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NOTE

Two new anthraquinones from the soil fungus *Penicillium purpurogenum* SC0070

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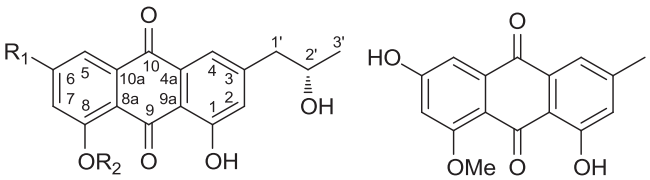
The genus *Penicillium* comprises >300 species,[1] which produce a variety of novel bioactive compounds. Well-known drugs from this genus are penicillin antibiotics produced by *P. chrysogenum*[2] and the antifungal metabolite griseofulvin produced by *P. griseofulvum*[3] and *P. patulum*.[4] As a part of an ongoing program aimed at exploring antibacterial natural products produced by the fungi obtained from South China,[5–7] we have investigated the bioactive compounds of the fungus *P. purpurogenum* SC0070 isolated from a soil sample collected under a palm grove. We recently reported that cultures of *P. purpurogenum* SC0070 produced a rearranged sterol with anti-inflammatory and cytotoxic properties, penicillitone, and a new related sterol, penicillisterol.[8] In this paper, continuing chemical investigation of the fungus has resulted in the isolation of two new anthraquinones, penipurdin A (**1**) and B (**2**), together with a known analog, questin (**3**). Details of the isolation, structure elucidation and biological activities of the compounds are presented here.

Solid-substrate fermentation cultures of *Aspergillus versicolor* SC0070 grown on wheat grains were extracted with 95% EtOH, and the resultant extract was sequentially partitioned with petroleum ether, $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$ and EtOAc extract were fractionated by silica gel column chromatography, followed by Sephadex LH-20 and/or reversed-phase HPLC, to afford compounds **1**–**3** (Figure 1).

Penipurdin A (**1**) was obtained as an amorphous organge-red solid ($[\alpha]^{20}_D$+33.3, *c* 1.14, MeOH). Its molecular formula was determined as $C_{18}H_{16}O_6$ from HRESIMS (found *m/z* 329.1022 $[M+H]^+$, calcd 329.1020) and NMR data. The ^1H and ^{13}C NMR spectra of **1** (Table 1) revealed the presence of two methyl groups including one *O*-methyl, one methylene, one *O*-methine, 12 aromatic carbons (four of which were protonated) and two conjugated ketone carbonyl groups (δ_C 182.6 and 186.6). These spectroscopic features suggested that **1** has the same anthraquinone skeleton as found in questin (**3**)[9] that was isolated from the crude extract of the same fungus. The substituents and their location on the anthraquinone ring were established by the analysis of the ^1H–^1H COSY, HMBC and NOESY spectra of **1** (Figure 2a). The chelated hydroxyl group at δ_H 13.22 exhibited HMBC correlations to C-1, C-2 and C-9a, establishing the location of this hydroxyl group at C-1. ^1H–^1H COSY connectivities of H_2-1' to H-2' (δ_H 3.88) and H-2' to H_3-3' suggested the presence of a 2-hydroxypropyl group. HMBC correlations of H_2-1' to C-2, C-3 and C-4 indicated that the 2-hydroxypropyl group was attached at C-1. For the left portion of **1**, a pair of protons [δ_H 7.21 and 6.84 (each 1H, d, J=2.0 Hz)] were located at C-5 and C-7, respectively, on the basis of HMBC correlations from H-5 (δ_H 7.21) to the carbonyl carbon C-10 (δ_C 182.6). The methoxyl group at δ_H 3.90 was placed on C-8 based on its correlation with H-7 (δ_H 6.84) in the NOESY spectrum. Consequently, the remaining phenolic hydroxyl group was assigned to C-6, which was further supported by the chemical shift for C-6 at δ_C 164.6. The absolute configuration at C-2' of **1** was determined by the modified Mosher method.[10] Treatment of **1** with (*S*)-MPA (methoxyphenylacetic acid) or (*R*)-MPA in the presence of DMAP (4-dimethylaminopyridine) afforded the (*S*)-MPA ester or (*R*)-MPA ester, respectively. The values of $\Delta\delta^{R,S}$ ($\delta_R-\delta_S$) in the ^1H NMR (Figure 2b) spectra suggested that the absolute configuration at C-2' of **1** was *S*. Therefore, the structure of **1** was proposed as shown in Figure 1, and named penipurdin A.

Penipurdin B (**2**) was isolated as a yellow powder. High-resolution ESIMS showed a peak at *m/z* 299.0916 $[M+H]^+$ (*m/z* calcd 299.0914)



1 R_1 = OH R_2 = Me
2 R_1 = R_2 = H

3

Figure 1 Structures of compounds **1–3** isolated from *P. purpurogenum* SC0070.

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Table 1 ^{1}H- and ^{13}C-NMR data of Penipurdin A (1) and B(2)

	*Penipurdin A (**1**)*[a]		*Penipurdin B (**2**)*[b]	
No.	*δ_C, type*	*δ_H, mult. (J in Hz)*	*δ_C, type*	*δ_H, mult. (J in Hz)*
1	161.6, C		163.2, C	
2	125.0, CH	7.14, d (1.2)	125.6, CH	7.27, d (1.2)
3	148.8, C		152.4, C	
4	119.9, CH	7.48, d (1.2)	122.2, CH	7.72, d (1.2)
4a	131.9, C		134.1, C	
5	107.2, CH	7.21, d (2.0)	120.3, CH	7.80, dd (8.2, 1.4)
6	164.6, C		138.3, CH	7.83, t (8.2)
7	105.1, CH	6.84, d (2.0)	125.1, CH	7.36, dd (8.2, 1.4)
8	163.8, C		163.3, C	
8a	112.9, C		116.9, C	
9	186.6, C		193.8, C	
9a	114.8, C		115.0, C	
10	182.6, C		182.3, C	
10a	137.1, C		134.8, C	
1'	45.1, CH_2	2.68, dd (12.9, 7.2)	46.5, CH_2	2.84, dd (13.4, 7.7)
		2.74, dd (12.9, 5.2)		2.91, dd (13.4, 4.8)
2'	66.8, CH	3.88, overlapped	68.2, CH	4.11, m
3'	23.5, CH_3	1.09, d (6.2)	23.9, CH_3	1.23, d (6.2)
2'-OH		4.70, brs		3.86, d (5.0)
1-OH		13.22, s		12.06[c]
6-OH		11.27, brs		
8-OMe	56.5, CH_3	3.90, s		
8-OH				11.95[c]

Abbreviation: No, number.
[a]In DMSO-d_6.
[b]In actone-d_6.
[c]These assignments are interchangeable.

corresponding to the molecular formula $C_{17}H_{15}O_5$. The ^{1}H and ^{13}C NMR data of **2** were greatly similar to those of **1** except for the absence of the methoxyl (8-OMe, **1**) and phenolic hydroxyl (6-OH, **1**) groups, which were replaced by a chelated hydroxyl group and an aromatic proton, respectively. The gross structure of **2** was further confirmed by COSY and HMBC experiments and the *S* configuration at C-2' was assigned by comparison of the sign of the optical rotation value of **2** (+14.2) with that of penipurdin A (**1**; +33.3).

The known compound **3** was identified as questin by comparison of its NMR and MS data with those reported.[9]

The cytotoxicities of compounds **1**, **2** and **3** were evaluated *in vitro* against the A549, HepG2 and Hela cell lines by MTT method.[11] Compound **2** exhibited cytotoxic activity with IC_{50} values of 74.7, 3.9 and 15.7 μM, respectively. The IC_{50} values of compound **3** were 39.5, 6.2 and 14.9 μM, respectively. Compound **1** showed no cytotoxic activity to the three tested cell lines.

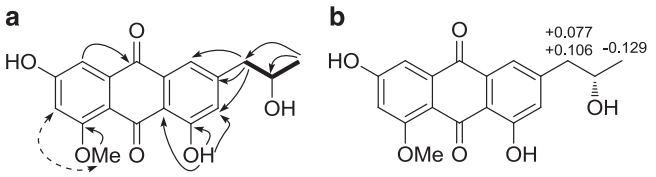

Figure 2 (**a**) Key HMBC (solid arrows), ^{1}H–^{1}H COSY (bond lines) and NOESEY (dashed arrow) correlations of **1**. (**b**) Observed chemical shift differences ($\Delta\delta = \delta_R - \delta_S$, p.p.m., 400 MHz) for the (*R*)- and (*S*)-MPA esters of **1**.

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