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APLYSINOPSIN DIMERS FROM A STONY CORAL. *TUBASTRAEA AUREA*

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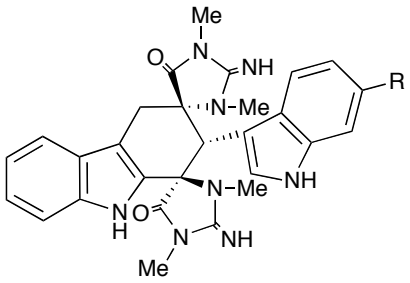
Abstract – Five novel bis(indole) alkaloids, which were composed of two molecules of aplysinopsin have been isolated from a stony coral, *Tubastraea aurea*. Their structures were elucidated on the basis of spectroscopic methods.

The secondary metabolites of the stony corals have not been studied so extensively as those of soft corals, based on the assumption that their calcareous bodies likely eliminate the requirement for defensive metabolites. However, they have been proven to be a source of many different types of compounds: anthraquinoid derivatives,[1] a phenol,[2] alkaloids,[3] macrolides,[4] and acetylenes.[5] Many of these compounds exhibited interesting biological activities, such as antiviral,[6] antifungal,[6] and cytotoxic activity.[7]

We examined the chemical constituents of the stony coral, *Tubastraea aurea*, collected in the Odomari area, Kagoshima Prefecture. A methanol extract of the animal was partitioned between CH_2Cl_2 and water, and then the aqueous part was extracted with *n*-BuOH. Neither extract exhibited biological activity (e.g. antibacterial, antifungal, or cytotoxic activity) in our initial screening. Nevertheless, we tried to isolate the aromatic compounds from the dichloromethane extract, because aromtic signals were observed in the ^{1}H NMR spectrum of the extract. The extract was subjected to silica gel chromatography and reversed-phase HPLC and yielded three unprecedented bis(indole) alkaloids named tubastrindoles A-C (**1**-**3**), dimers of aplysinopsin.[8] Further investigation has led to the isolation of five new bis(indole) alkaloids, tubastrindoles D-H (**4**-**8**). In this paper, we describe the isolation and structure elucidation of these compounds.

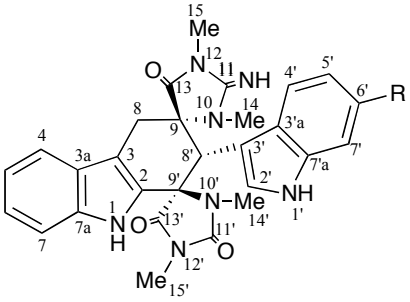
Tubastrindole D (**4**) was isolated as a pale yellow oil, and its molecular formula was established as $C_{28}H_{26}O_3N_7Br$ by HRFABMS and NMR spectra. The signal patterns in the ^1H NMR spectrum showed similarity to those of tubastrindole A (**1**) (Table 1). Thus, resonances due to four methyl protons (δ 2.63, 2.76, 2.87, 3.34, s, each 3H), isolated methylene protons (δ 3.55, 3.73, 1H each, AB, *J*= 17.4 Hz, H_2-8), a methine proton (δ 4.47, s, 1H, H-8'), and seven indole protons were observed. The latter protons were due to four consecutive phenyl protons (δ 7.60, 1H, d, *J*= 7.5 Hz, H-4, δ 7.14, 1H, t, *J*= 7.5 Hz, H-5, δ 7.26, 1H, t, *J*= 7.5 Hz, H-6, δ 7.37, 1H, d, *J*= 7.5 Hz, H-7), 1,2,4-substituted phenyl protons (δ 7.44, 1H, d, *J*= 8.7, H-4', δ 7.20, 1H, dd, *J*= 8.7, 1.5 Hz, H-5', δ 7.54, 1H, d, *J*= 1.5 Hz, H-7'), and an aromatic methine proton (δ 7.08, 1H, s, H-2'). The chemical shifts due to four methyl protons were similar to those of tubastrindole C (**3**). Therefore, compound (**4**) was assumed to be 6'-bromotubastrindole C, and this was confirmed by NOESY experiments (Figure 1): H-8'/H-8β (δ 3.73), H-4', Me-14', H-8α (δ 3.55)/H-4, Me-14, H-2'/ Me-14, Me-15', H-4'/ Me-14'. The presece of the 6-bromoindole moiety was also supported by comparing the ^{13}C NMR spectrum with that of **1** (Table 1).

The molecular formula $C_{28}H_{27}O_4N_7$ of tubastrindole E (**5**) had one more oxygen atom than that of **3**. The ^1H NMR spectrum was similar to that of **3**, except that a methine proton appeared at δ 5.99 (1H, s) replacing the methylene protons at C-8, suggesting a hydroxyl group at C-8. H-8 was situated on the same face (β) as H-8' (δ 4.58, 1H, s) on the basis of an NOE between H-8 and H-8'. The relative configuration of C-9, C-8', and C-9' was determined to be same as that of **4** by interpretation the NOE spectrum.



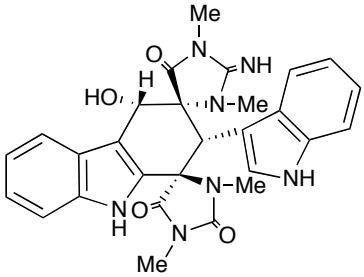
1 R=Br

2 R=H

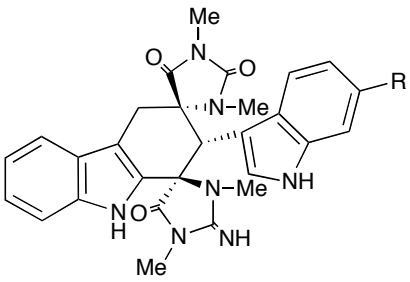


3 R=H

4 R=Br

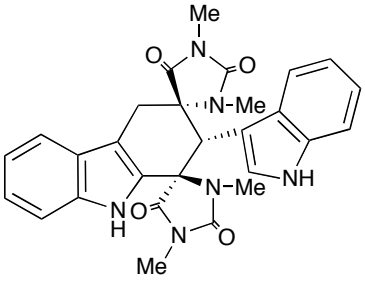


5



6 R=Br

7 R=H



8

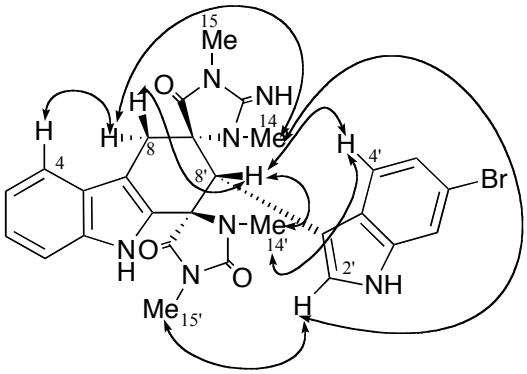


Figure 1. The key NOESY correlations for **4**.

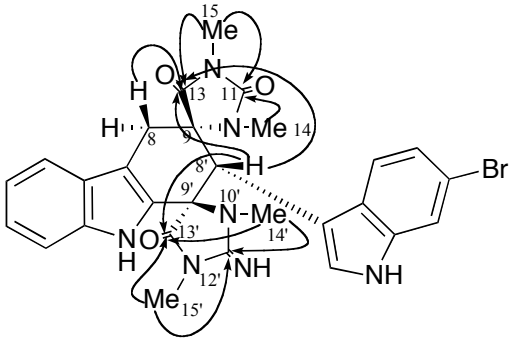


Figure 2. The key HMBC correlations for **6**.

Tubastrindole F (**6**), $C_{28}H_{26}O_3N_7Br$, was isomeric with **4**. The signal patterns of the ^{1}H NMR in relation to **4** indicated that the chemical shifts were somewhat different from each other. The positions of 1,3-dimethyl-imidazolidine-2,4-dione and 1,3-dimethyl-2-iminoimidazolidin-4-one were determined from the observation of the HMBC spectrum (Figure 2). Thus, H-8β (δ 3.70, 1H, AB, *J*= 16.9 Hz) and H-8' (δ 4.49, 1H, s) were correlated with an amido carbon (δ 175.9), which was thus assigned to C-13. Methyl protons at δ 2.57 (3H, s) were positioned at C-15, since they were correlated with C-13. Correlations of Me-15 and methyl protons at δ 3.07 (3H, s) to a urea-type carbon at δ 159.9 suggested the methyl group at N-10 and the urea-type carbon at C-11. H-8' showed a correlation with another amido carbon (δ 172.3), the latter of which was therefore positioned at C-13'. The position of the methyl group at δ 2.82 (3H, s) was determined to be at N-12' on the basis of a correlation to the methyl protons to C-13'. Correlations from Me-15' and methyl protons at δ 3.12 (3H, s) to an imino carbon (δ 158.7) indicated that the methyl group and the imino carbon were determined to be at N-10' and C-11', respectively. The relative stereochemistry was also elucidated by interpretation of the NOESY experiments: H-8α/H-4, Me-14, H-8β/H-8', Me-14/H-2', H-8'/H-4', Me-14'.

The ^{1}H NMR spectrum of tubastrindole G (**7**), $C_{28}H_{27}O_3N_7$, was similar to that of **6**, but resonances due to H-5' (δ 7.11, 1H) and H-7' (δ 7.38, 1H) were observed as a triplet (*J*= 7.3 Hz) and a doublet (*J*= 7.3 Hz), respectively. A new signal due to H-6' appeared at δ 7.17 (1H, t, *J*= 7.3 Hz).

This meant that bromine atom at C-6' in **6** was replaced by a hydrogen atom in **7**. This was also supported by comparing the ^{13}C and NOESY spectra with those of **6**.

Table 1. ^{1}H and ^{13}C NMR Data (CD_3OD, 400 Mz) for **5**-**9**.[a]

	4		**5**		**6**		**7**		**8**	
no.	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
2		126.9		124.9[††]		123.3		121.1		124.9
3		113.1		117.6		116.1		116.1		114.1
3a		126.8		126.2		126.5		126.6		127.0
4	7.60 d (7.5)	119.9	7.92 br d (8.0)	122.2	7.65 d (7.7)	120.2	7.66 d (8.0)	120.2	7.60 d (7.5)	119.9
5	7.14 t (7.5)	121.2	7.11 t (8.0)	121.0	7.16 t (7.7)	121.3	7.17 br t (8.0)	121.2	7.11 t (7.5)	120.9
6	7.26 t (7.5)	125.3	7.24 br t (8.0)	124.9	7.29 t (7.7)	125.6	7.29 br t (8.0)	125.6	7.23 t (7.5)	124.9
7	7.37 d (7.5)	112.9	7.36 d (8.0)	112.6[†]	7.37 d (7.7)	112.8	7.38 d (8.0)	112.7	7.34 d (7.5)	112.7
7a		139.6		139.5		139.5		139.7		113.9
8α	3.55 d (17.4)	27.4		69.6	3.46 d (16.9)	28.2	3.47 d (16.9)	28.3	3.37 d (16.9)	28.2
β	3.73 d (17.4)		5.99 s		3.70 d (16.9)		3.72 d (16.9)		3.65 d (16.9)	
9		72.5		78.6		70.1		70.2		70.4
11		161.6		161.9		159.9		159.9		160.2
13		174.9		173.8		175.9		176.1		177.0
14	3.34 s	33.1	3.41 s	34.7	3.07 s	29.7	3.09 s	29.7	3.13 s	29.7
15	2.63 s	26.4	2.77 s	26.5	2.57 s	25.2	2.56 s	25.2	2.47 s	24.9
2'	7.08 s	125.7	7.08 br s	125.0[††]	7.05 s	126.3	7.04 s	125.4	7.07 s	125.5
3'		105.6		105.1		105.4		104.9		106.1
3'a		128.3		129.1		128.0		129.0		129.7
4'	7.44 d (8.7)	120.3	7.50 br d (7.8)	118.4	7.44 d (7.9)	119.6	7.52 d (7.3)	118.0	7.49 d (7.3)	118.7
5'	7.20 dd (8.7, 1.5)	124.5	7.14 br t (7.8)	121.1	7.23 d (7.9)	124.4	7.11 br t (7.3)	121.2	7.04 t (7.3)	120.8
6'		117.0	7.24 br t (7.8)	123.5		116.7	7.17 br t (7.3)	123.4	7.10 t (7.3)	123.1
7'	7.54 d (1.5)	115.6	7.35 d (7.8)	112.5[†]	7.55 s	115.7	7.38 d (7.3)	112.8	7.31 d (7.3)	112.4
7'a		137.6		136.6		137.7		136.5		136.7
8'	4.47 s	44.0	4.58 s	42.5	4.49 s	44.2	4.55 s	44.4	4.51 s	43.6
9'		69.8		69.6		72.8		73.0		70.4
11'		157.6		157.2		158.7		158.7		157.2
13'		174.4		173.8		172.3		172.4		174.5
14'	2.87 s	25.6	2.87 s	25.6	3.12 s	28.3	3.14 s	28.3	2.89 s	25.6
15'	2.76 s	24.9	2.68 s	24.9	2.82 s	26.2	2.79 s	26.2	2.65 s	24.7

[a]Chemical shift values are in ppm, and *J* values (in Hz) are presented in parentheses.

[†, ††] These values may be interchangeable.

The molecular formula $C_{28}H_{26}O_4N_6$ of tubastrindole H (**8**) indicated that it had one more oxygen and one less nitrogen than that of **7**. The presence of two equivalents of 1,3-dimethyl-imidazolidine-2,4-dione was determined by comparing the NMR chemical shifts with corresponding shifts of **4** and **7**. The relative stereochemistry of the chiral centers was found to be similar to that of the related compounds described above by comparing the NOESY spectrum with those of **4** and **7**.

Previously, we reported that tubastrindoles A-C (**1**-**3**) could biogenetically be formed by an enzymatic Diels-Alder cycloaddition of two molecules of aplysinopsin. However, a series of new tubastrindoles, including tubastrindoles A-C,[9] had small absolute values of their optical rotations ranging from 1.4 to 14. This suggested that the dimers might be artifacts or be composed of both enantiomers in the almost same ratio.

EXPERIMENTAL

Animal Material. Specimens of *Tubastraea aurea* were collected at Odomari, Kagoshima prefecture. The reference sample (collection no. 253 and 275) was deposited at Department of Chemistry and Bioscience.

Extraction and Isolation. The organisms (wet weight: 8.7 kg) were chopped into small pieces and extracted with MeOH three times. The dried MeOH extract was suspended in H_2O and extracted with CH_2Cl_2. A portion (5.1 g) of the CH_2Cl_2 extract (37.8 g) was adsorbed on silica gel and subjected to chromatography on silica gel (100 g) packed in *n*-hexane. Fractions of 100 mL were collected as follows: 1-2 (CH_2Cl_2-*n*-hexane, 4:1), 3-4 (CH_2Cl_2), 5-6 (MeOH-CH_2Cl_2, 1:49), 7-8 (MeOH-CH_2Cl_2, 1:19), 9-11 (MeOH-CH_2Cl_2, 1:9), 12-13 (MeOH-CH_2Cl_2, 1:1), 14-18 (MeOH). Fractions 11-18 (3.1 g) were chromatographed on silica gel with MeOH-CH_2Cl_2 (3:97 to 3:17) to give a crude fraction, which was finally purified by HPLC (ODS) with MeOH-H_2O (3:17 to 3:7) including 0.1% TFA and CH_3CN-H_2O (3:7 to 1:4) including 0.1% TFA, yielding **1** (5.6 mg), **2** (12.6 mg), **3** (3.8 mg), **4** (1.5 mg), **5** (7.0 mg), **6** (2.5 mg), **7** (4.6 mg), and **8** (5.2 mg).

Tubastrindole D (**4**): pale yellow oil, $[\alpha]_D$ -1.4 (*c* 0.07, MeOH); UV (MeOH) λmax (log ε) 222 nm (4.59), 283 (3.89); IR (film) ν_{max} 3229, 1773, 1684, 1545 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRFABMS *m/z* 588.1375 $[M + H]^+$ (calcd for $C_{28}H_{27}O_3N_7{}^{79}Br$, 588.1359).

Tubastrindole E (**5**): pale yellow oil, $[\alpha]_D$ 16.7 (*c* 0.03, MeOH); UV (MeOH) λmax (log ε) 218 nm (4.49), 273 (3.84); IR (film) ν_{max} 3318, 1772, 1684 cm^{-1}; ^1H and ^{13}C NMR, see Table; HRFABMS *m/z* 526.2223 $[M + H]^+$ (calcd for $C_{28}H_{28}O_4N_7$, 526.2203).

Tubastrindole F (**6**): pale yellow oil, $[\alpha]_D$ -10.5 (*c* 0.10, MeOH); UV (MeOH) λmax (log ε) 222 nm (4.54), 270 (3.89); IR (film) ν_{max} 3677, 1773, 1684 cm^{-1}; ^1H and ^{13}C NMR, see Table; HRFABMS *m/z* 588.1353 $[M + H]^+$ (calcd for $C_{28}H_{27}O_3N_7{}^{79}Br$, 588.1359).

Tubastrindole G (**7**): pale yellow oil, $[\alpha]_D$ -10.4 (*c* 0.12, MeOH); UV (MeOH) λmax (log ε) 218 nm (4.63), 272 (4.00); IR (film) ν_{max} 1770, 1690 cm^{-1}; ^1H and ^{13}C NMR, see Table; HRFABMS *m/z* 510.2239 $[M + H]^+$ (calcd for $C_{28}H_{28}O_3N_7$, 510.2254).

Tubastrindole H (**8**): pale yellow oil, $[\alpha]_D$ -3.4 (*c* 0.07, MeOH); UV (MeOH) λmax (log ε) 218 nm (4.63), 280 (4.02); IR (film) ν_{max} 1770, 1701 cm^{-1}; ^1H and ^{13}C NMR, see Table; HRFABMS *m/z* 511.2097 $[M + H]^+$ (calcd for $C_{28}H_{27}O_4N_6$, 511.2093).

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9. Since we realized that the polarimeter had been broken, remeasurements were made; **1**: -14, (*c* 0.16, MeOH), **2**: -11 (*c* 0.07, MeOH), and **3**: +5 (*c* 0.04, MeOH).