

## Ceramicines E—I, New Limonoids from *Chisocheton ceramicus*

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Five new limonoids, ceramicines E—I (1–5), have been isolated from the bark of *Chisocheton ceramicus*. The structures and relative stereochemistry of them were fully elucidated based on 1D- and 2D-NMR data. Ceramicines E—I (1–5) exhibited moderate cell growth inhibitory activities on a range of cell lines (HL-60, A549, MCF7, and HCT116). The absolute structure of previously isolated ceramicine B (6) was also elucidated by circular dichroism (CD) and X-ray analysis.

**Key words** limonoid; *Chisocheton ceramicus*; ceramicine; cytotoxic activity

Confined to the plants from the order of Rutales, limonoids are known to be especially bountiful in plants from the Meliaceae family.<sup>1)</sup> The highly oxidative limonoids obtained from Meliaceae were known to possess various significant biological activities. Activities such as insecticidal, insect antifeedant, antibacterial, antifungal, antimalarial, anticancer, and antiviral have been reported.<sup>1–5)</sup>

In the course of our studies on the bark extract of *Chisocheton ceramicus*,<sup>6,7)</sup> we have isolated five new limonoids, ceramicines E—I (1–5). Here we sought to report the isolation, structure elucidation, and cytotoxic activity of 1–5 isolated from *C. ceramicus*. In addition, we also wish to report the absolute structure of the highly antiplasmodial ceramicine B (6)<sup>7)</sup> by using circular dichroism (CD) exciton chirality and X-ray crystallography methods.

The methanol extract from the bark of *C. ceramicus* was partitioned between hexane, ethyl acetate, *n*-butanol, and water. Subsequent purification by a silica gel column and high performance liquid chromatography (HPLC) led to the isolation of five new limonoids, ceramicines E (1, 2.0 mg, 0.029% yield), F (2, 9.5 mg, 0.14% yield), G (3, 5.6 mg, 0.08% yield), H (4, 0.3 mg, 0.004% yield), and I (5, 4.3 mg, 0.06% yield) together with the previously reported ceramicines B (6) and C.<sup>7)</sup>

Ceramicine E {1,  $[\alpha]_D^{28} +20$  ( $c=0.5$ , CHCl<sub>3</sub>)} was isolated as a colorless amorphous solid and had molecular formula,

C<sub>27</sub>H<sub>32</sub>O<sub>7</sub> as determined by high resolution-electrospray ionization-time-of-flight-mass spectra (HR-ESI-TOF-MS) [ $m/z$  469.2206 (M+H)<sup>+</sup>,  $\Delta -2.0$  mmu]. IR absorptions suggested the presence of a carbonyl (1718 cm<sup>-1</sup>) and a hydroxyl (3437 cm<sup>-1</sup>) groups. <sup>1</sup>H- and <sup>13</sup>C-NMR data (Tables 1, 2) revealed 27 carbon resonances due to two carbonyls, two *sp*<sup>2</sup> quaternary carbons, four *sp*<sup>3</sup> quaternary carbons, four *sp*<sup>2</sup> methines, seven *sp*<sup>3</sup> methines, four *sp*<sup>3</sup> methylenes, and four methyls. Among them, two *sp*<sup>2</sup> methines ( $\delta_C$  139.7, 142.6), four *sp*<sup>3</sup> methines ( $\delta_C$  52.9, 57.5, 65.3, 74.2), one *sp*<sup>3</sup> quaternary carbon ( $\delta_C$  57.1), and one *sp*<sup>3</sup> methylene ( $\delta_C$  47.7) are connected to an oxygen atom.

Five partial structures **a** (from C-2 to C3), **b** (from C-5 to C-7), **c** (from C-9 to C-12), **d** (from C-15 to C-17), and **e** (from C-22 to C-23) were deduced from <sup>1</sup>H–<sup>1</sup>H correlation spectroscopy (COSY) analysis of **1** in CDCl<sub>3</sub> (Fig. 1). The existence of two epoxides at C-2 and C-3, and C-4 and C-29 was deduced by the <sup>1</sup>H and <sup>13</sup>C chemical shifts (H-2:  $\delta_H$  3.35, C-2:  $\delta_C$  52.9; H-3:  $\delta_H$  3.01, C-3:  $\delta_C$  57.5; C-4:  $\delta_C$  57.1; H-29:  $\delta_H$  2.84 and 3.24, C-29:  $\delta_C$  47.7), which was confirmed by heteronuclear multiple bond connectivity (HMBC) analysis for H-3 of C-4, for H-5 of C-4 and C-29, and for H<sub>2</sub>-29 to C-3 as shown in Fig. 1. The HMBC correlations for H-2 and H<sub>3</sub>-19 of C-1 ( $\delta_C$  202.3), for H<sub>3</sub>-19 of C-5 ( $\delta_C$  31.8), C-9 ( $\delta_C$  32.0), and C-10 ( $\delta_C$  51.6), and for H<sub>3</sub>-30 of C-7 ( $\delta_C$  74.2), C-8 ( $\delta_C$  43.9), and C-9 bridged the partial structures of

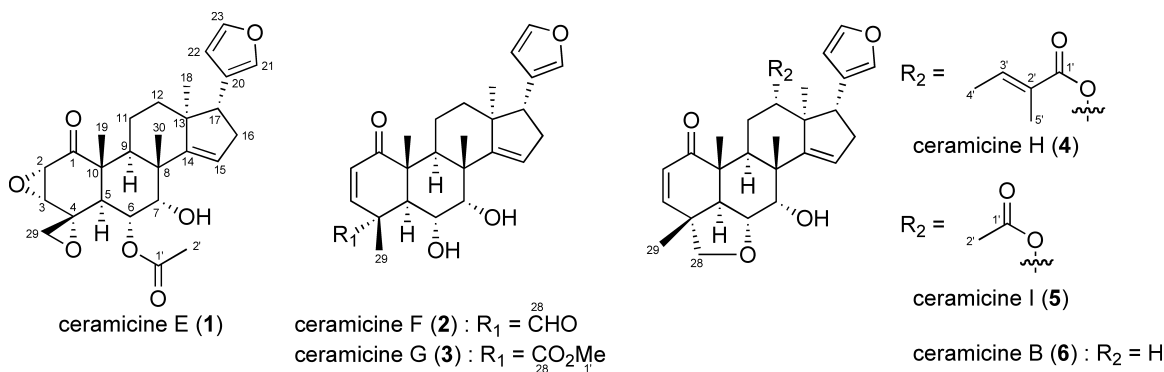


Chart 1

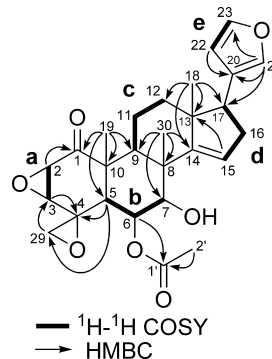
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Table 1.  $^1\text{H-NMR}$  Data ( $J$ , Hz) of Ceramicines E—I (1—5) in  $\text{CDCl}_3$  at 300 K

	1	2	3	4	5
1					
2	3.35 (d, 3.6)	5.95 (d, 10.1)	5.83 (d, 10.1)	5.82 (d, 9.6)	5.83 (d, 9.6)
3	3.01 (d, 3.6)	6.06 (d, 10.1)	6.32 (d, 10.1)	6.95 (d, 9.6)	6.96 (d, 9.6)
4					
5	3.18 (d, 11.7)	2.82 (d, 11.4)	3.35 (d, 11.6)	2.69 (d, 12.4)	2.69 (d, 12.4)
6	5.32 (dd, 11.7, 2.6)	4.09 (ddd, 12.1, 10.9, 3.1)	4.05 (br d, 11.9)	4.29 (dd, 12.4, 3.2)	4.26 (dd, 12.4, 3.2)
7	3.79 (br d, 2.6)	4.02 (dd, 3.1, 1.6)	3.99 (d, 2.4)	4.24 (d, 3.2)	4.24 (d, 3.2)
8					
9	2.68 m	2.52 m	2.52 m	2.59 m	2.60 m
10					
11a	1.60 m	1.63 m	1.58 m	2.27 m	2.30 m
11b	2.35 m	2.56 m	2.54 m	2.49 m	2.42 m
12a	1.60 m	1.59 m	1.58 m	5.10 (dd, 9.0, 7.0)	5.10 (dd, 9.0, 7.0)
12b	1.91 m	1.92 m	1.93 m		
13					
14					
15	5.52 (dd, 1.4, 3.4)	5.57 (dd, 3.6, 1.6)	5.57 (dd, 1.1, 3.4)	5.69 (dd, 1.2, 3.2)	5.68 m
16a	2.40 (ddd, 15.5, 7.5, 3.4)	2.41 (ddd, 15.6, 7.4, 3.6)	2.42 (ddd, 15.4, 7.3, 3.4)	2.45 m	2.47 m
16b	2.51 (ddd, 15.5, 10.9, 1.4)	2.51 m	2.52 m	2.55 m	2.53 m
17	2.85 (dd, 7.5, 10.9)	2.86 (dd, 10.9, 7.4)	2.86 (dd, 11.1, 7.3)	3.04 (dd, 11.0, 7.8)	3.04 (dd, 10.8, 8.0)
18	0.88 s	0.90 s	0.90 s	1.08 s	1.04 s
19	1.24 s	1.29 s	1.28 s	1.16 s	1.16 s
20					
21	7.25 m	7.26 s	7.26 br s	7.13 br s	7.21 br s
22	6.29 (dd, 1.6, 0.6)	6.29 (dd, 1.8, 1.0)	6.30 m	6.22 br s	6.26 br s
23	7.38 (dd, 1.6, 1.6)	7.39 (dd, 1.8, 1.6)	7.38 (dd, 1.7, 1.7)	7.27 (dd, 1.6, 1.6)	7.33 br s
28a		9.28 s		3.63 m	3.63 m
28b				3.79 (d, 6.8)	3.79 (d, 7.2)
29a	2.84 (dd, 3.7, 0.8)	1.54 s	1.61 s	1.33 s	1.34 s
29b	3.24 (d, 3.7)				
30	1.18 s	1.18 s	1.18 s	1.13 s	1.12 s
1'			3.70 s		
2'	2.13 s				1.93 s
3'				6.58 (br q, 7.6)	
4'				1.72 (br d, 7.6)	
5'				1.65 br s	

Table 2.  $^{13}\text{C-NMR}$  Data of Ceramicines E—I (1—5) in  $\text{CDCl}_3$  at 300 K

	1	2	3	4	5
1	202.3	202.8	203.3	202.4	202.4
2	52.9	128.8	127.0	130.0	130.0
3	57.5	143.9	146.4	151.0	151.1
4	57.1	51.8	47.9	41.8	41.8
5	31.8	44.1	43.3	47.6	47.6
6	65.3	66.3	66.6	73.7	73.7
7	74.2	74.6	75.0	72.0	72.0
8	43.9	44.0	44.2	46.5	46.4
9	32.0	33.2	33.6	36.5	36.2
10	51.6	47.9	48.6	47.2	47.1
11	18.2	17.9	17.9	26.9	26.8
12	33.2	32.8	32.9	77.2	77.2
13	46.9	47.0	47.1	51.7	51.4
14	159.1	160.4	160.7	156.7	156.6
15	120.5	120.2	120.0	122.6	122.6
16	34.3	34.2	34.3	36.4	36.4
17	51.9	51.8	51.9	50.6	50.5
18	21.6	21.3	21.4	16.0	15.9
19	14.3	16.4	16.2	14.1	14.1
20	124.4	124.3	124.4	124.3	124.4
21	139.7	139.6	139.6	140.4	140.2
22	111.0	110.9	111.0	111.8	111.7
23	142.6	142.6	142.6	141.8	142.1
28		200.9	175.9	79.9	79.9
29	47.7	15.5	17.0	20.1	20.1
30	25.6	26.1	26.4	26.5	26.5
1'	170.6		52.8	167.7	171.0
2'	21.4			128.6	21.4
3'				137.8	
4'				14.3	
5'				11.7	

Fig. 1. Selected  $^1\text{H-}^1\text{H}$  COSY and HMBC Correlations for Ceramicine E (1)

**a**, **b**, and **c** through C-1, C-8, and C-10. The HMBC correlations for H<sub>3</sub>-18 of C-12 ( $\delta_{\text{C}}$  33.2), C-13 ( $\delta_{\text{C}}$  46.9), C-14 ( $\delta_{\text{C}}$  159.1), and C-17 ( $\delta_{\text{C}}$  51.9), for H-15 of C-13, and for H<sub>3</sub>-30 of C-14 indicated the connectivity between structures **b**, **c**, and **d**. The connectivity of partial structure **e** ( $\beta$ -furyl ring) to **d** was shown by HMBC correlations for H-21 of C-17, C-20 ( $\delta_{\text{C}}$  124.4), and C-23 ( $\delta_{\text{C}}$  142.6), and for H-22 to C-20. Further analysis of HMBC correlations for H-6 and H-2' to C-1' ( $\delta_{\text{C}}$  170.6) indicated the presence of an acetate at C-6. Therefore, ceramicine E (**1**) was established as a new limonoid with a cyclopenta[ $\alpha$ ]phenanthrene ring system with a  $\beta$ -furyl ring at C-17, an acetate at C-6, and two epoxide rings.

The relative stereochemistry of **1** was elucidated by nu-

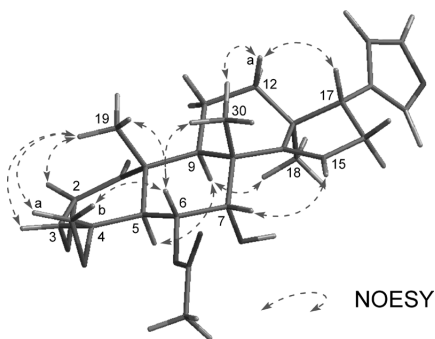


Fig. 2. Selected NOESY Correlations for Ceramicine E (1)

clear Overhauser effect spectroscopy (NOESY) correlations as shown in computer-generated 3D rendering (Fig. 2). NOESY correlations of H<sub>3</sub>-19/H-2, H-3, H-29a, and H-6, and H-6/H-29b and H<sub>3</sub>-30 suggested stereochemistry of two epoxides, H-6, H<sub>3</sub>-19, and H<sub>3</sub>-30 to take as shown in Fig. 2. The <sup>3</sup>J proton coupling constants (<sup>3</sup>J<sub>H-5/H-6</sub>=11.7 Hz and <sup>3</sup>J<sub>H-6/H-7</sub>=2.6 Hz) as well as NOESY correlations of H-9/H-5 and H<sub>3</sub>-18, H-7/H-15, and H-12a/H-17 and H<sub>3</sub>-30 indicated that H-6, H-7, and H-17 adopted a β-configurations, and H-5, H-9, and C-18 adopted an α-configurations as shown in Fig. 2.

Ceramicine F {**2**, [ $\alpha$ ]<sub>D</sub><sup>28</sup> +101 (*c*=0.5, CHCl<sub>3</sub>)} was isolated as a colorless amorphous solid and had molecular formula of C<sub>26</sub>H<sub>32</sub>O<sub>5</sub> as determined by HR-ESI-TOF-MS [*m/z* 447.2120 (M+Na)<sup>+</sup>, Δ -2.7 mmu]. IR absorptions showed the presence of carbonyl (1724, 1679 cm<sup>-1</sup>) and hydroxyl groups (3437 cm<sup>-1</sup>). <sup>1</sup>H- and <sup>13</sup>C-NMR data (Tables 1, 2) revealed 26 carbon resonance due to two carbonyls, two *sp*<sup>2</sup> quaternary carbons, four *sp*<sup>3</sup> quaternary carbons, six *sp*<sup>2</sup> methines, five *sp*<sup>3</sup> methines, three *sp*<sup>3</sup> methylenes and four methyls. In which, two *sp*<sup>2</sup> methines (δ<sub>C</sub> 139.6, 142.6), and two *sp*<sup>3</sup> methines (δ<sub>C</sub> 66.3, 74.6) are attached to an oxygen atom. The existence of an aldehyde group attached to the C-4 (δ<sub>C</sub> 51.8) was indicated by HMBC correlations for H<sub>3</sub>-29 of C-3 (δ<sub>C</sub> 143.9), C-4, C-5 (δ<sub>C</sub> 44.1), and C-28 (δ<sub>C</sub> 200.9). Based on the HR-ESI-TOF-MS and 2D-NMR data (Fig. 3), the structure of **2** was assigned to be similar to previously isolated ceramicine B (**6**)<sup>4</sup> except for the presence of a tetrahydrofuran ring at C-4, C-5, C-6, and C-28.

The relative stereochemistry of **2** was elucidated by NOESY correlations as shown in computer-generated 3D drawing (Fig. 3). As shown by Fig. 3, the relative stereochemistry was shown to be similar to **1**. NOESY correlations of H-6/H<sub>3</sub>-29, H<sub>3</sub>-19, and H<sub>3</sub>-30, and H-5/H-28, suggested the presence of the hydroxyl attached to C-6 and the aldehyde attached to C-4 to adopt the α-configuration.

Ceramicine G {**3**, [ $\alpha$ ]<sub>D</sub><sup>28</sup> -39 (*c*=0.5, CHCl<sub>3</sub>)} was isolated as a white powder and had molecular formula of C<sub>27</sub>H<sub>34</sub>O<sub>6</sub> as determined by HR-ESI-TOF-MS [*m/z* 447.2244 (M+Na)<sup>+</sup>, Δ -0.9 mmu], which was a CH<sub>2</sub>O unit larger than ceramicine F (**2**). <sup>1</sup>H- and <sup>13</sup>C-NMR data (Tables 1, 2) of **3** were analogous to those of **2**, but instead of an aldehyde at C-4, the presence of a methoxycarbonyl group at C-4 (δ<sub>C</sub> 47.9) was indicated by the HMBC correlations for H<sub>3</sub>-1', H<sub>3</sub>-29, H-3, and H-5 of C-28 (δ<sub>C</sub> 175.9) and H<sub>3</sub>-29 of C-3 (δ<sub>C</sub> 146.4), C-4, and C-5 (δ<sub>C</sub> 43.3). The NOESY correlations between H-6/H<sub>3</sub>-29, H<sub>3</sub>-19, and H<sub>3</sub>-30 suggested that methoxy-

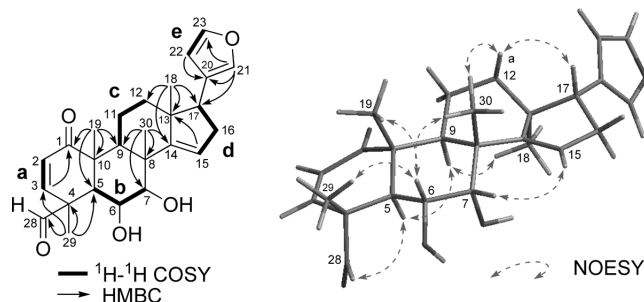


Fig. 3. Selected 2D-NMR Correlations for Ceramicine F (2)

carbonyl group to adopt the α-configuration.

Ceramicine H {**4**, [ $\alpha$ ]<sub>D</sub><sup>26</sup> +77 (*c*=0.2, MeOH)} was isolated as a colorless amorphous solid and had molecular formula of C<sub>31</sub>H<sub>38</sub>O<sub>6</sub> as determined by HR-ESI-TOF-MS [*m/z* 529.2565 (M+Na)<sup>+</sup>, Δ -0.1 mmu]. Whereas ceramicine I {**5**, [ $\alpha$ ]<sub>D</sub><sup>26</sup> +13 (*c*=0.3, MeOH)} possessing molecular formula of C<sub>28</sub>H<sub>35</sub>O<sub>6</sub> determined by HR-ESI-TOF-MS [*m/z* 489.2227 (M+Na)<sup>+</sup>, Δ -2.6 mmu] was isolated as a colorless amorphous solid. <sup>1</sup>H- and <sup>13</sup>C-NMR data (Tables 1, 2) of **4** and **5** were found to be similar to those of the previously isolated ceramicine C.<sup>7</sup> Based on the 2D-NMR (<sup>1</sup>H-<sup>1</sup>H COSY, heteronuclear single quantum coherence (HSQC), and HMBC) data, **4** was found to be ceramicine C<sup>7</sup> with a tiglate group attached to C-12 (δ<sub>C</sub> 77.2), whereas **5** was found with acetate group substituted to C-12 (δ<sub>C</sub> 77.2) instead of methacrylate at C-12 of ceramicine C, which was confirmed by HMBC correlations for H<sub>3</sub>-5' and H-12 of C-1' (δ<sub>C</sub> 167.7) and the chemical shifts<sup>8,9</sup> of H-3' (δ<sub>H</sub> 6.58) and C-5' (δ<sub>C</sub> 11.7) in **4** and for H<sub>3</sub>-2' and H-12 of C-1' (δ<sub>C</sub> 171.0) in **5**. The relative structures of **4** and **5** were shown to be similar to that of ceramicine B (**6**). The NOESY correlations between H-12/H-17 and H<sub>3</sub>-30 for **4** and **5** indicated that both the tiglate at C-12 and acetate at C-12 were to adopt the β-configurations.

Absolute stereostructures of a series of ceramicines are still remained to be solved. Hence, in this report the absolute configuration of ceramicine B (**6**), a major limonoid from *C. ceramicus*, was elucidated by using the exciton chirality CD method.<sup>10</sup> The CD spectrum of *p*-Br-benzoyl derivative of ceramicine B (**6**) was shown in Fig. 4A. At first position, [λ<sub>max</sub> 246 nm (Δε -9.89)] was of negative value, while at the second position [λ<sub>max</sub> 225 nm (Δε 14.52)] was of positive value. This indicates that the chirality of the two chromophores was a left-handed screw or oriented in a counter clockwise manner, as shown in Fig. 4B. Therefore, absolute configuration at C-7 could be assigned as *S*. Total structure of **6** including absolute stereochemistry was confirmed by the Flack parameter,<sup>11</sup> χ=-0.0(2) of X-ray analysis for the crystal obtained from MeOH/H<sub>2</sub>O (Fig. 5).

The current mortality from cancer is still unacceptably high.<sup>12</sup> This widespread mortality necessitates the development of new therapeutic agents against uncontrollable cell growth which ultimately triggers the onset of cancer. Investigations of any new possible lead compounds are therefore crucial in the struggle against cancer. Ceramicines G (**3**) and I (**5**) showed moderate cell growth inhibitory activity against HL-60, MCF7, and A549 cells (IC<sub>50</sub> for **3**: 26.1 μM for HL-60, 27.3 μM for MCF7, and 41.4 μM for A549; IC<sub>50</sub> for **5**:

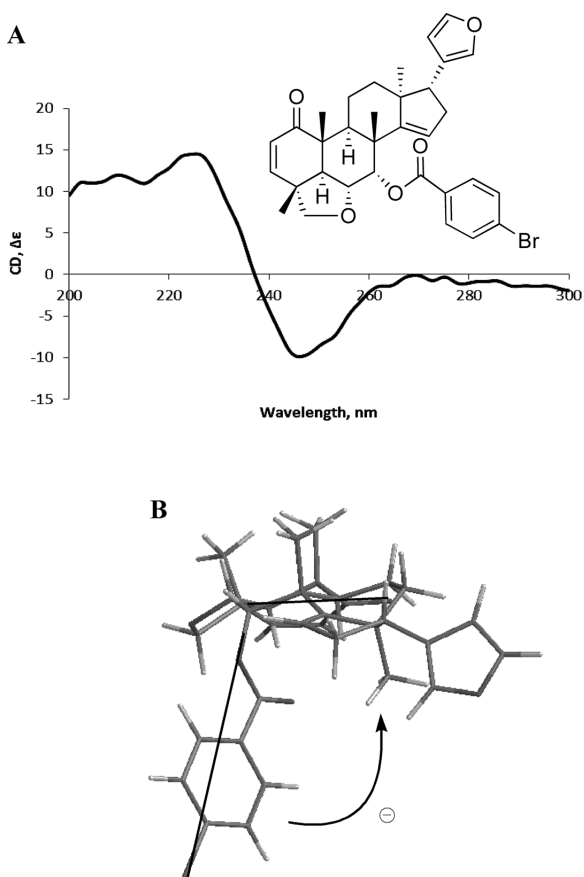


Fig. 4. CD Spectrum (A) and 3D Structure (B) of *p*-Br Benzoate of Ceramicine B (6)

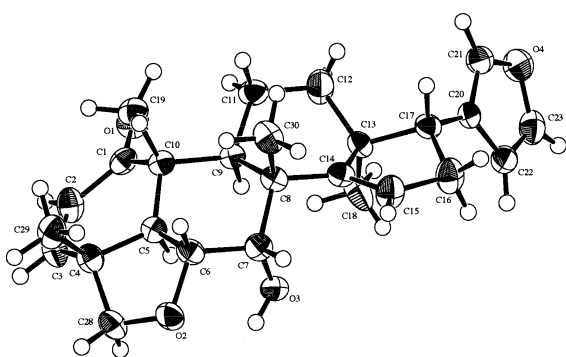


Fig. 5. Molecular Structure of Ceramicine B (6) Obtained by X-Ray Analysis [Flack Parameter:  $\chi = -0.0(2)$ ]

$IC_{50}$ : 42.2  $\mu M$  for HL-60, and 44.0  $\mu M$  for MCF7), whereas others did not show substantial inhibitory activity (each  $IC_{50} > 50 \mu M$ ).

## Experimental

**General Experimental Procedures** Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO Fourier transform/infrared (FT/IR)-4100 spectrophotometer. CD spectra were recorded on a JASCO J-820 polarimeter. High-resolution ESI-MS were obtained on a LTQ Orbitrap XL (Thermo Scientific, U.S.A.).  $^1H$ - and  $^{13}C$ -NMR spectra were measured on 400 MHz spectrometers at 300 K, while  $^{13}C$ -NMR spectra were documented with a 100 MHz spectrometer. Each ceramicine's NMR sample was prepared by dissolving with  $CDCl_3$  in 2.5 mm microcells (Kanto Chemicals Co., Inc., Japan) and the residual  $CHCl_3$  chemical shift used as

an internal standard are  $\delta_H$  7.26 and  $\delta_C$  77.0. Standard pulse sequences were used for the 2D- NMR experiments.  $^1H$ - $^1H$  COSY, and NOESY spectra were measured with spectral widths of both dimensions of 4800 Hz, and 8 scans with two dummy scans were accumulated into 1 K data points for each of 256  $t_1$  increments. NOESY spectra in the phase sensitive mode were measured with a mixing time of 800 and 30 ms. While for the HSQC spectra in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1 K data points were collected. The HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long range C-H coupling. Zero-filing to 1 K for  $F_1$  and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

**Material** The barks of *C. ceramicus* were collected from Pahang, Malaysia in 1996. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. Voucher specimens (Herbarium No. KL4648) are deposited in the Herbarium of Chemistry Department, University of Malaya.

**Extraction and Isolation** The dried ground barks of *C. ceramicus* (500 g) were extracted successively with methanol and 53 g of extract were obtained. Seven grams from the total extract was successively partitioned with hexane, ethyl acetate, *n*-butanol, and water. The hexane-soluble materials were further partitioned with a silica gel column (hexane/EtOAc, 10:0→1:1, and then  $CHCl_3/MeOH$ , 10:0→0:10). Fraction eluted from the silica gel column with  $CHCl_3/MeOH$  (7:3) was further purified using HPLC with Cadenza 5CD C18, 10×250 mm, under gradient elution (50—100% MeOH), flow rate 2.0 ml/min, UV: 210 nm to yield ceramicine E (1, 2.0 mg, 0.029% yield), and ceramicine F (2, 9.5 mg, 0.14% yield), both as colorless solid. Fraction eluted with  $CHCl_3/MeOH$  (1:1) was purified on HPLC with Cadenza 5CD C18, 10×250 mm, under gradient elution (70—100% MeOH), flow rate 2.0 ml/min, UV: 210 nm to obtain ceramicine G (3, 5.6 mg, 0.08% yield) as colorless solid. Whereas, fraction eluted with  $CHCl_3/MeOH$  (4:6) was purified on HPLC with Cadenza 5CD C18, 10×250 mm, under gradient elution (50—100% MeOH), flow rate 2.0 ml/min, UV: 210 nm to afford ceramicine H (4, 0.3 mg, 0.004% yield), and ceramicine I (5, 4.3 mg, 0.06% yield).

Ceramicine E (1): Colorless amorphous solid;  $[\alpha]_D^{28} + 20$  ( $c=0.5$ ,  $CHCl_3$ ); UV (MeOH)  $\lambda_{max}$  203 ( $\epsilon$  8640) nm; IR (KBr)  $\nu_{max}$  3437, 1718, 1506, 1389, 1372, and 1246  $cm^{-1}$ ;  $^1H$ - and  $^{13}C$ -NMR data (Tables 1, 2); ESI-MS  $m/z$  469 ( $M+H$ ) $^+$ ; HR-ESI-TOF-MS  $m/z$  469.2206 ( $M+H$ ; Calcd for  $C_{27}H_{33}O_7$ , 469.2226).

Ceramicine F (2): Colorless amorphous solid;  $[\alpha]_D^{28} + 101$  ( $c=0.5$ ,  $CHCl_3$ ); UV (MeOH)  $\lambda_{max}$  203 ( $\epsilon$  14592) nm; IR (KBr)  $\nu_{max}$  3437, 2980, 2920, 1724, 1679, 1448, and 1388  $cm^{-1}$ ;  $^1H$ - and  $^{13}C$ -NMR data (Tables 1, 2); ESI-MS  $m/z$  447 ( $M+Na$ ) $^+$ ; HR-ESI-TOF-MS  $m/z$  447.2120 ( $M+Na$ ; Calcd for  $C_{26}H_{32}O_5Na$ , 447.2147).

Ceramicine G (3): Colorless amorphous;  $[\alpha]_D^{28} - 39$  ( $c=0.5$ ,  $CHCl_3$ ); UV (MeOH)  $\lambda_{max}$  203 ( $\epsilon$  11000) nm; IR (neat)  $\nu_{max}$  3430, 2993, 1717, 1685, and 1260  $cm^{-1}$ ;  $^1H$ - and  $^{13}C$ -NMR data (Tables 1, 2); ESI-MS  $m/z$  477 ( $M+Na$ ) $^+$ ; HR-ESI-TOF-MS  $m/z$  477.2244 ( $M+Na$ ; Calcd for  $C_{27}H_{34}O_6Na$ , 477.2253).

Ceramicine H (4): Colorless amorphous solid;  $[\alpha]_D^{26} + 77$  ( $c=0.2$ , MeOH); UV (MeOH)  $\lambda_{max}$  203 ( $\epsilon$  29900) nm; IR ( $CCl_4$ )  $\nu_{max}$  3418, 2927, 2855, 1703, 1684, 1585, and 1260  $cm^{-1}$ ;  $^1H$ - and  $^{13}C$ -NMR data (Tables 1, 2); ESI-MS  $m/z$  529 ( $M+Na$ ) $^+$ ; HR-ESI-TOF-MS  $m/z$  529.2565 ( $M+Na$ ; Calcd for  $C_{31}H_{38}O_6Na$ , 529.2566).

Ceramicine I (5): Colorless amorphous solid;  $[\alpha]_D^{26} + 13$  ( $c=0.3$ , MeOH); UV (MeOH)  $\lambda_{max}$  203 ( $\epsilon$  14100) nm; IR ( $CCl_4$ )  $\nu_{max}$  2933, 1732, 1683, 1246, and 1030  $cm^{-1}$ ;  $^1H$ - and  $^{13}C$ -NMR data (Tables 1, 2); ESI-MS  $m/z$  489 ( $M+Na$ ) $^+$ ; HR-ESI-TOF-MS  $m/z$  489.2227 ( $M+Na$ ; Calcd for  $C_{28}H_{34}O_6Na$ , 489.2253).

***p*-Br-Benzoate of Ceramicine B (6)** To a solution of ceramicine B (6: 0.1 mg, 245  $\mu mol$ ) in  $CH_2Cl_2$  (100  $\mu l$ ) was added *p*-bromobenzoyl chloride with *N,N*-dimethyl-4-aminopyridine. The solution was stirred for 4 h in room temperature. Purification with column chromatography (hexane/ethyl acetate, 8:2) gave a pale yellow solid. CD (MeOH)  $\lambda_{max}$  225 ( $\Delta\epsilon + 14.52$ ) and 246 ( $-9.89$ ) nm;  $^1H$ -NMR (400 MHz,  $CDCl_3$ )  $\delta$ : 0.72 (3H, s), 1.24 (3H, s), 1.27 (3H, s), 1.34 (3H, s), 1.58 (1H, m), 1.90 (2H, m), 2.26 (2H, m), 2.50—2.65 (3H), 2.80 (1H, dd, 9.0, 9.0), 3.32 (1H, d, 7.2), 3.68 (1H, d, 7.2), 4.41 (1H, brd, 11.2), 5.53 (1H, brs), 5.84 (1H, brs), 5.89 (1H, brd, 9.4), 6.22 (1H, brs), 6.94 (1H, d, 9.4), 7.16 (1H, s), 7.33 (1H, brs), 7.53 (2H, d, 8.2), and 7.80 (2H, d, 8.2). ESI-MS 591 ( $M+H$ ) $^+$ .

**Cytotoxicity** HL-60, human promyelocytic leukemia cells were maintained in RPMI-1640 medium; A549, human lung adenocarcinoma; MCF7, human breast adenocarcinoma; and HCT116, human colorectal adenocarcinoma cells were maintained in Dulbecco's modified Eagle's medium

(DMEM) medium. Both growth medium were supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The cells ( $5 \times 10^5$  cells/well) were cultured in Nunc disposable 96-well plates containing 90  $\mu$ l of growth medium per well and were incubated at 37 °C in a humidified incubator of 5% CO<sub>2</sub>. Ten microliters of serially diluted samples (50, 25, 12.5, 6.25  $\mu$ M) were added to the cultures at 24 h of incubation. After 48 h of incubation with the samples, 15  $\mu$ l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/ml) were added to each of the wells. The cultures were incubated for another 3 h before the cells supernatant are removed. After the removal of the cells supernatant, 50  $\mu$ l of dimethyl sulfoxide (DMSO) was added to each well. The formed formazan crystal was dissolved by re-suspension by pipette. The optical density was measured using a microplate reader (Bio-Rad, U.S.A.) at 550 nm with reference wavelength at 700 nm. In all experiment, three replicates were used. Cisplatin was used as positive control (IC<sub>50</sub>: 0.87  $\mu$ M for HL-60, 27.7  $\mu$ M for MCF7, 27.8  $\mu$ M for A549, and 16.0  $\mu$ M for HCT116).

**X-Ray Crystallography** Measurement was made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-K $\alpha$  radiation. Crystal data of ceramicine B: A colorless platelet crystal, orthorhombic, C<sub>26</sub>H<sub>32</sub>O<sub>4</sub>, *M*=408.54, crystal dimensions 0.14 $\times$ 0.06 $\times$ 0.02 mm, space group *P*2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> (#9), *a*=7.30805(17) Å, *b*=13.4328(3) Å, *c*=21.6929(15) Å, *V*=2129.54 Å<sup>3</sup>, *Z*=4, *D*<sub>calc</sub>=1.274 g/cm<sup>3</sup>. Of the 25458 reflections that were obtained, 3899 were unique (*R*<sub>int</sub>=0.026). The structure was solved by direct methods. *R*<sub>1</sub>=0.0388 (*I*>2.00 $\sigma$ (*I*)). Flack parameter<sup>(11)</sup>=−0.0(2) (Fridel pairs=1651). All calculations were performed using the CrystalStructure crystallographic software package except for refinement, which was performed using SHELXL-97. The refined fractional atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). CCDC 802102 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via <http://www.ccdc.cam.ac.uk/submit>, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ,

U.K. (Fax: +44 1223 336 033; e-mail: [deposit@ccdc.cam.ac.uk](mailto:deposit@ccdc.cam.ac.uk)).

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