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CHEMICAL CONSTITUENTS OF *Chondrophycus papillosus* AND THEIR CYTOTOXICITY *IN VITRO*

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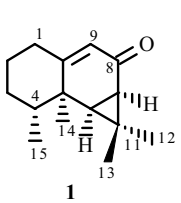
The red algae *Chondrophycus papillosus* belongs to the genus *Chondrophycus* of family Rhodomelaceae, which is widely distributed in the offshore area of Guangdong and Hainan Island in China. In previous reports, only one linear diterpene, one steroid, three benzene derivatives, and sulfated xylomannans have been isolated from *C. papillosus* [1, 2]. Recently, in the course of our search for bioactive substances from Chinese marine alga, the EtOAc crude extract of *C. papillosus* displayed strong *in vitro* activities against tumor cell lines K562, SGC-7901, and SMMC-7721. Further investigation on the chemical constituents of *C. papillosus* resulted in the isolation and identification of nine compounds, including three aristolane sesquiterpenes **1–3**, one snyderane sesquiterpene **4**, three polybrominated indoles **5–7**, one linear diterpene **8**, and one cholesterol derivative **9**. This paper describes the isolation, structure elucidation, and bioactivity evaluation of these compounds.

The structures of compounds **1–9** were determined to be aristolone (**1**) [3], 1(10)-aristolen-9β-ol (**2**) [4], 1(10),8-aristoladien-2-one (**3**) [5, 6], aplysistatin (**4**) [7], 2,3,5,6-tetrabromo-1-methyl-1*H*-indole (**5**) [8], 2,3,5,6-tetrabromoindole (**6**) [9], 3,5,6-tribromo-1*H*-indole (**7**) [10], phytol (**8**) [7, 11], and cholesterol (**9**) [12] on the basis of their ^{1}H NMR, ^{13}C NMR, and EI-MS spectral data and by comparison with those previously reported in the literature. Among these metabolites, compounds **1–7** were first isolated from *C. papillosus*.

The cytotoxic activity against tumor cell lines K562, SGC-7901, and SMMC-7721 of all the isolated compounds were evaluated. The bioassay results showed that compounds **2**, **5**, and **6** exhibited significant cytotoxicity against tumor cell lines K562, SGC-7901, and SMMC-7721. Compound **7** showed strong activity to K562, while compound **8** showed only weak activity to SGC-7901 (Table 1). From the cytotoxic activity analysis of polybrominated indoles **5–7**, we presume that the bromine group at C-2 could affect the potency of the compounds.

None of these compounds showed evident antibacterial activity against the tested bacterial strains of *Staphylococcus aureus* and MRSA. Compounds **5** and **6**, however, have been reported to show wide-spectrum activity against Gram-positive bacteria [13]. In addition, it was reported that compound **1** inhibited melanin synthesis [3], and compound **4** showed significant inhibitory activity against murine lymphocytic leukemia P-388 [14].



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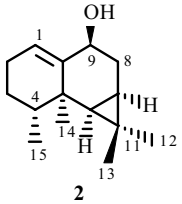

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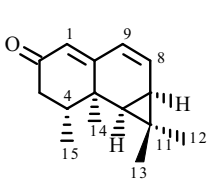

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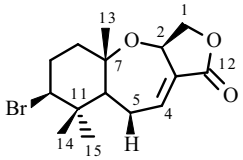

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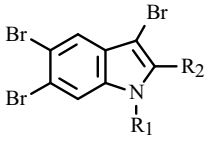

5 - 7

5: $R_1 = CH_3$, $R_2 = Br$
6: $R_1 = H$, $R_2 = Br$
7: $R_1 = R_2 = H$

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TABLE 1. Cytotoxicities of Compounds **2**, **5–8** (IC_{50} values, μg/mL)

Compound	K562	SGC-7901	SMMC-7721	Compound	K562	SGC-7901	SMMC-7721
2	7.6	11.8	9.2	**7**	12.0	–	–
5	4.2	8.0	7.5	**8**	–	50.0	–
6	2.0	3.5	6.3	Mitomycin C*	3.8	5.1	2.2

*Positive control.

General Methods. Optical rotation were measured on a Jasco-P-1020 digital polarimeter. NMR spectra were recorded on a Bruker AV-400 NMR spectrometer, and chemical shifts were recorded as δ values (400 MHz for ^1H and 100 MHz for ^{13}C). EI-MS were measured on VG Autosper-3000 mass spectrometer. Silica gel (200–300 mesh, Qingdao Haiyang Chemical Factory, Qingdao, China), octadecylsilyl (ODS) silica gel (45–60 mm; Merck KGaA, Darmstadt, Germany), and Sephadex LH-20 (Amersham Biosciences Inc., Piscataway, NJ, USA) were used. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 GF_{254} plates, 0.2 mm thick (Yantai Zifu Chemical Group Co., Yantai, China); spots were detected by spraying with 8% EtOH–H_2SO_4 reagent, followed by heating.

Plant Material. The red algae of *C. papillosus* was collected off the coast of Sanya, Hainan Island, China, in March 2006, and was identified by Prof. Xin-Zheng Li, Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, China. The voucher specimen (HN-SYM-20060040) was deposited in the Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China, Qingdao, China.

Extraction and Separation of Metabolites. The dried and powdered whole plant of *C. papillosus* (500.0 g) was extracted with 95% EtOH (three times, each 3 L) at room temperature. The ethanolic extract was evaporated *in vacuo* to yield a dark green residue (30.0 g), which was partitioned between H_2O and EtOAc. After removal of the solvent under reduced pressure, the EtOAc extract (13.0 g) was subjected to vacuum liquid chromatography (VLC) on silica gel and eluted with petroleum ether containing increasing amounts of EtOAc to afford 8 fractions (Fr.1–Fr.8). Fraction 1 was isolated by column chromatography on silica gel eluted with petroleum ether–acetone (10:1), and then subjected to Sephadex LH-20 eluted with EtOH to obtain compounds **3** (6.0 mg), **5** (25.0 mg) and **6** (90.0 mg). Fraction 2 was first subjected to silica gel chromatography to yield two subfractions (Fr.2-1–Fr.2-2) on the basis of TLC analysis. Fraction 2-1 was further purified by preparative HPLC (column: Kromasil 250 × 10 mm, 5 μm; mobile phase: MeOH–H_2O (80:20), UV detection 254 nm) to afford compounds **1** (25.1 mg) and **4** (15.0 mg). Fraction 2-2 was then subjected to repeated silica gel chromatography to obtain compounds **2** (12.0 mg) and **8** (7.5 mg). Fraction 3 was subjected to column chromatography on silica gel with petroleum ether–EtOAc (8:1) and further separated by Sephadex LH-20 eluted with $CHCl_3$–MeOH (1:1) to yield compounds **7** (10.0 mg) and **9** (8.0 mg).

Cytotoxic Assay. The crude extract of *C. papillosus* and the isolated compounds were screened for cytotoxic activity *in vitro* against K562, SGC-7901, and SMMC-7721 cell lines using the method of microculture tetrozolium assay (MTT) [15]. All three cell lines were maintained in RPMI 1640 (Gibco) containing 10% FBS (Gibco), 2 mg/mL sodium bicarbonate, 100 μg/mL penicillin sodium salt, and 100 μg/mL streptomycin sulfate. Cells were grown to 70% confluence, trypsinized with 0.05% trypsin-2 mM EDTA, and plated for experimental use. In all experiments, the cells were grown in RPMI-1640 medium with 10% FBS for 24 h prior to treatment. The compound was dissolved in DMSO at a concentration of 100 mM, then diluted in tissue culture medium and filtered before use. The cells (1.0×10^4) were seeded in 96-well tissue culture plates, treated with the tested compound or vehicle (0.1% DMSO) at various concentrations, and incubated at 37°C for 48 h followed by MTT assay at 570 nm. The IC_{50} values of the tested compound against different cell lines were obtained from the concentration-effect curves. Each experiment was repeated at least three times, and the combined data were analyzed using the Student paired *t* test.

Antibacterial Assay. The antibacterial activities of the isolated compounds were evaluated by the disc-diffusion method [16] with bacterial strains of *Staphylococcus aureus* and methicillin-resistant *staphylococcus aureus* (MRSA).

Aristolone (1): colorless needle ($CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.04 (3H, d, J = 6.6, H-15), 1.16 (3H, s, H-14), 1.18 (3H, s, H-12), 1.23 (3H, s, H-13), 1.34 (1H, m, H-2′), 1.36 (1H, d, J = 7.8, H-6), 1.41 (1H, m, H-3′), 1.53 (1H, m, H-3), 1.71 (1H, d, J = 7.8, H-7), 1.80 (1H, m, H-2), 1.82 (1H, m, H-4), 2.23 (1H, m, H-1′), 2.42 (1H, tdd, J = 16.0, 5.5, 2.2, H-1), 5.69 (1H, s, H-9). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 33.2 (C-1), 26.2 (C-2), 30.6 (C-3), 38.8 (C-4), 39.6 (C-5), 39.2 (C-6), 35.6 (C-7), 196.4 (C-8), 124.3 (C-9), 167.7 (C-10), 24.4 (C-11), 29.9 (C-12), 16.6 (C-13), 16.4 (C-14), 22.6 (C-15); EI-MS *m/z* (pos) 218 $[M]^+$.

1(10)-Aristolen-9β-ol (2): colorless needle ($CHCl_3$), $[\alpha]_D^{20}$ +55° (*c* 1.40, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.55 (1H, d, J = 9.2, H-6), 0.78 (1H, td, J = 9.5, 9.2, 3.5, H-7), 0.97 (3H, d, J = 6.8, H-15), 0.98 (3H, s, H-14), 1.00 (3H, s, H-12), 1.08 (3H, s, H-13), 1.23 (1H, ddd, J = 13.3, 11.8, 3.6, H-8′), 1.39 (2H, m, H-3, 3′), 1.69 (1H, br.s, OH), 1.76 (1H, m, H-4), 2.03 (2H, m, H-2, 2′), 2.23 (1H, ddd, J = 13.3, 9.5, 7.0, H-8), 4.29 (1H, tdd, J = 11.8, 7.0, 2.2, H-9), 5.54 (1H, dt, J = 4.5, 2.4, H-1). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 116.2 (C-1), 25.3 (C-2), 26.6 (C-3), 36.8 (C-4), 38.6 (C-5), 32.7 (C-6), 18.2 (C-7), 30.6 (C-8), 67.4 (C-9), 145.6 (C-10), 16.1 (C-11), 29.5 (C-12), 18.7 (C-13), 24.0 (C-14), 16.0 (C-15).

1(10),8-Aristoladien-2-one (3): yellow oil, $[\alpha]_D^{20}$ +23° (*c* 4.30, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.86 (3H, s, H-13), 1.09 (3H, s, H-12), 1.11 (3H, d, J = 5.9, H-15), 1.20 (3H, s, H-14), 1.43 (1H, m, H-4), 1.45 (1H, d, J = 1.3, H-6), 2.08 (1H, dd, J = 6.0, 1.3, H-7), 2.19 (1H, dd, J = 7.0, 1.4, H-3′), 2.22 (1H, dd, J = 7.0, 1.6, H-3), 5.67 (1H, s, H-1), 6.09 (1H, d, J = 9.6, H-9), 6.36 (1H, dd, J = 9.6, 6.0, H-8). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 122.6 (C-1), 199.4 (C-2), 43.2 (C-3), 38.0 (C-4), 36.2 (C-5), 34.0 (C-6), 26.1 (C-7), 137.1 (C-8), 125.1 (C-9), 162.7 (C-10), 27.9 (C-11), 14.7 (C-12), 22.1 (C-13), 28.9 (C-14), 15.1 (C-15).

Aplysistatin (4): colorless needle ($CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.96 (3H, s, H-14), 1.16 (3H, s, H-15), 1.29 (3H, s, H-13), 1.62 (1H, ddd, J = 13.2, 4.0, 3.5, H-8′), 1.78 (1H, ddd, J = 13.2, 13.2, 4.4, H-8), 2.04 (1H, dd, J = 9.8, 2.2, H-6), 2.11 (1H, dddd, J = 13.6, 4.5, 4.4, 3.5, H-9′), 2.27 (1H, dddd, J = 13.6, 13.2, 13.2, 4.0, H-9), 2.55 (2H, m, H-5), 3.86 (1H, dd, J = 9.0, 6.6, H-1′), 3.92 (1H, dd, J = 13.2, 4.5, H-10), 4.49 (1H, dd, J = 9.0, 8.6, H-1), 5.12 (1H, dd, J = 8.6, 6.6, H-2), 6.95 (1H, t, J = 3.3, H-4). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 70.0 (C-1), 66.9 (C-2), 132.0 (C-3), 143.2 (C-4), 27.3 (C-5), 51.3 (C-6), 79.1 (C-7), 37.7 (C-8), 32.5 (C-9), 65.2 (C-10), 41.1 (C-11), 169.0 (C-12), 21.8 (C-13), 18.1 (C-14), 30.8 (C-15).

2,3,5,6-Tetrabromo-1-methyl-1*H*-indole (5): colorless needle (petroleum). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm, J/Hz): 3.79 (3H, s, Me-N), 7.70 (1H, s, H-7), 8.09 (1H, s, H-4). ^{13}C NMR (100 MHz, DMSO-d_6, δ): 117.3 (C-2), 90.5 (C-3), 126.6 (C-3a), 121.8 (C-4), 115.3 (C-5), 118.3 (C-6), 115.9 (C-7), 135.6 (C-7a), 32.8 (Me-N); EI-MS (pos): 451/449/447/445/443 $[M]^+$ (1/4/6/4/1).

2,3,5,6-Tetrabromoindole (6): colorless needle (petroleum). ^{1}H NMR (400 MHz, acetone-d_6, δ, ppm, J/Hz): 7.70 (1H, s, H-7), 7.77 (1H, s, H-4), 11.5 (1H, br.s, NH). ^{13}C NMR (100 MHz, acetone-d_6, δ): 114.1 (C-2), 92.6 (C-3), 128.9 (C-3a), 123.2 (C-4), 117.2 (C-5), 118.6 (C-6), 116.6 (C-7), 136.6 (C-7a); EI-MS (pos): 437/435/433/431/429 $[M]^+$ (1/4/6/4/1).

3,5,6-Tribromo-1*H* indole (7): colorless needle (acetone). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.22 (1H, d, J = 2.5, H-2), 7.69 (1H, s, H-7), 7.84 (1H, s, H-4), 8.30 (1H, br.s, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 125.2 (C-2), 90.9 (C-3), 127.7 (C-3a), 123.6 (C-4), 116.3 (C-5), 118.6 (C-6), 116.1 (C-7), 134.8 (C-7a); EI-MS (pos): 357/355/353/351 $[M]^+$ (1/3/3/1).

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