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CAULERCHLORIN, A NOVEL CHLORINATED BISINDOLE ALKALOID WITH ANTIFUNGAL ACTIVITY FROM THE CHINESE GREEN ALGA *CAULERPA RACEMOSA*

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Abstract – Caulerchlorin (**2**), a novel chlorinated bisindole alkaloid with an eight-membered cyclic ring between two indole rings incorporated directly with a chlorine atom, together with three known related metabolites, caulerpin (**3**), monomethyl caulerpinate (**4**), and caulersin (**5**), has been isolated from the Chinese green alga *Caulerpa racemosa* (Forssk.) J. Agardh. The structure of **2** was determined on the basis of extensive spectroscopic analysis. Compounds **2**–**5** were evaluated for their antifungal activity against *Candida albicans* (SC5314), *Candida albicans* (Y0109), *Candida parapsilosis* (ATCC 22019), *Candida glabrata* (537), *Microsporum gypseum* (Cmccfmza), *Trichophyton rubrum* (Cmccftla), *Aspergillus fumigatus* (07544), and *Cryptococcus neoformans* (32609), and the result showed that only compounds **2** and **4** had a moderate/weak antifungal activity against *Cryptococcus neoformans* (32609) with MIC_{80} values of 16 and 64 μg mL^{-1}, respectively.

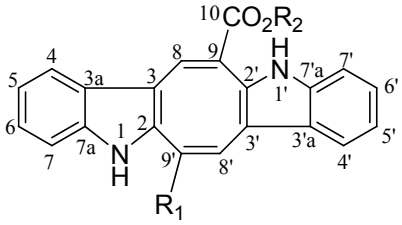
Indole alkaloids comprise a class of biologically active natural products biosynthesized by diverse range of organisms from both terrestrial and marine habitats. Within the marine environment, numerous indole alkaloids have been reported from sponges,[1] bryozoans,[2] coelenterates,[3] green algae[4] and red algae.[5] Adding to their interest is the fact that some of these metabolites display potent and diverse bioactivities, such as cytotoxic,[6] antifungal,[7] antibacterial,[7] antioxidant,[8] anti-inflammatory,[9] and antitumor properties.[10]

Green algae of the genus *Caulerpa* are well-known as a rich source of indole alkaloids. Caulerpin (**3**), the first bisindole pigment from the genus *Caulerpa*, whose structure was first proposed in 1970[11] and later

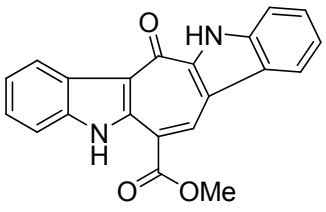
revised in 1978 on the basis of spectral data,[12] has been established as an inhibitor of mitochondrial respiration at complex I, suppressing hypoxic activation of HIF-1.[13] Furthermore, **3** acts as a plant growth regulator,[14] and has also been shown to be moderate antitumor activity[15] and to inhibit the multixenobiotic resistance (MXR) pump in algae.[16] In addition, two caulerpin analogues, caulerpin acid (**1**) and monomethyl caulerpinate (**4**), were isolated from *C. racemosa* collected off Visakhapatnam coast.[4a] In 1997, Su *et al*. reported the isolation a novel bisindole alkaloids, caulersin (**5**), with a central troponoid framework between two indole rings, from *C. serrulata* from the South China Sea.[4b]

In the course of our systematic investigations toward the isolation of secondary metabolites from Chinese marine organisms,[17-19] we have recently carried out a chemical study on the seaweed *C. racemosa*, collected from the Zhanjiang coastline, resulting in the discovery of a new polyacetylenic fatty acid and five known secondary metabolites.[20] Our continuing studies on the alkaloid fraction of the same specimen led to the isolation of a novel chlorinated bisindole alkaloid, caulerchlorin (**2**), with an eight-membered cyclic ring incorporated directly with a chlorine atom, along with three known related metabolites, caulerpin (**3**), monomethyl caulerpinate (**4**), and caulersin (**5**). We report herein the isolation, structural elucidation, and biological activity of this new compound.

The fresh algal material was exhaustively extracted with 95% ethanol. The EtOAc-soluble portion of this extract was subjected to separation by silica gel and Sephadex LH-20 column chromatography (CC), followed by purification with reversed-phase HPLC to afford a novel bisindole alkaloid, named caulerchlorin (**2**), along with three known related metabolites (**3**–**5**). The known alkaloids were readily identified as caulerpin (**3**), monomethyl caulerpinate (**4**), and caulersin (**5**) by comparing their NMR spectroscopic data with those reported in the literature.[4]



1 $R_1 = CO_2H$, $R_2 = H$
2 $R_1 = Cl$, $R_2 = Me$
3 $R_1 = CO_2Me$, $R_2 = Me$
4 $R_1 = CO_2H$, $R_2 = Me$



5

Figure 1. Chemical structures of compounds **1**-**5**

Caulerchlorin (**2**) was isolated as an orange-red amorphous solid, and its molecular formula, $C_{22}H_{15}ClN_2O_2$, was determined by HRESIMS at *m*/*z* 397.0723 $[M+Na]^+$ (calcd 397.0720), in combination with ^{13}C-NMR (DEPT) experiments, implying the presence of sixteen degrees of unsaturation. The

LRESIMS of **2** displayed a series of twin peaks, such as at *m*/*z* 375 and 377, corresponding to the $[M+H]^+$ isotopic peaks, respectively containing ^{35}Cl and ^{37}Cl, with the relative intensities of approximately 3:1 ratio, indicating the presence of one chlorine atom in the molecule. The IR data suggested the presence of amino or hydroxyl (3394 cm^{-1}) and a carbonyl moiety (1701 cm^{-1}) in **2**. Its UV spectrum showed absorption maxima at 232, 265 and 299 nm, according to an extensively conjugated aromatic system. The ^{13}C-NMR spectrum displayed singals for 22 carbons, which were attributed by DEPT experiment to one oxygen-bearing methyl (δ 52.6), ten sp^2 methines, and eleven sp^2 quaternary carbons. The ^1H-NMR spectrum of **2** showed two broad singlets at δ 8.35 (NH-1) and 8.89 (NH-1′), which disappeared in the presence of D_2O. Two olefinic singlet resonances at δ 7.06 (H-8′) and 8.06 (H-8) were assignable to the protons of two endocyclic trisubstituted double bonds. The pattern of aromatic signals at δ 7.09 (1H, ddd, J = 1.2, 7.2, 7.8 Hz, H-5′), 7.13 (1H, ddd, J = 1.2, 7.2, 7.8 Hz, H-5), 7.17 (1H, ddd, J = 1.2, 7.2, 8.4 Hz, H-6′), 7.22 (1H, ddd, J = 1.2, 7.2, 8.4 Hz, H-6), 7.29 (2H, dd, J = 1.2, 8.4 Hz, H-7, 7′), 7.44 (1H, dd, J = 1.2, 7.8 Hz, H-4′), and 7.46 (1H, dd, J = 1.2, 7.8 Hz, H-4) was characteristic of two indole rings. Finally, the ^1H-NMR spectrum was completed by signal at δ 3.92 (3H, s) due to a methoxyl group. The NMR data mentioned above were similar to those of the co-occurring caulerpin (**3**).[4a] However, the NMR spectra of **3** showed only 11 ^{13}C signals and 4 ^1H signals due to C_2 symmetry, while the NMR spectra of **2** contained 22 different ^{13}C signals and 13 ^1H signals, respectively, representing all of the carbon and hydrogen atoms in the molecule. Careful comparison of 1D and 2D NMR data of **2** and **3** revealed that the difference between them occurred only at C-9′ (-Cl in **2** and -COOMe in **3**), while the rest of the molecule was the same. All NMR data of **2** (Table 1) were unambiguously assigned by COSY, HMQC, and HMBC experiments (Figure 2). Therefore, according to all evidence presented above, caulerchlorin corresponds to structure **2** (Figure 1).

Table 1. ^1H (600 MHz) and ^{13}C NMR (150 MHz) data of caulerchlorin (**2**) in $CDCl_3$, δ in ppm, J in Hz

Pos.	δ_H	δ_C	HMBC[a] (C#)	Pos.	δ_H	δ_C	HMBC[a] (C#)
1 (N)	8.35 (1H, br s)	-	2, 3, 3a, 7a, 9′	1′ (N)	8.89 (1H, br s)	-	2′, 3′, 3′a, 7′a
2	-	133.3 s		2′	-	130.5 s	
3	-	113.5 s		3′	-	112.7 s	
3a	-	127.6 s		3′a	-	127.6 s	
4	7.46 (1H, dd, 1.2, 7.8)	118.7 d	6, 7a	4′	7.44 (1H, dd, 1.2, 7.8)	118.0 d	6′, 7′a
5	7.13 (1H, ddd, 1.2, 7.2, 7.8)	121.2 d	3a, 7	5′	7.09 (1H, ddd, 1.2, 7.2, 7.8)	120.3 d	3′a, 7′
6	7.22 (1H, ddd, 1.2, 7.2, 8.4)	124.3 d	4, 7a	6′	7.17 (1H, ddd, 1.2, 7.2, 8.4)	123.2 d	4′, 7′a
7	7.29 (1H, dd, 1.2, 8.4)	111.4 d	3a, 5	7′	7.29 (1H, dd, 1.2, 8.4)	111.3 d	3′a, 5′
7a	-	137.1 s		7′a	-	137.7 s	
8	8.06 (1H, s)	140.6 d	2, 3, 9, 10, 2′	8′	7.06 (1H, s)	128.2 d	2, 2′, 9′
9	-	126.3 s		9′	-	122.5 s	
10	-	166.8 s		10-OMe	3.92 (3H, s)	52.6 q	10

[a]All observed HMBC correlations are presented.

Compounds **2**–**5** were evaluated for their antifungal activity against *Candida albicans* (SC5314), *Candida albicans* (Y0109), *Candida parapsilosis* (ATCC 22019), *Candida glabrata* (537), *Microsporum gypseum* (Cmccfmza), *Trichophyton rubrum* (Cmccftla), *Aspergillus fumigatus* (07544), and *Cryptococcus neoformans* (32609), and the result showed that only compounds **2** and **4** had a moderate/weak antifungal activity against *Cryptococcus neoformans* (32609) with MIC_{80} values of 16 and 64 μg mL^{-1}, respectively. In addition, compounds **2**–**5** were also tested for anti-inflammatory effect,[21] and the result displayed that all of these compounds were inactive.

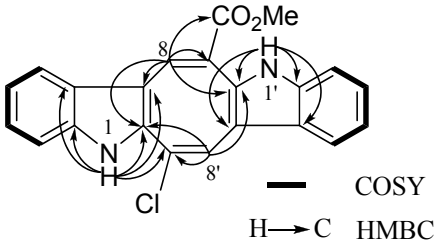


Figure 2. COSY and key HMBC correlations of compound **2**

EXPERIMENTAL

General Experimental Procedures. IR spectra were recorded on a Shimadzu FTIR-8400 spectrometer. UV spectra were obtained on a Beijin Puxi TU-1900 spectrophotometer. NMR spectra were measured on Bruker AV-600 spectrometer with the residual $CHCl_3$ (δ_H 7.26 ppm; δ_C 77.00 ppm) as an internal standard. ESIMS and HRESIMS spectra were recorded on a Finnigan-MAT-95 mass spectrometer. Reverse-phase HPLC [Agilent 1200 series liquid chromatography using a VWD G1314B detector at 210 nm and a semi-preparative ODS-A (10×250 mm, 5 μm) column] was employed for the purification. Commercial Si gel (Qing Dao Hai Yang Chemical Group Co., 200-300 mesh) was used for column chromatography, and precoated Si gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for analytical TLC. Sephadex LH-20 (Amersham Biosciences) was also used for column chromatography.

Biological Material. The alga *C. racemosa* was collected by hand from the Zhanjiang coastline, Guangdong Province, China, at a depth of 0.5-1 m, in June 2010, and the algal material was stored at –20 °C until processed. A voucher specimen (MP01-03) was deposited in the Herbarium of Department of Pharmacy, Nanchang University, Nanchang, China for inspection.

Extraction and Isolation. The fresh algal material (dry weight, 5.0 kg) was exhaustively extracted with EtOH (95%, v/v) three times for 24 h each time at room temperature. The extract was concentrated in vacuo to give a residue (350 g), which was partitioned sequentially with petroleum ether, EtOAc and *n*-BuOH, respectively. The EtOAc-soluble portion (150 g) was chromatographed by silica gel CC using

light petroleum ether with increasing amounts of acetone as the eluent to afford five fractions (Fr. A-Fr. E) on the basis of TLC analysis. Fraction C eluted with petroleum ether/acetone (8:2) was further subjected to CC on Sephadex LH-20 (petroleum ether-$CHCl_3$-MeOH 2:1:1) and followed by RP-HPLC purification (MeOH/H_2O 7.5:2.5 as the eluent) to afford compound **2** (51.0 mg). Fraction D eluted with petroleum ether/acetone (7:3) was further separated on silica gel CC (petroleum ether/acetone, 7.0:3.0, as the eluent), and then applied to Sephadex LH-20 eluted with $CHCl_3$-MeOH (1:1) to afford compounds **3** (280.0 mg) and **5** (25.0 mg). Fraction E eluted with petroleum ether/acetone (6.5:3.5) was subjected to repeated silica gel and Sephadex LH-20 chromatography leading to the isolation of compound **4** (5.8 mg). Caulerchlorin (**2**): orange-red amorphous solid; UV λ_{max} (MeOH) (log ε): 232 (2.03), 265 (1.87), 299 (1.89) nm; IR ν_{max} (KBr) 3394, 2924, 2851, 1701, 1624, 1493, 1454, 1420, 1319, 1258, 1142, 1049, 941, cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HRESIMS at *m/z* 397.0723 $[M+Na]^+$ (calcd for $C_{22}H_{15}ClN_2O_2Na$, 397.0720).

Antifungal Assay. The ATCC standard fungal strains *Candida albicans* (SC5314), *Candida albicans* (Y0109) and *Cryptococcus neoformans* (32609) and clinical fungal strains *Candida parapsilosis* (ATCC 22019), *Candida glabrata* (537), *Microsporum gypseum* (Cmccfmza), *Trichophyton rubrum* (Cmccftla), and *Aspergillus fumigatus* (07544) were used. Fungal strains were stored at –4 °C and grown at 35 °C on sabouraud dextrose agar (SDA) medium (10 g/L peptone; 40 g/L dextrose; 18 g/L agar; 0.1 g/L chloromycetin) with 1 mL of yeast extract-peptone-dextrose (YEPD) medium (10 g/L yeast extract; 20 g/L peptone; 20 g/L dextrose; 0.1 g/L chloromycetin). The suspension was diluted with RPMI 1640 to 1×10^3 to 5×10^3 units/mL, The alkaloids were dissolved in DMSO (6.4 g/L) and 2-fold diluted in the broth (64, 16, 4, 1, 0.25, 0.0625, 0.0156, 0.0039, 0.00097, and 0.00024 mg/L). Incubation was at 35 °C (24 h, 72 h, and one week), and the MIC was determined as the lowest concentration inhibiting fungal growth.

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REFERENCES

1. (a) A. Longeon, B. R. Copp, E. Quevrain, M. Roue, B. Kientz, T. Cresteil, S. Petek, C. Debitus, and M.-L. Bourguet-Kondracki, *Mar. Drugs*, 2011, **9**, 879; (b) M. Tsuda, Y. Takahashi, J. Fromont, Y. Mikami, and J.-i. Kobayashi, *J. Nat. Prod.*, 2005, **68**, 1277.
2. (a) L. Rahbk and C. Christophersen, *J. Nat. Prod.*, 1997, **60**, 175; (b) L. Peters, G. M. Koenig, H.

Terlau, and A. D. Wright, *J. Nat. Prod.*, 2002, **65**, 1633.

3. J. J. Fernandez, M. L. Souto, A. H. Daranas, and M. Norte, *Curr. Top. Phytochem.*, 2000, **4**, 105.
4. (a) A. S. R. Anjaneyulu, C. V. S. Prakash, and U. V. Mallavadhani, *Phytochemistry*, 1991, **30**, 3041; (b) J.-Y. Su, Y. Zhu, L.-M. Zeng, and X.-H. Xu, *J. Nat. Prod.*, 1997, **60**, 1043.
5. (a) D. Davyt, W. Entz, R. Fernandez, R. Mariezcurrena, A. W. Mombru, J. Saldana, L. Dominguez, J. Coll, and E. Manta, *J. Nat. Prod.*, 1998, **61**, 1560; (b) N.-Y. Ji, X.-M. Li, C.-M. Cui, and B.-G. Wang, *Helv. Chim. Acta*, 2007, **90**, 1731.
6. M. Shoeb, S. M. MacManus, M. Jaspars, J. Trevidu, L. Nahar, P. Kong-Thoo-Lin, and S. D. Sarker, *Tetrahedron*, 2006, **62**, 11172.
7. R. A. Hajare, R. M. Gaurkhede, P. P. Chinchole, and A. V. Chandewar, *Indian Pharm.*, 2011, **10**, 88.
8. C. L. Cardoso, I. Castro-Gamboa, D. H. S. Silva, M. Furlan, R. de A. Epifanio, A. da C. Pinto, C. M. de Rezende, J. A. Lima, and V. da S. Bolzani, *J. Nat. Prod.*, 2004, **67**, 1882.
9. K. Koyama, Y. Hirasawa, A. E. Nugroho, T. Hosoya, T. C. Hoe, K.-L. Chan, and H. Morita, *Org. Lett.*, 2010, **12**, 4188.
10. S. Wu, L. Wang, W. Guo, X. Liu, J. Liu, X. Wei, and B. Fang, *J. Med. Chem.*, 2011, **54**, 2668.
11. G. Aguilar-Santos, *J. Chem. Soc., Perkin Trans. 1*, 1970, **6**, 842.
12. B. C. Maiti, R. H. Thomson, and M. Mahendran, *J. Chem. Res. (S)*, 1978, 126.
13. Y. Liu, J. B. Morgan, V. Coothankandaswamy, R. Liu, M. B. Jekabsons, F. Mahdi, D. G. Nagle, and Y.-D. Zhou, *J. Nat. Prod.*, 2009, **72**, 2104.
14. M. F. Raub, J. H. Cardellina, II, and J. G. Schwede, *Phytochemistry*, 1987, **26**, 619.
15. S.-E. N. Ayyad and F. A. Badria, *Alexandria J. Pharm. Sci.*, 1994, **8**, 217.
16. H. C. Schroder, F. A. Badria, S. N. Ayyad, R. Batel, M. Wiens, H. M. Hassanein, B. Kurelec, and W. E. Muller, *Environ. Toxicol. Pharmacol.*, 1998, **5**, 119.
17. S.-C. Mao, Y.-W. Guo, R. van Soest, and G. Cimino, *J. Asian Nat. Prod. Res.*, 2011, **13**, 770.
18. S.-C. Mao, M. Gavagnin, E. Mollo, and Y.-W. Guo, *Biochem. Syst. Ecol.*, 2011, **39**, 408.
19. S.-C. Mao and Y.-W. Guo, *Zhongguo Tianran Yaowu*, 2010, **8**, 321.
20. S.-C. Mao, D.-Q. Liu, X.-Q. Yu, and X.-P. Lai, *Biochem. Syst. Ecol.*, 2011, **39**, 253.
21. S.-M. Li, X.-W. Yang, Y.-H. Shen, L. Feng, Y.-H. Wang, H.-W. Zeng, X.-H. Liu, J.-M. Tian, Y.-N. Shi, C.-L. Long, and W.-D. Zhang, *Phytochemistry*, 2008, **69**, 2200.