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Ceramicine A and walsogyne A, novel limonoids from two species of Meliaceae

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ABSTRACT

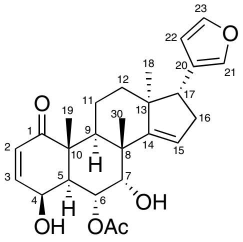
Two novel limonoids, ceramicine A (**1**) without any methyls at C-4, and walsogyne A (**2**) with a ring C-*seco* limonoid, have been isolated from the barks of *Chisocheton ceramicus* and *Walsura chrysogyne*, respectively, and the structures were fully elucidated on the basis of spectroscopic data. Ceramicine A (**1**) and walsogyne A (**2**) showed a moderate cytotoxic activity.

Limonoids from Meliaceae and Rutaceae have been thoroughly studied due to their significant biological activities such as antifeedants, insecticides, antitumor, and antimalarial activities[1,2] and their diverse structures with the oxidized backbone and the side chain moiety which bonded to ring D in the intact tetranortriterpene nucleus have attracted great interest.[3]

In continuation of our research on Meliaceae family,[4] we have found that the ethanol bark extracts of *Chisocheton ceramicus* and *Walsura chrysogyne* collected in Malaysia showed cytotoxic activity against P388 murine leukemia cells. We have isolated a novel limonoid, ceramicine A (**1**), with an unprecedented desmethylated skeleton at C-4 from *C. ceramicus* and walsogyne A (**2**) with a ring C–*seco* limonoid from *W. chrysogyne*. We now wish to report the isolation and structure elucidation of two novel limonoids, ceramicine A (**1**) and walsogyne A (**2**).

The barks of *C. ceramicus*, which were collected at Pahang, Malaysia, were extracted with ethanol and the extract was partitioned with 10% aq MeOH and $CHCl_3$. The $CHCl_3$-soluble materials were subjected to a silica gel column (hexane/EtOAc, 1:0→0:1), in which a fraction eluted with hexane/EtOAc (3:2) was further purified on a silica gel column with toluene/EtOAc (4:1) to afford ceramicine A (**1**, 25 mg, 0.0125% yield) as colorless solids.

The genus *Walsura* (Meliaceae) comprising about 40 species and varieties is mainly distributed in Southeast Asia and China. The barks of *W. chrysogyne*, which were collected at Mersing, Malaysia, were extracted with ethanol and the extract was partitioned with 10% aq MeOH and $CHCl_3$. The $CHCl_3$-soluble materials were subjected to a silica gel column (hexane/EtOAc, 1:0→0:1, and then $CHCl_3$/MeOH, 1:0→0:1), in which a fraction eluted with $CHCl_3$/MeOH (9:1) was further purified on a silica gel column with toluene/EtOAc (4:1), followed by ODS column with 70% MeOH to afford walsogyne A (**2**, 32 mg, 0.032% yield) as colorless solids.



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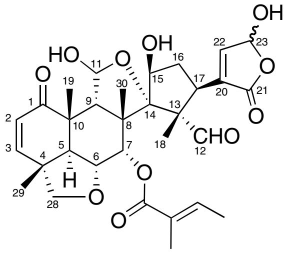

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The HRESIMS of ceramicine A (**1**),[5] displayed a pseudomolecular ion peak at 463.2093 $(M+Na)^+$, compatible to the molecular formula of $C_{26}H_{32}O_6$. IR absorptions indicated the presence of hydroxyl (3449 cm^{-1}) and carbonyl (1734, 1720, and 1686 cm^{-1}) groups, respectively. The ^{13}C NMR spectra revealed 26 carbon resonances 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. Among them, three sp^3 methines (δ_C 62.6, 69.8, and 73.2) and two sp^2 methines (δ_C 139.6 and 142.6) were ascribed to those bearing an oxygen atom (Table 1).

Four partial structures **a** (from C-2 to C-7), **b** (from C-9, C-11 to C-12), **c** (from C-15 to C-17), and **d** (from C-22 to C-23) were deduced from ^1H–^1H COSY analysis of **1** in $CDCl_3$ (Fig. 1). The presence of a 4-hydroxycyclohex-2-enone group in ring A was supported by HMBC correlations as shown in Figure 1. HMBC correlations for H-3 and H_3-19 of C-1 (δ_C 203.9) and for H-7, H_3-19, and H_3-30 of C-9 (δ_C 32.2) gave rise to the connectivity of partial structures **a** and **b** through C-8 and C-10 atoms. Connection between partial structures **b** and **c** could be assigned by HMBC correlations for H_3-18 of C-13 (δ_C 47.0) and C-14 (δ_C 159.8), for H_2-12 of C-17 (δ_C 51.7), and for H-15 of C-8 (δ_C 44.7). The presence of a β-furyl ring at

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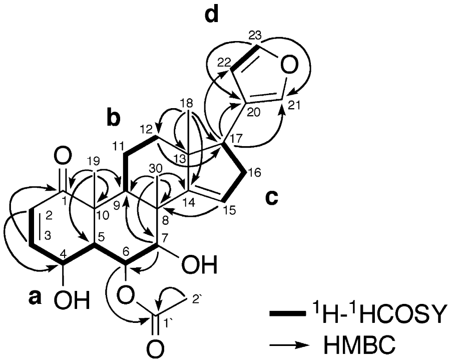


Figure 1. Selected 2D NMR correlations of ceramicine A (**1**).

C-17 was also assigned by the HMBC correlations as shown in Figure 1. In addition, HMBC correlations for H-6 and H$_3$-2′ of C-1′ (δ_C 172.5) indicated the presence of acetoxy group at C-6. Thus, ceramicine A (**1**) was concluded to be a unique limonoid possessing cyclopenta[*a*]phenanthren ring system without methyls at C-4 and with a β-furyl ring at C-17.

ROESY correlations of H-4, H-9/H-5, H-6/H$_3$-19, H-7, and H$_3$-30 indicated that the hydroxyl at C-4 was β-configuration and the acetoxy group at C-6 was α-configuration. Furthermore, the relative configurations at C-7, C-9, and C-17 were deduced from correlations of H-7/H-15, H-9/H$_3$-18, and H-17/H-12 and H$_3$-30 observed in the ROESY spectrum as shown in computer-generated 3D drawing (Fig. 2).

The HRESIMS of walsogyne A (**2**),[6] displayed a pseudomolecular ion peak at 609.2313 (M+Na)$^+$, compatible to the molecular formula of $C_{31}H_{38}O_{11}$. IR absorptions indicated the presence of hydroxyl (3439 cm^{-1}) and carbonyl (1759, 1719, and 1676 cm^{-1}) groups, respectively. UV spectra showed strong absorptions at 240 nm ascribable to conjugated chromophores. The ^{13}C NMR spectra revealed 31 carbon resonances due to four carbonyls, two sp^2 quaternary carbons, five sp^3 quaternary carbons, four sp^2 methines, eight sp^3 methines, two sp^3 methylenes, and six methyls. Among them, five methines (δ_C 71.3, 72.8, 80.4, 97.1, and 98.1), one methylene (δ_C 79.4), and one quaternary carbon (δ_C 98.8) were ascribed to those bearing an oxygen atom (Table 2).

Six partial structures **a** (from C-2 to C-3), **b** (from C-5 to C-7), **c** (from C-9 to C-11), **d** (from C-15 to C-17), **e** (from C-22 to C-23), and **f** (from C-3′ to C-4′) were deduced from ^{1}H–^{1}H COSY analysis of **2** in CDCl$_3$ (Fig. 3). The presence of a tiglate group in unit **f** at C-7 was supported by HMBC correlations as shown in Figure 3 and the chemical shifts of H-3′ (δ_H 6.65) and C-5′ (δ_C 12.2).[7] HMBC correlations for H$_3$-29 of C-3 (δ_C 153.7), H$_2$-28 and H-9 of C-5 (δ_C 45.4), H-5 and H$_3$-19 of C-1 (δ_C 203.9), and H-9 and H$_3$-30 of C-7 (δ_C 71.3) gave rise to the connectivity of partial structures **a**, **b**, and **c** through C-4, C-8, and C-10 atoms, constructing a decaline ring system. Connection between partial structures **c** and **d** could be assigned by HMBC correlations for H-11, H$_3$-18, and H$_3$-30 of C-14 (δ_C 98.8), H$_3$-18 of C-12 (δ_C 199.0) and C-17 (δ_C 39.0), and H-15 of C-13 (δ_C 59.1). The presence of a γ-hydroxy-α,β-unsaturated γ-lactone ring in unit **e** at C-17 was also assigned by the HMBC correlations as shown in Figure 3. Thus, walsogyne A (**2**) was concluded to be a unique ring C-*seco* limonoid possessing a tetrahydrofuran-2-ol ring produced by cleavage of C-11–C-12 bond and a γ-hydroxy-α,β-unsaturated γ-lactone ring at C-17.

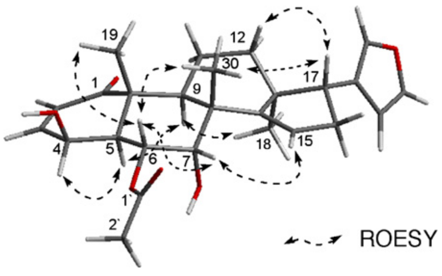


Figure 2. Selected ROESY correlations for ceramicine A (**1**).

Table 1

^{1}H [δ_H (*J*, Hz)] and ^{13}C NMR data (δ_C) of ceramicine A (**1**) in CDCl$_3$ at 300 K

	δ_H	δ_C
1		203.9
2	5.86 (1H, d, *J* = 9.6)	128.3
3	6.70 (1H, d, *J* = 9.6, 4.5)	142.6
4	4.30 (1H, t, *J* = 4.5)	62.6
5	2.57 (1H, m)	41.6
6	5.57 (1H, m)	69.8
7	4.09 (1H, br s)	73.2
8		44.7
9	2.40 (1H, m)	32.2
10		48.1
11	1.62 and 2.46 (each 1H, m)	17.7
12	1.58 and 1.90 (each 1H, m)	32.6
13		47.0
14		159.8
15	5.57 (1H, m)	120.6
16	2.38 and 2.52 (each 1H, m)	34.2
17	2.84 (1H, m)	51.7
18	0.87 (3H, s)	21.4
19	1.42 (3H, s)	16.8
20		124.3
21	7.24 (1H, s)	139.6
22	6.28 (1H, s)	110.9
23	7.37 (1H, s)	142.6
30	1.26 (3H, s)	26.0
1′		172.5
2′	2.20 (3H, s)	21.5

Table 2

^{1}H [δ_H (*J*, Hz)] and ^{13}C NMR data (δ_C) of walsogyne A (**2**) in CDCl$_3$ at 300 K

	δ_H	δ_C
1		203.9
2	6.02 (1H, d, 8.2)	130.0
3	7.12 (1H, d, 8.2)	153.7
4		41.5
5	2.48 (1H, d, 12.6)	45.4
6	4.33 (1H, br d, 12.6)	72.8
7	5.52 (1H, br s)	71.3
8		55.2
9	2.85 (1H, d, 6.5)	63.6
10		45.7
11	5.21 (1H, d, 6.5)	98.1
12	9.54 (1H, s)	199.0
13		59.1
14		98.8
15	4.83 (1H, d, 4.4)	80.4
16a	2.03 (1H, br d, 14.8)	34.7
16b	2.79 (1H, m)	
17	3.39 (1H, br d, 12.4)	39.0
18	1.25 (3H, s)	12.0
19	1.41 (3H, s)	21.9
20		134.3
21		172.9
22	6.76 (1H, d, 9.6)	149.0
23	6.02 (1H, d, 9.6)	97.1
28a	3.30 (1H, d, 7.1)	79.4
28b	3.71 (1H, d, 7.1)	
29	1.30 (3H, s)	19.9
30	1.66 (3H, s)	23.7
1′		166.4
2′		129.3
3′	6.65 (1H, q, 6.8)	137.5
4′	1.79 (3H, d, 6.8)	14.8
5′	1.82 (3H, s)	12.2

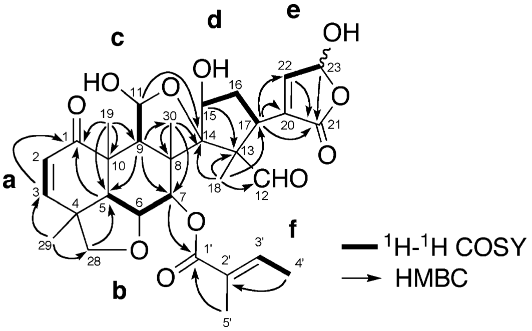


Figure 3. Selected 2D NMR correlations of walsogyne A (**2**).

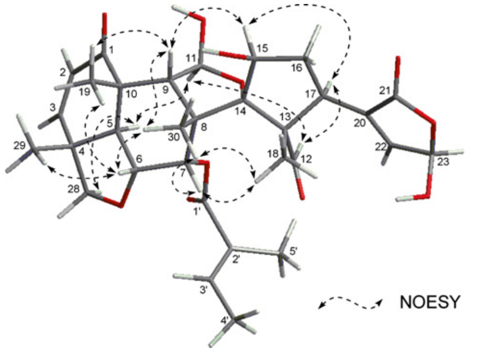
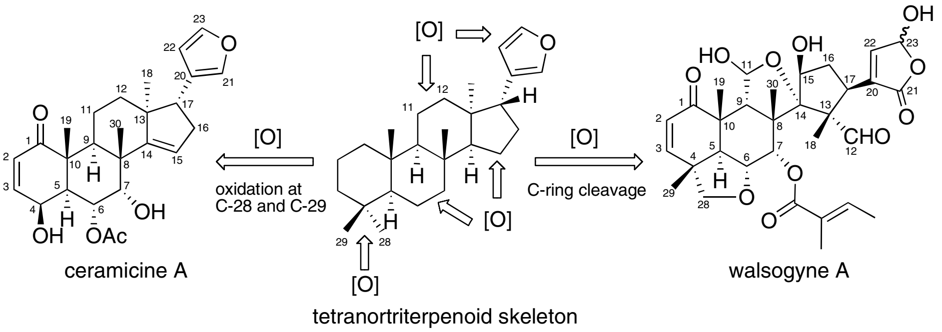


Figure 4. Selected NOESY correlations for walsogyne A (**2**).



Scheme 1. Plausible biogenetic path for ceramicine A (**1**) and walsogyne A (**2**).

NOESY correlations of H-6/H$_3$-19, H$_3$-29, and H$_3$-30, and H-7/H$_3$-18 and H$_3$-30 indicated that H-6 and H-7 were each β-configuration. The β-configuration of H-9 and α-configuration of H-5 and H-11 were assigned by the NOESY cross-peaks of H-9/H$_3$-19 and H$_3$-30, and H-5/H-11. Furthermore, the relative configurations at C-15 and C-17 were deduced from correlations observed in the NOESY spectrum as shown in computer-generated 3D drawing (Fig. 4). Stereochemistry at hemiacetal C-23 was to be epimerized because a pair of carbon signals (C-20, δ_C 134.3 and 134.4; C-22, δ_C 149.0 and 148.9; C-23, δ_C 97.1 and 97.0) appeared around C-23.

A plausible biogenetic pathway for ceramicine A (**1**) and walsogyne A (**2**) is proposed as shown in Scheme 1. Ceramicine A (**1**) could be transformed from tetranortriterpenoid skeleton derived from apotirucallane skeleton via oxidation at C-28 and C-29, followed by decarboxylation, whereas after oxidative cleavage of C-11–C-12 bond, walsogyne A (**2**) might be derived through keto-enol isomerization of aldehyde at C-9, followed by forming a tetrahydrofuran-2-ol as shown in Scheme 1.

Ceramicine A (**1**) and walsogyne A (**2**) showed a moderate in vitro cytotoxic activity on P388 cells (IC$_{50}$ 15 μg/ml for **1**; 5.0 μg/ml for **2**).

Acknowledgments

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5. Ceramicine A (**1**): colorless solid; $[\alpha]_D$ +30 (*c* 1.0, CHCl$_3$); IR (neat) ν_{max} 3449, 2928, 2868, 1734, 1720, 1686, 1459, 1376, and 1255 cm^{-1}; UV (MeOH) λ_{max} 203 nm (ε 22,000); ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 463 (M+Na)$^+$; HRESIMS *m/z* 463.2093 (M+Na; calcd for $C_{26}H_{32}NaO_6$, 463.2096).
6. Walsogyne A (**2**): colorless solid; $[\alpha]_D$ −31 (*c* 1.0, CHCl$_3$); IR (KBr) ν_{max} 3439, 2979, 2880, 1759, 1719, 1676, 1395, 1256, and 1071 cm^{-1}; UV (MeOH) λ_{max} 213 nm (ε 17,200); ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 609 (M+Na)$^+$; HRESIMS *m/z* 609.2313 (M+Na; calcd for $C_{31}H_{38}O_{11}Na$, 609.2312).
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