Notoamides A-D: prenylated indole alkaloids isolated from a marine-derived fungus, Aspergillus sp.

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Notoamides A-D: new prenylated indole alkaloids isolated from a marine-derived fungus, *Aspergillus* sp. **

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Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites, which are emerging as a significant new chemical resource for drug discovery.[1] During a search for natural products exhibiting pharmacologically interesting activities,[2] we screened extracts derived from marine organisms for cytotoxic activity. In this paper, we report the isolation, structure elucidation including absolute stereochemistry, and biological activities of four new doubly prenylated indole alkaloids, notoamides A-D (**1**-**4**), from a culture of marine-derived fungus, *Aspergillus* sp., isolated from the common mussel, *Mytilus edulis*. Biogenetically, notoamides are assumed to be related each other, and Williams *et al.* succeeded in the biomimetic synthesis of notoamides C (**3**) and D (**4**), which are reported in the successive paper.[3]

The fungus, *Aspergillus* sp., was separated from the mussel, *Mytilus edulis*, collected off Noto Peninsula in the Japan Sea. The fungus was fermented on agar plates and extracted with EtOH. The extract was concentrated under reduced pressure, and the aqueous residue was partitioned against EtOAc, and then *n*-BuOH. The EtOAc fraction was partitioned between hexane and 90% MeOH-H_2O, and cytotoxic activity was found in the aqueous MeOH and *n*-BuOH fractions. The two active fractions were combined and subjected to ODS chromatography with aqueous MeOH. The fraction eluted with 80% MeOH-H_2O was purified by ODS HPLC with 60% MeOH-H_2O to afford four new alkaloids, notoamides A (**1**, 3.4 mg), B (**2**, 2.1 mg), C (**3**, 7.9 mg), and D (**4**, 8.9 mg), along with the known compounds, sclerotiamide[4] (**5**, 2.9 mg), stephacidin A[5] (**6**, 6.1 mg), and deoxybrevianamide E[6] (**7**, 0.57 mg) (Figure 1).

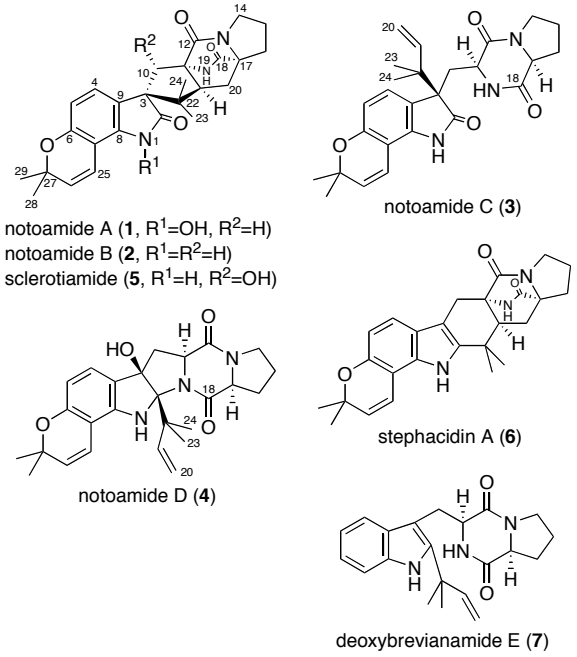

Figure 1. Structures of compounds isolated from *Aspergillus* sp.

The FABMS of notoamide C (**3**) showed a quasi molecular ion peak at *m/z* 450 $[M + H]^+$ and the molecular formula was determined as $C_{26}H_{31}N_3O_4$ on the basis of its HRFABMS, requiring thirteen degrees of unsaturation. The IR spectrum of **3** displayed amide absorption bands at 3500, 1700, and 1650 cm^{-1}. The presence of the amides was also supported by the carbonyl signals at δ 164.8 and 169.0 in the ^{13}C NMR spectrum. The UV absorption at 247.5 (log ε 4.3), 283.0 (3.9, sh), 294.0 (3.7, sh), and 319.5 nm (3.4, sh) was indicative of aromatic functionality with an extended conjugation. The ^1H NMR spectrum in acetone-d_6 revealed four singlet methyl signals, seven olefinic signals, and two exchangeable proton signals. The ^{13}C NMR spectrum in acetone-d_6 showed three carbonyl carbons, eight olefinic carbons, and four methyl signals. ^1H-^1H COSY revealed the presence of a 1,2,3,4-tetrasubstituted benzene ring and a 1,2-disubstituted *cis*-double bond (Figure 2 (A)). Interpretation of HMBC correlations in acetone-d_6, indicated the presence of a 5,6-disubstituted-2,2-dimethyl-2*H*-chromone moiety in which the benzene ring and double bond were incorporated. Connection of C-6 and C-27 via an oxygen atom was deduced by their chemical shifts. Analysis of 2D NMR spectra indicated the presence of a prenyl group (B), a partial structure (C), and a proline moiety (D) (Figure 2). Furthermore, HMBC correlations indicated that the NH was accommodated on a 2-oxindole ring of which the benzene ring was incorporated into the partial structure A and that the prenyl group (B) was attached to C-3. The HMBC correlations in DMSO-d_6 indicated that the proline moiety formed a diketopiperazine ring (Figure 2). NOE experiment of **3** in acetone-d_6 revealed a correlation between H-11 and H-17, indicating that the

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diketopiperazine ring was of the *cis*-configuration. Acid hydrolysis of **3** followed by analysis using TLC on a chiral stationary phase (CHIRALPLATE®) showed the presence of L-proline in the hydrolysate. Therefore, the stereogenic centers of **3** were determined as being 11*S*,17*S*. Although the absolute configuration of the C-3 stereogenic center was not determined by spectroscopic methods, the co-occurrence of notoamides A (**1**) and B (**2**) indicates the 3*R* configuration for **3** based on biogenetic considerations.

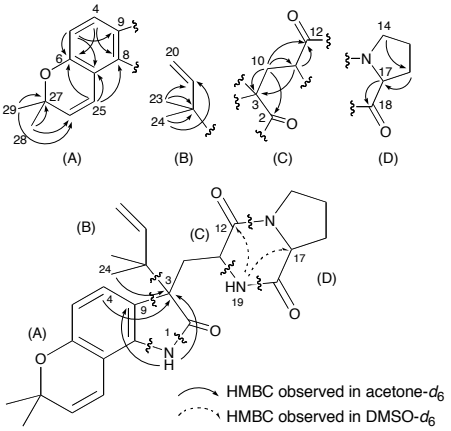

Figure 2. Key HMBC correlations for **3**.

Notoamide D (**4**) has the same molecular formula as **3** and revealed that ^{1}H and ^{13}C NMR spectra were very similar to those of **3**. Analysis of 2D NMR data revealed that **4** contained a pyrroloindol ring, and the positions of a hydroxy and a prenyl groups were indicated by HMBC correlations (Figure 3 (A)). The relative stereochemistry of **4** was established by through its NOE spectrum (Figure 3 (B)). Analysis of acid hydrolysate of **4** by TLC on the chiral stationary phase showed that the proline moiety that constitutes **4** is L-proline. Thus, the absolute stereochemistry of **4** was established as 2*S*,3*R*,11*S*,17*S*.

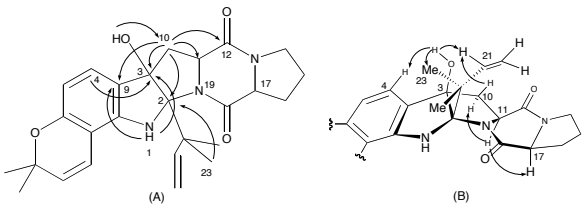

Figure 3. Key HMBC (A) and NOE (B) correlations for **4**.

The FABMS of notoamide B (**2**) showed a quasi molecular ion peak at *m/z* 448 $[M + H]^+$ and the molecular formula was determined as $C_{26}H_{29}N_3O_4$ on the basis of its HRFABMS, requiring thirteen degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **2** in acetone-d_6 were similar to those of **3** and **4**, except for the absence of olefinic signals for the isoprenyl group present in **3** and **4**. HMBC correlation indicated that **2** possessed a bicyclo[2.2.2]diazaoctane ring generated from a diketopiperazine ring and an isoprenyl group (Figure 4 (A)). NOE correlations observed in **2** indicated the relative stereochemistry as shown in Figure 4 (B). The CD spectrum of **2** correlates to relevant regions of the CD spectrum of brevianamide B[7] and revealed that the absolute stereochemistry of **2** should be 3*R*,11*S*,17*S*,21*S* (see Figure S5 in the Supporting Information). Williams *et al*. reported that the Cotton effect at 200-250 nm is due to an n-π* transition of the diketopiperazine amide bonds, which is diagnostic for the bicyclo[2.2.2]diaza octane diketopiperazine core. The absorption between 250~450 nm is diagnostic of the absolute stererochemistry at the *spiro*-oxindole stereogenic center and the sign of the Cotton effect for **2** correlated with that for (-)-paraherquamide B.[8] The structure of **2** was accordingly revealed to be that of 10-desoxy-sclerotiamide: sclerotiamide (**5**) was reported to be a metabolite of *Aspergillus sclerotiorum*,[4] for which its absolute stereochemistry was proposed by analogy to that of paraherquamide (**8**).[8] A molecular formula of notoamide A (**1**) $C_{26}H_{29}N_3O_5$ was established by HRFABMS, an oxygen atom more than **2**. The ^{1}H and ^{13}C NMR spectra of **1** were very similar to those of **2**, except for the absence of the exchangeable broad NH (H-1) signal in **1**. In addition, the differences of chemical shifts were observed for H-25 (δ 7.72 for **1** and δ 6.64 for **2**) and C-2 (δ 178.8 for **1** and δ 184.0 for **2**). Taken together, we have concluded that the structure of **1** to be the 1-hydroxy derivative of **2**. The CD spectrum of **1** reveals that the absolute stereochemistry is the same as that of **2** (Figure S5). This is the first report of a naturally occurring 1-hydroxy-2-oxindole alkaloid, although the synthesis of simple 1-hydroxy-2-oxindoles has been reported.[9]

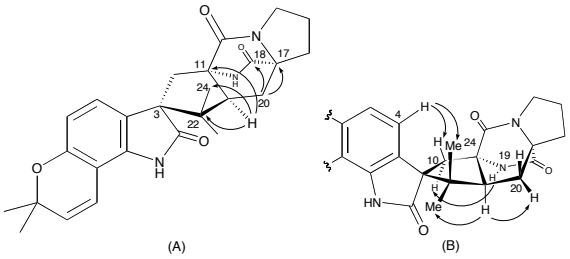

Figure 4. Key HMBC (A) and NOE (B) correlations for **2**.

Notoamides A-C (**1**-**3**) showed moderate cytotoxicity against HeLa and L1210 cells with IC_{50} values in the range of 22-52 μg/mL, but the IC_{50} value of notoamide D (**4**) was more than 100 μg/mL. Notoamide D (**4**) contains a pyrroloindol ring instead of a dihydroxypyrano-2-oxindole ring system common to notoamides A-C (**1**-**3**), which is likely responsible for the marked differences in cytotoxicity. It is further significant that we have found that **3** induced G2/M cell cycle arrest at a concentration of 6.3 μg/mL.

Notoamides A-D (**1**-**4**) possess the pyranoindole ring system which is also common to stephacidin A (**6**), stephacidin B and several members of paraherquamide family. Notoamides A (**1**) and B (**2**) contain the bicyclo[2.2.2]diazaoctane ring system, which is proposed to be biosynthetically generated from an oxidized diketopiperazine ring and an isoprenyl group by the Diels-Alder reaction.[3] In 2005, the isolation of structurally related compounds, which were named norgeamides, was reported by the Hans-Knöll Institute.[10] The norgeamides and notoamides C (**3**) and D (**4**) appear to be closely related in their biogenesis and are plausible pathway metabolites leading to the more complex alkaloids stephacidin A (**6**) and notoamides A (**1**) and B (**2**). It is highly significant that the *Aspergillus* sp. that we have investigated here, exhibits one of the most extensive co-metabolite profiles within the numerous families of prenylated indole alkaloids extant and suggests a provocative biosynthetic sequence involving deoxybrevianamide E (**7**) to stephacidin A (**6**) to notoamide B (**2**) and then branching to notoamide A (**1**) or sclerotiamide (**5**). Recently, Williams *et al*. have succeeded in the biomimetic synthesis of notoamides B, C, and D (**2**-**4**), which have further corroborated the structural and stereochemical assignments made herein. A more detailed discussion of plausible biogenetic pathways

for the norgeamides and notoamides including their biomimetic syntheses are reported in the accompanying paper.[3]

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[10] The structures of the norgeamides were published on the internet detailing some aspects of the research performed at the Hans-Knöll Institute. See: http://www.hki-jena.de/.