

Thaixylomolins A–C: Limonoids Featuring Two New Motifs from the Thai *Xylocarpus moluccensis*

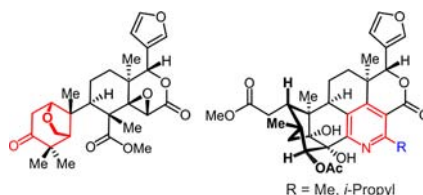
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ABSTRACT



Three limonoids named thaixylomolins A–C (1–3), featuring two new motifs, were isolated from the seeds of a Thai mangrove, *Xylocarpus moluccensis*. The absolute configurations of these limonoids were determined by extensive NMR investigations, single-crystal X-ray diffraction analysis, and circular-dichroism spectroscopy in combination with quantum-chemical calculations. Thaixylomolin B exhibited inhibitory activity against nitric oxide production in lipopolysaccharide and IFN- γ -induced RAW264.7 murine macrophages with an IC_{50} value of 84.3 μ M.

Research of limonoids has been becoming a hotspot in the field of natural product chemistry. The mangroves of the genus *Xylocarpus* are known to produce a variety of limonoids.¹ During the course of our search for potential lead structures from southeast Asian mangroves, three unprecedented limonoids, including thaixylomolin A (1),

which is a secomahoganin-type limonoid with a novel 6-oxabicyclo[3.2.1]octan-3-one motif, and thaixylomolins B–C (2–3), which are limonoids that contain a unique pentasubstituted pyridine scaffold (Figure 1), were obtained from the seeds of a Thai mangrove, *X. moluccensis*, collected in the Trang province. Herein we report the structural elucidation and absolute configuration assignment of thaixylomolins A–C (1–3).

Thaixylomolin A (1, 4.5 mg) was isolated as colorless crystals. Its molecular formula, $C_{27}H_{34}O_8$, was established by HRMS (ESI): m/z 487.2334, calcd for $[M + H]^+$ 487.2332. From this formula, it was determined that 1 had 11 degrees of unsaturation, of which, according to 1H and ^{13}C NMR data (Table S1, Supporting Information (SI)), five were due to three carbonyl groups (a ketone and two esters) and two $C=C$ double bonds; thus, the molecule

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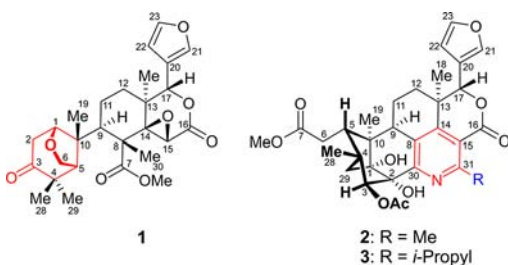


Figure 1. Structures of thaixylomolins A–C (**1**–**3**).

should be hexacyclic. DEPT experiments confirmed the presence of six methyl groups (one methoxy function and five tertiary methyls), four methylene groups (one oxygenated), eight methine groups (three olefinic and three oxygenated), and nine quaternary carbon atoms (one ketone, two esters, one olefinic, and one oxygenated). The NMR data of **1** and its 2D correlations (Figure 2) indicated the presence of a methoxycarbonyl moiety [$\delta_{\text{H}} = 3.72$ ppm (s)] and a typical β -substituted furyl ring [$\delta_{\text{H}} = 6.32$ (br s), 7.40 (br s), 7.40 ppm (br s)]. The aforementioned data suggested that **1** was a B- and D-ring *seco* limonoid containing a 14,15-epoxy ring.

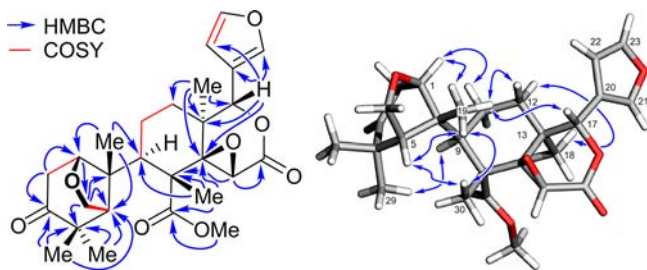


Figure 2. Selected ^1H – ^1H COSY, HMBC, and diagnostic NOE correlations for thaixylomolin A (**1**, MM2-optimized structure).

The NMR data of **1** were similar to those of secomahoganin,² except for the presence of a C1–O–C6 oxygen bridge, and the absence of a C₁=C₂ double bond and a 6-*O*-acetyl group. Two geminal protons of a methylene group [$\delta_{\text{H}} = 2.70$ ppm (2H, m)], exhibiting HMBC cross-peaks to C1 and C3, were identified as H₂-2, as corroborated by their ^1H – ^1H COSY interactions with H-1. Another pair of geminal protons [$\delta = 3.97$ (dd, $J = 9.6$, 4.4 Hz), 4.22 ppm (d, $J = 9.6$ Hz)] of an oxygenated methylene group, which had ^1H – ^1H COSY interactions with H-5 and exhibited HMBC correlations to C1, C4, C5, and C10, were assigned to H₂-6. The above observations revealed that C1 was connected with C6 by an oxygen

bridge to form a tetrahydrofuran substructure (C1, C10, C5, and C6), which was confirmed by HMBC cross-peaks from H-1 to C6 and, vice versa, from one of H₂-6 [$\delta = 4.22$ ppm (d, $J = 9.6$ Hz)] to C1. Moreover, the absence of HMBC correlations between H₂-6 and C7 indicated the cleavage of the C6–C7 bond, while the linkage of the 7-methoxycarbonyl moiety to C8 was established by a strong HMBC cross-peak from Me-30 to C7. Therefore, compound **1** was identified as a B- and D-ring *seco* limonoid that contained a 6-oxabicyclo[3.2.1]octan-3-one motif.

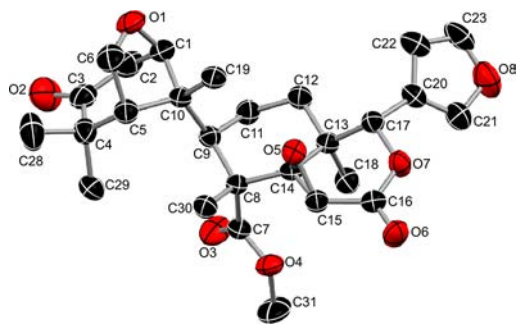


Figure 3. ORTEP plot (ellipsoids at 50% probability) of the X-ray structure of thaixylomolin A (**1**) (hydrogens have been omitted for reasons of clarity).

The full relative configuration of **1** was established by NOE interactions (Figure 2) and confirmed independently by single-crystal X-ray diffraction (Figure 3). In order to elucidate the absolute configuration of **1**, a conformational analysis using SCS-MP2/def2-TZVP//B97D/TZVP was performed on the enantiomers with the relative configuration found in the X-ray analysis. TDB2PLYP/def2-TZVP calculations of three conformers found within an energetical range of 3 kcal/mol yielded UV and CD spectra that were summed up after a Boltzmann statistical weighting. Comparison of the computed CD spectra of the possible enantiomers with the experimental curve³ showed a perfect match of the spectrum calculated for the 1*R*,5*R*,8*R*,9*R*,10*R*,13*S*,14*R*,15*S*,17*S*-configuration with the CD curve measured experimentally (Figure 4), unambiguously proving ($\Delta_{\text{ESI}} = 86\%$)⁴ that this is the correct absolute configuration of **1**.

Thaixylomolin B (**2**, 10.1 mg) was isolated as a white amorphous powder. HRMS (ESI) measurements suggested a molecular formula of C₃₁H₃₅NO₉ (m/z 566.2381, calcd for [$M + \text{H}$]⁺ 566.2385). From this formula, **2** was determined to contain 15 degrees of unsaturation, eight of which were due to three ester groups and five C=C double bonds,

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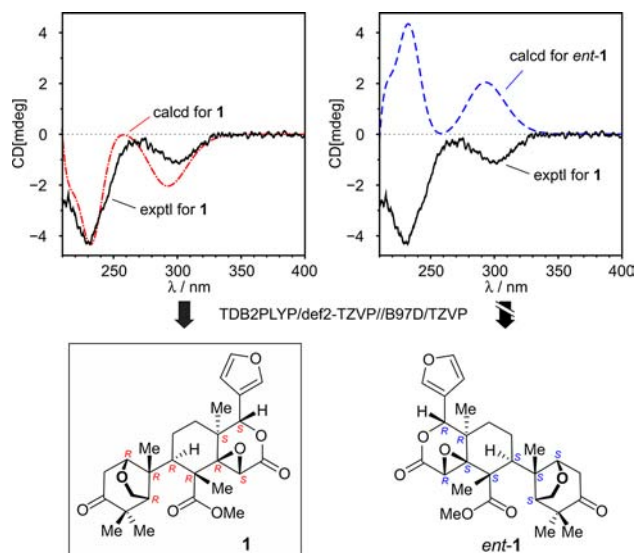


Figure 4. Determination of the absolute configuration of thaixylomolin A (**1**) by comparison of the calculated CD spectra with the experimental one.

according to the ^1H and ^{13}C NMR data (Table S1, SI); accordingly, the molecule should be heptacyclic. DEPT experiments confirmed the presence of six methyl units (one methoxy group, one acetyl group, and four tertiary methyl groups), four methylenes, seven methines (including three olefinic and two oxygenated groups), and fourteen quaternary carbon atoms (including three carbonyl groups, six olefinic and two oxygenated carbon atoms). In addition, the presence of a methoxycarbonyl moiety [$\delta_{\text{H}} = 3.65$ ppm (s)], an acetoxyl group [$\delta_{\text{H}} = 1.62$ ppm (s)], and a β -substituted furyl ring [$\delta_{\text{H}} = 6.54$ (br s), 7.48 (br s), 7.57 ppm (br s)] were evidenced by the analysis of NMR data of **2** and its 2D correlations (Figure 5).

The NMR data of **2** were similar to those of phragmalins, such as moluccensins A–C,^{1c} except for the presence of three additional olefinic quaternary carbon atoms and the absence of a C30 carbonyl moiety and a C1-*O*-substituted group. Two geminal protons of a methylene group [$\delta_{\text{H}} = 2.41$ ppm (2H, d, $J = 6.8$ Hz)], which showed HMBC correlations to C4, C5, and C7, were assigned to H₂-6, as corroborated by their ^1H – ^1H COSY interactions with H-5. Another pair of geminal protons [$\delta = 1.77$ (d, $J = 10.8$ Hz), 2.07 ppm (d, $J = 10.8$ Hz)] of a methylene group, which exhibited HMBC cross-peaks to C1, C2, C3, and C4, were assigned to be H₂-29. An oxygenated methine [$\delta_{\text{H}} = 5.16$ ppm (s)], which showed HMBC correlations to C13, C14, C16, C18, C20, C21, and C22, was assigned as CH-17. The second oxygenated methine [$\delta_{\text{H}} = 5.07$ ppm (s)], which exhibited HMBC correlations to C2, C-4, and C30, was identified as CH-3. A tertiary methyl group [$\delta_{\text{H}} = 1.18$ ppm (s)], which showed HMBC correlations to C12, C13, C14, and C17, was identified as Me-18. The second tertiary methyl group [$\delta_{\text{H}} = 1.25$ ppm (s)], which exhibited

HMBC cross-peaks to C1, C5, C9, and C10, was assigned to be Me-19. The third tertiary methyl moiety [$\delta_{\text{H}} = 0.88$ ppm (s)], which showed HMBC correlations to C3 and C4, was assigned to Me-28.

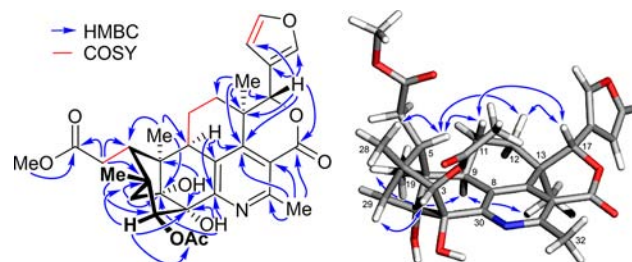


Figure 5. Selected ^1H – ^1H COSY, HMBC, and diagnostic NOE correlations for thaixylomolin B (**2**, B97D/TZVP-optimized structure).

Two exchangeable protons, showing no HSQC correlations to any carbon atoms, were revealed as the protons of two hydroxy groups, of which one [$\delta = 3.20$ ppm (br s)] was assigned as the proton of OH-1 due to its HMBC cross-peaks with C1, C2, and C10, while another [$\delta = 5.24$ ppm (br s)] was identified as the proton of OH-2 owing to its HMBC correlations with C1, C2, and C30. The remaining five olefinic quaternary carbons [$\delta_{\text{C}} = 156.2$ (qC), 124.9 (qC), 155.0 (qC), 116.2 (qC), 159.4 ppm (qC)] and the nitrogen atom, as deduced from the molecular formula of **2**, implied the presence of a pentasubstituted pyridine ring (C30, C8, C14, C15, C31), which was further corroborated by HMBC cross-peaks from H-9 to C8, C14, and C30, from H-3 to C30, and from H-17 and H₃-18 to C14. An aryl methyl group [$\delta_{\text{H}} = 2.88$ ppm (s)], showing HMBC correlations to C14, C15, C16, and C31, was assigned as Me-32, which, in turn, confirmed the existence of the pentasubstituted pyridine ring. Furthermore, the strong HMBC correlation between H-3 [$\delta = 5.07$ ppm (s)] and the carbonyl carbon ($\delta = 169.6$ ppm) of an acetyl group placed the latter moiety at C3. Thus, the structure of **2**, which contained a 2-methylpyridine ring, was identified as shown. The aforementioned analysis concluded that **2** was a novel limonoid, of which the backbone consisted of 28 carbon atoms and contained a pentasubstituted pyridine ring.

The relative configuration of **2** was established on the basis of NOE interactions (Figure 5). The significant NOE interaction from H-3 to H_{pro-R}-29 helped to assign the 3 α -H and the corresponding 3 β -acetoxyl group. NOEs between H-5 and H-11 β , H-12 β , and H₃-28, and between H-17 and the H-11 β and H-12 β , established the β -orientation of H-5 and H-17. Similarly, those between the H-9 and H₃-18 and H₃-19 indicated their mutual *cis*-relationship. Based on these results, the full relative configuration of **2** was established as shown.

Thaixylomolin C (**3**, 2.1 mg) had the molecular formula $\text{C}_{33}\text{H}_{39}\text{NO}_9$, as established by HRMS (ESI): m/z 594.2705, calcd for $[M + \text{H}]^+$ 594.2698. The NMR data

of **3** (Table S1, SI) were similar to those of **2**, except for the replacement of Me-31 by an isopropyl moiety, which was confirmed by ^1H – ^1H COSY interactions from H-32 to Me-33 and Me-34. HMBC cross-peaks from Me-33 and Me-34 to C31 placed the isopropyl group at C31 (Figure S2, SI). Thus, the structure of **3**, containing a 2-isopropylpyridine ring, was identified as a novel limonoid with a backbone of 30 carbon atoms.

The absolute configuration of thaixylomolin B (**2**) was determined by interpretation of the CD spectra, by a combination of the exciton chirality method and quantum-chemical calculations. The experimental CD spectrum showed a positive couplet at about 210 nm. This couplet occurs due to an interaction of the transition-dipole moments (TDM) of the nicotinic acid moiety and the furanyl substituent. This made it possible to elucidate the absolute configuration of the stereocenter at C-17 by using the exciton chirality method.⁵ To take into account the different conformations that the molecule might adopt, especially the furanyl group relative to the rest of the structure, a conformational analysis of **2** was performed based on the above established relative configuration again by using the SCS-MP2/def2-TZVP//B97D/TZVP method. For this purpose we first focused on the northeastern fragment because it is more relevant for the exciton chirality method. This moiety was found to be quite rigid, the furanyl substituent to always be in an equatorial position, and the main difference to be the dihedral angle C13–C17–C20–C21 of the axis. Two possibilities, one with the furanyl oxygen above and another with it below the ring plane, were found. But due to the near-orthogonal orientation of the furanyl TDM relative to the axis, nearly no difference was observed in the orientation of the important TDM and thus in both conformations a *P*-configuration was detected for the 17*R*-configuration (Figure 6) while the 17*S*-enantiomer showed an *M*-configuration (Figure S4, SI). As the relative configuration of **2** was known from the above-described NOESY investigations, the absolute configuration was finally established to be 1*R*,2*R*,3*S*,4*R*,5*S*,9*S*,10*R*,13*R*,17*R*. In addition, TDCAM-B3LYP/6-31G* calculations for the two possible enantiomers of **2** were performed and the good match of the CD spectrum that was calculated for 1*R*,2*R*,3*S*,4*R*,5*S*,9*S*,10*R*,13*R*,17*R*-**2** confirmed the above assigned absolute configuration (Figure 6, left bottom, and Figure S4, SI). Finally, the absolute configuration of thaixylomolin C (**3**) was found to be the same as that of **2** due to their very similar NOE interactions (Figure S3, SI) and CD spectra (Figure S5, SI).

Compounds **1** and **2** were tested for their anti-inflammatory activity. Whereas **1** was inactive, **2** exhibited inhibitory activity against nitric oxide production

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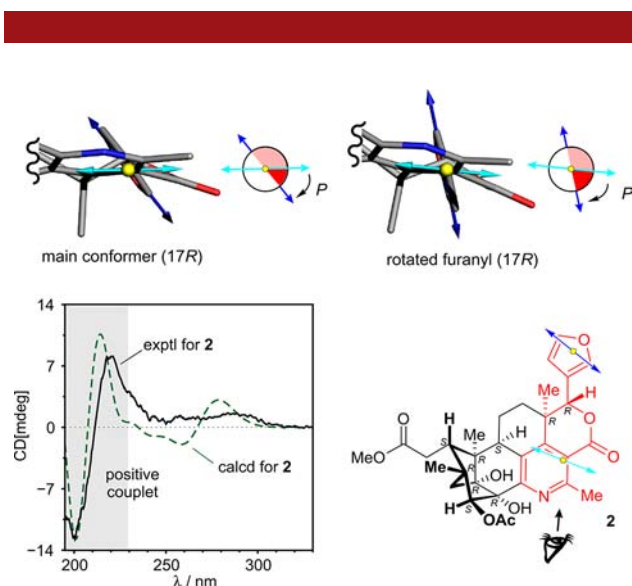


Figure 6. Elucidation of the absolute configuration of thaixylomolin B (**2**) using the exciton chirality method (positive couplet highlighted in gray) and quantum-chemical calculations. The important transition dipole moments of the furanyl part (blue arrow) and the nicotinic acid moiety (cyan arrow) have a *P*-orientation in the 17*R*-configured enantiomer of **2**.

in lipopolysaccharide and IFN- γ -induced RAW264.7 murine macrophages with an IC_{50} value of 84.3 μM .

To date, only five limonoids of the secomahoganin type have been reported.^{1d} Thaixylomolin A (**1**) is the first one containing a 6-oxabicyclo[3.2.1]octan-3-one motif. Thaixylomolins B–C (**2–3**) are the second type of *N*-containing limonoids, whereas the first type contains a central pyridine ring.^{1a,d} The complex structures of **1–3** also evidence the existence of new biosynthetic pathways to tetranortriterpenoids. The biosynthetic origin of **1–3** was proposed in Scheme S1, SI.

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Supporting Information Available. Experimental section, ^1H and ^{13}C NMR data, proposed biosynthetic origin, HR-ESIMS, 1D and 2D NMR spectra of **1–3**, ESI-MS² of **3**, single-crystal X-ray crystallographic data of **1**, the CD measurements of **2–3**, and quantum-chemical CD calculations of the enantiomer of **2**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.