HETEROCYCLES, Vol. 102, No. 7, 2021, pp. 1287 - 1293. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 3rd March, 2021, Accepted, 21st April, 2021, Published online, 17th May, 2021
DOI: 10.3987/COM-21-14447

# DEBROMOOSCILLATOXINS G AND I FROM THE CYANOBACTERIUM *MOOREA PRODUCENS*

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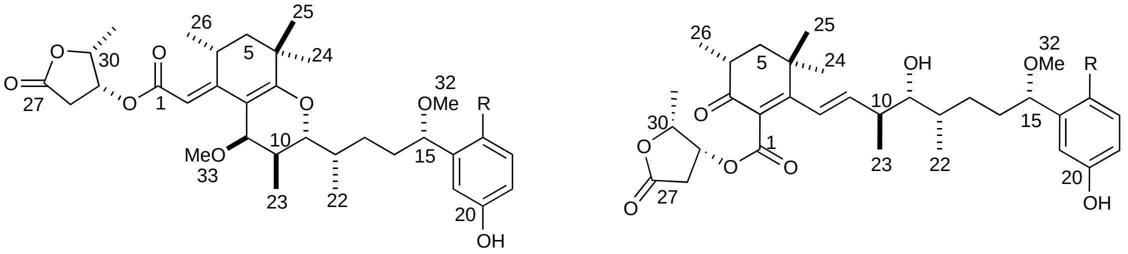
**Abstract** – Debromooscillatoxins G and I (**1** and **3**) were isolated from the marine cyanobacterium *Moorea producens* collected in Okinawa. Their structures were determined based on spectroscopic data, including nuclear magnetic resonance (NMR) and high resolution mass spectrometry (HRMS). Both the isolated compounds showed moderate cytotoxicity and diatom growth inhibition at a concentration of 10 µg/mL.

Aplysiatoxin is a cyanotoxin produced by some cyanobacteria species.[1,2] To date, approximately 50 aplysiatoxin-related compounds have been reported.[3-15] Aplysiatoxin and related compounds have been shown to be potent protein kinase C (PKC) activators and tumor promoters.[16-20] Simplified analogs of aplysiatoxins have also been demonstrated as potential leads for anticancer drug.[21-23] Recently, some aplysiatoxins have been reported to show potent blocking activity against potassium channel Kv1.5.[8-10,15] Thus, aplysiatoxins are interesting compounds owing to their unique structures and activities. In our previous studies, we have tried to identify new aplysiatoxins from the cyanobacterium.[11-13] In this article, the isolation, structure elucidation, and biological activities of two new aplysiatoxin analogs, debromooscillatoxins G and I (**1** and **3**, Figure 1) will be described.[24]



**1.** debromooscillatoxin G: R=H
**2.** oscillatoxin G: R=Br

**3**: debromooscillatoxin I: R=H
**4**: oscillatoxin I: R=Br

**Figure 1.** Structures of **1**–**4**.

High-resolution electrospray ionization mass spectrometry (HRESI-MS) indicated that the molecular formula of **1** was $C_{33}H_{46}O_8$ ($[M+H]^+$ *m/z* 571.3288, calcd for $C_{33}H_{47}O_8$ *m/z* 571.3265). The molecular formula and $^1H$ NMR spectrum suggested that **1** was a debromo analog of oscillatoxin G (**2**, Figure 1).[11,12] The $^1H$ NMR, $^{13}C$ NMR, and HSQC spectra (Table 1) revealed the presence of eight methyl and four methylene groups, nine methine (three aliphatic, five oxy, and one olefinic) carbons, six quaternary (an aliphatic, two carbonyl, and three olefinic) carbons, and a mono-substituted phenol. The proton connectivities $H_3$-26 to $H_2$-5, H-9 to H-15, H-17 to H-19, $H_2$-28 to $H_3$-31 were deduced from $^1H$-$^1H$ COSY and TOCSY analyses (Figure 2). The proton chemical shifts (Table 1) from H-2 to H-12 and $H_2$-28 to $H_3$-31 of **1** were in agreement with those of **2** (see Table S-1 in the Supplementary Information). The proton chemical shift ($\delta_H$ 4.08) for H-15 in **1** was observed at a higher field ($\delta_H$ 4.49) than that in **2** and was identical to that of debromooscillatoxins.[11,12] The partial structures were connected by an HMBC experiment. Key HMBC correlations H-2/C-1, C-4, C-8, $H_3$-26/C-3, $H_3$-24/C-5, C-6, C-7, $H_3$-25/C-5, C-6, C-7, H-9/C-7, C-8, H-15/C-17, C-21, H-29/C-1, $H_3$-32/C-15, and $H_3$-33/C-9 assigned the partial structures into the whole structure. The presence of γ-lactone in the molecule was deduced from the proton chemical shift ($\delta_H$ 4.83) of H-30. The HMBC correlations, H-2/C-8, H-9/C-7, and H-9/C-8, confirmed a C3-C11 fused ring structure analogous to **2**.[11] Agreements of $^1H$ and $^{13}C$ chemical shifts from C-1 to CH-12 and C-27 to $CH_3$-31 between **1** and **2** suggested that **1** had the same relative stereostructure as that of **2**. The proton coupling constants for H-5a (14.0, 2.7 Hz) and H-5b (14.2, 5.8 Hz) suggested the α orientation of $CH_3$-26. The proton coupling constant of H-4/H-5b in **1** was slightly larger than that (3.6 Hz) in **2** probably due to the distortion of the cyclohexene (C-3 to C-8). The small coupling constant ($J$ = 2.6 Hz) indicated the equatorial orientation of H-9. The large coupling constant ($J$ = 11.6 Hz) showed a diaxial orientation of H-10 and H-11. The small coupling constant ($J$ = 2.0 Hz) of H-11/H-12 indicated the $\alpha$ orientation of Me-22. All these analyses enabled us to elucidate the structure of **1**, as shown in Figure 1.

**Table 1.** NMR data for debromooscillatoxin G (**1**) and debromooscillatoxin I **(3)** in acetone-$d_6$ (600 MHz for $^1$H and 150 MHz for $^{13}$C)

| | **1** | | **3** | |
|---|---|---|---|---|
| No. | $\delta_H$ multi ($J$ in Hz) | $\delta_C$ type | $\delta_H$ multi ($J$ in Hz) | $\delta_C$ type |
| 1 | - | 165.5, C | - | 167.8, C |
| 2 | 5.73 s | 104.0, CH | - | 130.3, C |
| 3 | - | 164.5, C | - | 197.9, C |
| 4 | 4.01 m | 28.9, CH | 2.68 m | 38.2, CH |
| 5a | 1.62 dd (2.7, 14.0) | 42.1, $CH_2$ | 1.76 dd (13.8, 13.8) | 46.4, $CH_2$ |
| 5b | 1.75 dd (5.8, 14.2) | | 1.89 dd (4.8, 13.5) | |
| 6 | - | 34.3, C | - | 36.5, C |
| 7 | - | 166.8, C | - | 165.0, C |
| 8 | - | 106.0, C | 6.19 d (16.0) | 126.2, CH |
| 9 | 3.87 d (2.6) | 74.6, CH | 6.07 dd (8.6, 16.1) | 143.2, CH |
| 10 | 1.82 m | 34.4, CH | 2.48 m | 42.0, CH |
| 11 | 4.04 dd (2.0, 11.6) | 77.3, CH | 3.24 m | 78.5, CH |
| 12 | 1.79 m | 33.1, CH | 1.56 m | 36.9, CH |
| 13a | 1.54 m | 30.4, $CH_2$ | 1.38 m | 30.3, $CH_2$ |
| 13b | 1.61 m | | 1.47 m | |
| 14a | 1.69 m | 36.0, $CH_2$ | 1.62 m | 36.5, $CH_2$ |
| 14b | 1.69 m | | 1.79 m | |
| 15 | 4.08 dd (5.7, 7.3) | 84.0, CH | 4.08 dd (5.2, 7.5) | 84.9, CH |
| 16 | - | 144.5, C | - | 145.7, C |
| 17 | 6.78 d (7.5) | 117.3, CH | 6.80 d (7.5) | 118.8, CH |
| 18 | 7.17 dd (7.8, 7.8) | 129.2, CH | 7.20 dd (7.7, 7.7) | 130.3, CH |
| 19 | 6.75 d (8.1) | 114.3, CH | 6.77 d (7.9) | 115.2, CH |
| 20 | - | 157.6, C | - | 158.6, C |
| 21 | 6.82 s | 113.3, CH | 6.83 s | 114.3, CH |
| 22 | 0.85 d (6.7) | 11.9, Me | 0.94 d (6.7) | 14.8, Me |
| 23 | 1.05 d (6.7) | 12.7, Me | 1.05 d (6.8) | 18.3, Me |
| 24 | 1.26 s | 27.4, Me | 1.21 s | 29.3, Me |
| 25 | 1.06 s | 30.3, Me | 1.33 s | 25.6, Me |
| 26 | 1.19 d (7.3) | 21.5, Me | 1.10 d (6.6) | 15.2, Me |
| 27 | - | 174.3, C | - | 175.0, C |
| 28a | 2.49 dd (1.1, 17.9) | 36.3, $CH_2$ | 2.65 d (18.0) | 36.9, $CH_2$ |
| 28b | 3.09 dd (6.1, 18.0) | | 3.07 dd (6.0, 18.0) | |
| 29 | 5.52 m | 70.2, CH | 5.55 m | 73.5, CH |
| 30 | 4.83 m | 78.9, CH | 4.84 dd (4.0, 6.6) | 79.7, CH |
| 31 | 1.32 d (6.5) | 13.5, Me | 1.44 d (6.6) | 14.9, Me |
| 32 | 3.18 s | 55.7, Me | 3.19 s | 56.8, Me |

| | | | | |
|---|---|---|---|---|
| 33 | 3.45 s | 57.5, Me | - | - |
| 20-OH | 8.28 s | - | 8.28 s | - |
| 11-OH | - | - | 3.48 d (5.7) | - |

The molecular formula of **3** determined by HR ESI-MS was $C_{32}H_{44}O_8$ ($[M+H]^+$ *m*/*z* 557.3096, calcd for $C_{32}H_{45}O_8$ *m*/*z* 557.3108), indicating that **3** was also a debromo analog of oscillatoxins. The $^1H$ NMR spectrum of **3** resembled that of oscillatoxin I (**4**, Figure 1).[12] The $^{13}C$ NMR and HSQC spectra showed seven methyls, four methylenes, three methines possessing a methyl, four oxymethines, two olefinic methines, four aromatic methines in the phenol side-chain, and eight quaternary carbons. The $^1H$-$^1H$ COSY revealed four partial structures, from $H_3$-26 to $H_2$-5, H-8 to H-15, H-17 to H-19, and $H_2$-28 to Me-31. HMBC correlations from both $H_3$-24 and $H_3$-25 to C-5 and C-7, from H-8 to C-2, and from H-9 to C-7 confirmed a cyclohexenone structure. The HMBC correlations $H_3$-32/C-15, H-15/C-17, and C-21, and the proton chemical shift at $\delta_H$ 4.08 of H-15 confirmed the existence of the phenol side-chain. The $^1H$ and $^{13}C$ chemical shifts (Table 1), and the proton coupling constants from C-1 to CH-12 and C-27 to Me-31 suggested that the relative stereostructure of **3** was identical to that of **4** (see Table S-1 in the Supplementary Information).[12] The large coupling constant (13.8 Hz) for H-4/H-5a and the NOE correlation H-4/ $H_3$-25 indicated that H-4 was axially oriented as in **4**.[12] The observed NOE correlations $H_3$-23/H-11 and H-11/H-12 confirmed the stereostructure from C-10 to C-12. Although the correlation of the stereostructure at C-4, C-10, and C-29 could not be elucidated, the comparison of NMR spectra with those of **4** led to confirm the relative stereostructure of **3**. The *E* configuration of $\Delta^8$ was deduced from the large proton coupling constant (16 Hz) of H-8/H-9. Therefore, the structure of **3** was determined, as shown in Figure 1.

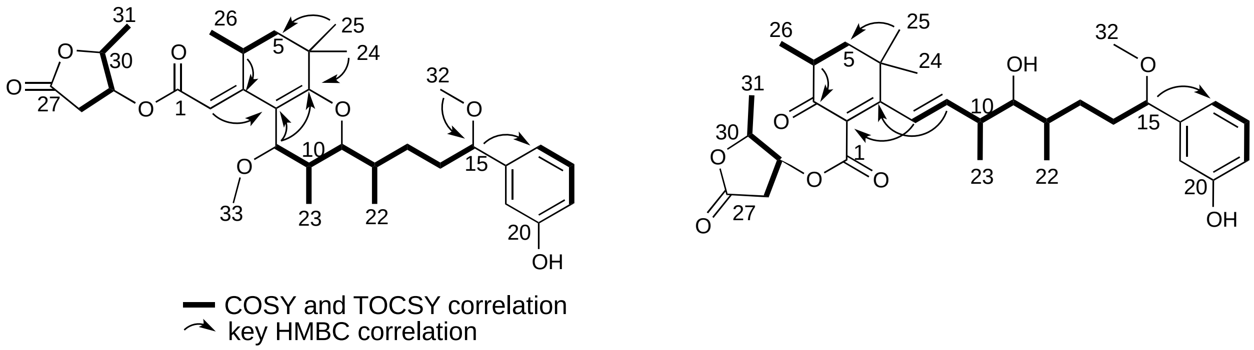


**Figure 2.** COSY, TOCSY, and HMBC correlations of **1** and **3**

## BIOLOGICAL ACTIVITIES

Debromooscillatoxins G (**1**) and I (**3**) showed mild cytotoxicity against mouse L1210 leukemia cells and diatom growth inhibition activity against the marine diatom *Nitzschia amabilis* at a concentration of 10 μg/mL (Table 2). The cytotoxicity of debromooscillatoxin G (**1**) was stronger than that of oscillatoxin G (**2**).[11] The cytotoxicity and diatom growth inhibition activity of debromooscillatoxin I (**3**) were weaker than those of oscillatoxin I (**4**).[12]

**Table 2.** Inhibition rates on cytotoxicity and diatom growth inhibition assays

| Compound | Cytotoxicity (%) | Diatom growth inhibition (%) |
|---|---|---|
| **1** | 40 | 30 |
| **3** | 30 | 50 |

L1210 mouse lymphoma cells were used for cytotoxicity assay. A marine diatom *Nitzschia amabilis* was used for diatom growth inhibition assay. The values indicate inhibition rates at a sample concentration of 10 μg/mL.

## ACKNOWLEDGEMENTS

This work was partly supported by JSPS KAKENHI Grant Number 19K06220 (H.N.) and 19H02720 (M.S.) from the Ministry of Education, Culture, Sport, Science, and Technology, Japan.

## REFERENCES AND NOTES

1. J. S. Mynderse, R. E. Moore, M. Kashiwagi, and T. R. Norton, *Science*, 1977, **196**, 538.
2. R. E. Moore, A. J. Blackman, C. E. Cheuk, J. S. Mynderse, G. K. Matsumoto, J. Clardy, R. W. Woodard, and J. C. Craig, *J. Org. Chem.*, 1984, **49**, 2484.
3. M. Entzeroth, A. J. Blackman, J. S. Mynderse, and R. E. Moore, *J. Org. Chem.*, 1985, **50**, 1255.
4. H. Nagai, T. Yasumoto, and Y. Hokama, *J. Nat. Prod.*, 1997, **60**, 925.
5. H. Nagai, Y. Kan, T. Fujita, B. Sakamoto, and Y. Hokama, *Biosci. Biotech. Biochem.*, 1998, **62**, 1011.
6. G. E. Chlipala, P. H. Tri, N. V. Hung, A. Krunic, S. H. Shim, D. D. Soejarto, and J. Orjala, *J. Nat. Prod.*, 2010, **73**, 784.
7. D. K. Gupta, P. Kaur, S. T. Leong, L. T. Tan, M. R. Prinsep, and J. J. H. Chu, *Mar. Drugs*, 2014, **12**, 115.
8. B. N. Han, T. T. Liang, L. J. Keen, T. T. Fan, X. D. Zhang, L. Xu, Q. Zhao, S. P. Wang, and H. W. Lin, *Org. Lett.*, 2018, **20**, 578.
9. Y. H. Tang, J. Wu, T. T. Fan, H. H. Zhang, X. X. Gong, Z. Y. Cao, J. Zhang, H. W. Lin, and B. N. Han, *RSC Adv.*, 2019, **9**, 7594.
10. T. T. Fan, H. H. Zhang, Y. H. Tang, F. Z. Zhang, and B. N. Han, *Mar. Drugs*, 2019, **17**, 652.

11. H. Nagai, M. Watanabe, S. Sato, M. Kawaguchi, Y. Y. Xiao, K. Hayashi, R. Watanabe, H. Uchida, and M. Satake, *Tetrahedron*, 2019, **75**, 2486.

12. H. Nagai, S. Sato, K. Iida, K. Hayashi, M. Kawaguchi, H. Uchida, and M. Satake, *Toxins*, 2019, **11**, 366.

13. M. Kawaguchi, M. Satake, B. T. Zhang, Y. Y. Xiao, M. Fukuoka, H. Uchida, and H. Nagai, *Molecules*, 2020, **25**, 457.

14. Y. H. Tang, T. T. Liang, T. T. Fan, L. J. Keen, X. D. Zhang, L. Xu, Q. Zhao, R. Zeng, and B. N. Han, *Nat. Prod. Res.*, 2020, **34**, 2151.

15. H. H. Zhang, X. K. Zhang, R. R. Si, S. C. Shen, T. T. Liang, T. T. Fan, W. Chen, L. H. Xu, and B. N. Han, *Toxins*, 2020, **12**, 733.

16. H. Fujiki, M. Suganuma, M. Nakayasu, H. Hoshino, R. E. Moore, and T. Sugimura, *Gann*, 1982, **73**, 495.

17. H. Fujiki, Y. Tanaka, R. Miyake, U. Kikkawa, Y. Nishizuka, and T. Sugimura, *Biochem. Biophys. Res. Commun.*, 1984, **120**, 339.

18. M. Suganuma, H. Fujiki, T. Tahira, C. Cheuk, R. E. Moore, and T. Sugimura, *Carcinogenesis*, 1984, **5**, 315.

19. J. P. Arcoleo and I. B. Weinstein, *Carcinogenesis*, 1985, **6**, 213.

20. H. Nakamura, Y. Kishi, M. A. Pajares, and R. R. Rando, *Proc. Natl. Acad. Sci. U.S.A.*, 1989, **86**, 9672.

21. Y. Nakagawa, R. C. Yanagita, N. Hamada, A. Murakami, H. Takahashi, N. Saito, H. Nagai, and K. Irie, *J. Am. Chem. Soc.*, 2009, **131**, 7573.

22. K. Irie and R. C. Yanagita, *Chem. Rec.*, 2014, **14**, 251.

23. T. Kobayashi, R. C. Yanagita, and K. Irie, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127657.

24. **General Experimental Procedures.**

Optical rotation was measured on a JASCO P-2100 polarimeter using a cell of 10 mm length. UV spectra were measured on a JASCO V-550 UV-spectrometer. NMR spectra were recorded using a Bruker AVANCE III 600 spectrometer ($\delta_H$ 2.05 and $\delta_C$ 206.26 for acetone-$d_6$). HRESI-MS spectral data were recorded using a Bruker micrOTOF QII mass spectrometer. HPLC was performed using a Hitachi Chromaster HPLC system. The bioassay results were obtained using a Bio-Rad Model 550 microplate reader.

**Marine Cyanobacterium.**

Samples of the marine cyanobacterium *Moorea producens* were collected from Kuba Beach, Nakagusuku, Okinawa, Japan, in July 2010. After freeze-drying, the samples were stored at –30 °C until the experiments were performed. The sample was identified via morphological observation under a microscope by Dr. Masayuki Fukuoka of Tokyo University of Marine Science and Technology.

**Extraction and Isolation.**

A frozen sample of the cyanobacterium *M. producens* (wet weight: 10.1 kg) was soaked for several days in EtOH at room temperature. After filtering the ethanol extract, it was extracted five times with MeOH and once with acetone. The extracts were then combined and concentrated in vacuo to yield a residue (37.8 g), which was partitioned between MeOH/water (4:1, v/v) and hexane. The solvent of the 80% MeOH-soluble layer was then removed, and the remaining sample was again partitioned using distilled water and ethyl acetate (EtOAc). The EtOAc layer was then evaporated to dryness. The EtOAc layer was separated using an open glass column (PEGASIL ODS, Senshu Co., Tokyo, Japan) measuring 20 × 120 mm with stepwise elution in 50, 70, 90, and 100% MeOH. The 70% MeOH eluate was then purified by HPLC using a reversed-phase column (Cosmosil 5C18-AR-II, 10 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan). Finally, debromooscillatoxin G (**1**, 1.7 mg) and debromooscillatoxin I (**3**, 0.6 mg) were isolated.

**Cytotoxicity and Diatom Growth Inhibition Assay.**

The details of the biological assay performed in this experiment were previously reported.[25,26]

**Debromooscillatoxin G (1):** isolated as a colorless amorphous solid; $[\alpha]^{19}_{D}$ −28.0 (c 0.01, MeOH); UV (MeOH) $\lambda$ max 220 nm ($\varepsilon$ 11210), 306 nm ($\varepsilon$ 10140); HRESI-MS $[M+H]^+$ *m/z* 571.3288 (calcd for $C_{33}H_{47}O_8$ *m/z* 571.3265); $^1$H and $^{13}$C NMR data are given in Table 1.

**Debromooscillatoxin I (3):** isolated as a colorless amorphous solid; $[\alpha]^{26}_{D}$ +8.0 (c 0.005, MeOH); UV (MeOH) $\lambda$ max 215 nm ($\varepsilon$ 12110), 274 nm ($\varepsilon$ 5990); HRESI-MS $[M+H]^+$ *m/z* 557.3096 (calcd for $C_{32}H_{45}O_8$ *m/z* 557.3108); $^1$H and $^{13}$C NMR data are given in Table 1.

25. T. Kawabata, D. J. Lindsay, M. Kitamura, S. Konishi, J. Nishikawa, S. Nishida, M. Kamio, and H. Nagai, *Fish. Sci.*, 2013, **79**, 487.

26. W. Jiang, T. Akagi, H. Suzuki, A. Takimoto, and H. Nagai, *Comp. Biochem. Phys. C*, 2016, **185**, 13.