Notoamide O, a Structurally Unprecedented Prenylated Indole Alkaloid, and Notoamides P−R from a Marine-Derived Fungus, *Aspergillus* sp.

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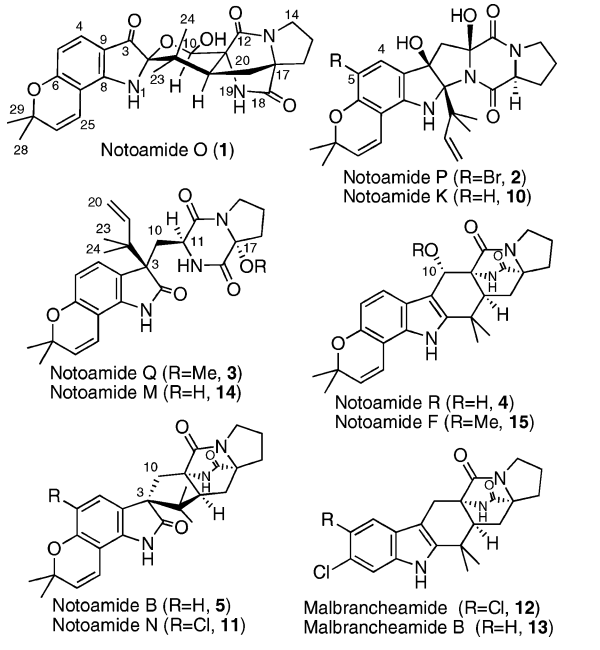
Notoamides O−R were isolated from a marine-derived *Aspergillus* sp. Notoamide O possesses a novel hemiacetal/hemiaminal ether functionality hitherto unknown among this family of prenylated indole alkaloids. The structure represents an unusual branch point for the oxidative modification of other members in the family of prenylated indole alkaloids in the biogenetic pathway.

Microorganisms are a prolific source of structurally interesting and biologically active metabolites.[1] Fungi of the genera *Aspergillus* and *Penicillium* are known to produce prenylated indole alkaloids with diverse ring systems that are derived from tryptophan, proline, and one or two isoprene units. The alkaloids possess a diketopiperazine or a characteristic bicyclo[2.2.2]diazaoctane ring and have become enticing targets for synthesis.[2] Recently, we reported structurally related metabolites, the notoamides, isolated from a marine-derived *Aspergillus* sp., along with several known metabolites.[3] The *Aspergillus* sp. that we have investigated exhibits an extensive co-metabolite profile representative of the structurally diverse ring systems in this family of prenylated indole alkaloids. In a subsequent chemical examination of the culture, we isolated a novel prenylated alkaloid, notoamide O (**1**), which possesses an unprecedented hemiacetal/hemiaminal ether functionality in this series of alkaloids, along with notoamides P (**2**), Q (**3**), and R (**4**). The details of the structure elucidation of **1** and a proposed biogenetic origin of the unusual hemiacetal/hemiaminal ether ring system are presented herein.



The molecular formula of **1**, $C_{26}H_{29}N_3O_6$, was established by HRFABMS and found to contain two additional oxygen atoms compared with notoamide B (**5**).[3a] The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) were similar to those of **5**, except for the absence of a methylene group, δ_H 2.21 and 3.04 (each d, J = 14.5 Hz, H_2-10)/δ_C 34.8 (C-10), in **5** and the presence of a methine group, δ_H 6.19 (s)/δ_C 104.8, in **1**. Another difference was reflected in the chemical shifts of a quaternary carbon at δ_C 62.6 (C-3) in **5** and δ_C 102.0 in **1**. Interpretation of the HMBC spectrum of **1** indicated a structure between the indole-derived ring and bicyclo ring moieties (Figure 1a). The HMBC correlations in **1**, δ_H 6.19 (H-10)/δ_C 48.6 (C-21), 61.3 (C-11), and 102.0 (C-2) and δ_H 0.90 (H_3-23) and 1.34 (H_3-24)/δ_C 102.0, showed the presence of a hemiacetal ring adjacent to the bicyclo[2.2.2]diazaoctane ring. The HMBC correlation between δ_H 7.45 (H-4) and δ_C 188.2 (C-3) unambiguously required the carbon at δ_C 102.0 to be C-2 of the indole-derived ring. Thus, the gross structure of **1** was determined, which was supported by the molecular formula and the chemical shifts. The NOE correlations observed in **1** indicated that H-1, H-10, and H-21 were on the same face of the central six-menbered ring (Figure 1b). Although the carbon at C-10 is incorporated into a hemiacetal ring, the signals for H-10 and C-10 appeared as individual resonances, which indicated that the structure might be the most stable isomer. The 11*S*,17*S* configuration of **1** was indicated by the Cotton effect at 200−250 nm that arises from an n−π* transition of the diketopiperazine amide bonds, which is diagnostic for the bicyclo[2.2.2]diazaoctane core (Figure S6, Supporting Information).[4] Therefore, the structure of **1** including the absolute configuration was established. The structure contains a hemiacetal ring connected to an indole-derived ring through a spiro hemiaminal ether carbon C-2 and is the only member of this family of prenylated indole alkaloids to have the C-2 and C-10 carbon atoms in the oxidation states shown. A proposed biogenetic pathway for the assembly of **1** is shown in Scheme 1. Stephacidin A (**6**) seems to be a plausible precursor for the construction of **1** via the intermediacy of aspergamide B (**7**),[5] an intermediate already implicated in the biosynthesis of stephacidin B from **6**. The immediate precursor of **7** could be **4**. Oxidative cleavage of the trisubstituted alkene in **7** would generate aldehyde **8**, which upon hydration yields the aldehyde hydrate **9**. Simple ring closure would afford **1**. Efforts are underway to detect **7** as well as precursor incorporation experiments to substantiate **6** as the key precursor to **1**.

The molecular formula of notoamide P (**2**) was determined as $C_{26}H_{30}{}^{79}BrN_3O_5$, indicating the replacement of a hydrogen atom with bromine. The ^{1}H NMR spectrum (Table 2) showed the presence of a single aromatic signal at δ 7.15 (H-4) instead of a pair of doublets for the aromatic protons at H-4 and H-5 as observed in other notoamides. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of notoamide K (**10**)[3b] except for δ_C 98.4 (C, C-5) in **2** and δ_H 6.07 (d, J = 8.0 Hz, H-5)/δ_C 106.6 (CH, C-5) in **10**. An

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Table 1. ^{1}H and ^{13}C NMR Data for Notoamide O (**1**)[a]

position	δ_C, mult.	δ_H, J in Hz	HMBC
1		7.83, br s	
2	102.0, C		
3	188.2, C		
4	126.0, CH	7.45, d (8.4)	3, 6, 8
5	117.5, CH	6.77, d (8.4)	6, 7, 9
6	162.2, C		
7	112.9, C		
8	158.3, C		
9	118.4, C		
10	104.8, CH	6.19, s	2, 11, 12
11	61.3, C		
12	167.0, C		
14	44.2, CH_2	3.49, m	15, 16, 17
		3.38, m	15, 16
15	25.1, CH_2	2.05, m	17
		1.87, m	14, 17
16	29.6, CH_2	2.64, m	14, 15, 17, 18
		1.86, m	14, 15, 17, 18, 20
17	68.0, C		
18	173.1, C		
20	30.2, CH_2	2.13, dd (13.0, 10.4)	11, 17, 21
		1.93, dd (13.0, 7.6)	17, 18, 21, 22
21	48.6, CH	2.93, dd (10.4, 7.6)	11, 12, 20, 22, 23, 24
22	40.0, C		
23	22.0, CH_3	0.90, s	2, 21, 22, 24
24	14.8, CH_3	1.34, s	2, 21, 22, 23
25	117.5, CH	6.84, d (10.1)	6, 7, 8, 27
26	131.5, CH	5.90, d (10.1)	7, 27, 28, 29
27	79.4, C		
28	28.7, CH_3	1.51, s	26, 27, 29
29	28.4, CH_3	1.48, s	26, 27, 28
10-OH		7.34, s	

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

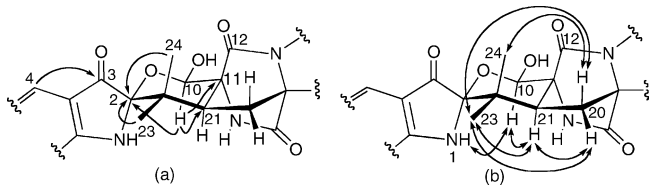

Figure 1. (a) Key HMBC and (b) NOE correlations for **1**.

analysis of HMBC correlations established **2** as 5-bromonotoamide K. Although prenylated indole alkaloids containing chlorine atoms have been isolated, such as notoamide N (**11**),[3d] malbrancheamide (**12**), and malbrancheamide B (**13**),[6] compound **2** is the first brominated derivative to have been discovered in this family of prenylated indole alkaloids. It is of considerable interest to determine what type of halogenating enzyme is involved in the production of **2** and **11** by our marine-derived *Aspergillus* sp.

The FABMS spectrum of notoamide Q (**3**) showed a quasi molecular ion peak at m/z 480 $[M + H]^+$, and the molecular formula was determined as $C_{27}H_{33}N_3O_5$ on the basis of HRFABMS. The ^{1}H and ^{13}C NMR spectra of **3** (Table S1) were similar to those of notoamide M (**14**)[3d] except for the presence of a methoxy signal at δ 3.01 (3H, s), which was attached to C-17, as shown on the basis of an HMBC correlation between 17-OMe and C-17. Thus, **3** is a 17-*O*-methyl derivative of **14**. The NOE correlation between δ 4.22 (H-11) and the methoxy signal (17-OMe) established the 17*R* configuration for **3**. Although the absolute configuration at C-17 of **14** could not be determined by spectroscopic methods,[7] the 17*R* configuration is likely based on biogenetic considerations.

The molecular formula of notoamide R (**4**), $C_{26}H_{29}N_3O_4$, was established by HRFABMS, and the analysis of NMR data (Table S2) showed that **4** was a demethyl derivative of notoamide F (**15**).[3b]

In conclusion, we have isolated a structurally unprecedented prenylated indole alkaloid, notoamide O (**1**), along with three new alkaloids, notoamides P−R (**2**−**4**), from a marine-derived *Aspergillus* sp. The structure of **1** possesses a novel hemiacetal/hemiaminal ether functionality hitherto unknown among this family of prenylated indole alkaloids. A number of prenylated indole alkaloids containing a bicyclo[2.2.2]diazaoctane ring have been so far isolated from fungi of the genera *Aspergillus* and *Penicillium*,[2,3,8] and the study of the biosynthetic pathways to these alkaloids has recently become an area of significant interest.[3c,d,8−10] Compound **1** represents another fascinating structural and biogenetic puzzle worthy of further investigation and may represent another unusual branch point for the oxidative modification of stephacidin A (**6**). Notably, a series of three pairs of enantiomeric prenylated indole

Scheme 1. Possible Biogenetic Pathway to **1** from **6**

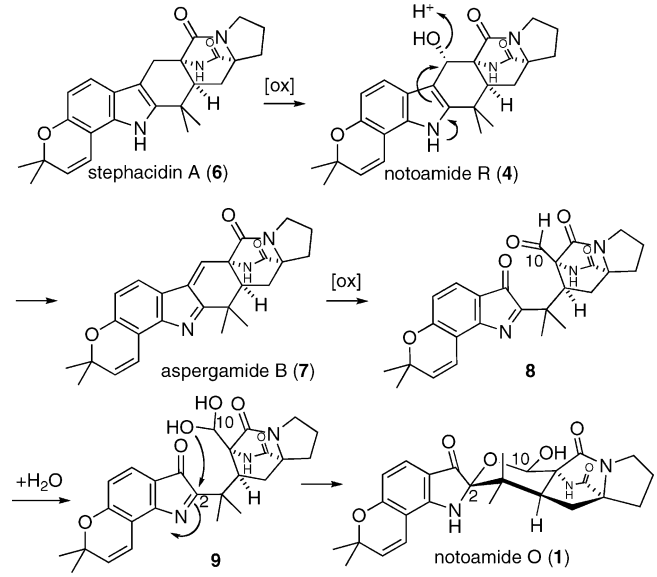

Table 2. ^{1}H and ^{13}C NMR Data for Notoamide P (**2**)[a]

position	δ_C, mult.	δ_H, J in Hz	HMBC
1		7.81, br s	
2	110.4, C		
3	90.0, C		
4	127.0, CH	7.15, s	3, 5, 6, 8
5	98.4, C		
6	150.8, C		
7	105.4, C		
8	143.9, C		
9	127.6, C		
10	48.5, CH	3.33, d (13.8)	2, 3, 9, 11, 12
		2.52, d (13.8)	2, 3, 9, 11, 12
11	90.5, C		
12	163.7, C		
14	46.1, CH_2	3.40, ddd (11.9, 11.4, 7.5)	15
		3.27, ddd (11.9, 8.7, 3.7)	16
15	23.3, CH_2	1.90, m	16, 17
		1.85, m	
16	29.0, CH_2	2.25, m	15
		1.97, m	17, 18
17	59.7, CH	4.31, t (7.8)	16, 18
18	172.4, C		
20	113.5, CH_2	5.06, dd (17.7, 1.5)	21, 22
		4.99, dd (11.0, 1.5)	22
21	145.4, CH	6.26, dd (17.7, 11.0)	
22	45.4, C		
23	22.2, CH_3	1.24, s	2, 22, 24
24	23.9, CH_3	1.25, s	2, 22, 23
25	118.2, CH	6.46, d (9.0)	6, 7, 8, 27
26	129.4, CH	5.62, d (9.0)	7, 27, 28, 29
27	77.5, C		
28	27.8, CH_3	1.392, s	26, 29
29	27.9, CH_3	1.395, s	26, 28
3-OH		4.78, br s	
11-OH		5.78, br s	

[a] Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6.

alkaloids, notoamide B (**5**), stephacidin A (**6**), and versicolamide B, are produced by distinct species of the genus *Aspergillus*.[3a,3d,8,10] Interestingly, all three pairs of these alkaloids were isolated from the respective organisms in their optically pure forms. These alkaloids are hypothesized to arise via biosynthetic intramolecular Diels−Alder (IMDA) reactions, implying that each *Aspergillus* species possesses enantiomerically distinct Diels−Alderases.[10] In addition to notoamide M (**14**),[3d] the isolation of notoamide Q (**3**), both of which contain an oxygenated C-17 carbon, provides indirect, provocative support for a potential pathway to an azadiene species that culminates in the construction of the unique bicyclo[2.2.2]-diazaoctane ring system.[11] Notoamide P (**2**) is the first brominated member of this family of prenylated indole alkaloids. The existence of a halogenase system in our marine-derived *Aspergillus* sp. is an important finding, which we are pursuing in the context of identifying the biochemical class of this halogenase. Our laboratories are continuing studies to elucidate the origin of the fascinating enantio-divergence manifest in the natural production of notoamides, stephacidins, and versicolamides. The biosynthetic pathway leading to **1**, a new interesting metabolite reported in this study, is also an attractive subject to be resolved.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Jasco P-1020 polarimeter in MeOH. UV spectra were measured on a Shimadzu UVmini-1240 spectrophotometer in MeOH. CD spectra were measured on a JASCO J-720WI spectropolarimeter in MeOH at 24 °C. IR spectra were measured on a JASCO FT-IR230 spectrophotometer. NMR spectra were recorded on a Bruker Avance 500 NMR spectrometer in acetone-d_6 or CD_3OD. Chemical shifts were referenced to the residual solvent peaks (δ_H 2.04 and δ_C 29.8 for acetone-d_6; δ_H 3.30 and δ_C 49.0 for CD_3OD), and multiplicities of carbon resonances were determined by HMQC spectra. Mass spectra were measured on a JMS AX-500 or JMS HX-110 mass spectrometer. The ODS chromatography was performed with Cosmosil 140C_{18}-PREP (Nacalai Tesque), and HPLC was carried out with Inertsil ODS-3 (Φ 20 × 250 mm, GL Sciences Inc.) or Luna 5u Phenyl-Hexyl (Φ 21.2 × 250 mm, Phenomenex).

Source Organism. The fungus *Aspergillus* sp. was separated from the mussel *Mytilus edulis galloprovincialis*, which was collected off Noto Peninsula in the Sea of Japan.[3a]

Culture Conditions and Isolation of Notoamides O−R (1−4). Conditions for the growth and isolation of notoamides O−R were the same as previously reported.[3b] The fraction that eluted from the ODS column with 80% MeOH−H_2O was purified by reversed-phase HPLC with MeOH−H_2O to afford notoamides O (**1**, 0.9 mg), P (**2**, 1.1 mg), Q (**3**, 3.8 mg), and R (**4**, 4.4 mg).

Notoamide O (1): $[\alpha]^{20}_D$ +190 (*c* 0.63, MeOH); UV (MeOH) λ_{max} (log ε) 248 (4.1, sh), 265 (4.2), 320 (3.7), 365 (3.0, sh) nm; CD (0.74 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 208 (−3.7), 228 (13.9), 246 (−3.7), 261 (3.2), 284 (−8.8), 322 (−3.2), 353 (8.1), 367 (7.3) nm; IR (film) ν_{max} 3400, 2970, 2920, 1700, 1650, 1580 cm^{-1}; NMR data (acetone-d_6), see Table 1; NOESY cross-peaks H-10/H-1, H-21; H-26/H_3-28, H_3-29; H-21/H_3-23; H-20α (δ 2.13)/H_3-23; H-20β (δ 1.93)/H_3-23, H_3-24; HRFABMS $[M + H]^+$ *m/z* 480.2137 (calcd for $C_{26}H_{30}N_3O_6$, 480.2135).

Notoamide P (2): $[\alpha]^{19}_D$ −140 (*c* 0.73, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.3), 288 (3.8), 346 (3.7) nm; CD (0.56 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 207 (0.85), 224 (−0.11), 234 (0.062), 256 (−1.1), 346 (0.14) nm; IR (film) ν_{max} 3400, 2970, 2920, 1680 cm^{-1}; NMR data (acetone-d_6), see Table 2; NOE OH-11/H_3-23, H_3-24; FABMS *m/z* 544 $[M + H]^+$, 546 $[M + 2 + H]^+$ (1:1); HRFABMS $[M + H]^+$ *m/z* 544.1464 (calcd for $C_{26}H_{31}{}^{79}BrN_3O_5$, 544.1447).

Notoamide Q (3): $[\alpha]^{20}_D$ +82 (*c* 2.2, MeOH); UV (MeOH) λ_{max} (log ε) 247 (4.3), 281 (3.8, sh), 318 (3.2, sh) nm; CD (0.60 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 228 (14), 253 (−3.9), 274 (4.4) nm; IR (film) ν_{max} 3400, 2970, 2930, 1700 cm^{-1}; NMR data (acetone-d_6), see Table S1; NOE H-11/17-OMe; H-26/H_3-28, H_3-29; H-1/H-25; H-4/H_3-23, H_3-24, H-10 (δ 2.83); H-20 (δ 4.98)/H_3-23, H_3-24; H-21/H_3-23, H_3-24, H-10 (δ 2.66); HRFABMS $[M + H]^+$ *m/z* 480.2475 (calcd for $C_{27}H_{34}N_3O_5$, 480.2498).

Notoamide R (4): $[\alpha]^{14}_D$ +38 (*c* 0.50, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.4), 306 (3.8), 332 (3.4, sh) nm; CD (1.3 mM, MeOH) λ_{ext} ($\Delta\varepsilon$) 203 (−9.6), 222 (8.3), 248 (−0.47), 257 (0.26) nm; IR (film) ν_{max} 3400, 2970, 2930, 1680 cm^{-1}; NMR data (CD_3OD), see Table S2; HRFABMS $[M + H]^+$ *m/z* 448.2238 (calcd for $C_{26}H_{30}N_3O_4$, 448.2236).

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Supporting Information Available: 1D and 2D NMR and CD spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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