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CHISOMICINES D AND E, TWO NEW LIMONOIDS FROM *CHISOCHETON CERAMICUS*

Ibrahim A. Najmuldeen,[a] A. Hamid A. Hadi,[a,]* Khalit Mohamad,[b] Khalijah Awang,[a] Kamal Aziz Ketuly,[c] Mat Ropi Mukhtar,[a] Hairin Taha,[a] Noraziah Nordin,[a] Marc Litaudon,[d] Françoise Guéritte,[d] Alfarius Eko Nugroho,[e] and Hiroshi Morita[e,]*

[a]Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia, [b]Department of Pharmacy, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia, [c]CENAR, Department of Chemistry, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia, [d]Institut de Chimie des Substances Naturelles, 1, Avenue de la Terrasse, CNRS, 91198 Gif-sur-Yvette, France, [e]Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan

Abstract – Two new limonoids, chisomicines D and E (**1** – **2**) have been isolated from the bark of Malaysian *Chisocheton ceramicus* (Miq.) C. DC. Their structures were elucidated on the basis of 1D and 2D-NMR data analysis.

Meliaceae or the mahogany family comprises over 1400 species in 51 genera of woody plants distributed in subtropical and tropical regions worldwide. Within this family, two major subfamilies,[1] Melioideae and Swietenioideae, have been recognized based on seed morphology,[2] wood anatomy and molecular data.[3] Melioideae forms a monophyletic group that includes 73% of the genera and 87% of the species of the family.[3] Plants belonging to the Meliaceae family are known to produce a group of highly oxidized triterpenes named limonoids.

Information about a wide range of biological activities, such as antimurine P388 leukemia cell lines especially those with a 14,15-epoxy D ring,[4] antifeedants,[5] insecticides,[6] antitumor,[7] and antimalarial and growth regulating activities,[8] as well as a variety of medicinal effects in animals and humans was shown for limonoids.[9, 10] We have found the alcoholic extract of the bark of *Chisocheton ceramicus* (Miq.) C. DC. (Meliaceae) is a rich source of interesting limonoids, named as a series of ceramicines and chisomicines.[11-14] In continuation of our research on the Meliaceae family,[14-16] we have isolated two new

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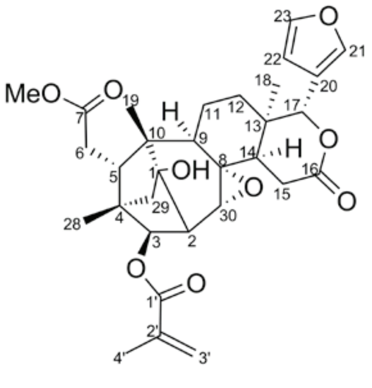
[†]Dedicated to Prof. Dr. Albert Padwa, Emory University on the occasion of the 75th birthday.

phragmalin-type limonoids, chisomicines D (**1**) and E (**2**) from the bark of *C. ceramicus*. We now wish to report isolation and structure elucidation of **1** and **2**.



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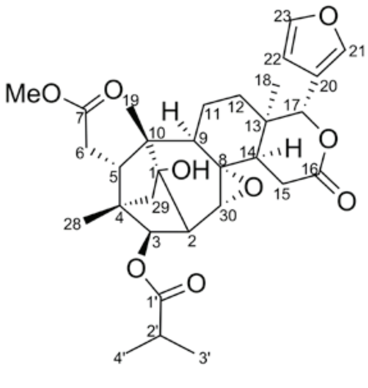

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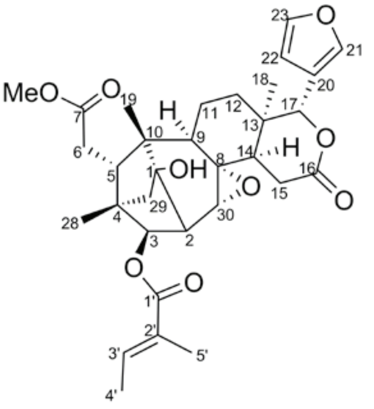

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Chisomicine D (**1**) was obtained as white amorphous solid and the HRESIMS displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 555.2551 corresponding to the molecular formula of $C_{31}H_{38}O_9$ and IR absorptions (1735 and 1703 cm^{-1}) implied the presence of carbonyl functionalities.

The ^{1}H-NMR spectrum ($CDCl_3$ 700MHz) of **1** displayed signals for four tertiary methyls at δ_H 1.14 (H_3-18), 1.02 (H_3-19), 0.93 (H_3-28), and 1.95 (H_3-4') as well as one methoxy at δ_H 3.71. The signals typical for a furan moiety at δ_H 6.45 (H-22), 7.42 (H-23), and 7.70 (H-21) were observed at downfield region.

The ^{13}C NMR spectrum of **1** revealed the presence of thirty one carbon atoms in the molecule, and the observed signals were very similar to those of chisomicine B (**3**).[14] Further analysis of the ^{13}C NMR data indicated that the following signals at δ_C 135.3 (C-2'), δ_C 127.1 (C-3'), and δ_C 21.5 (C-4') in place of δ_C 128.0 (C-2'), δ_C 139.3 (C-3'), δ_C 14.2 (C-4'), and δ_C 12.1 (C-5') were observed, which implies the presence of a methacrylate group in **1** instead of a tiglate group in chisomicine B.

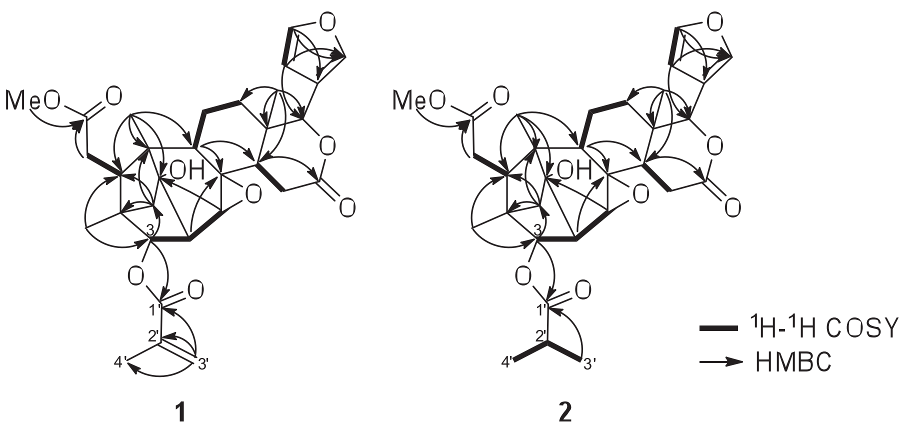

Figure 1. Selected 2D-NMR Correlations of **1** and **2**

The previously mentioned assumption as well as the planar structure of **1** was confirmed by using the 2D-NMR correlations (HSQC, ^{1}H–^{1}H COSY, and HMBC) as shown in Figure 1. The HMBC correlations of H_2-3' to C-1', C-2', and C-4' verified the presence of a methacrylate moiety, while the HMBC correlation of H-3 to C-1' confirmed attachment of the methacrylate moiety to C-3.

Table 1. ^{1}H & ^{13}C NMR Spectral Data of Chisomicines D and E (**1** and **2**) in $CDCl_3$

	δ_H (J, Hz)		δ_C	
	1	**2**	**1**	**2**
1			80.4	80.2
2	2.99 (1H, dd, 11.0, 3.3)	2.88 (1H, dd, 11.0, 3.3)	43.6	43.2
3	4.85 (1H, d, 11.0)	5.18 (1H, d, 11.0)	78.7	76.7
4			45.1	46.0
5	3.05 (1H, dd, 11.0, 2.4)	2.95 (1H, dd, 11.0, 2. 4)	39.2	39.3
6	2.28 (2H, m)	2.28 (2H, m)	34.1	34.1
7			173.7	173.6
8			61.3	61.3
9	1.88 (1H, m)	1.90 (1H, m)	41.6	41.4
10			44.7	44.9
11a	1.90 (1H, m)	1.90 (1H, m)	21.5	21.5
11b	1.88 (1H, m)	1.88 (1H, m)		
12	1.70 (1H, m)	1.70 (1H, m)	33.5	33.6
	1.48 (1H, d, 11.0)	1.48 (1H, d, 11.0)		
13			36.0	36.1
14	2.08 (1H, dd, 6.2, 1.3)	2.18 (1H, dd, 6.2, 1.3)	44.9	45.1
15	2.58 (1H, dd, 18.6, 7.2)	2.67 (1H, dd, 18.6, 7.2)	27.3	27.4
	2.37 (1H, dd, 18.6, 1.9)	2.38 (1H, dd, 18.6, 1.9)		
16			169.5	169.8
17	5.46 (1H, s)	5.70 (1H, s)	77.7	77.7
18	1.14 (3H, s)	1.11 (3H, s)	22.2	22.1
19	1.02 (3H, s)	1.03 (3H, s)	18.1	18.3
20			120.7	120.4
21	7.70 (1H, s)	7.85 (1H, s)	141.3	141.8
22	6.45 (1H, s)	6.51 (1H, s)	109.6	109.6
23	7.42 (1H, br. s)	7.44 (1H, br. s)	143.1	143.3
28	0.93 (3H, s)	0.85 (3H, s)	15.0	15.1
29	2.04 (1H, d, 11.0)	2.04 (1H, d, 11.0)	43.4	43.3
	1.40 (1H, dd, 11.0, 1.7)	1.40 (1H, dd, 11.0, 1.7)		
30	3.28 (1H, d, 3.0)	3.31 (1H, d, 3.0)	59.5	59.9
OMe	3.71(3H, s)	3.67 (3H, s)	52.0	51.9
1`			167.8	177.7
2`		2.65 (1H, m)	135.3	33.7
3`a	6.30 (1H, s)	1.12 (3H, br. s)	127.1	18.8
3`b	5.40 (1H, s)			
4`	1.95 (3H, s)	1.05 (3H, br. s)	21.5	19.8

The relative structure of **1** was presumed to be the same as chisomicine B because of the high similarity of the NMR chemical shifts of the backbone skeleton, and the NOESY correlations further supports the presumption. NOESY correlations (Figure 2) of H-2/H-29b and H-3/H_3-28 and H-29b suggested β orientation of H_3-28 and α orientation of H-2 and H-3 while those of H-5/H-14 and H-3'b and H-14/H-30 indicated β orientation of H-5, H-14, and H-30. And finally the relative configurations of C-10, C-13, and C-14 were deduced from the NOESY correlations of H-14/H-10 and H_3-18, implying α orientation of H-10, H-14, and H_3-18.

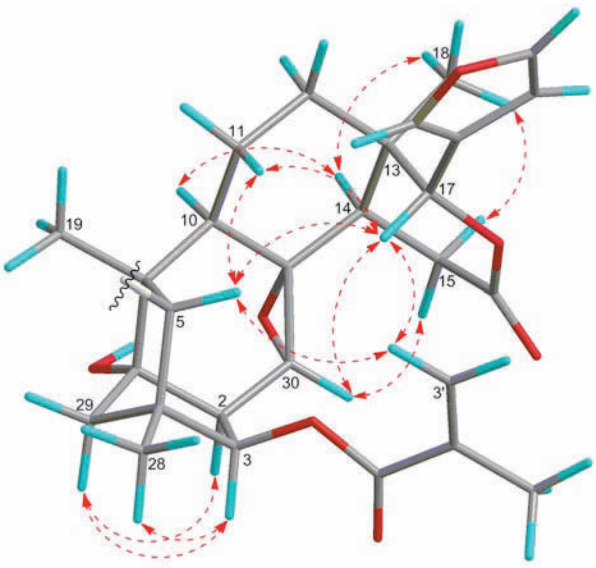

Figure 2. Selected NOESY Correlations of **1**. The side chain at position 5 (C-6 and C-7) was omitted for clarity

Chisomicine E (**2**) was obtained as white amorphous solid and the HRESIMS displayed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 557.2693 corresponding to the molecular formula of $C_{31}H_{40}O_9$, and IR absorptions (1735 cm^{-1}) implied the presence of carbonyl functionalities. The ^1H and ^{13}C NMR spectra of **2** are similar to those of **1** except for the chemical shifts of a side chain at position 3 (Table 1). Based on the NMR data and the HRESIMS data, the structure of **2** was presumed to be chisomicine E with an isobutyrate group at C-3 instead of a methacrylate group. The COSY correlations of H-2'/H_3-3' and H_3-4' and the HMBC correlations of H_3-3' and H-3 to C-1' confirmed the existence of the isobutyrate unit at C-3 (Figure 1).

The relative structure of **2** was assumed to be the same as chisomicine B based on the chemical shifts similarity, and was also confirmed by the observed NOESY correlations which are similar to those of **1**.

The isolated compounds were tested for cytotoxicity against HL-60 cell line. Both compounds were found to be inactive ($IC_{50} > 50$ μM).

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. UV spectra were obtained on an Ultrospec 2100 pro spectrophotometer and IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer. High-resolution ESI MS were obtained on a LTQ Orbitrap XL (Thermo Scientific). ^{1}H and 2D NMR spectra were recorded on a Bruker AV700 spectrometer and chemical shifts were referenced to the residual solvent peaks (δ_H 7.26 and δ_C 77.0 for chloroform-*d*). Standard pulse sequences were employed 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 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY spectra in the phase sensitive mode were measured with a mixing time of 800 ms. For HSQC spectra in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C-H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

Plant Material. The barks of *C. ceramicus* were collected in 2000 from Hutan Simpan Bukit Enggang, Malaysia. The plant species was identified by Mr. Teo Leong Eng, with a voucher specimen (No. KL 4973) and the herbarium specimen was deposited in the herbarium of the Chemistry Department, University of Malaya.

Extraction and Isolation. The dried and powdered bark of *C. ceramicus* (900 g) was extracted successively with methanol and the methanol extract (200 g) was partitioned with 10% MeOH aq. and EtOAc. EtOAc soluble materials (10 g) were subjected to a silica gel column (hexane/ EtOAc, 1:0→ 0:1), in which a fraction eluted with hexane/EtOAc (1:4) was further purified on a silica gel column with the mixture of EtOAc/acetone/hexane (65:10:25). The first sub-fraction has been subjected to an π-NAP HPLC (H_2O/MeCN 77:23, 2.4 mL/min, 210 nm) to give chisomicine D (**1,** 0.5 mg 0.005%) and E (**2,** 0.5 mg 0.005%).

Chisomicine D (1): white amorphous solid; $[\alpha]_D^{20}$ -15° (*c* 0.3, MeOH); IR (KBr) ν_{max} 3391, 2972, 1735, 1703 and 1268 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (3.82) and 274 (2.69); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 226 (0.03), 230 (0) and 244 (0.04); ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 577 $(M+Na)^+$; HRESIMS *m/z* 555.2551 (M+H; calcd for $C_{31}H_{39}O_9$, 555.2589).

Chisomicine E (2): white amorphous powder; $[\alpha]_D^{20}$ +18° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3391, 2972, 1735 and 1268 cm^{-1}; UV (MeOH) λ_{max} (log ε) 203 (3.77) and 272 (2.92); CD (MeOH) λ_{max} ($\Delta\varepsilon$) 222 (0.27), 257 (0.04), 275 (0) and 299 (0.07); ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 579 $(M+Na)^+$; HRESIMS *m/z* 557.2693 (M+H; calcd for $C_{31}H_{41}O_9$, 557.2745).

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