Moluccensins H−J, 30-Ketophragmalin Limonoids from *Xylocarpus moluccensis*

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Three new phragmalin limonoids, moluccensins H−J (**1**−**3**), were isolated from seed kernels of the cedar mangrove, *Xylocarpus moluccensis*. Their structures were established by extensive spectroscopic analysis. Compound **2** displayed weak antibacterial activity against *Staphylococcus hominis* and *Enterococcus faecalis*.

Limonoids are moderately polar, insoluble in water, but soluble in hydrocarbons, alcohols, and ketones. In the plant kingdom, they are most abundant in the order Sapindales and especially mahogony (Meliaceae) and citrus (Rutaceae). Limonoid examination of the Meliaceae family (meliacins) is of growing interest due to a range of biological activities, such as insect antifeedants and growth regulators, and antibacterial, antifungal, antimalarial, anticancer, and antiviral activities in humans.[1−3] These chemically unique limonoids include the phragmalins, the ring B,D-seco limonoids with characteristic tricyclo[3.3.$1^{2,10}$.$1^{1,4}$]decane and tricyclo[4.2.$1^{10,30}$.$1^{1,4}$]decane ring systems, which characterize the structural diversity and potential biological significance.[4−6] They occur with greater frequency in the Meliaceae rather than the Rutaceae.[7,8] Indeed, several new phragmalins have been isolated from *Xylocarpus granatum* in recent years.[9−12]

Limonoid derivatives have been found in all *Xylocarpus* plants studied, but their distribution and content varies between different species and between parts, or geocultivars, of the same species. These unique characteristics prompted us to investigate another plant in this genus, the cedar mangrove or puzzlenut, *Xylocarpus moluccensis* (Lamk.) Roem. *X. moluccensis* seeds have been used as a cure for elephantiatis, scabies, and kudis and is reported to have antifilarial activity.[13−15] They also affect the central nervous system.[16] The bark has been used for abdominal problems such as dysentery and diarrhea and inhibits diverse Gram-negative and Gram-positive bacteria.[17] Additionally, this species produces a number of limonoids[18−20] including the recent isolation of seven new phragmalins with a conjugated C-30 carbonyl group.[21]

We have isolated three new 30-ketophragmalin limonoids, moluccensins H−J (**1**−**3**), from the kernel seeds of *X. moluccensis*. These feature a characteristic α,β-unsaturated ketone moiety at C-30. We report the isolation and structural elucidation of compounds **1**−**3**.

Moluccensin H (**1**) was isolated as a colorless gum, with molecular formula $C_{29}H_{32}O_{10}$ by HRESIMS (*m*/*z* 563.1888 [M + Na]$^+$, calcd 563.1893), indicating 14 degrees of unsaturation. The IR absorptions at 3452, 1737, and 1682 cm^{-1} implied hydroxy and ester groups. The ^{1}H NMR spectrum (Table 1) displayed resonances of a β-substituted furanyl ring (δ_H 7.49, 7.44, and 6.45), an olefinic proton (δ_H 7.00), three tertiary methyl (δ_H 1.22, 0.99, and 0.97), an *O*-methyl (δ_H 3.70), and an *O*-acetyl (δ_H 1.96) group. In the ^{13}C NMR spectrum, 29 nonequivalent carbon resonances were observed, including four carbonyl carbons (δ_C 194.6, 173.4, 169.5, and 165.5), eight olefinic carbons (δ_C 168.9, 152.3, 143.1, 141.3, 121.8, 119.9, 115.2, and 110.0), and five methyl carbons (δ_C 52.2, 20.6, 16.7, 16.1, and 15.7). The remaining carbons were assigned to four methylenes, three methines, and five quaternary carbons, based on the results of an HSQC experiment. These NMR data indicated that eight of the 14 units of unsaturation come from four carbon−carbon double bonds and four carbonyls. Therefore, the remaining six degrees required **1** to comprise a hexacyclic core. The data from decouplings and the subsequent 2D NMR studies (HMBC and HSQC) suggested that **1** was a phragmalin limonoid. Two protons at δ_H 1.94 and 1.87 correlating in the HSQC spectrum to a methylene signal at δ_C 41.7 were indicative of the H-29 protons of the characteristic 4,29,1-ring bridge of phragmalin limonoids.[8−11] This was confirmed by the HMBC correlations (Figure 1) observed from the H-29 protons to the tertiary carbon at δ_C 43.5 (C-5) and to the quaternary carbons at δ_C 86.6 (C-1), 45.1 (C-4), and 48.4 (C-10). The HMBC correlations between C-7 (δ_C 173.4) and H_2-6 (δ_H 2.52 and 2.40) and the *O*-methyl protons at δ_H 3.70 also confirmed the typical C-6−C-7 appendage of phragmalins.[22−25] A proton singlet at δ_H 5.02 was assignable to H-17 by correlations with the furanyl carbon at δ_C 119.9 (C-20) and the C-18 methyl carbon at δ_C 15.7. A δ-lactone ring was corroborated by the HMBC cross-peaks from H-17 to both bridgehead carbons, C-13 (δ_C 36.4) and C-14 (δ_C 152.3), and the carbonyl carbon at δ_C 165.5. The vinylic proton at δ_H 7.00 assigned to H-15 also exhibited significant HMBC correlation to C-13 and C-14 and the lactone carbonyl carbon (δ_C 165.5). Further, this α,β-unsaturated δ-lactone was conjugated to the $\Delta^{8,9}$ double bond to form a conjugated diene lactone system, which was confirmed by the HMBC correlation of H-15/C-8 and Me-19/C-9. The $\Delta^{8,9}$ double bond was also conjugated to the C-30 ketone carbonyl carbon, responsible for the high-field carbon signal at δ_C 194.6. This carbonyl carbon was assigned to C-30 due to the HMBC cross-peak from the D_2O exchangeable proton at δ_H 4.95, which correlated to C-2 at δ_C 80.6. The above analyses, and other 1D and 2D NMR information, led us to suggest the gross structure of **1** (Figure 1) as a new structure with a characteristic diene lactone-conjugated ketone moiety at C-30.

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Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) Data of Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		86.6		84.7		84.4
2		80.6		92.2	3.44, s	63.6
3	4.90, s	87.6	4.92, s	82.8	4.95, s	83.7
4		45.1		40.1		40.6
5	2.79, m	43.5	2.37, t (10.0)	39.6	2.13, m	40.5
6	2.40, m	33.1	2.25, d (12.0)	34.2	2.22, dd (2.8, 15.6)	34.2
	2.52, dd (5.6, 17.2)		2.43, m		2.38 dd (4.4, 15.6)	
7		173.4		172.9		172.9
8		121.8		133.9		135.2
9		168.9	2.46, m	46.9	2.43 br, m	48.1
10		48.4		55.4		56.4
11	2.43, m	25.2	1.43, m	18.7	1.40, m	19.5
			1.74, m		1.73, m	
12	1.49, m	30.5	1.41, m	31.4	1.38, m	31.9
	1.67 br, m		1.49, m		1.52, m	
13		36.4		40.8		40.4
14		152.3		139.2		132.1
15	7.00, s	115.2	3.75, m	33.0	3.37, d (19.2)	35.3
					3.89, d (19.2)	
16		165.5		169.9		171.0
17	5.02, s	80.4	5.17, s	80.2	5.17, s	80.9
18	0.99, s	15.7	1.00, s	17.1	0.97, s	17.0
19	1.22, s	16.1	1.03, s	15.1	0.96, s	14.7
20		119.9		120.4		120.4
21	7.49, s	141.3	7.45, s	141.2	7.43, s	141.2
22	6.45, s	110.0	6.40, s	110.0	6.39, s	110.1
23	7.44, s	143.1	7.40, s	143.0	7.38, s	142.9
28	0.97, s	16.7	0.96, s	19.7	0.94, s	19.1
29	1.87, dd (2.0, 11.2)	41.7	1.72, m	43.7	1.94, d (12.8)	44.1
	1.94, d (11.2)		2.20, d (13.2)		2.12 br, s	
30		194.6		203.5		205.1
7-OCH_3	3.70, s	52.2	3.65, s	51.8	3.63, s	51.7
3-OAc		169.5		170.1		170.5
	1.96, s	20.6	2.15, s	20.5	2.10, s	20.6
2-OCH_3			3.40, s	55.1		
2-OH	4.95, s					
1-OH	2.88 br, s		2.93 br, s		3.12 br, s	

The relative configuration of **1** was elucidated by NOESY data (Figure 1). Limonoids are stereochemically homogeneous compounds since they have a prototypical structure that either contains or is derived from a precursor with a 4,4,8-trimethyl-17-furanyl-steroid skeleton.[26] The orientation of H-17 had been found to be exclusively β in all known phragmalins.[9−12] Thus, the cross-peaks in the NOESY spectrum from H-17 and H-5 to H-12β and from H-5 to Me-28 indicated a β-orientation of these protons. NOESY correlations of Me-18 with H-12α, 1-OH with Me-19, and H-3 with H-29 and 2-OH all suggested that Me-18, Me-19, H-3, H_2-29, 1-OH, and 2-OH were α-oriented. Thus, the relative configuration of **1** is, accordingly, correct.

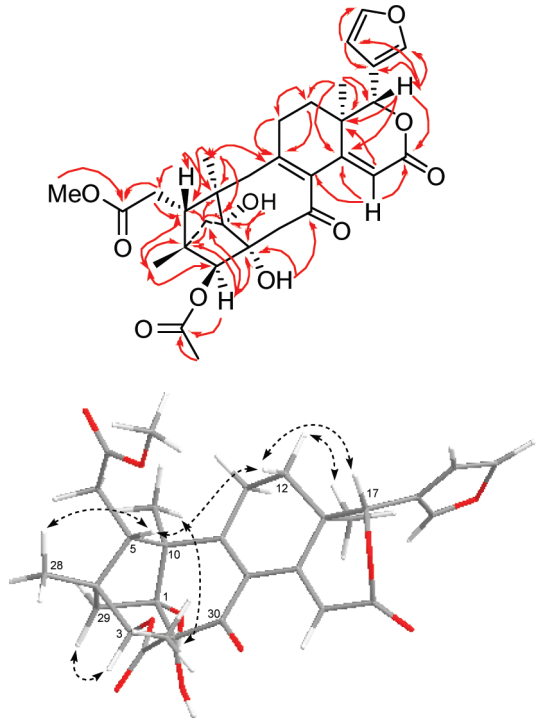

Figure 1. Key HMBC and NOESY correlations of **1**.

Moluccensin I (**2**) was isolated as a light yellow gum with molecular formula $C_{30}H_{36}O_{10}$ as determined by the HRESIMS ion at m/z 579.2201 $[M + Na]^+$ (calcd 579.2206). Thus, 13 unsaturations are present in compound **2**. The ^{1}H and ^{13}C NMR, as well as the 2D NMR spectra, suggested that **2** is also a 30-ketophragmalin limonoid with the same basic skeleton as **1**. The obvious difference was the absence of the olefinic proton at C-15 in **1** and the presence of only one double bond between C-8 and C-14, confirmed by the HMBC correlations of Me-18/C-14, H_2-15/C-8, and H_2-11/C-8. Without the extended conjugative effect in **1**, the ketone carbonyl at C-30 was significantly shifted downfield to δ_C 203.5. Furthermore, analysis of NMR data revealed the presence of the *O*-methyl group (δ_H 3.40 and δ_C 55.1) at C-2 in place of the hydroxy group of **1**. The similar NOESY correlations between **2** (Figure 2) and **1** (Figure 1) indicated the same stereochemistry for the core skeleton of **2** compared to **1**. The key NOE cross-peak in **2** between Me-19 and H-9 confirmed the α-orientation of H-9.

Moluccensin J (**3**) was isolated as a light yellow gum with molecular formula $C_{29}H_{34}O_9$ indicated by the HRESIMS ion at m/z 549.2100 $[M + Na]^+$ (calcd 549.2101). The ^{1}H and ^{13}C NMR data

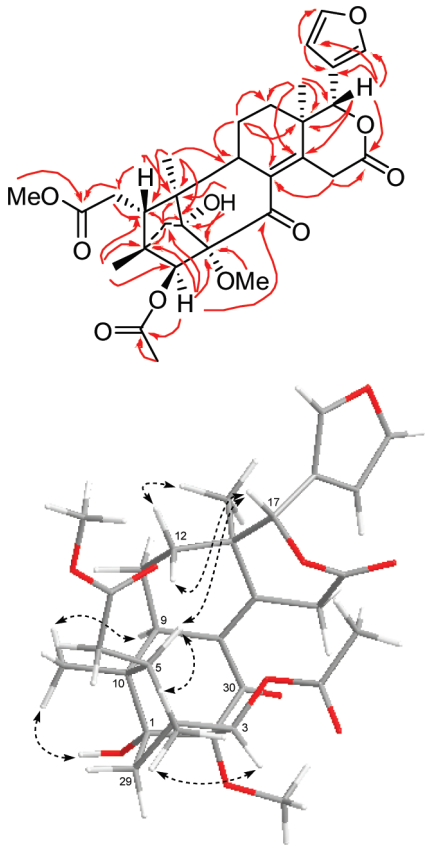

Figure 2. Key HMBC and NOESY correlations of **2**.

of **3** were virtually identical to those of **2**. The absence of the *O*-methyl signal, along with a resonance indicating an additional methine proton at δ_H 3.44 coupled to the carbon resonance at δ_C 63.6 in the HSQC spectrum, suggested that the methoxy group had been replaced by a hydrogen at C-2. This was confirmed by the $^1H-^1H$ COSY cross-peak of H-2/H-3 and the HMBC correlations from H-2 at δ_H 3.44 to C-30 at δ_C 205.1 and C-1 at δ_C 84.4. The relative configuration was determined to be the same as **2** by NOESY.

Compounds **1**−**3** were tested for their cytotoxic effects against five human tumor cell lines (BT474, CHAGO, Hep-G2, KATO-3, and SW-620) as well as their antibacterial properties against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus hominis*, *Staphylococcus epidermidis, Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Salmonella typhimurium*. All compounds were inactive against the cell lines (IC_{50} > 10 μg/mL). Only **2** displayed weak antibacterial activity against *Staphylococcus hominis* and *Enterococcus faecalis,* with a MIC at 256 μg/mL.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter at 589 nm, and UV data were recorded on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded on a Bruker Vector 22 Fourier transform spectrophotometer. HRESIMS spectra were obtained with a Bruker micrOTOF. The NMR spectra were recorded in $CDCl_3$ using a Varian YH400 spectrometer at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR using TMS as internal standard.

Plant Material. Fruits of *X. moluccensis* were collected from Samutsongkram Province, Thailand, in May 2009. Plant materials were identified by Royal Forest Department, Bangkok, Thailand. A voucher specimen (BKF 159046) was deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Extraction and Isolation. The kernel seeds (1.50 kg) of *X. moluccensis* were extracted with MeOH (2 L × 2, each for two days) at room temperature. After removing the solvent in vacuo, the combined MeOH crude extract was suspended in H_2O (250 mL) and partitioned with EtOAc (500 mL × 3) to obtain the EtOAc crude extract (56.8 g). This was chromatographed on a silica gel column eluted with a gradient of acetone−*n*-hexane (from 1:0 to 0:1) to yield seven fractions (I−VII). Fraction IV (10.2 g) was subjected to silica gel column chromatography and eluted with a gradient system of acetone−benzene to give 15 subfractions (IV.1−IV.15). Subfraction IV.8 (2.1 g) was further subjected to column chromatography over silica gel using a mixture of acetone−*n*-hexane (1:2) to afford nine fractions, IV.8.1−IV.8.9. Fraction IV.8.5 was further chromatographed on a silica gel column, eluted with acetone−*n*-hexane (1:2), to yield **1** (60.8 mg), while fraction IV.8.9 was purified by column chromatography with MeOH−CH_2Cl_2 (3:97) to give **2** (128.3 mg) and **3** (156.0 mg), respectively.

Moloccensin H (1): colorless gum; $[\alpha]^{20}_D$ +140 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 265 (4.16) nm; IR (KBr) ν_{max} 3452, 2957, 1737, 1682, 1636, 1376, 1227, 1029, and 875 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 563.1888 $[M + Na]^+$ (calcd $C_{29}H_{32}O_{10}Na$, 563.1893).

Moloccensin I (2): light yellow gum; $[\alpha]^{20}_D$ −12 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 215 (4.12) nm; IR (KBr) ν_{max} 3458, 2944, 1743, 1504, 1460, 1373, 1228, 1163, 1111, 1024, 913, 875, and 731 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 579.2201 $[M + Na]^+$ (calcd $C_{29}H_{32}O_{10}Na$, 579.2206).

Moloccensin J (3): light yellow gum; $[\alpha]^{20}_D$ −4 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 290 (3.13) nm; IR (KBr) ν_{max} 3452, 2944, 1741, 1505, 1461, 1437, 1372, 1236, 1164, 1025, 912, 875, and 731 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 549.2100 $[M + Na]^+$ (calcd. $C_{29}H_{34}O_9Na$, 549.2101).

Cytotoxicity Bioassays.[27,28] All stock cultures were grown in T-25 flasks. Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 5000 cells per well with compounds added from DMSO-diluted stock. After three days in culture, attached cells were stained with MTT (3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium] bromide). Absorbency at 540 nm was measured using a microplate reader after solubilizing the bound dye. The mean IC_{50} is the concentration of agent that inhibits cell growth by 50% under the experimental conditions and is the average from at least six independent determinations that were reproducible and statistically significant at the 5% level. The following human tumor cell lines were used in the assay: human breast ductol carcinoma ATCC No. HTB 20 (BT474), undifferentiated lung carcinoma (CHAGO), liver hepatoblastoma (Hep-G2), gastric carcinoma ATCC No. HTB 103 (KATO-3), and colon adeno carcinoma ATCC No. CCL 227 (SW-620). All cell lines were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, and were cultured in RPMI-1640 supplemented with 25 mM HEPES, 0.25% (w/v) sodium bicarbonate, 5% (v/v) fetal bovine serum, and 100 μg/mL kanamycin.

Antibacterial Assays. A broth microdilution method was used to determine the MIC according to the NCCLS protocol.[29] Five reference Gram-positive bacteria, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Staphylococcus hominis* ATCC 27844, *Staphylococcus epidermidis* ATCC 12228, and *Enterococcus faecalis* ATCC 29212, and four Gram-negative bacteria, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, and *Salmonella typhimurium* ATCC 13311, were tested. All tests were performed in Mueller Hinton broth. Serial doubling dilutions of the compound, prepared in a 96-well microtiter plate, ranged from 0.5 to 256 μg/mL. The final concentration of each strain was adjusted to 5×10^5 cfu/mL. The MIC was defined as the lowest concentration of the compound at which the microorganism does not demonstrate visible growth as determined by turbidity.

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Supporting Information Available: IR, HRESIMS, and ^1H and ^{13}C NMR spectra of compounds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

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