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NOTES

Aureoquinone, a New Protease Inhibitor from *Aureobasidium* sp.

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Searching for new bioactive metabolites of basidiomycetes we isolated recently the new metabolite aureoquinone (**1**) (Fig. 1) as an inhibitor of several proteases from surface cultures of *Aureobasidium* sp. grown on a synthetic medium. The strain was originally isolated from a fruiting body of *Crepidotus* sp. collected in the *Picea*-forest near Radison (Quebec, Canada). *Aureobasidium* sp. forms smooth colonies with a dark brown colour covered with slimy masses of conidia on malt agar. The mycelium displays vegetative hyphae of approximately 2.5 μm width. The conidiogenous hyphae are often constricted at the septa with up to 5 μm length. The blastoconidia are hyaline, slender cylindrical to ellipsoidal and possess variable shape and size often 3~4/1 to 5/2 μm. These characteristics suggested that the strain is a representative of the genus *Aureobasidium*[1].

It was propagated as inoculum on agar plates at 24°C for 15 days using a medium composed of (g/liter): malt extract 40, yeast extract 4, agar 15, deionized water, pH 6.0. Thereafter 0.5~1 cm^2 areas of the agar plate cultures were used to inoculate 100 ml of a liquid surface culture medium in 500 ml Erlenmeyer flasks composed as follows (g/liter): malt extract 20, glucose 10, yeast extract 1, pH 6.0. Cultivation was carried out for three weeks at 24°C.

Aureoquinone (**1**) was extracted from 60 liters of surface culture of *Aureobasidium* sp. by 20 liters of ethyl acetate. The extract was dried and evaporated to dryness. The residue (5 g) was subjected to column chromatography on silica gel 60 (0.063~0.1 mm, Merck, column 600×30 mm). Stepwise elution occurred by a gradient of $CHCl_3$ - MeOH (1 : 0, 9 : 1, 8 : 2, 1 : 1, v/v) yielding 10 fractions. Fraction 8 containing the major amount of **1** as detected by brownish to reddish color and intensity of *m/z* 249 during ESI-MS. **1** was purified by repeated chromatography on silica gel 60. Yield 60 mg (brown needles). The physico-chemical properties of **1** are shown in Table 1.

The molecular weight and the chemical formula ($C_{12}H_{10}O_6$) of the novel metabolite **1** were determined from the HREI mass spectrum (Table 1). Supporting evidence

Fig. 1. Structure of aureoquinone (**1**).

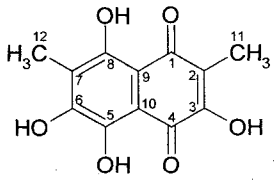


Table 1. Physico-chemical properties of aureoquinone (**1**).

Melting point[a]	268 - 269 °C
Appearance	brownish needles
Chemical formula	$C_{12}H_{10}O_6$
HREI-MS *m/z*	250.0480 $[M]^+$; calcd. 250.0477
IR (KBr) cm^{-1}	3360, 2920, 1620, 1550, 1472, 1318, 1288
UV λ^{MeOH}_{max} (ε)	234 (16200), 328 (12000)

[a] Büchi Melting Point Apparatus B540

Table 2. Assignments ^{1}H and ^{13}C NMR chemical shift and coupling data of aureoquinone (**1**) (in DMSO-d_6, chemicals shifts in ppm, (s: singlet)).

Position	δ_C	δ_H
C-1	191.5	
C-2	120.9	
C-3	153.5	
C-4	191.5	
C-5	172.3	13.8 (1H, s, OH)
C-6	165.2	
C-7	120.9	
C-8	172.3	13.8 (1H, s, OH)
C-9	102.5	
C-10	108.4	
C-11	8.3	2.0 (3H, s, CH_3)
C-12	8.3	2.0 (3H, s, CH_3)

The DMSO-d_6 ^{1}H and ^{13}C peaks at 2.49 ppm and 39.5 ppm, respectively, were used as reference signals.

was supplied by the negative ion mode ESI-MS due to *m/z* 249.1 ($[M-H]^-$).

The chemical constitution of **1** was characterized by one- and two-dimensional NMR experiments (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC). The ^{1}H NMR spectrum showed the presence of two methyl groups at 2.0 ppm. Two additional hydroxyl protons appeared at 13.8 ppm suggesting the formation of H-bonds to the quinone carbonyls.

The ^{13}C NMR and DEPT spectra displayed only eight carbon atoms because of the symmetrical arrangement of functional groups in the upper and the lower part of the molecule, respectively.

The assignments of ^{1}H and ^{13}C chemical shift data of **1** are shown in Table 2. The structure of aureoquinone as shown in Fig. 1 was confirmed unambiguously by X-ray crystallographic analysis. **1** forms a layer structure stabilized by hydrogen bonds in the solid state (Fig. 2).

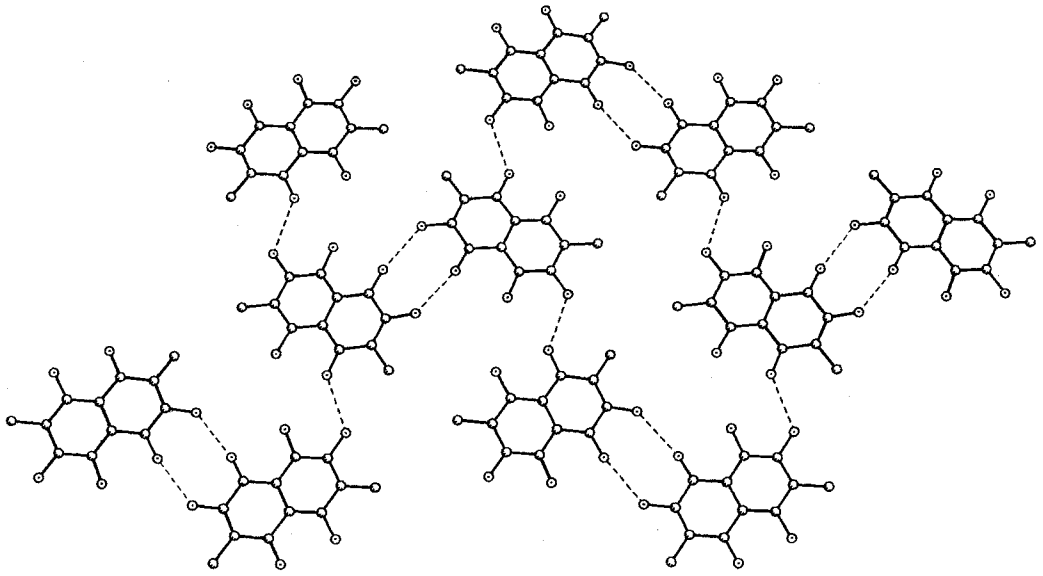
The intensity data of aureoquinone (**1**) was collected on a Nonius KappaCCD diffractometer, using graphite-monochromated Mo-K_α radiation. Data were corrected for Lorentz and polarization effects, but not for absorption[2].

The structure was solved by direct methods (SHELXS)[2] and refined by full-matrix least squares techniques against Fo^2 (SHELXL-97)[3]. The hydrogen atoms of compound **1** were located by difference Fourier synthesis and refined isotropically. All non-hydrogen atoms were refined anisotropically[3]. XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

Crystal Data of aureoquinone (**1**)[4]: $C_{12}H_{10}O_6$, Mr= 250.20 $gmol^{-1}$, dark red prism, size 0.34×0.32×0.26 mm^3, monoclinic, space group $P2_1/c$, a=15.9429(6), b=19.390(1), c=6.8944(4) Å, β=95.398(3)°, V=2121.8(2) $Å^3$, T=−90°C, Z=8, $\rho_{calcd.}$=1.566 gcm^{-3}, μ(Mo-K_α)= 1.28 cm^{-1}, F(000)=1040, 7366 reflections in h(−19/19), k(−22/24), l(−8/8), measured in the range 3.15°≤Θ≤ 26.35°, completeness Θ_{max}=99.6%, 4314 independent reflections, R_{int}=0.066, 2278 reflections with $F_o>4\sigma(Fo)$, 405 parameters, 0 restraints, R_{1obs}=0.064, wR^2_{obs}=0.134, R_{1all}=0.146, wR^2_{all}=0.166, GOOF=1.000, largest difference peak and hole: 0.248/−0.290 e $Å^{-3}$.

Metabolite **1** inhibited a series of proteases such as trypsin (IC_{50}: 11.4 μg/ml), papain (IC_{50}: 14.5 μg/ml), thermolysin (IC_{50}: 17.8 μg/ml), collagenase (IC_{50}: 7.1 μg/ml) and zinc-protease (IC_{50}: 8.7 μg/ml). Detection of protease activity was carried out by BODIPY dye-labeled casein substrate assay with direct fluorescence

Fig. 2. Layer structure of aureoquinone (**1**) stabilized by H-bondings.



measurement[6].

Moreover, aureoquinone (**1**) displayed moderate antimicrobial activity against Gram-positive bacteria such as *Bacillus subtilis* ATCC 6633 (MIC: 120 μg/ml)[7].

Experimental

Mass spectra (HREI-MS) were recorded on a double-focusing sector field mass spectrometer AMD-402 (AMD Intectra; Harpstedt, Germany) and a quadrupole mass spectrometer Quattro 400 (Electrospray ionization MS; Fisons; VG Biotech; Altrincham, U.K.). ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance DRX 500 spectrometer. UV-VIS spectra were measured in methanol using a Beckmann instrument DU 600. IR spectra were recorded in KBr (IR-470 spectrometer; Shimadzu, Japan).

Acknowledgements

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