Moluccensins H–J, 30-Ketophragmalin Limonoids from Xylocarpus moluccensis

Khanitha Pudhom,*,†,‡ Damrong Sommit,§ Paulwatt Nuclear,‡ Nattaya Ngamrojanavanich,†,‡ and Amorn Petsom†,‡

Research Centre for Bioorganic Chemistry, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand, Center for Petrochemicals, Advanced Materials, Chulalongkorn University, Bangkok, 10330, Thailand, Department of Chemistry, Faculty of Science, Mahanakorn University of Technology, Bangkok 10530, Thailand, and Program in Biology, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok, 10120, Thailand

Received September 19, 2009

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 mahogany (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[3.3.1.2.10.30.11.4]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 elephantiasis, 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 C29H32O10 by HRESIMS (+ [M + Na]+, calc 563.1893), indicating 14 degrees of unsaturation. The IR absorptions at 3452, 1737, and 1682 cm−1 implied hydroxy and ester groups. The 1H NMR spectrum (Table 1) displayed resonances of a β-substituted furanyl ring (δH 7.49, 7.44, and 6.45), an olefinic proton (δH 7.00), three tertiary methyl (δH 1.22, 0.99, and 0.97), an O-methyl (δH 3.70), and an O-acetyl (δH 1.96) group. In the 13C NMR spectrum, 29 nonequivalent carbon resonances were observed, including four carbonyl carbons (δC 194.6, 173.4, 169.5, and 165.5), eight olefinic carbons (δC 168.9, 152.3, 143.1, 141.3, 121.8, 119.9, 115.2, and 110.0), and five methyl carbons (δC 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 HMQC) suggested that 1 was a phragmalin limonoid. Two protons at δH 1.94 and 1.87 correlating in the HSQC spectrum to a methylene signal at δC 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 δC 43.5 (C-5) and to the quaternary carbons at δC 86.6 (C-1), 45.1 (C-4), and 48.4 (C-10). The HMBC correlations between C-7 (δC 173.4) and H-6 (δH 2.52 and 2.40) and the O-methyl protons at δH 3.70 also confirmed the typical C-6–C-7 appendage of phragmalins.22–25

A proton singlet at δH 5.02 was assignable to H-17 by correlations with the furanyl carbon at δC 119.9 (C-20) and the C-18 methyl carbon at δC 15.7. A δ-lactone ring was corroborated by the HMBC cross-peaks from H-17 to both bridgehead carbons, C-13 (δC 36.4) and C-14 (δC 152.3), and the carbonyl carbon at δC 165.5. The vinylic proton at δH 7.00 assigned to H-15 also exhibited significant HMBC correlation to C-13 and C-14 and the lactone carbonyl carbon (δC 165.5). Further, this α,β-unsaturated δ-lactone was conjugated to the Δ8,9 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 Δ8,9 double bond was also conjugated to the C-30 ketone carbonyl carbon, responsible for the high-field carbon signal at δC 194.6. This carbonyl carbon was assigned to C-30 due to the HMBC cross-peak from the D2O exchangeable proton at δH 4.95, which correlated to C-2 at δC 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.
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 \(\beta\) in all known phragmalins.\(^{9-12}\) Thus, the cross-peaks in the NOESY spectrum from H-17 and H-5 to H-12/\(\beta\) and from H-5 to Me-28 indicated a \(\beta\)-orientation of these protons. NOESY correlations of Me-18 with H-12,\(\alpha\)-OH with Me-19, and H-3 with H2-29, 1-OH, and 2-OH all suggested that Me-18, Me-19, H-3, H2-29, 1-OH, and 2-OH were \(\alpha\)-oriented. Thus, the relative configuration of 1 is, accordingly, correct.

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, H2-15/C-8, and H2-11/C-8. Without the extended conjugative effect in 1, the ketone carbonyl at C-30 was significantly shifted downfield to \(\delta_C\) 203.5. Furthermore, analysis of NMR data revealed the presence of the O-methyl group (\(\delta_H\) 3.40 and \(\delta_C\) 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 \(\alpha\)-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 1. Key HMBC and NOESY correlations of 1.](image-url)
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 $\delta_H$ 3.44 coupled to the carbon resonance at $\delta_C$ 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 $^1$H-$^1$H COSY cross-peak of H-2/H-3 and the HMBC correlations from H-2 to $\delta_H$ 3.44 to C-30 at $\delta_C$ 205.1 and C-1 at $\delta_C$ 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 g/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 microOTOF. The NMR spectra were recorded on a Shimadzu UV-160 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 341 polarimeter at 589 nm, and UV data 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 g/mL. The final concentration of each strain was adjusted to 5 x 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.

Acknowledgment. This work was supported by the Thailand Research Fund (MRG4990028). We thank Miss S. Chokpaiboon and Miss P. Pompeng of the Program in Biotechnology, Faculty of Science, Chulalongkorn University, for cytotoxic and antibacterial assays. Additional funding was provided in part by the Thailand Research Fund (MRG4980028). We thank Miss S. Chokpaiboon and Miss P. Pompeng of the Program in Biotechnology, Faculty of Science, Chulalongkorn University, for cytotoxic and antibacterial assays. Additional funding was provided in part by the Thailand Research Fund (MRG4980028).


