Manauealides,[1] Some of the Causative Agents of a Red Alga *Gracilaria coronopifolia* Poisoning in Hawaii

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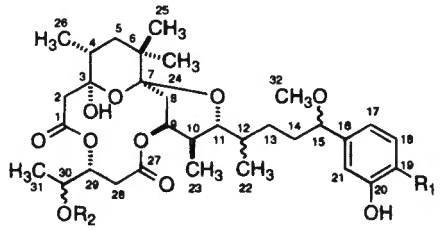
Manauealides A–C (**1**–**3**), compounds related to debromoaplysiatoxin (**5**), were isolated and characterized from a red alga *Gracilaria coronopifolia*. Compounds **1** and **2** are presumed to be the causative toxins of *G. coronopifolia* food poisoning in Hawaii. Manauealide A (**1**) and C (**3**) are new macrolides, whereas manauealide B (**2**) is a known semisynthetic product of **5**.

Successive food poisonings from ingestion of a red alga *Gracilaria coronopifolia* J. Agardh (Gracilariaceae) occurred in Hawaii in September 1994.[2,3] These were the first reported cases of seaweed poisoning in Hawaii, although the local residents had been eating algae, including *G. coronopifolia*, regularly for years. The characteristic symptoms of these poisonings were vomiting, diarrhea, and a burning sensation in the mouth and throat.[2] Some poisonings, including fatal cases, by ingestion of *Gracilaria* species have been reported.[4–8] Prostaglandin E_2 has been suspected as a culprit in Japanese poisoning cases.[9,10] In algal poisoning cases of Guam,[6] polycavernosides, novel glycosidic macrolides, were isolated as causative agents of poisoning.[11,12] In the poisoning cases of Hawaii, the symptoms were different from the former reported cases; thus, the causative agents of Hawaiian cases were thought to be different.

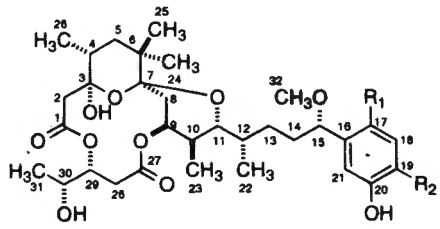
Our study on the toxic agents of Hawaiian poisoning cases revealed that the main causative agents were aplysiatoxin (**4**) and debromoaplysiatoxin (**5**).[3] These toxins had been previously isolated from the digestive gland of the sea hare *Stylocheilus longicauda*.[13,14] Debromoaplysiatoxin (**5**) had also been isolated from a mixture of two blue-green algae *Schizothrix calcicola* and *Oscillatoria nigroviridis*, along with oscillatoxin A, which is 31-nor debromoaplysiatoxin, and related compounds.[15,16,17] Aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) discovered in a blue-green alga *Lyngbya majuscula* were also identified as the causative agents of a severe contact dermatitis (swimmers' itch) that sometimes affects swimmers in Hawaiian waters.[16,18] The *G. coronopifolia* poisonings in Hawaii in 1994, however, were the first reported cases of the implication of aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) in food poisoning.[3]

We have conducted a continuous study on the toxins associated with the toxic specimen and have recollected *G. coronopifolia* from the site of the original toxic specimen. In this report we describe the isolation and characterization of the two previously undescribed toxic



1 R_1 = Cl R_2 = H
3 R_1 = H R_2 = $COCH_3$

Figure 1.



2 R_1 = H R_2 = Br
4 R_1 = Br R_2 = H
5 R_1 = H R_2 = H

Figure 2.

macrolides manauealide A and C (**1**, **3**; Figure 1), and the isolation of manauealide B (**2**; Figure 2), which was obtained for the first time as a natural product. We also completely assigned ^{13}C chemical shifts for aplysiatoxin (**4**) and debromoaplysiatoxin (**5**). Furthermore, we report the seasonable variability of toxic components in *G. coronopifolia*.

G. coronopifolia samples were extracted with acetone and methanol and purified by liquid–liquid partition and by HPLC. Purification of the toxin was monitored by mouse toxicity; the compounds which caused diarrhea in mice were isolated.

Manauealide A (**1**) was obtained as a white solid from sample A (see the Experimental Section). The molecular formula $C_{32}H_{47}ClO_{10}$ was derived from HRFABMS data. ^1H NMR spectra showed that **1** has three singlet methyls (δ 0.73, 0.81, 3.16) and four doublet methyls (δ 0.69, 0.78, 0.85, 1.11). ^{13}C NMR spectra suggested the presence of two carboxylic esters (δ 169.2, 170.3) and two acetals (δ 98.8, 100.6). ^1H and ^{13}C NMR spectra of **1** resembled very closely those of debromoaplysiatoxin (**5**).[3,16] Prominent differences in the ^1H and ^{13}C values between **1** and **5** were observed only in the aromatic

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region; only three protons (δ 6.87, 7.08, 7.28) were observed in the aromatic region of **1**, while debromoaplysiatoxin (**5**) has four protons on the aromatic ring. These results allowed us to deduce that compound **1** was a debromoaplysiatoxin-related compound in which an aromatic proton had been substituted by a chlorine. Interpretation of ^{1}H−^{1}H COSY, TOCSY, HMBC, and HSQC data for **1** led to the elucidation of its planar structure. In an attempt to determine the stereostructure, a ROESY experiment was performed. The following correlations were observed; H-24−H-8ax, H-24−H-5ax, H-25−H-4, H-2β−H-4, H-29−H-11, and H-29−H-23. No correlation was observed between H-24 and H-4. From these results and ^{1}H coupling constants, the stereochemistries around the two oxane rings and a macrolide part of **1** were established to be identical with those of **5**. Insufficient material hampered the determination of the stereochemistries of H-12, H-15, and H-30. However, the ^{1}H and ^{13}C chemical shifts and ^{1}H coupling constants of **1** and those of **5** are almost identical except for the aromatic region, and the CD spectrum of **1** showed a positive Cotton effect, $[\theta]_{281}$ +825°, which was comparable with the one reported for **5**, $[\theta]_{269}$ +902°.[16] These data suggest that the absolute stereochemistry of **1** and **5** are the same. Manauealide A (**1**) is the first example of a chlorinated aplysiatoxin.

Manauealide B (**2**) was isolated as a white solid from sample A (see the Experimental Section). The positive ion HRFABMS revealed that **2** had the same molecular formula, $C_{32}H_{47}BrO_{10}$, as aplysiatoxin (**4**). The planar structure of **2** was elucidated by interpretation of spectral data. Compound **2** had been previously synthesized from debromoaplysiatoxin (**5**) by bromination.[16] Comparison of reported ^{1}H chemical shifts and coupling constants for **2** with those for manauealide B revealed that manauealide B has the same stereochemistry as **2**. The CD spectrum of **2** showed a positive Cotton effect at $[\theta]_{279}$ +386° as did debromoaplysiatoxin (**5**), $[\theta]_{269}$ +902°. Thus, the absolute stereochemistry of manauealide B was deduced to be the one shown in structure **2**. The ^{13}C assignments for **2** (Table 1) had not been previously reported. Manauealide B (**2**) was obtained as a natural product for the first time in this study.

Manauealide C (**3**) was isolated as a white solid from sample B (see the Experimental Section). The molecular formula $C_{34}H_{50}O_{11}$ was derived from HRFABMS data. Examination of the spectral data revealed that the planar structure of manauealide C (**3**) was identical to that of debromoaplysiatoxin (**5**) except that C-30 had an acetoxy group instead of a hydroxyl group. The stereostructure of the macrolide portion of the molecule, including the spiroacetal ring, was determined to be as shown by structure **3** from a ROESY experiment and ^{1}H coupling constants. The elucidation of the stereochemistries at H-12, H-15, and H-30 were hampered by small sample amounts; however, the CD spectrum of **3** also showed a positive Cotton effect at $[\theta]_{276}$ +2705°. Furthermore, the ^{1}H and ^{13}C chemical shifts and ^{1}H coupling constants for **3** and **5** were almost identical, except for those of the protons and carbons in the vicinity of the acetoxy group. Thus, the absolute stereochemistry of **3** was suggested to be identical with that of **5**.

The complete ^{13}C assignment for aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) (Table 2) were established by

Table 1. ^{1}H NMR (500 MHz) Data for Compounds **1** and **3**[a]

proton	1	3
H-2β	2.49 (d, 12.5 Hz)	2.53 (d, 12.5 Hz)
H-2α	2.77 (d, 12.5 Hz)	2.79 (d, 12.5 Hz)
H-4	1.82 (m)	1.87 (m)
H-5ax	1.58 (t, 13.0, 13.0 Hz)	1.62 (t, 12.7, 13.0 Hz)
H-5eq	1.04 (dd, 3.6, 13.4 Hz)	1.06 (dd, 3.4, 13.4 Hz)
H-8ax	1.69 (dd, 3.6, 14.8 Hz)	1.70 (dd, 3.6, 14.6Hz)
H-8eq	2.65 (dd, 2.8, 14.6 Hz)	2.67 (dd, 2.9, 14.6 Hz)
H-9	5.22 (m, 2.8, 3.6, 4.1 Hz)	5.23 (m)
H-10	1.69 (m)	1.71 (m)
H-11	3.88 (dd, 1.9, 10.7 Hz)	3.92 (dd, 2.2, 10.8 Hz)
H-12	1.51 (m)	1.52 (m)
H-13	1.28 (m)	1.32 (m)
H-13′	1.36 (m)	1.39 (m)
H-14	1.58 (m)	1.64 (m)
H-14′	1.95 (m)	1.95 (m)
H-15	3.99 (t, 6.5, 6.6 Hz)	3.99 (t, 6.5, 6.5 Hz)
H-17	6.87 (dd, 1.8, 8.2 Hz)	6.83 (d, 7.5 Hz)
H-18	7.28 (d, 8.0 Hz)	7.13 (t, 7.7, 7.8 Hz)
H-19		6.71 (ddd, 1.8, 1.9, 7.7 Hz)
H-21	7.08 (d, 1.8 Hz)	6.90 (t, 1.8, 1.9 Hz)
3H-22	0.78 (d, 6.8 Hz)	0.79 (d, 6.8 Hz)
3H-23	0.69 (d, 6.9 Hz)	0.72 (d, 6.9 Hz)
3H-24	0.81 (s)	0.84 (s)
3H-25	0.73 (s)	0.82 (s)
3H-26	0.85 (d, 6.7 Hz)	0.87 (d, 6.7 Hz)
H-28α	2.93 (dd, 11.7, 18.1 Hz)	2.98 (dd, 11.8, 18.0 Hz)
H-28β	2.86 (dd, 2.0, 18.2 Hz)	2.83 (dd, 2.7, 17.6 Hz)
H-29	5.19 (m, 2.0, 3.6, 11.7 Hz)	5.39 (m)
H-30	4.02 (m)	5.13 (m)
3H-31	1.11 (d, 6.6 Hz)	1.21 (d, 6.6 Hz)
CH_3O	3.16 (s)	3.14 (s)
CH_3CO		2.04 (s)

[a] Spectra determined in acetone-d_6; data reported in ppm.

interpretation of ^{1}H−^{1}H COSY, TOCSY, HMBC, HSQC, and ROESY data. Unambiguous ^{13}C assignment for aplysiatoxin (**4**) had not been previously reported. In an earlier report of ^{13}C chemical shifts for debromoaplysiatoxin (**5**),[19] the chemical shifts of C-3 and C-7 and those of C-24 and C-25 should be interchanged.

In our studies, the toxic substances, manauealide A (**1**) and B (**2**), were identified along with aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) in the toxic specimen (sample A) of *G. coronopifolia* which caused food poisoning in Hawaii.[2] A 1 μg injection of either **1** or **2** caused diarrhea in mice for a period of 3−4 h. A 10 μg injection of **1** also caused diarrhea in mice for a 3−4 h period, but a 10 μg injection of **2** caused diarrhea in mice and death within 12 h. The symptoms of *G. coronopifolia* poisoning in Hawaii included diarrhea.[2] Thus, manaueallides A and B (**1**, **2**) may act as causative agents of the poisoning along with the major toxins aplysiatoxin (**4**) and debromoaplysiatoxin (**5**). Manauealide C (**3**) was obtained from the algal sample (sample B) recollected 1 week after the incidents from the same place where the initial toxic specimen had been collected. Manaueallide C (**3**) also showed toxicity to mice. One and ten microgram injections of **3** caused diarrhea in mice for 2−3 h and 3−4 h periods, respectively. This algal sample also contained prominent amounts of aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) (see the Experimental Section). However, **1** and **2** could not be detected in sample B. *G. coronopifolia* (sample C) was recollected 3 months after the incidents at the same site of the toxic specimen collection. Interestingly, aplysiatoxin (**4**), debromoaplysiatoxin (**5**), and manauealides A−C (**1**−**3**) could not be detected in sample C.

These results suggested that the toxic components contained in *G. coronopifolia* were seasonally variable.

Table 2. ^{13}C NMR (125 MHz) Data for Compounds 1–5[a]

position	1[b]	2[b]	3[b]	4[b]	5[b]
1	169.2 (0)	169.3 (0)	168.9 (0)	169.1 (0)	169.2 (0)
2	47.0 (2)	47.0 (2)	46.8 (2)	46.8 (2)	46.9 (2)
3	98.8 (0)	98.8 (0)	98.7 (0)	98.8 (0)	98.8 (0)
4	35.7 (1)	35.7 (1)	35.7 (1)	35.6 (1)	35.7 (1)
5	41.1 (2)	41.1 (2)	41.1 (2)	41.1 (2)	41.1 (2)
6	39.0 (0)	38.9 (0)	38.9 (0)	39.0 (0)	39.0 (0)
7	100.6 (0)	100.7 (0)	100.8 (0)	100.8 (0)	100.8 (0)
8	33.6 (2)	33.6 (2)	33.7 (2)	33.6 (2)	33.6 (2)
9	73.2 (1)	73.2 (1)	73.6 (1)	73.2 (1)	73.2 (1)
10	35.4 (1)	35.4 (1)	35.4 (1)	35.4 (1)	35.4 (1)
11	69.6 (1)	69.6 (1)	69.9 (1)	69.7 (1)	69.8 (1)
12	34.2 (1)	34.2 (1)	34.1 (1)	34.4 (1)	34.2 (1)
13	31.0 (2)	31.0 (2)	31.3 (2)	30.7 (2)	31.2 (2)
14	36.0 (2)	36.0 (2)	36.1 (2)	35.6 (2)	36.1 (2)
15	85.2 (1)	85.2 (1)	85.8 (1)	83.6 (1)	85.8 (1)
16	144.9 (0)	145.6 (0)	145.9 (0)	144.3 (0)	145.9 (0)
17	120.4 (1)	120.8 (1)	119.3 (1)	113.1 (0)	119.3 (1)
18	130.2 (1)	133.4 (1)	129.8 (1)	133.7 (1)	129.8 (1)
19	119.6 (0)	110.0 (0)	114.9 (1)	117.2 (1)	115.0 (1)
20	153.7 (0)	156.9 (0)	158.3 (0)	158.2 (0)	158.3 (0)
21	116.2 (1)	115.9 (1)	114.6 (1)	116.1 (1)	114.6 (1)
22	13.6 (3)	13.6 (3)	13.5 (3)	13.5 (3)	13.6 (3)
23	13.0 (3)	13.0 (3)	13.0 (3)	13.0 (3)	13.0 (3)
24	26.7 (3)	26.7 (3)	26.8 (3)	26.8 (3)	26.8 (3)
25	23.4 (3)	23.5 (3)	23.7 (3)	23.7 (3)	23.6 (3)
26	16.5 (3)	16.5 (3)	16.4 (3)	16.5 (3)	16.5 (3)
27	170.3 (0)	170.4 (0)	169.7 (0)	170.4 (0)	170.4 (0)
28	34.6 (2)	34.7 (2)	36.0 (2)	34.6 (2)	34.6 (2)
29	74.3 (1)	74.3 (1)	71.6 (1)	74.2 (1)	74.3 (1)
30	67.1 (1)	67.1 (1)	70.4 (1)	67.0 (1)	67.0 (1)
31	17.7 (3)	17.7 (3)	15.8 (3)	17.6 (3)	17.7 (3)
32	56.7 (3)	56.7 (3)	56.6 (3)	56.8 (3)	56.6 (3)
33			170.4 (0)		
34			30.2 (3)		

[a] Spectra determined in acetone-d_6; data reported in ppm. [b] Carbon types determined by a DEPT experiment and reported as 0 (quaternary), 1 (CH), 2 (CH_2), or 3 (CH_3).

In our previous paper, we suggested that epiphytic organisms, possibly blue-green algae, may be the true producers of the causative toxins found in the *G. coronopifolia* which caused poisoning in Hawaii.[3] The results obtained in this study also support this proposal. Spotty distribution and variability of the toxic components in *G. coronopifolia* may be the result of different dominant epiphytic strains. Such a variability in toxin productivity among strains is known in the blue-green alga *Lyngbya majusucula*.[20] Furthermore, the seasonal changes of the toxicity of *L. majuscula* were also reported.[18] Aplysiatoxin (**4**) and debromoaplysiatoxin (**5**) have been shown to be activators of protein kinase C and to be potent tumor promoters.[21,22] It has been reported that tumor promoters containing blue-green algae sometimes grow epiphytically on edible seaweeds,[23] and many epiphytes including blue-green algae grow on *Gracilarlia*.[3,24] Therefore, in some cases of reported *Gracilaria* sp. poisonings, it is possible that the causative agents originate from the epiphytes on the algae and not from the *Gracilaria* sp. itself.

Experimental Section

Instruments. UV spectra were recorded on a Shimadzu UV-250 spectrophotometer. ^1H- and ^{13}C-NMR spectra were measured on JEOL GSX 400, Varian Unity 500 plus and Bruker DMX500 spectrometers, using acetone-d_6 as a solvent. FAB mass spectra were obtained on a JEOL JMS-HX/HX110A spectrometer. Optical rotations were determined on a JASCO DIP-1000 instrument.

Algal Material. Sample A: A sample of *Gracilaria coronopifolia* which caused poisonings in seven persons from Kona, Hawaii, and one from Wailuku, Maui, was collected from Waiehu, Maui, in September of 1994, and the remaining portion of the toxic specimen (2.2 kg, wet weight) was transferred to the University of Hawaii laboratory in a frozen condition and kept at −15 °C until extraction could be carried out. Sample B: *G. coronopifolia* (4.8 kg, wet weight) was recollected 1 week after the poisonings by sample A at the same site in Waiehu, Maui, as sample A. Sample C: *G. coronopifolia* (0.4 kg, wet weight) was recollected on December 1994 at the same site as sample A. These samples were transferred to the University of Hawaii while cooled with ice and then kept at −15 °C.

Extraction and Isolation. The toxic algal specimen (sample A) was processed according to a previously described procedure.[3] In short, the sample was extracted with acetone and MeOH, the extracts were partitioned between H_2O and $CHCl_3$, and the water phase was extracted with EtOAc. The EtOAc fraction was subjected to HPLC purification on a TSK-GEL ODS 120-T column (7.8 × 300 mm; TOSOHAAS Inc., Japan) with 80 or 90% CH_3CN in H_2O. The toxin purification was guided by a mouse toxicity assay. From sample A, **1** (1.1 mg), **2** (0.6 mg), **4** (1.8 mg), and **5** (2.0 mg) were isolated. Sample B was processed according to the same method as described above. From sample B, **3** (1.25 mg), **4** (6.7 mg) and **5** (3.2 mg) were isolated. Sample C was processed according to the same method as described above; compounds **1**–**5** could not be detected in this sample.

Mouse Toxicity Test. The sample was dissolved in 0.8 mL of 1% Tween 60 solution and intraperitoneally injected into a *ddY* male mouse (18–20 g).

Manauealide A (1): UV (MeOH) λ_{max} nm (ϵ) 233 (776), 279 (738); $[\alpha]_D$ = +13.8° (*c* 0.55, MeOH); CD (EtOH) $[\theta]_{281}$ +825°; ^1H NMR (acetone-d_6) see Table 1; ^{13}C NMR (acetone-d_6) see Table 2; FABMS (positive ion) *m/z* 649 $[M + Na]^+$, 631 $[M + Na - H_2O]^+$, 609 $[M + H - H_2O]^+$; HRFABMS *m/z* 649.2787 (calcd for $C_{32}H_{47}{}^{35}ClNaO_{10}$, 649.2756).

Manauealide B (2): UV (MeOH) λ_{max} nm (ϵ) 232 (740), 278 (704); $[\alpha]_D$ = +21.3° (*c* 0.3, MeOH); CD (EtOH) $[\theta]_{279}$ +386°; ^1H NMR (acetone-d_6, 500 MHz) 2.47 (1H, d, J = 12.6 Hz, H-2β), 2.77 (1H, d, J = 12.6 Hz, H-2α), 1.82 (1H, m, H-4), 1.61 (1H, t, J = 13.0, 13.0 Hz, H-5ax), 1.05 (1H, dd, J = 3.6, 13.4 Hz, H-5eq), 1.70 (1H, dd, J = 3.5, 14.5 Hz, H-8ax), 2.68 (1H, dd, J = 2.9, 14.6 Hz, H-8eq), 5.23 (1H, m, J = 2.7, 2.8, 3.5 Hz, H-9), 1.72 (1H, m, H-10), 3.90 (1H, dd, J = 2.1, 10.8 Hz, H-11), 1.52 (1H, m, H-12), 1.30 (1H, m, H-13), 1.36 (1H, m, H-13′), 1.60 (1H, m, H-14), 1.94 (1H, m, H-14′), 4.0 (1H, t, J = 6.4, 6.5 Hz, H-15), 6.83 (1H, dd, J = 1.8, 8.2 Hz, H-17), 7.44 (1H, d, J = 8.1 Hz, H-18), 7.07 (1H, d, J = 1.8 Hz, H-21), 0.79 (3H, d, J = 6.8 Hz, H-22), 0.71 (3H, d, J = 6.9 Hz, H-23), 0.83 (3H, s, H-24), 0.77 (3H, s, H-25), 0.86 (3H, d, J = 6.9 Hz, H-26), 2.92 (1H, dd, J = 11.6, 18.3 Hz, H-28α), 2.85 (1H, dd, J = 1.9, 18.3 Hz, H-28β), 5.22 (1H, m, H-29), 4.03 (1H, m, H-30), 1.12(3H, d, J = 6.5 Hz, H-31), 3.18 (3H, s, H-32); ^{13}C NMR (acetone-d_6) see Table 2; FABMS (positive ion) *m/z* 693 $[M + Na]^+$, 695, 675 $[M + Na - H_2O]^+$, 677, 653 $[M + H - H_2O]^+$, 655; HRFABMS *m/z* 693.2282 (calcd for $C_{32}H_{47}{}^{79}BrNaO_{10}$, 693.2250).

Manauealide C (3): UV (MeOH) λ_{max} nm (ϵ) 231 (554), 276 (582); $[\alpha]_D$ = +33.1° (*c* 1.0, MeOH); CD (EtOH) $[\theta]_{276}$ +2705°; ^{1}H NMR (acetone-d_6) see Table 1; ^{13}C NMR (acetone-d_6) see Table 2; FABMS (positive ion) *m/z* 635 $[M + H]^+$, 657 $[M + Na]^+$, and 617 $[M + H - H_2O]^+$; HRFABMS *m/z* 657.3271 (calcd for $C_{34}H_{50}NaO_{11}$, 657.3250).

Aplysiatoxin (4): ^{1}H NMR (acetone-d_6, 500 MHz) 2.62 (1H, d, *J* = 12.7 Hz, H-2β), 2.74 (1H, d, *J* = 12.6 Hz, H-2α), 1.88 (1H, m, H-4), 1.62 (1H, t, *J* = 13.1, 13.1 Hz, H-5ax), 1.05 (1H, dd, *J* = 3.6, 13.4 Hz, H-5eq), 1.72 (1H, dd, *J* = 3.6, 14.7 Hz, H-8ax), 2.69 (1H, dd, *J* = 2.8, 14.6 Hz, H-8eq), 5.24 (1H, m, *J* = 2.8, 3.1, 3.6 Hz, H-9), 1.73 (1H, m, H-10), 3.94 (1H, dd, *J* = 2.1, 10.8 Hz, H-11), 1.57 (1H, m, H-12), 1.42 (1H, m, H-13), 1.48 (1H, m, H-13′), 1.53 (1H, m, H-14), 2.00 (1H, m, H-14′), 4.48 (1H, t, *J* = 6.2, 6.2 Hz, H-15), 7.33 (1H, d, *J* = 8.8 Hz, H-18), 6.70 (1H, dd, *J* = 3.0, 8.7 Hz, H-19), 7.11 (1H, d, *J* = 3.1 Hz, H-21), 0.82 (3H, d, *J* = 6.8 Hz, H-22), 0.73 (3H, d, *J* = 6.9 Hz, H-23), 0.85 (3H, s, H-24), 0.86 (3H, s, H-25), 0.85 (3H, d, *J* = 5.0 Hz, H-26), 2.91 (1H, dd, *J* = 10.7, 18.2 Hz, H-28α), 2.87 (1H, dd, *J* = 3.2, 18.1 Hz, H-28β), 5.20 (1H, m, *J* = 3.2, 3.9, 10.7 Hz, H-29), 4.0 (1H, m, H-30), 1.10 (3H, d, *J* = 6.4 Hz, H-31), 3.19 (3H, s, H-32); ^{13}C NMR (acetone-d_6) see Table 2.

Debromoaplysiatoxin (5): ^{1}H NMR (acetone-d_6, 500 MHz) 2.52 (1H, d, *J* = 12.7 Hz, H-2β), 2.76 (1H, d, *J* = 12.5 Hz, H-2α), 1.86 (1H, m, H-4), 1.62 (1H, t, *J* = 13.1, 13.1 Hz, H-5ax), 1.05 (1H, dd, *J* = 3.6, 13.4 Hz, H-5eq), 1.71 (1H, dd, *J* = 3.6, 14.8 Hz, H-8ax), 2.68 (1H, dd, *J* = 3.0, 14.7 Hz, H-8eq), 5.23 (1H, m, H-9), 1.71 (1H, m, H-10), 3.93 (1H, dd, *J* = 2.1, 10.9 Hz, H-11), 1.52 (1H, m, H-12), 1.31 (1H, m, H-13), 1.39 (1H, m, H-13′), 1.63 (1H, m, H-14), 1.97 (1H, m, H-14′), 4.0 (1H, t, *J* = 6.5, 6.5 Hz, H-15), 6.84 (1H, dt, *J* = 1, 1, 7.9 Hz, H-17), 7.13 (1H, t, *J* = 7.8, 7.8 Hz, H-18), 6.71 (1H, dt, *J* = 1, 1, 7.9 Hz, H-19), 6.92 (1H, t, *J* = 1, 1 Hz, H-21), 0.79 (3H, d, *J* = 6.8 Hz, H-22), 0.71 (3H, d, *J* = 6.9 Hz, H-23), 0.83 (3H, s, H-24), 0.80 (3H, s, H-25), 0.86 (3H, d, *J* = 6.8 Hz, H-26), 2.92 (1H, dd, *J* = 11.1, 18.2 Hz, H-28α), 2.87 (1H, dd, *J* = 2.8, 18.1 Hz, H-28β), 5.23 (1H, m, H-29), 4.03 (1H, m, H-30), 1.12 (3H, d, *J* = 6.4 Hz, H-31), 3.17 (3H, s, H-32). ^{13}C NMR (acetone-d_6) see Table 2.

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References and Notes

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