6 mg) and **3** (5 mg).

2.3.1. Spirioiridoconfal A (1)

White colloidal solid; $[\alpha]_D^{22}$ = +69.47 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 241 (4.12) nm; IR (KBr) ν_{max} 3425, 1653, 1451, 1383, 1290, 1053 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)-HRESIMS *m/z* 547.3257 $[M + HCOO]^-$ (calcd for $C_{30}H_{46}O_6HCOO$, 547.3276).

2.3.2. Spirioiridoconfal B (2)

White colloidal solid; $[\alpha]_D^{22}$ = −11.11 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 202 (2.86) nm; IR (KBr) ν_{max} 3440, 1630, 1384 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)-HRESIMS *m/z* 547.3249 $[M + HCOO]^-$ (calcd for $C_{30}H_{46}O_6HCOO$, 547.3276).

2.3.3. Spirioiridoconfal C (3)

White colloidal solid; $[\alpha]_D^{22}$ = +25.47 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 252 (4.04) nm; IR (KBr) ν_{max} 3426, 1657, 1383, 1289, 1203, 1058 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)-HRESIMS *m/z* 531.3337 $[M + HCOO]^-$ (calcd for $C_{30}H_{46}O_5HCOO$, 531.3327).

2.3.4. Isobelamcandal (4)

White colloidal solid; $[\alpha]_D^{22}$ = +51.30 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 250 (4.12) nm; IR (KBr) ν_{max} 3430, 1722, 1656, 1383, 1237, 1053 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (−)-HRESIMS *m/z* 573.3395 $[M + HCOO]^-$ (calcd for $C_{32}H_{48}O_6HCOO$, 573.3433).

2.3.5. 17-hydroxyl-27-ene-iridal (5)

White colloidal solid; $[\alpha]_D^{22}$ = −12.63 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 202 (3.23) nm; IR (KBr) ν_{max} 3427, 1630, 1384, 582 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; (+)-HRESIMS *m/z* 479.3441 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496).

Table 1
The ^1H NMR data of compounds **1–5** in CD_3OD (δ_H in ppm, *J* in Hz).

No.	Compounds				
	1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]
1	10.14 (1H, s)	10.20, (1H, s)	10.17 (1H, s)	1.81(3H, s)	10.18 (1H, s)
3	3.46–3.50 (1H, m)	3.48–3.50, (1H, m)	3.50–3.51 (1H, m)	3.46–3.50 (1H, m)	3.44–3.50 (2H, m)
	3.41–344 (1H, m)	3.40–3.43, (1H, m)	3.45–3.49 (1H, m)	3.42–3.45 (1H, m)	
4	1.28–1.31 (2H, m)	1.28–1.30, (2H, m)	2.55–2.30 (1H, m)	2.14–2.22 (1H, m)	1.16–1.24 (2H, m)
			1.91–1.95 (1H, m)	1.95–2.01 (1H, m)	
5	2.14–2.18 (1H, m)	2.17–2.20 (1H, m)	2.17–2.20 (1H, m)	2.14–2.22 (1H, m)	2.78 (1H, m)
	2.02–2.07 (1H, m)	2.01–2.05 (1H, m)	1.50–1.60 (1H, m)	1.27–1.32 (1H, m)	2.26–2.34 (1H, m)
6	3.53 (1H, d, 10.2)	3.66 (1H, d, 10.3)	3.52–3.54 (1H,m)	3.03–3.05 (1H, m)	3.34 (1H, br.s)
8	2.75–2.78 (1H, m)	2.73–2.76 (1H, m)	2.79–2.82 (1H, m)	3.25–3.28 (1H, m)	1.83–1.89 (1H, m)
	2.52 (1H, br.d 13.8)	2.53 (1H, br.d, 13.2)	2.54 (1H, br.d, 14.4)	2.75–2.80 (1H, m)	1.59 (1H, overlapped)
9	1.75–1.77 (1H, m)	1.71–1.73 (2H, m)	1.70–172 (1H, m)	1.79–1.81 (1H, m)	2.39–2.42 (1H, m)
	1.65–1.67 (1H, m)		1.63–1.65 (1H, m)	1.65 (1H, overlapped)	2.26–2.34 (1H, m)
12	1.51–1.55 (1H, m)	1.36–1.40 (m, 2H)	1.33–1.40 (m, 2H)	1.32–1.38 (2H, m)	1.59 (m, overlapped)
	1.42–1.46 (1H, m)				1.16–1.24 (1H, m)
13	1.82–1.86 (1H, m)	1.96–1.99 (1H, m)	1.91–1.95 (1H, m)	1.95–2.01 (2H, m)	1.68–1.73 (1H, m)
	1.62–1.65 (1H, m)	1.61 (1H, overlapped)	1.57–1.60 (1H, m)	1.67 (1H, overlapped)	1.37–1.45 (1H, m)
14	2.75 (1H, m)	2.79–2.81 (1H, m)	2.83–2.82 (1H, m)	3.06–3.08 (1H, m)	5.08 (1H, m)
16	4.26 (1H, d, 6.9)	4.11 (1H, d, 5.0)	4.79 (1H, d, 8.3)	6.26 (1H, d, 11.2)	2.19–0.2.22 (1H, m)
					1.98–2.00 (1H, m)
17	5.84 (1H, dd, 15.9, 7.0)	5.89 (1H, dd, 15.8, 6.6)	5.96 (1H, dd, 5.6, 16.0)	6.62 (1H, dd, 15.2, 11.2)	4.38 (1H, m)
18	6.30 (1H, d, 15.2)	6.33 (1H, d, 15.8)	6.39 (1H, d, 16.0)	6.31 (1H, d, 15.2)	5.08 (1H, m)
20	2.24–2.26 (1H, m)	2.25–2.27 (2H, m)	2.55–2.30 (1H, m)	2.25–2.32 (1H, m)	1.94–1.97 (2H, m)
			2.17–2.20 (1H, m)	1.41–1.47 (1H, m)	
21	2.18–2.20 (2H, m)	2.19 (2H, m)	2.17–2.20 (1H, m)	1.27–1.32 (2H, m)	2.02–2.09 (2H, m)
			1.31–1.43 (1H, m)		
22	5.16 (1H, tt)	5.16(1H, tt)	5.16–5.19 (1H, m)	5.13 (1H, m)	5.09 (1H, m)
24	1.67 (3H, s)	1.67 (3H, s)	1.70 (3H, s)	1.67 (3H, s)	1.66 (3H, s)
25	1.79 (3H, s)	1.79 (3H, s)	1.82 (3H, s)	10.20 (1H, s)	1.81 (3H, s)
26	5.00 (1H, s)	4.60 (1H, d, 6.5)	4.57 (1H, d, 8.2)	4.44 (1H, d, 4.0)	1.15 (3H, s)
27	1.28 (3H, s)	1.28 (3H, s)	1.17 (3H, s)	1.26 (3H, s)	4.98 (1H, br.s)
					4.76 (1H, br.s)
28	3.67 (1H, d, 11.6)	3.49 (2H, s)	5.22 (1H, br.s)	4.78 (1H, d, 13.6)	1.60 (3H, s)
	3.48 (1H, s)		4.99 (1H, br.d, 8.1)	4.71 (1H, d, 13.6)	
29	5.00 (1H, s)	5.05 (1H, s)	5.04 (br.s)	5.05 (1H, br.s)	1.58 (3H, br.s)
	4.96 (1H, s)	5.00 (1H, s)	4.99 (1H, br.d, 8.1)	5.00 (1H, br.s)	
30	1.61 (3H, s)	1.60 (3H, s)	1.62 (3H, s)	1.58 (3H, s)	1.55 (3H, s)
2′				2.01 (3H, s)	

[a] ^1H NMR data calculated in 800 MHz.
[b] ^1H NMR data calculated in 600 MHz.