Cooliatin, an Unprecedented Natural Dioxocyclononane from Dinoflagellate *Coolia monotis* from South China Sea

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Cooliatin, an unprecedented dioxocyclononane, was isolated from the dinoflagellate *Coolia monotis* from South China Sea. Its structure was identified on the basis of spectroscopic evidence.

Key words cooliatin; dioxocyclononane; *Coolia monotis*; dinoflagellate

Coolia, a small genus of dinoflagellates belonging to the family Ostreopsidaceae, comprises four species, *viz. C. areolata*, *C. canariensis*, *C. monotis*, and *C. tropicalis*, among which the latest species *C. canariensis* was established in 2008.[1—4] It was reported in 1995 that *C. monotis* could produce a toxin, which was named cooliatoxin.[5] However, its structure has not yet been characterized. Later, Holms and his coworkers proposed it as an analogue of yessotoxin with a molecular weight of 1063.[5—7] Moreover, a ceramide with a novel branched-chain was identified from *C. monotis* in 1998.[8,9] In the current paper, cooliatin, an unprecedented dioxocyclononane, was obtained from the chloroform extract of *C. monotis* (Cm LHT01) cells and its structure was elucidated on the basis of spectroscopic methods, including ^{1}H–^{1}H COSY, HSQC, HMBC and NOESY spectra. The dinoflagellate *C. monotis* was isolated from coastal seaweeds of Hainan island and its cells were artificially mass-cultured for chemical analysis.

Cooliatin was obtained as colorless oil. Its UV maxima were observed at 232 nm and 196 nm (sh). Its electrospary ionization (ESI)-MS (positive ion mode) showed pseudo-molecular peaks at *m/z* 289 $[M-O+Na]^+$, 305 $[M-O+K]^+$ and 321 $[M+K]^+$, which proposed that cooliatin had the molecular weight of 282. The molecular formula of cooliatin was determined as $C_{15}H_{22}O_5$ (unsaturation values of 5) by the HR-ESI-MS spectrum (*m/z* 289.1405, Calcd for $[M-O+Na]^+$ 289.1410). The ^{1}H- and ^{13}C-NMR data (Table 1) revealed that cooliatin had five methyls, two methylenes, two methines and six quaternary carbons. In addition, the NMR spectral data showed the presence of an acetoxy group (δ_H 1.99 s, δ_C 21.7, 170.9) and two conjugated carbonyls (δ_C 198.1, 209.2). Two substructures **1a** (from C3 to C8 and C10 to C13) and **1b** (including C1, C2, C9, C1′ and C2′) (Fig. 1) were determined by analysis of the ^{1}H–^{1}H COSY, HSQC, and HMBC spectral data of cooliatin. Substructure **1a** was elucidated by starting from a double bond (δ_C 101.4 CH, 119.7 qC) conjugated with two neighboring carbonyls (δ_C 198.1, 209.2). Their connections could be corroborated by HMBC correlations from H-5 to C-6, C-7 and C-8. Protons of methyl (H_3-10) connected to C-6 was suggested by HMBC correlations from its protons to C-5 and C-7. A quaternary carbon (C-4) bearing two methyl singlet resonances at δ_H 1.43 (H_3-11) and 1.16 (H_3-12), respectively, was connected to C-5 and this fragment was confirmed by HMBC correlations between H_3-12/C-4, H_3-11/C-4, H_3-11/C-12, H_3-12/C-11 and H-5/C-4 (Fig. 1). Moreover, an oxygenated quaternary carbon (C-3) bearing a methyl singlet resonance at δ_H 1.42 (H_3-13) was connected to the above quaternary carbon (C-4) and these connections were corroborated by HMBC correlations between H_3-13/C-3, H_3-13/C-4 and H-5/C-3 (Fig. 1). C-4, bearing two methyl groups, should be situated between C3 and C5, being confirmed by the HMBC cross-peak between

Table 1. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) NMR Data of Cooliatin in Acetone-d_6

Position	δ_H (J=Hz)	δ_C
1	5.40, m	68.6 CH
2a	1.55, t, 12.5	46.7 CH_2
2b	2.24, m	
3		72.4 qC
4		37.1 qC
5	5.73 s	101.4 CH
6		119.7 qC
7		198.1 qC
8		209.2 qC
9a	1.46, t, 12.0	46.4 CH_2
9b	1.97, m	
10	2.16, s	27.1 CH_3
11	1.43, s[a)]	31.2 CH_3
12	1.16, s	32.5 CH_3
13	1.42, s[a)]	29.7 CH_3
1′		170.9 qC
2′	1.99, s	21.7 CH_3
3-OH	4.23, s	

a) Overlapped signals assigned by ^{1}H–^{1}H COSY, HSQC and HMBC spectra.

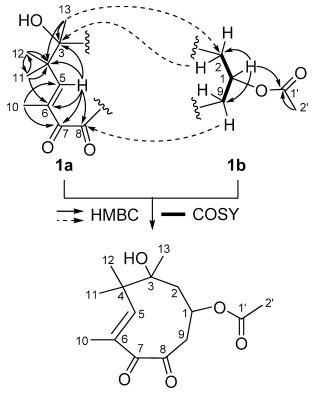

Fig. 1. Selected HMBC, ^{1}H–^{1}H COSY Correlations for Cooliatin

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H_3-11/C-6. Taken together, the above data gave substructure **1a**. The second substructure **1b**, could be assembled by starting from an oxygenated methine (C-1), which was situated between two methylenes. The fragment $–CH_2–CH(O)–CH_2–$ was corroborated by $^1H–^1H$ COSY correlations between H-2/H-1 and H-1/H-9. An acetoxy group (δ_H 1.99 s, δ_C 21.7, 170.9) was connected to C-1 and its connection was confirmed by the HMBC correlation from H-1 (δ_H 5.40 m) to the carbonyl (δ_C 170.9) of this acetoxy group. From these data, the second substructure was unambiguously deduced as **1b**. Furthermore, HMBC cross-peaks between H-2/C-3, H_3-13/C-2 and H-9/C-8, connected substructures **1a** and **1b** together through the carbon–carbon bonds of C2–C3 and C8–C9. From all these observations, the planar structure of cooliatin was identified as shown in Fig. 1. The geometry of the double bound between C-5 and C-6 could be determined as *E* by NOE correlations between H_3-10/H_3-11 and H_3-10/H_3-12 in Fig. 2. Based on the above results, cooliatin was identified as (*E*)-3-hydroxy-3,4,4,6-tetramethyl-7,8-dioxocyclonon-5-enyl acetate. However, the configuration of C-1 and C-3 has not been established due to the flexibility of the cyclononane ring.

The base peak *m*/*z* 289 in the positive mode ESI-MS of cooliatin, was derived *via* the intermediate *m*/*z* 284 by loss of a water molecule and adding of a sodium ion (Chart 1). The existence of this intermediate was supported by the observation of the high peak *m*/*z* 283 $[284-H]^-$ in the negative mode ESI-MS of cooliatin. Then the base peak *m*/*z* 289 was converted into *m*/*z* 229 by the loss of one molecule acetic acid. Afterwards, the simultaneous cleavage between C-3/C-4 and C-8/C-9 afforded *m*/*z* 145 (Chart 1). The above ESI-MS fragmentation of cooliatin was further confirmed by ESI-MS^2.

In conclusion, cooliatin, an unprecedented dioxocyclononane, was isolated from the dinoflagellate *Coolia monotis* from South China Sea and its structure was identified as (*E*)-3-hydroxy-3,4,4,6-tetramethyl-7,8-dioxocyclonon-5-enyl acetate. To our knowledge, cooliatin was the first cyclononane derivative found in nature. This study demonstrates that *C. monotis* is a rewarding new source for the production of secondary metabolites with novel carbon frameworks.

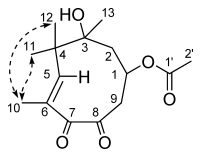

Fig. 2. Selected NOE Correlations for Cooliatin

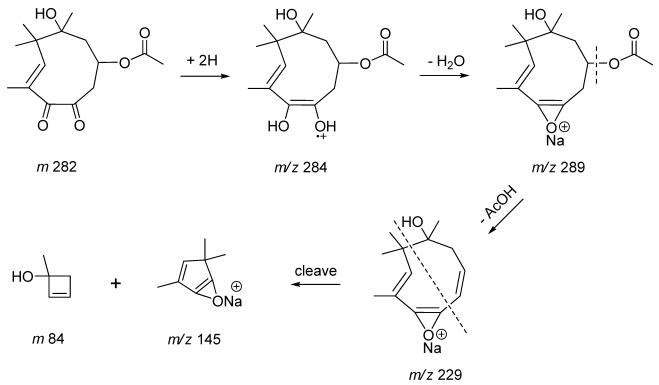

Chart 1. Positive ESI-MS Fragments of Cooliatin

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Experimental

General Experimental Procedures NMR spectra were recorded on a Bruker Avance 500 spectrometer (^1H: 500 MHz, ^{13}C: 125 MHz). Positive and negative mode ESI-MS were carried out on MDS SCIEX API 2000 LC-MS. Fragmentation and HR-ESI-MS spectra were acquired with a Bruker Bio TOF Q LC-MS instrument. Column chromatography (CC) was performed on silica gel (200—300 mesh; Qindao Hai Yang Chemical Factory), Sephadex LH-20 gel (Amersham Pharmacia Biotech) and HPLC was carried on Waters 2998 with YMC ODS series. The combination of fractions was guided by TLC (silica gel GF, Qindao Hai Yang Chemical Factory) results.

Alga Material *Coolia monotis* was isolated from coastal seaweeds of Hainan island and was identified by Mr. Ji-Lin Liang. Cells with the wet weight 3.5 kg was harvested after artificial and indoor mass culture in winter 2006. The species numbered CM LHT01 is preserved in our laboratory and the live cells are stably cultured.

Extraction and Isolation Cell (3.5 kg) disruption of *Coolia monotis* was performed by ultrasonication in $CHCl_3$: MeOH (1 : 1, v/v) and the resulting solvent was then evaporated under reduced pressure. The residue was suspended in water and extracted in turn with *n*-hexane, $CHCl_3$, AcOEt and *n*-BuOH. The $CHCl_3$ extract (5.1 g) was subjected to a silica gel CC and eluted with a gradient solvent system of $CHCl_3$: MeOH (100 : 0 to 0 : 100, v/v) to afford 91 fractions. Fractions 28—37 (60 mg) were combined and subjected to a Sephadex LH-20 CC and eluted with 100% MeOH to afford 15 subfractions. Subfractions 9—15 were combined and subjected to RP-HPLC (YMC ODS column 250×10 mm, 5 μm) and eluted with a gradient of 20 to 50% aqueous MeCN at flow of 3 ml/min to yield cooliatin (1.8 mg, t_R=21.5 min).

HPLC (YMC ODS column 250×4.6 mm, 5 μm) analysis of the chloroform extract of *Coolia monotis* eluted with 26% CH_3CN confirmed that cooliatin (t_R=29.6 min), characterized by its diagnostic UV spectrum, was a real natural product of this marine dinoflagellate.

Cooliatin, (*E*)-3-Hydroxy-3,4,4,6-tetramethyl-7,8-dioxocyclonon-5-enyl Acetate: Colorless oil. UV λ_{max} 232 and 196 nm (sh). ^1H- and ^{13}C-NMR see Table 1; ESI-MS (positive-ion mode) *m*/*z*: 555 $[2(M-O)+Na]^+$, 321 $[M+K]^+$, 305 $[M+Na]^+$, 289 $[M-O+Na]^+$, 266 $[M-O]^+$; ESI-MS (negative-ion mode) *m*/*z*: 283 $[M+2H-H]^-$; HR-ESI-MS (positive-ion mode) *m*/*z*: 289.1405 $[M-O+Na]^+$ (Calcd for $C_{15}H_{22}O_4Na$: 289.1410).

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