

## Tetranortriterpenoids from the Leaves of *Cipadessa cinerascens*

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Seven known and six new tetranortriterpenoids, cineracipadesins A–F (**1**–**6**), were isolated from the leaves of *Cipadessa cinerascens*. Compound **1** has a mexicanolide-type structural skeleton with a rare 9 $\alpha$ ,11 $\alpha$ -epoxide ring; compound **2** has a methyl angolensate-type structure with 9,11-dihydroxy groups, representing the first example of a precursor of a trijugin-type limonoid; and **3** is the first reported methyl angolensate-type limonoid with a ketone group at ring C. Their structures were determined with extensive spectroscopic analysis. X-ray crystallography confirmed the structure of **1**. The ability of compounds **1**–**7** to inhibit the growth of the P-388 murine leukemia cell line was evaluated.

The leaves, bark, and roots of *Cipadessa cinerascens* (Pellegr) Hand.-Mazz have been used in traditional Chinese medicine to treat stomachache, dysentery, rheumatism, malaria, scald, and skin itch.<sup>1</sup> *C. cinerascens*, a shrub belonging to the tribe Trichileae of the subfamily Melioideae (Meliaceae), is mainly distributed in the southwest of China.<sup>2</sup> Except for flavonoids and their glucosides,<sup>3–5</sup> 13 B,D-seco-type limonoids (including four with two novel skeletons) were isolated by the efforts of our group and other teams.<sup>6–10</sup> In the continuing search for novel limonoids from *C. cinerascens*, six new tetranortriterpenoids, cineracipadesins A–F (**1**–**6**), and a new natural product, methyl 2 $\beta$ ,3 $\beta$ -diacetoxy-3-deoxoangolensate (**7**),<sup>11</sup> were discovered to coexist with six known products: cipadesin E,<sup>7</sup> cipatrijugin B,<sup>8</sup> cipatrijugin C,<sup>8</sup> cipatrijugin D,<sup>8</sup> cipadesin A,<sup>6</sup> and cipadesin B.<sup>6</sup> It is noteworthy that the isolated tetranortriterpenoids have four different structural frameworks. We describe here the isolation, structural determination, and cytotoxicity of compounds **1**–**7**.

### Results and Discussion

Cineracipadesin A (**1**) was isolated as colorless crystals (MeOH/CHCl<sub>3</sub>). The HR-ESIMS spectrum indicated a molecular formula of C<sub>32</sub>H<sub>40</sub>O<sub>10</sub>, with a pseudomolecular ion peak at *m/z* 607.2529 [M + Na]<sup>+</sup> (calcd 607.2519), suggesting 12 degrees of unsaturation. The IR spectrum showed the presence of a carbonyl group (1736 cm<sup>-1</sup>). The observation of proton signals for a  $\beta$ -substituted furan ring ( $\delta_{\text{H}}$  7.32 [s, H-21], 6.18 [s, H-22], and 7.41 [t, 1.6 H-23]), a carbomethoxy group ( $\delta_{\text{H}}$  3.73 [s]), four instead of five methyl singlets ( $\delta_{\text{H}}$  1.11 [s, H-18], 0.83 [s, H-19], 0.93 [s, H-28], and 0.82 [s, H-29]), and a characteristic low-field H-17 proton at  $\delta$  5.01 (s) in the <sup>1</sup>H NMR spectrum strongly suggest that **1** is a B,D-seco mexicanolide-type limonoid.<sup>12</sup> The <sup>13</sup>C and DEPT spectra revealed two double bonds and three carbonyls, showing **1** to be heptacyclic. The observed significant downfield shifts of C-8, C-9, C-11, and C-30 in the <sup>13</sup>C NMR spectrum indicate that **1** has 8,30-epoxide and 9,11-epoxide rings, together with the normally occurring five-carbon rings of the mexicanolide-type skeleton, accounting for the necessary seven-ring system. The planar structure of **1** was further confirmed by detailed 2D NMR analysis, as shown in Figure 1, in which a 2-methylbutyryl group was located at C-3 by the HMBC correlations of H-3/C-1'. Single-crystal X-ray diffraction (Figure

2) confirmed the planar structure of **1** and allowed the determination of its relative configuration. Furthermore, there are two conformational isomers in the crystal, and normal van der Waals contacts were observed in the packing of the molecules (see Supporting Information for further details). Cineracipadesin A (**1**), with both a 9,11-epoxide ring and an 8,30-epoxide ring, is the second example of such a limonoid found in nature.<sup>13</sup>

The molecular formula C<sub>31</sub>H<sub>40</sub>O<sub>12</sub> was assigned to cineracipadesin B (**2**) from its HR-ESIMS peak at *m/z* 627.2427 [M + Na]<sup>+</sup> (calcd 627.2417). The IR spectrum showed strong absorption bands at 3451, 1746, and 1249 cm<sup>-1</sup>, suggesting the presence of hydroxy, carbonyl, and ether functionalities, respectively. The <sup>1</sup>H and <sup>13</sup>C NMR spectra (Tables 2 and 3) showed the presence of four tertiary methyls ( $\delta_{\text{H}}$  0.99 [s, H<sub>3</sub>-18], 0.96 [s, H<sub>3</sub>-19], 1.13 [s, H<sub>3</sub>-28], 0.81 [s, H<sub>3</sub>-29];  $\delta_{\text{C}}$  16.3, 16.5, 21.8, 27.0), a methoxy ( $\delta_{\text{H}}$  3.77 [s],  $\delta_{\text{C}}$  52.0), an exocyclic methylene group ( $\delta_{\text{H}}$  5.55 [s], 5.19 [s],  $\delta_{\text{C}}$  113.5), and a  $\beta$ -substituted furan ring ( $\delta_{\text{H}}$  7.44 [s, H-21], 6.41 [s, H-22], 7.44 [s, H-23];  $\delta_{\text{C}}$  121.1, 140.0, 109.4, 143.1), suggesting that compound **2** is a methyl angolensate-type limonoid.<sup>14</sup> 2D NMR analysis confirmed this suggestion and provided detailed information on its structure. The HMBC correlations of H-2/C-2-OAc and H-3/C-3-OAc identified two *O*-acetyl groups at C-2 and C-3, respectively. Two singlets ( $\delta_{\text{H}}$  3.60 and 2.06), which had no correlations with any carbon signals in the HSQC spectrum, were assigned as OH-9 and OH-11, respectively, due to HMBC correlations of OH-9/C-9, C-10, and C-11, and OH-11/C-9 and C-11. The planar structure of **2** was thus established as shown in Figure 2.

The relative configuration of **2** was deduced from the analysis of its ROESY correlations. As shown in Figure 2, the observed correlations of H-1/H-2, H<sub>3</sub>-19, and H-30a, and H-2/H<sub>3</sub>-19 and H<sub>3</sub>-28, together with H-3/H<sub>3</sub>-28 and H-30b/H<sub>3</sub>-18, indicated that H-1, H-2, H-3, Me-18, Me-19, and Me-28 are cofacial and were arbitrarily assigned as  $\alpha$ -oriented, which determined the  $\beta$ -orientation of Me-29 accordingly. The cross-peaks from H<sub>3</sub>-29 to H-5, from H-5 to H-11 and H-12 $\beta$ , and from H-12 $\beta$  to H-17 indicated the  $\beta$ -orientations of H-5, H-11, and H-17. The hydroxy group at C-9 was determined to be  $\alpha$ -oriented by its ROESY correlation of OH-9/H-30a, and the hydroxy group at C-11 was assigned as  $\alpha$ -oriented by the  $\beta$ -assignment of H-11. Therefore, the structure of **2**, methyl 9 $\alpha$ ,11 $\alpha$ -dihydroxy-2 $\beta$ ,3 $\beta$ -diacetoxy-3-deoxoangolensate, was established as shown in Figure 2 and is the first identified precursor of a trijugin-type limonoid in nature.<sup>15–19</sup> The proposed biogenetic relationship between cineracipadesin B (**2**) and cipadesin E, a trijugin-type limonoid, is shown in Figure 3.

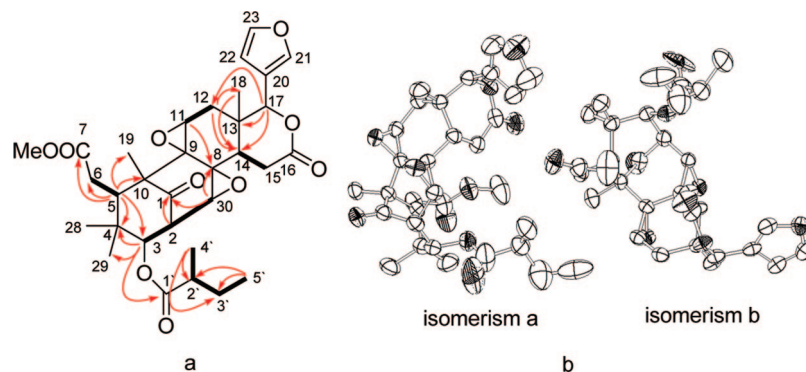
Cineracipadesin C (**3**), a white, amorphous powder, has the molecular formula C<sub>29</sub>H<sub>36</sub>O<sub>10</sub>, as established by HR-ESIMS (*m/z*

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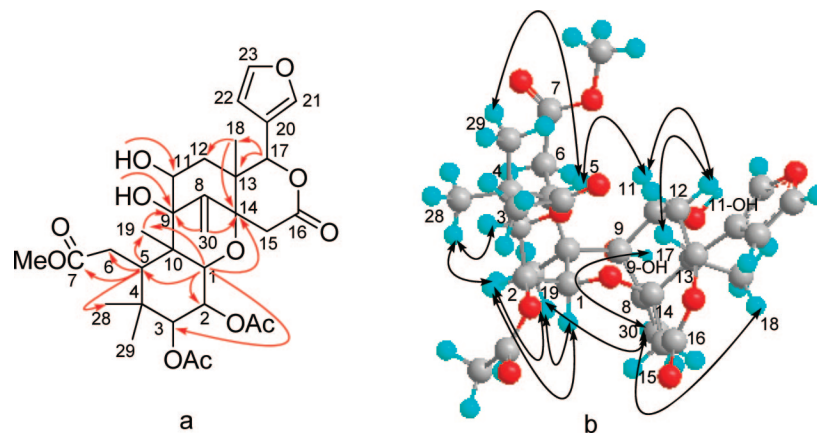
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**Figure 1.**  $^1\text{H}$ – $^1\text{H}$  COSY (–) and selected HMBC (→) correlations of **1** (a); single-crystal X-ray structure of **1** (b).



**Figure 2.** Selected HMBC (→) (a) and ROESY (↔) correlations (b) of **2**.

**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR Data of Cineracipadesin A (**1**) in  $\text{CDCl}_3$  at 400 MHz

C	$\delta_{\text{H}}$ (mult, $J$ )	$\delta_{\text{C}}$	C	$\delta_{\text{H}}$ (mult, $J$ )	$\delta_{\text{C}}$
1		208.8	16		169.1
2	3.69 <sup>a</sup>	48.7	17	5.01, s	82.7
3	5.10, d (9.5)	77.3	18	1.11, s	23.3
4		38.5	19	0.83, s	10.3
5	3.31, dd (8.0, 5.6)	43.1	20		121.8
6a	2.45, m	32.0	21	7.32, s	140.5
6b	2.40, m		22	6.18, s	109.2
7		173.6	23	7.41, t (1.6)	143.6
8		62.5	28	0.93, s	24.5
9		59.3	29	0.82, s	16.8
10		49.8	30	3.26, d (2.8)	60.5
11	3.23, t (1.6)	56.3	OMe	3.73, s	52.5
12 $\alpha$	2.00, d (15.0)	27.5	1'		175.5
12 $\beta$	2.42 <sup>a</sup>		2'	2.56, m	41.1
13		34.0	3'a	1.78, ddd (21.2, 14.8, 7.6)	26.8
14	1.65, m	38.5	3'b	1.62, ddd (21.2, 14.8, 7.6)	
15a	3.73 <sup>a</sup>	28.9	4'	0.97, t (7.6)	11.6
15b	2.74, dd (18.4, 6.8)		5'	1.26, d (7.2)	16.8

<sup>a</sup> Overlapped, without denoting multiplicity.

found 567.2195  $[\text{M} + \text{Na}]^+$ , calcd 567.2206). Its IR spectrum showed absorption bands similar to those of **2**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for **3** also showed close similarity to those of **2**, except for the absence of an acetyl group and two oxygenated methines in the latter and the presence of an additional methylene ( $\delta_{\text{H}}$  2.19 [ddd, 16.0, 4.5, 3.0], 2.03 [dt, 16.0, 2.0],  $\delta_{\text{C}}$  27.8) and a ketone group ( $\delta_{\text{C}}$  208.7) in the former. HMBC correlations from H-12 and 9-OH to the ketone group, as well as from the methylene to C-3, C-4, and C-10, confirmed that their positions should be at C-11 and C-2, respectively. Detailed 2D NMR analysis identified the planar and stereo structures of **3**, cineracipadesin C, which is the first methyl angolensate-type limonoid identified with a ketone group at ring C.

Cineracipadesin D (**4**) was obtained as a white, amorphous powder. The HR-ESIMS spectrum of **4** indicated a molecular formula of  $\text{C}_{31}\text{H}_{40}\text{O}_{10}$  from the pseudomolecular ion peak at  $m/z$  595.2505  $[\text{M} + \text{Na}]^+$  (calcd 595.2519). The IR spectrum showed strong absorption bands at 1736 and 1243  $\text{cm}^{-1}$ , suggesting the presence of carbonyl and ether functionalities, respectively. Comparison of the 1D spectroscopic data of **4** with those of **3** showed an overall similarity, except that the hydroxy group at C-9 is replaced with a methine in the latter ( $\delta_{\text{H}}$  2.14 [br s],  $\delta_{\text{C}}$  54.9) and the C-11 ketone is replaced with an acetyl group. The HMBC correlations of the methine proton with C-8, C-30, C-14, C-10, C-11, and C-12, together with the NOE correlations of the proton with H<sub>3</sub>-19, supported the first observation and demonstrated the  $\alpha$ -orientation of H-9. The HMBC correlations of H-11/C-8, C-12, and C-11-OAc confirmed the second observation, and the ROE correlation of H-11/H-5 assigned an  $\alpha$ -orientation to 11-OAc. Consequently, cineracipadesin D (**4**) was deduced to be methyl 3 $\beta$ ,11 $\alpha$ -diacetoxy-3-deoxoangolensate.

Cineracipadesin E (**5**) was obtained as a white, amorphous powder with a molecular formula of  $\text{C}_{33}\text{H}_{42}\text{O}_{13}$ , in agreement with the HR-ESIMS analysis ( $m/z$  669.2552  $[\text{M} + \text{Na}]^+$ , calcd 669.2523). Its IR spectrum resembles that of **2**. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for **5** were similar to those of **2**, with the only exception being that 11-OH in **2** is substituted with 11-OAc in **5**, as suggested by the upfield-shifted C-11 signal ( $\Delta$  3.4 ppm) and downfield-shifted H-11 resonance ( $\Delta$  1.2 ppm) in **5**. The relative configuration of **5** was suggested to be the same as that of **2**, on the basis of similar chemical shifts and ROESY data, thus identifying the structure of **5**.

Cineracipadesin F (**6**) was isolated as a white, amorphous solid. Its molecular formula  $\text{C}_{33}\text{H}_{40}\text{O}_{13}$  was determined by the  $[\text{M} + \text{Na}]^+$  ion peak 667.2388  $[\text{M} + \text{Na}]^+$  (calcd 667.2366) on HR-ESIMS. Its IR spectrum closely resembles that of **3**. The  $^{13}\text{C}$  NMR data for **6** showed obvious downfield-shifted signals for C-12, C-13, and

**Table 2.** <sup>1</sup>H NMR Data of Cineracipadesins B–F (2–6)

no.	2	3	4	5	6
1	3.54, d (4.6)	3.55, dd (4.5, 2.0)	3.21, d (4.0)	3.58, d (4.5)	4.12, d (2.3)
2 $\alpha$	5.19, m	2.19, ddd (16.0, 4.5, 3.0)	2.07 <sup>a</sup>	5.22, dd (4.5, 3.0)	5.24, t (3.6)
2 $\beta$		2.03, dt (16.0, 2.0)	1.90 <sup>a</sup>		
3	5.05, d (2.5)	4.80, t (3.0)	4.73, t (2.0)	5.10, d (3.0)	5.13, d (3.6)
5	2.69, d (10.0)	2.79, d (9.0)	2.71, d (10.0)	2.82, d (9.0)	3.12, t (4.4)
6 $\alpha$	3.13, d (17.5)	2.69, d (18.0)	2.47, dd (17.0, 10.0)	3.05, d (14.0)	2.26, dd (18.6, 4.4)
6 $\beta$	2.44, dd (17.5, 10.0)	2.32, dd (18.0, 9.0)	2.70, d (17.0)	2.46, dd (14.0, 9.0)	2.79, d (18.6)
9			2.14, br s		
11	4.67, s		5.57, br t (2.0)	5.80, s	
12 $\alpha$	1.69, d (14.5)	2.45, d (14.1)	1.49, d (14.5)	1.75, dd (11.6, 1.6)	1.95, d (15.1)
12 $\beta$	2.59, m	3.60, d (14.1)	2.43, dd (14.5, 4.5)	2.60, dd (11.6, 3.2)	3.14, d (15.1)
15 $\alpha$	2.55, d (17.7)	2.82, d (18.2)	2.93, d (18.0)	2.59, d (14.0)	2.79, d (17.2)
15 $\beta$	2.91, d (17.7)	3.00, d (18.2)	2.62, d, (18.0)	2.94, d (14.0)	2.86, d (17.2)
17	5.78, s	5.94, s	5.75, s	5.80, s	6.38, s
18	0.99, s	0.76, s	0.98, s	0.94, s	0.92, s
19	0.96, s	0.91, s	0.85, s	0.99, s	1.14, s
21	7.44, s	7.48, s	7.41, s	7.46, s	7.57, s
22	6.41, s	6.41, s	6.39, s	6.43, s	6.47, s
23	7.44, s	7.46, t (1.6)	7.41, s	7.46, s	7.45, s
28	1.13, s	0.78, s	0.99, s	1.16, s	1.07, s
29	0.81, s	0.97, s	0.83, s	0.89, s	0.88, s
30a	5.55, s	5.70, s	5.13, s	5.53, s	5.59, s
30b	5.19, s	5.15, s	5.03, s	5.18, s	5.46, s
OMe	3.77, s	3.68, s	3.79, s	3.77, s	3.72, s
2-OAc	2.00, s			2.04, s	2.14, s
3-OAc	2.14, s	2.14, s	2.09, s	2.17, s	2.11, s
11-OAc			1.95, s	2.03, s	2.08, s
9-OH	3.60, s	4.21, s		2.40 s	
11-OH	2.06, s				

<sup>a</sup> Overlapped, without denoting multiplicity.**Table 3.** <sup>13</sup>C NMR Data of Cineracipadesins B–F (2–6)

position	2	3	4	5	6
1	74.4	74.3	72.8	74.4	73.8
2	66.2	27.8	27.5	66.2	65.4
3	76.1	75.6	76.1	76.0	74.2
4	39.4	38.1	37.7	39.3	39.0
5	36.7	37.3	36.5	36.2	36.8
6	30.9	30.2	31.4	30.9	29.3
7	175.5	174.1	174.9	174.3	173.7
8	145.2	144.9	142.3	145.3	144.2
9	79.6	87.7	54.9	78.4	202.3
10	50.6	52.9	44.8	51.7	55.9
11	71.5	208.7	71.7	75.1	86.6
12	37.7	46.6	34.1	34.8	44.4
13	40.4	42.8	40.9	40.3	44.8
14	81.5	80.6	79.9	81.3	91.4
15	33.6	34.1	33.5	33.6	33.8
16	169.3	169.0	170.4	169.3	168.2
17	79.7	78.2	79.5	79.6	79.1
18	16.3	14.6	15.5	15.6	18.0
19	16.5	16.7	21.8	16.6	19.0
20	121.1	120.2	120.9	120.8	121.6
21	140.0	140.3	140.1	140.1	139.8
22	109.4	109.2	109.6	109.4	108.5
23	143.1	143.5	143.0	143.2	143.7
28	21.8	21.6	21.4	21.8	22.8
29	27.0	26.3	26.9	27.0	27.3
30	113.5	114.5	114.4	112.9	115.5
OMe	52.0	51.9	52.0	52.1	52.0
2-OAc	170.7			170.6	169.7
	20.7			20.7	21.3
3-OAc	170.1	169.9	170.1	170.0	170.4
	21.3	21.6	21.6	21.3	20.8
11-OAc			170.0	169.4	170.8
			21.3	21.3	20.4

C-14 relative to those of **2**, **3**, **4**, and **5**. These facts, combined with the presence of a C-9 ketone established by HMBC correlations of H<sub>3</sub>-19/C-9 and H-12/C-9, strongly suggest that **6** is a trijugin-type limonoid, characterized by a contracted five-membered ring C with an exocyclic carbonyl at C-9.<sup>12</sup> Comparison of the <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for **6** with those for cipadesin E (**8**)<sup>4</sup> suggested

that their structures are closely related; the major difference was that 11-OH in **8** was acetylated in **6**, as indicated by an additional acetyl group ( $\delta_{\text{H}}$  2.08 [s],  $\delta_{\text{C}}$  20.4, 170.8) and analysis of the 2D NMR data for **6**. Therefore, the acetyl group at C-11 is tentatively assigned as  $\alpha$ -oriented from biosynthetic considerations,<sup>20</sup> because of the lack of corresponding correlation information from the ROESY spectrum. Thus, the structure of **6** was established.

The structures of known compounds were identified by comparison of their physical data with those in the literature, and compound **7** was isolated from nature for the first time.

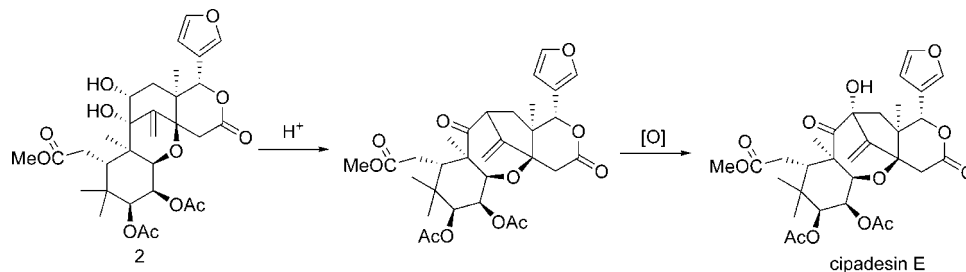
The genus *Cipadessa* has previously been placed by Harms<sup>2</sup> in the tribe Turraeeae, as sister group to *Munronia* Wight, but ultimately in the tribe Trichileae of the subfamily Melioideae based on its vegetative, floral, fruit, and pollen characteristics.<sup>21</sup> Phytochemical analysis of the genus revealed its 15 unexpected mexicanolide-type limonoids, which predominantly occur in the subfamily Swietenioideae.<sup>22–26</sup> These findings led to debate regarding its position in the Meliaceae family.<sup>27</sup> However, our report of the co-occurrence of 13 limonoids with four types of skeletons that must be produced via several biosynthetic routes in *C. cinerascens* is consistent with the evolutionary trend of limonoid chemistry in the subfamily Melioideae,<sup>28</sup> which supports the position of the genus in the subfamily despite the new-found mexicanolide-type limonoids in the genus. In addition, the isolation of trijugin-type limonoids, first found in and common among the genus *Trichilia* P. Browne,<sup>15,29,30</sup> also shows that *C. cinerascens* is closely related to the genus *Trichilia* and confirms its placement in the Trichileae tribe. Thus, our phytochemical investigation confirmed the taxonomy proposed by Pennington and Styles.<sup>21</sup>

Cineracipadesins A–F (**1–6**) and methyl 2 $\beta$ ,3 $\beta$ -diacetoxo-3-deoangolensate (**7**) were tested for *in vitro* cytotoxicity against the P-388 murine leukemia cell line using the MTT method.<sup>31</sup> However, none of the compounds showed activity against the tumor cells (50% effective dose for clonal inhibition, ED<sub>50</sub> > 5  $\mu$ g/mL).

## Experimental Section

**General Experimental Procedures.** The melting point of **1** was obtained on an X-4 apparatus and is uncorrected. Optical rotations were





**Figure 3.** Proposed biosynthetic relationship between cineracipadesin B (2) and cipadesin E.

measured with a Perkin-Elmer model 241 polarimeter. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer with a KBr disk. <sup>1</sup>H and 2D NMR spectra were recorded on a Bruker DRX-500 instrument, and <sup>13</sup>C NMR spectra on a Bruker AM-400 spectrometer. Chemical shifts were reported using TMS as the internal standard. ESIMS and HR-ESIMS spectra were measured with a Finnigan MAT 90 instrument and VG Auto Spec-3000 spectrometer, respectively. The X-ray data were collected on a MAC DIP-2030K diffractometer with a graphite monochromator ( $\omega$  scan,  $2\theta_{\max} = 50.0^\circ$ ) with Mo K $\alpha$  radiation. Column chromatography was performed on silica gel (90–150  $\mu\text{m}$ ; Qingdao Marine Chemical Inc.), Sephadex LH-20 (40–70  $\mu\text{m}$ ; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep RP-18 gel (40–63  $\mu\text{m}$ ; Merck, Darmstadt, Germany). Semipreparative HPLC was performed on a Zorbax SB-C-18 column (i.d. 9.4  $\times$  250 mm; Agilent Co. Ltd., USA). Precoated silica gel GF254 and HF254 plates (Qingdao Haiyang Chemical Plant, Qingdao, People's Republic of China) were used for thin-layer chromatography.

**Plant Material.** The leaves of *C. cinerascens* were collected in July 2007 from Mengla, Yunnan Province, People's Republic of China. The plant was identified by Prof. Jing-Yun Cui of the Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (no. KUN 0596223) was deposited at the Kunming Institute of Botany, Kunming, People's Republic of China.

**Extraction and Isolation.** The air-dried powder of the plant material (11.5 kg) was extracted three times with 95% EtOH. The extracts were combined and concentrated, then suspended in H<sub>2</sub>O. The water layer was further extracted with petroleum ether (PE), CHCl<sub>3</sub>, and *n*-BuOH. The CHCl<sub>3</sub> extract (500 g) was subjected to silica gel column chromatography, eluted with PE/EtOAc (from 1:0 to 1:1) and then PE/EtOAc/CH<sub>3</sub>OH (from 1:1:0 to 1:1:1), yielding 10 fractions (A1–A10). Fraction A2 (5 g) was applied to an MCI gel column (eluted with MeOH/H<sub>2</sub>O from 5:5 to 10:0), further purified on Sephadex LH-20, and applied to a silica gel column (eluted with acetone/CHCl<sub>3</sub> from 10:1 to 9:1) to yield **1** (20 mg), **3** (5 mg), and **4** (6 mg). Fraction A5 (18 g) was applied to a C<sub>18</sub> column (eluted with MeOH/H<sub>2</sub>O from 1:9 to 10:0), and the fraction eluted with 40% MeOH was purified further on Sephadex LH-20 (CHCl<sub>3</sub>/MeOH, 1:1) to yield fraction B1. Fraction B1 was then further purified by semipreparative HPLC to yield **2** (3 mg), **5** (4 mg), and **6** (4 mg).

**Cineracipadesin A (1):** colorless crystals (MeOH/CH<sub>2</sub>Cl<sub>2</sub>); mp 210–212 °C;  $[\alpha]_D^{25} -110.0$  (*c* 0.005, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3460 (water), 3412, 2949, 1736, 1460, 1262, 1182, 1118, 1024 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 1; <sup>13</sup>C NMR data, see Table 1; positive-ion ESIMS *m/z* 585.3 [M + H]<sup>+</sup>; HR-ESIMS *m/z* 607.2529 [M + Na]<sup>+</sup>, calcd 607.2519.

**Cineracipadesin B (2):** white, amorphous solid;  $[\alpha]_D^{25} +19.5$  (*c* 0.105, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3451, 2978, 1745, 1686, 1378, 1248, 1165, 1083, 1048, 1021 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 2; <sup>13</sup>C NMR data, see Table 3; positive-ion ESIMS *m/z* 627.5 [M + Na]<sup>+</sup>; HR-ESIMS *m/z* 627.2427 [M + Na]<sup>+</sup>, calcd 627.2417.

**Cineracipadesin C (3):** white, amorphous powder;  $[\alpha]_D^{25} -94.2$  (*c* 0.020, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3436, 2951, 1744, 1433, 1375, 1247, 1176, 1081, 1060, 1029 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 2; <sup>13</sup>C NMR data, see Table 3; positive-ion ESIMS *m/z* 567.5 [M + Na]<sup>+</sup>; HR-ESIMS *m/z* 567.2195 [M + Na]<sup>+</sup>, calcd 567.2206.

**Cineracipadesin D (4):** white, amorphous powder;  $[\alpha]_D^{25} -38.3$  (*c* 0.005, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3445 (water), 2951, 1736, 1462, 1372, 1243, 1086, 1054, 1034 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 2; <sup>13</sup>C NMR data, see Table 3; positive-ion ESIMS *m/z* 573.3 [M + H]<sup>+</sup>, 595.5 [M + Na]<sup>+</sup>; HR-ESIMS *m/z* 595.2505 [M + Na]<sup>+</sup>, calcd 595.2519.

**Cineracipadesin E (5):** white, amorphous powder;  $[\alpha]_D^{25} -54.8$  (*c* 0.035, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3468, 1740, 1375, 1246, 1049 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 2; <sup>13</sup>C NMR data, see Table 3; positive-ion ESIMS *m/z* 647.6 [M + H]<sup>+</sup>; HR-ESIMS *m/z* 669.2552 [M + Na]<sup>+</sup>, calcd 669.2523.

**Cineracipadesin F (6):** white, amorphous solid;  $[\alpha]_D^{25} 0.0$  (*c* 0.012, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3445, 1745, 1375, 1235, 1075, 1038 cm<sup>-1</sup>; <sup>1</sup>H NMR data, see Table 2; <sup>13</sup>C NMR data, see Table 3; positive-ion ESIMS *m/z* 667.4 [M + H]<sup>+</sup>; HR-ESIMS *m/z* 667.2388 [M + Na]<sup>+</sup>, calcd 667.2366.

**Cytotoxicity Bioassays.** Cytotoxicity of compounds **1–7** against the P-388 murine leukemia cell line was evaluated by the methylthiazol-tetrazolium method. Cells were plated in a 96-well plate 24 h before treatment and continuously exposed to different concentrations of compounds (100, 10, 1, and 0.1  $\mu\text{M}$ ) for 72 h. After compound treatment, cells were counted as described in the literature.<sup>30</sup>

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**Supporting Information Available:** X-ray crystallographic data for cineracipadesin A (**1**); 1D, 2D NMR spectra for cineracipadesins A–F (**1–6**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

- Editorial Committee of Administration Bureau of Traditional Chinese Medicine. *Chinese Materia Medica*; Shanghai Scientific and Technical Press: Shanghai, 1998; Vol. 5, pp 3860–3861.
- Delectis Florae Reipublicae Popularis Sinicae Agenda Academiae Sinicae Edita. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, 1997; Vol. 43 (3), pp 58–60.
- Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1990**, *21*, 2–4.
- Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1991**, *22*, 6–8.
- Liang, L.; Zhong, C.-C.; Xiao, Z.-Y. *Zhongcaoyao* **1994**, *25*, 236–237.
- Yuan, X. H.; Li, B. G.; Zhou, M.; Qi, H. Y.; Zhang, G. L. *Org. Lett.* **2005**, *7*, 5051–5053.
- Yuan, X. H.; Li, B. G.; Xu, C. X.; Zhou, M.; Qi, H. Y.; Zhang, G. L. *Chem. Pharm. Bull.* **2007**, *55*, 902–904.
- Di, Y. T.; He, H. P.; Liu, H. Y.; Yi, P.; Zhang, Z.; Ren, Y. L.; Wang, J. S.; Sun, Q. Y.; Yang, F. M.; Fang, X.; Li, S. L.; Zhu, H. J.; Hao, X. J. *J. Nat. Prod.* **2007**, *70*, 1352–1355.
- Ren, Y. L.; Tang, Q. R.; Di, Y. T.; He, H. P.; Zhang, Z.; Li, S. L.; Hao, X. J. *Helv. Chim. Acta* **2007**, *38*, 764–768.
- Fang, X.; Di, Y. T.; He, H. P.; Liu, H. Y.; Zhang, Z.; Ren, Y. L.; Gao, Z. L.; Gao, S.; Hao, X. J. *Org. Lett.* **2008**, *10*, 1905–1908.
- Kehrli, A. R. H.; Taylor, D. A. H. *J. Chem. Soc., Perkin Trans 1* **1990**, 2067–2070.
- Saad, M. M. G.; Iwagawa, T.; Doe, M.; Nakatani, M. *Tetrahedron* **2003**, *59*, 8027–8033.
- Coomes, P. H.; Mulholland, D. A.; Randrianarivoelosia, M. *Phytochemistry* **2005**, *66*, 1100–1107.
- Chan, W. R.; Magnus, K. E.; Speight, P.; Mootoo, B. S. *J. Chem. Soc.* **1967**, *C*, 171–177.

- (15) Purushothaman, K. K.; Venkatanarasimhan, M.; Sarada, A.; Connolly, J. D.; Rycroft, D. S. *Can. J. Chem.* **1987**, *65*, 35–37.
- (16) Mulholland, D. A.; Iourine, S. E. *Phytochemistry* **1998**, *47*, 1357–1361.
- (17) Zhang, H. P.; Wu, S. H.; Shen, Y. M.; Ma, Y. B.; Wu, D. G.; Qi, S. H.; Luo, X. D. *Can. J. Chem.* **2003**, *81*, 253–257.
- (18) Ismail, I. S.; Ito, H.; Hatano, T.; Taniguchi, S.; Yoshida, T. *Phytochemistry* **2003**, *64*, 1345–1349.
- (19) Ismail, I. S.; Ito, H.; Hatano, T.; Taniguchi, S.; Yoshida, T. *Chem. Pharm. Bull.* **2004**, *52*, 1145–1147.
- (20) Lei, C.; Huang, S. X.; Chen, J. J.; Yang, L. B.; Xiao, W. L.; Chang, Y.; Lu, Y.; Huang, H.; Pu, J. X.; Sun, H. D. *J. Nat. Prod.* **2008**, *71*, 1228–1232.
- (21) Pennington, T. D.; Styles, B. T. *Blumea* **1975**, *22*, 419–540.
- (22) Luo, X. D.; Wu, S. H.; Ma, Y. B.; Wu, D. G. *Phytochemistry* **2000**, *55*, 867–872.
- (23) Marpaung, L.; Nakamura, N.; Kaduda, H.; Hattori, M. *J. Nat. Med.* **2001**, *55*, 220.
- (24) Leite, A. C.; Fernandes, J. B.; Da Silva, M. F.; das, G. F.; Vieira, P. C. *Z. Naturforsch* **2005**, *60b*, 351–356.
- (25) Leite, A. C.; Bueno, F. C.; Oliveira, C. G.; Fernandes, J. B.; Vieira, P. C.; Da Silva, M. F.; das, G. F.; Bueno, O. C.; Pagnocca, F. C.; Hebling, M.J. A.; Mauricio, B. J. *J. Braz. Chem. Soc.* **2005**, *16*, 1391–1395.
- (26) Gan, L. S.; Wang, X. N.; Wu, Y.; Yue, J. M. *J. Nat. Prod.* **2007**, *70*, 1344–1347.
- (27) Mulholland, D. A.; McFarland, K.; Randrianarivojosia, M. *Biochem. Syst. Ecol.* **2006**, *34*, 365–369.
- (28) Da Silva, M. F.; das, G. F.; Gottlieb, O. R.; Dreyer, D. L. *Biochem. Syst. Ecol.* **1984**, *12*, 299–310.
- (29) Venkatanarasimhan, M.; Kundu, A. B.; Patra, A. *Indian J. Chem.* **1978**, *29B*, 970.
- (30) Wang, X.-N.; Fan, C., Q.; Sheng, Y.; Gan, L. S.; Yue, J. M. *Phytochemistry* **2008**, *69*, 1319–1327.
- (31) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

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