

A New Halogenated Biindole and A New *Apo*-carotenone from Green Alga *Chaetomorpha basiretorsa* Setchell

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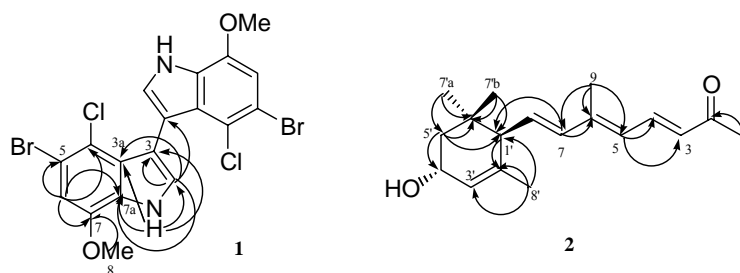
Abstract: A new halogenated biindole and a new *apo*-carotenone have been isolated from the ethanolic extract of the green alga *Chaetomorpha basiretorsa* Setchell. On the basis of chemical and spectroscopic methods including 2D NMR technique, their structures have been elucidated as 4,4'-dichloro-5,5'-dibromo-7,7'-dimethoxy-2,2'-bi-1H-indole and 1'S*,4'R*-8-(4'-hydroxy-2',6',6'-trimethylcyclohex-2-enyl)-6-methyloct-3E,5E,7E-trien-2-one, respectively.

Keywords: Green alga, *Chaetomorpha basiretorsa* Setchell, 4,4'-dichloro-5,5'-dibromo-7,7'-dimethoxy-2, 2'-bi-1H-indole, 1'S*,4'R*-8-(4'-hydroxy-2',6',6'-trimethylcyclohex-2-enyl)-6-methyloct-3E,5E,7E-trien-2-one.

Marine organism is a favorable source of halogenated metabolites and terpenoids. As part of our program to assess systematically the chemical and biological diversity of seaweeds distributed in China sea¹⁻³, we collected the green alga *Chaetomorpha basiretorsa* Setchell from the Naozhou Island, Zhanjiang province. *C. basiretorsa* (Cladophoreae) widely distributed along the coast of the south China sea⁴ and there is no chemical constituent reported before. In our chemical investigation, a new halogenated biindole **1** and a new *apo*-carotenone derivative **2** were isolated from this green alga. In this paper, we report the isolation and structural elucidation of **1** and **2**. Although polybrominated indoles have been isolated previously from marine red algae *Rhodophyllis membranacea*⁵ and *Laurencia brongniartii*^{6,7}, as well as the blue green alga *Rivularia firma*^{8,9}, this is the first report of halogenated biindole from green algae.

The air-dried green alga *C. basiretorsa* (14.3 Kg) was powdered and extracted with 95% EtOH at room temperature for 4 times. The solvent was removed under reduced pressure at below 40°C to give a residue (574 g). The residue was suspended in water and then partitioned with EtOAc. The EtOAc fraction (370 g) was subjected to column chromatography over silica gel, eluting with a gradient increasing ethyl acetate (0~100%) in light petroleum. The fractions eluted by 10% and 20% ethyl acetate in light petroleum were separately purified by Sephadex LH-20 with light petroleum-CH₂Cl-MeOH (5:5:1)

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Figure 1 The structures and key HMBC correlations of compounds **1** and **2**

as eluent, and then by preparative HPLC with a RP-18 column (250×22 mm, particle size 10 μ m) and 75% MeOH in H₂O as the mobile phase to yield **2** (24 mg) and **1** (8 mg), respectively.

Compound **1** was obtained as amorphous powder. The IR spectrum (KBr) of **1** showed a strong absorption band for N-H group (3431 cm^{-1}) and a characteristic band for conjugated system (1653 cm^{-1}). In the higher mass region the EI mass spectrum exhibited a molecular ion cluster at m/z 522/520/518/516 with an intensity ratio of 36:100:92:28, indicating that **1** was polyhalogenated. The molecular formula C₁₈H₁₂O₂N₂Cl₂Br₂ was deduced from the HREIMS at m/z 515.8645 [M]⁺ (calcd. 515.8642). Both the ¹H and ¹³C NMR spectra contained only half the number of signals expected from the molecular formula, suggesting that **1** was a symmetrical dimer. The ¹H NMR spectrum in DMSO-*d*₆ displayed four signals including one exchangeable broad singlet at δ 11.68 (2H, NH-1 and NH-1'), two aromatic proton singlets at δ 7.28 (2H, H-2 and H-2') and 6.97 (2H, H-6 and H-6'), and a methoxyl singlet at δ 3.99 (6H, MeO-7 and MeO-7'). The ¹³C NMR and DEPT spectrum showed nine carbon signals including six quaternary carbons, two sp² methines and one methoxyl group (see **Table 1**). These data revealed that **1** was a biindole with a symmetrical substitution pattern of two bromine atoms, two chlorine atoms and two methoxyl group⁸.

In order to determine positions of the dimerization and the substituents, the HMBC and NOE difference experiments of **1** were carried out. In the HMBC spectrum (see **Figure 1**), two- and three- bond correlations from N-H to C-2, C-3, C-3a and C-7a, and from H-2 to C-3, C-3', C-3a, C-7a, in combination with chemical shifts of these carbons, established the dimerization position at C-3. This was confirmed by an NOE enhancement of N-H in the NOE difference spectrum when H-2 was irradiated. In addition, correlations from the methoxyl protons to C-7, and from H-6 to C-4, C-5, C-7 and C-7a in the HMBC spectrum of **1** and comparison of the chemical shift values of these carbons with those of related compounds in literatures^{8,9}, demonstrated that the substitution pattern of the half structure of **1** was 4-chloro-5-bromo-7-methoxy-2-1H-indolyl. This was also confirmed by the NOE difference experiment that showed an enhancement of H-6 by irradiation of the methoxyl protons. Consequently, the structure of **1** was determined as 4,4'-dichloro-5,5'-dibromo-7,7'-dimethoxy-2,2'-bi-1H-indole.

Table 1 ^1H and ^{13}C NMR data of compound **1** and **2** (δ ppm)^a

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1			2.24 (3H, s)	26.7 q
2	7.28 (1H, s)	123.3 d		197.2 s
3		104.3 s	6.15 (1H, d, 15.5 Hz)	130.4 d
3a		125.1 s		
4		122.2 s	7.60 (1H, dd, 11.5, 15.5 Hz)	138.8 d
5		116.3 s	6.26 (1H, d, 11.5 Hz)	128.1 d
6	6.97 (1H, s)	105.9 d		144.2 s
7		146.0 s	6.30 (1H, d, 15.5 Hz)	137.0 d
7a		124.2 s		
8	3.99 (3H, s.)	55.7 q	5.79 (1H, dd, 10.5, 15.5 Hz)	134.0 d
9			2.03 (3H, s)	12.8 q
1'			2.46 (1H, d, 10.5 Hz)	55.2 d
2'	7.28 (1H, s)	123.3 d		135.2 s
3'		104.3 s	5.54 (1H, s)	126.5 d
3'a		125.1 s		
4'		122.2 s	4.17 (1H, dd, 6.0, 7.0 Hz)	64.5 d
5'		116.3 s		
5'a			1.35 (1H, dd, 7.0, 13.0 Hz)	
5'b			1.78 (1H, dd, 6.0, 13.0 Hz)	44.9 t
6'	6.97 (1H, s)	105.9 d		34.0 s
7'		146.0 s		
7'a		124.2 s	0.85 (3H, s)	23.9 q
7'b			0.98 (3H, s)	29.2 q
8'	3.99 (3H, s.)	55.7 q	1.58 (3H, s)	22.3 q

^aNMR data were measured at 500 MHz for proton and at 125 MHz for carbon in DMSO- d_6 for **1** and in acetone- d_6 for **2**. The assignments were based on DEPT, HMQC and HMBC experiments.

Compound **2** was obtained as colorless oil, its molecular formula $\text{C}_{18}\text{H}_{26}\text{O}_2$ was deduced from a combination of the EIMS at m/z 274 $[\text{M}]^+$ and NMR data (see **Table 1**). The IR spectrum suggested the presence of hydroxy (3408 cm^{-1}) and conjugated carbonyl (1714 and 1660 cm^{-1}) groups. The ^1H NMR spectrum of **2** showed signals attributed to five methyl singlets at δ 0.85, 0.98, 1.58, 2.03 and 2.24 (each 3H, s), six olefinic protons at δ 5.54 (, 1H, H-3'), 5.79 (dd, 1H, $J=10.5$ and 15.5 Hz , H-8), 6.15 (d, 1H, $J=15.5\text{ Hz}$, H-3), 6.26 (d, 1H, $J=11.5\text{ Hz}$, H-5), 6.30 (d, 1H, $J=15.5\text{ Hz}$, H-7), and 7.60 (dd, 1H, $J=11.5$ and 15.5 Hz , H-4), an ABX spin system at δ 1.35 (dd, 1H, $J=7.0$ and 13.0 Hz , H-5'a), 1.78 (dd, 1H, $J=6.0$ and 13.0 Hz , H-5'b) and 4.17 (dd, 1H, $J=6.0$ and 7.0 Hz , H-4'), in addition to a deshielded methine proton at δ 2.46 (d, 1H, $J=10.0\text{ Hz}$, H-1'). The ^{13}C NMR and DEPT spectra of **2** displayed eighteen carbon signals including six methyls, one methylene, eight methines (six sp^2 hybrid, $\delta >120.0\text{ ppm}$) and four quaternary carbons (one carbonyl at δ 197.2 and two sp^2 hybrid, $\delta >135.0\text{ ppm}$). These spectroscopic data suggested that **2** was an *apo*-13- β -carotenone derivative¹⁰. The partial structures of the cyclohexene moiety and the polyenone chain in **2** were established by 2D NMR including ^1H - ^1H gCOSY, gHSQC and gHMBC experiments. The protonated carbon and their corresponding proton signals were unambiguously assigned by the gHSQC experiment (see **Table 1**). The ^1H - ^1H gCOSY spectrum revealed the presence of two AMX spin systems consisting of two pairs of *trans* coupling

olefinic protons (H-3 and H-4, and H-7 and H-8) with H-5 and H-1', respectively, in addition to the ABX spin system. This indicated that the double bond of the cyclohexene moiety was not between C-1' and C-2' as usual¹⁰. In the gHMBC spectrum of **2** long range correlations of C-1' with H-5'a, H-5'b, H₃-7'a, H₃-7'b and H₃-8', C-5' with H₃-7'a and H₃-7'b, as well as correlations of a pair of double bond carbons C-2' and C-3' with H₃-8' demonstrated that the double bond of the cyclohexene moiety was between C-2' and C-3'. Meanwhile, HMBC correlations of C-2 with H₃-1, H-3 and H-4, C-5 with H-3, H-4, H-7 and H₃-9, as well as C-6 with H-4, H-5, H-7, H-8 and H-9 established that the polyenone chain of **2** was 6-methyloct-3,5,7-trien-2-one. In addition, in gHMBC of **2** correlations from H-7 to C-1' and from H-8 to C-1', C-2' and C-6' confirmed the connectivity between the cyclohexene moiety and the polyenone chain. The coupling pattern and chemical shifts of the olefinic protons revealed that the relative geometry of the polyenone moiety was all-*E*^{10,11}, while the coupling patterns of H-1', H-3' and H-4' indicated that the relative stereochemistry of the cyclohexene moiety was identical to lactucaxanthin¹¹. Therefore, the structure of compound **2** was assigned as 1'*S**,4'*R**-8-(4'-hydroxy-2', 6', 6'-trimethylcyclohex-2-enyl)-6-methyloct-3*E*, 5*E*, 7*E*-trien-2-one.

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