Fungal Metabolites

DOI: 10.1002/anie.200604381

Notoamides A–D: Prenylated Indole Alkaloids Isolated from a Marine-Derived Fungus, *Aspergillus* sp.**

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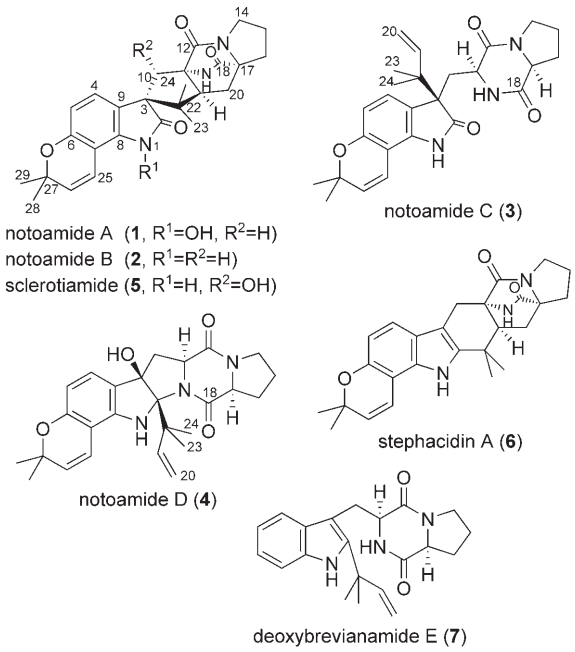
Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites, which are emerging as a significant resource for new chemicals in drug discovery.[1] During a search for natural products that exhibit pharmacologically interesting activities,[2] we screened extracts derived from marine organisms for cytotoxic activity. Herein we report the isolation, structure elucidation with the absolute stereochemistry, and biological activities of four new doubly prenylated indole alkaloids, the notoamides A–D (**1**–**4**). These alkaloids were obtained from a culture of marine-derived fungus, *Aspergillus* sp., which was isolated from the common mussel, *Mytilus edulis*. Biogenetically, the notoamides are assumed to be related to each other, and Williams and co-workers report their successful biomimetic syntheses of notoamides C (**3**) and D (**4**) in a following Communication.[3]

The fungus *Aspergillus* sp. was separated from the mussel *Mytilus edulis*, which was collected off Noto Peninsula in the Sea of Japan. The fungus was fermented on agar plates and extracted with EtOH. The extract was concentrated under reduced pressure and the aqueous residue extracted with EtOAc, and then with *n*BuOH. The EtOAc fraction was partitioned between hexane and MeOH/H_2O (9:1), and cytotoxic activity was found in the aqueous MeOH and *n*BuOH fractions. These two fractions were combined and subjected to reverse-phase chromatography on a octadecasilane (ODS) with aqueous MeOH as the eluent. The fraction eluted with MeOH/H_2O (4:1) was purified by HPLC (ODS; MeOH/H_2O 3:2) 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, Scheme 1).



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

The FAB mass spectrum of **3** shows a quasi molecular ion peak at *m*/*z* 450 ([*M*+H]$^+$), and the molecular formula was determined to be $C_{26}H_{31}N_3O_4$ on the basis of its high-resolution FAB mass spectrum; thus **3** must have 13 degrees of unsaturation. The IR spectrum of **3** displays absorption bands at 3500, 1700, and 1650 cm^{-1}, which correspond to amide groups. The presence of the amide groups was also evident by the signals at $\delta = 164.8$ and 169.0 ppm in the ^{13}C NMR spectrum. The UV absorptions at 247.5 (log ε 4.3), 283.0 (3.9, sh), 294.0 (3.7, sh), and 319.5 nm (3.4, sh) are indicative of aromatic functionality with an extended conjugation. The ^{1}H NMR spectrum recorded in [D_6]acetone

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[**] This work was supported by Grants-in-Aid for Scientific Research (No. 16590005) and for Scientific Research on Priority Areas (No. 18032033) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, as well as by a grant from the Research Foundation for Pharmaceutical Sciences. Support from the NIH is also gratefully acknowledged (CA70375 to R.M.W.).

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reveals four singlet signals corresponding to methyl groups, seven olefinic signals, and two exchangeable-proton signals. The ^{13}C NMR spectrum recorded in [D$_6$]acetone reveals that **3** contains three carbonyl and eight olefinic carbon atoms, as well as four methyl groups. The ^{1}H–^{1}H COSY spectrum reveals the presence of a 1,2,3,4-tetrasubstituted benzene ring and a *cis* 1,2-disubstituted double bond A (Figure 1). Inter-

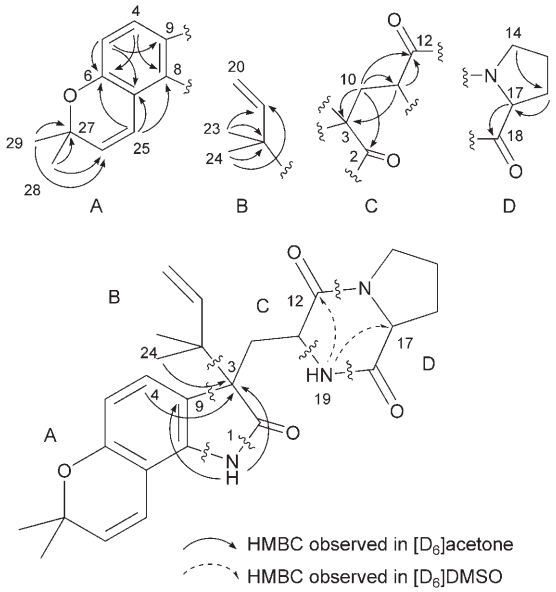

Figure 1. Significant HMBCs for **3** showing the *cis* 1,2-disubstituted double bond A, a prenyl group B, a partial structure C, and a proline moiety D.

pretation of HMBCs in [D$_6$]acetone, indicated the presence of a 5,6-disubstituted 2,2-dimethyl-2*H*-chromone moiety in which the benzene ring and the double bond are incorporated. The connection between C6 and C27 with an oxygen atom was deduced by their corresponding chemical shifts. Analysis of 2D NMR spectra indicates the presence of a prenyl group B, a partial structure C, and a proline moiety D (Figure 1). Furthermore, HMBCs indicate that one NH group belongs to a 2-oxindole ring, which also includes the benzene ring for the partial structure A, and to which the prenyl group B was attached at the C3 position. The HMBCs in [D$_6$]DMSO indicate that the proline moiety forms a diketopiperazine ring (Figure 1). NOE experiments of **3** in [D$_6$]acetone reveal a correlation between H11 and H17, which indicates that the diketopiperazine ring is of *cis* configuration. Acid hydrolysis of **3**, followed by analysis by 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 stereogenic center at C3 was not determined by spectroscopic methods, the co-occurrence of notoamides A (**1**) and B (**2**) indicates the configuration 3*R* for **3** on the basis of biogenetic considerations.

Notoamide D (**4**) has the same molecular formula as **3** and the ^{1}H and ^{13}C NMR spectra were very similar to those of **3**. Analysis of 2D NMR data revealed that **4** contains a pyrroloindole ring, and the positions of a hydroxy and a prenyl group were indicated by HMBCs (Figure 2a). The relative stereochemistry of **4** was established from the NOE

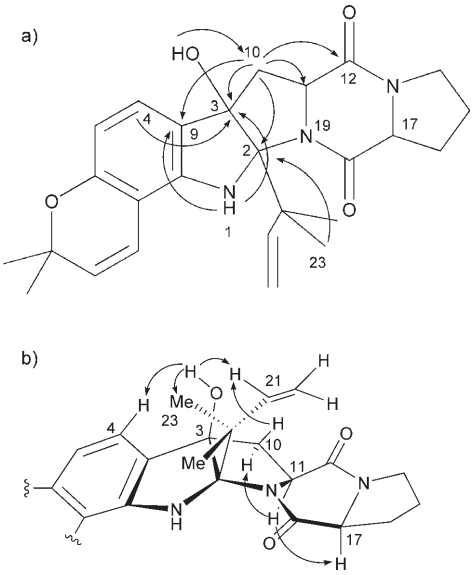

Figure 2. a) Significant HMBCs and b) NOE correlations for **4**.

spectrum (Figure 2b). Analysis of acid hydrolysate of **4** by TLC on a chiral stationary phase (CHIRALPLATE) showed again the presence of L-proline in **4**. Thus, the absolute stereochemistry of **4** was established as 2*S*,3*R*,11*S*,17*S*.

The FAB mass spectrum of **2** shows 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 the high-resolution FAB mass spectrum; thus **2** must have 14 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra of **2** in [D$_6$]acetone are similar to those of **3** and **4**, except for the absence of olefinic signals for the isoprenyl group present in **3** and **4**. HMBC indicates that **2** possesses a bicyclo-[2.2.2]diazaoctane ring, which is generated from a diketopiperazine ring and an isoprenyl group (Figure 3a). NOE correlations observed in **2** indicate that the relative stereochemistry of **2** is as shown in Figure 3b. The CD spectrum of **2** correlates to relevant regions of that 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 arises from an n-π^* transition of the diketopiperazine amide bonds, which is diagnostic for the bicyclo-[2.2.2]diazaoctane diketopiperazine core.[7] The absorption between 250–450 nm is diagnostic of the absolute configuration at the stereogenic center of the spirooxindole, 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 to be that of paraherquamide (**8**) by analogy.[8] The molecular formula of **1** ($C_{26}H_{29}N_3O_5$) was established by high-resolution

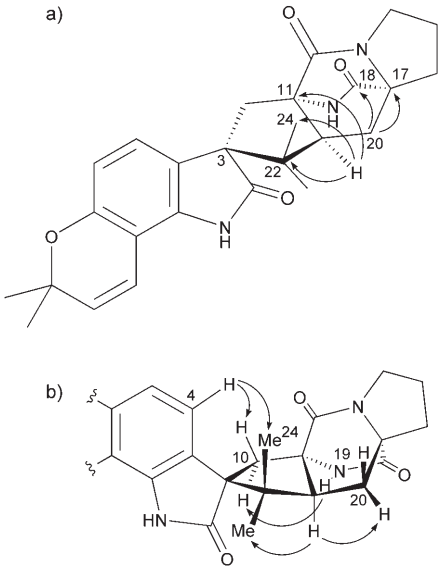

Figure 3. a) Significant HMBCs and b) NOE correlations for **2**.

FABMS and was found to have an additional oxygen atom compared with **2**. The ^{1}H and ^{13}C NMR spectra of **1** are very similar to those of **2**, except for the absence of the exchangeable broad NH1 signal in **1**. In addition, differences were observed in the chemical shifts for H25 ($\delta = 7.72$ ppm for **1** and $\delta = 6.64$ ppm for **2**) and C2 ($\delta = 178.8$ ppm for **1** and $\delta = 184.0$ ppm for **2**). Taken together, we have concluded that the structure of **1** is the 1-hydroxy derivative of **2**. The CD spectrum of **1** reveals that the absolute stereochemistry is the same as that of **2** (see Figure S5 in the Supporting Information). 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]

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

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. Compounds **1** and **2** contain the bicyclo[2.2.2]diazaoctane ring system, which could be generated biosynthetically 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 metabolites along the pathway that leads to the more complex alkaloids stephacidin A (**6**) and notoamides A (**1**) and B (**2**). It is highly significant that *Aspergillus* sp., which we have investigated here, exhibits one of the most extensive co-metabolite profiles within the numerous families of prenylated indole alkaloids extant. The profile suggests a possible biosynthetic sequence that involves: deoxybrevianamide E (**7**)→stephacidin A (**6**)→notoamide B (**2**), and then branching to notoamide A (**1**) or sclerotiamide (**5**). Recently, Williams et al. have successfully carried out 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 and their biomimetic syntheses are reported in the following Communications.[3]

Received: October 26, 2006
Revised: December 7, 2006
Published online: February 16, 2007

Keywords: indoles · metabolites · natural products · NMR spectroscopy · structure elucidation

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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/.

