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ISOLATION, STRUCTURE ELUCIDATION, CRYSTAL STRUCTURE, AND BIOLOGICAL ACTIVITY OF A MARINE NATURAL ALKALOID, VIRIDICATOL

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Functionalized 4-arylquinolin-2(1*H*)-ones constitute a valuable class of biologically active molecules, including several fungal metabolites such as viridicatin [1] and 3-*O*-methylviridicatin [2]. In the search for new bioactive natural products from marine microorganisms, we have embarked on the study of the metabolites of marine fungi, including those from mangroves from the South China Sea, and this has yielded a lot of novel and bioactive secondary metabolites [3–6]. In this paper, we describe the isolation, structure elucidation, crystal structure, and biological activity of the marine 4-aryl-quinolin-2-one alkaloid, viridicatol (**1**).

Viridicatol was isolated as colorless crystals. The molecular formula $C_{15}H_{11}NO_3$ (eleven degrees of unsaturation) was determined by EI-MS analysis together with NMR data. The numbers of hydrogen and carbon atoms observed in the ^1H and ^{13}C NMR spectra were in agreement with the molecular formula. In the ^{13}C NMR spectrum and the DEPT spectrum, one carbonyl carbon signal (δ_C 158.3) and 14 olefinic carbon signals, including eight methine signals (δ_C 129.5, 126.5, 124.5, 122.2, 120.4, 116.8, 115.3, 114.7), were observed. In the ^1H NMR spectrum, there are three exchangeable proton signals observed at δ_H 12.16, 9.55, and 9.06. The proton signals and the coupling constants at δ_H 7.34 (1H, dd, J = 8.0, 1.0 Hz), 7.32 (1H, ddd, J = 8.0, 8.0, 1.0 Hz), 7.09 (1H, dd, J = 8.0, 1.0 Hz), and 7.07 (1H, ddd, J = 8.0, 8.0, 1.0 Hz) indicated the presence of an *ortho*-disubstituted benzene system. Another four proton signals at δ_H 7.29 (1H, dd, J = 8.0, 8.0 Hz), 6.82 (1H, m), 6.72 (1H, ddd, J = 8.5, 1.5, 1.0 Hz), and 6.71 (1H, dd, J = 2.5, 1.5 Hz) were assigned to the 1,3-disubstituted benzene ring. According to the above data, the structure of the title alkaloid was determined as viridicatol (**1**) [7, 8]. To the best of our knowledge, this is the first time viridicatol was reported as the secondary metabolite of mangrove endophytic fungus.

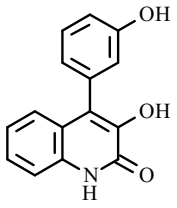

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TABLE 1. Selected Bond Lengths (Å) and Bond Angles (°)

Bond	Dist.	Bond	Dist.	Bond	Dist.
O1-C9	1.239 (2)	O2-C8	1.352 (2)	O3-C4	1.356 (2)
N1-C9	1.352 (2)	N1-C10	1.390 (2)	C6-C7	1.495 (2)
Angle	(°)	Angle	(°)	Angle	(°)
C9-N1-C10	124.98 (15)	O3-C4-C3	117.81 (16)	O3-C4-C5	122.21 (17)
C3-C4-C5	119.97 (17)	O2-C8-C7	120.72 (16)	O2-C8-C9	116.64 (16)
O1-C9-N1	121.76 (17)	O1-C9-C8	122.60 (18)	N1-C9-C8	115.61 (16)

TABLE 2. Hydrogen Bond Lengths (Å) and Bond Angles (°)

D–H···A	d (D–H)	d (H···A)	d (D···A)	∠ DHA
O2–H2···O1i	0.82	1.97	2.672(2)	142.5
Intra O2–H2···O1	0.82	2.30	2.7365(19)	114.2
O2–H2···O1ii	0.82	1.90	2.719(2)	173.4
N1–H1A···O4iii	0.86	2.19	3.048(2)	171.3
C16–H16C···O2	0.96	2.59	3.439(3)	148
Intra C17–H17C···O4	0.96	2.44	2.770(4)	100

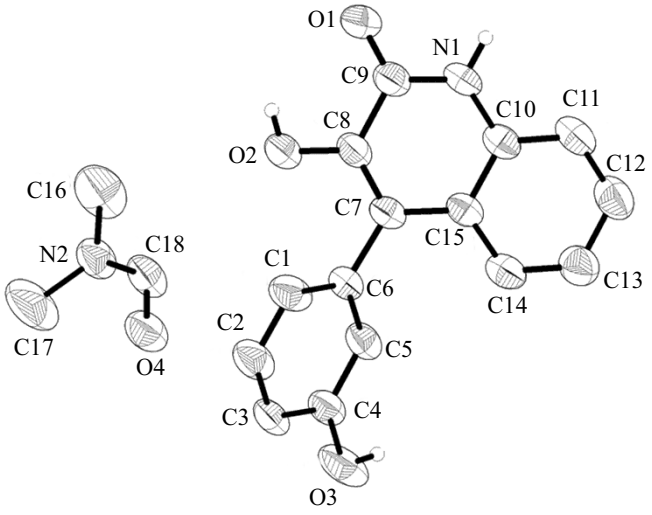

Fig. 1. Molecular structure of viridicatol solvate with DMF at 30% ellipsoid probability.

Crystals of viridicatol were obtained by slow evaporation of DMF solvent. X-ray structural analysis indicates that the asymmetric unit contains one title molecule and one DMF molecule. An ORTEP drawing of the title compound showing the molecular conformation and atom-labeling scheme is depicted in Fig. 1. The selected bond lengths and angles for the title compound are listed in Table 1. It is worthy noting that the bond distance of C1–O9 is 1.239 (2) Å, which shows typical features of C=O double bonds [9], while the bond distances of N1–C9 and N1–C10 are 1.352 (2) Å and 1.390 (2) Å, respectively, indicating a single bond character. The above results confirm that the quinolin-2(1H)-one unit of viridicatol is in the lactam form and not in the tautomeric quinolin-2-ol conformation. Other bond lengths and bond angles of the title compound are in normal ranges and close to the corresponding values observed in other similar compounds [10]. In the crystal structure, the deviations of the N1, C7, C8, and C9 atoms from the edge-fused benzene ring plane are 0.0069, 0.0060, 0.0114, and 0.0164 Å, respectively, indicating that the ten atoms C7, C8, C9, N1, C10, C11, C12, C13, C14, and C15 are nearly coplanar. The results are different from other quinolin-2(1*H*)-one compounds [10]. Furthermore, two benzene rings make a dihedral angle of 66.2 (6)°.

Analysis of the crystal packing indicates that the tile molecules and solvent molecules take on N-H···O and O-H···O hydrogen bonds and C-H···O interactions in the unit cell (for details, see Table 2), which result in the formation of a two-dimensional layered structure. Within this assembly, the two tile molecules form a dimer through the formation of O2-H2···O1 hydrogen bonds (O···O: 2.672 (3) Å). Meanwhile, the dimers are connected to two DMF molecules via O3-H3···O4 and N1-H1A···O4 hydrogen bonds, forming four-component aggregates. It is noted that the adjacent tetramers are further linked to each other through the formation of weak C16-H(16C)···O2 interactions (C···O: 3.493 (3) Å), generating an infinite one-dimensional chain. Furthermore, the neighboring 1-*D* chains pack on each other via an edge-to-face type of C-H···π stacking interactions, which further consolidates the crystal packing. The characteristics of the C-H···π interactions are H···π distance 3.655 (3) Å and C13-H13···π angleis 130°. For clarity, Cg (2) is the C-H···π acceptor ring [C1-C2-C3-C4-C5-C6], and C13-H13 [1] acts as the C-H···π donor.

The crystallographic data for viridicatol have been deposited at the Cambridge Crystallographic Data Centre (CCDC No. 738934).

In the primary bioassay, the title compound showed cytotoxicity toward KB, KBv200, A549, hepG2, MCF7, K562, SMMC7721, and SGC7901 tumor cell lines with IC_{50} values of 25.0, 16.5, 60.0, 85.0, 45.0, 25.0, 80.2, and 80.0 μg/mL, respectively.

Melting point was determined on an X-4 micro-melting point apparatus and is uncorrected. Mass spectra were measured with a VG-ZABHS mass spectrometer. NMR spectra were performed on a Varian Inova 300 NB NMR spectrometer using TMS as an internal standard. X-ray data were generated on a Bruker Smart 1000 CCD system diffractometer.

Fungus Material and Culture Conditions. The fungus *Penicillium* sp. was isolated from the the bark of *Acanthus ilicifolius* Linn. (endophyte) of Shenzhen, Guangdong. This isolate was deposited in the School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, China. Starter cultures (from Prof. Shi-ning Zhou) were maintained on cornmeal seawater agar. Plugs of agar supporting mycelial growth were cut and transferred aseptically to a 500 mL Erlenmeyer flask containing 300 mL of liquid medium (glucose 1%, peptone 0.2%, yeast extract 0.1%, NaCl 0.25%, pH 7.0). The flask was incubated at 30°C on a rotary shaker for 3 days. The mycelium was aseptically incubated at 30°C for 5 weeks. The cultures (70 L) were filtered through cheesecloth. The filtrate was concentrated to 3 L below 55°C and extracted several times by shaking with twofolds volumes of ethyl acetate. The combined extracts were concentrated to give a yellow oil and chromatographed on silica gel using gradient elution from petroleum to ethyl acetate to obtain the compound viridicatol (**1**) (110.0 mg) from the 100% ethyl acetate–petroleum ether fraction.

Viridicatol (1): colorless crystals. mp 253°C. ^{1}H NMR (300 MHz, acetone-d_6, δ, ppm, J/Hz): 12.16 (1H, s, OH), 9.55 (1H, s, OH), 9.06 (1H, s, NH), 7.34 (1H, dd, J = 8.0, 1.0), 7.32 (1H, ddd, J = 8.0, 8.0, 1.0), 7.29 (1H, dd, J = 8.0, 8.0), 7.09 (1H, dd, J = 8.0, 1.0), 7.07 (1H, ddd, J = 8.0, 8.0, 1.0), 6.82 (1H, m), 6.72 (1H, ddd, J = 8.5, 1.5, 1.0), 6.71 (1H, dd, J = 2.5, 1.5).

^{13}C NMR (75 MHz, acetone-d_6, δ, ppm): 158.3 (C), 157.3 (C), 142.2 (C), 134.9 (C), 133.1 (C), 129.5 (CH), 126.5 (CH), 124.5 (CH), 124.2 (C), 122.2 (CH), 120.9 (C), 120.4 (CH), 116.8 (CH), 115.3 (CH), 114.7 (CH).

ESI-MS (*m*/*z*, I_{rel}, %): 254.1 (98) $[M + H]^+$, 236.2 (100), 208.1 (15), 198.1 (13), 132.1 (15). Mass spectrum ESI-MS (*m*/*z*, I_{rel}, %): 252.2 (9) $[M - H]^-$, 234.2 (25), 224.2 (100), 208.2 (15), 181.2 (10).

It crystallized in the triclinic system, space group P-1 with a = 9.0294 (15) Å, b = 9.7857 (16) Å, c = 10.649 (3) Å, α = 102.402 (3)°, β = 103.878 (3)°, γ = 109.372 (2)°, $C_{18}H_{17}N_2O_4$, Mr = 325.34, V = 816.0 (3) Å^3, *Z* = 2, Dc = 1.324 g/cm^3, F (000) = 342, μ = 0.095 mm^{-1}, final R = 0.0494, and wR = 0.1336 for 6125 observed reflections (I > 2 σ(I)). The crystallographic data for viridicatol deposited at the Cambridge Crystallographic Data Centre (CCDC No. 738934) can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44 1223 336033; email: deposit@ccdc.cam.ac.uk).

Crystallographic Data Collection and Structure. Crystals of the title compound suitable for single crystal X-ray diffraction analysis were obtained by slow evaporation of DMF. A colorless plate of the title compound, approximately 0.45 × 0.29 × 0.18 mm, was mounded on a glass fiber. The unit cell and data collection were performed on a Bruker SMART 1000CCD system diffractometer with graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). At 291 (2) K, a total of 6125 reflections (R_{int} = 0.0174) was collected in the range 2.33 ≤ θ ≤ 25.50°, of which 3018 observed reflections with $I \geq 2\sigma$ (I) were used in the succeeding structure determination and refinements. All empirical absorption corrections were applied by using the SADABS program [11]. The crystal structure was solved using direct method, which yielded the positions of all nonhydrogen atoms. These were refined first isotropically and then anisotropically. All the hydrogen atoms of the title compound were placed in calculated positions with fixed isotropic thermal parameters and included in structure factor calculations in the final stage of full-matrix least-squares refinement. All calculations were performed using the SHELXTL system of computer

programs [12]. The final refinement converged at $R = 0.0448$, $wR = 0.1090$ ($w = 1/[\sigma^2 (Fo^2) + (0.0539P)^2 + 0.20P]$, where $P = (Fo^2 + 2Fc^2)/3$; S = 1.033; (Δ/σ)max = 0.000. The largest peak and deepest hole on the final difference Fourier map were 0.155 and -0.213 e/Å^3, respectively.

Cytotoxic Activity. The title compound was tested *in vitro* against KB, KBv200, A549, hepG2, MCF7, K562, SMMC7721, and SGC7901 cell lines. All eight 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 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′s paired *t* test.

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