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Iridals from *Iris tectorum* and *Belamcanda chinensis*

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Abstract

Three iridals, iridotectorals A and B, and iridobelamal A, were isolated from rhizomes of *Iris tectorum and Belamcanda chinensis*, respectively, along with five known iridals. Their structures were elucidated on the basis of spectral evidence. The human promyelocytic leukemia (HL-60) cell-adhesion activity of the eight iridals is also discussed.

Keywords: *Iris tectorum*; *Belamcanda chinensis*; Iridaceae; Iridal; Iridotectorals A, B; Iridobelamal A

1. Introduction

Iridal-type triterpenoids are characteristic components of Iridaceous plants, and are typically monocyclic or bicyclic seco-ring A compounds (Marner, Krick, Gellrich & Jaenicke, 1982; Krick, Marner & Jaenicke, 1983). Recently, a few biological activities for the iridals, including piscicidal (Miyake, Ito & Yoshida, 1997; Ito, Onouce, Miyake & Yoshida, 1999) and antiulcer (Muto et al., 1994) activities, have been reported. In our previous studies, methanolic extracts of rhizomes of *Iris tectorum* Maxim. and *Belamcanda chinensis* DC. have been found to stimulate morphological change of HL-60 cells involving adhesion to culture plates (Takahashi, Ishino, Hoshino, Tokumaru & Suzuki, 1993). HL-60 cell-adhesion activity-directed fractionation of the methanolic extract of *I. tectorum* afforded an iridal-type triterpene, 28-deacetybelamcandal (Takshashi, Hano, Suganuma, Okabe & Nomura, 1999). This was also found to be a tumor-promoting compound, whose action is mediated through the activation of protein kinase C (PKC) in a two-stage carcinogenesis on mouse skin (Takahashi et al., 1999). In our survey of the tumor-promoting iridals, further fractionation of the methanolic extract of *I. tectorum* as well as *B. chinensis* were examined. This paper describes the isolation and characterization of the iridals of both Iridaceous plants.

2. Results and discussion

The methanolic extract of dried rhizomes of *I. tectorum* was suspended in water, then extracted with chloroform to give a chloroform-soluble portion. The chloroform extract was subjected to column chromatography on silica gel, and subsequent purification by repeated silica gel column chromatography and by LiChroprep RP-18 afforded iridotectorals A (**1**) and B (**2**) along with two known iridals, 28-deacetylbelamcandal (**3**) (Abe, Chen & Yamauchi, 1991) and (6*R*,10*S*,11*R*)-26-ξ-hydroxy-(13*R*)-oxaspiroirid-16-enal (**4**) (Miyake et al., 1997; Marner, Littek, Arold, Seferiadis & Jaenicke, 1990a). An analogous isolation procedure on the methanolic extract of *B. chinensis* afforded iridobelamal A (**5**) along with five known iri-

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dals, **3**, **4**, (6*R*,10*S*,11*S*,14*S*,26*R*)-26-hydroxy-15-methylidene-spiroirid-16-enal (**6**) (Marner, Karime-Nejed & Jaenicke, 1990b), 16-*O*-acetyl-*iso*-iridogermanal (**7**) (Abe et al., 1991), and *iso*-iridogermanal (**8**) (Krick et al., 1983). The known iridals were identified by comparison of their physical and NMR spectral data with those reported in the literature.

Iridotectral A (**1**) was obtained as white glassy substance and showed a pseudomolecular ion $(M + Na)^+$ peak at *m*/*z* 509 in the ESIMS. The ^{1}H- and ^{13}C-NMR spectra of **1** indicated an equilibrium mixture of two anomers (**1a**:**1b** = 2:1 on the basis of signal area in the ^{1}H-NMR spectrum) related to that of **4** (Miyake et al., 1997; Marner et al., 1990a). Detailed analysis of the 2D-NMR spectra, including the ^{13}C–^{1}H COSY and HMBC spectra, revealed **1** to be an oxaspiroiridal-type triterpenoid similar to **4** Tables 1–3). However, upfield shift of H-6 (Δ 0.50 in the major component, Δ 0.56 in the minor component) and downfield shift of one H-8 proton (Δ 0.74 in the major component, Δ 0.71 in the minor component) as compared to **4** clearly indicated that **1** is a geometric isomer of **4** at the α,β-unsaturated aldehyde moiety (Tables 1 and 2) (Miyake et al., 1997). NOE cross peaks between H-6 and 2-CH_3 and between H-8 and 2-CHO in the 2D NOESY spectrum further supported this conclusion. Thus, the structure of iridotectoral A is represented by formula **1**.

Iridotecoral B (**2**), a white glassy substance, $[\alpha]_D$ + 67° (EtOH), showed an $(M + Na)^+$ ion peak at *m*/*z* 509 in the ESIMS. The ^{1}H- and ^{13}C-NMR spectra were similar to those of **3** (Tables 1 and 3). In the ^{1}H-NMR specrum, upfield shift (Δ 0.51) of the H-6 signal and downfield shift (Δ 0.49) of one H-8 proton as compared with the corresponding protons of **3** suggested that **2** is a geometric isomer of **3** at the α,β-unsaturated aldehyde moiety (Tables 1 and 2) (Miyake et al., 1997). The ^{13}C-NMR spectrum also supported the structural features of **2** (Table 3). The structure of iridotectoral **B** is thus represented by formula **2**.

Iridobelamal A (**5**), a colorless oil, $[\alpha]_D + 51°$ (EtOH), showed an $(M + Na)^+$ ion peak at *m*/*z* 497 in the ESIMS. The ^{1}H- and ^{13}C-NMR spectra were essentially the same as those of *iso*-iridogermanal (**8**)

Table 1
^{1}H-NMR spectral data for **1**, **2**, and **3** (δ $CDCl_3$)[a]

Position	**1a** (major)	**1b** (minor)	**2**	**5**
1	1.88 *br s*	1.83 *br s*	1.81 *br s*	1.81 *br s*
3	3.61 *m*	3.61 *m*	3.69 *m*	3.61 *t* (6)
	3.68 *m*	3.68 *m*	3.49 *m*	
4	1.4–1.5 *m*	1.4–1.5 *m*	1.3–1.5 *m*	1.2 *m*
				1.37 *m*
5	1.75 *m*	2.03 *m*	2.2–2.4 *m*	−2.10 *m*
	2.05 *m*	2.25 *m*		
6	3.20 *br d* (10)	2.72 *br d* (10)	3.07 *br d* (10)	2.79 *br d* (9)
8	2.68 *td* (13, 6)	2.67 *td* (13, 6)	2.52 *br d* (13)	2.59 *br dt* (14, 4)
	3.22 *br d* (13)	3.19 *br d* (13)	3.22 *td* (13, 6)	3.22 *br d* (14)
9	1.5 *m*	1.45 *m*	1.67 *m*	
		1.7 *m*	1.6–1.7 *m*	1.83 *m*
12	1.35 *dd* (7, 14)	1.58 *dd* (8, 14)	1.24 *m*	
		1.74 *m*	1.65 *m*	1.95 *m*
13	5.19 *q* (8)	4.62 *q* (8)	2.03 *m*	
14	5.49 *br d* (8)	5.50 *br d* (8)	3.22 *dt* (13, 4)	5.26 *br t* (7)
16	6.12 *d* (15)	6.13 *d* (15)	6.23 *br d* (11)	3.94 *br t* (7)
17	6.44 *dd* (11, 15)	6.44 *dd* (11, 15)	6.49 *dd* (11, 16)	2.24 *dt* (13, 7)
				2.27 *dt* (13, 7)
18	5.88 *br d* (11)	5.88 *br d* (11)	6.32 *d* (16)	5.08 *m*
20	2.05–2.15 *m*	2.05–2.15 *m*	2.2–2.3 *m*	2.03 *m*
21	2.05–2.15 *m*	2.05–2.15 *m*	2.07 *m*	
22	5.09 *m*	5.09 *m*	5.04 *m*	5.06 *m*
24	1.68 *br s*	1.68 *br s*	1.69 *s*	1.68 *br s*
25	10.23 *s*	10.23 *s*	10.23 *s*	10.23 *s*
26	5.71 *s*	5.20 *s*	4.37 *d* (4)	1.08 *s*
27	1.21 *s*	1.45 *s*	1.29 *s*	1.16 *s*
28	1.83 *br s*	1.82 *br s*	4.00 *d* (12)	1.60 *br s*
			4.17 *d* (12)	
29	1.79 *br s*	1.79 *br s*	5.05 *br s*	1.62 *br s*
			5.08 *br s*	
30	1.60 *br s*	1.60 *br s*	1.59 *s*	1.59 *br s*

[a] Value in parantheses denotes coupling constant (*J* in Hz); abbreviations: *b* = broad, *s* = singlet, *d* = doublet, *m* = multiplet.Table 2

(Tables 1–3). However, upfield shift (Δ 0.52) of the H-6 and downfield shift (Δ 0.68) of one H-8 proton in the ^{1}H-NMR spectrum as compared with the corresponding protons of **8**, and further downfield shift (Δ 3.6) of C-6 and upfield shift (Δ 4.1) of C-8 in the ^{13}C-NMR spectrum as compared to **8** indicated that **5** was a geometric isomer of **8** at the α,β-unsaturated aldehyde moiety (Tables 1 and 2) (Miyake et al., 1997). The structure of iridobelamal A was thus determined as **5**.

The HL-60 cell-adhesion activity was examined, for **1**–**8**, as the minimum concentration required to cause the adhesion of all living cells (100% adhesion). Of these, 28-deacetylbelamcandal (**3**), (6*R*,10*S*,11*R*)-26-ξ-hydroxy-(13*R*)-oxaspiroirid-16-enal (**4**), (6*R*,10*S*,11*S*,14*S*,26*R*)-26-hydroxy-15-methylidene-spiroirid-16-enal (**6**), and 16-*O*-acetyl-*iso*-iridogermanal (**7**) were active substances which induced 100% HL-60 cell-adhesion and were 0.5, 1.3, 2.5 and 5 μM, respectively. The potencies were 1/160–1/3000 of TPA (100%-adhesion: 3 nM), a potent inducer and a tumor-promoter. Iridals **1**, **2** and **5** from the present study were inactive within the cell death range (10–40 μM) (Scheme 1).

3. Experimental

3.1. General procedures

^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL EX-400 FTNMR (400 MHz) spectrometer in $CDCl_3$, using TMS as internal standard. ESIMS were measured on an LCQ mass spectrometer (Finnigan MAT) with a direct injection system. UV and IR spectra were recorded on a Shimadzu UV-2200 and Shimadzu FTIR 8100A, respectively. Optical rotations were recorded on a JASCO DIP-370. Wakogel C-200

Table 2
^{1}H-NMR spectral data for **4**, **5**, and **8** (δ $CDCl_3$)[a]

Position	**3**	**4a** (major)	**4b** (minor)	**8**
1	10.15 *s*	10.28 *s*	10.21 *s*	10.18 *s*
3	3.43 *m*	3.59 *m*	3.59 *m*	3.61 *t* (6)
	3.63 *m*	3.67 *m*	3.67 *m*	
4	1.3–1.5 *m*	1.4–1.5 *m*	1.4–1.5 *m*	1.27 *m*
				1.37 *m*
5	2.0–2.2 *m*	1.9–2.0 *m*	2.2 *m*	1.83 *m*
				2.02 *m*
6	3.58 *br d* (10)	3.70 *br d* (11)	3.28 *br d* (10)	3.31 *br d* (9)
8	2.48 *br d* (13)	2.48 *br d* (13)	2.48 *br d* (13)	2.54 *br d* (14)
	2.73 *td* (13, 6)	2.71 *td* (13, 6)	2.71 *td* (13, 6)	2.60 *td* (14, 4)
9	1.65–1.70 *m*	1.45 *m*	1.45 *m*	1.67 *m*
		1.70 *m*	1.7 *m*	1.85 *m*
12	1.3–1.5 *dd* (9, 14)	1.33 *dd* (7, 14)	1.57 *m*	1.17 *m*
		1.8 *m*	1.65 *m*	1.35 *m*
13	1.65–1.79 *m*	5.18 *ddd* (7, 8, 9)	4.60 *dd*d (7, 8, 9)	1.92 *m*
	2.0–2.2 *m*			2.00 *m*
14	3.26 *dt* (13, 4)	5.49 *br d* (9)	5.49 *br d* (9)	5.24 *br t* (7)
16	6.21 *br d* (11)	6.13 *d* (15)	6.13 *d* (15)	3.92 *dd* (5, 7)
17	6.47 *dd* (11, 16)	6.43 *dd* (11, 15)	6.44 *dd* (11, 15)	2.21 *dt* (13, 7)
				2.33 *dt* (13, 7)
18	6.30 *d* (16)	5.87 *br d* (11)	5.87 *br d* (11)	5.07 *m*
20	2.3–2.4 *m*	2.0–2.1 *m*	2.0–2.15 *m*	2.03 *m*
21	2.0–2.2 *m*	2.10–2.15 *m*	2.1–2.15 *m*	2.07 *m*
22	5.11 *m*	5.08 *m*	5.08 *m*	5.06 *m*
24	1.70 s	1.68 *br s*	1.68 *br s*	1.68 *br s*
25	1.81 *s*	1.84 *br s*	1.84 *br s*	1.83 *br s*
26	4.36 *d* (4)	5.72 *s*	5.18 *s*	1.09 *s*
27	1.32 *s*	1.22 *s*	1.46 *s*	1.15 *s*
28	3.98 *d* (11)	1.81 *br s*	1.80 *br s*	1.55 *br s*
	4.16 *d* (11)			
29	5.03 *br s*	1.79 *br s*	1.79 *br s*	1.62 *br s*
	5.06 *br s*			
30	1.59 *s*	1.60 *br s*	1.60 *br s*	1.59 *br s*

[a] Value in parantheses denotes coupling constant (*J* in Hz); abbreviations: *b* = broad, *s* = singlet, *d* = doublet, *m* = multiplet.

(Wako Pure Chemical Industry, Osaka, Japan) was used for column chromatography.

3.2. Plant material

The rhizomes of *Iris tectorum* Maxim. and *Belamcanda chinensis* DC. were purchased from Nakajima Pharmaceutical (Omiya, Saitama, Japan) in November 1996. Authentic specimens (IT 9601 and BC 9601) were deposited at the Department of Food Chemistry, Saitama Prefectural Institute of Public Health.

3.3. Extraction and isolation

The individual MeOH extracts of dried rhizomes of *I. tectorum* (500 g) and *B. chinensis* (500 g) were partitioned between water and $CHCl_3$. The $CHCl_3$ extracts were subjected to silica gel column chromatography (CC) with $CHCl_3$–AcOEt (2:8–8:2). Repeated silica gel CC and further separation by liquid chromatography was carried out in turn on: LiChroprep RP-18 (Merck, Darmstadt, Germany, 25 mm i.d. × 310 mm, MeOH–H_2O = 80:20) and LiChrospher RP-18(e) (Merck, 10 mm i.d. × 250 mm, MeCN–H_2O = 65:35–80:20), to yield **1** (15 mg), **2** (6 mg), **3** (60 mg), and **4** (50 mg) from *I. tectorum*, and **5** (12 mg), **3** (40 mg), **4** (30 mg), **6** (35 mg), **7** (40 mg), and **8** (30 mg) from *B. chinensis*, respectively. The known iridals **3**, **4**, **6**, **7**, and **8**, were identified by comparison of their physical and NMR data with those reported in the literature.

3.4. Iridotectoral A (*1*)

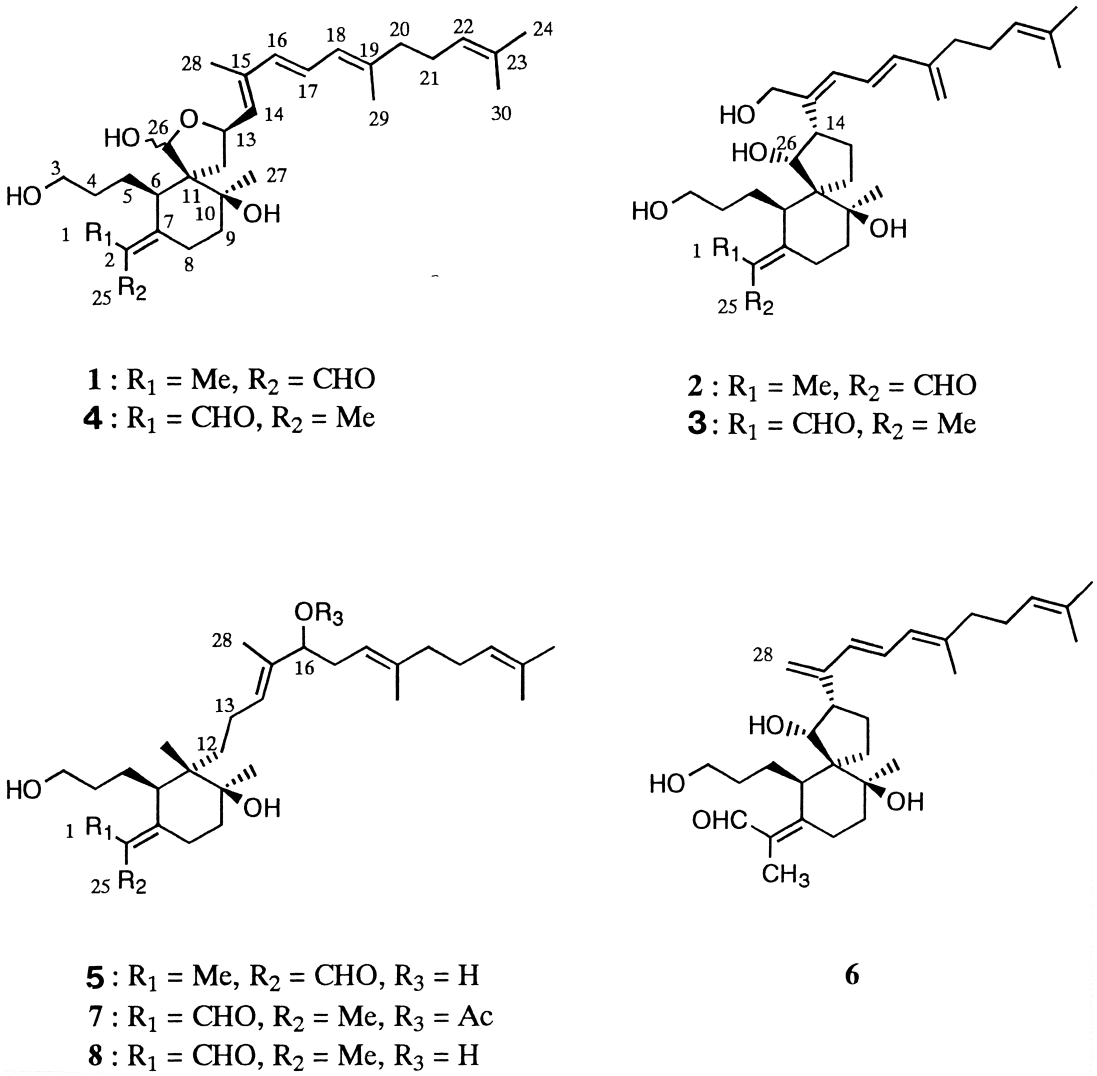
A white glassy substance, UV (EtOH) λ_{max} (log ε): 260 infl. (4.56), 271 (4.62), 281 (4.67), 290 sh (4.55); CD (EtOH): $[\theta]_{272}$ ($\Delta\epsilon$ −4.3), $[\theta]_{234}$ ($\Delta\epsilon$ +3.5); IR ν_{max} (KBr): 3370, 2970, 2930, 2860, 1660, 1610, 1450, 1380, 1290, 1210, 1100, 1050, 1010, 920; ESIMS *m*/*z*: 509 $(M + Na)^+$.

3.5. Iridotectoral B (*2*)

A white glassy substance, $[\alpha]_D$ +67° (*c* 0.12, EtOH); UV (EtOH) λ_{max} (log ε): 252 infl. (4.31), 265 (4.33), 276 (4.31), 290 sh (4.16); CD (EtOH): $[\theta]_{276}$ ($\Delta\epsilon$ -3.9), $[\theta]_{247}$ ($\Delta\epsilon$ +7.2); IR λ_{max} ($CHCl_3$): 3410, 2950, 2940, 2860,

Table 3
^{13}C-NMR spectral data of **1**, **2**, **3**, **4**, **5**, and **8** (δ $CDCl_3$)

C No.	**1a** (major)	**1b** (minor)	**2**	**5**	**3**	**4a** (major)	**4b** (minor)	**8**
1	11.5	11.3	11.5	11.6	191.3	190.8	190.2	189.7
2	131.8	131.9	132.9	132.7	132.8	132.7	133.1	132.7
3	62.0	62.4	60.9	62.9	61.1	62.1	62.4	63.0
4	30.8	31.6	30.6	31.7	30.6	31.0	32.0	32.7
5	28.5	27.0	29.0	26.8	29.2	28.6	27.0	26.6
6	46.6	50.2	46.4	47.0	42.3	42.8	46.4	43.4
7	162.0	161.4	163.4	163.1	163.4	161.9	161.4	162.8
8	19.7	19.6	19.8	19.7	24.1	23.9	23.9	23.8
9	39.2	39.2	38.9	37.7	38.0	38.4	38.2	37.0
10	73.7	74.6	74.7	74.8	74.1	73.9	74.8	75.0
11	59.9	54.9	58.7	44.9	58.7	59.9	54.6	44.7
12	41.7	42.9	34.1	36.6	34.9	42.7	43.8	36.9
13	73.3	69.5	25.4	22.6	25.5	73.6	69.4	21.8
14	129.5	128.9	48.1	125.4	48.4	129.9	129.2	125.0
15	137.0	136.9	137.3	136.6	137.8	137.2	137.4	136.7
16	133.7	133.5	131.2	76.4	131.3	134.0	134.0	76.7
17	125.0	125.2	123.0	33.9	123.5	125.4	125.6	34.2
18	124.6	124.6	136.5	119.6	136.7	125.0	125.0	119.6
19	139.6	139.7	145.3	138.5	145.7	139.9	140.6	138.4
20	39.8	39.8	32.0	39.5	32.3	40.1	40.1	39.8
21	26.3	26.3	26.5	26.2	26.8	26.6	26.6	26.5
22	123.6	123.6	123.8	123.8	123.9	123.9	123.9	123.8
23	131.5	131.5	131.8	131.3	132.1	131.8	131.8	131.3
24	25.4	25.4	26.0	25.4	25.7	25.7	25.7	25.7
25	190.7	190.4	190.8	190.4	11.0	11.1	11.2	10.9
26	99.1	104.8	76.0	17.5	76.3	99.3	105.2	17.9
27	27.4	27.3	27.5	26.0	27.9	27.9	27.8	26.3
28	12.9	12.8	65.7	11.6	65.9	13.3	13.1	11.9
29	16.6	16.6	116.9	16.0	117.0	16.9	16.9	16.4
30	17.4	17.4	17.5	17.4	17.9	17.7	17.7	17.7



Scheme 1. Iridals from the rhizones of *I. tectorum* and *B. chinensis*

1650, 1610, 1450, 1380, 1200, 1060, 960; ESIMS *m/z*: 509 $(M + Na)^+$.

3.6. Iridobelamal A (5)

A colorless viscous oil, $[\alpha]_D$ +51° (*c* 0.08, EtOH); UV (EtOH) λ_{max} (log ε): 254 (4.14); CD (EtOH): $[\theta]_{252}$ (Δε +3.8); ESIMS *m/z*: 497 $(M + Na)^+$.

3.7. Induction of HL-60 cells adhesion

Induction of HL-60 cell-adhesion was examined as described previously (Takahashi et al., 1993, 1999). Briefly, HL-60 cells were cultivated in RPMI-1640 medium (Gibco, NY, USA) containing 10% fetal bovine serum. The cells (3.2×10^4 cells, 0.1 ml) were incubated with test compound for 48 h. Adherent cells accompanied by macrophage-like differentiation were observed by a microscope (×100).

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