Two polyhydroxy xanthones and their antiviral activity from gorgonian coral-derived fungus *Arthrillum* sp.

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**Abstract:** Objective To study the chemical structures and antibacterial and antiviral activities of 2,3,6,8-tetrahydroxy-1-methylxanthone (1) and 2,3,4,6,8-pentahydroxy-1-methylxanthone (2) isolated from a gorgonian coral-derived fungus. **Methods** The chemical structures of 1 and 2 were studied by using the modern spectrum analysis methods and X-ray crystallographic techniques. The biological activities including antimicrobial activity and antiviral activity were evaluated by using their evaluation models. **Results** Compound 1 was further confirmed by X-ray single-crystal diffraction analysis for the first time. Compound 1 showed significant anti HSV activity in vitro with an IC₅₀ value of 27.40 μmol/L, which was stronger than that of the positive control ribavirin (IC₅₀ 313.00 μmol/L). **Conclusion** The data of single-crystal and bioactivity possessed reference value for the systematic study of xanthones and their analogues in the future.

**Key words:** gorgonian coral; *Arthrillum* sp.; polyhydroxy xanthone; antiviral activity; X-ray

花柳珊瑚来源的真菌 *Arthrillum* sp. 中的 2 个多羟基氧杂蒽酮类化合物及其抗病毒活性研究△

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**摘要:** 目的 研究从花柳珊瑚类附生真菌 *Arthrillum* sp. 分离得到的 1-甲基-2,3,6,8-四羟基氧杂蒽酮 (1) 和 1-甲基-2,3,4,6,8-五羟基氧杂蒽酮 (2) 的化学结构及其抗菌和抗病毒活性。方法 利用现代波谱解析方法和 X-ray 单晶衍射技术, 对化合物 1 和 2 的化学结构进行研究; 利用抗菌和抗病毒活性模型对其进行活性评价。结果 化合物 1 的结构首次通过单晶数据进一步确定, 体外化合物 1 显著抗病毒活性, 化合物 1 的 IC₅₀ 值为 27.40 μmol/L, 强于阳性对照利巴韦林的抗 H1N1 活性 (IC₅₀ 313.00 μmol/L)。结论 化合物 1 的单晶数据, 以及活性数据对今后系统研究珊瑚菌类化合物具有参考价值。

**关键词:** 花柳珊瑚; *Arthrillum* sp.; 多羟基氧杂蒽; 抗病毒活性; X-ray

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Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites that have become interesting and significant resources for drug discovery\(^{[1]}\). Some fungal species such as *Pentillium griseofulvum*\(^{[2]}\), *Phomopsis* sp.\(^{[3]}\), *Xyleri* sp.\(^{[4]}\), and *Arthrinium* sp.\(^{[5]}\), are well known sources of xanthone derivatives. Xanthones are also to be found in nature as the metabolites of many different species within the plant, and bacterial kingdoms\(^{[6]}\). They have polyketide origin\(^{[7]}\) and show various bioactivities, such as COX 2 in hibition\(^{[8]}\), cytotoxic activity\(^{[9]}\), antimicrobial\(^{[10]}\), antitumor\(^{[11]}\), and antioxidant\(^{[12]}\). As part of our ongoing investigation on new and bioactive natural products from marine fungi in the South China Sea\(^{[13]}\), two polyhydroxy xanthones, 2,3,6,8-tetrahydroxy-1-methylxanthone (1) and 2,3,4,6,8-pentahydroxy-1-methylxanthone (2)\(^{[14]}\), were firstly obtained from the marine derived fungus *Arthrinium* sp. (RA05 09) isolated from the gorgonian coral *Anthorgorgia caerulea* collected from the South China Sea. Both compounds 1 and 2 belonged to polyhydroxy xanthones with a relatively rare methyl group at C 1\(^{[15]}\). Herein, we report the cultivation, isolation, structure determination, crystal structure, and biological evaluation of compounds 1 and 2.

![Chemical structures of compounds 1 and 2](image)

Fig. 1  Chemical structures of compounds 1 and 2

1 EXPERIMENTAL SECTION

1.1 Instruments

NMR spectra were recorded on a JEOL JEM-FEC 260MHz spectrometer (500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C). Chemical shifts \(\delta\) were reported in ppm, using TMS as internal standard and coupling constants \(J\) were in Hz. EIMS spectra were measured on a Thermo DSQ EIMS spectrometer. Silica gel (Qing Dao Hai Yang Chemical Group Co. ; 200 - 300 mesh), octadeylsilica gel (Unicorn; 45 - 60 \(\mu\)m) and Sephadex LH-20 (GE Healthcare) were used for column chromatography (CC). Precautional silica gel plates (Yan Tai Zhi Fu Chemical Group Co.; G60, F 254) were used for thin layer chromatography (TLC).

1.2 The isolation of compounds 1 and 2

Fungal Material. The fungus *Arthrinium* sp. was isolated from a piece of fresh tissue from the gorgonian coral *A. caerulea* collected from the South China Sea. The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qing dao, PR China, with the GenBank accession number KR492515.

Fermentation, Extraction and Isolation. The fungal strain *Arthrinium* sp. (RA05 09) was cultivated in 50L liquid medium (20.0 g of glucose, 200.0 g of potato, 10.0 g of sea salt in 1 L of water, in a 500 mL Erlenmeyer flasks each containing 200 mL of culture broth) at 27 °C without shaking for 28 days. The whole fermented broth (50 L) was filtered through cheesecloth to separate into culture broth and mycelia. The fungal mycelia were concentrated under reduced pressure to about a quarter of the original volume, then extracted three times with an equivalent volume of EtOAc to give an EtOAc solution.

The mycelia were extracted three times with methanol, then concentrated and extracted with EtOAc to give another EtOAc solution. The combined EtOAc solutions were concentrated to give an EtOAc extract (10.0 g). The EtOAc extract was subjected to vacuum liquid chromatography (VLC) on silica gel using a step gradient elution with petroleum ether and EtOAc then with EtOAc MeOH to afford 7 fractions (Fr. 1 - Fr. 7). Fr. 3 was isolated by column chromatography (CC) on silica gel eluted with petroleum ether...
EtOAc (v/v, 7:3) to obtain six sub-fractions, Fr. 3-3 was subjected to Sephadex 1H-20 CC with petroleum ether — C11H8O — MeOII (v/v/v 2:1:1), to yield compounds 1 (5.0 mg) and 2 (4.5 mg).

1.3 Crystal cultivation

By slow crystalization from MeOII, single crystals of 1 suitable for X-ray diffraction analysis were obtained, which further confirmed the structure of 1 (Fig. 2).

Fig. 2 The structure of compound 1, with atom labels and 50% probability displacement ellipsoids for non-H atoms

1.4 Biological assays

The antibacterial activities against nine bacterial strains, *Shigella dysenteriae*, *Bacillus subtilis*, *B. Cereus*, *Escherichia coli*, *B. Megaterium*, *Micrococcus lysodeikticus*, *M. Luteus*, *Enterobacter aerogenes*, *Bacterium paratyphosum B*, were determined by a serial dilution technique using 96-well microtiter plates. The compounds were dissolved in DMSO to give a stock solution. Bacterial species were cultured overnight at 37°C in LB broth and diluted to 10⁵ cfu/mL when used. LB broth was used as a blank control, and DMSO was used as a negative control, while ciprofloxacin was used as a positive control. The plates were incubated at 37°C for 24 h. The results were observed with a Multi-skran Mk3 (Thermo Labsystems) at 630 nm.

The cytotoxic activities against human cervical carcinoma (HeLa) and human lung carcinoma (A549) cell lines were evaluated by the SRB method. The antiviral activities of compounds of 1 and 2 against respiratory syncytial virus (RSV), herpes simplex virus (HSV), coxsackie B3 virus (CoxB3), enterovirus 71 (EV71), and H1N1 were determined by the CPE inhibition assay, according to established procedures. Ribavirin was used as a positive control.

2 RESULTS AND DISCUSSION

2.1 Crystal data

Crystal data for 1: C11H10O6, Mr 310.25, triclinic, cell a = 7.123(4) Å, b = 8.069(4) Å, c = 11.596(6) Å, α = 93.86(3)°, β = 93.56(3)°, γ = 102.58(3)°, V = 646.99 (505) Å³, Z 2, D = 1.593 g/cm³, F(000) = 324, μ = 1.14 mm⁻¹, final R = 0.0962 and wR = 0.2206 for 1408 observed reflections (I>2σ(I)).

The crystallographic data for 1 have been deposited at the Cambridge Crystallographic Data Center (CCDC NO. 1062826).

2.2 Structural identification

Compound 1 was obtained as yellow crystals. Its molecular formula of C11H10O6 (ten degrees of unsaturation) was determined by EI-MS analysis together with NMR data. 1H NMR (500 MHz, acetone-δ, δ ppm, J/Hz): 13.60 (111, s, 11.8), 6.80 (111, s, 11.4), 6.28 (111, d, J = 1.5 Hz, H-5), 6.17 (1H, d, J = 1.5 Hz, H-7), 2.78 (311, s, 11.1). 13C NMR (125 MHz, acetone-δ, δ ppm, J/Hz): 183.5 (C, C-9), 165.1 (C, C-6), 154.8 (C, C-8), 158.1 (C, C-10a), 153.9 (C, C-4a), 152.6 (C, C-3), 141.8 (C, C-2), 125.7 (C, C-11), 112.5 (C, C-1a), 103.9 (C, C-9a), 100.9 (C, C-14), 98.5 (C, C-7), 93.6 (CH, C-5), 13.9 (CH₃, C-11).

Compound 2 was obtained as yellow crystals. The molecular formula C11H10O6 (ten degrees of unsaturation) was determined by EI-MS analysis together with NMR data, 1H NMR (500 MHz, acetone δ, δ ppm, J/Hz): 13.58 (111, s, H-8), 6.32 (1H, d, J = 2.0 Hz, H-5), 6.16 (1H, d, J = 2.0 Hz, H-7), 2.73 (1H, s, H-1), 13C NMR (125 MHz, acetone δ, δ ppm, J/Hz):
Two polyhydroxy xanthones and their antiviral activity from gorgonian coral-derived fungus *Arthrinium* sp.

<table>
<thead>
<tr>
<th>Compd</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
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<td>&gt;200</td>
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<td>200</td>
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<td>50</td>
<td>&gt;200</td>
<td>100</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.030</td>
<td>0.022</td>
<td>0.313</td>
<td>0.156</td>
<td>0.025</td>
<td>1.250</td>
<td>0.078</td>
<td>0.078</td>
<td>0.156</td>
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</table>


Data were expressed in MIC values (μmol/L); Ciprofloxacin was used as a positive control.

<table>
<thead>
<tr>
<th>Compd</th>
<th>RSV</th>
<th>HSV</th>
<th>CoxB3</th>
<th>EV71</th>
<th>H1N1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} (μmol/L)</td>
<td>ST</td>
<td>IC_{50} (μmol/L)</td>
<td>ST</td>
<td>IC_{50} (μmol/L)</td>
</tr>
<tr>
<td>1</td>
<td>15.1</td>
<td>35.7</td>
<td>21.4</td>
<td>19.7</td>
<td>16.6</td>
</tr>
<tr>
<td>2</td>
<td>8.1</td>
<td>9.6</td>
<td>—</td>
<td>—</td>
<td>62.5</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>78</td>
<td>&gt;128</td>
<td>313</td>
<td>&gt;32</td>
<td>39</td>
</tr>
</tbody>
</table>

No activity, ST—TC_{50}/IC_{50}.

Compounds 1 and 2 were evaluated for nine pathogenic bacteria (Table 1)[17]. Only 2 showed antimicrobial activity against *M. luteus* with an MIC value of 50 μmol/L. Besides, compounds 1 and 2 were tested for their cytotoxic activities against HeLa and A549 cell lines at a concentration of 100 μmol/L, with weak cytotoxic activities were observed. More importantly, in our current antiviral research (Table 2), 1 showed strong antiviral activity against RSV, HSV, CoxB3, and EV71 with the MIC values of 15.1, 21.4, 16.6, and 27.3 μmol/L, respectively, which was stronger than that of the positive control Ribavirin (MIC 78, 313, 39, and 418 μmol/L). Especially, 1 showed significant anti HSV and anti EV71 activity *in vitro*, which was almost 11 times stronger than that of the positive control Ribavirin. Compound 2 also showed various degrees of antiviral activity against RSV, EV71, and CoxB3, especially exhibited potent anti EV71 (IC_{50} 14.43 μmol/L), which was about 26 times stronger than Ribavirin (IC_{50} 418.00 μmol/L).

3 CONCLUSION

In conclusion, two polyhydroxy xanthones derivatives (1 and 2) were isolated from the gorgonian coral derived *Arthrinium* sp. fungus for the first time. The structure of 1 was further determined by the single crystal X-ray diffraction analysis for the first time. Interestingly, compounds 1 and 2 showed potent antiviral activities against RSV, HSV, CoxB3, and EV71 and represent a promising new class of antiviral agent. Unfortunately, limited amounts of 1 and 2 prevented further structural modification as well as structure activity relationships (SAR) studies. Further studies on polyhydroxy xanthones, including the synthesis of analogues and SAR, are in progress.

REFERENCES


