

## Nasimaluns A and B: *neo*-Clerodane Diterpenoids from *Barringtonia racemosa*

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An ethanolic extract of the roots of *Barringtonia racemosa* afforded two novel *neo*-clerodane-type diterpenoids, methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate (nasimalun A, **1**) and dimethyl-15,16-epoxy-3,13(16),14-*neo*-clerodatrien-17,18-dicarboxylate (17-carboxymethylhardwickiic acid methyl ester, nasimalun B, **2**) by NMR and MS analyses and by comparison of their spectral data with related compounds. The relative stereochemistry of the asymmetric centers in **1** and **2** was determined by selective 1D NOESY experiments.

*Barringtonia* is an important Old World genus of the family Lecythidaceae and consists of 20 species distributed from tropical Africa to Formosa, Polynesia, and northern Australia.<sup>1</sup> *B. racemosa* Blume (Bengali name, "Mohasomudra") is an evergreen tree that grows in the Sunderban of Bangladesh, Sri Lanka, and the west coast of India.<sup>2</sup> Several species found in India are used as folk medicines. The seeds are aromatic and useful in colic and ophthalmia.<sup>3</sup> *Barringtonia* species have been shown to contain polyhydroxylated triterpenoids<sup>1,4</sup> and saponins.<sup>5–7</sup> We have examined *B. racemosa*, and in this paper we report the structures of two new *neo*-clerodane diterpenes (**1**, **2**).

The concentrated ethanolic extract of *B. racemosa* was diluted with H<sub>2</sub>O and then extracted with CHCl<sub>3</sub>. Fractionation of the CHCl<sub>3</sub>-soluble materials by vacuum liquid chromatography (VLC) and TLC afforded two *neo*-clerodane diterpenes (**1**, **2**). HRFABMS of compound **1** established its molecular formula as C<sub>21</sub>H<sub>24</sub>O<sub>6</sub>. Its IR spectrum displayed bands indicating a furan ring ( $\nu$  1562, 1507, 873 cm<sup>-1</sup>) and three carbonyl groups ( $\nu$  1770, 1730, 1669 cm<sup>-1</sup>). The <sup>1</sup>H and <sup>13</sup>C NMR spectra of **1** closely resembled those of tambalin (**3**), an insect antifeedant *neo*-clerodane isolated from *Tanacetum balsamita*,<sup>8</sup> and bacchasmacranone (**4**) obtained from *Baccharis macraei*.<sup>9</sup> Typical low-field signals in the <sup>1</sup>H NMR spectrum of **1** at  $\delta$  6.73 (H-14), 7.42 (H-15), and 8.01 (H-16) suggested the presence of a 3-substituted furan ring. The downfield resonance of H-16 revealed that the furan ring was conjugated with a carbonyl group,<sup>10</sup> which was confirmed by the HMBC correlation of H-14 with the ketone carbon at  $\delta_C$  193.6 (C-12). This structural fragment was identical to that found in 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide.<sup>11</sup> The <sup>1</sup>H NMR spectrum of **1** further showed signals for an  $\alpha,\beta$ -unsaturated 18,19-clerodanolide at  $\delta$  6.74 (H-3), 3.93 (H-19a), and 4.33 (H-19b).<sup>9</sup> HMBC correlations from H-3 to C-5 and C-18 and from H-19b to C-4, C-5, C-6, and C-18 confirmed the 18,19-olide functionality in **1**. The AB quartet centered at  $\delta$  2.83 and 3.04 assignable to H<sub>2</sub>-11 demonstrated connectivities over two bonds to  $\delta_C$  39.6 (C-9) and C-12 and over three bonds to  $\delta_C$  48.7 (C-8), 46.7 (C-10), and 19.2 (C-20) in the HMBC spectrum. The last correlation placed the single methyl group resonating at  $\delta$  0.82 on C-9. The doublet of doublets at  $\delta$  3.21 was attributed to H-8, and the HMBC correlation from this proton to the ester carbonyl at  $\delta$  174.0 placed the carboxymethyl substituent ( $\delta_H$  3.60;  $\delta_C$  51.4) on C-8.

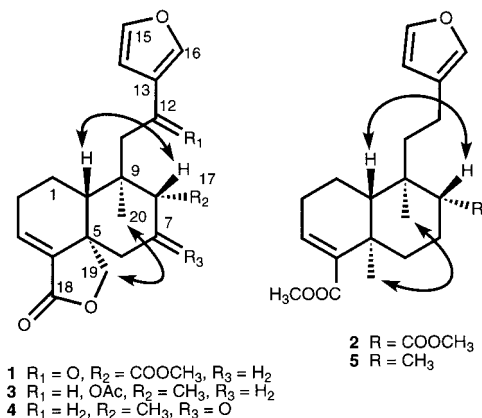
It was possible to trace all of the proton–proton spin systems in **1** with data from a COSY-45 experiment. Heteronuclear correlation experiments (HMBC and HMQC) allowed unambiguous assignment of all <sup>1</sup>H and <sup>13</sup>C NMR resonances in **1**. The relative stereochemistry of the chiral centers in **1** was determined by selective 1D NOESY experiments. Irradiation at the resonance frequency of H-10 produced significant NOESY correlations with H-1eq, H-6ax, and H-8, while H<sub>3</sub>-20 showed strong NOESY interactions with H-1ax, H-7ax, H<sub>2</sub>-19, and both of the C-11 methylene proton resonances. This established a *cis* relationship between H-8 and H-10 and between the methyl group at C-9 and H<sub>2</sub>-19. Thus, the side chain at C-9 was assigned the equatorial configuration. The key NOE correlations are depicted on the structure **1**. On this basis, the new diterpene was identified as methyl-15,16-epoxy-12-oxo-3,13(16),14-*neo*-clerodatrien-18,19-olide-17-carboxylate, for which we have proposed the trivial name nasimalun A (**1**).

The molecular formula for **2** was established as C<sub>22</sub>H<sub>30</sub>O<sub>5</sub> from HRFABMS. The <sup>13</sup>C NMR spectrum showed 22 signals, while DEPT and HMQC experiments confirmed that 16 out of the 22 carbons in **2** were attached to protons. The <sup>1</sup>H and <sup>13</sup>C NMR spectral data of **2** were, in part, identical to those reported for hardwickiic acid methyl ester (**5**),<sup>12</sup> suggesting a close structural similarity between these two compounds. However, resonances appropriate for a secondary methyl group in **5** were absent in the spectra of **2** and were replaced with resonances indicative of a carboxymethyl residue. In fact, the <sup>1</sup>H and <sup>13</sup>C NMR spectra of **2** displayed signals for two carboxymethyl groups, as compared to one in case of hardwickiic acid methyl ester (**5**). The second carboxymethyl moiety in **2** was placed at C-8 as determined by HMBC experiments. The relative stereochemistry of compound **2** was also established by 1D NOESY experiments as shown. NOESY data established the *cis* relationship between H-8 and H-10 and between H<sub>3</sub>-19 and H<sub>3</sub>-20. Thus, compound **2** was identified as dimethyl-15,16-epoxy-3,13(16),14-*neo*-clerodatrien-17,18-dicarboxylate (17-carboxymethylhardwickiic acid methyl ester, **2**) for which the trivial name nasimalun B has been proposed.

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp (589 nm). UV and IR spectra were obtained on V-500 UV/VIS (JASCO) and IR-230 (JASCO) spectrophotometers, respectively. <sup>1</sup>H NMR spectra were obtained in CDCl<sub>3</sub> on a FG 2Hx54 T=25 A600 instrument operating at 600 MHz,

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Arrows indicate key NOESY interactions in **1** and **2**.

while the <sup>13</sup>C NMR spectra were obtained in the same instrument at 150 MHz using TMS as internal standard. The chemical shifts ( $\delta$ ) and coupling constants ( $J$ ) are expressed in parts per million (ppm) and hertz (Hz), respectively. Inverse-detected heteronuclear correlations were measured using the HMQC (optimized for  $^1J_{CH} = 145$  Hz) and HMBC (optimized for  $^nJ_{CH} = 8.3$  Hz) pulse sequences with a pulsed-field gradient. 1D NOESY experiments were carried out with the gNOESY pulse sequence using the excitation bandwidth of 20 Hz. EIMS and FABMS were recorded on Hitachi-U and Harata-013 spectrometers, respectively.

**Plant Material.** Roots of *B. racemosa* were collected from Khulna, Bangladesh, in November 1998. The sample was identified by Mr. Manzurul Quader Mian, Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB accession no. 28 062).

**Extraction and Isolation.** Air-dried roots (2.0 kg) were cut into small pieces and extracted with 95% EtOH at room temperature. Evaporation of solvent under reduced pressure gave a semisolid mass (30.0 g). A portion (4.0 gm) of this residue was diluted with H<sub>2</sub>O and extracted with CHCl<sub>3</sub> (200 mL  $\times$  3). The CHCl<sub>3</sub>-soluble material was subjected to fractionation by VLC over Si gel (60 H) using petroleum ether (60–80 °C), EtOAc, and MeOH in order of increasing polarity. The eluates were combined on the basis of TLC analysis to provide a total of 18 fractions. Evaporation of solvents from fraction 7, followed by washings of the crystalline deposits with petroleum ether (60–80 °C) and EtOAc mixture yielded **1** (12.0 mg). Repeated preparative TLC of fraction 6 over Si gel PF<sub>254</sub>, using toluene–EtOAc (95:5) as the mobile phase, afforded 8.0 mg of compound **2**.

**Nasimalun A (1):** white amorphous powder;  $[\alpha]_D -85^\circ$  (c 0.2, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  253 (log  $\epsilon$  3.54), 207 (log  $\epsilon$  4.34) nm; IR (film)  $\nu_{max}$  3146, 2946, 1770, 1730, 1669, 1562, 1507, 1460, 1433, 1411, 1394, 1366, 1322, 1277, 1194, 1144, 1118, 1036, 1016, 981, 940, 915, 873, 823, 807, 771, 753 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  8.01 (1H, s, H-16), 7.42 (1H, d,  $J = 2.0$  Hz, H-15), 6.74 (1H, dd,  $J = 8.0, 2.0$  Hz, H-3), 6.73 (1H, d,  $J = 2.0$  Hz, H-14), 4.33 (1H, d,  $J = 8.0$  Hz, H-19b), 3.93 (1H, dd,  $J = 8.0, 2.0$  Hz, H-19a), 3.60 (3H, s, COOCH<sub>3</sub>), 3.21 (1H, dd,  $J = 13.5, 4.5$  Hz, H-8ax), 3.04 (1H, d,  $J = 18.0$ , H-11b), 2.83 (1H, d,  $J = 18.0$ , H-11a), 2.73 (1H, dd,  $J = 11.0, 2.0$  Hz, H-10ax), 2.28 (1H, m, H-2eq), 2.22 (1H, m, H-2ax), 2.03 (1H, dddd,  $J = 13.5, 13.5, 13.5, 4.5$  Hz, H-7ax), 1.98 (1H, ddd,  $J = 13.5, 4.5, 3.0$  Hz, H-6eq), 1.87 (1H, dddd,  $J = 13.5, 4.5, 4.5,$

3.0 Hz, H-7eq), 1.64 (1H, dddd,  $J = 11.0, 2.0, 2.0, 2.0$  Hz, H-1eq), 1.36 (1H, dddd,  $J = 13.5, 13.5, 4.5, 2.0$  Hz, H-6ax), 1.09 (1H, dddd,  $J = 11.0, 11.0, 11.0, 4.0$  Hz, H-1ax), 0.82 (3H, s, H-20); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  193.6 (s, C-12), 174.0 (s, C-17), 169.0 (s, C-18), 147.1 (d, C-16), 144.3 (d, C-15), 137.8 (s, C-4), 136.2 (d, C-3), 128.6 (s, C-13), 108.5 (d, C-14), 71.4 (t, C-19), 51.4 (q, COOCH<sub>3</sub>), 48.7 (d, C-8), 46.7 (d, C-10), 46.5 (t, C-11), 45.1 (s, C-5), 39.6 (s, C-9), 33.2 (t, C-6), 27.3 (t, C-2), 22.1 (t, C-7), 20.1 (t, C-1), 19.2 (q, C-20); HRFABMS  $m/z$  [MH]<sup>+</sup> 373.1640 (calcd 373.1651 for C<sub>21</sub>H<sub>25</sub>O<sub>6</sub>).

**Nasimalun B (2):** white gum;  $[\alpha]_D -95^\circ$  (c 0.1, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{max}$  205 (log  $\epsilon$  4.27) nm; IR (film)  $\nu_{max}$  2952, 1733, 1706, 1558, 1506, 1456, 1436, 1362, 1252, 1188, 1143, 1067, 1023, 743, 873, 770, 760 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  7.36 (1H, dd,  $J = 2.0, 1.0$ , H-15), 7.22 (1H, s, H-16), 6.65 (1H, dd,  $J = 5.0, 3.0$  Hz, H-3), 6.28 (1H, d,  $J = 2.0$  Hz, H-14), 3.71 (3H, s, COOCH<sub>3</sub>-4), 3.68 (3H, s, COOCH<sub>3</sub>-8), 2.59 (1H, dd,  $J = 13.5, 4.0$  Hz, H-8ax), 2.53 (1H, ddd,  $J = 13.5, 13.5, 3.5$  Hz, H-12a), 2.44 (1H, ddd,  $J = 13.5, 3.0, 3.0$  Hz, H-6eq), 2.33 (1H, ddd,  $J = 20.0, 5.0, 5.0$  Hz, H-2eq), 2.21 (1H, m, H-12b), 2.19 (1H, m, H-2ax), 2.05 (1H, dddd,  $J = 13.5, 13.5, 13.5, 3.0$  Hz, H-7ax), 1.77 (1H, ddd,  $J = 18.0, 13.5, 5.0$  Hz, H-11a), 1.70 (1H, dddd,  $J = 13.5, 5.0, 5.0, 5.0$  Hz, H-1eq), 1.66 (1H, m, H-7eq), 1.53 (1H, dddd,  $J = 13.5, 13.5, 13.5, 5.0$  Hz, H-1ax), 1.45 (1H, m, H-11b), 1.43 (1H, dd,  $J = 12.0, 6.0$  Hz, H-10ax), 1.34 (3H, s, H-19), 1.13 (1H, ddd,  $J = 13.5, 13.5, 4.0$  Hz, H-6ax), 0.95 (3H, s, H-20); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150 MHz)  $\delta$  174.9 (s, C-17), 167.5 (s, C-18), 142.7 (d, C-15), 141.8 (s, C-4), 138.5 (d, C-16), 137.2 (d, C-3), 125.1 (s, C-13), 111.0 (d, C-14), 51.2 (q, COOCH<sub>3</sub>-4), 51.1 (q, COOCH<sub>3</sub>-8), 49.1 (d, C-8), 46.4 (d, C-10), 40.9 (t, C-11), 38.7 (s, C-9), 37.3 (s, C-5), 34.9 (t, C-6), 26.9 (t, C-2), 21.6 (t, C-7), 20.8 (q, C-19), 19.9 (q, C-20), 18.1 (t, C-12), 17.2 (t, C-1); HRFABMS  $m/z$  [MH]<sup>+</sup> 375.2167 (calcd 375.2171 for C<sub>22</sub>H<sub>31</sub>O<sub>5</sub>).

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