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Three novel bis(indole) alkaloids from a stony coral, *Tubastraea* sp.

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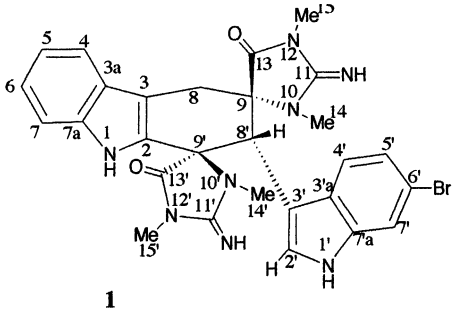
Abstract—Three novel bis(indole) alkaloids with an unprecedented skeleton have been isolated from a stony coral, *Tubastraea* sp. These indoles could biogenetically be derived from two molecules of aplysinopsin via a Diels–Alder reaction.

The investigation of stony corals for secondary metabolites has not been so extensive as that for metabolites in soft corals. Most investigators assumed that the secondary metabolites were produced to protect soft corals that have no physical defenses against predators and therefore calcareous hard corals, not requiring such mechanisms, do not contain many secondary metabolites. Recently, the stony corals have, however, been reported to be a source of different types of compounds: anthraquinoid derivatives,[1] a phenol,[2] alkaloids,[3] macrolides,[4] and acetylenes.[5] Many of them exhibited interesting biological activities, such as antiviral, antifungal, and cytotoxic activity. We have isolated three novel bis(indole) alkaloids named tubastrindoles A–C from a stony coral, *Tubastraea* sp., collected in the Odomari area, Kagoshima Prefecture. In this paper we describe the isolation and structure elucidation of these compounds. A methanol extract of the animal (8.7 kg wet wt) was partitioned between CH_2Cl_2 and water, and then the aqueous part was extracted with *n*-BuOH. Neither extract exhibited biological activity, such as antibacterial, antifungal, or cytotoxic activity in our



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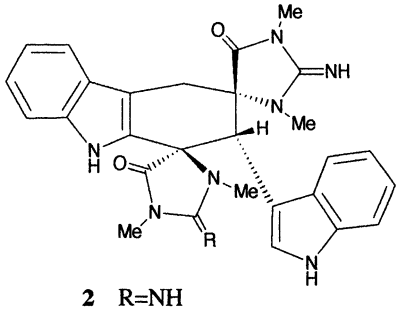
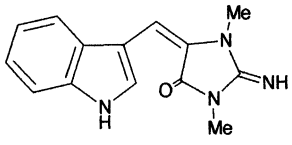


2 R=NH

3 R=O



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Keywords: stony coral; *Tubastraea* sp.; tubastrindoles; bis indoles; aplysinopsin; tryptophan; guanidine.
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Table 1. ^{1}H and ^{13}C NMR spectral data of **1**, **2**, and **3**

	1		**2**		**3**	
C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		123.3		123.5		126.8
3		115.0		115.0		112.9
3a		126.4		126.4		126.7
4	7.64 d (7.9)	120.1	7.65 d (7.8)	120.1	7.60 d (8.0)	119.7
5	7.19 t (7.9)	121.5	7.19 overlapped	121.5	7.14 br t (8.0)	121.0
6	7.32 t (7.9)	125.9	7.31 t (7.8)	125.9	7.25 br t (7.7)	125.0
7	7.40 d (7.9)	113.0	7.41 d (7.8)	112.9[a]	7.37 d (8.3)	112.7
7a		139.5		139.5		139.4
8	3.63 d (17.4)	27.6	3.63 d (17.4)	27.6	3.55 d (17.3)	27.4
	3.81 d (17.4)		3.82 d (17.4)		3.73 d (17.3)	
9		72.3		72.3		72.5
11		161.6		161.5		161.2
13		174.0		174.1		174.7
14	3.30 s	33.1	3.32 s	33.0	3.35 s	33.1
15	2.71 s	26.6	2.68 s	26.6	2.57 s	26.4
2′	7.06 s	125.6	7.03 s	124.7	7.06 s	124.6
3′		104.4		103.8		104.9
3′a		127.7		128.7		129.0
4′	7.47 d (8.6)	119.7	7.54 d (7.9)	118.0	7.50 d (8.0)	118.5
5′	7.25 dd (1.6, 8.6)	124.8	7.13 t (7.9)	121.5	7.08 br t (8.0)	121.0
6′		117.3	7.20 overlapped	123.9	7.14 br t (7.6)	123.5
7′	7.59 d (1.6)	115.9	7.41 d (7.9)	113.0[a]	7.35 d (8.3)	112.5
7′a		137.4		136.7		136.7
8′	4.53 s	44.5	4.57 s	44.7	4.52 s	44.2
9′		72.3		72.4		69.8
11′		159.0		158.9		157.3
13′		172.5		172.6		174.2
14′	3.12 s	28.4	3.14 s	28.4	2.88 s	25.7
15′	2.94 s	26.5	2.91 s	26.5	2.75 s	24.9

[a] These values may be interchangeable.

initial screening, but we pursued the chemical study as one of them showed interesting NMR resonances in the aromatic region. The dichloromethane extract was subjected to vacuum chromatography on Kieselgel 60H with CH_2Cl_2–MeOH. The fractions eluted with MeOH–CH_2Cl_2 (1:9 to 1:4) were further chromatographed on Kieselgel 60 with CH_2Cl_2–MeOH. The final purification was done by HPLC on μ-Bondapack C_{18} with CH_3CN–H_2O–TFA (30:70:0.1) to give optically active tubastrindoles A–C (**1**–**3**)[6] in the yields of 3.5, 9.5, and 7.0 mg, respectively, along with aplysinopsin (**4**).[7]

Tubastrindole A (**1**) was determined to have the molecular formula $C_{28}H_{27}BrN_8O_2$ on the basis of (+)-HRFABMS data [m/z 587.1520 (M+H), Δ +0.2 mmu]. The UV absorptions at 284 nm (ε 2800) and 294 nm (ε 2100) suggested the presence of indole chromophore(s). In the ^{1}H NMR spectrum, four methyls (δ 2.71, s, 2.94, s, 3.12, s, 3.30, s, each 3H), a methylene (δ 3.63 and 3.81, 1H each, AB, $J=17.4$ Hz), a methine (δ 4.53, s, 1H), and seven aromatic proton resonances were observed (Table 1). The presence of a 2,3-disubstituted and a 3,6-disubstituted indole moiety was evident from the ^{1}H–^{1}H COSY NMR spectrum: H-4/H-5, H-5/H-6, H-6/H-7, H-4′/H-5′, H-5′/H-7′. The chemical shift of C-6′ (δ 117.3, s) suggested the location of the bromine atom on this carbon.[8]

The ^{13}C NMR spectrum indicated the presence of four methyls, an sp^3 methylene, an sp^3 methine, two guanidines, and two amide carbonyls as well as those corresponding to the carbon atoms of two indole ring skeletons (Table 1). Therefore, three more rings should be present in **1** to account for the unsaturation requirement of the molecular formula. One of them was confirmed to be a six-membered ring by HMBC experiments: H-8/C-2, C-3, C-9; H-8/C-2, C-9, H-8′/C-9 and/or C-9′ (Fig. 1). The carbons 9 and 9′ are parts comprising of two 5,5-disubstituted 1,3-dimethyl-2-iminoimidazolidin-4-one moieties, respectively, which were determined as follows. The 14-methyl protons (δ 3.30),

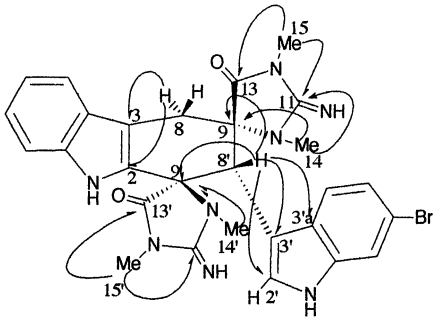

Figure 1. Selected HMBC correlations for **1**.

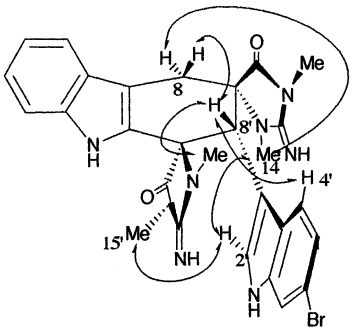

Figure 2. Selected NOE correlations for **1**.

which were assumed to be attached to a nitrogen atom from the chemical shift value, were correlated with the quaternary carbon at C-9 and guanidine carbon at C-11 (δ 161.6, s) and 15-methyl protons (δ 2.71) with C-13 (δ 174.0, s) and C-11. The presence of another imidazolidine moiety was established by similar HMBC correlation as shown in Figure 1. The connectivity of the 6-bromoindol-3-yl moiety to C-8′ was shown by the HMBC observation of H-8′/C-2′, C-3′, C-3′a. The relative stereochemistry of the chiral centers was elucidated from NOESY experiments on **1** (Fig. 2). An NOE between H-8 (δ 3.81) and H-8′ indicated that these hydrogens are in the same face (β) of the ring. NOEs between H-8α (δ 3.63) and H-14 and between H-8′ and H-14′ indicated the stereochemical orientation of the two spiro-junctions as shown in Figure 2. Therefore, the structure of tubastrindole A is represented by **1**. It is noted that the two five-membered imidazolidinone rings and the 3,6-disubstituted indole are situated approximately perpendicular to the six-membered ring as incicated by the NOEs from H-8′ to H-4′ and H-14′ and from H-2′ to H-14 and H-15′.

The molecular formula of tubastrindole B (**2**) was deduced as $C_{28}H_{28}N_8O_2$ from the (+)-HRFABMS data [*m*/*z* 509.2411 (M+H), Δ −0.2 mmu]. The ^{1}H NMR spectrum was similar to that of **1**, except for the presence of an additional proton signal at δ 7.20 (1H, overlapped) in **2**. The proton was located at C-6′ in the place of the bromine atom in **1** by the signals for H-5′ (δ 7.13, t, J=7.9 Hz) and H-7′ (δ 7.41, d, J=7.9 Hz). This was further supported by the ^{13}C NMR signal at δ 123.9 which appeared as a doublet. The relative stereochemistry of **2** was concluded to be the same as that of **1** on the basis of the NOESY data. Thus, the structure of tubastrindole B was established as 6′-debromotubastrindole A.

The molecular formula of tubastrindole C (**3**), $C_{28}H_{27}N_7O_3$ as established by the (+)-HRFABMS data [*m*/*z* 510.2254 (M+H), Δ +0.0 mmu] had one more oxygen and one less nitrogen and hydrogen atoms than **2**. The ^{1}H NMR spectrum was essentially identical to that of **2**, the difference being that several peaks were overlapped or separated. Comparison of the ^{13}C NMR spectrum of **3** with that of **2** indicated that the signals for C-2, C-3, C-11′, and C-13′ in **3** were shifted 1.6–3.3 ppm from those of **2**. This implied that one of the 1,3-dimethyl-2-iminoimidazolidin-4-one moieties was replaced with a 1,3-dimethylimidazolidine-2,4-dione. The same relative stereochemistry of **3** was also by NOE observation. Therefore, the structure of tubastrindole C was assigned as shown in **3.**

There have been several reports on metabolites isolated from the stony coral, *Tubastraea* sp., that proposed indole derivative was condensed with a 2-iminoimidazolidin-4-one or imidazolidine-2,4-dione derivative. However, this is the first isolation of bis(indole) metabolites with an unprecedented skeleton, consisting of two indoles and two imidazolidinones. The compounds (**1**–**3**) could biogenetically be formed from an enzymatic Diels–Alder cycloadditon of two molecules of aplysinopsin, which was probably derived from tryptophan and guanidine, followed by some modifications.

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