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Putrescine Bisamides from *Aglaia gigantea*

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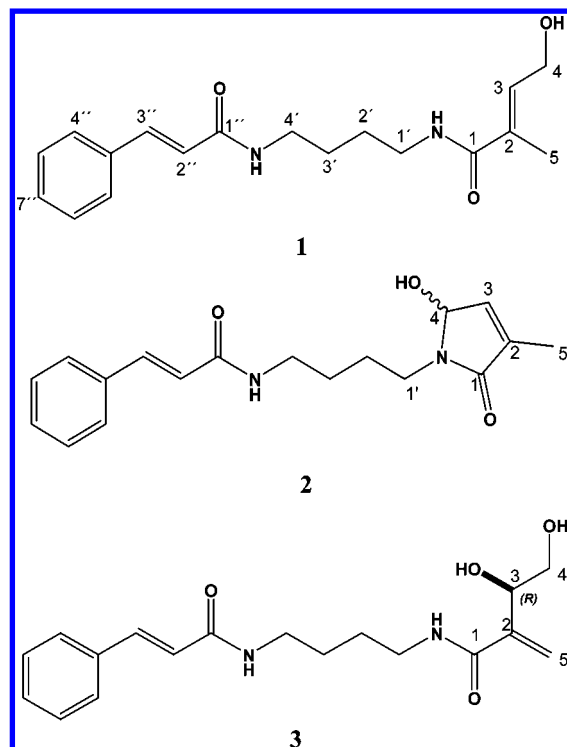
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Phytochemical analysis of the leaves of *Aglaia gigantea* collected in Vietnam yielded three cinnamoyl putrescine bisamide derivatives, which included the known compound dasyclamide (1), as well as two new natural products, gigantamide A (2) and grandiamide D (3). In this study, the structure of dasyclamide (1) was confirmed by X-ray crystallography. The structures of the two new alkaloids, gigantamide A (2) and grandiamide D (3), were elucidated through extensive 1D and 2D NMR spectroscopy and analysis of their mass spectrometric (ESIMS, HRQTOFMS) data. The absolute configuration of grandiamide D (3) was determined via Mosher ester derivatization.

The plant genus *Aglaia*, which belongs to the Meliaceae family, has been found to yield a variety of different classes of compounds such as bisamides, flavonoids, lignans, rocaglate derivatives, sesquiterpenoids, triterpenoids, and tetraterpenoids.^{1,2} Phytochemical analysis of the leaves of *Aglaia gigantea* collected in Vietnam yielded three cinnamoyl bisamide derivatives, including the known compound dasyclamide (1) and two new natural products, gigantamide A (2) and grandiamide D (3). Dasyclamide (1), also known as aglaurubine, has been isolated previously from *A. roburghiana*³ and *A. dasyclada*.⁴ Putrescine bisamide derivatives have been hypothesized to be one of the building blocks or precursors in the biosynthesis of the rocaglamides. Rocaglamides are cyclopenta-[b]tetrahydrobenzofurans and exhibit strong insecticidal activity against *Spodoptera littoralis*, which is comparable in potency with azadirachtin,⁵ and also have displayed pronounced antiproliferative activity against human cancer cells.⁶

Leaves of *A. gigantea* were collected in Catba, an island near Haiphong, Vietnam. Samples were ground and successively extracted with *n*-hexane, CH₂Cl₂, and MeOH. The CH₂Cl₂ extract was chromatographed over a vacuum-liquid chromatography (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to normal-phase column chromatography, and the isolated compounds were purified by semi-preparative HPLC. Identification of the isolated known compound, dasyclamide (1), was confirmed by comparison of its ¹H and ¹³C NMR data with those published in the literature, and its structure was confirmed by X-ray crystallography. The structures of the new putrescine bisamide alkaloids, gigantamide A (2) and grandiamide D (3), were elucidated unambiguously by extensive spectroscopic analysis (1D and 2D NMR experiments that included COSY, HMQC, and HMBC) and mass spectrometric measurements (LC-ESIMS and HRQTOFMS) as well as through Mosher chemical derivatization in order to obtain the absolute configuration, in the case of grandiamide D (3).



Compound 1 was obtained as white needles, which were recrystallized from EtOAc–MeOH (4:1) at room temperature. The positive ESIMS showed pseudomolecular ion peaks at *m/z* 317.3 [M + H]⁺, 339.3 [M + Na]⁺, and 655.3 [2M + Na]⁺, while in the negative-mode, peaks at *m/z* 315.9 [M – H][–], 361.8 [M + HCOO][–], and 677.9 [2M + HCOO][–] were observed. The molecular weight, in combination with 1D and 2D NMR data, is identical to the data obtained for dasyclamide which was previously isolated by our group from *A. dasyclada* collected in mainland China.⁴ In this study, the structure of dasyclamide was substantiated for the first time by X-ray crystallography (see Figure 1).

Compound 2 was isolated as a white amorphous residue. The positive-mode ESIMS showed pseudomolecular ion peaks at *m/z* 315.2 [M + H]⁺, *m/z* 629.3 [2M + H]⁺, and *m/z* 651.2 [2M + Na]⁺, while the negative-mode ESIMS exhibited peaks at *m/z* 313.8

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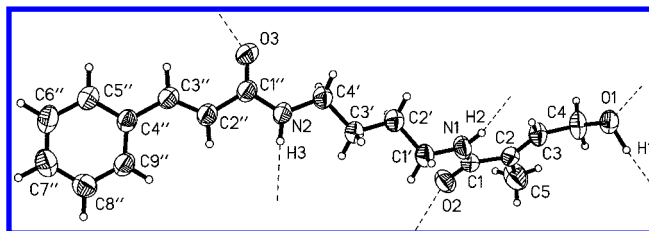


Figure 1. Molecular structure of dasyclamide (**1**) in the crystal. Displacement ellipsoids are drawn at the 50% probability level, radii of hydrogen atoms are chosen arbitrarily, and most of the hydrogen atom labels are omitted for clarity. Dashed lines indicate the directions of intermolecular hydrogen bonding: O1–H1...O3a (O...O 2.6471(17) Å), N1–H2...O1b (N...O 2.919(2) Å), N2–H3...O2c (N...O 2.8898(19) Å). Symmetry codes: a $x + 1, y, z + 1$; b $-x + 2, -y + 1, -z + 1$; c $x, -y + 3/2, z - 1/2$.

[M – H][–], m/z 359.4 [M + HCOO][–], and m/z 672.8 [2M + HCOO][–]. The molecular weight is compatible with the molecular formula, C₁₈H₂₂N₂O₃, as confirmed through HRQTOFMS which gave a pseudomolecular ion peak at m/z 337.151 [M + Na]⁺. The ¹H NMR spectrum of compound **2** in CD₃OD was very similar to that of dasyclamide. However, the molecular weight of compound **2** was 2 mass units less than that of **1**. The multiplet signal integrating for five protons between δ 7.55 and 7.36 indicated the presence of a monosubstituted phenyl system. A typical *trans* pair of doublet (J = 15.7 Hz) protons at δ 6.58 and 7.53 were detected as well as four methylene groups for a putrescine moiety at δ 3.53, 3.30, 1.67, and 1.60. When the ¹H NMR data of **2** were compared with those of **1**, major differences were detected for the hydroxytylgic acid moiety. The H-4 signal in compound **2** was shifted 1.10 ppm downfield to δ 5.33, and integrated for one proton and resonated as a broad doublet that coupled to the broad triplet found at δ_{H-3} 6.62, with a coupling constant of 1.6 Hz instead of 6.0 Hz as found in dasyclamide. Inspection of the HMQC spectrum of **2** showed the presence of a methine carbon at δ 82.4 (C-4) replacing the methylene signal at δ 60.0 found in **1**. Taken together, this was considered a conclusive evidence for the cyclization between C-4 of the terminal hydroxytylgic acid unit and the NH of the putrescine unit found in dasyclamide (**1**) to form a pyrrolidinone ring. The ¹H–¹H COSY spectrum showed a homoallylic correlation of H-3 (⁴*J*) with the H₃-5 methyl as well as a correlation between H-4 and H-3 within the pyrrolidinone ring system. A similar COSY spectrum acquired in DMSO-*d*₆ showed a correlation of H-4 to a hydroxyl doublet at δ 6.10 (6.7 Hz), confirming the presence of a free hydroxyl group at C-4. In the HMBC spectrum, H-3 at (δ 6.62) showed a correlation with C-1 (δ 170.9) while the methylene protons at δ 3.53 (H₂-1') correlated with C-1 and the oxygenated methine carbon C-4 at δ 82.4, confirming both the presence and the position of attachment of the pyrrolidinone ring. Furthermore, the H₃-5 methyl signal at 1.89 ppm also gave cross-peaks with carbon signals at δ 170.9 (C-1), 130.2 (C-2), and 137.5 (C-3). Compound **2** was identified as a new cinnamoyl putrescine bisamide that included a terminal pyrrolidinone ring and has been named gigantamide A. The absolute stereochemistry at C-4 could not be determined due to the small yield of the compound isolated.

Compound **3** was isolated as a white amorphous residue. The negative-mode ESIMS showed pseudomolecular ion peaks at m/z 331.8 [M – H][–], 377.5 [M + HCOO][–], and 709.6 [2M + HCOO][–]. The HRQTOFMS also showed a pseudomolecular ion peaks at m/z 333.1810 [M + H]⁺ and m/z 355.1630 [M + Na]⁺, revealing the molecular formula to be C₁₈H₂₄N₂O₄. Close inspection of the ¹H and ¹³C NMR data obtained for **3** indicated that it is structurally related to grandiamide C, which was previously isolated from *A. grandis*.⁷ The structure of grandiamide C has been unambiguously established by synthesis.⁸ The molecular weight of **3** was 16 mass units larger than that of grandiamide C and suggested the presence of an additional hydroxyl group in the molecule. The ¹H and ¹³C NMR data of compound **3** were comparable to those of dasyclamide (**1**) and again comprised resonances for a cinnamoyl moiety and a putrescine bisamide unit. Compared to **1**, H-3 in **3** was shifted

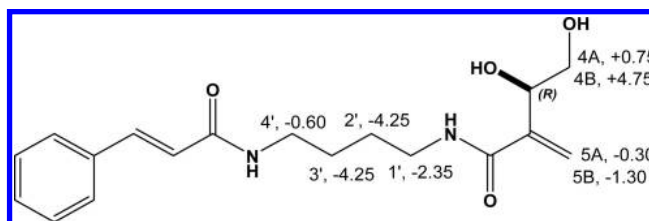


Figure 2. Mosher analysis of compound **3**, $\Delta\delta_H$ ($\delta_S - \delta_R$) obtained for the MTPA esters. $\Delta\delta$ values are expressed in Hz (500 MHz).

upfield to 4.40 ppm (dd, J = 3.8, 6.3 Hz) due to the loss of the double bond between C-3 and C-2, while its chemical shifts gave evidence of a hydroxyl substituent at C-3. In addition, the signals for the methylene protons at C-4 were shifted upfield and resonated separately at δ 3.55 (dd, J = 11.4, 4.4 Hz) and 3.40 (dd, J = 11.4, 6.9 Hz), indicating their proximity to a chiral center. As in **4**, an exomethylene function was identified by two broad singlets at δ 5.69 (H-5A) and 5.52 (H-5B). In the HMBC spectrum, these protons showed strong correlations with C-1 (δ 170.0), C-2 (δ 145.5), and C-3 (δ 71.5) and correlations of H-3 with C-2 and C-4 (δ 65.5). The absolute configuration of the asymmetric center at C-3 of **3** was determined via the Mosher method.⁹ From the observed changes in chemical shifts¹⁰ and by comparison of the NMR spectra of the corresponding bis-(*R*)- and bis-(*S*)-MTPA-Cl with those described in the literature,¹¹ it was evident that C-3 could be assigned the *R* configuration (Figure 2). Thus, compound **3** was completely characterized and was named grandiamide D.

Among the putrescine bisamide derivatives isolated from the genus *Aglaia*, odorine and odorinol have been found to be biologically active. Odorine and odorinol were shown to exhibit antileukemic activity^{12,13} and were also recently described to strongly inhibit both the initiation and promotion stages of skin carcinogenesis in mice.⁷ Pyramidatine is another bisamide congener obtained from *A. pyramidata* and was described to possess the potential to reverse drug resistance in cancer cell cultures.¹⁴ The two new bisamide congeners isolated in this study were found to be devoid of biological activity when compared to the rocaglamides with regard to their cytotoxicity and insecticidal activity. However, their detection nonetheless is important from a phytochemical point of view since gigantamide A (**2**) and grandiamide D (**3**) represent putative biogenetic precursors of both the pharmacologically active rocaglamides and the inactive aglains.¹

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer-241 MC polarimeter. 1D and 2D NMR spectra (chemical shifts in ppm) were recorded on Bruker DRX 500 spectrometers using the standard Bruker software referenced to the residue signals of CD₃OD and DMSO-*d*₆. ESIMS were obtained on a Thermo-Finnigan LCQ DECA ion trap system coupled to an Agilent 1100 HPLC additionally equipped with a photodiode array detector. HRESIMS were determined on a Micromass QToF 2 mass spectrometer. For HPLC analysis, samples were injected into an HPLC system with a photodiode-

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