7-Epi-30-methyloscillatoxin D from an Okinawan cyanobacterium Okeania hirsuta

Original Research Paper

7-*Epi*-30-Methyloscillatoxin D From an Okinawan Cyanobacterium *Okeania hirsuta*

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Abstract

Some aplysiatoxin-related compounds have been isolated from an Okinawan cyanobacterium *Okeania hirsuta*. The structure of a natural product isolated as 30-methyloscillatoxin D (**1a**) from this cyanobacterium in our previous report was re-investigated and revised to be a 7-epimer of 30-methyloscillatoxin D (**4a**) using precise NMR analysis. The synthesis of *O*-Me-7-*epi*-30-methyloscillatoxin D (**4b**) led to confirmation of the stereochemistry of **4a**. This is the first report of a 7-*epi*-type aplysiatoxin-related compound from a natural source.

Keywords

cyanobacteria, aplysiatoxin, oscillatoxin, polyketide, cytotoxicity

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Introduction

Moore et al, in 1985, isolated 30-methyloscillatoxin D (**1a**) for the first time along with debromoaplysiatoxin (**3**) from a mixture of marine cyanobacteria *Schizothrix calcicola* and *Oscillatoria nigroviridis*.[1] In July 2010, an outbreak of the marine cyanobacterium *Okeania hirsuta* (formerly reported as *Moorea producens*, see Supplemental Material) occurred in Okinawa, Japan. The sample used in this study was collected at that time and at that site. In 2019, we reported the re-isolation of 30-methyloscillatoxin D (**1a**) from this cyanobacterium.[2] Recently Nishikawa et al reported the total synthesis of 30-methyloscillatoxin D (**1a**) and *O*-Me-30-methyloscillatoxin D (**1b**).[3,4] Comparison of the ^{1}H NMR data of the synthesized **1a**[3] with our reported **1a**[2] showed a significant difference. Thus, the structure of the compound which was formerly reported by us as **1a**[2] was re-investigated and revised to be 7-*epi*-30-methyloscillatoxin D (**4a**). Finally, the structure of **4a** was confirmed by comparison of ^{1}H and ^{13}C NMR data of the natural compound **4a** and synthesized compound **4b**. This is the first report of the isolation of a 7-*epi*-type aplysiatoxin-related compound from a natural source. In this study, we also isolated **1a** itself and 17-bromo-30-methyl-oscillatoxin D (**2**) from the same cyanobacterium sample.

Results and Discussion

7-*Epi*-30-methyloscillatoxin D (**4a**) was isolated as a white solid ($[\alpha]_D^{17}$ + 31.4 (*c* 0.7, MeOH)). The UV maxima observed at 199 nm (ϵ = 13376), 215 nm (ϵ = 6259), and 275 nm (ϵ = 2021) indicated the presence of an aromatic group. To determine the molecular formula of $C_{32}H_{44}O_8$, high-resolution electrospray ionization (ESI) mass spectrometric analysis was used (Supplemental Figure S1) ($[M + H]^+$ at m/z 557.3064, calcd. for $C_{32}H_{45}O_8$, 557.3109), and the results agreed with those of **1a**. However, the proton chemical shifts from H-2 to H-12 of this compound differed from **1a** (Table 1). The ^{1}H NMR (Supplemental Figure S2), ^{13}C NMR (Supplemental Figure S3), and HSQC (Supplemental Figure S4) spectra of **4a** showed seven methyl groups (two singlets, four doublets, and a methoxy), four methylenes, four aliphatic methines, four oxygenated methines, two olefinic methines, four aromatic protons, and seven quaternary carbons (one aliphatic, one oxygenated, two

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Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data (in Acetone-d_6) for 7-*epi*-30-Methyloscillatoxin D (**4a**), *O*-Me-7-*epi*-30-Methylocsillatoxin D (**4b**), and 30-Methyloscillatoxin D (**1a**) (δ, ppm; J, Hz).

	δ_H (*J* in Hz)			δ_C		
No	**4a**[(1)] (600 MHz)	**4b** (400 MHz)	**1a** (600 MHz)	**4a**[(1)] (150 MHz)	**4b** (100 MHz)	**1a** (150 MHz)
1				166.7	166.7	169.2
2	3.96 s	3.96, s	4.01 s	62.6	62.7	65.2
3				205.3	205.3	205.7
4	2.65 m	2.65, m	2.81 m	41.6	41.6	41.4
5a	1.54 dd (13.1, 6.5)	1.51, dd (13.0, 6.5)	1.37 m	45.9	46.0	44.0
5b	1.76 m	1.74, t (13.0)	1.72 m			
6				41.5	41.5	41.3
7				84.3	84.3	84.7
8	5.81 s	5.81, s	5.77 d (10.5)	137.4[(2)]	137.4[(3)]	134.6
9	5.81 s	5.81, s	5.51 dd (3.0, 10.8)	125.1[(2)]	125.1[(3)]	126.9
10	2.36 m	2.31-2.42, m	2.15 m	30.5	30.4	31.0
11	3.28 dd (9.5, 1.7)	3.27, dd (9.5, 1.5)	3.15 m	81.1	81.2	79.0
12	1.64 m	1.58–1.67, m	1.72 m	35.5	35.6	34.3
13a	1.36 m	1.36, m	1.39 m	31.8	31.8	31.6
13b	1.36 m	1.36, m	1.48 m			
14a	1.61 m	1.58–1.67, m	1.55 m	37.4	37.5	36.8
14b	1.78 m	1.79, m	1.72 m			
15	4.02 dd (6.6, 6.6)	4.08, t (6.5)	4.02 dd (6.6, 6.6)	85.2	85.2	84.7
16				145.6	145.6	145.6
17	6.74 m	6.80–6.90, m	6.79 br.d (7.4)	118.9	120.0	118.8
18	7.15 dd (7.5, 7.8)	7.25, t (8.0)	7.17 dd (7.4, 7.8)	130.2	130.3	130.1
19	6.73 m	6.80–6.90, m	6.74 br.d (8.4)	115.3	113.7	115.1
20				158.6	161.0	158.4
21	6.78 dd (1.9, 1.9)	6.80–6.90, m	6.83 m	114.3	113.1	114.3
22	0.86 d (6.9)	0.86, d (7.0)	0.89 d (7.2)	13.7	13.7	13.3
23	0.84 d (7.3)	0.84, d (7.0)	0.88 d (7.2)	17.2	17.3	16.9
24	0.95 s	0.93, s	0.92 s	27.2	27.3	25.1
25	1.32 s	1.32, s	1.31 s	24.7	24.8	22.6
26	0.93 d (6.4)	0.92, d (6.5)	0.99 d (6.2)	14.9	14.8	14.5
27				175.1	175.1	174.8
28a	2.38 d (18.1)	2.38. brd (18.0)	2.53 d (17.4)	37.6	37.6	37.4
28b	3.02 dd (18.0, 5.9)	3.01, dd (18.0, 6.0)	3.02 dd (18.0, 5.9)			
29	5.41 m	5.41, dd (5.0, 4.5)	5.37 m	72.0	72.1	73.6
30	4.75 m	4.75, qd (6.5, 4.0)	4.80 m	79.4	79.5	79.4
31	1.27 d (6.5)	1.27, d (6.5)	1.41 d (6.4)	14.9	15.0	14.9
32	3.15 s	3.16, s	3.15 s	56.7	56.8	56.6
20-OH	8.22 s		8.25			

(1) By re-assignment in this study, the chemical shifts of **4a** were slightly revised from formerly reported values (**4a** had been incorrectly assigned as 30-methyloscillatoxin D at that time).[2]
(2), (3) ^{13}C chemical shifts were interchangeable.

aromatics in a phenol moiety, two esters, and a ketone). The proton connectivities of H-4 (H_3-26) to H_2-5, H-10 to H-15, H-17 to H-19, and H_2-28 to H_3-31 were assigned using ^{1}H-^{1}H COSY (Supplemental Figure S5). The proton signals at H-8 and H-9 measured in acetone-d_6 had the same chemical shifts and were observed as a singlet peak due to second-order coupling. The partial structures were assembled using HMBC (Supplemental Figure S6) correlations from H-2 to C-1, C-3 and C-7, H_3-26 to C-3, H_3-24 to C-7 and C-8, H_3-25 to C-7, and H-15 to C-16. The position of γ-lactone was deduced from the proton chemical shift at H-29 (δ_H 5.41). The planar structure of **4a** was the same as that of **1a,** suggesting that **4a** is a stereoisomer of **1a**.

The stereostructure around C-7 in **4a** was deduced from the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments. The rotating-frame Overhauser effect (ROE) correlations of H-2/H-4, H-2/H_3-25, and H-4/H_3-25 indicated that H-2 (δ_H 3.96), H-4 (δ_H 2.65), and H_3-25 (δ_H 1.32) were oriented in the β position. The ROE correlations, H-8 (δ_H 5.81)/H-2 and H-8/H_3-25, show that C-7 has an *R*-configuration (Figure 1). Therefore, **4a** was deduced to be a C-7 epimer of **1a**. The large coupling constant (9.5 Hz) between H-10 (δ_H 2.36) and H-11 (δ_H 3.28) and the ROE correlation H-11/H_3-23 (δ_H 0.84) indicates the anti-position of H-10/H-11. The observed small coupling constant (1.7 Hz) of H-11/H-12 (δ_H 1.64) was typical for oscillatoxins, probable

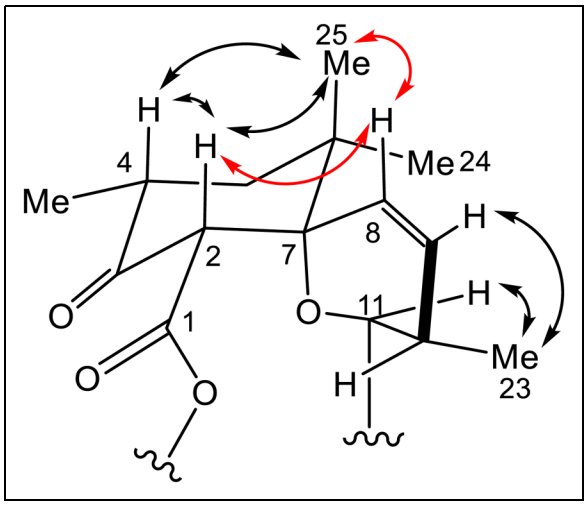

Figure 1. Rotating-frame Overhauser effect (ROE) correlations of compound **4a**.

due to the gauche conformation of H-11/H-12. Therefore, the configuration at C-12 was suggested to be *S*. The configurations of C-15, C-29, and C-30 were deduced from the proton chemical shifts and coupling constants. Therefore, the configuration, except for C-7 in **4a**, was the same as that of **1a**. These observations elucidate the structure of **4a** (Figure 2).

The stereochemical assignment (Table 1) was confirmed by comparison of ^{1}H and ^{13}C NMR data of **4a** and **4b**. Compound **4b** was synthesized from a stereochemically-defined intermediate **7** (Figure 3),[4] which was previously obtained as a byproduct in the synthesis of *O*-Me-30-methyloscillatoxin D (**1b**).[4] Transesterification of **7** with lactone **8**[5] was carried out in the presence of DMAP under toluene reflux conditions to provide **4b** in 27% yield. The ^{1}H and ^{13}C NMR spectra around C-7 of the natural compound **4a** are in good agreement with those of the synthetic compound **4b**, except for the aromatic moiety (Table 1).

The isolation of **1a** and **4a** from the same sample (Supplemental Figures S11 and S12) confirms that **4a** was not

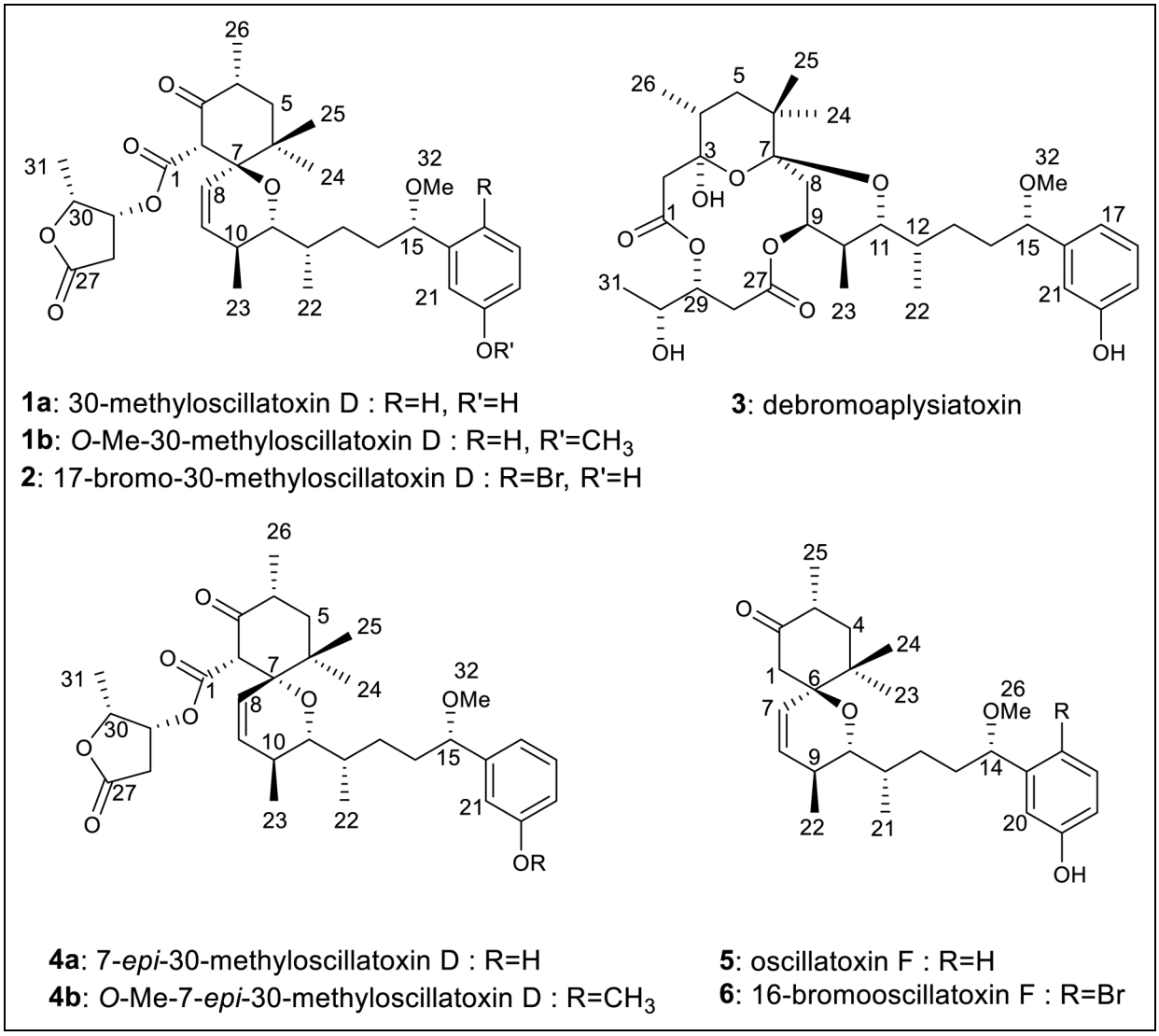

Figure 2. Structures of compounds **1a** to **6**.

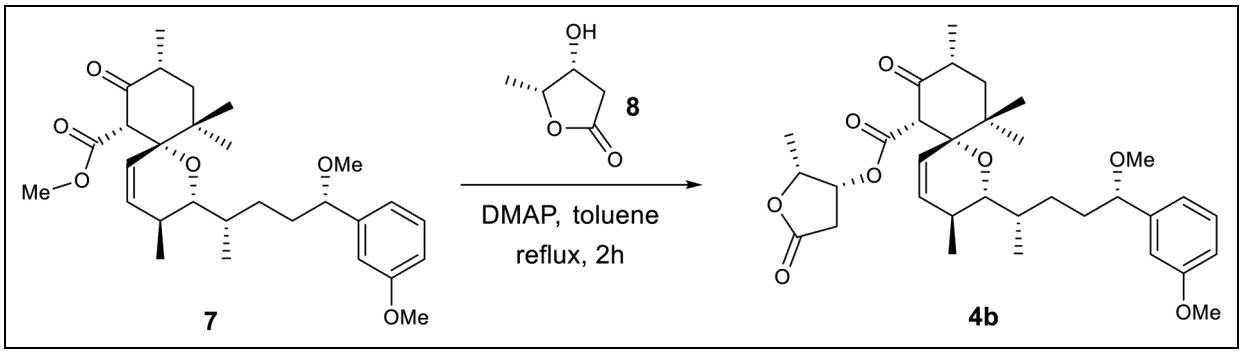

Figure 3. Synthesis of **4b** from **7**.

an artifact produced during isolation. In our previous report, we mistakenly described the cytotoxicity of **4a** as 90% at the tested dose (10 μg/mL).[2] However, after re-examination, the cytotoxicity of **4a** was corrected to 25% inhibition at the tested dose (10 μg/mL). Unfortunately, we could not examine the cytotoxicity of the newly isolated **1a** because of its instability. Isolated **1a** and **2** were rapidly converted to oscillatoxin F (**5**)[6] and 16-bromooscillatoxin F (**6**) (see Supplemental Material) within a month under dry, dark, and refrigerated conditions. Compound **6** has not been obtained as a natural product, so far. Interestingly, **4a** was stable for over two years under the same conditions.

Previously, the cyanobacterium sample used in this study had been identified as *Moorea producens* by morphological observations using microscopy.[7] However, this cyanobacterium was found in this study to be *Okeania hirsuta* by re-identification using gene analysis methods (see Supplemental Material). This *O hirsuta* sample strain (20100713-a) is a chemically rich species. Over 40 compounds have been isolated from this *O hirsuta* sample, of which more than 20 were new compounds.[2,7–12]

Conclusion

In this study, 30-methyloscillatoxin D (**1a**), 17-bromo-30-methyloscillatoxin D (**2**), and 7-*epi*-30-methylocsillatoxin D (**4a**) were isolated and identified from the cyanobacterium *O hirsuta* sample strain (20100713-a). The synthesis of *O*-Me-7-*epi*-30-methyloscillatoxin D (**4b**) confirmed the stereochemistry of 7-*epi*-30-methylocsillatoxin D (**4a**). Compound **4a** is the first report of a 7-*epi*-type aplysiatoxin-related compound from a natural source.

Experimental

General

Optical rotations were measured using either a JASCO P-2100 or a JASCO DIP-370 (JASCO Co., Tokyo) using a 10 mm length cell. Infrared spectra (IR) were recorded on a JASCO FT/IR-4100 (JASCO Co., Tokyo). NMR spectra were recorded in acetone-d_6 using either a Bruker AVANCE III 600 spectrometer or a Bruker AVANCE-400 (400 MHz; Bruker Co., Billerica) spectrometer. UV spectra were measured using a HITACHI U-3000 spectrometer (Hitachi High-Tech Science Co., Tokyo). HPLC was performed using a Hitachi Chromaster HPLC System (Hitachi High-Tech Science Co., Tokyo). HR-ESI-MS spectral data were determined using either a Bruker micrOTOF QII (Bruker Co., Bremen) mass spectrometer or an Agilent 6220 Accurate-Mass TOF (Agilent Technologies, Santa Clara). Bioassay results were recorded on a Model 550 microplate reader (Bio-Rad, California).

Biological Material

Samples of the marine cyanobacterium *Okeania hirsuta* (20100713-a) were collected from Kuba Beach, Nakagusuku, Okinawa, Japan in July 2010. *O hirsuta* was a dominant cyanobacterial species in the sample. The sample also contained some unidentified diatoms. The cyanobacterium was identified by 16S rRNA sequence analysis and morphological and chemotaxonomic observations (see Supplemental Material). This biological material had previously been incorrectly identified as *Moorea producens* by morphological observations using microscopy.[2,7–12]

Extraction and Isolation

Extraction and purification of the frozen *O hirsuta* sample (wet weight: 9.7 kg) was performed in the same manner as described in a previous paper.[2] The EtOAc layer was evaporated to dryness. The EtOAc layer was fractionated using an open glass column measuring 40 × 400 mm and packed with ODS resin (Cosmosil 75C18-OPN, Nacalai Tesque Inc., Kyoto) with stepwise elution with 50%, 70%, 90%, and 100% methanol. The 70% methanol eluate was then purified via HPLC using a reversed-phase column (Cosmosil 5C18-AR-II, 10 × 250 mm, Nakalai Tesque Inc., Kyoto). 7-*Epi*-30-methyloscillatoxin D (**4a**, 0.9 mg) had been already isolated and incorrectly identified as 30-methyloscillatoxin D in a former paper.[2] Finally,

30-methyloscillatoxin D (**1a**, 1.7 mg, see Supplemental Material), and 17-bromo-30-methyloscillatoxin D (**2**, 1.3 mg, see Supplemental Material) were also isolated.

Synthesis of O-*Me-7*-epi-*30-Methyloscillatoxin D (**4b**)*

To a solution of spiro ether **7** (14.0 mg, 28.8 μmol) and lactone **8** (14.4 mg, 0.115 mmol) in toluene (1.5 mL) was added DMAP (3.4 mg, 28 μmol). Then, the solution was refluxed for 2 h and diluted with saturated NH_4Cl solution (10 mL) after cooling to room temperature. The mixture was extracted with EtOAc (10 mL × 3). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc/hexane = 1/10 to 1/1) to afford **4b** (4.5 mg, 27%) as a colorless oil. $[\alpha]_D^{23}$ + 97 (*c* 0.23, $CHCl_3$). IR (film): ν_{max} (cm^{-1}) 2965, 2933, 1786, 1767, 1716, 1487, 1457, 1261, 1164, 1120, and 1055. HR-MS (ESI, positive): calcd. For $C_{33}H_{46}NaO_8$ $[M + Na]^+$, 593.3085; Found 593.3078. ^1H and ^{13}C NMR chemical shifts of **4b** are shown in Table 1.

Biological Test

Cytotoxicity assays against mouse L1210 leukemia cells were carried out for **4a**. Bioactive assays were performed using the XTT colorimetric reaction method, as previously reported.[13]

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

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Informed Consent

Not applicable.

Statement of Human and Animal Rights

Not applicable.

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Supplemental Material

Supplemental material for this article is available online.

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