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ANTIMICROBIAL ACETOPHENONE AND PHENALENONE DERIVATIVES FROM A SOIL-DERIVED FUNGUS *PENICILLIUM VERRUCISPORUM* JX1

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Abstract – A new acetophenone derivative, named veracetophenone (**1**), along with six known compounds communol F (**2**), clavatol (**3**), (+)-scleroquinone (**4**), (–)-trypethelone (**5**), (–)-sclerodin (**6**), and (–)-scleroderolide (**7**), were isolated from a soil-derived fungus *Penicillium verrucisporum* JX1. Their structures were identified on the basis of spectral analysis of 1D, 2D-NMR, and HRESIMS. The configuration of **4** was depicted based on spectroscopic data as well as optical property. Their antibacterial and antifungal activities were also evaluated.

Natural products or natural product-derived compounds, nowadays, still play an important role in drug discovery.[1,2] As fungus is a prolific producer of natural products with a broad diversity of skeletons and bioactivities, many attentions were refocused on it after the finding and activation of biosynthetic gene clusters in fungal genome.[3,4] During our ongoing investigation of active scaffolds, a soil-derived fungus, *Penicillium verrucisporum* JX1, attracted our attention owing to its antimicrobial activity during the preliminary screening tests. In order to discover the bioactive constituents, a scale-up fermentation (12 L) of strain JX1 was performed, followed by extraction and separation, to yield a new acetophenone derivative (**1**), together with two known acetophenone derivatives communol F (**2**) and clavatol (**3**) as well as four known phenalenone derivatives (+)-scleroquinone (**4**), (–)-trypethelone (**5**), (–)-sclerodin (**6**), and (–)-scleroderolide (**7**) (Figure 1). Antimicrobial bioassays indicated their potential antibacterial and antifungal activities, which were coincident with the literature.[5] This study also highlights the first reported antifungal activity of communol F[6] (**2**) and antimicrobial activity of (+)-scleroquinone (**4**). In this paper, the isolation, structural identification, and biological tests of these compounds were described.

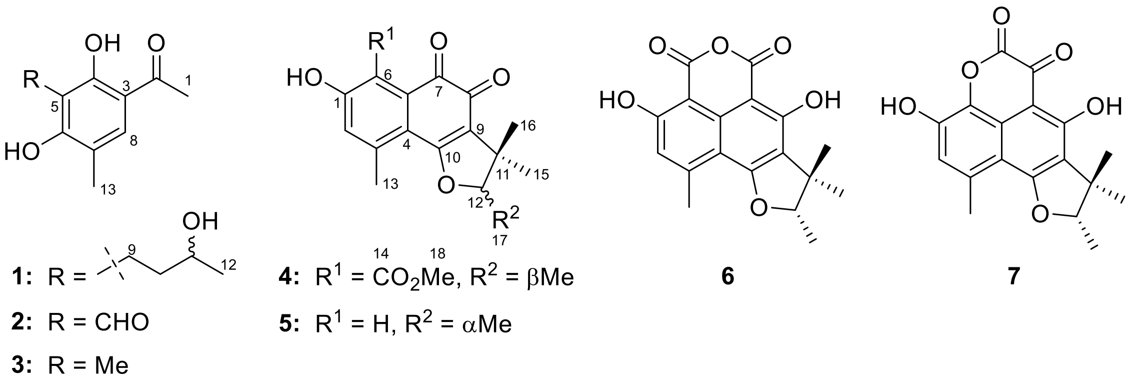

Figure 1. Chemical structures of compounds **1-7**

Compound **1** had the molecular formula of $C_{13}H_{18}O_4$ on the basis of the HRESIMS data at *m/z* 237.1131 [M - H]$^-$ (calcd for 237.1121, $C_{13}H_{17}O_4$). The ^{13}C and ^1H NMR data (Table 1) of **1** were very similar with those of clavatol (**3**), with the presence of the additional signals for an oxygenated methine (δ_C 65.5/δ_H 3.51) and two methylenes (δ_C 37.8/δ_H 1.48 and δ_C 18.8/δ_H 2.54, 2.63) in **1** based on the analysis of ^1H, ^{13}C NMR and HSQC spectra. Further investigations on the 2D NMR spectra, including the COSY cross peaks of H_2-9 (δ_H 2.54, 2.63)/H_2-10 (δ_H 1.48), H_2-10/H-11 (δ_H 3.51), and H-11/H_3-12 (δ_H 1.06), and the HMBC correlations of H_2-9, H_2-10 and H_3-12 with C-11 (δ_C 65.5), H_3-12, H-11, and H_2-9 with C-10 (δ_C 37.8), as well as H-11 and H_2-10 with C-9 (δ_C 18.8), suggested a 3-hydroxybutyl substituent in **1** (Figure 2). And it was connected to C-5 (δ_C 114.7) based on HMBC correlations from H_2-9 and H_2-10 to C-5. A downfield hydroxyl group (δ_H 12.99) correlated with C-3 (δ_C 111.3), C-4 (δ_C 160.7), and C-5 in HMBC spectrum was thereby determined to be attached at C-4. Similarly, a methyl group (δ_C 16.3/δ_H 2.10) was deduced to be sited at C-7 (δ_C 116.5) based on its HMBC correlations with C-7, C-8 (δ_C 130.3). The unassigned carbon signal (δ_C 162.8) was confirmed as C-6 by HMBC correlation with H-8 (δ_H 7.48). H-8 was correlated with C-2 (δ_C 202.1), C-13 (δ_C 16.3), and C-4 as well. It was apparent that the ketone group [a keto (δ_C 202.1) and a methyl group (δ_C 26.0/δ_H 2.49)] was linked to C-3 according to the HMBC correlations of H_3-1 (δ_H 2.49) with C-2 and C-3. Hence, the structure of compound **1** was elucidated and named as veracetophenone (Figure 1).

Table 1. ^{13}C and ^{1}H NMR spectroscopic data of compounds **1** and **4**

Position	**1**$^{a)}$			**4**			
	δ_C	type	δ_H (mult., J in Hz)	$\delta_C$$^{b)}$	type	$\delta_H$$^{b)}$ (mult., J in Hz)	$\delta_H$$^{c)}$ (mult., J in Hz)
1	26.0	Me	2.49 (s)	163.2	C		
2	202.1	C		126.0	CH	6.82 (s)	6.97 (s)
3	111.3	C		143.5	C		
4	160.7	C		115.2	C		
5	114.7	C		131.8	C		
6	162.8$^{d)}$	C		126.6	C		
7	116.5	C		184.1	C		
8	130.3	CH	7.48 (s)	175.4	C		
9	18.8	CH_2	2.54 (m); 2.63 (m)	122.1	C		
10	37.8	CH_2	1.48 (m)	175.6	C		
11	65.5	CH	3.51 (m)	43.8	C		
12	23.3	Me	1.06 (d, 6.1)	94.4	CH	4.72 (q, 6.6)	4.63 (q, 6.7)
13	16.3	Me	2.10 (s)	22.7	Me	2.54 (s)	2.58 (s)
14				170.6	C		
15				20.5	Me	1.23 (s)	1.25 (s)
16				25.9	Me	1.40 (s)	1.42 (s)
17				14.9	Me	1.48 (d, 6.7)	1.44 (d, 6.6)
18				53.1	Me	3.90 (s)	3.87 (s)
OH			12.99 (s)				

a) Measured in DMSO-d_6 at 125 MHz (^{13}C) and 500 MHz (^{1}H); b) Measured in CD_3OD at 200 MHz (^{13}C) and 800 MHz (^{1}H); c) Measured in $CDCl_3$ at 500 MHz (^{1}H); d) deduced by HMBC correlations

Compound **4** displayed a quasimolecular ion at *m/z* 353.0993 [M + Na]$^+$ (calcd for 353.0996, $C_{18}H_{18}O_6Na$) in the HRESIMS, consistent with the molecular formula $C_{18}H_{18}O_6$. Analysis of 1D, 2D NMR (Figure 2) as well as HRESIMS and EIMS spectra of compound **4** helped to determine the structure as scleroquinone,[7] whose carbon data and optical value were not reported yet. Hence, the carbon chemical shifts of compound **4** were assigned for the first time (Table 1). The stereochemistry at C-12 could be deduced as *R*-configuration on the basis of its optical value of + 156.5, due to the right rotation of such frameworks was in *R*-configuration and *vice versa*.[8-13] The configuration of compound **4** was thus depicted and named as (+)-scleroquinone (Figure 1).

Five other known compounds were identified as communol F (**2**),[6] clavatol (**3**),[14] (–)-trypethelone (**5**),[12] (–)-sclerodin (**6**),[15] and (–)-scleroderolide (**7**),[13] respectively, by comparison with MS and NMR data reported in the literatures.

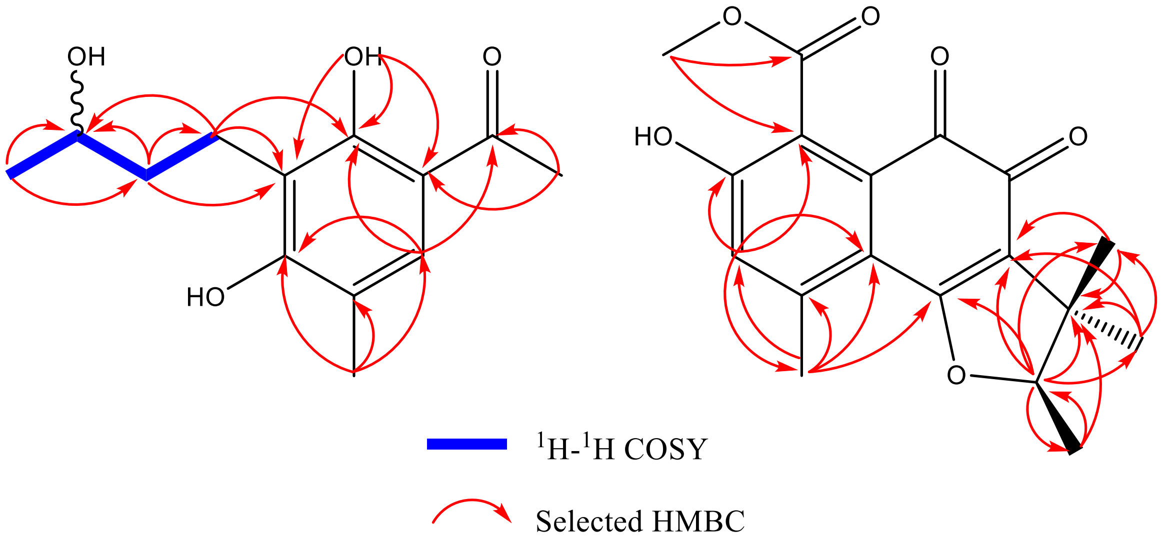

Figure 2. ^{1}H-^{1}H COSY and key HMBC correlations for compounds **1** and **4**

The biological activity tests *in vitro* were performed using two bacterial strains, *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative), and a fungus strain of *Saccharomyces cerevisiae* (Table 2). Compound **1** showed moderate inhibitory effect on *B. subtilis* (MIC: 64 μg/mL). Compound **2** displayed apparent inhibition against both *B. subtilis* and *S. cerevisiae* with the same MIC value of 16 μg/mL, as well as moderate inhibition against *E. coli* (MIC: 64 μg/mL). From the point of view of structure-activity relationship, the aldehyde group in **2** might be the key substituent for the antibacterial and antifungal bioactivity. Moreover, compound **4** was firstly reported its moderate inhibition against all three tested strains.

Table 2. *In vitro* antimicrobial activities of compounds **1-7** (MIC: μg/mL)

Compound	Test microorganisms		
	B. subtilis	*E. coli*	*S. cerevisiae*
1	64	>256	>256
2	16	64	16
3	32	128	128
4	64	128	32
5	128	>256	64
6	64	>256	128
7	32	128	64
Gentamicin	1	2	-
Amphotericin B	-	-	2

EXPERIMENTAL

General Experimental Procedures

Optical rotation was obtained using a Rudolph Research Autopol III automatic polarimeter. UV spectra were measured with a Shimadzu 2401A spectrophotometer. 1D and 2D NMR spectra were recorded on Bruker AVANCE AV500 and Bruker AVANCE 800 AV spectrometer with $SiMe_4$ as internal standard. Liquid chromatography-mass spectrometry (LC-MS) was conducted with an Agilent 6120 Quadrupole MSD mass spectrometer. EIMS was recorded on a QP2010 mass spectrometer. High resolution electrospray mass (HRESIMS) data were performed with an Agilent Technologies 6210 LC/TOF mass spectrometer. Preparative HPLC was performed on a Shimadzu LC-6AD equipped with PDA detector and a Develosil ODS-HG Column (10.0 × 250 mm, 5 μm). Column chromatography (CC) was carried out on silica gel H (100-200 and 200-300 mesh, Qingdao Marine Chemical Ltd.), Gel ODS-A (50 μm, YMC), and Sephadex LH-20 (25-100 μm, Pharmacia). *Bacillus subtilis* (CGMCC 1.9086), *Escherichia coli* (CGMCC 1.8745) and *Saccharomyces cerevisiae* (CGMCC 2.3888) were purchased from China General Microbiological Culture Collection Center.

Fungal material

Strain JX1 was isolated from a soil sample collected from Jingxi conservation of Guangxi (Coordinates: N 22° 53' 29.58" and E 106° 29' 39.81"). Strain isolation procedure was mainly followed the reported protocol.[16] A soil sample was kept at 75 °C for 10 min to restrict nonsporulating bacteria. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3}) were prepared from adding 0.5 g of soil in 1.0 mL of sterile water. A 100 μL aliquot was spread on M2 agar (glucose, 4.0 g/L; malt extract, 10.0 g/L; yeast extract, 4.0 g/L; $CaCO_3$, 2.0 g/L; and agar, 15.0 g/L) plates with nalidixic acid (75 μg/mL). After incubation for 3-7 days at 28 °C, a number of colonies were observed on media plates, which were purified by subculturing on the M2 agar plates for several times. Strain JX1 was displayed 99.6% identity as *Penicillium verrucisporum* based on its morphological characteristics and ITS gene sequences (see Supporting Information, Figure S1. GenBank Accession No. MZ914656). A voucher strain has been deposited in our laboratory at –80 °C.

Fermentation, Extraction and Isolation

Strain JX1 was cultivated on M2 agar plates at 28 °C for 4 days. To prepare the seed culture, chunks of agar with the fully-grown strain were used to inoculate six 250 mL conical flasks, each containing 50 mL of medium Bran (corn flour, 40.0 g/L; glucose, 10.0 g/L; gluten powder, 5.0 g/L; $K_2HPO_4 \cdot 3H_2O$, 0.5 g/L; bran, 10.0 g/L; $CaCO_3$, 2.0 g/L; and $(NH_4)_2SO_4$, 1.0 g/L).[17] After 3 days of incubation (200 rpm, 28 °C), the seed cultures were transferred to inoculate 120 flasks (250 mL) containing 100 mL of medium Bran on a rotary shaker (200 rpm, 28 °C) for 7 days. The fermented whole broth (12 L) was centrifuged (8000 rpm, 15 min) at 4 °C and filtered through gauze to obtain filtrate and mycelia. The filtrate was subjected to a XAD-16N resin column (6 × 50 cm) and washed with water (5 L) and MeOH (5 L) successively. The

MeOH fraction was evaporated under reduced pressure to give 8.8 g of crude extract. The mycelia were extracted with MeOH (3 × 5 L) using sonication for 30 min. The MeOH solution was then evaporated to yield 27.8 g of crude extract. Both crude extracts were combined according to similar characters of main metabolites on the basis of HPLC and TLC analyses.

The combined crude extract (36.6 g) was subjected to a silica gel column (5 × 70 cm, 100–200 mesh) with a gradient elution of petroleum ether-EtOAc (9:1, 8:2, 7:3, 6:4 and 5:5; each 3 L) to afford five fractions (A-E) based on TLC analyses. Fraction A (19.7 g) was rechromatographed on a silica gel column (4 × 60 cm, 200-300 mesh) eluted with petroleum ether-EtOAc (95:5; 8 L) to give six subfractions (A1-A6). By recrystallization in EtOAc, subfractions A1, A2, and A4 yielded compounds **6** (832 mg), **2** (6.3 mg), and **3** (15.7 mg), respectively. Fraction B (2.2 g) was separated on a Sephadex LH-20 column (2 × 120 cm) eluted with CH_2Cl_2-MeOH (1:1) to give Fr. B1-B5. Subfraction B2 (267 mg) was further divided into six parts (Fr. B2-1-B2-6) using an ODS column (2.5 × 30 cm) elution with 90% aqueous MeOH. Fr. B2-2 (88 mg) was purified by semipreparative HPLC using 40% aqueous MeCN as eluant to yield compound **1** (3.7 mg). Similarly, fraction D (2.6 g) was first purified by a Sephadex LH-20 [CH_2Cl_2-MeOH (1:1)], and then loaded onto an ODS column (80% aqueous MeOH), and subsequently prepared using semipreparative HPLC (35% aqueous MeCN) to yield compounds **4** (1.3 mg), **5** (2.1 mg), and **7** (12.1 mg).

Compound **1**: pale powder; $[\alpha]_D^{25}$ + 6.1 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε): 218 (4.33), 283 (4.17), 330 (3.79) nm; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1; (+)-ESIMS *m/z*: 239.2 $[M + H]^+$, 261.2 $[M + Na]^+$; (–)-ESIMS *m/z*: 237.2 $[M - H]^-$; (–)-HRESIMS *m/z*: 237.1131 $[M - H]^-$ (calcd for 237.1121, $C_{13}H_{17}O_4$).

Antimicrobial activity assays

Two bacterial strains, *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative), and a fungus strain of *Saccharomyces cerevisiae* were carried out as model strains for biological activity according to the method[18] with minor adjustment. Bacterial strains and fungus strain were grown in liquid or on agar plates using LB medium (yeast extract, 5 g/L; peptone, 10 g/L; and NaCl 10 g/L) and YPDA medium (yeast extract, 10.0 g/L; peptone, 20.0 g/L; glucose, 20.0 g/L; and adenine sulfate, 0.03 g/L), respectively. Individual stains were grown in 5 mL of medium for 16 h at 37 °C with shaking (200 rpm). And then, the liquid cultures were diluted using homologous sterile liquid medium until OD_{600} 0.02 by calculation. Aliquots (200 μL) of each diluted culture were subsequently transferred into the individual well of a 96-well plate supplied with 2 μL of the samples dissolved in DMSO (final concentration range from 256 μg/mL to 2 μg/mL). After incubated at 37 °C for 16 h with shaking (200 rpm), the OD_{600} of each well was recorded whose value was normalized to the negative control (1% DMSO). Finally, a 5 μL aliquot of resazurin solution was added to each well and the plates were then incubated at 37 °C for another 3 h with shaking (200 rpm) to allow resazurin to convert to resorufin by viable cells. The minimal concentration of the test compound that inhibited visible microbial growth was defined as the MIC value. Commercial gentamicin and amphotericin B were set as the positive controls for bacteria and fungus, respectively.

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