J Nat Med (2012) 66:566–570
DOI 10.1007/s11418-011-0616-9

NOTE

Ceramicines J–L, new limonoids from *Chisocheton ceramicus*

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Received: 25 October 2011 / Accepted: 20 November 2011 / Published online: 13 December 2011

Abstract Three new limonoids, ceramicines J (**1**), K (**2**), and L (**3**), were isolated from the hexane layer of *Chisocheton ceramicus* bark extract. Their structures were elucidated from 1D and 2D NMR data. Ceramicines J–L (**1**–**3**) exhibited dose-dependent moderate cytotoxicity against the HL-60 cell line.

Keywords Limonoids · *Chisocheton ceramicus* · Ceramicines J–L · Cytotoxic activity

Introduction

The wide-ranging activities of limonoids are of interest in the field of plant natural products. Limonoids with insecticidal, insect antifeedant, antibacterial, antifungal, antimalarial, anticancer, and antiviral activities [1–5] are known to be especially bountiful in plants from the Meliaceae family [1].

In our continued effort to gain a deeper understanding of secondary metabolites from plants of the Meliaceae family [6–8], the studies of *Chisocheton ceramicus* bark extract have yielded three new limonoids, ceramicines J–L (**1**–**3**). In this paper, we describe the isolation, structure elucidation, and cytotoxic activity of **1**–**3** isolated from *C. ceramicus*.

Results and discussion

The methanol extract from the bark of *C. ceramicus* was sequentially partitioned between hexane, ethyl acetate, *n*-butanol, and water. Subsequent purification by a silica gel column and HPLC led to the isolation of three new limonoids, ceramicines J (**1**, 1.0 mg, 0.0002% yield), K (**2**, 0.6 mg, 0.00012% yield), and L (**3**, 0.8 mg, 0.00016% yield).

Ceramicine J {**1**, $[\alpha]_D^{25}$ −88 (*c* 0.5, $CHCl_3$)} was isolated as a colorless amorphous solid and had molecular formula, $C_{26}H_{32}O_5$ as determined by high-resolution–electrospray ionization–time-of-flight mass spectroscopy (HR-ESI-TOF-MS) [*m/z* 425.2328 $(M + H)^+$, Δ −0.3 mmu]. IR absorptions suggested the presence of two carbonyls (1724 and 1675 cm^{-1}). ^1H and ^{13}C NMR data (Tables 1, 2) revealed 26 carbon resonances due to two carbonyls, one sp^2 quaternary carbons, four sp^3 quaternary carbons, five sp^2 methines, six sp^3 methines, four sp^3 methylenes, and four methyls. Among them, two sp^2 methines (δ_C 140.2 and 142.8), two sp^3 methines (δ_C 73.6 and 69.4), and one sp^3 methylene (δ_C 79.8) are connected to an oxygen atom (Fig. 1).

^1H and ^{13}C NMR data (Tables 1, 2) of **1** were analogous to those of previously reported ceramicine B [7], but instead an olefin between C-14 and C-15 was replaced by a carbonyl. On the basis of data from COSY analysis of **1** in $CDCl_3$ (Fig. 2), five partial structures **a** (from C-2 to C-3), **b** (from C-5 to C-7), **c** (from C-9 to C-12), **d** (from C-16 to

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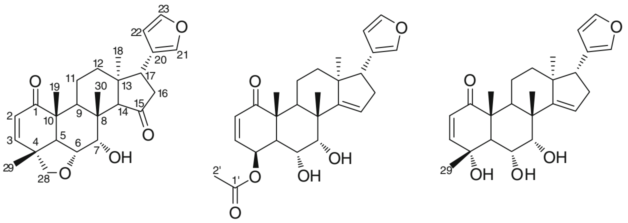
Table 1 ^{1}H (J, Hz) data of ceramicines J–L (**1**–**3**) in $CDCl_3$ at 300 K

	1	**2**	**3**
1			
2	5.84 (d, 9.7)	5.93 (d, 10.0)	5.75 (d, 10.3)
3	6.95 (d, 9.7)	6.77 (ddd, 17.1, 10.0, 4.6)	6.41 (d, 10.3)
4		5.65 (t, 4.6)	
5	2.61 (d, 12.5)	2.46 (dd, 9.0, 4.6)	2.63 (d, 11.6)
6	4.20 (dd, 12.5, 2.9)	4.12 (t, 9.0)	4.39 m
7	4.17 brs	4.05 brs	3.98 (d, 2.4)
8			
9	1.73 m	2.49 m	2.50 m
10			
11a	1.71 m	1.62 m	1.55 m
11b	2.54 m	2.52 m	2.55 m
12a	1.32 (d, 14.6)	1.60 m	1.59 m
12b	1.94 (dt, 14.6, 2.8)	1.93 m	1.92 (dt, 13.0, 2.9)
13			
14	2.91 s		
15		5.58 s	5.58 s
16a	2.52 (2H, m)	2.43 m	2.42 m
16b		2.55 m	2.54 m
17	3.49 (t, 9.9)	2.87 (dd, 11.0, 7.4)	2.87 (dd, 10.9, 7.3)
18	0.78 s	0.88 s	0.89 s
19	1.17 s	1.33 s	1.25 s
20			
21	7.27 s	7.26 s	7.26 s
22	6.30 s	6.30 s	6.30 s
23	7.40 s	7.39 s	7.39 s
28a	3.60 (d, 7.1)		
28b	3.79 (d, 7.1)		
29	1.32 s		1.62 s
30	1.07 s	1.22 s	1.19 s
1’			
2’		2.15 s	

Table 2 ^{13}C NMR data of ceramicines J–L (**1**–**3**) in $CDCl_3$ at 300 K

	1	**2**	**3**
1	202.5	203.7	203.8
2	130.0	130.1	125.6
3	151.0	139.2	149.8
4	41.8	65.2	71.5
5	47.1	43.6	50.9
6	73.6	65.0	68.4
7	69.4	74.8	75.0
8	44.1	47.2	44.3
9	43.7	32.7	33.5
10	46.4	48.0	49.1
11	19.1	17.9	17.8
12	34.2	32.7	33.1
13	42.1	44.2	47.1
14	60.7	160.7	160.6
15	219.1	120.0	120.3
16	43.4	34.3	34.3
17	37.7	52.0	51.9
18	27.6	21.3	21.5
19	15.0	16.0	15.8
20	123.0	124.4	124.4
21	140.2	139.7	139.7
22	110.8	111.0	111.0
23	142.8	142.7	142.7
28	79.8		
29	20.0		23.8
30	17.7	26.3	26.4
1′		170.5	
2′		21.1	



ceramicine J (1) ceramicine K (2) ceramicine L (3)

Fig. 1 Structures of ceramicines J–L (**1**–**3**)

C-17), and **e** (from C-22 to C-23) were deduced. The HMBC correlations for H_3-29 of C-3 to C-5; H-2 of C-4 and C-10; H_3-19 of C-1, C-5, C-9; H_3-30 of C-7, C-9, and C-14; and H_3-18 of C-12 to C-14 bridged the partial structure of **a**, **b**, and **c** through C-1, C-8, and C-10, thus forming a phenanthrene ring system. The relation between partial structure **c** and **d** could be assigned by HMBC correlations for H_3-18 of C-12 to C-14, and C-17; H_2-16 and H-14 of C-15, such that a cyclopentanone was deduced to be attached to the phenanthrene ring system through C-13 and C-14. The connectivity of partial structure **e** (β-furyl ring) to **d** was shown by HMBC correlations for H-20 of C-17, H-21 of C-20, and C-22. Further analysis of HMBC correlations of H_3-29 of C-3 and C-5, and H_2-28 of C-6, indicated the presence of a tetrahydrofuran ring at C-4–C6 and C-28. Therefore, ceramicine J (**1**) was established as a new limonoid with a cyclopentanone[α]phenanthrene ring system with a β-furyl ring at C-17, and a tetrahydrofuran ring.

The relative stereochemistry of **1** was elucidated by ROESY correlations as shown in the computer-generated 3D rendering (Fig. 2). ROESY correlations of H_3-29/H-6, and H_3-19 suggested the stereochemistry of the C-29 methyl group as shown in Fig. 2. The ^{3}J proton coupling constants ($^{3}J_{H\text{-}5/H\text{-}6} = 12.5$ Hz and $^{3}J_{H\text{-}6/H\text{-}7} \approx 0$ Hz) as

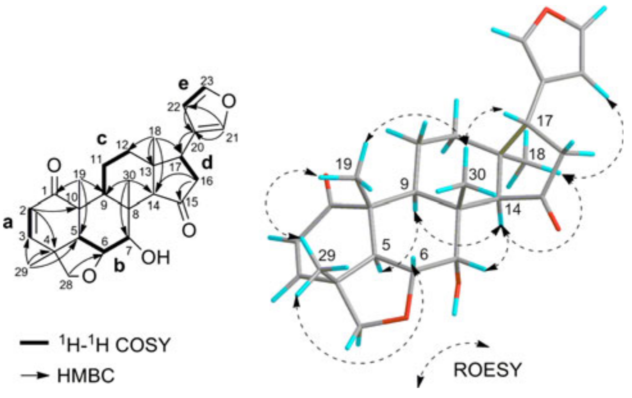


Fig. 2 Selected 2D NMR correlations for ceramicine J (**1**)

well as ROESY correlations of H-7/H-6 and H-14, and H_3-18/H-14 indicated that H-6 and H-7 adopted *β*-configurations, and H-5, H-9, and C-18 adopted α-configurations as shown in Fig. 2.

Ceramicine K {**2**, $[\alpha]_D^{26}$ +6 (*c* 0.4, $CHCl_3$)} was isolated as a colorless amorphous solid and had molecular formula of $C_{26}H_{32}O_6$ as determined by HR-ESI-TOF-MS [*m/z* 463.2136 (M + Na)$^+$, Δ +3.9 mmu], which contained one oxygen more than ceramicine J (**1**). IR absorptions showed the presence of two carbonyls (1747 and 1691 cm^{-1}). ^{1}H and ^{13}C NMR data (Tables 1, 2) revealed 26 carbon resonance due to two carbonyls, two *sp*^{2} quaternary carbons, three *sp*^{3} quaternary carbons, six *sp*^{2} methines, six *sp*^{3} methines, three *sp*^{3} methylenes, and four methyls. Of these, two *sp*^{2} methines (δ_C 139.7 and 142.7) and three *sp*^{3} methines (δ_C 65.2, 65.6, and 74.8) are attached to an oxygen atom. Further observation analysis of the ^{13}C NMR data showed the presence of an olefin on C-14 and C-15 (δ_C 160.7 and 120.0, respectively) in place of the carbonyl on C-15 (δ_C 219.1) as seen in ceramicine J (**1**). In addition, HMBC correlations for H-4 and H_3-2′ of C-1′ included the presence of an acetate at C-4 (δ_C 65.2). On the basis of these NMR data including 2D NMR (COSY, HSQC, and HMBC) as well as HR-ESI-TOF-MS, the planar structure of **2** was elucidated as shown in Fig. 3.

The relative stereochemistry of **2** was elucidated by ROESY correlations as shown in the computer-generated 3D drawing (Fig. 3). The relative stereochemistry was similar to that of **1**. ROESY correlations of H-6/H_3-19, and H_3-30; H-7/H-15, and H_3-30 suggested the presence of the hydroxyls attached to C-6 and C-7 adopted the α-configuration. In contrast, the ^3J proton coupling constants ($^3J_{H\text{-}4/H\text{-}5}$ = 4.6 Hz), along with ROESY correlation of H-4/H-5 implied, the acetate attached to C-4 adopted the *β*-configuration.

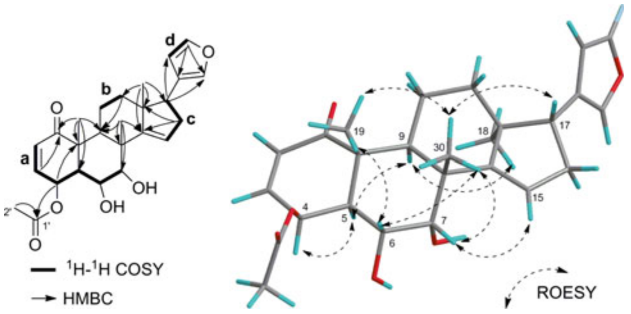


Fig. 3 Selected 2D NMR correlations for ceramicine K (**2**)

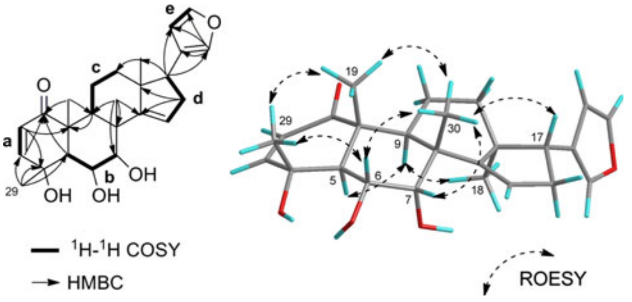


Fig. 4 Selected 2D NMR correlations for ceramicine L (**3**)

Ceramicine L {**3**, $[\alpha]_D^{27}$ +14 (*c* 0.3, $CHCl_3$)} was isolated as a colorless amorphous solid with molecular formula of $C_{25}H_{32}O_5$ as established by HR-ESI-TOF-MS [*m/z* 435.2147 (M + Na)$^+$, Δ +2.6 mmu], which was smaller than that of ceramicine K (**2**) by a CO unit. ^{1}H and ^{13}C NMR data (Tables 1, 2) of **3** were analogous to those of **2**, but instead of an acetate attached at C-4 in **2**, the presence of an *sp*^{3} quaternary carbon for C-4 with a methyl and a hydroxy group was suggested by the HMBC correlations H_3-29 to C-3, C-4 (δ_C 71.5), and C-5. The ROESY correlations between H-6/H_3-29 and H_3-30, H-7/H_3-30, and H_3-29/H_3-19 suggested that all three hydroxyls adopted the α-configuration (Fig. 4).

Ceramicines J–L (**1**–**3**) from *C. ceramicus* were subjected to cytotoxic activity studies using the MTT assay [9]. Results showed that **1**, **2**, and **3** exhibited dose-dependent but weak cytotoxicity against the HL-60 cell line (36, 33, and 25% inhibition at 50 μM, respectively). Cisplatin was used as a positive control (IC_{50} at 0.87 μM for HL-60).

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 an

LTQ Orbitrap XL (Thermo Scientific, USA). ^{1}H and 2D NMR spectra were measured on 700-MHz spectrometers at 300 K, whereas ^{13}C NMR spectra were measured with a 175-MHz spectrometer. Each ceramicine's NMR sample was prepared by dissolving with $CDCl_3$ in 2.5-mm micro-cells (Kanto Chemicals Co., Inc., Japan) and the residual $CHCl_3$ chemical shifts used as an internal standard are δ_H 7.26 and δ_C 77.0. Standard pulse sequences were used for the 2D NMR experiments. ^{1}H–^{1}H 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. ROESY spectra in the phase-sensitive mode were measured with a mixing time of 800 and 30 ms. 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 pulsed field gradient (PFG), a 50-ms delay time was used for long-range C–H coupling. Zero-filling 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 the Chemistry Department, University of Malaya.

Extraction and isolation

The dried ground barks of *C. ceramicus* (500 g) were extracted successively with methanol and 49 g of extract was obtained. The total extract was successively partitioned with hexane, ethyl acetate, *n*-butanol, and water. The hexane-soluble materials were preliminary partitioned with a silica gel column (hexane/EtOAc, 1:0 → 0:1). The fraction eluted from the silica gel column with hexane/EtOAc (1:9) was further purified a silica gel column (toluene/EtOAc, 6:1 → 4:1). The fraction eluted with toluene/EtOAc (4:1) was finally purified using HPLC with Cosmosil πNAP, 10 × 250 mm, under isocratic elution [MeOH/H_2O (78%/22%)], flow rate 2.0 ml min^{-1}, UV 210 nm, to yield ceramicine J (**1**, 1.0 mg, 0.0002% yield) as a colorless amorphous solid. The fraction eluted with 100% EtOAc from the preliminary silica gel column was purified with another silica gel column with toluene/EtOAc (6:1) under isocratic condition. Eluent nos. 12 and 13 were finally purified using HPLC with Cosmosil πNAP, 10 × 250 mm, under isocratic elution [MeOH/H_2O (78%/22%)], flow rate 2.0 ml min^{-1}, UV 210 nm, to yield ceramicine K (**2**, 0.6 mg, 0.00012% yield) and ceramicine L (**3**, 0.8 mg, 0.00016% yield) both also as colorless amorphous solids.

Ceramicine J (**1**): colorless amorphous solid. $[\alpha]_D^{25}$ −88 (*c* 0.5, $CHCl_3$). IR (CCl_4) cm^{-1}: 2928, 1724, 1675, 1574. UV λ_{max} (MeOH) nm (ε): 204 (13600). ^{1}H and ^{13} C NMR (Tables 1, 2). ESI-MS *m/z*: 425 (M + H)$^+$. HR-ESI-MS *m/z*: 425.2325 (M + H: calcd for $C_{26}H_{33}O_5$, 425.2328).

Ceramicine K (**2**): colorless amorphous solid. $[\alpha]_D^{26}$ +6 (*c* 0.4, $CHCl_3$). IR (CCl_4) cm^{-1}: 2927, 1747, 1691, 1653. UV λ_{max} (MeOH) nm (ε): 203 (8760). ^{1}H and ^{13} C NMR (Tables 1, 2). ESI-MS *m/z*: 463 (M + Na)$^+$. HR-ESI-MS *m/z*: 463.2136 (M + Na: calcd for $C_{26}H_{32}O_6$, Na, 463.2097).

Ceramicine L (**3**): colorless amorphous solid. $[\alpha]_D^{27}$ +14 (*c* 0.3, $CHCl_3$). IR (CCl_4) cm^{-1}: 2926, 2854, 1689, 1653. UV λ_{max} (MeOH) nm (ε): 203 (13240). ^{1}H and ^{13} C NMR (Tables 1, 2). ESI-MS *m/z*: 435 (M + Na)$^+$. HR-ESI-MS *m/z*: 435.2173 (M + Na: calcd for $C_{25}H_{32}O_5Na$, 435.2147).

Cytotoxicity

Human promyelocytic leukemia cells (HL-60) were maintained in RPMI-1640 medium. Growth medium were supplemented with 10% fetal calf serum and 1% penicillin–streptomycin. The cells (5×10^3 cells/well) were cultured in Nunc disposable 96-well plate containing 90 µl of growth medium per well and were incubated at 37°C in a humidified incubator of 5% CO_2. At 24 h of incubation, 10-µl aliquots of serially diluted samples (50, 25, 12.5, 6.25, and 3.125 µM) were added to the cultures. After 48 h of incubation with the samples, 15 µl of MTT (5 mg ml^{-1}) was added to each of the wells. The cultures were incubated for another 3 h before the cells' supernatant was removed. Thereafter 50 µl 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, USA) at 550 nm with reference wavelength at 700 nm. In all experiments, three replicates were used. Cisplatin was used as positive control (IC_{50} 0.87 µM for HL-60).

Acknowledgments This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant from the Open Research Center Project.

References

1. Roy A, Saraf S (2006) Limonoids: overview of significant bioactive triterpenes distributed in plants kingdom. Biol Pharm Bull 29:191–201
2. Zhang H, Wang X, Chen F, Androulakis XM, Wargovich MJ (2007) Anticancer activity of limonoids from *Khaya senegalensis*. Phytotherapy Res 21:731–734

3. Carpinella MC, Defago MT, Valladares G, Palacios SM (2003) Antifeedant and insecticide properties of a limonoids from *Melia azedarach* (Meliaceae) with potential use for pest management. J Agric Food Chem 51:369–374
4. Bray DH, Warhurst DC, Connolly JD, O'Neill MJ, Philipson JD (1990) Plants as sources of antimalarial drugs. Part 7. Activity of some species of meliaceae plants and their constituent limonoids. Phytotherapy Res 4:29–35
5. Koul O, Singh G, Singh R, Singh J, Danielwski WM, Berlozecki S (2004) Bioefficacy and mode-of-action of some limonids of salannin group from *Azadirachta indica* A. Juss and their role in a multicomponent system against lepidopteran larvae. J Biosci 29:409–416
6. Mohamad K, Hirasawa Y, Lim CS, Awang K, Hadi AHA, Takeya K, Morita H (2008) Ceramicine A and walsogyne A, a novel limonoids from two species of Meliaceae. Tetrahedron Lett 49:4276–4278
7. Mohamad K, Hirasawa Y, Litaudon M, Awang K, Hadi AHA, Takeya K, Ekasari W, Widyawaruyanti A, Zaini NC, Morita H (2009) Ceramicine B–D, a new antiplasmodial limonoids from *Chisocheton ceramicus*. Bioorg Med Chem 17:727–730
8. Wong CP, Shimada M, Nagakura Y, Nugroho AE, Hirasawa Y, Kaneda T, Awang K, Hadi AHA, Mohamad K, Shiro M, Morita H (2011) Ceramicine E–I, new limonoids from *Chisocheton ceramicus*. Chem Pharm Bull 59:407–411
9. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxic assay. J Immunol Methods 65:55–63