Neo-debromoaplysiatoxin C, with new structural rearrangement, derived from debromoaplysiatoxin

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ABSTRACT

Neo-debromoaplysiatoxin C (1), a new member of the aplysiatoxin family, was isolated from the marine cyanobacterium \textit{Lyngbya} sp. The structure of 1 was elucidated based on spectroscopic data, and its stereochemistry was determined from NOESY spectrum and biosynthetic considerations. This new compound presents an intriguing 10-membered lactone ring skeleton derived from debromoaplysiatoxin by structural rearrangement, which is the first example in the aplysiatoxin family. Its biological properties were evaluated for cytotoxicity, PKC\textsuperscript{\delta} activation and inhibitory effects on potassium channel.

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1. Introduction

Marine cyanobacteria, known as blue-green algae, are ancient prokaryotic microorganisms that have been able to yield biologically active and structurally diverse natural products owing to their extremely competitive living environment (Dixit and Suseela 2013). The \textit{Lyngbya} genus of cyanobacteria have become known as exceptional producers of such metabolites, which have helped the organisms to compete and thrive (Liu and Rein 2010). Among these metabolites, the aplysiatoxins (ATXs), have attracted researchers’ attentions. Several ATXs (Ashida et al. 2016) were found to be activators
of protein kinase C (PKC). Since PKC is involved in numerous signal transduction pathways, we can presume the toxins to be potential targets in the search for new treatments for intractable diseases, especially cancer (Teicher 2006). Since the first ATXs, aplysiatoxin and debromoaplysiatoxin (Kato and Scheuer 1974), were isolated from the sea hare Stylocheilus longicauda in 1974, approximately 27 naturally occurring aplysiatoxins (Kato and Scheuer 1974, 1975; Mynderse and Moore 1978; Moore et al. 1984; Entzeroth et al. 1985; Nagai et al. 1997; Chlipala et al. 2010; Gupta et al. 2014; Han et al. 2018) have been disclosed so far. Further chemical searching for ATXs analogues from marine cyanobacterium Lyngbya sp. collected off the coast of Hainan Island, China led to the isolation of neo-debromoaplysiatoxin C (1) (Figure 1). This new compound, containing a single 10-membered lactone ring, is the first example seen in the aplysiatoxin family. Herein, we elaborated the structure elucidation and plausible biosynthetic pathway of neo-debromoaplysiatoxin C (1).

2. Results and discussion

Neo-debromoaplysiatoxin C (1) was obtained as a white solid. The molecular formula of C_{32}H_{44}O_{9} with eleven degrees of unsaturation was deduced from HRESIMS data (m/z 595.2866 [M + Na]^{+}). The $^1$H NMR spectrum of 1 indicated the presence of four aromatic protons (δH 7.16, 6.79, 6.76 and 6.74), four singlet methyls (δH 3.18, 1.80, 1.13 and 1.01) and three doublet methyls (δH 1.37, 0.93 and 0.87). The $^{13}$C, DEPT and HSQC NMR spectra of 1 indicated the presence of 32 carbons, including one keto carbon (δC 205.2) and three carbonyl carbons (δC 175.6, 174.7 and 169.2), four sp$^2$ quaternary carbons (δC 156.4, 143.8, 138.5 and 134.2), one aliphatic quaternary carbon (δC 46.0), six methyl carbons, one oxygenated methyl carbon, six methylene carbons, two sp$^3$ and four sp$^2$ methine carbons (δC 129.4, 119.4, 114.9 and 113.5) and four oxygenated methine carbons (δC 84.3, 80.1, 79.1 and 72.0) (Table S1). Apart from these spectroscopic signatures, there were two degrees of unsaturation, means two rings in the structure of 1.

The COSY correlations, H$_2$-28/H-29/H-30/H$_3$-31, H-12/H-11/H-10/H$_3$-23 and H-10/H-9β, H$_2$-13/H$_2$-14/H-15 and H-17/H-18/H-19, established the four partial structures: C$_{28}$ – C$_{29}$ – C$_{30}$ – C$_{31}$, C$_{23}$ – C$_{10}$ – C$_{11}$ – C$_{12}$ and C$_{10}$ – C$_9$, C$_{13}$ – C$_{14}$ – C$_{15}$, and C$_{17}$ – C$_{18}$ – C$_{19}$ (Figure S1). HMBC cross-peaks from H$_3$-25 to C-1, C-2, C-3 and C-24, from H$_3$-26 to C-3, C-4, C-5, C-6, from H$_2$-6 to C-4, C-5, C-7 and C-8, from H$_2$-9 to C-8,
from H$_3$-23 to C-9, C-10 and C-11, and a key cross-peak between H-11 and C-1 undoubtedly established the 10-membered ring as well as the positions of the methyl CH$_3$-24 and CH$_3$-25 at the C-2, the methyl CH$_3$-26 at C-4, the methylene CH$_2$-6 at C-5 and CH$_2$-23 at C-10. The side chain (C12–C15, C-22 and C-32) at C-11 was confirmed by the correlations of H$_3$-22/C-11, C-12 and C-13, H$_2$-14/C-12, C-13 and C-15, and H-15/C-32. The $^1$H NMR spectra ($^\delta_H$ 7.16 (t, 7.7), 6.79 (m, 2.0), 6.76 (overlap) and 6.74 (overlap)) and $^{13}$C NMR spectra ($^\delta_C$ 156.4, 143.8, 129.4, 119.4, 114.9 and 113.5) revealed the molecule exhibited a 1, 3-disubstituted benzene ring, which was connected to the side chain at C-15 supported by the HMBC correlations from H-15 to C-17 and C-21 (Figure S1). Moreover, HMBC correlations of H$_2$-6/C-4, C-5, C-7 and C-8 uncovered that carbonyl carbon C-7 was connected to the 10-membered ring via C-6. Further, the $\gamma$-lactone ring was determined by consecutive $^1$H–$^1$H COSY correlations of H$_2$-28/H-29/ H-30/H$_3$-31 combined with the HMBC correlations from H$_2$-28 to C-27($^\delta_C$ 174.7), where C-27 and C-30 ($^\delta_C$ 79.1) were possibly connected by an ester bond on basis of the $^1$H and $^{13}$C NMR shift data as well as the last unsaturation of this molecule. The $\gamma$-lactone ring was connected to C-7 through an ester linkage with C-7 ($^\delta_C$ 169.2) and C-29($^\delta_C$ 72.0) by the HMBC correlation from H-29 to C-7. Accordingly, the planar structure of neo-debromoaplysiatoxin C was assigned (Figure S1).

The NOESY experiment and vicinal coupling constants were utilized to speculate on the relative configuration of 1. In the 10-membered ring units, the Z-configuration of $\Delta^{4,5}$ double bond was supported by the NOESY correlation between H$_2$-6 and H$_3$-26. The anti relationship of H-10/H-11 and the gauche relationship of H-12/H-11 were established from the coupling constants of H-11 ($J = 10.8, 1.0$ Hz), the presence of a NOESY correlation of H-11/H-12 and the absence of a NOESY correlation between H-10 and H-11, which indicated $J_{H_{10}, H_{11}} = 10.8$ Hz and $J_{H_{11}, H_{12}} = 1.0$ Hz. The anti relationship of H-9$\beta$ and H-10 was speculated from the large coupling constant of H-9$\beta$ ($J = 15.0, 11.2$ Hz) (Table S1). The NOESY cross-peaks of H-9$\beta$/H-11/H$_3$-23 indicated these protons were coficially oriented and assigned as $\beta$-orientation. The NOESY correlations from H-10 to H-9$\alpha$ positioned these protons in the $\alpha$-orientation, the NOESY correlations of H-11/H$_2$/H$_3$-23, H-10/H$_3$-22 and the small coupling constant between H-12 and H-11($J_{H_{11}, H_{12}} = 1.0$ Hz) established the relative stereochemistry of C-12(Figure S1). Thus, the relative stereochemistry of C-10, 11, 12 was established as $10S^*, 11R^*, 12S^*$ (Newman projections analysis for C11/C12 and C10/C11 in Figure S13). The coupling constants of H and the chemical shifts of C and H at $\gamma$-lactone ring in 1 were in accordance with those of 30-methylosciellatoxin D (Entzeroth et al.1985) indicating the same relative configuration of 1 and the known ATXs at C-29 and C-30, which established a relative stereochemistry of 29R* and 30R* (Xianjin et al. 2018). Neo-debromoaplysiatoxin C and neo-debromoaplysiatoxin A-B (Han et al. 2018) were obtained from the same specimen. From their structural similarities and hypothetical biosynthesis (Scheme 1 and Han et al. 2018), it is likely that these three compounds have a common biosynthetic origin and derived from debromoaplysiatoxin, thus the stereochemistry of C-15 remained unchanged and was assigned as S. Accordingly, the stereochemistry of neo-debromoaplysiatoxin C was assigned as $10S^*, 11R^*, 12S^*, 15S, 29R^*, 30R^*$ or $10S^*, 11R^*, 12S^*, 15S, 29S^*, 30S^*$. 
The cytotoxicities of neo-debromoaplysiatoxin C (1) against PC-9 and HepG2 cell lines were investigated, and the compound did not show apparent cytotoxicity at 10 μM within 48 h. Subsequently, we evaluated 1 for interactions with PKC and effects on voltage-gated potassium channels as previously reported (Han et al. 2018). The results indicated no significant effect on any of these assays, which is possibly due to the structural frame of this compound differing wildly from the original group of ATXs as it has been noted that the structure of debromoaplysiatoxin contains a recognition domain (Ashida et al. 2016) as a vital role in intermolecular hydrogen bonding with the PKCδ C1B domain. Through a sequence of reactions (Scheme 1) such as alcoholy-sis, oxidation and nucleophilic reaction, debromoaplysiatoxin was converted into compound 1, which no longer contains the PKC activation recognition domain.

3. Experimental

3.1. General experimental procedures

Optical rotation was obtained with a Jasco P-2000 polarimeter. UV spectra and infrared spectra measurements were conducted on a Thermo UV/EV300 spectrometer and a Thermo Scientific Nicolet 6700 instrument respectively. NMR spectra were measured on Agilent 600 MHz spectrometer with CDCl3 (δH 7.26 and δC 77.16) as the solvent and internal standard. HRESIMS data were collected with a Waters Q-Tof micro YA019 mass spectrometer. Silica gel 60 (200–300 mesh; Yantai) and ODS (50 μm, YMC Co.) were used for column chromatography. TLC was carried on silica gel 60 F254 plates. A YMC-Pack Pro C18 column (250 × 10 mm, 5 μm) and a Waters 1525 series HPLC pump with a Waters 2998 photodiode array detector were used in the Reversed-phase HPLC experiment.

3.2. Plant material

The cyanobacterium *Lyngbya* sp. was gathered off the harbor of Sanya Hainan, China, in November 2016. The sample was identified by Prof. Bing-Nan Han (Zhejiang Sci-Tech University). Then the specimen was transported to the laboratory after frozen. Morphological and molecular identification of cyanobacterium have been reported in detail in our previous paper (Han et al. 2018). A voucher specimen (voucher number: BNH-201606; genebank accession numbers: MH636576) has been well preserved in Zhejiang Sci-Tech University.

3.3. Extraction and isolation

Thawed cyanobacterium *Lyngbya* sp. (800 g, wet weight) was extracted with MeOH/CH2Cl2 (2:1, v/v) to obtain crude extract. The extract was dissolved in 1 L MeOH – H2O (9:1, v/v) and extracted with 1L CH2Cl2 three times. The CH2Cl2 fraction (20 g) was divided into seven fractions (F.A – G) on VLC using PE/EtOAc (5:1, 2:1, 1:1, 1:2, 1:5, 0:1, v/v). Subsequently, Fraction F.D (800 mg) was separated by ODS (10–100% MeCN/H2O, 180 min, flow rate 20 mL/min,) to yield twenty-one fractions (F.D.1–21). RP HPLC
(Waters SunFire Prep C18, 42% MeCN/H$_2$O, 8.0 mL/min, UV detection at 190 nm) was used to purify the thirteenth fraction (F.D.13) to afford 1 (6.0 mg, $t_R = 58$ min).

### 3.4. Spectroscopic data of neo-debromoaplysiatoxin C (1)

Neo-debromoaplysiatoxin C (1): white solid; $[\alpha]^{25}_D = +56.6$ (c 0.07, MeOH); CD (MeOH) $\lambda_{\text{max}}$ (Δε) 232 (+12.09) nm; UV (MeOH)$\lambda_{\text{max}}$ (logε) 218 (5.09) nm; IR (KBr) $v_{\text{max}}$ 3427, 2926, 1631, 1584, 1466, 1401, 1384, 1337 cm$^{-1}$; $^1$H and $^{13}$C NMR data see Table S1; HRESIMS $m/z$ 595.2866 [M + Na]$^+$ (calcd for C$_{32}$H$_{44}$O$_9$Na, 595.2883).

### Declaration of interest statement

The authors declare no competing financial interest.

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