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Anti-influenza Virus Polyketides from the Acid-Tolerant Fungus *Penicillium purpurogenum* JS03-21

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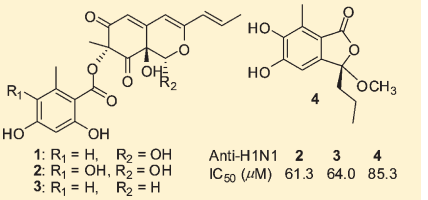
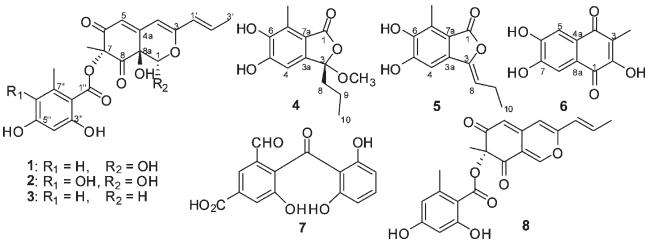
Supporting Information

ABSTRACT: Fractionation of the ethyl acetate extract of an acid-tolerant fungus, *Penicillium purpurogenum* JS03-21, resulted in the isolation of six new compounds, purpurquinones A−C (**1**−**3**), purpuresters A and B (**4** and **5**), and 2,6,7-trihydroxy-3-methylnaphthalene-1,4-dione (**6**), together with three known compounds, TAN-931 (**7**), (−)-mitorubrin (**8**), and orsellinic acid. The structures of **1**−**6** were elucidated primarily by NMR experiments. The absolute configurations of **1**−**4** were assigned on the basis of CD and NOESY data. Compounds **2**−**4** and **7** exhibited significant antiviral activity against H1N1, with IC_{50} values of 61.3, 64.0, 85.3, and 58.6 μM, respectively.



Influenza is a terrible disease that has caused many deaths in the past few years[1] and has attracted the attention of biologists and chemists seeking new approaches and medicines. However, many efforts in developing anti-influenza drugs have been frustrated by the rapid mutations of the influenza virus resulting in resistance. In our search for new drugs against the influenza virus from extremophiles, an acid-tolerant fungal strain JS03-21, identified as *Penicillium purpurogenum*, was isolated from the local red soil collected from Jianshui, Yunnan, China. The fungus *P. purpurogenum* has been reported to produce purpactins[2] and 3′,4′-dihydroxyisoflavone.[3] It has been reported that in general the secondary metabolites can be regulated by external pH.[4,5] In some cases, the pH effects were larger than carbon and nitrogen effects,[4] and certain silent genes can be activated by low pH.[6] Fungi capable of surviving at low pH conditions have attracted much attention in recent years for their diverse secondary metabolites with a range of biological activities,[7−9] such as the novel berkelic acid, with inhibitory activity against caspase-1 and MMP-3 and selective cytotoxicity against OVCAR-3 cells with a GI_{50} of 91 nM.[7,9] The secondary metabolites contained in the extract of strain JS03-21 fermented at pH 2 showed more chemical diversity and a stronger anti-H1N1 activity at 100 μg/mL than those grown at pH 7 (Figure S32). A chemical investigation of *P. purpurogenum* JS03-21 fermented at pH 2 resulted in the isolation of six new compounds, purpurquinones A−C (**1**−**3**), purpuresters A and B (**4** and **5**), and 2,6,7-trihydroxy-3-methylnaphthalene-1,4-dione (**6**). Three known compounds, TAN-931 (**7**),[10] (−)-mitorubrin (**8**),[11] and orsellinic acid,[12] were also isolated. Antiviral activity was observed for compounds **2**−**4** and **7** against influenza A (H1N1), with IC_{50} values of 61.3, 64.0, 85.3, and 58.6 μM, respectively.

Purpurquinone A (**1**) isolated as a yellow power was assigned the molecular formula $C_{21}H_{20}O_9$ on the basis of HRESIMS, consistent with 12 degrees of unsaturation. The IR spectrum showed the presence of a hydroxy (3134 cm^{-1}), a conjugated



carbonyl (1642 cm^{-1}), and a benzene nucleus (1589 and 1445 cm^{-1}). The ^1H NMR spectrum showed two *meta*-coupled aromatic protons at δ_H 6.16/6.26, four olefinic proton signals at δ_H 6.40/6.12/6.10/5.85, and three methyl signals at δ_H 2.47/1.85/1.78. These data, combined with the two conjugated ketone carbonyls at δ_C 200.0/191.7 (Table 1), suggested the presence of an azaphilone core and an orsellinic acid[12] moiety for **1**, analogous to (−)-mitorubrin (**8**).[11] The differences in NMR data between **1** and **8** could be explained by the replacement of a double bond in **8** with an oxygenated methenyl ($\delta_{H/C}$ 5.71/93.7) and an oxygenated quaternary carbon (δ_C 68.6) in **1**, indicating that **1** is the dihydroxylated derivative of **8**. The key HMBC correlations from OH-8a (δ_H 7.16) to C-4a/8a (δ_C 149.9/68.6), from OH-1 (δ_H 7.70) to C-8a/1 (δ_C 68.6/93.7), and from H-1 (δ_H 5.71) to C-4a/8 (δ_C 149.9/200.0) revealed that the dihydroxylation occurs at C_1 and C_{8a} (Figure 1). The NOESY correlations among H-1, H_3-7 (δ_H 1.78), and OH-8a suggested their *cis*- configuration (Figure S33). According to the literature, the absolute configuration at C-7 of the azaphilones can be determined from the specific rotation and the CD curve. The positive and the negative Cotton effects at the longest wavelength (340−380 nm) correspond to 7*S* and 7*R* absolute configurations, respectively.[13,14] The negative CD Cotton effect ($\Delta\varepsilon$ −6.2) at

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds 1−3 (^{1}H 600 MHz, ^{13}C 150 MHz, DMSO-d_6, TMS, δ ppm)

	1		2		3	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	93.7, CH	5.71 d (5.5)	93.7, CH	5.71 d (3.3)	69.8, CH_2	4.62 d (12.1), 3.87 d (12.6)
3	155.4, qC		155.4, qC		159.7, qC	
4	101.9, CH	5.85 s	101.9, CH	5.85 s	102.1, CH	5.92 s
4a	149.9, qC		149.8, qC		149.8, qC	
5	120.0, CH	6.12 s	120.0, CH	6.11 s	117.4, CH	6.04 s
6	191.7, qC		191.9, qC		191.8, qC	
7	84.8, qC		84.6, qC		84.3, qC	
7-CH_3	25.1, CH_3	1.78 s	25.1, CH_3	1.77 s	24.1, CH_3	1.81 s
8	200.0, qC		200.1, qC		200.4, qC	
8a	68.6, qC		68.6, qC		66.5, qC	
1′	126.7, CH	6.10 dd (15.5, 1.4)	126.4, CH	6.10 dd (15.4, 1.7)	125.8, CH	6.10 dd (15.4, 1.6)
2′	133.9, CH	6.40 m	133.8, CH	6.40 m	134.8, CH	6.45 m
3′	18.7, CH_3	1.85 dd (6.8, 0.9)	18.7, CH_3	1.86 dd (7.1, 1.1)	18.7, CH_3	1.85 dd (6.9, 1.6)
1″	168.2, qC		168.3, qC		168.4, qC	
2″	104.7, qC		104.6, qC		105.1, qC	
3″	163.9, qC		155.9, qC		163.4, qC	
4″	101.1, CH	6.16 d (1.9)	101.0, CH	6.11 s	101.2, CH	6.16 d (2.2)
5″	163.1, qC		152.5, qC		162.9, qC	
6″	111.9, CH	6.26 d (1.9)	137.4, qC		111.8, CH	6.24 d (1.4)
7″	143.2, qC		126.7, qC		143.0, qC	
7″-CH_3	23.6, CH_3	2.47 s	14.8, CH_3	2.38 s	23.3, CH_3	2.44 s
1-OH		7.70 d (5.5)		7.67 d (4.4)		
8a-OH		7.16 s		7.13 s		7.10 s
3″-OH		10.51 brs		9.88 brs		10.31 brs
5″-OH		10.35 brs		10.34 brs		10.31 brs
6″-OH				8.01 brs		

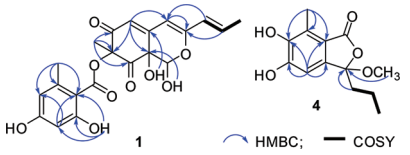

Figure 1. Selected $^{1}H-^{1}H$ COSY and HMBC correlations for **1** and **4**.

358 nm of **1** (Figure S1) clearly indicated the 7*R*-configuration. Thus, the structure of compound **1** was elucidated as (1*S*,7*R*,8a*S*)-1,8a-dihydroxy-7-methyl-6,8-dioxo-3-(*E*-prop-1-enyl)- 6,7,8,8a-tetrahydro-1*H*-isochromen-7-yl 2,4-dihydroxy-6-methylbenzoate.

The molecular formula of purpurquinone B (**2**) was determined to be $C_{21}H_{20}O_{10}$ on the basis of HRESIMS, with one oxygen atom more than that of **1**. Its 1D NMR spectra were similar to those of **1** except for the presence of an additional phenolic proton and relatively upfield shifts for C-3″, C-5″, C-6″, and 7″-CH_3 (Table 1). These data indicated that **2** is the C-6″ hydroxylated derivative of **1**, which was supported by the HMBC data. The key HMBC correlations of the only aromatic proton (δ_H 6.11) with C-2″ (δ_C 104.6), C-3″ (δ_C 155.9), C-5″ (δ_C 152.5), and C-6″ (δ_C 137.4) indicated that the additional hydroxy group (δ_H 8.01) was connected to C-6″. The NOESY correlations among H-1 (δ_H 5.71), H_3-7 (δ_H 1.77), and OH-8a (δ_H 7.13) suggested their *cis*- configuration (Figure S33). The similar CD Cotton effects at 361 nm ($\Delta\varepsilon$ −10.3), 320 nm ($\Delta\varepsilon$ +7.7), and 214 nm ($\Delta\varepsilon$ +5.5) (Figure S1) indicated that compound **2** has the same absolute configuration as **1**. Thus, the structure of **2** was elucidated to be (1*S*,7*R*,8a*S*)-1,8a-dihydroxy-7-methyl-6,8-dioxo-3-(*E*-prop-1-enyl)-6,7,8,8a-tetrahydro-1*H*-isochromen-7-yl 2,4,5-trihydroxy-6-methylbenzoate.

Compound **3** was obtained as a yellow power and has the molecular formula $C_{21}H_{20}O_8$ on the basis of HRESIMS, with one oxygen atom less than that of **1**. Analysis of the ^{1}H and ^{13}C NMR data for **3** revealed the presence of similar structural features to those found in **1**, except that an oxymethine (δ_H/δ_C 5.71/93.7) was replaced by a methylene (δ_H/δ_C 3.87, 4.62/69.8). In addition, C-3 was shifted downfield, whereas C-5 and C-8a were shifted upfield, indicating that **3** is the C-1 dehydroxy analogue of **1**. The NOESY correlations among OH-8a (δ_H 7.10) and H_3-7 (δ_H 1.81) suggested a *cis*-configuration between OH-8a and H_3-7 (Figure S33). The same (7*R*, 8a*S*)-configuration was deduced from the similar CD Cotton effects at 355 nm ($\Delta\varepsilon$ −4.6), 306 ($\Delta\varepsilon$ +3.9), and 221 nm ($\Delta\varepsilon$ +3.2) (Figure S1). Thus, the structure of compound **3** was determined as (7*R* and 8a*S*)-8a-hydroxy-7-methyl-6,8-dioxo-3-(*E*-prop-1-enyl)-6,7,8,8a-tetrahydro-1*H*-isochromen-7-yl 2,4-dihydroxy-6-methylbenzoate.

Compound **4** was assigned a molecular formula of $C_{13}H_{16}O_5$ on the basis of HRESIMS. The IR spectrum showed the presence of hydroxy groups (3138 cm^{-1}), a conjugated carbonyl group (1741 cm^{-1}), and benzene ring (1518 and 1459 cm^{-1}). Analysis of the ^{1}H, ^{13}C, and DEPT NMR data (Table 2) for **4** revealed the

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Table 2. ^{1}H and ^{13}C NMR Data for Compounds 4−6 (^{1}H 600 MHz, ^{13}C 150 MHz, DMSO-d_6, TMS, δ ppm)

	4		5		6	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	168.9, qC		167.5, qC		180.5, qC	
2					155.5, qC	
3	109.5, qC		143.7, qC		118.9, qC	
3-CH_3					9.0, CH_3	1.88 s
3-OCH_3	50.8, CH_3	2.90 s				
3a	121.6, qC		117.3, qC			
4	108.2, CH	6.69 s	108.6, CH	6.76 s	185.0, qC	
4a					126.6, qC	
5	151.5, qC		151.6, qC		113.2, CH	7.29 s
6	159.1, qC		158.1, qC		151.8, qC	
7	127.3, qC		123.7, qC		150.2, qC	
7-CH_3	9.7, CH_3	2.26 s	9.8, CH_3	2.28 s		
7a	115.1, qC		114.9, qC			
8	39.0, CH_2	2.05 m	109.9, CH	5.59 t (7.7)	112.9, CH	7.29 s
8a					123.5, qC	
9	17.0, CH_2	1.20 m, 0.99 m	19.5, CH_2	2.31 m		
10	14.3, CH_3	0.79 dd (7.4, 7.3)	14.8, CH_3	1.04 t (7.7)		
2-OH						10.23 brs
5-OH		9.95 s		10.04 s		
6-OH		9.97 s		10.56 s		10.54 brs
7-OH						10.62 brs

presence of three methyl groups, two methylenes, one methenyl, six aromatic carbons (one protonated), and one carbonyl carbon. These data accounted for an isobenzofuranone moiety with a pentasubstituted aryl ring.[15] Analysis of $^{1}H-^{1}H$ COSY correlations established a propyl moiety. The key HMBC correlations (Figure 1) from the methoxy protons and H-8 to C-3 (δ_C 109.5) indicated that methoxy and propyl are connected to C-3. The HMBC correlations from CH_3 (δ_H 2.26) and H-4 (δ_H 6.69) to C-6 (δ_C 159.1) and C-7a (δ_C 115.1) indicated the positions of the methyl and two hydroxy groups. The *S*-configuration of **4** was deduced from similar CD Cotton effects to pestaphthalides A[15] at 280 nm ($\Delta\varepsilon_{max}$ −0.3) and 212 nm ($\Delta\varepsilon_{max}$ +2.0) and the same optical direction as cytosporone E ($[\alpha]^{24}_{D}$ −90.7).[16] The structure of **4** was thus determined to be (*S*)-5,6-dihydroxy-3-methoxy-7-methyl-3propyl isobenzofuran-1(3*H*)-one.

Compound **5** was obtained as a yellowish powder and was assigned the molecular formula $C_{12}H_{12}O_4$ from HRESIMS. Analysis of the ^{1}H and ^{13}C NMR data (Table 2) for **5** revealed a structure similar to **4**, except that the C-3−C-8 fragment was replaced by an olefin. The relatively upfield shift of H-8/C-8 ($\delta_{H/C}$ 5.59/109.9) indicated the *Z*-configuration of the double bond at C-3 and C-8.[17] Thus, the structure of **5** was established as (*Z*)-5,6-dihydroxy-7-methyl-3-propylideneisobenzofuran-1(3*H*)-one.

Compound **6** was obtained as a red powder and was assigned the molecular formula $C_{11}H_8O_5$ on the basis of HRESIMS and NMR data (Table 2). The IR spectrum showed the presence of hydroxy groups (3134 cm^{-1}), conjugated carbonyl groups (1738 cm^{-1}), and a benzene ring (1569 cm^{-1}). The NMR spectra resonances displayed a methyl group, two quinoid carbonyls (δ_C 185.0/180.5), six sp^2 quaternary carbons (three oxygenated), and two sp^2 methines (Table 2), together accounting for a naphthoquinone structure. The two aromatic proton signals at δ_H 7.292/7.287 corresponded to two contrapuntal protons in a nearly symmetrical molecular structure. On the basis of these NMR data and three exchangeable proton signals at δ_H 10.23/10.54/10.62, **6** was established as 2,6,7-trihydroxy-3-methyl naphthalene-1,4-dione.

The activity of compounds **1**−**9** against influenza A virus (H1N1) was evaluated by the CPE inhibition assay.[18,19] Compounds **2**−**4** and **7** exhibited stronger anti-H1N1 activity than ribavirin (positive control) with IC_{50} values of 61.3, 64.0, 85.3, 58.6, and 100.8 μM, respectively, while other compounds were inactive (IC_{50} > 200 μM). New anti-influenza compounds **2** and **3** with anti-H1N1 activity were produced by *P. purpurogenum* JS03-21 only at low pH, demonstrating that acid-tolerant fungi might be a promising source for drug discovery.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Waters 2487 absorbance detector. CD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were taken on a Nicolet NEXUS 470 spectrophotometer as KBr disks. ^{1}H and ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer for compounds **1**−**6** using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS was measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min]. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF254 (10−40 μm) and over silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Sweden), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory).

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Fungal Material. The fungal strain *Penicillium purpurogenum* JS03-21 was isolated from the local red soil used for manufacturing purple pottery from Jianshui, Yunnan Province, China. It was identified according to its morphological characteristics and analyses of its 18S rRNA sequence (GenBank accession No. HQ434765) by Prof. C. X. Fang, China Center for Type Culture Collection. A voucher specimen is deposited in our laboratory at −80 °C. The working strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. *P. purpurogenum* JS03-21 was grown under static conditions at 22 °C for 35 days in 143 1000-mL conical flasks containing liquid medium (300 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), and yeast extract (3 g/L) after adjusting its pH to 2.0. The fermented whole broth (45 L) was filtered through cheesecloth to separate the filtrate from the mycelia. The filtrate was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with an equivalent volume of EtOAc to give an EtOAc solution, which was concentrated under reduced pressure to give EtOAc extract A (29.1 g). The mycelia were extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with an equivalent volume of EtOAc to give another EtOAc solution, which was concentrated under reduced pressure to give EtOAc extract B (68.2 g).

Purification. Extract A (29.1 g) and extract B (68.2 g) were subjected to VLC using step gradient elution with MeOH−CH_2Cl_2 (0−100%) to give 11 fractions (A1−A11) and four fractions (B1−B4), respectively. Fraction A6 was subjected to CC over Sephadex LH-20 eluting with MeOH to afford six subfractions (A6.1−A6.6). Fraction A6.6 was further purified on semipreparative HPLC (61% MeOH−H_2O) to give compound **8** (42.0 mg, t_R 18.2 min). Fraction A7 was fractionated on a silica gel column eluting with CH_2Cl_2−MeOH (50:1−30:1), and then fractionationed on Sephadex LH-20 eluting with MeOH to give seven subfractions (A7.1−A7.7). Fraction A7.6 was purified by Sephadex LH-20 with CH_2Cl_2−MeOH (1:1) and was further separated by HPLC (60% MeOH−H_2O) to give **5** (40 mg, t_R 14.5 min) and **3** (44 mg, t_R 17 min). Fraction A7.7 was purified by Sephadex LH-20 with CH_2Cl_2−MeOH (1:1) and was further purified by HPLC (35% MeOH−H_2O + 0.2% CF_3CO_2H) to yield orsellinic acid (14.4 mg, t_R 18 min), **7** (7 mg, t_R 23 min), and **6** (1.9 mg, t_R 28.5 min). Fraction A9 was subjected to CC eluting with CH_2Cl_2−MeOH (50:1−0:100) to afford four subfractions (A9.1−A9.4). Fractions A9.1 and A9.2 were respectively purified by Sephadex LH-20 eluting with MeOH to provide five subfractions (A9.1.1−A9.1.5) and fractions A9.2.1−A9.2.5. Then fraction A9.1.3 was further purified by Sephadex LH-20 with CH_2Cl_2−MeOH (1:1) to provide four subfractions (A9.1.3.1−A9.1.3.4). Compounds **4** (45.0 mg, t_R 16 min) and **2** (132.0 mg, t_R 12.3 min) were purified from fractions A9.1.3.4 and A9.2.4 by semipreparative HPLC eluting with 50% MeOH−H_2O and 45% MeOH−H_2O, respectively. Fraction B1 was chromatographyed over Sephadex LH-20 eluting with MeOH to afford four subfractions (B1.1−B1.4). Fraction B1.3 was further purified on semipreparative HPLC (55% MeOH−H_2O, 4 mL/min) to give compound **1** (255 mg, t_R 9.5 min).

Purpurquinone A (1): yellow powder; $[\alpha]^{23}_D$ −152.2 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.09), 257 (3.84), 351 (4.24) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 358 (−6.2), 306 (+6.6), 270 (−0.4), 253 (+0.3), 238 (−0.2), 215 (+3.5) nm; IR (KBr) ν_{max} 3134, 1742, 1642, 1589, 1445, 1397, 1266, 1170, 1106, 839, 746 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 439.1015 $[M + Na]^+$ (calcd for $C_{21}H_{20}O_9Na$, 439.1005).

Purpurquinone B (2): yellow powder; $[\alpha]^{23}_D$ −576.2 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.89), 230 (3.95), 266 (3.85), 351 (4.25) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 361 (−10.3), 320 (+7.7), 272 (−0.4), 252 (+0.9), 239 (−0.1), 214 (+5.5) nm; IR (KBr) ν_{max} 3148, 1744, 1642, 1587, 1397, 1347, 1286, 1175, 1095, 949, 767 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 431.0965 $[M - H]^-$ (calcd for $C_{21}H_{19}O_{10}$, 431.0978).

Purpurquinone C (3): yellow powder; $[\alpha]^{23}_D$ −604.1 (*c* 0.8, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.20), 264 (4.13), 353 (4.42) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 355 (−4.6), 306 (+3.9), 273 (−0.6), 221 (+3.2) nm; IR (KBr) ν_{max} 3114, 1741, 1642, 1584, 1561, 1445, 1440, 1324, 1274, 1173, 1101, 845, 746 cm^{-1}; ^1H and ^{13}C NMR data see Table 1; HRESIMS *m/z* 399.1061 $[M - H]^-$ (calcd for $C_{21}H_{19}O_8$, 399.1080).

Purpurester A (4): yellow solid; $[\alpha]^{23}_D$ −7.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.26), 248 (3.97), 315 (3.96) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 280 (−0.3), 212 (+2.0) nm; IR (KBr) ν_{max} 3138, 2964, 1741, 1518, 1459, 1388, 1268, 1216, 1140, 1025, 902, 860 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 251.0907 $[M - H]^-$ (calcd for $C_{13}H_{15}O_5$, 251.0919).

Purpurester B (5): yellow powder; UV (MeOH) λ_{max} (log ε) 213 (3.82), 238 (3.95), 349 (3.59) nm; IR (KBr) ν_{max} 3158, 2971, 1724, 1611, 1400, 1325, 1206, 1128, 1035, 999, 856, 780 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 219.0663 $[M - H]^-$ (calcd for $C_{12}H_{11}O_4$, 219.0657).

2,6,7-Trihydroxy-3-methylnaphthalene-1,4-dione (6): red powder; UV (MeOH) λ_{max} (log ε) 217 (3.43), 268 (3.89), 305 (3.62) nm; IR (KBr) ν_{max} 3134, 1738, 1569, 1518, 1399, 1320, 1193, 1147, 1020, 966 cm^{-1}; ^1H and ^{13}C NMR data see Table 2; HRESIMS *m/z* 219.0296 $[M - H]^-$ (calcd for $C_{11}H_7O_5$, 219.0293).

■ ASSOCIATED CONTENT

Supporting Information. Bioassay protocol used, the NMR spectra of **1**−**6**, and 18S rRNA sequence of *P. purpurogenum* JS03-21. These materials are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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