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A New Cytotoxic Compound from *Penicillium auratiogriseum*, Symbiotic or Epiphytic Fungus of Sponge *Mycale plumose*

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Abstract: A new compound, (*S*)-2, 4-dihydroxy-1-butyl (4-hydroxy) benzoate (**1**), and a known compound, fructigenines A (**2**), were isolated from fungus *Penicillium auratiogriseum* derived from sponge *Mycale plumose*, by bioassay-guided fractionation. Their structures were established by spectroscopic and chemical methods. Both compounds showed cytotoxic activity against tsFT210 cells.

Keywords: *Penicillium auratiogriseum*, *Mycale plumose*, aromatic ester, alkaloid, anti-tumor activity.

To investigate the bioactive metabolites from marine microbes, the fungus *Penicillium auratiogriseum* was isolated from the sponge *Mycale plumose* collected in Qingdao, China. The ethyl acetate extract of the fermentation broth of *P. auratiogriseum* showed significant cytoxicity in the mouse cdc2 mutant cell line (tsFT210). Thus 5 L of liquid medium was inoculated with the purely isolated fungus and incubated at 25 ˚C for 9 days. The cultured broth was extracted three times with the equivalent volume of ethyl acetate and evaporated under reduced pressure to yield an active gum. The gum (3.5 g) was subjected to a repeated flash column chromatography over silica gel H, Sephadex LH-20 and HPLC separation to afford a new aromatic ester, (*S*)-2, 4-dihydroxy-1-butyl (4-hydroxy) benzoate (**1**, 6 mg), and a known diketopiperazine alkaloid, fructigenines A (**2**, 18 mg) (**Figure 1**)[1]. A cytotoxic test against tsFT210 cell was performed to screen the active ingredient during the course of purification.

Compound **1**, obtained as a amorphous white powder, UV λ_{max}^{MeOH} nm: 224, 254, 262; IR ν (KBr, cm^{-1}): 3731, 3625 (OH), 1678 (conjugated ester CO), 1608, 1512 (benzene ring), 760; $[\alpha]_D^{22}$ -8.36 (*c* 0.013, CH_3OH). The negative HRESIMS exhibited the molecular ion peak at *m/z* 225.2409 $[M-H]^-$ corresponding to the molecular formula $C_{11}H_{14}O_5$ (calcd. 225.2179 for M-H). ^{1}H-NMR spectra at δ 7.95 (d, 2H, J=8.82 Hz) and 7.01 (d, 2H, J=8.82 Hz) and ^{13}C NMR (DEPT) spectra at δ 164.4 (s), 132.6 × 2 (d), 123.4 (s) and 115.4 × 2 (d) implied a moiety of 1, 4-disubstituted benzene ring in the molecule. ^{1}H-NMR spectra at δ 4.11 (m), 4.03(dd, J=4.05, 9.66 Hz), 3.97 (dd, J=6.42, 9.66 Hz),

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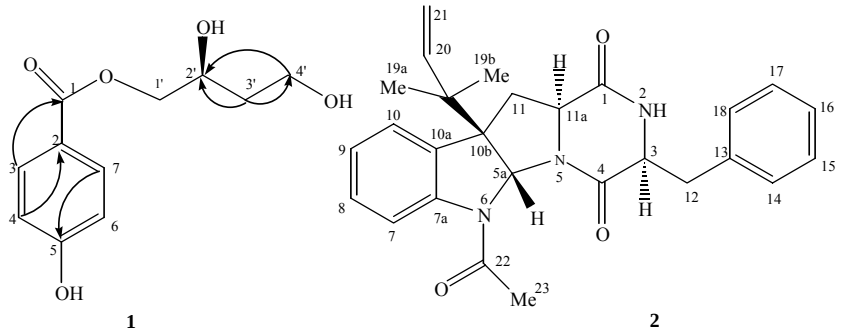
3.75 (dd, 2H, J=5.82, 6.96 Hz), 1.84 (m) and 1.75 (m) and ^{13}C-NMR (DEPT) spectra at δ 168.5 (s), 73.6 (t), 68.2 (d), 59.7 (t) and 37.1 (t) corresponded to the ester carbonyl, two oxygenated methylene, one oxymethine and one high field methylene group (**Table 1**). ^{1}H-^{1}H COSY spectra further revealed that these groups were correlated each other, forming $HOCH_2$-CH(OH)-CH_2-CH_2-OH moiety. 4-Hydroxybenzoic acid (**1a**) and (*S*)-1

Figure 1 The structures of **1** and **2** and the key HMBC correlations of **1**



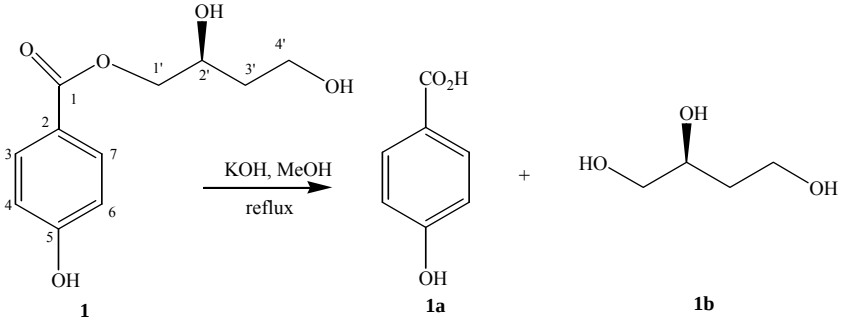
1 **2**

Table 1 The NMR spectra data of compound **1** (CD_3OD, δ ppm) [a]

position	δ_H	δ_C	HMBC [b]	^{1}H-^{1}H COSY
1	/	168.5s	/	/
2	/	123.7s	/	/
3, 7	7.95 (d, 2H, J=8.82 Hz)	132.6d	C-1, C-5, C-7	4, 6
4, 6	7.01 (d, 2H, J=8.82 Hz)	115.4d	C-2, C-6	3, 7
5	/	164.4s	/	/
1′	4.03 (dd, J=4.05, 9.66 Hz), 3.97 (dd, J=6.42, 9.66 Hz)	73.6t		2′
2′	4.11 (m)	68.2d		1′, 3′
3′	1.84 (m), 1.75 (m)	37.1t	C-2′, C-4′	4′
4′	3.75 (dd, 2H, J=5.82, 6.96 Hz)	59.7t	C-2′, C-3′	3′, 2′

[a] ^{1}H, ^{13}C NMR and HMBC, ^{1}H-^{1}H COSY spectra were obtained at 600, 150 and 600 MHz, respectively.

Figure 2 The hydrolysis of compound **1** by KOH



1 **1a** **1b**

2, 4-butanetriol (**1b**) were obtained after hydrolysis of **1** by 10% solution of KOH in MeOH (**Figure 2**). The structures of **1a** and **1b** were identified by comparing their ^{1}H spectra and optical rotation with those of authentic samples[2, 3]. Besides, the following key long-range correlations between ^{1}H and ^{13}C were observed in the HMBC experiments of **1**: between H-3 (δ 7.95) and C-1 (δ 168.5, s), between H-4 (δ 7.01) and C-2 (δ 123.4, s), between H-3' (δ 1.84 and 1.75) and C-2' (δ 68.2, d) and C-4' (δ 59.7, t), between H-4' (δ 3.75) and C-2' (δ 68.2, d). Thus, the structure of compound **1** was unambiguously elucidated as (*S*)-2, 4-dihydroxy-1-butyl (4-hydroxy) benzoate.

In addition, compounds **1** and **2** were tested for their antitumor activity in tsFT210 cells line. Both of them showed potent cytotoxic effects in tsFT210 cells, with maximum inhibitory effect observed at 8.0 and 22.0 μg/mL, respectively.

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References and Notes

1. K. Arai, K. Kimura, T. Mushiroda, Y. Yamamoto, *Chem. Pharm. Bull.,* **1989**, *73*: 2937.
2. 4-Hydroxybenzoic acid (**1a**) was obtained as amorphous powder. Positive ESIMS *m/z* (%): 139.13 $[M+H]^+$; ^{1}H NMR (600 HMz, d_6-acetone, in ppm): 7.91 (d, 2H, J=8.76 Hz), 6.91 (d, 2H, J=8.76 Hz).
3. a. P. J. Kocienski, C. Yeates, S. D. A. Street, S. F. Campbell, *J. Chem. Soc*., *Perkin Trans. I*, **1987**, 2183.
   b. (*S*)-1, 2, 4-butanetriol (**1b**) was obtained as oil; $[\alpha]_D^{22}$-28.6 (*c* 0.025, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$, in ppm): 3.65 (m, 2H), 3.54 (m, 2H), 3.40 (m, 1H), 1.25 (m, 2H).

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